OSBI Forensic Biology Units' Training Manual
A Training Program for Forensic Biology
Revision 10
Effective December 1, 2023

ATTENTION:

This training manual provides general information for new analysts and technicians to become qualified to perform independent casework analysis in the areas for forensic serology and forensic DNA. The information presented here is not intended to be policy, but for informational purposes only. The protocols and requirements for specific tests can be found in the OSBI Forensic Biology Units' Policy Manual and should be followed when performing specific tests and procedures. Although this manual is written for analysts in the Forensic Biology Units, the training for a technician in any of these units is very similar with the exception of interpretation of results and report writing. Therefore, technicians will follow the applicable sections of this manual for their training, with the exception of areas indicated with an asterisk (*) in the table of contents below. Additionally, in the body of the manual, sections specific to technicians are indicated with *italicized font* and an asterisk (*). Analysts and technicians may or may not be trained in all areas, depending on the needs of the OSBI Forensic Biology Discipline at the current time.

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General Training

1 Overview of Training Program

1.1 Purpose and Scope

1.1.1 The purpose of this document is to provide a training program for the Forensic Biology Units within the Oklahoma State Bureau of Investigation (OSBI) Criminalistics Services Division. This training program supplements successful completion of college course work in biochemistry, molecular biology, and genetics. The OSBI Forensic Biology Unit requires, as per the FBI's Quality Assurance Standards (QAS), that all Forensic DNA Analysts complete course work in genetics, biochemistry, molecular biology (molecular genetics or recombinant DNA technology) or other courses which provide a basic understanding of the foundation of forensic DNA analysis, as well as course work and/or training in statistics as it applies to forensic DNA analysis in accordance with QAS. The course work must be successfully completed before the Analyst will be qualified to perform DNA casework. The OSBI Forensic Biology Discipline uses the date of hire/appointment/promotion as the defined date to use for determining the applicable version of the QAS for requirements to assess education, experience, and training of individuals.

1.1.2 This program will provide exposure to methods, techniques, and procedures presently used and accepted by the courts and forensic DNA analysts/serologists. This training will focus on the methods that the OSBI Forensic Biology Units currently utilize to allow proficiency to be developed using both known and simulated casework samples. Trainees are required to successfully complete a competency test prior to performing any activity on evidence. This competency may be for a single method or a combined competency for a set of methods. The training will expose the trainee to court procedures and assist in developing the skills necessary for effective expert witness testimony.

1.1.3 Throughout the training, oral and practical examination and/or informal mock sessions related to case approach and identification of biological substances will be conducted to ensure the trainee comprehends the information and develops the appropriate skills necessary to perform casework analysis.

1.1.4 The training program encompasses both serological and DNA analysis; however, normally serology training will be completed first, with a period of time to perform serological casework prior to completing the DNA portion of the Training Manual. There may be separate or combined mock trials on the serology and DNA portions of the Training Manual. It will be up to the supervisor/Technical Manager which portion of the Training Manual will be completed first or in conjunction and to what order it will be completed, based on the experience of the trainee and the current needs of the Unit.

1.1.5 Upon completion of training, the trainee will undergo a practical examination consisting of a mock case and a mock trial as well as a comprehensive oral examination. The practical examination will consist of a fabricated case, used to evaluate the trainee's technical skills and abilities to perform casework. The trainee will testify to the results on the practical examination, thus likening this test to an actual courtroom situation. The comprehensive oral examination/competency is used to access the trainee's technical knowledge of the aspects associated with performing casework in the OSBI Forensic Biology Units. Satisfactory performance in all areas is required prior to the trainee being released as a qualified Analyst for the Forensic Biology Units. The comprehensive oral examination/competency can be completed prior to or after the mock courtroom testimony and does not have to be completed on the same day – this is decided by the Technical Manager and trainee.

1.2 Coordination of the Training Program

The Forensic Biology Technical Manger will have the overall responsibility of training any new Forensic Biology Analysts. The Technical Manger may assign a trainer to a specific trainee in coordination with the trainee's Supervisor.

1.3 Training Period

The training program will be divided into two areas; serology training and DNA training. Typically, it is estimated that the serology training will be about 4 to 6 months. The Analyst will then be released for independent serology casework for a period of time (typically two to six months). Then the Analyst will begin their DNA training, which is estimated to be complete within 6 to 12 months – with a minimum training period of 6 months in accordance with QAS requirements for at least 6 months of documented forensic human DNA laboratory experience. Some individuals may require less time to complete their training than others, depending on the experience and education of the trainee. The qualifications of the trainee will be evaluated and modifications will be made to the training program as appropriate. Any modifications will be approved and documented by the Technical Manager. The length of the training period will be the discretion of the Technical Manager and the trainee's Supervisor.

1.4 Training Location

Whenever practical, the bulk of an individual's training will occur in the laboratory to which the trainee will be assigned. If this is not possible, the training will be conducted at the most convenient laboratory. These arrangements will be made through the FBU Technical Manager and in conjunction with the appropriate Supervisor and/or Administrator. Oversight and direction of the training will be provided by the Technical Manager.

1.5 Instructions for the Trainer

1.5.1 The intent of the training program is to ensure that every trainee is provided with the basic principles and fundamentals necessary for the complete education of a Forensic Biologist. All of the listed topics will be incorporated into the training program; however, education and prior experience of the trainee will be used to guide the amount of time devoted to any one topic. The sequence in which the tasks are presented in the Manual is not a mandatory order for the instruction. Exposure to certain goals (i.e., testimony) may be continuous throughout the training despite the successful completion of a specific training section. The Technical Manager will issue a memorandum to the trainee and trainer regarding the commencement of the training and the assignment of the trainer.

1.5.2 The trainer will document the completion of each required training task by the trainee on the checklist for that aspect of training.

1.5.2.1 The checklist will be retained by the trainee in the appropriate section of the trainee's notebook.

1.5.2.2 The completed final checklist will be retained in the trainee's final notebook once they are released for independent casework.

1.5.3 The trainee will be evaluated on their performance during the course of the training program. The trainer will submit a monthly evaluation of the trainee's progress to the Technical Manager, trainee, trainee's Supervisor, and Administrator. The monthly training evaluation is due to the Administrator for each trainee by the fifth working day of the month.

1.5.4 The monthly training evaluation will be in the form of a memorandum and include the following items:

1.5.4.1 A summation of the progress made during the month

1.5.4.2 An evaluation of the trainee's notebook

1.5.4.3 An evaluation of the progress during the month, to include:

1.5.4.3.1 Problem areas, as applicable, and their solution or proposed solutions

1.5.4.3.2 Trainee's strong points.

1.5.4.3.3 Trainee's weak points and suggested remedies.

1.5.4.3.4 Statement concerning trainee's overall performance.

1.5.4.4 Plans for the upcoming month

1.5.5 The monthly training memorandum will become part of the training history of the trainee and will be used to document the trainee's progress toward qualification. There will be one memorandum per trainee. The monthly training memorandum format is provided in Appendix C.

1.5.6 The trainer will discuss the monthly evaluation with the trainee and/or their Supervisor if appropriate. Any comments will be included in the monthly training memorandum.

1.5.7 When the trainee has satisfactorily completed all training requirements, a memorandum will be issued by the Technical Manager stating that the trainee will be released to perform the duties of a qualified analyst. If the trainee cannot meet the criteria expected of them during the period allowed for training in each of the areas, steps will be taken to effect the appropriate action.

1.5.8 At the conclusion of the training, the trainee should have the following:

1.5.8.1 Knowledge of the principles and practices of forensic serology and DNA as they relate to the analysis of case material.

1.5.8.2 Knowledge of the theory and application of instrumentation and specialized techniques used to examine biological evidence.

1.5.8.3 The ability to perform accurate forensic analysis independently and proficiently, to accurately document the findings of all analyses in accordance with the appropriate policies and procedures, and to accurately generate a report on those findings.

1.6 Instructions for the Trainee

1.6.1 The trainee is expected to keep a training notebook on all work completed. A digital training notebook will be maintained with the use of Microsoft Word and Adobe PDF Professional (recommended). The training notebook will consist of an organized folder(s) containing the training material and will be retained on either the trainee's computer or a network location or both (for reviews by the trainer and Technical Manager). The

completed checklist for each training topic and the monthly training memorandums will be included in the notebook.

1.6.2 The notebook should be organized by Training Manual Sections. Within each Section folder, the trainee must include the following, as applicable:

1.6.2.1 Assignment completion checklist

1.6.2.2 Assignment

1.6.2.3 Graded quizzes or exercises (if applicable)

1.6.2.4 Testing Results

1.6.2.5 Supporting documentation (if applicable)

1.6.2.6 Any additional documentation as necessary

1.6.2.7 A summary of the applicable assignment (if applicable)

1.6.3 For applicable assignments (i.e., serological analyses), the trainee will provide a summary regarding the results of the tests performed and the theory behind the tests for each testing procedure used in this training manual where assigned. The summary should include the following areas:

1.6.3.1 Principle

1.6.3.2 Procedure outline (to include the purpose of critical reagents) or reference the appropriate procedure in the FBU Policy Manual.

1.6.3.3 Sensitivity

1.6.3.4 Specificity

1.6.3.5 Interpretation of results

1.6.3.6 Possible interferences/problems

1.6.3.7 Comments and/or general discussion/observations of procedure/method

1.6.4 The trainee will keep a daily log of all activities (training, casework, quality control, etc.). This daily training log will help the trainer provide the monthly training memorandums for the trainee's monthly activities. The daily log is not required to be part of the trainee's training notebook.

1.6.5 A list of study terms is located in Appendix A of this training manual.

1.6.6 The training program provides the trainee with exposure to various types of samples. Similar samples have been grouped together. Each group of samples can be worked simultaneously, although they may be at different stages of the procedure.

1.6.7 The minimum passing score for all exams, quizzes, competency tests, etc. is an 80% unless otherwise noted and documented by the Technical Manager.

1.7 General Guidelines for Comprehensive Oral Examination/Competency (Technical Question) Session

1.7.1 A comprehensive oral examination will be given to the trainee either at the conclusion of the mock trial or at another date chosen by the trainee and Technical Manger.

1.7.2 The technical questions will be limited to technical aspects of the training and should be used to ascertain whether the goals of the training program have been achieved.

1.7.3 The format of the technical questions will be a board style. This format will include a set of general knowledge questions and a set of scenarios given to the trainee. Any clarification needed to these questions can be attained by follow up questions posed by the panel. This will ensure that the trainee fully understands the technical nature of the Forensic Biologist.

1.7.4 The technical questions may be stopped at any time upon the request of any of the involved parties.

1.7.5 The technical questions will not exceed two hours.

1.7.6 The technical questions may be recorded to be observed at a later date for training purposes.

1.7.7 An evaluation of the trainee will be conducted each panel member. This evaluation will be provided to the Technical Manager and an average score of the trainee will be given.

1.7.8 Immediately following the technical questions, the trainee will be excused, and the panel will discuss and evaluate the trainee's performance. The trainee will then be notified of the results of the technical questions.

1.7.9 The outcome of the technical questions will be:

1.7.9.1 Satisfactory

1.7.9.2 Not Satisfactory

1.7.9.2.1 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

1.7.9.2.2 The technical evaluation must be retaken in its entirety without knowledge of which questions were missed

1.7.9.2.3 If the second technical evaluation is not satisfactory, remedial training and/or progressive discipline will be initiated.

1.8 General Guidelines for the Mock Trial

1.8.1 Every case that a Forensic Biologist analyzes has the potential of testifying in court as an expert witness. It will be the trainee and trainer's responsibility to ensure that the trainee is thoroughly prepared for legal questioning. This can be accomplished by a combination of practice mock trials, question and answer sessions, literature review, and observation of courtroom testimony given by experienced Analysts.

1.8.2 A mock case (fabricated case serving as a competency test) will be prepared and assigned to the trainee, so that the trainer and Technical Manager know the expected

results. This mock case will also serve as the trainee's display in applying the techniques and procedures to actual casework examinations. The trainee will receive the mock case when all applicable sections of the training manual have been satisfactorily completed.

1.8.3 A mock trial (oral practical) will incorporate all aspects of the training and will be held after the trainee has completed their final competency test (mock case).

1.8.4 The mock trial will be formal and will be conducted in a manner as close to a real courtroom atmosphere as possible, including conduct and procedures.

1.8.5 The mock trial will cover standard procedural questions (i.e., qualifying the witness and general laboratory procedures), chain of custody questions, and questions over the reported results.

1.8.6 The Technical Manager and appropriate Supervisor, or an appropriate designee(s), will be included in the trainee's mock trial. The Technical Manager will serve as the "Judge" and the Supervisor will observe or participate in the mock trial process. There will be at least one qualified analyst to act as the "prosecutor" and one to act as the "defense". It will be up to the discretion of the Technical Manager to have other qualified analysts acting as the assisting "prosecuting" and "defense" attorneys. (Note: every effort should be made to have both FBU Technical Manager and appropriate Supervisor observe/participate in the trainee's mock trial; however, if special circumstances arise a designated Lead Analyst may be selected)

1.8.7 The mock trial will not exceed three hours. The "prosecutor" and "defense" attorney may agree to selected items to be introduced at the trial to remain within the set time constraints.

1.8.8 Harassment of the expert witness by the "prosecutor" or "defense" counsel will be kept to the minimum necessary to achieve the common pressure situation(s) encountered during courtroom testimony. Questioning by both the "prosecutor" and "defense" counsel should be relevant and realistic.

1.8.9 The mock trial may be stopped at any time upon the request of any of the involved parties.

1.8.10 The mock trial will be recorded and the recording will be viewed by the trainee at a later date to identify weaknesses and strengths in the trainee's testimony.

1.8.11 Each mock trial panel member will evaluate the trainee's testimony on an evaluation form and submit to the Technical Manager to determine an average score for the trainee.

1.8.12 Immediately following the mock trial, the trainee will be excused and the mock trial panel members will discuss and evaluate the trainee's performance. The trainee will then be notified of the results of the mock trial.

1.8.13 The outcome of the mock trial will be:

1.8.13.1 Satisfactory

1.8.13.2 Not Satisfactory

1.8.13.2.1 If the panel determines that the trainee's performance was not satisfactory, steps must be taken to effect the appropriate action.

1.8.13.2.2 A new mock trial will be conducted. Trainee may be assigned a new mock case, or a previous mock case may be utilized for the second mock trial

1.8.13.2.3 If the second mock trial is not satisfactory, remedial training and/or progressive discipline will be initiated.

1.9 Transition from Trainee to Qualified Analyst

1.9.1 After the trainee has successfully completed all training, there is a period of adjustment. The Supervisor must ensure that the transition from trainee to qualified Analyst takes place as smoothly as possible with appropriate and necessary guidance.

1.9.2 For a period of time, all newly qualified Analysts' reports must be reviewed by the FBU Technical Manager, or designee, prior to closing the case. The Analyst's reports must be monitored closely for at least six months. Once the Analyst has been under review for a period of time (dependent on the number of cases worked by the Analyst, problem(s) encountered, etc.), the Technical Manager will issue a memo releasing the Analyst from Technical Manager, or designee, review of all cases before closing of the case by the Analyst.

1.9.3 The Supervisor, Technical Manager, or designee will accompany the newly qualified Analyst to court for the first few cases.

1.10 Training on Additional Techniques/Methodology for Qualified Analysts

1.10.1 Any new techniques, new methodology, or procedure modifications in the Unit may require additional training for qualified Analysts. The training necessary as a result of the implementation of new techniques, methodologies, and/or procedure modifications will be documented by the Technical Manager before implementation of the new procedure.

1.11 Training on Legacy Data Re-interpretation

1.11.1 Analysts are not to reinterpret any data generated with a legacy amplification test kit on which they are not current or previously qualified and for which they are currently proficiency tested. Per the SWGDAM document titled "*SWGDAM Clarification on the Reinterpretation of Data Typed with Legacy Amplification Test Kits*" (June 2016), assessing/evaluating allele calls, genotype calls (to include potential allelic drop-out), a change in the assumptions used, or removing alleles (or entire loci) from statistical estimates from legacy amplification test kit data, are not considered reinterpretation.

If legacy data needs to be reinterpreted:

If the analyst is sufficiently trained, has reviewed the validation and standard operating procedures for the appropriate application of analytical procedures, (thresholds, peak height ratios), the analyst may review and reinterpret legacy data in accordance with the following:

1) The analyst must be currently qualified (trained and proficiency tested) in the legacy amplification test kit or

2) The analyst must be currently qualified in an amplification test kit (trained and proficiency tested) and the analyst was previously qualified (trained and proficiency tested) in the legacy amplification test kit within the last two calendar years or

3) If the analyst is currently qualified in an amplification test kit (trained and proficiency tested), but was never previously qualified in the legacy amplification test kit, the analyst can reinterpret the legacy amplification test kit data if the analyst is trained in the legacy amplification test kit protocols by a previously qualified analyst. This training must be documented and approved by the Technical Manager and the documentation shall contain sufficient detail for an auditor to confirm the analyst is qualified for the reinterpretation of the legacy amplification test kit data.

- If a previously qualified analyst is not available to train a currently qualified analyst on the legacy amplification test kit, the current analyst should not reinterpret legacy data.

NOTE: If the analyst must reinterpret the legacy amplification test kit data without training from a previously qualified analyst, then the analyst and the Technical Manger shall train in the legacy test kit interpretation protocols, review all validation data, and standard operating procedures for the legacy amplification test kit. The analyst will be given an interpretation competency test. The training and successful completion of the competency test will be documented and approved by the Technical Manager and the documentation shall contain sufficient detail for an auditor to confirm the analyst is qualified for the reinterpretation of the legacy amplification test kit data.

NOTE: Technical Reviewers of reinterpretations of legacy data are required to have the same qualifications as the analyst reinterpreting the data.

As a result of these requirements, the OSBI FBU will have at least one analyst that meets the above specifications for the review of legacy data when necessary.

1.12 Re-Training of Previously Qualified Analysts

1.12.1 Opportunities for re-training of previously qualified analysts may present due to extended absences, disqualification in a technique/method/category of testing or an indication that training was not effective, transfer between disciplines, etc. If a previously qualified analyst needs re-training, the training program will be structured in accordance with the parameters of OSBI CSD QP 19 – Training section II.F. as well as this Training Manual, and may be modified, as needed, at the discretion of the Technical Manager.

2 Orientation to the OSBI

2.1 Goals

2.1.1 To ensure the trainee is familiar with the laboratory facility and other laboratory personnel prior to performing any work within the laboratory.

2.1.2 To ensure the trainee understands the standard administrative documentation/reports that must be submitted to their Supervisor.

2.1.3 To introduce the trainee to the organizational structure of the Agency, Division, Unit, and be familiar with the chain of command.

2.1.4 Introduce the trainee to the location and become familiar with the content within the agency policy and procedure manual.

2.1.5 To introduce the trainee the topic of accreditation and the location of the protocols.

2.2 Organizational Structure of the OSBI

2.2.1 Chain of command is the line of authority through which the employee reports. The chain of command for a Forensic Biologist proceeds from lowest to highest as follows: Supervisor, Criminalistics Administrator, Criminalistics Services Division (CSD) Director, OSBI Deputy Director, and OSBI Director. If possible, report to your Supervisor first. In the absence of your immediate Supervisor, proceed up the chain of command. Issues should be handled at the lowest level of the chain of command as possible and in compliance with any applicable unit, division, and/or agency policy.

2.2.2 An agency-wide organizational chart for the OSBI is available via a link present on the OSBI intranet (http://osbinet) homepage.

2.2.3 An organizational chart for the OSBI Criminalistics Services Division is available as an attachment to the OSBI CSD Quality Manual.

2.3 Orientation to the OSBI Laboratory System

2.3.1 The trainee will be given a tour of his/her assigned laboratory facility by his/her Supervisor or their designee. If the trainee is assigned to a regional laboratory, the trainee will also tour the Forensic Science Center in order to be introduced into each discipline within the OSBI Criminalistics Services Division.

2.3.2 OSBI Criminalistics Services Division Disciplines

2.3.2.1 Forensic Biology Units – The OSBI Forensic Biology Units examine evidence in order to identify potential biological fluids and/or hairs present. Appropriate biological samples are subjected to DNA analysis through the polymerase chain reaction (PCR) using capillary electrophoresis in order to compare crime scene (forensic unknown) DNA profiles with DNA profiles developed from known reference samples. DNA profiles are forwarded for entry into the Combined DNA Index System (CODIS) database, if eligible.

2.3.2.2 CODIS Unit – The Combined DNA Index System (CODIS) Unit is responsible for maintaining the State's CODIS database. Offender samples are analyzed in order to input known DNA profiles into the CODIS database. Eligible forensic and Legal DNA profiles forwarded from the Forensic Biology Units are input into

the CODIS database. Upon receiving a CODIS "hit" or match of two profiles in the database, verification is performed by re-analyzing the offender sample. Law enforcement agencies are notified once a hit has been verified.

2.3.2.3 Controlled Substances (Seized Drugs) - This Unit is used to aid officers in the investigation of drug offenses. They typically identify drugs (i.e., marijuana, cocaine, etc.) that are not found in the body.

2.3.2.4 Firearms and Toolmarks Unit – This Unit is a specializes in the identification of fired ammunition components (bullets and cartridge cases), firearms functionality, Integrated Ballistics Identification System (IBIS) entry, serial restoration, distance determination, and toolmark identification.

2.3.2.5 Latent Evidence Unit – This Unit specializes in latent print, footwear, and tire impression examination. The duties of a latent print examiner include crime scene processing, laboratory processing of evidence, collecting inked impressions of deceased individuals, and comparing unknown impressions to known impressions in order to effect identifications. Some of the tools utilized in accomplishing these tasks include the Automated Fingerprint Identification System (AFIS), the FBI's Next Generation Identification (NGI), digital imaging equipment, and various chemical and physical development techniques.

2.3.2.6 Toxicology Unit – The objective of the Toxicology Unit is to provide forensic toxicology services to state, county, and local law enforcement agencies throughout the State of Oklahoma where laboratory services are not available. Toxicology services are provided for agencies submitting blood samples in cases involving violation of Title 47 of the Oklahoma State Statute. Most of the cases in the Toxicology Unit are the identification of chemicals in the blood or urine of an individual, as well as determining blood alcohol concentration.

2.3.2.7 Trace Evidence – This Unit offers analysis in fibers, paint, physical match, gunshot residue, ignitable liquids, and several others. A trace evidence Analyst spends years training in these specified fields. Each sub-discipline takes dedication and hundreds of hours in training in order to become proficient.

2.3.3 Introduction to Laboratory Security

2.3.3.1 The OSBI CSD maintains the integrity and prevents contamination of evidence and ensures the confidentiality of records by limiting access to restricted areas to authorized personnel.

2.3.3.2 The security of the laboratory is governed under QP 20 in the Quality Manual.

2.3.3.3 The issuance of any security devices and the responsibility of the trainee for maintaining those security devices will be mandated according to the policies set forth in QP 20.

2.3.4 Monthly Documentation

2.3.4.1 Hours spent associated with training (# hours presenting, # hours preparing for presentation, and # hours attending training) as well as the number of hours spent associated with Court appearances (# of appearances for jury trials, preliminary hearing, etc.) will be provided to Supervisors for inclusion in quarterly management reports.

2.3.4.2 The trainee will be required to submit a list of activities (Activity Log) performed during the month to the trainer to provide the monthly training memorandum as described above. This is due to the trainer on the third working day of the month.

2.3.5 Accreditation

2.3.5.1 Accreditation is the status that has been achieved through the completion and maintenance of certain quality requirements that have been enacted by a governing body.

2.3.5.2 The OSBI CSD has adopted the standards in ISO/IEC 17025 (set forth by the American National Standards Institute (ANSI) – American Society of Quality (ASQ) National Accreditation Board (ANAB) accreditation program).

2.3.5.2.1 The International Standards are accredited with the International Organization for Standardization and the International Electrotechnical Commission (ISO/IEC) 17025 standards.

2.3.5.3 The accreditation is required to be renewed every four years and with accreditation with the ISO/IEC 17025 standards there is an annual surveillance visit that is required.

2.3.5.4 The OSBI Laboratory has been accredited with the 17025 ASCLD/Lab legacy standards since 2001, with re-accreditation in 2006. The laboratory system has been accredited with the 17025 international standards since January 20, 2012. In January 2019, the OSBI Laboratory was re-accredited by ANAB to the 17025 international standards.

2.3.6 Proficiency Testing

2.3.6.1 A Forensic Biologist is required to be proficiency tested annually in serology and semi-annually in DNA. Analysts qualified in more than one DNA technology[1] (e.g., STR, Y-STR, etc.) shall be proficiency tested in each technology at least once per calendar year. Typically, one of the DNA proficiency tests also includes a serology proficiency test and can count as a serology and DNA proficiency test. The Analyst (or Technicians) must enter the proficiency testing cycle within six months of their qualification date.

2.4 Tasks

2.4.1 Read the following references:

2.4.1.1 Read the Quality Manual sections: Foreword, OSBI Mission, OSBI Vision, and QM 5.0 and sub-sections Organization, 8.0 and sub-sections Management System and Quality Procedures section, QP 1 Responsibilities and Authority, QP 20 Lab Security, and QP 30 Proficiency Tests.

2.4.1.2 Read the following Forensic Biology Policy Manual sections: CWQM_3 Quality Assurance Program, CWQM_4 Organization & Management, CWQM_5 Personnel, CWQM_6 Training, CWQM_7 Facilities & Evidence Control, and CWQM_13 Proficiency Testing.

[1] *Technology* is used to describe the type of forensic DNA analysis performed in the laboratory, such as RFLP, STR, Y-STR, XSTR, SNP, microhaplotypes or mitochondrial DNA.

2.4.1.3 Read the following agency policies: Intro 2, Intro 3, Intro 4, Intro 7, Intro 8, and any other applicable agency policies throughout the training program.

2.4.2 Complete the OSBI CSD New Employee General Training Manual (on QMS).

2.5 Evaluation

2.5.1 The trainer and/or designee will review the information with the trainee and ensure they have demonstrated knowledge of the above topics.

2.5.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the above tasks.

2.5.3 Documentation of the completion of the OSBI CSD New Employee General Training Manual (and any associated assignments) will be maintained in the trainee's training notebook.

3 Safety

3.1 Goals

3.1.1 To provide the trainee with information covering the laboratory's environmental health and safety program.

3.1.2 To provide the trainee with the appropriate personal protective equipment (PPE).

3.1.3 To familiarize the trainee with the safety equipment location and procedures within the laboratory.

3.2 Chemical Hygiene

3.2.1 A chemical hazard is any chemical that is a physical or health hazard.

3.2.2 Hazardous materials consist of:

3.2.2.1 Acids and bases

3.2.2.2 Chloroform

3.2.2.3 O-tolidine

3.2.2.4 Carcinogens, teratogens, and mutagens

3.2.3 All trainees must have received and maintain access to Oklahoma State Department of Health (OSDH) Form 207.

3.2.4 All trainees must be provided the proper Personal Protective Equipment (PPE).

3.2.5 The Safety Coordinator or designee will provide the trainee instruction on the safety equipment throughout the laboratory, to include the following:

3.2.5.1 Location and operation of showers, eyewashes, and fire extinguishers

3.2.5.2 Location and procedures for biohazard trash

3.2.5.3 Location and procedures for re-usable PPE (laboratory coats, masks, and goggles)

3.2.6 The trainee will review all applicable OSBI Policies and Procedures on safety to include the following:

3.2.6.1 OSBI Policy and Procedure Policy 121; to include all subsections

3.2.6.2 OSBI Policy and Procedure Accident Report Form 121.5A

3.2.6.3 OSDH Form 207: Any injury, chemical exposure, or biohazard risk exposure should be reported immediately to the employee's Supervisor; any procedural guidelines and medical recommendation will be followed, if necessary.

3.3 Bloodborne Pathogens

3.3.1 All trainees will attend training on bloodborne pathogens as organized by the Safety Coordinator.

3.3.2 A Forensic Biologist must be acutely aware of the potential hazards to themselves and others during the examination of biological specimens and the use of hazardous materials.

3.3.3 A biohazard is defined as a material of biological composition, especially those that are infectious in nature that constitutes a threat to man or to his environment.

3.3.4 These hazards include, but are not limited to the following:

3.3.4.1 Bloodborne Pathogens

3.3.4.1.1 Hepatitis

3.3.4.1.2 AIDS

3.3.4.1.3 Sexually Transmitted Diseases

3.3.4.1.4 Parasites

3.3.4.1.5 Bacterial Infections

3.4 Safety Procedures

3.4.1 Personal Protection

3.4.1.1 Use gloves, masks, safety glasses, and other protective clothing and equipment as appropriate

3.4.1.2 Avoid production of aerosols

3.4.1.3 No mouth pipetting

3.4.1.4 Material Safety Data Sheets (MSDS) and Chemical Information List (CIL)

3.4.1.4.1 A Material Safety Data Sheet (MSDS) is a written information sheet concerning a chemical. Each MSDS is prepared in accordance with 20 CFR 1910.1200. An MSDS typically consists of information prepared by the manufacturer and details potential hazards, characteristics, and precautions relevant to the product.

3.4.1.4.2 The Chemical Information List is a complete list of all chemicals in the work place or unit.

3.4.1.4.3 All trainees will know the location of the MSDS and CIL for the laboratory. The MSDS and CIL are maintained electronically and the trainees will have access to the MSDS and CIL from their computers.

3.4.2 Biosafety Practices

3.4.2.1 Follow the appropriate procedures for cleaning yourself, your work areas, and equipment.

3.4.2.2 All biological materials and containers/supplies that have come in contact with biological materials and/or hazardous chemicals will be placed in biohazard bags, which will be disposed of properly.

3.4.2.2.1 **Trainee will be made aware of the hazards of co-mingling elements of the PrepFiler kits with other biohazard trash containing bleach. Separate biohazard containers for each will be maintained and properly labeled.**

3.4.2.3 All glassware for disposal will be placed in the appropriate glass containers or sharps containers and will be properly disposed of.

3.4.2.4 Hazardous chemicals will be retained in appropriately labeled containers in a hood until picked up by a disposal company.

3.5 Universal Precautions

3.5.1 The term “Universal Precautions” refers to the practice of avoiding contact with bodily fluids by means of wearing the appropriate personal protective equipment. Under universal precautions all bodily fluids are considered potentially infectious.

3.5.2 Universal precautions are designed to prevent the transmission of diseases.

3.5.3 All biological materials encountered in the Forensic Biology Units will be handled using universal precautions.

3.6 Tasks

3.6.1 Read the following references and complete the training modules:

3.6.1.1 OSBI Policy and Procedure Policy 121

3.6.1.2 Bloodborne pathogens training

3.6.1.3 Chemical Hygiene/HazCom training

3.7 Evaluation

3.7.1 The trainer and/or designee will review the information with the trainee and ensure they have demonstrated knowledge of the above topics.

3.7.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the above tasks.

4 Forensic Biology Unit Code of Ethics

4.1 Goals

4.1.1 To reinforce the importance of ethics as it relates to forensic biology.

4.1.2 To reinforce the importance of ethics at it relates to testimony.

4.2 Code of Ethics

4.2.1 Ethics is defined as a principle of right or good conduct; or a system of moral principles or values. A code of ethics for the forensic scientist represents standards that every analyst should strive to meet. The ethical conduct of Forensic Biologists is of the utmost importance.

4.2.2 Forensic Biologists should strive to maintain the highest standards of professional practice. The Analyst keeps abreast of new developments in scientific methods and in all cases views them with an open mind. It is both proper and advisable that the scientific worker seeks knowledge in new fields; however, will not be hasty to apply such knowledge before they have adequate training and experience.

4.2.3 Forensic Biologists should always remain totally objective. A Forensic Biologist should be inquisitive, inquiring, progressive, logical, and most importantly, unbiased. The scientific mind must be unbiased and must refuse to be swayed by evidence or matters outside the specific material under consideration. The Forensic Biologist will be alert to recognize the significance of a test result as it may relate to the investigative aspects of a case. The Analyst, however, will scrupulously avoid confusing scientific fact with investigative theory in their interpretations.

4.2.4 Scientific method demands that the individual be aware of their own limitations and refuse to extend beyond them. Where test results are capable of being interpreted to the advantage of either side of a case, the Forensic Biologist will not choose that interpretation favoring the side by which they are employed merely as a means of justifying their employment. It is both wise and proper that the Forensic Biologist be aware of the various possible implications of their opinions and conclusions and be prepared to weigh them, if called upon to do so. In any such case, however, the Analyst will clearly distinguish between that which may be regarded as scientifically demonstrated fact and that which is speculative.

4.2.5 Forensic Biologists should always thoroughly examine and analyze the evidence, conduct examinations based on established scientific principles and protocols, and render opinions based on facts and observations. Scientific analyses will not be conducted on physical evidence without first being validated as scientifically sound. A proper scientific method demands reliability of validity in the samples analyzed and the processes used to analyze those samples.

4.2.6 Conclusions drawn in casework must not be based upon un-validated processes and procedures. Valid conclusions call for the application of proven methods. Only those methods/protocols/procedures developed, recognized, and validated in the field of forensic science will be used to analyze physical evidence.

4.2.7 Scientific analyses are designed to disclose facts, and all interpretations shall be consistent with that purpose and will not be knowingly distorted. Where appropriate to the correct interpretation of a test, experimental controls shall be used as verification that

the analyses are working properly. Where test results are inconclusive or indefinite, any conclusions drawn shall be fully explained and based on scientific facts.

4.2.8 A Forensic Biologist should never intentionally withhold or omit any findings or opinions discovered during an examination that would cause the facts of a case to be misinterpreted or wrongfully reported. They will not, merely for the sake of bolstering their conclusions, utilize unwarranted and superfluous tests in an attempt to give apparent greater weight to their results. Conclusions will not be drawn from materials which themselves appear unrepresentative, atypical, or unreliable. A truly scientific method requires that no generally discredited or unreliable procedure be utilized in the analysis. This is not to say that they should not be critical of untried or unproven methods, but they will recognize superior methods if and when they are introduced.

4.2.9 Forensic Biologist should never misrepresent their credentials, education, training, experience, or memberships.

4.2.10 Forensic Biologists should be forever vigilant of the importance of their role and conduct themselves only in the most professional manner at all times.

4.3 Ethics in the Courtroom

4.3.1 The expert witness is one who has substantially greater knowledge of a given subject or science than has the average person. An expert opinion is properly defined as “the formal opinion of an expert.” Ordinary opinion consists of one’s thoughts or beliefs on matters, generally unsupported by detailed analysis of the subject under consideration. Expert opinion is also defended as the considered opinion of an expert or a formal judgment. It is to be understood that an “expert opinion” is an opinion derived only from a formal consideration of a subject within the expert’s knowledge and experience.

4.3.2 The ethical expert does not take advantage of their privilege to express opinions by offering opinions on matters within his field of qualification to which they have not given formal consideration.

4.3.3 Regardless of legal definitions, the Forensic Biologist will realize that there are degrees of certainty represented under the single term of “expert opinion.” They will not take advantage of the general privilege to assign greater significance to an interpretation than is justified by the available data.

4.3.4 Where circumstances indicate it to be proper, the expert will not hesitate to indicate that while they have an opinion, derived of study and judgment within their field, the opinion may lack the certainty of other opinions he/she might offer. By this or other means, they take care to leave no false impressions in the minds of the jurors or the court.

4.3.5 In all respects, the Forensic Biologist will avoid the use of terms and opinions that will be assigned greater weight than are due them. Where an opinion requires qualification or explanation, it is not only proper but also incumbent upon the witness to offer such qualification.

4.3.6 The expert witness should keep in mind that the lay juror is apt to assign greater or less significance to ordinary words of a scientist than to the same words when used by a lay witness. The Forensic Biologist, therefore, will avoid such terms as may be misconstrued or misunderstood.

4.3.7 It is not the object of the Forensic Biologist’s appearance in court to present only that evidence which supports the view of the side that employs them. They have a moral

obligation to see to it that the court understands the evidence as it exists and to present it in an impartial manner.

4.3.8 The Forensic Biologist will not by implication, knowingly or intentionally, assist the contestants in a case through such tactics that will implant a false impression in the minds of the jury.

4.3.9 The Forensic Biologist, testifying as an expert witness, will make every effort to use understandable language in their explanations and demonstrations in order that the jury will obtain a true and valid concept of the testimony. The use of unclear, misleading, circuitous, or ambiguous language with a view of confusing an issue in the minds of the court or jury is unethical.

4.3.10 A Forensic Biologist will answer all questions put to them in a clear, straightforward manner and refuse to extend themselves beyond their field of competence.

4.3.11 Where the expert must prepare photographs or offer oral "background information" to the jury in respect to a specific type of analytic method, this information shall be reliable and valid, typifying the usual or normal basis for the method. The instructional material shall be of that level which will provide the jury with a proper basis for evaluating the subsequent evidence presentations, and not such as would provide them with a lower standard than the science demands.

4.3.12 Any and all photographic displays or court exhibits shall be made according to acceptable practice, and shall not be intentionally altered or distorted with a view to misleading court or jury.

4.3.13 By way of conveying information to the court, it is appropriate that the expert witness utilize any of a variety of demonstrative materials and methods. Such methods and materials shall not however, be unduly sensational.

4.4 Read the ANAB Guiding Principles of Professional Responsibility for Forensic Service Providers and Forensic Personnel at the following location:
https://anab.qualtraxcloud.com/ShowDocument.aspx?ID=6732

4.5 Tasks

4.5.1 Review the Code of Ethics and Ethics in the Courtroom sections above.

4.5.2 Review the Guiding Principles at the link above.

4.6 Evaluation

4.6.1 The trainee will complete the above tasks and will initial and date the checklist, thereby recognizing the importance of abiding by the Code of Ethics.

5 Quality

5.1 Goals

5.1.1 To allow the trainee to become familiar with the concept of quality assurance and quality control.

5.1.2 To introduce the trainee to the Quality Assurance Standards (QAS).

5.1.3 To ensure the trainee has an adequate working knowledge of the content within the CSD and FBU Quality Manuals.

5.2 General

5.2.1 Specific procedures for general QC practices will be followed according to the FBU Policy Manual and the Quality Manual and Quality Procedures of the OSBI Laboratory.

5.2.2 Quality refers to adhering to generally recognized standard of good laboratory practice.

5.2.3 Quality control refers to the day-to-day operational techniques or activities required to consistently provide accurate and reliable analytical results that fulfill the requirements for quality work.

5.2.4 Quality assurance is a program conducted by a laboratory to ensure accuracy and reliability of tests performed.

5.2.5 The Quality Manager (QM) is the Criminalistics Administrator assigned the responsibility of overseeing quality operations including proficiency testing, auditing, reviewing quality policies and procedures.

5.2.6 Non-conforming work does not meet the standards set forth in policy, procedure, protocol, or does not meet the needs of the customer. This may occur due to protocol drift or due to a quality or technical problem with a reagent, supply, or instrument.

5.2.7 Performance checks are actions taken to ensure analysis methods perform as intended.

5.2.8 Evidence is properly sealed only if its contents cannot readily escape and only if opening the container would result in obvious damage/alteration to the container or its seal.

5.2.9 An important part of the quality assurance system is the routine quality control checks such as temperature monitoring, decontamination, and safety equipment checks.

5.2.10 It is imperative that the Analyst be able to recognize problems, troubleshoot if necessary, and notify the appropriate personnel.

5.2.11 Quality control in the DNA laboratory begins with the Analyst/Technician. The Analyst/Technician must ensure that all laboratory reagents, laboratory instruments, and laboratory practices meet the standards set forth in the FBU Policy Manual.

5.2.12 Following proper quality control measures will greatly reduce the likelihood of contamination, poor reagent quality, and poor instrument quality.

5.2.13 Proper personal protective equipment (PPE) will be utilized. Gloves, masks, and lab coats are required to be worn at all times in the Forensic Biology laboratory and when analyzing Forensic Biology evidence.

5.3 Serology

5.3.1 Work areas will be cleaned with a 1:10 bleach solution before and after use.

5.3.2 Each separate biological sample shall be handled on a clean sheet of paper or benchkote-like material to minimize the potential for work surface contamination. Analysts shall keep in mind that secondary DNA transfer can occur from the outer surfaces of the packaging to the clean work surface; therefore, the work surface needs to be changed prior to placement of the evidence if the work surface is exposed to the outer surface of the packaging. Gloves should be changed between handling packaging and the evidence item.

5.3.3 Questioned samples must always be processed before known samples.

5.3.3.1 An exception to this would be an additional questioned sample received after the processing of a known sample for the case.

5.3.4 Instruments used to process biological materials shall be cleaned with a 1:10 bleach solution or alcohol before and after each sample is handled.

5.3.5 Only one item of evidence will be opened and analyzed at a time.

5.3.6 Preservation of biological material should be a priority.

5.3.6.1 Size of staining and substrate should be considered when choosing the assay for serological analysis (e.g., potential for false positives/negatives).

5.3.6.2 The potential for downstream analysis (e.g., DNA analysis, analyses by other Units, applicable laws) should be considered.

5.3.7 Proper positive and negative controls will be performed for the appropriate tests used to analyze the evidence.

5.3.7.1 A positive control utilizes a known substance that will produce a positive result to ensure that the test is functioning properly (i.e., known blood with Takayama).

5.3.7.2 A negative control is a sample that will produce a negative result. This is used to ensure that the test you are using is functioning properly and does not give false positives.

5.4 DNA

5.4.1 It is critical that good laboratory practices and techniques be utilized while in the laboratory. Adverse quality control procedures and lab practices can not only affect your results, but those of the other Analysts in the laboratory as well.

5.4.2 All pipette tips used in DNA analysis must be disposable, non-aerosol, and filtered.

5.4.3 All work spaces will be cleaned with a 1:10 bleach solution or alcohol before and after use.

5.4.4 Tubes (except PrepFiler tubes/column assemblies), supplies, and most reagents (except Proteinase K and DTT) will be exposed to UV radiation (Crosslinker) for at least 15 minutes prior to use.

5.4.5 Each separate biological sample shall be handled on a clean sheet of paper or benchkote-like material to minimize the potential for work surface contamination. Analysts shall keep in mind that secondary DNA transfer can occur from the outer surfaces of the packaging to the clean work surface; therefore, the work surface needs to be changed prior to placement of the evidence if the work surface is exposed to the outer surface of the packaging. Gloves should be changed between handling packaging and the evidence item.

5.4.6 Questioned samples must always be processed before known samples.

5.4.6.1. An exception to this would be an additional questioned sample received after the processing of a known sample for the case.

5.4.7 Tube de-cappers should always be used to open microcentrifuge tubes and amplification tubes.

5.4.8 Only one tube should be open at one time, as a general rule.

5.4.8.1 An exception to this rule is in the preparation of dilutions or normalized dilutions from extracts or when combining extracts from the same sample source. Transfers occurring from plate to plate are also an exception.

5.4.9 Ensure tubes are properly labeled with unique identifiers (i.e., case number, item number, etc.) to ensure samples are properly tracked throughout the analysis process.

5.4.10 Contamination Prevention and Troubleshooting

5.4.10.1 Contamination is the undesirable transfer of material to the evidence samples from another source.

5.4.10.2 Routine quality control practices are employed when handling evidence, reagents, supplies, equipment, and instruments. Regardless of the policy mechanisms within the laboratory, contamination events still occur in the laboratory.

5.4.10.3 A Forensic Biologist should be aware of methods to prevent or significantly reduce contamination and to troubleshoot the likely cause of the contamination event.

5.4.10.4 A trainee must develop good laboratory practices for sample handling consisting of proper tube labeling and organization. In short, the trainee must develop an efficient work flow for analyzing the samples, while ensuring steps are taken to minimize contamination.

5.4.10.5 Types of Contamination

5.4.10.5.1 Sample contamination from the environment

5.4.10.5.2 Sample to sample contamination

5.4.10.5.3 Sample contamination with PCR product carryover (unamplified sample DNA contaminated with amplified DNA)

5.4.10.6 Sample contamination from the environment can occur at various stages of the evidence analysis process. These stages include, but are not limited to, stain collection, DNA extraction, and set-up of samples for normalization and quantitation and PCR amplification. The ability to detect the exogenous DNA depends on the concentration of the evidence DNA sample. In other words, a touch DNA sample or negative control is more susceptible to detect the exogenous DNA than a neat bloodstain.

5.4.10.6.1 Some examples of this type of contamination would be: evidence that was handled at crime scene by officer without the use of gloves or inadvertently depositing saliva at the crime scene; an analyst inadvertently contaminating a sample in serology by not using proper techniques; or laboratory personnel contaminating work surfaces in the laboratory by not wearing the gloves while in the laboratory.

5.4.10.7 Sample-to-sample contamination occurs when one sample is introduced into another sample. This can occur at sample collection or analysis of the sample in the laboratory. Due to this possibility, it is important to generally analyze samples of suspected lower quantities of DNA (touch DNA) before those containing higher amounts of DNA (neat blood).

5.4.10.7.1 Some examples of sample-to-sample contamination are as follows: having multiple tubes open at one time and Analyst pipettes a sample into wrong tube (i.e., making dilutions and pipettes sample 1A into 1B dilution tube); having multiple items of evidence open at one time and the biological material is transferred to another item; or not properly changing gloves when analyzing or collecting the items of evidence. Failing to change pipette tips when pipetting sample or when setting up a plate to run on the instrument are other examples when this contamination can occur.

5.4.10.8 Sample contamination with PCR product carryover (unamplified sample DNA contaminated with amplified DNA) occurs when amplified DNA comes into contact with DNA that has not yet been amplified. Due to the high concentration of DNA in amplified product, subsequent amplification of the contaminated sample would likely lead to the detection of the contaminant profile instead of the sample profile. This would create the potential for reporting erroneous results. Therefore, it is important that a separate amplification area be maintained in the forensic DNA laboratory. Strict physical isolation should be maintained between areas that contain amplified DNA and non-amplified DNA.

5.4.10.8.1 An example of this would be if an analyst aerosolizes PCR product onto a tube rack, pipettor, or another moveable supply during genetic analysis setup and then removes the supply to another (pre-amplification) area in the laboratory without proper decontamination. The Analyst then unknowingly contaminates other pre-amplification samples.

NOTE: As a general rule, once a DNA Analyst handles amplified DNA product, the Analyst should NOT handle other evidence for the remainder of the day or until the Analyst is able to clean any potential amplified DNA from their clothes and person.

5.5 Tasks

5.5.1 The trainee and/or designee will review the information with the trainee and ensure they have demonstrated knowledge of the above topics.
** Technicians complete Technician Assignment 0 General Maintenance & QC, as applicable.*

5.5.2 Read the following references:

5.5.2.1 CWQM_3 Quality Assurance Program and General Review of Quality Control Procedures

5.5.2.2 General Review of Quality Manual and Procedures to ensure how they are organized and where information can be obtained.

5.5.2.3 The trainee will review and become familiar with the Quality Assurance Standards, associated Audit Document, and Guidance Document.

5.6 Evaluation

5.6.1 The trainee and trainer will initial and date the trainee checklist documenting completion of the above tasks.

6 Receiving and Handling Physical Evidence

6.1 Goals

6.1.1 To obtain a working knowledge of factors influencing the deterioration of evidence as it relates to proper vs. improper packaging, handling, and storage.

6.1.2 To develop a thorough understanding of evidence handling procedures, including preservation of chain of custody, use of the laboratory information management system (LIMS), and intra/interlabortory transfer of evidence.

6.1.3 To develop a knowledge of court procedures involving identification and introduction of evidence.

6.1.4 To develop a thorough understanding of the necessity for:

6.1.4.1 Detailed and comprehensive notes

6.1.4.2 Adequate labeling of evidentiary material

6.1.4.3 Drawings/Photographs

6.2 Laboratory Information Management System (LIMS)

6.2.1 A laboratory information management system (LIMS) is a computer program that the laboratory utilizes to track and maintain evidence within the laboratory system.

6.2.2 The LIMS that is utilized in the OSBI Criminalistics Services Division is commonly referred to as the BEAST and was developed by Porter Lee.

6.2.3 This system tracks the chain of custody of the items of evidence within the laboratory and provides a system to take notes and generate reports on the cases.

6.3 Submission of Evidence to the OSBI Laboratory System (QP 5 Evidence Intake)

6.3.1 Any law enforcement agency can submit evidence to the laboratory for analysis. Civil cases will not be accepted for analysis.

6.3.2 The Physical Evidence Technicians (and Criminalists in the regions, as needed) will accept evidence from the law enforcement agencies and enter the items into the LIMS. The system will generate a unique OSBI Laboratory case number and a unique item number for each package of evidence will be given. The case number typically begins with the year that the evidence is submitted and a dash followed by a sequential number (i.e., 2018-003546, 2018-003547, etc.). Each package (i.e., evidence envelope, brown paper sack, etc.), will be given a container letter. The containers start with “A” and go to “Z” for the case. If there are more containers than letters in the alphabet, the containers will proceed after “Z” with “AA”, “AB”, etc.

6.3.3 Typically the law enforcement agencies will have a Request for Laboratory Examination (RFLE) form that they fill out and bring with the evidence for every case submitted. The original RFLE will be scanned and provided in the Documents tab of the BEAST. The RFLE will have the items of evidence submitted, suspect and victim information, and should include a brief description of the types of analysis requested by the agency. The BEAST will also generate an RFLE when the case is made and can be viewed by

accessing the case in the BEAST on the Submissions tab by clicking on the Receipt button.

6.3.4 At the Forensic Science Center, when the Evidence Technicians have completed taking the evidence in for submission, the evidence will be taken to the target Unit's property room awaiting the Analysts to check the evidence out when the case is pulled for analysis. At Regional Laboratories, the evidence will be placed into an evidence vault holding pending evidence awaiting the Analysts to check the evidence out when the case is pulled for analysis.

6.3.5 Firearms that are submitted for analysis must be unloaded and should be indicated on the RFLE and the container that they are unloaded. Sharp objects should be packaged in a sharps container and labeled as containing sharp objects.

6.3.6 The submission of evidence to the OSBI Laboratory system is governed by QP 5 Evidence Intake in the OSBI CSD Quality Manual and Procedures.

6.4 Checking Out Evidence for Analysis (QP 6.1 Evidence Handling)

6.4.1 Before an Analyst can take custody of evidence, they will need to be given a username and password for access to the LIMS. The Supervisor will obtain the username and initial password and provide it to the Analyst.

6.4.2 When the Analyst is ready to check out evidence for a case, they will access the property room, locate the evidence that is needed, log onto the LIMS program, and check out the evidence to their custody.

6.4.2.1 This is accomplished by scanning the barcode of the person to whom evidence is being checked out.

6.4.2.2 The barcodes on the packages of evidence will then be scanned individually.

6.4.2.3 The Analyst will then press the save button (or scan the Process barcode) and type in their password. The evidence will now reflect the Analyst's possession.

6.4.2.4 The Analyst can perform a custody inquiry to ensure that the evidence is in their possession.

6.4.3 The evidence will be stored in the Analyst's individual evidence cabinet or in the property room.

6.5 Documentation of Evidence within the Forensic Biology Unit

6.5.1 The proper handling of physical evidence is one of the most important aspects of a case. The evidence must be handled in such a way to prevent contamination, loss, or deleterious change. The integrity of the evidence lays the foundation for reliable results.

6.5.2 The Forensic Biology discipline is not viewed solely by the finished work product, but also by the examinations and interpretations made during the course of the analysis. Any opinions rendered through a written report or in court room testimony must be based on fact. The Analyst bears the responsibility for justifying their opinion and the work that has led to this opinion. In general, documentation to support conclusions must be such that in the absence of the Analyst, another qualified Analyst could evaluate what was done and interpret the data.

6.5.3 Initial documentation of evidence

6.5.3.1 Upon receipt of evidence from the property room, it is the responsibility of the Analyst to ensure the evidence is properly packaged and sealed and this should be documented in the Analyst's case record.

6.5.3.1.1 An evidence container is "properly sealed" only if its contents cannot readily escape and only if opening the container would result in obvious damage/alteration of the container or its seal. Staples alone cannot provide a sealed condition on evidence packaging. It is acknowledged that not all evidence can be sealed inside a container. A proper seal would constitute tape sealing, heat-sealing, or lock sealing and initialing the seal. A date on the seal is also recommended.

6.5.3.2 The evidence package should contain the OSBI laboratory case number, as well as the requesting agency, and other pertinent information.

6.5.3.3 Itemization of Evidence (QP 6.1 Evidence Handing)

6.5.3.3.1 Item numbers will be assigned to the evidence at the time of submission (i.e., container A will be Item 1, container B will be Item 2, etc.). This is typically referred to as the parent item number.

6.5.3.3.2 Sub-items will be given to evidence that contains multiple items within a package or when it is necessary to swab or individualize staining on items.

6.5.3.3.2.1 Example: Three shirts within a brown paper sack: The first shirt would be Item 1A, second Item 1B, and third Item 1C.

6.5.3.3.2.2 Example: One shirt with five stains that are tested: First stain would be 1A, second 1B, third 1C, and so forth.

6.5.3.3.2.3 It may be necessary to create sub-items of sub-items; this works in the same manner. Example would be Item 1A (shirt) with stains 1A1, 1A2, etc.

6.5.3.3.3 The sub-items of the evidence should be added to the items tab of the LIMS.

6.5.3.3.3.1 This process is outlined in QP 6.1 Evidence Handling.

6.5.3.3.3.2 Sub-items must be separate in the items tab if the sub items are going to be separated from the parent item.

6.5.3.4 The Analyst will put the case number, item number(s), their initials, and on the outside of the packaging, if possible.

6.5.3.4.1 The item number listed on the outer packaging will be the parent item. Sub-items will be listed if they are individual evidence items,

but is not necessary if the sub-items are only stains on an item of evidence.

6.5.3.5 Opening of Evidence Containers

6.5.3.5.1 After documenting the information pertaining to the container, the Analyst opens the container. If possible, the original seal should not be compromised by opening at that position.

6.5.3.5.2 The evidence should be carefully removed from its package to prevent flaking blood from being dispersed into the air, loss of trace evidence (hairs, fibers, debris, etc.), and exposure to the Analyst.

6.5.3.5.3 The inside of the package should be examined to ensure all of the evidence in the container is inventoried and addressed appropriately.

6.5.3.6 Documentation of Evidence (FBU CASE_1)

6.5.3.6.1 An overall visual inspection of the item of evidence will be documented that includes, size, color(s), and condition of evidence.

6.5.3.6.1.1 A photograph can be used to document the overall size, color, and condition of the evidence if you choose.

6.5.3.6.2 The dates of analysis and item numbers will be documented in the case notes.

6.5.3.6.3 Hairs and/or debris will be documented as observed/collected in the case notes.

6.5.3.6.4 A description of the staining present on an item will be documented to include the size/amount and color of the stain (for any positive stain).

6.5.3.6.5 Use of any aids in searching the evidence will be documented, such as flashlights, Crimescopes, alternate light sources (ALS), stereomicroscopes, microscopes, etc. This does not need to be documented if it is required to complete the analysis (i.e., cell search, Takayama, etc.).

6.5.3.6.6 Any pertinent information from the item of evidence will be documented in the case notes.

6.5.3.6.7 If there are liquid samples, the amount of the liquid and color of the liquid will be documented in the case notes. If the liquid is stained out, the number and/or size of the stains will be documented.

6.5.3.6.8 In the event that the entire sample shall be consumed in testing the following points will need to be completed (QP 16.2 Contents of Case Records):

6.5.3.6.8.1 A letter to consume (LTC) shall be obtained that is signed by the appropriate District Attorney detailing what item(s) of evidence will need to be consumed.

NOTE: For property crime with no listed suspect, a LTC may not be necessary.

6.5.3.6.8.2 The evidence will be photographed and a scale will be included in the picture, if possible.

6.5.3.6.8.3 The DNA cuttings and extracts for the sample will be retained, if volume remains.

6.5.3.6.9 If possible, the Analyst will put their initials, date, case number, and item number on the item of evidence. If this is not possible, the information will be put on the next closest container to the item of evidence.

6.6 Transferring Evidence (QP 7 Evidence Transactions)

6.6.1 There are times when evidence will need to be transferred to another Unit for analysis. For these items of evidence, special precautions or handling may be required to ensure the integrity of the evidence for future analyses (e.g. for items that may be forwarded on to the Latent Evidence Unit an Analyst may wear double gloves or cotton gloves under latex/nitrile gloves for the handling evidence, minimize evidence handling of an item to preserve any latent evidence, avoid marking on an item that could disturb any latent evidence, marking only the proximal container instead of the item to minimize the potential for disturbing latent evidence).

6.6.2 The evidence that needs to be transferred will be properly packaged and sealed prior to transferring the item to the other Unit.

6.6.3 In the LIMS, an assignment for that unit will need to be created (if it does not already exist) and a hard copy Transfer Form will be filled out if the evidence is being transferred to another Unit outside Forensic Biology.

6.6.4 The evidence will be returned to the property room and placed on the appropriate shelf with a copy of the Transfer Form on the package.

6.7 Returning Evidence after Analysis

6.7.1 If any items of evidence in the original package were removed and placed into a new package, the item numbers of the evidence removed must be listed on the outside of the package, along with the date removed and the Analyst's initials who removed them.

6.7.2 The evidence will be properly packaged and sealed prior to returning the items of evidence to the property room.

6.7.3 The Analyst will scan the property room barcode then scan the barcode(s) of all the items that will be returned. The Analyst will then press the save button (or scan the Process barcode) and enter his/her password.

6.7.4 At the FSC, the items of evidence will be placed on the return shelves, so that the Evidence Technicians can retrieve them and take them to the working property room in preparation for return to the original requesting agency.

At Regional Laboratories, the items of evidence will be placed into the Return Vault for return to the original requesting agency.

6.7.5 At the FSC, if there are any items of evidence that will need to be retained at the OSBI for long term storage, a piece of red tape will be placed in the upper right-hand corner of the package and the word “Retain” will be written on the tape.

At Regional Laboratories, if there are items of evidence that will need to be retained at the OSBI for long term, they will be placed in the “retained evidence” boxes in the vault.

6.7.6 Any items of evidence that require refrigeration will be placed in the appropriate location in the refrigerator for evidence return.

6.7.7 At the FSC, if DNA products (cuttings, extracts, etc.) are retained, they must be kept refrigerated while in the property room.

At Regional Laboratories, if DNA products are retained, they will be placed directly into the freezers in the vault.

The package of the DNA extracts should be labeled “Keep Frozen”, indicating to the Evidence Technicians to keep this package frozen in long term storage. At the FSC, the package for the DNA products will need the red tape with the word “Retain” on the upper right-hand corner, as well.

6.8 Tasks

6.8.1 Observe a qualified Analyst obtain evidence from the property room, document the appropriate information for the packaging and items of evidence, and return evidence to the property room.

6.8.2 Read the following references:

6.8.2.1 QP 5 Evidence Intake, QP 6.1 Evidence Handling, QP 7 Evidence Transactions

6.8.2.2 CASE_1 Notes, CASE_2 Photography, CASE_8 Retention of Samples, and CWQM_7 Facilities & Evidence Control

6.9 Evaluation

6.9.1 The trainee and trainer will initial and date the trainee checklist documenting completion of the above tasks.

7 Proper Swabbing Technique for Items of Evidence

7.1 Goals

7.1.1 To obtain a working knowledge of when to swab items of evidence.

7.1.2 To develop the proper technique for swabbing items of evidence.

7.2 If possible, a cutting should be taken for DNA analysis or retention. However, there are instances when taking a cutting is not practical or best for the needs of the case.

7.2.1 There are some surfaces where it would not be possible to take a cutting or where it may be too difficult and cumbersome to take a cutting.

7.2.2 Some items of evidence need to be swabbed for brief or extended contact or “touch” DNA.

7.3 Whenever possible the staining or item should be swabbed with at least two swabs.

7.3.1 This will ensure that there is plenty of sample available for DNA analysis or further testing.

7.3.2 Whenever possible, when swabbing an item of evidence for contact DNA, the swabs should be taken at the same time. Every attempt should be made to ensure the entire swab heads are swabbed on the item of evidence.

7.3.3 Care should be taken to ensure that one is not “combining” potential areas of staining and thereby creating a mixture (e.g., swabbing a handle and a blade of a knife onto one set of swabs). Analysts should consult the Quality Manager and/or Technical Manager prior to swabbing if they have questions during analysis.

7.3.4 There are some instances where it will not be possible or practical to take two swabs; in these instances, leaving approximately ½ of one swab will suffice for additional analysis.

7.4 Procedure for swabbing the items of evidence.

7.4.1 Sterile swabs that are sealed should be used whenever possible. If packaged sterile swabs cannot be used, then the swab must be crosslinked prior to swabbing the item of evidence.

7.4.2 Swabbing of a dry stain usually requires the swabs be wet with a small amount of sterile H_2O prior to swabbing the stain or item. For a hard, non-porous item, one wet swab followed by one dry swab may be used. For a more porous surface, two wet swabs may be used. Depending on the target of the swabbing (swabbing DNA off an item such as a bottle v. swabbing DNA from an item of clothing looking for DNA other than the wearer), a difference in pressure will be required.

7.4.2.1 Example: swabbing a drinking can for DNA can use more pressure than trying to swab a pair of underwear from a victim looking for foreign male DNA from a digital molestation case. In the second example, the target would be the skin cells from the top layer of the clothing and skin cells from the individual wearing the item of clothing.

7.4.3 After the swabs are taken, they should be allowed to dry for a period of time.

7.4.4 When the swabs are dry they should be packaged, ensuring the Analyst's initials, case number, item number, and date are on the packaging. At this point, if the swabs will be used for additional analysis, they do not need to be sealed. If used immediately for analysis, the packaging will be completed when the evidence is being returned after the analysis is complete.

7.4.5 When swabbing stains, it is important to remember that multiple stained areas cannot be collected onto one swab.

7.4.5.1 If there are two distinct staining areas that are being swabbed, each stain will be swabbed on a separate swab (two swabs can be taken of the same stain).

7.4.6 The swabs should be given a sub-item number of the parent item they are swabbed from.

7.4.6.1 Example: If a shirt is swabbed for contact DNA that is Item 2, and two swabs are taken from the shirt, both swabs would be given a sub-item number of 2A.

7.4.6.2 Example: If there is an item with two distinct blood stains that are being swabbed and the stains are item numbers 1A and 1B. The swabs from each staining would be given an item number of 1A1 and 1B1, respectively, if staining from 1A and 1B remain on the item; conversely, if entire stains from 1A and 1B are collected onto the swabs, the swabs may be referred to as the stains, 1A and 1B.

7.4.6.3 Example: There are some instances, that the entire swab will be consumed in testing when items are swabbed for contact DNA testing. In this case, a sub-item number of the swab is not necessary as long as there is no swab remaining after testing.

7.5 Tasks

7.5.1 Observe a qualified Analyst swab an item(s) of evidence.

7.6 Evaluation

7.6.1 The trainee and trainer will initial and date the trainee checklist documenting completion of observing an analyst.

8 Introduction to the Pipettor and Dilutions

8.1 Goals

8.1.1 To introduce the trainee to basic laboratory equipment.

8.1.2 To ensure that the trainee follows the appropriate safety precautions when using the pipettor in the laboratory.

8.1.3 To ensure the trainee understands the proper use of pipettors and can prepare dilutions of liquids in the laboratory.

8.2 A typical micropipette is designed to manipulate liquids that have similar density to water. Not all liquids encountered in the laboratory will behave the same as water. It is essential that an Analyst become familiar with a good and proper technique for handling all varieties of liquids that could be encountered in a typical forensic laboratory.

8.3 A dilution is a solution that is sequentially diluted to a specific ratio. This is done by starting with a known concentration and using a specific volume of buffer to dilute the solution in a new tube. An example of this in forensic biology laboratories is making the standards used to prepare the standard curve for quantitation.

8.4 When using a specific STR or Y-STR kit there will be a target range in which the DNA concentration will produce optimal results. It is essential to be able to calculate and prepare dilutions of your resulting DNA extract to achieve this approximate range.

8.5 Types of Mechanical Pipettors

8.5.1 Adjustable Pipettor

8.5.1.1 There are mechanical pipettors that deliver a certain range of liquids with a high degree of accuracy. The most common ranges that are used in the laboratory are:

8.5.1.1.1 0.1 to 2 µL

8.5.1.1.2 0 to 10 µL

8.5.1.1.3 10-100 µL

8.5.1.1.4 20-200 µL

8.5.1.1.5 200-1000 µL

8.5.1.2 Digital pipettors that have 1 mL and 5 mL tips, which can pipette from 1 µL to 1000 µL for the 1 mL pipette. There are also multichannel pipettors that can be used.

8.5.1.3 The minimum volume the pipettor can effectively handle is either listed on the pipettor or 10% of its maximum volume (i.e., 10-100 µL pipettor, can effectively dispense volumes as low as 10 µL; 100 µL x 0.10= 10 µL).

8.5.2 Repeat Pipettors

8.5.2.1 They are designed to deliver a fixed volume of liquid over a number of times.

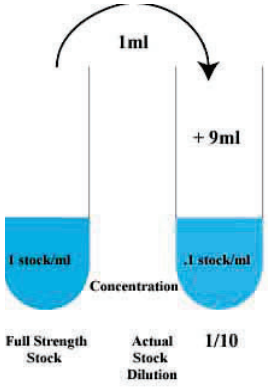
8.5.2.2 There are both digital and mechanical repeat pipettors available.

8.6 Dilutions

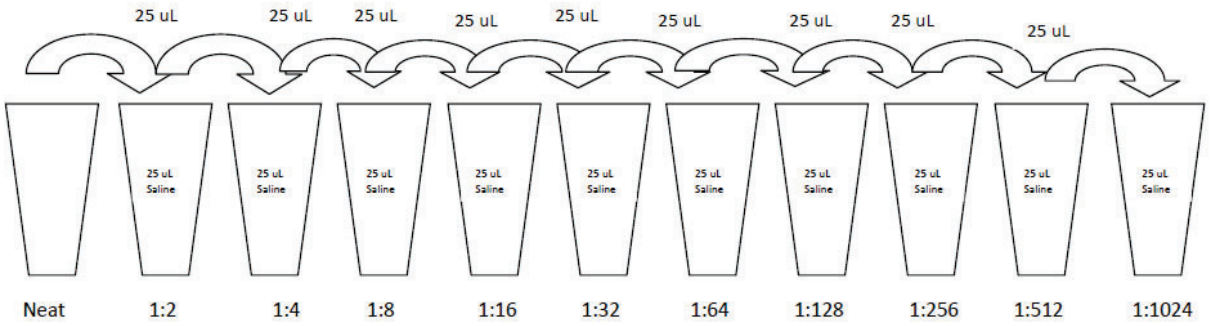
8.6.1 A dilution is the process of making a substance less concentrated by adding a diluent like water or phosphate buffered saline (PBS).

8.6.2 A serial dilution is the stepwise dilution of a substance in solution. Usually, the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. Serial dilutions are used to accurately create highly diluted solutions.

8.6.2.1 An example of making a dilution from stock solution: Take a known volume of stock solution and place it into a volume of diluent to produce the desired concentration. For instance, take 1 µL of stock solution and place it into 9 µL of saline, to produce a 1/10 dilution of the stock solution.



8.6.2.2 An example of a serial double solution. How would you prepare a 50 µL of a serial dilution to approximately 1:1024? To make the dilution sets you would add 25 µL of phosphate buffered saline to each of your dilution tubes. Then you would add 50 µL of your solvent to the first tube and this would be your neat tube. Then from the neat tube you would take 25 µL of solution from your neat tube and add it to the 1:2 dilution tube (containing 25 µL of PBS), mix, then take 25 µL of this solution and add to the next dilution tube and repeat for all dilutions.



8.6.2.3 Example: How would you prepare a 100 mL of a 1X GA buffer solution from a 10X stock solution?

$M_1V_1=M_2V_2$ ⇨ (1X * 100 mL)/10X = 10 mL
$V_1= M_2V_2/ M_1$

8.6.2.4 Example: How would you prepare a 1.0 L solution of 0.10 M NaCl from stock NaCl (MW=58.4 g)?

(0.10 M NaCl) * (58.4 g/mol)* (1000 mL) = 5.84 g
M = mol/1000 mL

8.7 Quality Control of Pipettors (FBU Policy Manual QC_14 Pipettors)

8.7.1 A performance check of all pipettors is required to be completed annually.

8.7.2 This is done by calculating the percent error for each pipettor.

8.7.3 If the pipettor fails this performance check, it will be adjusted in-house or may be sent out for calibration and maintenance.

8.8 Tasks

8.8.1 Complete the pipetting exercise. This exercise will include transferring a series of volumes of water onto an analytical balance and reporting the average volumes transferred.

8.8.2 Complete the dilution exercise, which will allow the trainee to be able to setup and make dilutions for the remainder of the training.

8.8.3 Read FBU Policy Manual QC_6 Pipettors and CWQM_10 Equipment.

8.9 Evaluation

8.9.1 The successful completion of the dilution exercise and the pipetting exercise.

8.9.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the tasks.

9 Introduction to the Microscope

9.1 Goals

9.1.1 To introduce the trainee to the various microscopes used in the laboratory and the nomenclature of the various items on the microscope.

9.1.2 To learn the proper care and maintenance of the microscope.

9.2 A microscope is an instrument used to see objects that are too small for the naked eye. The science of investigating small objects using such an instrument is called microscopy. Microscopic means invisible to the eye unless aided by a microscope.

9.3 Types of microscopes

9.3.1 Stereoscope

9.3.1.1 The stereoscope allows the Analyst to view large items that may not need microscopic analysis, but need some level of assistance to search the item properly.

9.3.1.2 The stereoscope acts as a large hands-free magnifying glass and can help the Analyst to observe staining that may be masked by the color or type of substrate.

9.3.1.3 Some typical items that may require a stereoscope to aid in searching includes shoes, weapons, or clothing.

9.3.2 Compound Microscope

9.3.2.1 The microscope is a tool used to magnify items otherwise too small to see with the unaided eye.

9.3.2.2 The microscope in the forensic laboratory is typically used in Takayama, Sperm Cell Search, and Hair Analysis.

9.3.3 Polarizing Microscope

9.3.3.1 When a beam of light passes through certain types of crystalline structures, it emerges vibrating in only one plane.

9.3.3.2 Light that is confined to a single plane of vibration is said to be plane-polarized and the device that polarizes light in this manner is called a polarizer.

9.3.3.3 A microscope can be outfitted with a polarizer and analyzer to allow the viewer to detect polarized light; this is known as a polarizing microscope.

9.3.3.4 Birefringence is the difference in the two indices of refraction exhibited by most crystalline substances.

9.4 Magnification and Resolution

9.4.1 By using multiple lenses, microscopes can magnify by a larger amount but this doesn't always mean that more detail can be seen.

9.4.2 The amount of detail depends on the resolving power of a microscope, which is the smallest separation at which two objects can be distinguished or resolved.

9.4.3 Magnification is how much bigger a sample appears to be under the microscope than it is in real life. Overall magnification is calculated by taking the objective lens magnification and multiplying by the eyepiece lens magnification.

9.4.4 Resolution is the ability to distinguish between two points on an image (amount of detail).

9.4.4.1 The resolution of an image is limited by the wavelength of radiation used to view the sample.

9.4.4.2 When objects in the specimen are much smaller than the wavelength of radiation being used, they do not interrupt the waves and so they are not detected.

9.4.4.3 The wavelength of light is much larger than the wavelength of electrons, so the resolution of the light microscope is a lot lower.

9.4.4.4 Using a microscope with a more powerful magnification will not increase this resolution any further; it will increase the size of the image, but objects closer than 200nm will still only be seen as one point.

9.5 Illumination

9.5.1 Effective illumination is required for efficient magnification and resolving power. Since the intensity of daylight is an uncontrolled variable, artificial light from a tungsten lamp is the most commonly used light source in microscopy.

9.5.2 The light passes through the condenser located beneath the stage. The condenser contains two lenses that are necessary to produce a maximum numerical aperture. The height of the condenser can be adjusted with the condenser knob.

9.5.3 Between the light source and the condenser is the iris diaphragm, which can be opened and closed by means of a lever, regulating the amount of light entering the condenser.

9.5.4 Excessive illumination may actually obscure the specimen because of lack of contrast. The amount of light entering the microscope differs with each objective lens used.

9.6 Light Microscopy

9.6.1 This is the oldest, simplest, and most widely-used form of microscopy. The specimens are illuminated with light, which is focused using glass lenses and viewed using the eye or photographic means.

9.6.2 The specimens can be living or dead, but often need to be stained with a colored dye to make them visible.

9.6.3 All light microscopes today are compound microscopes, which means they use several lenses to obtain high magnification.

9.6.4 Light microscopy has a resolution of about 200nm, which is sufficient to see cells but not the details of cell organelles.

9.7 Koehler Illumination

9.7.1 This type of illumination was named after August Koehler (1866-1948).

9.7.2 It is a method of specimen illumination used for transmitted and reflected light optical microscopy. It acts to generate an extremely even illumination of the sample and ensures that an image of the illumination source is not visible in the resulting image.

9.8 Care of the Microscope

9.8.1 Ensure the microscope stays clean, by replacing the dust cover on the microscope. The smallest bit of dust, oil, or lint can decrease the efficiency of the microscope.

9.8.2 The microscope lens and eyepieces can be cleaned with lens tissue paper.

9.8.3 If you experience issues with the microscope, the Supervisor and Technical Manager should be notified so that the appropriate adjustments and/or repairs can be made.

9.9 Tasks

9.9.1 The microscope will be used at various stages of the training program; the trainee will become familiar with the operation of the microscope for the particular tests that are used in the FBU.

9.9.2 Read the following references:

9.9.2.1 Saferstein, Richard. Criminalistics: An Introduction to Forensic Science, 8th Edition, Chapter 7 (pages 169-185).

9.9.2.2 Microscope PDF Handout

9.10 Evaluation

9.10.1 The successful completion of the above tasks.

9.10.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

10 Blood Training

10.1 Goals

10.1.1 To familiarize the trainee with identifying suspected blood stains.

10.1.2 To develop a basic understanding of the use of presumptive and confirmatory tests.

10.1.3 To familiarize the trainee with the proper uses and limitations of the Hemastix® test.

10.1.4 To familiarize the trainee with the proper uses and limitations of the Takayama test.

10.1.5 To familiarize the trainee with the proper uses and limitations of the RSID™-Blood test.

10.2 Identification of potential bloodstains

10.2.1 The study of serology involves the examination and analysis of body fluids, including blood.

10.2.2 Forensic serology deals with a variety of body fluids (blood, saliva, semen, and urine); samples are often in stain form (not typically liquid) and are often degraded or deteriorated, making detection and analysis more difficult.

10.2.3 Blood

10.2.3.1 Blood refers to a highly complex mixture of cells, enzymes, proteins, and inorganic substances.

10.2.3.2 The fluid portion of blood is called plasma, which is composed principally of water and accounts for approximately 55% of blood content.

10.2.3.3 Suspended in the plasma are solid materials consisting mainly of cells - red blood cells (erythrocytes), white blood cells (leukocytes), and platelets. This solid portion accounts for approximately 45% of blood content.

10.2.3.4 Blood clots when a protein in plasma, known as fibrin, traps and entangles red blood cells. If one were to remove the clotted material, a pale yellowish liquid known as serum would be left.

10.2.3.5 Functionally, red blood cells transport oxygen from the lungs to the body tissues and, in turn, remove carbon dioxide from tissues by transporting it back to the lungs, where it is exhaled.

10.2.3.6 On the surface of the red-blood cell there are millions of characteristic chemical structures called antigens.

10.2.3.7 Antigens impart blood-type characteristics to the red blood cells. Blood antigens are grouped into systems depending on their relationship to one another. This is the ABO blood typing with the Rh system.

10.2.3.8 The serum contains certain proteins known as antibodies.

10.2.4 The first step to identify potential bloodstains is a careful visual examination of the evidence item to locate any stains or material visibly characteristic to blood.

10.2.4.1 Blood may appear brown to red-brown based on how dilute the stain is.

10.2.4.2 Generally, the more dilute the blood, the lighter of an appearance it will have.

10.2.4.3 Depending on which substrate the blood is deposited on, it may have different appearance.

10.2.4.4 The shape of the staining (smears, ninety-degree blood drops, swipes, etc.) can be taken into consideration during examination.

10.2.5 A flashlight, stereoscope, or sometimes Crimescope can be useful to aid in the visual examination helping to identify potential bloodstains.

10.3 Presumptive Testing

10.3.1 A presumptive test is a chemical test which, by production of color or light, indicates the presence of a body fluid of forensic interest (blood, semen, etc.). It is usually more sensitive and less specific than a confirmatory test.

10.3.2 A catalytic test employs the chemical oxidation of a chromogenic substance by an oxidizing agent catalyzed by the presence of blood or specifically the hemoglobin or red pigment of blood.

10.3.3 Those tests that produce color reactions are usually carried out by first applying a solution of the chromogen to a sample of the suspected material/stain followed by addition of the oxidizing agent.

10.3.4 The catalyst of the reaction is the peroxidase-like activity of the heme group of hemoglobin.

10.3.5 A rapid color development, characteristic of the chromogen used, constitutes a positive result.

10.3.6 Tetramethylbenzidine (TMB) (Hemastix® Test)

10.3.6.1 Tetramethylbenzidine (TMB) is the 3, 3', 5, 5' tetramethyl derivative of benzidine

10.3.6.2 The Hemastix® Test (Miles Laboratories) has been adopted by a number of laboratories, and is also sometimes used at crime scenes.

10.3.6.3 The test itself consists of a plastic strip with a reagent treated filter paper tab at one end.

10.3.6.4 The tab contains TMB, diisopropylbenzene dihydroperoxide, buffering materials, and non-reactants.

10.3.6.5 Testing the bloodstain is accomplished by rubbing the stain with the Hemastix® pad and then adding distilled water to the tab.

10.3.6.6 The reagent tab is originally yellow and a color change to green or blue-green indicates the presence of blood.

10.3.6.7 OSBI FBU Individual Steps in Protocol – Hemastix (SER_1)

10.3.6.7.1 There are two methods that can be used to perform the test, a dry method and wet method.

10.3.6.7.1.1 Dry Method: brush the dry pad on the suspected stain and add de-ionized water to the pad. There should be an immediate color change within 5 seconds to a green or blue-green color. The test is negative if no color change is observed within 5 seconds. There is a possibility the longer the pad sits exposed to air, that a color change will develop.

10.3.6.7.1.2 Wet Method: add de-ionized water to the reagent pad of the Hemastix and then lightly brush the moistened Hemastix pad on the suspected stain. The pad will change color within 5 seconds if a positive result is obtained. This method may leave a green mark on the stain itself.

10.3.7 Additional Presumptive tests that are used at other laboratories.

10.3.7.1 Benzidine (Adler Test)

10.3.7.1.1 The reaction is normally carried out in an ethanol/acetic acid solution, results in a characteristic blue to dark blue color. The blue, in turn, may eventually turn to a brown color.

10.3.7.2 Phenolphthalein (Kastle-Meyer Test)

10.3.7.2.1 This test uses the simple acid-base indicator phenolphthalein. This test produces a bright pink color when blood is indicated. The reagent consists of reduced phenolphthalein in alkaline solution, which is oxidized by peroxide in the presence of hemoglobin in blood.

10.3.7.3 o-Tolidine

10.3.7.3.1 ortho-tolidine is the 3, 3’ dimethyl derivative of benzidine. The reaction, similar to that of benzidine, is conducted under acidic conditions and produces a blue color reaction resembling that of benzidine when testing blood.

10.3.7.4 Leucomalachite Green (LMG)

10.3.7.4.1 LMG oxidation is catalyzed by heme to produce a green color; the reaction is usually carried out in an acid (acetic acid) medium with hydrogen peroxide as the oxidizer.

10.3.8 Tests that use Chemiluminescence and Fluorescence

10.3.8.1 Chemiluminescence is the process by which light is emitted as a product of the chemical reaction. No additional light is required for the reaction to take place.

10.3.8.2 Fluorescence occurs when a chemical substance is exposed to a particular wavelength of light (usually short waves, such as ultraviolet) and light energy is emitted at longer wavelengths.

10.3.8.3 Often the presence of blood is suspected at crime scenes but cannot be seen under normal lighting conditions. Some examples of these would be a drag pattern across a floor that has been cleaned up or a washed spatter pattern on a wall.

10.3.8.4 These types of tests are usually presumptive and involve spraying a chemical mixture on a suspected bloodstained area, usually *in situ* and observing (sketching, photographs, etc.) the result in darkness or with the aid of alternate light source (ALS).

10.3.8.5 The observed result is a production of light which often enables the observer to determine the limits, shape, and some degree of detail in the original blood-stained area, often including an enhancement of blood patterns already present.

10.3.8.6 These types of tests are used for locating and defining blood rather than specifically identifying it.

10.3.8.7 Common Tests

10.3.8.7.1 Luminol

10.3.8.7.1.1 It will luminesce after oxidation in acid or alkaline solution. It involves spraying a mixture of luminol and a suitable oxidant in aqueous solution over an area thought to have traces of blood present. A resultant blue-white to yellow-green glow will indicate the presence of blood. Outlines and details are often visible for up to 30 seconds before additional spraying is required. It is sensitive up to dilutions of 1 in 10,000,000. The reaction can be documented with photography.

10.3.8.7.2 Fluorescein

10.3.8.7.2.1 It is typically used for larger area applications at crime scenes. Its purpose is to define or enhance patterns not visible but thought to be present. Any visible blood that is desired for subsequent analysis should be covered to shield it from chemical contamination or secured before the area is tested.

10.3.8.7.2.2 Fluorescein is reduced in alkaline solution over zinc to fluorescin which is then applied to the suspected bloodstained area. The catalytic activity of the heme then accelerates the oxidation by hydrogen peroxide of the fluorescin to Fluorescein which will fluoresce when treated

with ultraviolet light. The use of an ALS is required to visualize the fluorescence.

10.3.8.7.3 Bluestar® Forensic

10.3.8.7.3.1 A reagent whose purpose is to reveal bloodstains that have been washed out, wiped off, or which are invisible to the naked eye. This product is intended for crime scene investigators.

10.3.8.7.3.2 Based on chemiluminescence, its unique formula is based on Luminol, but comes in pre-formulated tablets which avoid the weighing of chemical components. Bluestar is a chemically enhanced version of Luminol with a longer lasting chemiluminescent emission. The product states it does not interfere with downstream DNA analysis, but as with any liquid added to an already dilute bloodstain, the additional liquid will likely further dilute any DNA present in the stain.

10.4 Confirmatory Testing

10.4.1 A confirmatory test is a test that verifies the presence of a specific compound. It is typically less sensitive than a presumptive test, but more specific.

10.4.2 Crystal tests are regarded as confirmatory tests for blood. These tests involve the non-protein heme group of hemoglobin, the oxygen carrying protein of erythrocytes that belong to a class of compounds called porphyrins. The heme structure contains a hexavalent iron atom. Nitrogen atoms within the ring structure bind four of the iron coordination positions and one is bound to histidine nitrogen in the globin protein. In hemoglobin, the remaining coordination position is normally bound by water or, in oxygenated hemoglobin, oxygen. In dried bloodstains, these last two positions are used in the crystal formation that is the basis for confirming the presence of blood.

10.4.3 Takayama Test

10.4.3.1 Heme gently heated with pyridine under alkaline conditions in the presence of a reducing sugar (i.e., glucose) can form crystals of pyridine ferroprotoporphyrin or hemochromogen. The reaction was examined by Takayama (1912) and found the best results with a reagent containing water, saturated glucose solution, sodium hydroxide (10%), and pyridine in a ratio of 2:1:1:1 by volume.

10.4.3.2 The normal procedure would be to take a small cutting (or flake) of the stain and place it under a cover slip and allow the reagent to flow under the cover slip and saturate the sample. After a brief heating period, the crystals are viewed microscopically. This test will confirm that blood is present, but will not confirm that human blood was present. A positive result will be observed regardless if the blood is human or animal.

10.4.3.3 An improvement to the test was offered by Hatch in 1993. Oxygen and pyridine compete for the same binding site on the heme molecule. Hatch used Cleland's reagent, dithiothreitol (DTT), in the reagent to reduce this

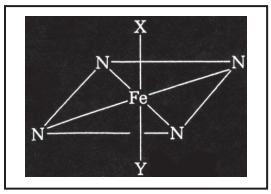
completion and shift the reaction in favor of pyridine, increasing the rate of hemochromogen crystal formation.

10.4.3.4 OSBI FBU Individual Steps in Protocol – Takayama Test (SER_2)

10.4.3.4.1 There are two options when performing Takayama, Option A which uses water and heat versus Option B which uses DTT.

10.4.3.4.2 The Takayama reagent is prepared in the following manner:

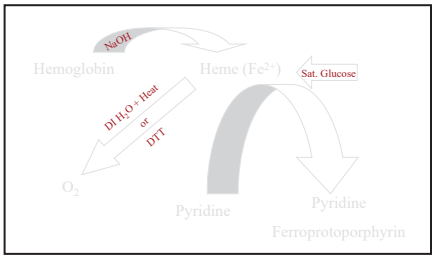
10.4.3.4.2.1 1 part 10% Sodium Hydroxide (separates the heme and globin subunits and frees the fifth ordinate position)



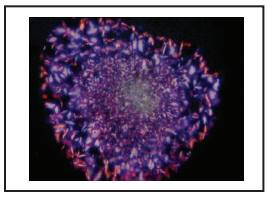
1 part saturated glucose (reduces metheme to heme)

1 part pyridine (with the 5th and 6th ordinate positions on the iron free, binds and forms the pyridine ferroprotoporphyrin)

2 parts de-ionized water (Option A) or 2 parts 0.05M dithiothreitol (Option B) (drives off O_2 from the sixth ordinate position)



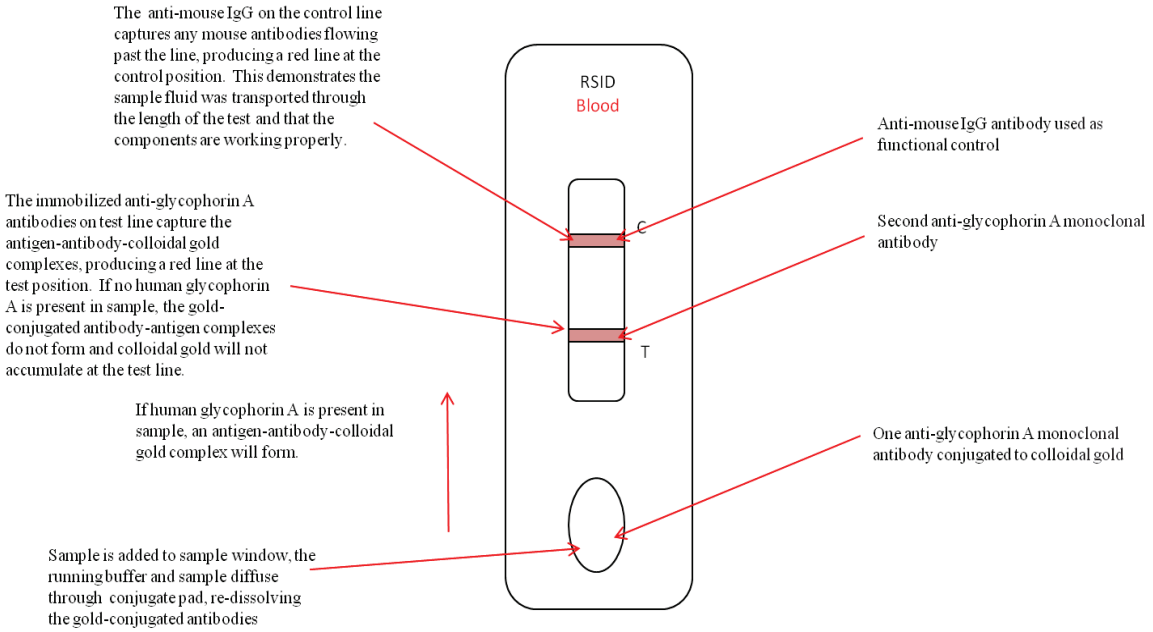
10.4.3.4.2.2 Takayama is positive when the analyst observes pink or red rhomboid or needle shape crystals (microcrystalline characteristics) that exhibit birefringence under plane polarized light.



10.4.4 Teichmann Test

10.4.4.1 This test was described by Teichmann (1853) and consists of heating dried blood in the presence of glacial acetic acid and a halide (usually chloride) to form the hematin derivative. The crystals that are formed are observed microscopically and are rhombic in shape and are brownish in color.

10.4.5 Rapid Stain Identification (RSID) – Blood



10.4.5.1 Rapid Stain Identification (RSID™)-Blood is an immunological based test that is specific for human blood. The test detects a red blood cell membrane antigen, glycophorin A, with two anti-glycophorin A monoclonal antibodies in a lateral flow immunochromatographic test strip.

10.4.5.2 Glycophorin A, a human specific antibody, is abundantly expressed on human red blood cell membranes where it functions to prevent cellular aggregation.

10.4.5.3 In this assay, one of the antibodies is conjugated to colloidal gold and is deposited on a conjugate pad beneath the sample window. The other antibody is striped onto the "Test Line" of a membrane attached to the conjugate pad. The "Control Line" on the membrane consists of anti-mouse IgG antibody and is used as the functional control. Attached to the other end of the membrane is the wick, which absorbs the tested fluid and running buffer at the completion of the test, preventing backflow of the sample.

10.4.5.4 When sample is added to the sample window, the running buffer and sample diffuse through the conjugate pad, re-dissolving the gold-conjugated antibodies. If human glycophorin A is present in the sample, an antigen-antibody-colloidal gold complex will form. Sample and antibodies (complexed and free) are transported to the membrane phase of the strip test. The immobilized anti-glycophorin A antibodies on the test line capture the glycophorin A antigen-antibody-colloidal gold complexes, producing a red line at the test position. If no human glycophorin A is present in the sample, the gold-conjugated antibody-antigen complexes do not form and colloidal gold will not accumulate at the test line. The anti-mouse IgG on the control line captures any mouse antibodies flowing past the line, producing a red line at the control position. The control line demonstrates that the sample fluid was transported through the length of the test and that the components are working correctly.

10.4.5.5 When the test should be used.

10.4.5.5.1 RSID™-Blood should be used to confirm human blood.

10.4.5.5.2 Can be used where there is indication of dilute blood (i.e., Hemastix® positive only, Takayama was tried but failed and the Analyst thinks the stain may be blood).

10.4.5.5.3 The test is to be used at Analyst's discretion and should be used in conjunction with Takayama, not as a replacement.

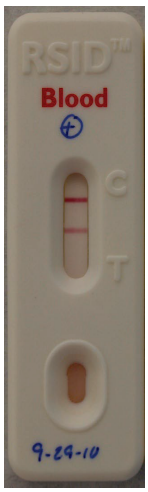
10.4.5.5.4 Can be used on samples that have contaminants that interfere with the Takayama test (i.e., dirt).

10.4.5.6 OSBI FBU Individual Steps in Protocol – Testing for Human Blood (SER_3)

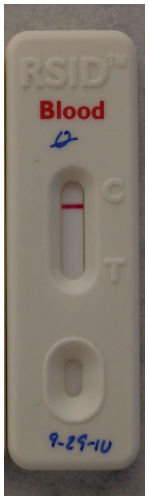
10.4.5.6.1 The positive and negative control can be made and stored refrigerated and used as long as the components (buffers) have not expired. These do not need to be made on a daily basis.

10.4.5.6.2 A cutting of the stain will be placed in tube and the Extraction Buffer added and incubated for 1 hour. The cutting is then removed and placed in the perforated cap and spun down to get the liquid out of the cutting. Then a portion of extract is combined with running buffer and added to the cassette window. The results are recorded at approximately ten minutes.

10.4.5.6.3 A positive result has the formation of two lines, one at the control and the other at the test line. A negative result is the formation of a line only at the control region; this ensures the test is functioning properly.



Positive Result



Negative Result

10.4.6 The ABAcard HemaTrace or Seratec HemeDirect tests are similar to the RSID™ - Blood test, except that these tests function to detect the presence of hemoglobin. The detection is based on the determination of human hemoglobin in the sample by a specific antigen/antibody reaction. The result is interpreted visually by the appearance of a red

test result line in hemoglobin positive samples. These tests will detect blood, but will also cross-react with higher order primate blood and some with ferret blood.

10.5 Tasks

10.5.1 Observe a qualified analyst(s) perform all phases of blood testing (including Hemastix, Takayama, and RSID, as/if available and as applicable). The trainee may observe as many analysts as they feel necessary to become comfortable with the processes.

10.5.2 Complete the Assignment 1 Blood Serology. **Technicians complete Technician Assignment 1 RSID-Blood Serology Assistance*.

10.6 Evaluation

10.6.1 Take and pass the blood assignment quiz.

10.6.2 The successful completion of the above tasks.

10.6.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

10.6.4 **Technician Mock Trial Assignment*

10.6.4.1 **Following the successfully completion of the technician competency test for a given sample set (in conjunction with a qualified analyst), the trainee will complete an informal mock trial evaluation with the assigned trainer and/or Technical Manager.. The technician will be released for technician duties in casework by Memorandum from the Technical Manager after successful completion of this assignment.*

11 Sexual Assault Training

11.1 Goals

11.1.1 To familiarize the trainee with the operation of the Crimescope for searching for body fluids.

11.1.2 To introduce the trainee to the theory of presumptive semen testing.

11.1.3 To familiarize the trainee with the AP Spot test.

11.1.4 To introduce the trainee to the theory of confirmatory testing of p30.

11.1.5 To familiarize the trainee with the p30 test.

11.1.6 To introduce the trainee to the theory of confirmatory semen testing through the identification of spermatozoa.

11.1.7 To familiarize the trainee with the identification of spermatozoa with the microscope.

11.2 Seminal Fluid

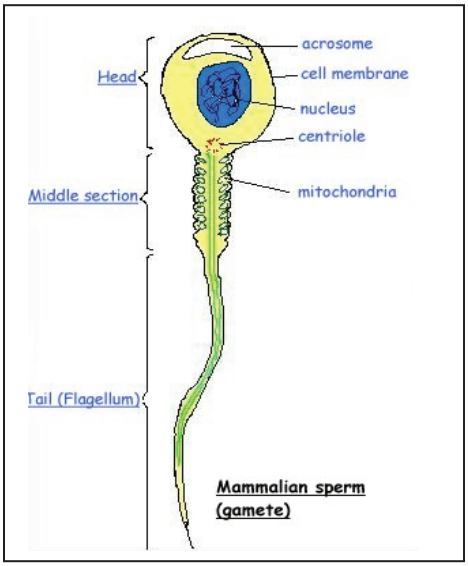
11.2.1 Semen is produced by post pubescent males and ejaculated following stimulation. It is a semi fluid mixture of cells, amino acids, sugars, salts, ions, and other organic and inorganic materials elaborated as a heterogeneous gelatinous mass contributed by the seminal vesicles, prostate gland, and Cowper's gland.

11.2.2 The formation of spermatozoa is initiated within the seminiferous tubules of the testis. Immature spermatozoa, incapable of mobility or fertilization, exit the testis and enter the epididymis to complete their maturation. Mature cells enter the vas deferens and reside in this structure until expelled during ejaculation. The secretions of the accessory glands comprise seminal plasma and upon emission from the body, the suspension of spermatozoa in seminal plasma forms semen which may also be referred to as seminal fluid. Upon exit from the body, semen immediately begins to coagulate. Human semen is unique in that after 20 to 30 minutes post ejaculation, the coagulated semen begins to re-liquefy. The composition of semen varies during the ejaculatory process. The initial orgasmic contraction is rich in spermatozoa and prostatic fluid and subsequent contractions within the same ejaculation lowers the cell concentration.

11.2.3 A normospermic male's semen contains sperm cell concentrations from approximately 50 million to 150 million sperm cells per milliliter. Oligospermic individuals elaborate semen in which the sperm cell count is 20 million sperm cells per milliliter or less. Azoospermia refers to individuals who are unable to produce semen containing spermatozoa.

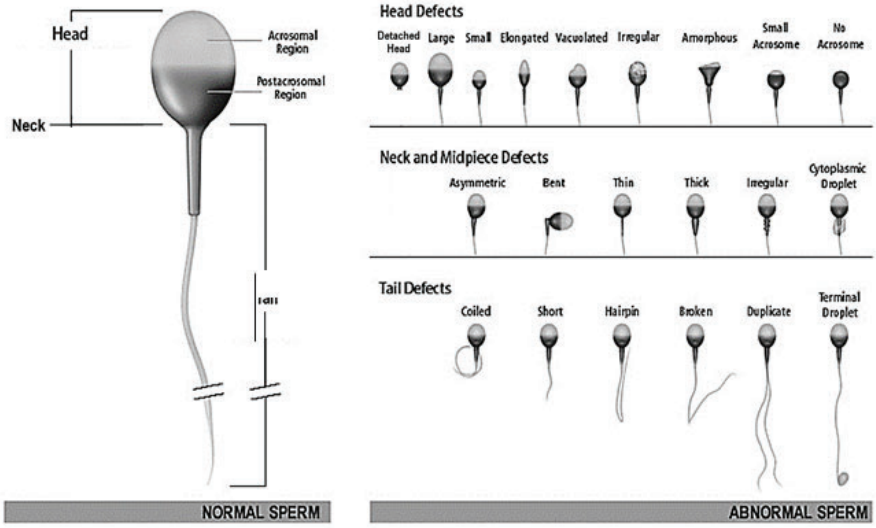
11.2..4 Ejaculate volumes of human males range from 2 to 6 mL and typically contain between 100 and 150 million sperm cells per milliliter. Certain diseases, genetic conditions, excessive abuse of alcohol or drugs, prolonged exposure to certain chemicals, and elective surgery procedures may result in a drastically reduced sperm count or complete absence of sperm cells from semen.

11.3 Sperm Cell



11.3.1 The principal cellular component of semen is the spermatozoan or sperm cell, a specialized, flagellated structure approximately 55 micrometers in length. The human sperm cell head is typically ovoid in shape with approximate dimensions of 4.5 micrometers in length, 2.5 micrometers in width, and 1.5 micrometers in thickness. The head contains the cell nucleus which is packed with deoxyribonucleic acid (DNA). The anterior portion is capped with the acrosome. This structure is rich in enzymes to assist in penetrating the cell wall of the female egg during fertilization. A flagellated tail is attached to the head via a short mid-piece and accounts for about 90% of the total length of a sperm cell.

11.3.2 Some sperm cells may exhibit atypical morphology as depicted in the diagram below. If sperm with this morphology are observed during sexual assault casework, consultation with the Technical Manager is recommended.



11.4 Alternate Light Source (ALS)

11.4.1 The first step in serological analysis is a visual examination of items using a good light source to detect any visible staining. Seminal fluid stains may appear off-white to yellow with a texture different than the substrate itself.

11.4.2 Alternate light sources (ALS) use different wavelengths of light that may cause a body fluid to be more visible through fluorescence.

11.4.3 Dried semen stains possess substances capable of fluorescing in the visible light region when irradiated with a UV wavelengths. Studies suggest these fluorescent properties are the result of two processes: One source is the conversion of non-protein substances to compounds that are capable of fluorescence and the second is the growth of the bacterium *Pseudomonas fluorescens* on the semen stains which elaborates the

fluorescence during growth. Fluorescent appearance of stains will differ depending upon the substrate that it is on. On most materials, semen stains will appear bluish white to a purplish color against a dark background.

11.5 Presumptive Testing

11.5.1 Acid phosphatase (AP) is a class of enzymes that can catalyze hydrolysis of certain organic phosphates. AP cleaves the ester-linked phosphate groups from a substance. There are three types of AP found in the body; seminal, vaginal, and erythrocytic.

11.5.2 These enzymes are ubiquitous in nature and may be found in materials as diverse as mammalian liver and cauliflower stem juice. In 1935, Kutscher and Wolbergs discovered that human semen contains uniquely high levels (20 to 400 times higher) of seminal acid phosphatase (SAP) compared with other body fluids and plant tissues.

11.5.3 Ten years later, Lundquist suggested utilizing this fact as the scientific basis of the presumptive identification of semen.

11.5.4 In males, puberty stimulates the large-scale synthesis of SAP by secretory epithelial cells that line the prostate gland. SAP levels remain high until the age of about 40, after which it gradually declines.

11.5.5 No correlation exists between the level of SAP and the number of sperm cells present in ejaculate and no variation has been found between males with normal sperm counts and those who are clinically infertile or who underwent vasectomies.

11.5.6 Acid Phosphate (AP) Spot Test

11.5.6.1 The basic theory of the AP Spot test is that there is a substrate containing a phosphate group in a dilute acid is reacted with a stain suspected to be semen. The substrate is alpha-naphthyl phosphate. If semen is present in the suspected stain, the acid phosphatase removes the phosphate group from the substrate. A diazonium salt reacts with the dephosphorylated substrate to from an azo dye complex which produces a color change. The diazonium salt from this test is Fast Blue B salt and the color change would indicate the presence of acid phosphatase.

11.5.6.2 The test can be performed by either rubbing filter paper or a swab over the suspected stain or by taking a cutting of the suspected stain. The solution is than added to the rubbing or cutting. If a purple color change is observed within two minutes, the test is positive.

11.5.6.3 This is a presumptive test for the presence of acid phosphatase and if a positive result is observed then a confirmatory test of cell search (and possibly p30) is required. If a negative result is observed then no further testing is required. In instances where all stains tested are negative for AP, then additional p30 and cell search testing is necessary. Additional testing must be performed in consultation with the Technical Manger and/or Lead Analyst to determine how many stains require further testing.

11.5.6.4 Vaginal acid phosphatase (VAP) can produce a pink color change that *may* be distinguishable from that produced by seminal AP.

11.5.6.5 Some false positives for AP spot test include vaginal AP, fecal material, some plant matter, spermicides, and some feminine hygiene products. Some false

negatives include interference by contaminants and dilution of semen beyond the detection limits of the test.

11.5.6.6 OSBI FBU Individual Steps in Protocol – AP Spot Test (SER_4)

11.5.6.6.1 Use whichever method you prefer and rub the suspected stain and add ~ 3 drops of the AP Spot Test Solution. The color change should be within two minutes. A positive result will develop a purple color and a negative result is when there is no color change or a color other than purple.

11.5.6.6.2 Solutions A and B are stored separately and frozen. When they are thawed the two solutions are mixed together when performing the test.

11.5.7 There are several other presumptive tests for SAP; one previously used at the OSBI was called STMP (Sodium Thymolphthalein Monophosphate).

11.5.8 General time frames for AP

11.5.8.1 High levels of AP may be detected in the vagina up to 72 hours post-coitus.

11.5.8.2 Based on observations made at the OSBI, AP can be detected after an alleged assault up to 24 hours in vaginal region, 12 hours in the anal region, and 2-3 hours in the oral region.

11.6 P30 or Prostate Specific Antigen (PSA) Immunoassay Test

11.6.1 In 1978, Sensabaugh identified p30 or PSA as a suitable marker for demonstrating the presence of semen. A positive p30 test is confirmatory for p30. The only confirmatory test for spermatozoa is the sperm cell search.

11.6.2 Seminal fluid p30 (PSA) is a monomeric glycoprotein, which has a molecular weight of about 30,000 Daltons. Histological studies show the origin of p30 in the epithelial cells that line the ducts of the prostate gland. p30 is secreted into the seminal plasma by the prostate gland and varies in concentration from 300 to 4,000 nanograms per milliliter. The main function of p30 is to liquefy the seminal fluid and the levels of p30 are unrelated to the levels of acid phosphatase or sperm production.

11.6.3 Since the prostate gland lies distal to the customary point of interruption in a vasectomy this procedure has no effect on the elaboration of p30 into semen.

11.6.4 The urine and serum of males, breast milk, and sweat contain levels of p30 that are usually below the limits of detection for this immunoassay. However, there are instances of faint p30 positive (i.e., oral swabs); analyst discretion should be used when deciding to move these samples on for DNA analysis.

11.6.5 p30 is also found in elevated levels in males with prostate cancer, but this is of little consequence forensically.

11.6.6 Classically, the detection of p30 was achieved using immunoelectrophoresis. This method was time consuming, has limited sensitivity, and has been superseded by newer methods.

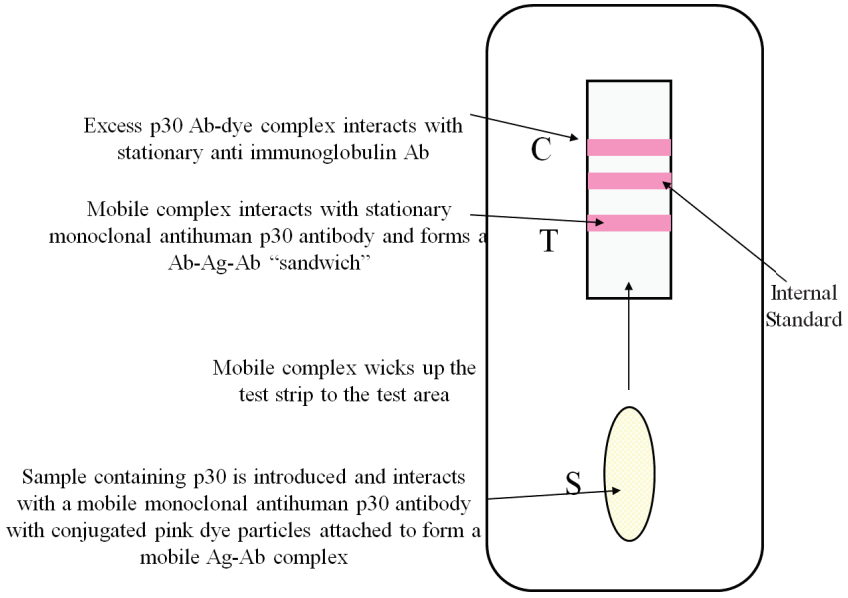
11.6.7 This test is based on an antibody-antigen reaction, but is quick, sensitive, and simple to use.

11.6.8 Seratec PSA Semiquant Test.

11.6.8.1 The test is a chromatographic immunoassay for the rapid semi-quantitative determination of PSA in body fluids.

11.6.8.2 The test utilizes two monoclonal murine anti-PSA antibodies. One of the anti-PSA antibodies is immobilized at the test region on the membrane. The upstream control region and the region of the internal standard contain immobilized polyclonal goat anti-mouse antibodies, which are antibodies against the mobile murine anti-PSA antibody present in the sample loading region. A glass fiber pad downstream of the membrane is used for sample loading and transmission to a second fiber pad with the dried gold labeled second monoclonal murine anti-PSA antibody. PSA at the sample will bind to the re-mobilized gold-labeled antibody and form a PSA-gold-labeled-anti-PSA-antibody complex.

11.6.8.3 The reaction mixture, including the complex, is carried upwards with the fluid through capillary effect of the membrane. In any case the colored gold labeled anti-PSA-antibody will bind to the anti-mouse-antibody at the control region and the region of the internal standard thus developing two pink lines. These two pink lines are independent of the existence of PSA in the sample and indicate only the correct execution of the test. If the sample contains PSA, the PSA-gold-labeled anti-PSA-antibody complex will bind to the immobilized monoclonal antibody at the test result region, because it recognizes another epitope on the PSA molecule, thereby forming an antibody-antigen-antibody "sandwich." The binding is indicated by the formation of an additional line pink line in the test area; hence, providing positive results.



11.6.8.4 False positives of the test include some urine in males and some human breast milk. Some false negatives include samples containing semen diluted

beyond the detection limits of the test, the high dose hook effect, samples masked by certain contaminants, and sampling error.

11.6.8.4.1 The test is forensically designed to detect the higher levels of p30, commonly found in males rather than those levels of p30 found in females.

11.6.8.4.2 Sampling error can occur due to the fact that the semen does not spread evenly throughout a stain.

11.6.8.4.3 The high dose hook effect results when there is an excess of p30; this causes all of the binding sites at the test area of the cartridge to be full, and when any complexes migrate to the test region, they cannot bind with the immobilized monoclonal antibody due to the previous blockage; therefore, no pink line will form, resulting in a false negative result.

11.6.8.3 General time frames for p30

11.6.8.3.1 p30 is rarely detected in the vagina 24 hours or more after intercourse.

11.6.8.3.2 Based on observations made at the OSBI p30 can be detected up to 12-16 hours in the vaginal region, 6-9 hours in the anal region, and 2-3 hours in the oral region after the alleged assault.

11.6.8.4 OSBI FBU Individual Steps in Protocol – p30 Detection (SER_5)

11.6.8.4.1 A cutting is taken from the suspected stain and placed in a tube. Between 250µL to 750µL of phosphate buffered saline (PBS) is added to the tube. Most analysts use ~500µL of PBS when they perform the test.

11.6.8.4.2 The sample is then incubated for approximately 30 minutes at room temperature. The cutting is then removed from the tube and placed into the perforated tube cap (or spin basket). Holes are poked into the tube cap with forceps prior to removing the cutting, to allow the liquid to flow back into the tube when centrifuged. The tube with the cutting in the cap is centrifuged at maximum speed for approximately five minutes.

11.6.8.4.3 Typically the p30 test and the cell search test are performed at the same time. At this point, the supernatant will be removed from this tube and placed into another tube, leaving the sperm pellet. Analysts have an option, depending on the sample type, to remove the entire volume of supernatant to a tube for use in p30 testing and then approximately 10µL of PBS will be added back to the sperm pellet in the original tube for the SER_6 procedure; this amount will subsequently be added to the sperm slide OR to remove all but approximately 10 µL of supernatant for use in p30 testing. The remaining 10µL (including the sperm pellet) in the tube is then placed onto a sperm slide for the SER_6 procedure.

11.6.8.4.4 The supernatant will be used for the p30 testing. Approximately 200µL of this will be added to the p30 card well for testing. The results will be read at approximately ten minutes.

11.6.8.4.5 A pink line at the test area, the internal standard area, and the control region will indicate a positive result. When there is no line at the test area this is a negative result for p30.

11.6.8.4.6 The internal standard area is used to correlate a concentration of the p30. The internal standard is approximately 4 ng/mL of PSA and based on the color intensity of the line, you can estimate a concentration of p30. This is not used at the OSBI laboratory and is provided for information only.

11.7 Microscopic Identification of Spermatozoa

11.7.1 Microscopic identification of sperm cells provides unambiguous proof that a stain under scrutiny contains semen.

11.7.2 It is unusual for a forensic scientist to examine semen in which sperm cells are motile since motility is lost within 3 to 6 hours of ejaculation.

11.7.3 Established staining techniques greatly assist the trained eye to easily distinguish sperm cells from extraneous material such as epithelial cells.

11.7.4 The most commonly encountered staining technique uses picroindigocarmine (Stain B) and nuclear fast red (Stain A) dyes and is colloquially referred to as the Christmas tree stain.

11.7.5 It was developed specifically for sperm cell visualization and different parts of the sperm structures are singularly colored and contrast well with the colors taken up by the epithelial cells.

11.7.6 Sperm cell heads are ovoid and exhibit characteristic differential staining. The anterior portion is pink or red in color and the posterior is dark red or purple and often appears shining, this portion is the acrosomal cap and typically looks like a clear area under the microscope.

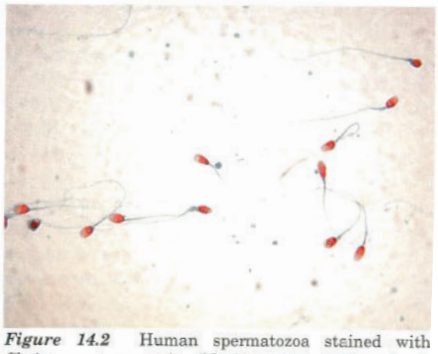


Figure 14.2 Human spermatozoa stained with Christmas tree stain (Nuclear Fast Red and picroindigocarmine). (Original magnification × 1000.)

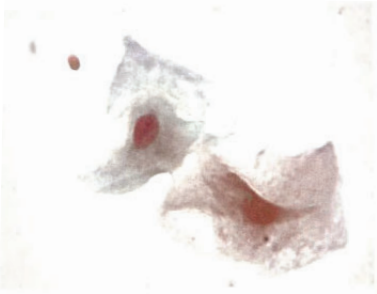


Figure 14.3 Human spermatozoon and nucleated epithelial cells stained with Christmas tree stain (Nuclear Fast Red and **picroindigocarmine**). (Original magnification × 1000.)

11.7.7 Sperm cell tails stain green and the mid-piece stains blue (this is usually not seen when visualizing sperm under the microscope).

11.7.8 Epithelial cells also take up the stain and appear blue-green with red nuclei (refer to photo marked Figure 14.3 above).

11.7.9 Morphologies of sperm cells of other animals are documented and reference collections are available. It is unusual to encounter the spermatozoa of other animals in casework and the possible presence should be apparent in the case history. Review the animal spermatozoa available in the lab or on the network; note the differences between human and animal sperm.

11.7.10 Some false positive of this test include some higher order primate sperm, which may not be able to distinguished from human sperm. Some false negatives include samples containing semen that are outside the routine detection limits of the test, samples that are masked by certain contaminants, and sampling error. Sampling error can occur due to the fact that semen does not evenly spread throughout the stain.

11.7.11 General time frames for Sperm

11.7.11.1 The generally accepted maximum times for sperm heads to be detected in living persons are 7 days in the vaginal cavity, 2 to 3 days in the anus and rectum, and 24 hours in the oral cavity. Intact (tail attached) spermatozoa are commonly observed on vaginal swabs taken between 0 to 26 hours after intercourse and are rarely observed in oral or anal samples after 5 hours.

11.7.11.2 Based on observations made at the OSBI sperm can be detected 8-12 hours in the vaginal region, 6-9 hours in the anal region, and 2-3 hours in the oral region after the alleged assault.

11.7.12 OSBI FBU Individual Steps in Protocol – Microscopic ID of Sperm (SER_6)

11.7.12.1 Typically the p30 test and the cell search test are performed in conjunction with each other.

11.7.12.2 A cutting is taken from the suspected stain and placed in a tube. Between 250µL to 750µL of phosphate buffered saline (PBS) is added to the tube. Most analysts use ~500µL of PBS when they perform the test.

11.7.12.3 The sample is then incubated for approximately 30 minutes. During the incubation period the samples may be sonicated for 15 minutes to help release the sperm from the substrate. It is required for cloth substrates and is analyst discretion as to whether other substrates should be sonicated.

11.7.12.4 The cutting is then removed from the tube and placed into the tube cap (or spin basket). Holes are poked into the tube cap prior to removing the cutting with forceps to allow the liquid to flow back into the tube when centrifuged. The tube with the cutting in the cap is centrifuged at maximum speed for approximately five minutes.

11.7.12.5 At this point, the supernatant will be removed from this tube and placed into another tube, leaving the sperm pellet. Analysts have an option, depending on the sample type, to remove the entire volume of supernatant to a tube for use in p30 testing and then approximately 10µL of PBS will be added back to the sperm pellet in the original tube for the SER_6 procedure; this amount will subsequently be added to the sperm slide OR to remove all supernatant for use in p30 testing with the exception of approximately 10µL. The remaining

10µL in the extraction tube is then placed onto a sperm slide for the SER_6 procedure.

11.7.12.6 The slide will then be heat fixed, once all the samples are placed in the appropriate wells. This is done by placing the slide on the hot plate. Be careful as not to overheat the sample and burn it. The slide should be taken off of the hot plate once the well is dried.

11.7.12.7 The wells of the microscope slide are then stained with the Nuclear Fast Red (Stain A) for approximately 5 to 15 minutes. It is analyst discretion how long the stain is left on but, typically most analysts leave it on for 8 to 10 minutes. The slide is then rinsed with de-ionized water at the sink.

11.7.12.8 Then the wells for the microscope slide are stained with Picroindigocarmine Stain (Stain B) for 5 to 30 seconds. The slide is then rinsed with ethanol or methanol to remove the excess stain and the slide is allowed to dry briefly.

11.7.12.9 The slide is then covered with a cover slip, which covers all the wells that contain samples. It is attached permanently by adding a small amount of the Permount to the slide in several locations and the slide cover is placed on the slide, ensuring that the Permount is evenly distributed throughout the cover slip. This process is done in the fume hood. The slide should be allowed to dry to ensure the cover slip will not move when looking at the slide.

11.7.12.10 Each well of the slide is then viewed with the microscope looking for spermatozoa. The presence of spermatozoa must be confirmed with the 40x objective, but the wells can be searched with the 20x objective.

11.7.12.10.1 A positive result is when there is intact (head with attached tail) or partial (head only) sperm observed.

11.7.12.10.2 A negative result is when there are no sperm cells observed.

11.7.12.10.3 Case notes must include slide number and well number should be designated for each well on the slide that has a sample (i.e., for the first slide and first well you could put 1-1). For positive results the number of sperm per field of view should be recorded as well as the Vernier numbers to record the location on the slide of where the sperm where located at (i.e., 4 x 98.5, 111.0).

11.7.13 Other staining methods that are used to stain sperm slides are Florence Stain and Auramine-O.

11.7.14 There are also some fluorescence stains like Sperm Hy-Liter that use a fluorescent microscope to visualize the sperm heads.

11.8 Tasks

11.8.1 Observe a qualified analyst(s) perform all phases of sexual assault testing (including AP, Seratec, Sperm Search, as/if available and as applicable). The trainee may observe as many analysts as they feel necessary to become comfortable with the processes.

11.8.2 Complete Assignment 2 Sexual Assault Serology. **Technicians complete Technician Assignment 2 Sexual Assault Serology Assistance.*

11.9 Evaluation

11.9.1 Take and pass the sexual assault assignment quiz.

11.9.2 The successful completion of the above tasks.

11.9.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

11.9.4 **Technician Mock Trial Assignment*

11.9.4.1 **Following the successfully completion of the technician competency test for a given sample set (in conjunction with a qualified analyst), the trainee will complete an informal mock trial evaluation with the assigned trainer and/or Technical Manager.. The technician will be released for technician duties in casework by Memorandum from the Technical Manager after successful completion of this assignment.*

12 Evaluation of Hairs

12.1 Goals

12.1.1 To become familiar with the microscopic structures of hair.

12.1.2 To become familiar with the basic differences in human and animal hairs.

12.1.3 To recognize the characteristics of human hair roots in the various growth stages.

12.1.4 To recognize the types of hair roots that are suitable for STR typing.

12.1.5 To become familiar with the mounting of hairs for microscopic examination.

12.1.6 To become familiar with the documentation of hairs.

12.2 West Virginia University of Forensic Science Initiative “Hair Evaluation of DNA Analysis”

12.2.1 The trainee will complete the units obtained in the WVU Hair Evaluation Training. There are short quizzes after each unit that will be completed; these quizzes are open book.

12.2.2 The following Units are in the WVU Training:

12.2.2.1 Unit 1 Why Conduct Hair Exams?

12.2.2.2 Unit 2 Recovery and Collection of Hair Evidence

12.2.2.3 Unit 3 Basic Microscopy

12.2.2.4 Unit 4 Microscopical Hair Properties

12.2.2.5 Unit 5 Is it Human or Animal?

12.2.2.6 Unit 6 Race, Ancestry, Ethnicity

12.2.2.7 Unit 7 Body Area (Somatic Origin)

12.2.2.8 Unit 8 Damage, Disease, Treatment

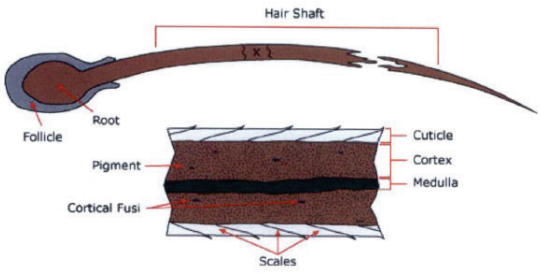
12.2.2.9 Unit 9 Suitability

12.2.2.10 Unit 10 Examination of Hair and DNA

12.2.2.11 Unit 11 Legalities of Hair Evidence

12.3 General Information on Hairs

12.3.1 Hair is an appendage of the skin that grows out of an organ known as the hair follicle. The length of the hair extends from its root or bulb embedded in the follicle, continues into a shaft, and terminates at the tip end. It is the shaft, which is composed of three layers-the cuticle, cortex, and medulla-that is subjected



to the most intense examination by the forensic scientist.

12.3.2 The cuticle is the scale structure covering the exterior of the hair. The cuticle is formed by overlapping scales that always point toward the tip end of each hair. The scales are formed from specialized cells that have hardened (keratinized) and flattened in progressing from the follicle. The scales of most animal hairs are described as having the appearance of shingles on a roof, which makes it an important feature for species identification.

12.3.3 The cortex is contained within the protective layer of the cuticle and is made up of spindle-shaped cortical cells that are aligned in a regular array, parallel to the length of the hair. The cortex derives its major forensic importance from the fact that it is embedded with the pigment granules that impart hair with color. The color, shape, and distribution of these granules provide important points of comparison among the hairs of different individuals.

12.3.4 The medulla is a collection of cells having the appearance of a central canal running through a hair. In many animals, this canal is a predominant feature, occupying more than half of the hair's diameter. Generally, for human hairs the medulla is less than one third of the diameter of the hair shaft and in animals it is typically ½ or greater. The presence and appearance of the medulla varies from individual to individual and even among hairs of a given individual. Not all hairs have a medulla and when they do exist, the degree of medullation can vary. Medulla can be classified as being continuous, interrupted, fragmented, or absent.

12.3.5 The root and other surrounding cells contained within the hair follicle provide the tools necessary to produce hair and continue its growth. Human head hair grow in three developmental stages and the shape and size of the hair root is determined by the growth phase in which the hair happens to be. The three phases of hair growth are anagen, catagen, and telogen phases.

12.3.5.1 Anagen Phase: The initial growth phase during which the hair follicle is actively producing the hair. The root bulb is a flame shaped appearance. A follicular tag is a translucent piece of tissue surrounding the hair's shaft near the root. It contains the richest source of DNA associated with the hair.

Anagen

12.3.5.2 Catagen Phase: A transition stage between the anagen and telogen phases of hair growth. Catagen roots are typically an elongated appearance as the root bulb shrinks in size and is being pushed out of the hair follicle.

Catagen

12.3.5.3 Telogen Phase: The final growth phase in which hair naturally falls out of the skin. The root is a club-shaped appearance and during a two to six month period the hair will be pushed out the follicle causing the hair to be shed naturally.

Telogen

12.4 Documentation and Collection of Hair Evidence

12.4.1 Hairs should be collected when analyzing other pieces of evidence such as sheets, clothing, hats, etc. Typically, these are collected, bindled, and kept with the evidence and documented as possible hairs being collected from the item. Typically, these types of hairs are not analyzed any further at this time.

12.4.2 Hair analysis will be conducted following the FBU Policy Manual SER_7 protocol. The macroscopic characteristics that should be documented are the approximate color and shade, hair form (straight, curved, wavy, loose curl, tight curl, etc.), and the approximate length of the hair.

12.4.3 Microscopic examinations characteristics that should be documented are the characteristics of the medulla (note the diameter relative to the hair shaft), any distinguishing characteristics (color, shade, cuticle scaling, pigment, etc.), the presence or absence of a root, and if the root is present if there is cellular material present. These should be documented in your case notes. A photograph will help document these characteristics.

12.4.4 Factors to keep in mind when determining if a hair is human or animal: the color and pigmentation of the hair, the medulla, the root shape, the diameter of the hair, and the scaling of the hair.

12.5 Tasks

12.5.1 Complete the West Virginia University Forensic Science Initiative “Hair Evaluation of DNA Analysts” training unit modules.

12.5.2 Complete Hair Evaluation assignment.

12.5.3 Complete the Hair Competency – assigned and graded by the Technical Manager or designee.

12.6 Evaluation

12.6.1 Successful completion of the Hair Competency.

12.6.2 The successful completion of the above tasks.

12.6.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

13 Casework Approach and Documentation for Serology Casework

13.1 Goals

13.1.1 To become familiar with documentation of serology cases in the Analysts' notes.

13.1.2 To become familiar with the format and report wording of analysts within the Forensic Biology Units.

13.2 Digital Photography

13.2.1 In several cases, it may be necessary to take photos of the item of evidence to help describe them or document certain conditions of the evidence. In these instances, the photo is not considered part of the evidence and does not need to be handled in any specific way.

13.2.2 Typically, these types of photos that document the evidence are included in the case record by inserting the picture into the BEAST Image Vault. The analyst may also wish to include photo(s) in the BEAST worksheet. Photos added to the BEAST Image Vault must bear the case number and Item number in the file name.

13.2.3 In cases where the evidence is being consumed, it is required to photograph the evidence before consumption. For example, if consuming a swab, a photo will be taken of the swab prior to taking the cutting, ensuring that the swab staining can be seen in the photograph. In this case, at minimum, the case number, item number, and description of the evidence should be in the file name. These photos should be available in the BEAST Image Vault.

13.2.4 Every attempt should be made when photographing evidence to include the case number, item number, date, Analyst's initials, and a scale in the photo. Some exceptions to this requirement are when taking photos of hairs on the microscope at magnification and close-up pictures of particular staining, etc.

13.3 General Guidelines for Documentation of Evidence in Serology Notes

13.3.1 A description of the outer packaging should be included in your notes (i.e., condition of seal, how the package is labeled, and any other pertinent information).

13.3.2 The date that the analysis is performed should be documented in your notes.

13.3.3 An overall visual description of the evidence should include size, color, and condition of the evidence if applicable.

13.3.4 If debris and/or hairs are collected, this should be documented in your case notes.

13.3.5 A description of the size and staining of any positive stains should be documented. In addition, the location of the stain on the item of evidence should be documented.

13.3.6 If a stereomicroscope and/or microscope, flashlight, Crimescope, etc. are used this should be documented in the notes, with the exception of the tests that require routine use of a microscope (i.e., Takayama, sperm cell search, hair analysis, etc.).

13.3.7 The lot numbers and expiration dates should be documented in the case notes. If multiple lot numbers of the same reagent are used, the Analyst should document which lot number was used for which day of testing.

13.3.8 Serology controls must be tested prior to/or in conjunction with the evidence samples and the results should be documented in the notes.

13.3.9 When batching cases together (i.e., sexual assault kits and cases) all the case numbers comprising a batch should be clearly represented in the notes. This is usually put in the "Case Remarks" panel in the BEAST.

13.3.10 **If a technician is utilized in casework the analyst must clearly identify who performed the assistance and what the technician did. This is accomplished by putting the technician's initials or name in the "Case Remarks" section in the BEAST and having them initial this section. The notes can be printed off and initialed by hand or with the electronic initial in PDF. If an electronic version is used, then it should be uploaded to the Documents tab in the BEAST. In serology cases, this is most typically done when the technician does the p30 extraction and prepares the slide(s) for the analyst. There are other instances where a technician could be used as well (i.e., RSID-Blood testing).*

13.4 General Guidelines for Serology Report Writing

13.4.1 The procedure for generating the Analytical Reports is covered under QP 28 Report Writing.

13.4.2 The reports should have the title "Criminalistics Examination Report" and will include the date the report is issued.

13.4.3 All the header information will be automatically generated by the BEAST, when you generate the report.

13.4.4 The report will reflect the date the evidence was first received by the laboratory.

13.4.5 The report will include a description of the evidence submitted and an unambiguous identification of the items tested.

13.4.6 If known, the requesting agency's item number should be included in the description of evidence.

13.4.7 The report should contain a signature block with the title of the analyst issuing the report and an electronic signature, when issued through the BEAST.

13.4.8 The requesting agency case number should be in the appropriate location on the report.

13.4.9 The analysis of evidence section will include an identification of the items tested and the results of those tests.

13.4.10 When associations are made, the significance of the association shall be clearly communicated and when there is an elimination, the elimination shall be clearly stated in the report.

13.4.11 When there are inconclusive results, the report should clearly state why no conclusions can be drawn.

13.4.12 The reports will have the page number and total number of pages.

13.4.13 The reports will follow the proper rules of grammar and correct spelling.

13.4.14 The reports will be in Times New Roman font and preferable be font size 10 or 12. The certification block and any footer can have a font size of less than 10; minimum font size should be no less than 7.

13.4.15 If there are multiple submittals, the date submitted should be listed before those items in the description of evidence section.

13.5 Criminalistics Statistics

13.5.1 Every case the Analyst works will require them to track how many items were submitted, the time it took to work the case, and the number of examinations for the case.

13.5.2 This is recorded in the Notes tab of the BEAST.

13.5.3 The Forensic Biology Units utilize an Excel spreadsheet that will assist in calculating the number of examinations. This Excel spreadsheet is "Biology_Number_of_Exams_Stats_Calculator_v1.0" and is located at \\pm-fsc16273s\Biology

13.5.4 The Analyst can maintain their own personal spreadsheet to track the cases they have worked.

13.5.5 In the notes section, the Analyst will indicate what type of analysis it is by putting a 1 in the block for doing this type of case and a 0 if this type of analysis was not done in this case (serology, DNA, Y-STR).

13.6 Report Wording

13.6.1 The report wording is covered in CASE_7 Report Wording of the FBUs' Policy Manual.

13.6.2 Any testing performed must be reported.

13.6.3 The wording described in the protocol is a guideline for the report wording and does not have to be followed exactly; however, every attempt should be made to keep the wording in the report consistent with other Analysts, so as to limit the confusion to the customer when reading reports from multiple Analysts.

13.6.4 Every case is different and there is no set report wording for every circumstance.

13.7 Tasks

13.7.1 The trainee will review at least 5 cases from different Analysts to become familiar with how Analysts put their serology case files together. The cases must be a mixture of both blood cases and sexual assault cases (i.e., 3 blood cases and 2 sexual assault cases, etc.).

13.7.2 Read CWQM_11 Reports, CASE_1 Case Notes, CASE_2 Digital Photography, CASE_3 Serology Analysis, CASE_7 Report Wording, Quality Manual QP 28 Report Writing, and QP 29 Criminalistics Statistics.

13.7.3 The trainer will advise the trainee on how to use the BEAST; this can be done by the trainee observing the trainer work a case, generate the notes, write the report, route/complete the technical review process, and fill in the statistics in the BEAST.

13.8 Evaluation

13.8.1 The successful completion of the above tasks.

13.8.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

14 Administrative and Technical Review Training for Serology

14.1 Goals

14.1.1 To introduce the trainee to the requirements for reviewing a serology case file.

14.1.2 Familiarize the trainee with the administrative (AR) and technical review (TR) process.

14.2 General Information

14.2.1 Administrative and Technical Reviews are governed by QP 31 Reviews and CWQM_12 Review.

14.2.2 The review process does not shift the responsibility of the Analyst's findings to the reviewer; each Analyst is responsible for their own work.

14.2.3 Each Analyst should review their own cases thoroughly before submitting the case for review.

14.2.4 When the Analyst has reviewed their case, they will press the sign button in the BEAST and then route the case assignment to the reviewer and designate in the appropriate designation code (i.e., TR).

14.2.5 Administrative and technical reviews can be done separately by different reviewers or at the same time. Typically, in the FBU, one reviewer will do both the administrative and technical reviews. The list of qualified individuals to perform these reviews is maintained by the Technical Manger and is available on the network.

14.2.6 When the reviewer has completed the review, they will route the case back for any corrections, as necessary, by using the routing tab and putting the designation for corrections (C). If the reviewer has questions or comments, they may route the case back using the routing tab and putting the designation for question or comment (RQC). If there are no corrections or questions or comments, the case will be routed for approval by selecting the appropriate designator (RA).

14.2.7 When the necessary corrections are made, the Analyst will again sign the report and route it for review. Once the reviewing Analyst has verified all corrections are made, they will route it back for approval. At this point the Analyst can "approve" the case, which will close the assignment and affix the Analyst's signature to the report.

14.2.8 Analysts may route cases in the BEAST at any time for training purposes (RTP) to allow trainees to review cases prior to an official technical review.

14.2.9 All serology cases will be 100% administratively and technically reviewed by a qualified analyst.

14.3 Administrative Reviews

14.3.1 An administrative review is an evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness.

14.3.2 General Guidelines for Administrative Reviews

14.3.2.1 Review the report for spelling and grammatical accuracy.

14.3.2.2 Ensure that all hardcopies in the case file have the analyst's initials and case number on them.

14.3.2.3 Ensure the report has all the necessary information and all required sections are included in the report.

14.3.2.4 Ensure the original RFLE and BEAST information is accurate.

14.4 Technical Reviews

14.4.1 A technical review is an evaluation of reports, notes, data, and other documents to ensure there is an appropriate and sufficient basis for the scientific conclusions drawn.

14.4.2 General Guidelines for Technical Reviews

14.4.2.1 Ensure that all lot numbers and expirations dates are properly documented.

14.4.2.2 Ensure that all dates of analysis are documented.

14.4.2.3 Ensure that all appropriate testing was done as dictated by case circumstances and what was requested by submitting agency.

14.4.2.4 Ensure that the conclusions are supported by the data from the analysis of evidence.

14.4.3 A review of the chain of custody will be conducted by verifying the item's location, submittal dates and where the item has traveled since being submitted to the lab on the Custody tab in the LIMS.

14.5 Tasks

14.5.1 Read QP 31 Reviews and CWQM_12 Review.

14.5.2 The trainer or designee will give the trainee a serology case they are reviewing from another Analyst, before or after they initially review it and without telling the trainee what corrections were found; the trainer will ask the trainee to review it and note any corrections they may have. This will be done with at least 2 blood cases and 2 sexual assault cases. The Technical Manager will determine if more cases should be completed.

14.5.3 Upon being released to perform independent casework in serology, the trainee will be given cases from other Analysts to review. The trainee will review these cases log any findings. After the official TR has been completed, the trainee will compare the official TR finding to their own and forward this log to the Technical Manager for review. The Technical Manager will determine if there are any problems with the review. If, after a number of cases as determined by the Technical Manager, there are no problems, the trainee will be released to perform independent reviews. If there are problems, the Technical Manager will develop a plan to remediate the cause of the problem.

14.6 Evaluation

14.6.1 The successful completion of tasks 14.5.1 - 14.5.3.

14.6.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the above tasks.

15 Mock Cases

15.1 Goals

15.1.1 To become familiar with the case working requirements for forensic serology analysis according to the policies and procedures of the OSBI Laboratory.

15.1.2 To ensure the trainee is capable of independent case work analysis and techniques used in serology.

15.1.3 To ensure the trainee has developed good laboratory practices.

15.1.4 To ensure the trainee can compile a comprehensive, accurate, and straightforward report that meets the report writing requirements.

15.2 Mock Cases

15.2.1 There will be ~five mock cases that are to be provided by the Technical Manager and completed by the trainee; these will generally consist of at least 1 blood only, 2 sexual assault only, and 2 blood and sexual assault combined.

15.2.2 These cases will be completed in a manner that will resemble actual casework analysis, which will include all the required documentation in the LIMS.

15.2.3 A final mock case that includes blood, sexual assault, and hair analysis will be used as the case for the mock trial.

15.3 Tasks

15.3.1 The mock cases will be completed with the skills that were obtained throughout the serology training.

15.3.2 Complete Serology Mock Cases Assignment.

15.4 Evaluation

15.4.1 The successful completion of the above tasks.

15.4.2 The successful completion of final mock case that will serve as the practical competency test.

15.4.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the above tasks.

15.5 **Technician Competency Assignment*

15.5.1 ***The technician will be assigned a set of competency samples for which the technician must successfully complete the duties of a technician for the sample set working in conjunction with a qualified analyst. Both the technician and the assigned qualified analyst will provide results to the Technical Manager for evaluation.*

16 Testimony and Expert Witness Qualification

16.1 Goals

16.1.1 To be aware of the etiquette required in the Oklahoma and Federal Court Systems.

16.1.2 To become familiar with the legal terms, procedures, and some of the pertinent legal developments in both Federal and State jurisdictions as they apply to forensic biology.

16.1.3 To become skilled at expressing results and conclusions to a court of law in both jury and technical formats.

16.1.4 To allow the trainee the opportunity to practice testimony prior to the mock trial for serology.

16.2 There are many legal terms and procedures that will be encountered through the interaction with the court system. It is important to develop knowledge of some of these legal terms. Some of the more common legal terms that the trainee may encounter are as follows:

16.2.1 American Board of Criminalistics (ABC): A group of regional and national organizations that certify forensic practitioners through a voluntary process of peer review and testing. The ABC offers certification in criminalistics, forensic biology, drug chemistry, fire debris analysis, and trace evidence.

16.2.2 American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB): An accrediting organization for forensic science laboratories absorbed by the American National Standards Institute (ANSI) National Accreditation Board (ANAB), which provides internationally recognized accreditation for ISO/IEC 17025 testing, calibration, and forensic laboratories.

16.2.3 Bench Trial: In the United States, a trial that has a single judge who decides issues of both law and fact.

16.2.4 Burden of Proof: The prosecution must prove beyond a reasonable doubt that the defendant is guilty of the crime charged.

16.2.5 Chain of Custody: A documentary method for keeping track of physical and tangible items of evidence during the dispute resolution process.

16.2.6 Credibility: The believability of a witness.

16.2.7 Cross-Examination: The phase of the dispute resolution process in which opposing counsel asks questions of witnesses in order to test the truth, accuracy, or thoroughness of direct testimony.

16.2.8 Defendant: A person or entity against whom a civil or criminal action has been brought.

16.2.9 Deposition: Testimony given outside the presence of the trier of fact in the presence of a court reporter, counsel, and the parties, for the purpose of finding out what you know, determining what sort of witness you will be, and locking you into a position.

16.2.10 Direct Examination: The procedure during a trial or hearing that first presents a witness's testimony to the trier of fact.

16.2.11 Discovery: Those processes used before trial in order to uncover the facts of a case.

16.2.12 Expert: An individual who, by knowledge, skills, experience, training, and/or education, may testify in the form of an opinion or otherwise to scientific, technical, or other specialized knowledge.

16.2.13 Expert Opinion: A major exception to the general rule of evidence that otherwise requires testimony based upon personal knowledge. Because of their knowledge, training, and experience, experts are allowed to render opinions about what happened rather than merely recite what their senses recorded.

16.2.14 Expert Testimony: Presentation of verbal or written evidence in the dispute resolution process in a scientific, professional, technical, or specialized field, usually beyond the knowledge of a layperson.

16.2.15 Expert Witness: A person who by reason of education, training, and/or experience has special knowledge not held by the general public.

16.2.16 Federal Rules of Criminal Procedure: Those organized processes that govern trials and preliminary matters in the U.S. courts involving criminal proceedings.

16.2.17 Federal Rules of Evidence: The rules that govern the admissibility of evidence in the U.S. Federal court system.

16.2.18 *Frye* rule: A law of court that provides that in order for an expert witness to testify concerning scientific, technical, professional, or specialized matters, the opinion testimony must be based upon a reasonable degree of acceptance within the scientific, technical, professional, or specialized field of the process utilized by the witness to reach the conclusions rendered.

16.2.19 Grand Jury: A group of citizens who determine if enough evidence exists for a criminal trial. If so, an indictment is filed with a court in the appropriate jurisdiction. Grand juries do not exist outside the United States and are not universal within the country (Connecticut, Pennsylvania, and District of Columbia do not use grand jury indictments); each state that uses them has its own set of grand jury procedures.

16.2.20 Hearing: An organized process by which the contesting parties present their evidence and testimony.

16.2.21 Hearsay: A statement of conduct made by a witness outside the presence of the trier of fact with no opportunity for cross-examination by the opposition. There are certain exceptions to the hearsay exclusion that are based on indicators of reliability attending such declarations or conduct which make them admissible.

16.2.22 Hearsay Rule: A legal rule that prevents testimony by people who heard someone else say something; the person who heard the statement cannot be cross examined on the substance of what they heard but only that they heard it.

16.2.23 International Organization for Standardization (ISO, derived from the Greek “isos” meaning equal): An international standard-setting body composed of representatives from various national standards organizations. Founded in 1947, the organization promulgates worldwide industrial and commercial standards.

16.2.24 Jury: A sworn body of persons convened to render a verdict and a finding of fact on a legal question officially submitted to them or set a penalty or judgment on a convicted person or persons.

16.2.25 Mistrial: The cancellation of trial prior to a verdict.

16.2.26 National Institute of Standards and Technology (NIST): A non-regulatory agency of the U.S. Department of Commerce whose mission is to promote U.S. innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve quality of life.

16.2.27 Opinion: A person's ideas and thoughts on something; it is an assessment, judgment, or evaluation of something.

16.2.28 Opinion Witness: A forensic or expert witness who is entitled to render opinions, as opposed to testifying about factual matters based upon the senses.

16.2.29 Prosecutor: The attorney who represents the people (prosecution) in a criminal case.

16.2.30 Protocol: A series of steps, processes, or procedures usually followed in a scientific, technical, professional, or specialized area.

16.2.31 Rules of Evidence: Rules that determine what is an is not admissible in various dispute resolution processes. They may be enacted by the legislative bodies, determined by appellate decision, or controlled by court order.

16.2.32 Scientific Revolution: The deconstruction of a dominant paradigm in science and its replacement by a new, significantly different paradigm. The shift from Newtonian to Einsteinium physics is a good example of a scientific revolution.

16.2.33 Statute: An enactment by a legislative body that constitutes the law of a particular state or country.

16.2.34 Technique: A standard method that can be taught.

16.2.35 Testimony: The process of conveying information from witness to a judge, jury, arbitration, or other hearing panel.

16.2.36 Testing: A means of analysis, examination, or diagnosis.

16.2.37 Trial: The method by which disputes are resolved in the court system, with either a jury or judge as the trier of fact.

16.2.38 Trier of Fact: The judge, jury, administrative body, board, or arbitration panel that determines the fact issues of the controversy in the dispute resolution process.

16.2.39 U.S. Court of Appeals: Also known as circuit courts, these are the intermediate appellate courts of the U.S. federal court system. A court of appeals decides appeals from the district courts within its federal judicial circuit.

16.2.40 Verdict: A trial decision by a jury in a criminal or civil case.

16.2.41 *Voir dire*: This French term means "to speak the truth," and is a process by which attorneys or the court are allowed to question jurors as to their fitness to serve as impartial triers of fact. Also, the examination conducted by an attorney or the court in

a trial or hearing by which a witness or a document is tested for reliability. Expert witnesses are sometimes subjected to *voir dire* examination before being allowed to render opinions.

16.3 Testimony

16.3.1 Testimony is one of the most important parts of a forensic scientist's job. Testimony is highly structured, interactive, narrowly focused, of indeterminate length, and directed by someone who is not the speaker.

16.3.2 Expert Testimony is not a simple conversation.

16.3.2.1 Expert testimony may be one of the most difficult forms of spoken communication human conduct.

16.3.2.2 Expert testimony requires a simplification of very technical information to an audience who does not necessarily have any knowledge of the topic.

16.3.2.3 The rules governing testimony are layered and intricate, spanning English grammar and vocabulary through scientific methodology and into federal, state, or local laws, and even "house rules" of particular judges or jurisdictions may apply.

16.3.2.4 The question-and-answer format is done in an adversarial setting; even if the attorney asking you the question is the one who called you to testify, opposing counsel is listening and may be looking for openings and missteps.

16.3.3 Analyst's Report

16.3.3.1 The report should be organized, neat, and clear.

16.3.4 Preparation for Testimony

16.3.4.1 The attorney may want to meet with you prior to testifying to go over your testimony and explain the tests and procedures to them.

16.3.4.2 Typically introductory questions will come first, such as your name and title, place of employment, education, training, and experience. Observations and testing will come next, followed by your opinions and reasoning.

16.3.4.2.1 At trial, your testimony is typically divided into three phases.

Phase1: Your qualifications as an expert witness to render opinion testimony (*voir dire)*.

Phase 2: Direct Examination, which includes your work and how it was performed, findings of fact based on your analysis, your expert opinions and interpretations and the reasons that support your conclusions.

Phase 3: Cross-Examination concerning facts in evidence and your direct examination.

16.3.5 Dress and Demeanor

16.3.5.1 When you testify you are representing more than yourself. You are representing your laboratory, your government, and your profession. You should dress like you are on a job interview. Typically for males, a suit and tie will be the appropriate dress. For females, typically a suit is the appropriate dress.

16.3.5.2 Refer to the OSBI Policy 108 on the appropriate dress requirements.

16.4 Responding to Questions

16.4.1 It is necessary for the trainee to become familiar with how to properly communicate with attorneys, judges, and juries. Many times, the terms and amount of detail used will vary when explaining an item, test, or result to these different groups of people.

16.4.2 Jury Answer

16.4.2.1 When speaking to a jury, the answer to the question should be concise and as uncomplicated as possible. You should try to refrain from using technical terms as much as possible and many times analogies are used to equate a particular test with an everyday item or process most people can understand. If more information is required, then it may be necessary to provide more detail about the process. It is important that if pressed, the Analyst understands and can verbalize a technical answer to the question.

16.4.3 Technical Answer

16.4.3.1 A technical answer is a more detailed answer and included the technical terms and procedural elements. This is the answer that can be provided if either all the court participants have a good understanding of the issue being discussed or if more information is needed on the jury answers. These answers should be understandable and concise, but they may be longer and more in depth than any jury-friendly answer.

16.5 Tasks

16.5.1 The trainee should observe other qualified Analysts testify in court. Preferably the trainee should observe a blood and sexual assault testimony, but it will depend on the frequency of the qualified Analysts testifying in court and to what analysis methods to which they are testifying. The trainee should document any testimony observed on the trainee checklist and/or the trainee notebook.

16.5.2 The trainee and trainer or designee should have several practice mock court sessions that cover the various aspects of testimony.

16.5.3 The trainee will complete a formal mock trial and technical question session over the areas of serology.

16.5.4 Read the following references.

16.5.4.1 Appendix 4 and Chapter 18: Legal Aspects of DNA Testing and the Scientific Expert in Court from Advanced Topics in Forensic DNA Typing: Methodology by John Butler.

16.5.4.2 Chapters 1, 3, 4, and 5 from Feder's Succeeding as an Expert Witness 4th Edition

16.5.4.3 Twenty-Five Suggestions to a Court Witness document, Oklahoma District Attorney's Council

16.6 Evaluation

16.6.1 The successful completion of the above tasks.

16.6.2 The successful completion of a mock trial and technical answer question session covering the areas for forensic serology.

16.6.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

16.6.4 **Technician Mock Trial Assignment*

16.6.4.1 **Following the successfully completion of the duties of a technician for a given sample set (in conjunction with a qualified analyst), the trainee will complete an informal mock trial evaluation with the assigned trainer and/or Technical Manager. Technicians will be released for technician duties in casework by Memorandum from the Technical Manager after successful completion of this assignment.*

17 History of Forensic DNA Analysis

17.1 Goals

17.1.1 To introduce the concepts of the development of the forensic DNA field.

17.1.2 To provide the trainee with a review of the knowledge required for general DNA and forensic DNA analysis.

17.1.3 To introduce the trainee to the development and implantation of Quality Assurance in forensic DNA analysis.

17.2 Brief History

17.2.1 “DNA Fingerprinting” or DNA typing (profiling) was first described in 1985 by English geneticist named Dr. Alec Jeffreys.

17.2.2 He found that certain regions of DNA contained DNA sequences that were repeated over and over again next to each other.

17.2.3 He also found that the number of repeated sections present in a sample could differ from individual to individual.

17.2.4 By developing a technique to examine the length variation of these DNA repeat sequences, Dr. Jeffreys created the ability to perform identity tests.

17.2.5 These DNA repeat regions became known as variable number of tandem repeats (VNTRs).

17.2.6 The technique Dr. Jeffreys used was called restriction fragment length polymorphism (RFLP); which involves the use of a restriction enzyme to cut the regions of DNA surrounding the VNTRs.

17.2.7 The RFLP method was used to help in an English immigration case and to solve a double murder case (1986).

17.2.8 Around 1985, Kary Mullis invented the polymerase chain reaction (PCR) as a means to make copies of any desired region of DNA. This method allows for the sensitive detection needed in forensic applications. In the mid 1980’s there were no polymorphic genetic marker systems characterized yet for use with the amplification power of PCR and it would be several years before PCR would be used in forensic applications.

17.2.9 The first PCR based DNA test kit detected sequence variation at the Human Leukocyte Antigen (HLA) DQA1 gene found on chromosome 6.

17.2.10 Recognizing the need for greater power of discrimination than could be provided by the 28 detectable genotypes on DQA1, the AmpliType PM+DQA1 kit was developed. This kit co-amplified a portion of the HLA DQA1 gene along with five other DNA segments located on human chromosomes 4, 7, 11, and 19.

17.2.11 The amplified fragment length polymorphism that gained the greatest use in the mid-1990s was D1S80 (a PCR amplified VNTR), a minisatellite on chromosome 1 containing a 16 bp repeat unit and alleles spanning the range of 14 to 41 repeat units.

17.2.12 Microsatellites or short tandem repeats (STRs) were being developed during this time, which had shorter repeat units (in the range of 2 to 7 bp) and it was becoming apparent this methodology would be the better for the future use in forensic DNA testing.

17.2.13 In 1994, the first commercial kit for silver-stain analysis became available.

17.2.14 Colored fluorescent dyes were developed to give the capability of simultaneously detecting STR alleles in the same size range by labeling the potentially overlapping PCR products with different colored fluorescent dyes.

17.2.15 The United Kingdom's Forensic Science Service launched the first national DNA database in April 1995 using 6 STR loci.

17.2.16 In November 1997, the FBI Laboratory selected 13 core loci for data sharing throughout the United States (the original 13 core CODIS loci).

17.2.17 From here, the multiplex kits were developed with an increasing number of loci and enhanced into the different kits that are commercially available today.

17.3 Historical Development of the Quality Assurance Standards

17.3.1 The Technical Working Group on DNA Analysis Methods (TWGDAM) was established in November of 1988 under FBI laboratory sponsorship to aid forensic DNA scientists. Since 1998, TWGDAM has been known as SWGDAM (Scientific Working Group on DNA Analysis Methods). It is a group of approximately 50 scientists to bring recommendations on various aspects of forensic DNA analysis.

17.3.2 The DNA Advisory Board (DAB) was a congressionally mandated organization that was created and funded by the US Congress DNA Identification Act of 1994. The first meeting was held on May 12, 1995. The DAB consisted of thirteen voting members that included scientists from state, local, and private forensic laboratories, molecular geneticists and population geneticists not affiliated with a forensic laboratory, a representative from NIST, the chair of TWGDAM, and a judge. The DAB was created for a five-year period to issue standards for the Forensic DNA community. When DAB's responsibilities ended in 2000, SWGDAM was designated as the group responsible for offering recommendations to the forensic community.

17.3.3 Quality Assurance Standards (QAS):

17.3.3.1 Forensic DNA laboratories in the US are mandated by congress to follow strict quality assurance standards.

17.3.3.2 In October 1998, the FBI DAB issued the Quality Assurance Standards (QAS) that define how forensic DNA laboratories are required to conduct business. The QAS was revised a decade later and the revision was in effect on July 1, 2009, and some additional revisions were done in 2011. In 2020, the QAS was further revised and reorganized with the discussion portions moved to an associated Guidance Document.

17.3.3.3 DNA laboratories are governed by the QAS and regularly audited for their compliance to these standards.

17.3.3.4 There are seventeen topics covered by the QAS:

(1) Scope & Applicability
(2) Definitions
(3) Quality Assurance Program
(4) Organization and Management
(5) Personnel
(6) Training
(7) Facilities and Evidence Control
(8) Validation
(9) Analytical Procedures
(10) Equipment
(11) Reports
(12) Review
(13) Proficiency Testing
(14) Corrective Action
(15) Audits
(16) Professional Development
(17) Outsourcing Ownership

17.4 Tasks

17.4.1 Read the following references

17.4.1.1 Fundamentals of Forensic DNA typing, by John Butler Chapter 1, Chapter 2, and Chapter 3.

17.4.1.2 Advanced Topics in Forensic DNA Typing: Methodology, by John Butler, Chapter 1 and Chapter 7 (pages 167-176 only).

17.4.2 Review the above sections.

17.4.3 Complete the DNA Preparation of Training Samples in Assignment 0 (as applicable and directed by the trainer).

17.5 Evaluation

17.5.1 Complete the above tasks.

17.5.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

18 Y-Screen Assay

18.1 Goals

18.1.1 To introduce the trainee to the Y-screen assay for the detection of male DNA in forensic sexual assault samples.

18.1.2 To introduce the trainee the theory behind the extraction and quantitation techniques utilized in the Y-screen assay.

18.1.3 To introduce the trainee to the quality control practices associated with the Y-screen assay.

18.1.4 To ensure the trainee fully understands the precautions taken to prevent contamination during the Y-screen assay.

18.1.5 To introduce the trainee to the importance of reagent blanks and controls for the Y-screen assay.

18.1.7 To introduce the trainee to the various maintenance requirements for the instrumentation and equipment used in the Y-screen assay.

18.1.8 Understand how the Y-screen assay is utilized in casework.

18.2 Y-Screen Assay

18.2.1 SWGDAM recommends that laboratories screen swab samples with a sensitive test for male DNA, such as the Y-screen assay, and use serology tests, which are generally limited to one biological fluid source, only when necessary.

18.2.2 The Y-screen assay is a “Direct to DNA” approach. The Y-screen assay is a confirmatory test that is widely accepted and used throughout the forensic DNA community to screen sexual assault evidence for the presence of male DNA, even in the presence of high concentrations of female DNA.

18.2.2.1 OSBI uses the Y-screen assay to screen intimate forensic orifice swabs only (questioned items only) that are included in standard sexual assault kits, packages designated as sexual assault kits, or collected from the Office of the Chief Medical Examiner in instances where a sexual assault is suspected.

18.2.2.2 Intimate external (non-orifice) body swabs (not including external genitalia swabs), etc. are more vulnerable to sampling variability and may produce false-negative Y-screen assay results.

18.2.2.3 Underwear, clothing, bedding, tampons, maxi pads, etc. (including swabs of such substrates) have shown variability in substrate composition and fabric dyes, where applicable, that may affect (inhibit) amplification by real-time PCR. Because the Y-screen assay makes use of a crude lysis, PCR inhibitors cannot be removed during the assay. These sample types are also vulnerable to sampling variability and may produce false-negative Y-screen assay results.

18.2.3 The Y-screen assay may be used in conjunction with, or independent from, other laboratory screening methods to assist in deciding whether to proceed with DNA extraction procedures.

18.2.4 The Y-screen assay is an independent protocol validated with the laboratory's quantification kit and quantification system/software. It allows for the screening of up to 96 samples at once within a couple hours using current DNA techniques (extraction and quantification) and potentially eliminates the need for time-consuming and labor-intensive traditional serological screening methods, such as p30 and sperm cell search tests, thereby increasing throughput capabilities for processing sexual assault kits.

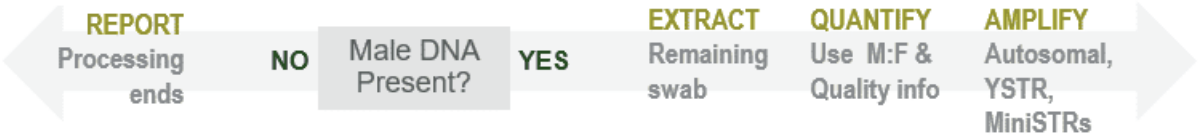
18.2.5 The OSBI Y-screen assay employed utilizes a crude extraction step, which rapidly lyses cells (including sperm), and the Quantifiler Trio kit. The assay provides the following information to aid in determining whether downstream DNA analysis is necessary:

- Whether the sample contains sufficient male DNA to obtain useful STR results
- Relative quantities of human male and female DNA that might be expected from a larger sample of the swab

18.2.6 Due to the crude lysis NaOH extraction used, any resulting quantification data should not be used for additional downstream decisions regarding STR and/or Y-STR analysis. Upon detection of human male DNA using the Y-screen assay, the remaining sample or a portion thereof must be extracted and quantified using downstream DNA analysis methods. Only then will quantitation values, ratios, and degradation index information be interpreted to assess sample viability for STR and/or Y-STR analysis.

18.2.7 The Y-screen assay provides information indicating the test has failed using the internal positive control (IPC), which is unavailable in many common serology tests.

18.2.8 As shown by the OSBI internal validation, the Y-screen assay is more sensitive than the STR/Y-STR kits. Therefore, for samples tested with the Y-screen assay that are negative, DNA analysis may be terminated (i.e., no downstream DNA analysis is necessary). Similarly, no serological analyses would be necessary for negative Y-screen assay samples, as such samples would not provide probative male DNA profiles based on the OSBI internal validation. Samples tested with the Y-screen assay that are positive (male DNA indicated) are candidates for further downstream DNA testing. The workflow is depicted as follows:



18.2.9 If male DNA is not detected with the Y-screen assay, the IPC amplification plot must be assessed to confirm the reaction did not fail due to the presence of inhibitors.

18.2.10 The IPC system template DNA is present at a consistent concentration across all reactions on the plate. Therefore, the IPC (JUN dye) C_T should be relatively constant in typical reactions. Normally, the C_T value for the IPC results should be between 20 and 30. However, the presence of PCR inhibitors and/or higher concentrations of

DNA can increase the IPC C_T relative to the average IPC C_T of the quantification standards on the same plate. With regards to the YS assay, it is possible for inhibitors to be present in the real-time PCR reaction due to the crude lysis utilized for the assay. High levels of inhibitors can cause the reaction to fail, which may be detected by Quantifiler Trio kit IPC. Likewise, if there is a high level of human (female) DNA present (≥ 10 ng/µL), which is routinely encountered when screening SA kit swabs with YS, competition between the human and IPC reactions may suppress the IPC amplification for a particular sample.

18.2.10.1 Generally, an IPC value of ≥ 30 can indicate that the sample is inhibited; however, as the amount of DNA in a sample increase, the value obtained for the IPC may also increase due to slight inhibition because of the high amount of (female) DNA present and not necessarily due to other PCR inhibitors. When encountering an elevated IPC C_T value associated with a sample that appears to be the result of elevated (female) DNA present and not due to a possible unknown PCR inhibitor affecting the YS sample, evaluate the IPC amplification plot and compare the IPC C_T value of the sample to the IPC C_T values of the quantification standards with similar concentrations. Remember, inhibition is generally assessed as having an IPC C_T value of undetermined or elevated by >1 C_T relative to the IPC C_T values of the quantification standards on the same plate with similar concentrations. If the IPC C_T is determined to be acceptable (no apparent reaction failure due to PCR inhibition), then the YS result for the sample in question may be reported as negative; otherwise, if the IPC C_T is not acceptable (inhibition due to PCR inhibitor), the YS result for the sample in question must be reported as inconclusive.

18.3 Tasks

18.3.1 Review the above sections and read section 19.3 (Quantitation) in this training manual.

18.3.2 Review the above information and following training materials:

18.3.2.1 Teachback Unit 2 Y-Screen Assay Background

18.3.2.2 Teachback Unit 3 OSBI Y-Screen Internal Validation

18.3.3 Observe a qualified Analyst perform the Y-screen assay (extraction and quantitation). The trainee may observe as many analysts as they feel necessary to become comfortable with the processes.

18.3.4 Complete the Y-Screen Assay Assignment.

18.3.4.1 Trainee should complete this assignment in conjunction with the training in Section 19 pertaining to quantitation to ensure the trainee fully understands the quantitation step of the Y-screen assay. * *Technicians complete Technician Assignment 4 Y-Screen Assay Assistance.*

18.3.5 Take the associated Y-Screen Assay quiz.

18.4 Evaluation

18.4.1 Successfully pass the quiz.

18.4.2 The successful completion of the above tasks.

18.4.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

18.4.4 **Technician Mock Trial Assignment*

18.4.4.1 **Following the successfully completion the technician competency test for a given sample set (in conjunction with a qualified analyst), the trainee will complete an informal mock trial evaluation with the assigned trainer and/or Technical Manager.. The technician will be released for technician duties in casework by Memorandum from the Technical Manager after successful completion of this assignment.*

19 Forensic DNA Analysis Process (STRs)

19.1 Goals

19.1.1 To introduce the trainee to the techniques utilized in the OSBI Forensic Biology Units to successfully recover, isolate, quantitate, amplify, analyze (capillary electrophoresis [CE]), and interpret DNA profiles.

19.1.2 To introduce the trainee the theory behind the extraction and purification, quantitation, amplification, and genetic analysis techniques utilized in the Forensic Biology Units.

19.1.3 To introduce the trainee to the quality control practices associated with DNA analysis.

19.1.4 To ensure the trainee fully understands the precautions taken to prevent contamination throughout the DNA process.

19.1.5 To introduce the trainee to the importance of reagent blanks and controls throughout the DNA process.

19.1.6 To introduce the trainee to the need for dedicated pre-amplification and post-amplification rooms and all requirements associated with the separate rooms.

19.1.7 To introduce the trainee to the various maintenance requirements for the different instrumentation and equipment used in the DNA laboratory.

19.1.8 To introduce the trainee to the various artifacts that are encountered when analyzing DNA profiles.

19.1.9 To define and understand Hardy-Weinberg Equilibrium (HWE), linkage equilibrium (LE) and the equations derived from each.

19.1.10 To understand the significance of the statistical calculation and be able to explain what the statistics do and do not mean in court.

19.1.11 To define and understand reference of population database.

19.1.12 To understand how the OSBI STATS Excel Spreadsheet and the population databases are used in casework.

19.1.13 To understand the equations and how to use them in casework applications.

19.2 DNA Extraction

19.2.1 Biological samples contain a number of substances besides DNA which can inhibit the ability to analyze the DNA. The extraction methods are developed to separate proteins and other cellular materials from the DNA molecules. Ideally, the DNA extraction process removes inhibitors that reduce the efficiency of or prevent polymerase chain reaction (PCR) amplification. The extraction process should also produce a stable solution containing high-quality DNA that will not degrade over time during sample storage.

19.2.2 The goals of the DNA extraction process are to: (1) lyse cells to release the DNA molecules, (2) separate DNA molecules from other cellular material, and (3) to isolate/purify the DNA to be able to perform PCR amplification.

19.2.3 All samples should be carefully handled to avoid sample-to-sample contamination or introduction of extraneous DNA. The extraction process is where the DNA sample is more susceptible to contamination in the laboratory than any other time in the forensic DNA analysis process.

19.2.4 The evidence samples (questioned) will be processed at separate times and/or separate locations from reference samples (knowns).

19.2.5 Organic Extraction (Standard Extraction)

19.2.5.1 Organic extraction was used for the longest period of time and for many years was the most widely used method for DNA extraction. High molecular weight DNA may be obtained most effectively with organic extraction. In 2016, the Organic Extraction procedure was taken off-line at the OSBI. New extraction methods have been developed that use chemicals that are less toxic than phenol.

19.2.5.2 Organic Extraction involves the serial addition of several chemicals. First sodium dodecylsulfate (SDS) and Proteinase K (Pro K) are added to break open the cell membranes and to break down the proteins that protect the DNA molecules while they are in chromosomes. The OSBI FBU used a stain extraction buffer (SEB), which is a detergent buffer that maintains a stable environment for the DNA and also breaks bonds and lyses open cells. The SDS is contained within the SEB, as well as other chemicals.

19.2.5.3 The sample was then placed through a Phenol/Chloroform/Isoamyl (PCI) step to remove the digested proteins and lipids, leaving the extracted DNA. The DNA is more soluble in the aqueous portion of the organic-aqueous mixture. When centrifuged, the unwanted proteins and cellular debris are separated away from the aqueous phase and double-stranded DNA molecules can be cleanly transferred for analysis. The aqueous layer was collected and placed in a centrifugal filter device. The OSBI used Microcon filters for the purification of DNA.

19.2.5.4 The Microcon filter devices were used to purify and concentrate the DNA. The samples are purified by removing salts, inhibitors, and low molecular weight solutes. Using Ultracel low adsorption, DNA accumulates on the surface without penetrating the membrane; the membrane has different properties based on the angle of the rotor and is hydrophilic. The membrane readily absorbs water and is made of regenerated nylon membranes so the device has the ability to retain molecules above a specified molecular weight. There is a dead-stop associated with the device that will prevent the membrane from drying out and causing potential sample loss.

19.2.5.5 The organic extraction method works well for recovery of high molecular weight DNA, but it is time consuming, involves the use of hazardous chemicals, and requires sample to be transferred between multiple tubes, which could increase the risk of error or contamination.

19.2.5.6 Standard organic extraction was used for samples that do not have an indication of containing sperm cells (i.e., blood, touch, known buccal swabs, teeth, bone, cigarette butts, etc.)

19.2.6 Organic Differential Extraction

19.2.6.1 Differential extraction is the modified version of the organic extraction method that separates epithelial and sperm cells. Differential extraction was first described in 1985 by Peter Gill. Differential extraction is used to isolate DNA from samples testing presumptive or confirmatory positive for seminal fluid.

19.2.6.2 This method is commonly used to isolate the female and male fractions in sexual assault cases that contain a mixture of male and female DNA. By separating the male fraction away from the victim's DNA profile, it is much easier to interpret the perpetrator's DNA profile in sexual assault cases.

19.2.6.3 The organic differential extraction procedure involves preferentially breaking open the female epithelial cells with incubation in an SDS/Pro K mixture. Sperm nuclei are subsequently lysed by treatment with an SDS/Pro K/dithiothreitol (DTT) mixture. The DTT breaks down the protein disulfide bonds that make up sperm cells.

19.2.6.4 Differential extraction works because sperm nuclei are impervious to digestion without DTT.

19.2.6.5 Differential extraction works well in most sexual assault cases to separate female and male fractions from one another. However, there are some perpetrators of sexual assaults that have had vasectomies in which case there is an absence of spermatozoa. Azoospermic semen cannot be separated from the female fraction with the differential extraction technique. In this case, the use of Y chromosome specific markers permit male DNA profiles to be developed in the presence of excess female DNA.

19.2.6.6 The OSBI FBU used a buffer (TNE), sarkosyl detergent, and Pro K to lyse the epithelial cells and proteins for the epithelial fraction (female portion). The epithelial fraction was incubated at 37˚C for about 2 hours. The epithelial fraction was then removed to a separate tube and kept, resulting in the sperm pellet left in the extraction tube. The sperm pellet was washed three times with sperm wash buffer.

19.2.6.7 The sperm pellet was then treated with a detergent buffer (TNE), sarkosyl, DTT, and Pro K to extract the sperm cells. The sperm fraction was incubated at 37˚C for 2 to 24 hours.

19.2.6.8 Both the epithelial and sperm fractions were taken through the remainder of the PCI and Microcon portions of the standard organic procedure as described in 19.2.5.3 and 19.2.5.4.

19.2.7 Solid-Phase DNA Extraction Methods (AutoMate Express using PrepFiler Chemistry)

19.2.7.1 In this type of extraction method the DNA is bound to a substrate such as silica particles. The DNA is retained while proteins and other cellular components are washed away. The DNA is then released in purified form.

19.2.7.2 In general, the way the magnetic silica particle technology for extraction works is as follows. The DNA molecules are reversibly bound to the magnetic particles in solution with a pH more acidic than pH of 7.5. A magnet is used to draw the silica-coated magnetic particles to the bottom

or side of the tube leaving any impurities in solution. These solution impurities (proteins, cell debris, etc.) are easily removed by drawing the liquid off of the particles. The magnetic particles with DNA attached can be washed several times to thoroughly clean the DNA. DNA is then released back into solution; the volume is determined by the amount of buffer the analyst/instrument (depending on protocol) adds in the elution volume.

19.2.7.3 The OSBI is using the PrepFiler Express and PrepFiler Express BTA Chemistry to be used in conjunction with the AutoMate Express to extract forensic samples.

19.2.7.4 The PrepFiler kit uses a combination of uniquely structure magnetic particles and a multi-component surface chemistry optimized to provide extremely efficient DNA binding capacity and maximum DNA recovery. The magnetized DNA complex remains stable during wash steps while removing inhibitors and enables the efficient release of DNA during elution to deliver a high yield of high-quality DNA. According to Life Technologies, the bead design cannot be saturated.

19.2.7.5 PrepFiler Workflow

19.2.7.5.1 Lysis: There is a PrepFiler Lysis Buffer that uses a special formulation of detergents and chaotropic salts that create better lysis conditions for optimal liberation of DNA from the biological material. Optimized protocols, with the option to use larger volumes of lysis solution, enable more efficient DNA extraction from a range of sample types and substrates, including highly absorbent material.

19.2.7.5.2 Substrate Removal: The PrepFiler Lysep™ Column or PrepFiler Filter Plate has dedicated plastics designed to easily separate substrate (swab, cloth, etc.) from the lysed sample; it is provided in single and 96-well format.

19.2.7.5.3 DNA Binding: The PrepFiler polymer-embedded magnetic particles are much smaller in comparison to other commonly used magnetic extraction technologies, resulting in a larger surface area with higher DNA binding capacity and more easily distributed particles. This maximizes the interface between the PrepFiler reagents and the magnetic particles, facilitating more effective DNA capture. Uniquely designed reagent components, in combination with the composite structure of the magnetic particles, enable maximum DNA capture and stable DNA/particle complex formation.

19.2.7.5.4 Wash/Purify: The PrepFiler Wash Buffer is a specially formulated wash solution maximizes the removal of most PCR inhibitors while minimizing the loss of DNA during the wash step.

19.2.7.5.5 DNA Elution: PrepFiler Elution buffer is a combination of unique polymer-embedded magnetic particles and PrepFiler reagent components enable effective DNA release into the

elution buffer. Elution conditions have been optimized for maximum elution efficiency and recovery of purified DNA.

19.2.7.6 The PrepFiler BTA Kit is a kit is specifically designed for challenging samples such as bones, teeth, and adhesive-based samples such as cigarette butts and tape lifts. It was designed to specifically improve the quantity and quality of DNA isolated from these forensic sample types, thereby increasing the potential to obtain maximum information from downstream STR analysis.

19.2.7.7 The AutoMate Express Forensic DNA Extraction System provides an easy to use, robust, bench-top instrument that utilizes the same PrepFiler and PrepFiler BTA chemistries packaged in pre-filled, foil sealed cartridges. The AutoMate processes 1 to 13 samples/blanks in a single run.

19.2.7.8 The standard extraction procedure for using the PrepFiler Express Chemistries with the Automate Express is covered under FBU Policy Manual DNA_2.

19.2.7.9 The differential extraction procedure for using the PrepFiler Express Chemistries with the Automate Express is covered under FBU Policy Manual DNA_3.

19.2.7.9.1 Function of each chemical component of the differential extraction process includes:

- E-Cell Digest Buffer (1 M Tris-HCl, 20% SDS, 0.5 M EDTA, 5 M NaCl, and Ultrapure DI water) - serves as a buffer to protect the DNA by providing a stable environment and breaks open epithelial cells
  - 1 M Tris-HCl – a stabilizing buffer.
  - 20% SDS – a detergent that lyses open the cells.
  - 0.5 M EDTA – a metal chelator that binds the magnesium and calcium ions that are present in solution. These ions are cofactors for nucleases. The chelating of these ions prevents the DNA from being destroyed by nucleases.
  - 5 M NaCl – a sodium ion that increases the amount of energy necessary to break the bonds of the exposed negatively charged DNA strands by shielding/neutralizing the repulsive nature of the DNA strands.
  - Ultrapure DI water – places the reagents in the appropriate concentrations for the step and adds volume to the reagent mix.
- Autoclaved Water - used for the sperm pellet wash steps
- Proteinase K - indiscriminately destroys proteins in the cell

- 1.0M DTT (dithiothreitol) - is a reducing agent which breaks any disulfide bonds. Disulfide bonds are characteristically found in sperm cell membranes. DTT is the only reagent used for the sperm fraction that is not used for the epithelial fraction. Addition of DTT is necessary for the extraction of DNA from sperm cells.

19.2.7.10 General Guidelines to be kept in mind when using the PrepFiler Chemistries and Automate Express:

19.2.7.10.1 The lysis buffers from the PrepFiler Express and PrepFiler Express BTA kit are different and cannot be interchanged.

19.2.7.10.2 When loading the tips, load them into the tray prior to placing the tray back into the instrument.

19.2.7.10.3 Ensure the beads in the cartridges are mixed thoroughly (no settling) prior to placing them on the instrument.

19.2.7.10.4 Ensure that both tubes (Lysep™ column and the sample collection tube) are labeled.

19.2.7.10.5 Ensure that the two cartridge types are not placed on the same instrument at the same time, the instrument can only run one type of analysis at a time.

19.2.7.10.6 When extracting bone and teeth samples, the screw top cap is used, not the Lysep™ column.

19.2.7.10.7 Do not open the door when the instrument is running. If it needs to be opened, pause the instrument first.

19.2.7.10.8 Bleach is not to be used on any of the components of the instrument. Bleach will cause a toxic gas to buildup. A special waste container will be needed for the waste from the PrepFiler Chemistry components.

19.2.7.10.9 Do not UV the Lysep™ columns, as it will damage them. The Lysep™ columns are treated with Ethylene Oxide and do not need to be cross-linked.

19.2.7.10.10 The piercing units should be cleaned with an alcohol wipe after each instrument run.

19.2.7.10.11 When opening the doors of the instrument, ensure they are opened evenly and gently as to not cause damage to the instrument.

19.2.7.10.12 On the tip and tube rack, row T1 is not used. It is there for potential future chemistry kits.

19.2.8 Quality Control of Extraction

19.2.8.1 The reagents for extraction need to be verified that they are working properly after each lot that is prepared.

19.2.8.2 This QC is done by taking two different bloodstains that are NIST traceable and extracting, quantitating, amplifying, and profiling them. The samples that are used have been previously profiled and the profiles that are developed with the new reagent must match the employee profile spreadsheet from previous analyses of the samples. The reagent blanks and controls must be free from contamination.

19.2.8.3 If the process fails, the reagents will be troubleshot and the technical manager will be notified before proceeding with the course of action.

19.2.8.4 Extraction reagents made in-house expire after six months after the preparation date. The PrepFiler kits expire as listed by the manufacturer.

19.3 DNA Quantitation

19.3.1 To ensure that DNA recovered from an extraction is human rather than another source such as bacteria, the FBI's Quality Assurance Standards require human specific DNA quantitation of forensic samples.

19.3.2 Only after DNA in a sample has been isolated can its quantity and quality be reliably assessed.

19.3.3 The primary purpose of DNA quantitation in forensic casework is to determine the appropriate amount of DNA template to include in PCR amplification of STR loci in order to avoid off-scale or low-level data and associated artifacts.

19.3.4 PCR amplification of too much DNA results in overblown electropherograms that make interpretation of results more challenging and time-consuming to review. Too little DNA can result in loss of alleles due to stochastic amplification and failure to equally amplify the STR alleles present in the sample.

19.3.5 Normalization is the process of achieving a DNA concentration that fits into the optimal window for analysis (typically 0.5 ng to 1.0 ng for GlobalFiler and Yfiler Plus). This process involves diluting the sample down to the desired range or concentrating it by removing excess volume (by use of a vacuum centrifuge - such as DNA Vacufuge).

19.3.6 DNA quantitation can serve as a gateway to potential DNA testing options. An example is a determination of the total human DNA levels compared to the male DNA levels; this can be used to assess whether autosomal STRs or Y-STRs should be used as the first course of action with the evidentiary samples.

19.3.7 Real-Time Quantitative PCR (qPCR)

19.3.7.1 Real Time PCR Assays provide an assessment of both DNA yield and quality for amplification purposes.

19.3.7.2 Real time quantitative PCR is sometimes referred to as “kinetic analysis” because it analyzes the cycle-to-cycle change in fluorescence signal resulting from amplification of a target sequence during PCR.

19.3.7.3 Typically there are two approaches to monitoring this; one and most commonly, is the use of a fluorogenic 5’ nuclease assay (better known as TaqMan) or the use of an intercalating dye (such as SYBR Green, that is highly specific for double-stranded DNA molecules). The TaqMan approach monitors change in fluorescence due to displacement of a dual dye-labeled probe from a specific sequence within the target region, while the SYBR Green assay detects the formation of any PCR product. The OSBI uses the TaqMan quantitative Real-Time PCR technology.

19.3.7.4 The OSBI currently uses the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument, and prior to it, the OSBI used the Applied Biosystems 7500 Real-Time PCR Instrument.

19.3.7.5 The 5’ Nuclease Activity (TaqMan)

19.3.7.5.1 TaqMan probes are labeled with two fluorescent dyes that emit signals at different wavelengths. The probe sequence is intended to hybridize specifically in the DNA target region of interest between two PCR primers.

19.3.7.5.2 Typically the probe is designed to have a slightly higher annealing temperature compared to the PCR primers so that the probe will be hybridized when extension (polymerization) of the primers begins.

19.3.7.5.3 A minor groove binder is used near the 3’ end of the TaqMan probes to enable the use of shorter sequences that have higher annealing temperatures than would be expected for sequences of equivalent length.

19.3.7.5.4 The reporter (R) dye, usually FAM or VIC, is attached at the 5’ end of the probe sequence while the quencher (Q) dye is synthesized on the 3’ end.

19.3.7.5.5 The Quantifiler® Trio DNA Quantification Kit uses multiple-copy target loci for improved detection sensitivity. The human-specific target loci (small autosomal, large autosomal, and Y-chromosome targets) each consist of multiple copies dispersed on various autosomal chromosomes (small autosomal and large autosomal), or multiple copies on the Y-chromosome.

19.3.7.5.6 To maximize the consistency of quantification results, genomic targets were selected with conserved primer-and probe-binding sites within individual genomes and also with minimal copy number variability between different individuals and population groups. As a result, the detection sensitivity of the Quantifiler® Trio assay is improved over previous quantification kits. Assay chemistry has been optimized for more efficient multiplexing, faster PCR cycle times (1 hour), and increased

inhibitor tolerance. With these improvements the quantification kit better matches the newer STR kits.

19.3.7.5.7 The Quantifiler® Trio Kit used by the OSBI is designed to simultaneously quantify the total amount of amplifiable human DNA and human male DNA in a sample. The primary quantification targets (small autosomal and Y) consist of short amplicons ranging in size from 75-80 bases in order to detect degraded DNA samples. In addition, a large autosomal target of more than 200 bases is included to aid in determining if a DNA sample is degraded.

19.3.7.5.8 Quantifiler® Trio Assay:

19.3.7.5.8.1 The assay combines four 5' nuclease assays:

- Two separate target-specific human assays (one short PCR amplicon and one long PCR amplicon)
- A target-specific human male DNA assay
- An internal PCR control (IPC) assay.

Each target consists of PCR primers and dye-labeled TaqMan® probes with non-fluorescent quenchers (NFQ) for the amplification of multi-copy loci. The table below provides information about the targets for the quantification kit.

Target	Amplicon length	Ploidy	Copy Number	Dye/Quencher
Human Target, small autosomal	80 bases	Diploid	Multi-copy	VIC® dye with MGB
Human Target, large autosomal	214 bases	Diploid	Multi-copy	ABY® dye with QSY®
Human Male Target	75 bases	Haploid	Multi-copy	FAM™ dye with MGB
Internal PCR Control	130 bases	NA	Synthetic IPC template is included in the primer	JUN® dye with QSY® quencher

The Quantifiler® Trio Assay targets serve the following functions:

Small Autosomal (SA) Target: The primary quantification target for total human genomic DNA. Its smaller amplicon size (80 bp) is aligned with the sizes of typical "mini" STR loci and makes it better able to detect degraded DNA samples.

Large Autosomal (LA) Target: Used mainly as an indicator of DNA degradation, by comparing the ratio of its quantification result with that of the SA target. NOTE: The large autosomal target often gives an overestimate of the DNA present when compared to the small autosomal and Y-chromosome result.

Y Chromosome (Y) Target: Allows the quantification of a sample's human male DNA component, and is particularly useful in assessing mixture samples of male and female DNA.
NOTE: The Y-chromosome target often gives an overestimate of the DNA present when compared to the small autosomal result.

The differences in the estimations listed above may be attributable to both copy number variation between the targets and quantification variation.

Male:Female Ratio (M:F ratio)
This ratio is automatically calculated by the HID Real-Time PCR Analysis Software. The quantity value for human DNA present is based on the small autosomal target value obtained for the sample using the following equation:
Male DNA : Female DNA – Quantity of Male DNA / Quantity of Male DNA : (Quantity of Human DNA – Quantity of Male DNA) / Quantity of Male DNA (in ng/µL).

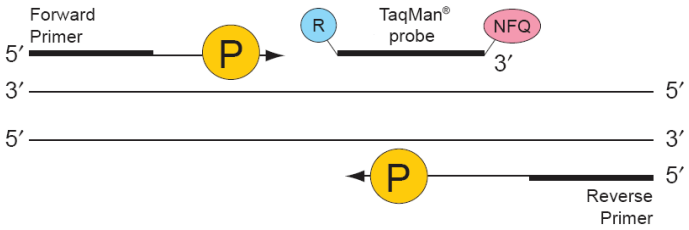
Example: Male DNA = 2ng/µL
Human DNA = 8ng/µL
2/2 : (8-2)/2 = 1:3

As the ratio of female DNA increases relative to male DNA, the ability to detect the minor male component may be limited with Autosomal STR analysis. In these instances, Y-STR analysis may be considered and/or more appropriate depending on the case circumstances. In general, Male:Female ratios of 1:10 or greater may be better suited for Y-STR analysis.

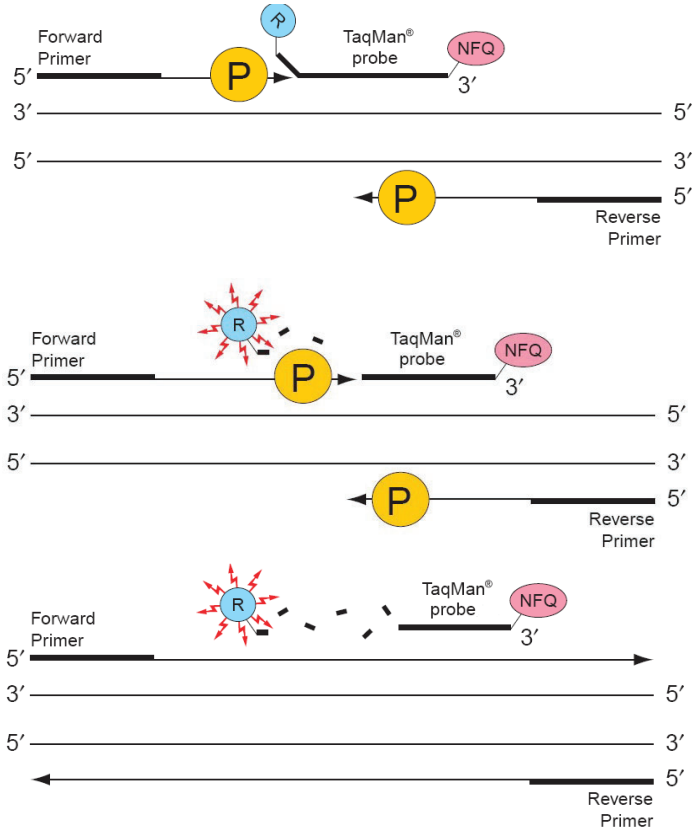
The IPC system consists of IPC template DNA (synthetic), primers for amplifying the 130 base IPC template DNA, and a TaqMan® probe dye quencher (JUN® dye with QSY® quencher). The IPC enables verification that the components and the detection instrumentation are working correctly. It also helps to confirm the validity of negative results and aides in identifying inhibitors that may be present.

19.3.7.5.8.2 The AB Quantifiler® Trio Kit uses the TaqMan® Probe with a non-fluorescent quencher (NFQ). The TaqMan® MGB probes contain a reporter dye (FAM™ or VIC®) linked to the 5' end of the probe, a minor groove binder (MGB) and NFQ at the 3' end of the probe. The MGB with NFQ allows the instrument to measure reporter dye fluorescence more precisely as the NFQ does not fluoresce. The MGB increases the melting temperature of the probe as it is shorter resulting in greater differences in the melting temperature between matched and mismatched probes. The TaqMan QSY® probes have a reporter dye (ABY® or JUN®) linked to the 5' end of the probe and a NFQ (QSY®) at the 3' end of the probe.

19.3.7.5.9 When the probe is intact and the reporter dye is in close proximity to the quencher dye, no fluorescence will result because the suppression of the reporter fluorescence due to Forster-type energy transfer.



19.3.7.5.10 When the reporter dye molecule is released from the probe and is no longer in close proximity to the quencher dye, it can begin to fluoresce. Increase in florescent signal results when the target sequence is complementary to the TaqMan® probe. It is important to note that mismatches between the DNA template sequence and the TaqMan probe can cause failure to detect DNA template appropriately.



19.3.7.5.11 The IPC is labeled with a reporter dye and hybridizes to a synthetic template added to each reaction. The TaqMan® probe for detecting the target region of interest is labeled with a reporter dye and is therefore spectrally resolvable from the reporter dye.

19.3.7.5.12 The Quantifiler® Trio kit also has a Mustang Purple® Passive Reference Standard dye that is placed in each well of the plate

to adjust for well-to-well differences across a plate through background subtraction.

19.3.7.6 Real-Time PCR Analysis

19.3.7.6.1 There are three distinct phases that define the PCR process: geometric or exponential amplification, linear amplification, and the plateau region.

19.3.7.6.2 During exponential amplification, there is a high degree of precision surrounding the production of new PCR products. When the reaction is performing close to 100% efficiency, then a doubling of amplicons occurs with each cycle. A plot of cycle number versus log scale of DNA concentration should result in a linear relationship during the exponential phase of PCR amplification.

19.3.7.6.3 A linear phase of amplification follows the exponential phase as one or more components fall below critical concentration and amplification efficiency slows down to an arithmetic increase rather than a geometric one in the exponential phase. Since components such as deoxynucleotide triphosphates (dNTPs) or primers may be used up at slightly different rates between the reactions, the linear phase is not as consistent from sample-to-sample and therefore is not as useful for comparison purposes.

19.3.7.6.4 The final phase of PCR is the plateau region where accumulation of PCR product slows to a halt as multiple components have reached the end of their effectiveness in the assay. The fluorescent signal observed in the plateau phase levels out. The accumulation of PCR product generally ceases when its concentration reaches approximately 10^{-7} mol/L.

19.3.7.6.5 The optimal place to measure fluorescence versus cycle number is in the exponential phase of PCR where the relationship between the amount of product and input DNA is more likely to be consistent.

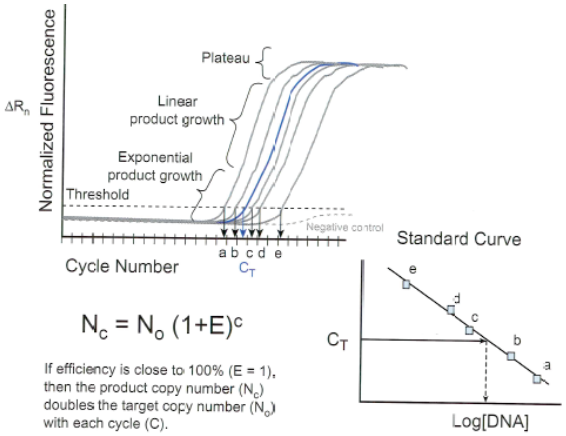


FIGURE 3.5 Real-time PCR output and example standard curve used to determine quantity of input DNA.

19.3.7.6.6 Real-time PCR instruments use what is termed the cycle threshold (C_T) for calculations. The C_T value is the point in terms of PCR amplification cycles when the level of fluorescence exceeds some arbitrary threshold, such as 0.2, that is set by the real-time PCR software to be above the baseline noise observed in the early stages of PCR.

19.3.7.6.7 The fewer cycles it takes to get to a detectable level of fluorescence (i.e., to cross the threshold set by the software), the greater the initial number of DNA molecules put into the PCR reaction. Thus a plot of the log of DNA concentrations versus the C_T value for each sample results in a linear relationship with a negative slope.

19.3.7.6.8 The cleavage of the TaqMan® probes results in an increase in fluorescence signal. This rise in fluorescence can be correlated to the initial DNA template amounts when compared with samples of known DNA concentration. The known concentrations are used to generate a standard curve based on their measured C_T values. Provided that the PCR amplification conditions are consistent from sample-to-sample, a sample with an unknown DNA quantity can be compared to this standard curve to calculate its initial DNA template concentration.

19.3.7.7 Correlation of DNA Quantity and STR Amplification

19.3.7.7.1 With past quantitation kits, there were times when “zero” quantitative values are observed and STR/Y-STR typing results were obtained. While there was sometimes DNA present in such samples, the qPCR result was very low or zero due to the PCR primers failing to find sufficient target to amplify. With the upgrading of quantitation kits to Quantifiler® Trio, the estimate of the amount of amplifiable DNA is now shown with the use of large and small autosomal targets during quantitation. A true “cut-off” may be able to be established through internal validation and casework experience in-house.

19.3.7.7.2 Pipetting accuracy may be a factor. A mis-pipetting of the DNA sample going into the qPCR assay could make a result appear lower than it really is. Reduction of volume to save money with qPCR assays could exacerbate pipetting accuracy issues as well as effectively concentrate PCR inhibitors.

19.3.7.7.3 As the amount of DNA in the sample increases, the value obtained for the IPC will increase due to slight inhibition because of the high amount of DNA.

19.3.7.8 AB Quantifiler® Trio Quantification Kit

19.3.7.8.1 The kit contains the following components:

Reagent	Contents	Quantity	Storage†
Quantifiler® THP PCR Reaction Mix	dNTPs, buffer, enzyme, Mustang Purple® Passive Reference Standard, and stabilizers	4 tubes, 1 mL/tube	–15 to –25°C upon receipt 2 to 8°C after initial use Store protected from light
Quantifiler® Trio Primer Mix	Target-specific primers, ABY®, JUN®, VIC®, and FAM™ dye-labeled probes, and Internal PCR Control (IPC) template	4 tubes, 0.8 mL/tube	–15 to –25°C upon receipt 2 to 8°C after initial use Store protected from light
Quantifiler® THP DNA Dilution Buffer	Genomic DNA Standard dilution buffer	2 tubes, 1.8 mL/tube	–15 to –25°C upon receipt 2 to 8°C after initial use
Quantifiler® THP DNA Standard	Genomic DNA Standard formulated at 100 ng/µL to generate standard curves	1 tube, 0.12 mL	–15 to –25°C upon receipt 2 to 8°C after initial use

19.3.7.8.2 To perform the assay, a master mix of PCR reaction mix and primer mix is prepared. The amount of each is based upon the number of samples you have.

19.3.7.8.2.1 Addition of Quantifiler Automation Enhancer to the Quantifiler Trio PCR Reaction Mix prevents bubbles from being introduced during robotic mixing and basic pipeting.

19.3.7.8.2.2 1µL of Quantifiler™ Automation Enhancer is added to each 1mL tube of thawed Quantifiler™ THP PCR Reaction Mix (1:1000) in the Quantifiler™Trio kit prior to use. The Reaction Mix tubes are each marked with an “E” after addition of the Automation Enhancer.

19.3.7.8.2.3 The lot number and expiration of the Quantifiler™ Automation Enhancer is recorded on the outside of the Quantifiler™Trio kit box. The date the Automation Enhancer was added to each Reaction Mix tube is recorded on the outside of the Quantifiler™Trio kit box (i.e., working lot number) along with the expiration of the working lot, which is 6 months from the date of addition of Automation Enhancer to the Quantifiler™ THP PCR Reaction Mix or the date of either kit if they have an earlier expiration date.

19.3.7.8.3 A ten-fold dilution series with 5 concentration points ranging in concentration from 50 ng/µL (Standard A) to 0.005 ng/µL (Standard E) is prepared.

Standard	Concentration (ng/µL)	Example Volumes	Dilution Factor
Std. A	50.000	10 µL [100 ng/µL stock] + 10 µL Quantifiler® THP DNA dilution buffer	2×
Std. B	5.000	10 µL [Std. 1] + 90 µL Quantifiler® THP DNA dilution buffer	10×
Std. C	0.500	10 µL [Std. 2] + 90 µL Quantifiler® THP DNA dilution buffer	10×
Std. D	0.050	10 µL [Std. 3] + 90 µL Quantifiler® THP DNA dilution buffer	10×
Std. E	0.005	10 µL [Std. 4] + 90 µL Quantifiler® THP DNA dilution buffer	10×

19.3.7.8.3.1 When using the Quantifiler® THP DNA Dilution Buffer, you can store prepared DNA quantification standards in low-binding tubes for up to two weeks at 2 to 8^0 C.

To prepare DNA quantification standards dilution series:

1. Label to tubes with the appropriate information
2. Dispense the required amount of THP DNA Dilution Buffer to each tube (see table above)
3. Vortex the DNA standard stock for 3 to 5 seconds, add the appropriate amount of stock standard to the first DNA standard tube, and mix thoroughly.
4. Add the appropriate volume for the first standard to the next standard tube, and mix thoroughly.
5. Repeat steps for the remaining standards.

Troubleshooting the Standard Curve
The table below provides common errors that can result from incorrect quantities or tasks not being properly set.

Observation	Possible Cause	Recommended Action
Slope for the standard curve is outside the typical range *or* R^2 value is significantly less than 0.98	When defining quantification values for the standards, an incorrect quantity was applied.	1. From the analysis section, move the cursor over the well and verify that the quantity is correct. 2. Update with the correct values and reanalyze, if necessary.
At each concentration, the standard curves for all targets are not shown	For the standard curve samples, the task was set to unknown for one of the targets in the assay.	1. From the analysis section, move the cursor over the well and verify that the task is set to "S" for all of the standard curve samples. 2. Update and reanalyze, if necessary.
Slope value for standard is outside the expected range (see "Slope" on page 44)	Standards have not been properly stored, or are older than 2 weeks.	Prepare fresh standards.
A failed standard is incorporated into the standard curve.	Standard DNA not loaded in well.	Exclude failed standard from standard curve analysis. (Select **Plate Setup ▸ Define Samples and Targets**, then change the Sample Type from Standard to Unknown), then reanalyze.

19.3.7.8.3.2 To prepare the reaction:

1. Calculate the volume for each component needed to prepare the reactions (see table below) by multiplying the total number of samples (plus a correction factor of about 10-15% to allow for pipetting errors by the volume of the primer mix (8 µL) and reaction mix (10 µL)).

For the Quantifiler® Trio DNA Quantification Kit:

Component	Volume Per Reaction (µL)
Quantifiler® Trio Primer Mix	8
Quantifiler® THP PCR Reaction Mix	10

2. Dispense 18 µL of the master mix into each reaction well.
3. Add 2 µL of each sample, standard, and control to the appropriate wells.
4. Seal the reaction plate with the Optical Adhesive Cover.
5. Remove bubbles, while the plate is inside the base by tapping the base on the bench top to bring the bubbles to the surface of the liquid in the well. Lift the

plate from the base and inspect each well for bubbles. To remove remaining bubbles, tap each well with a marker, pen, or gloved fingertip. **It is critical to ensure the bubbles are removed to avoid noise in the fluorescence single that bubbles can cause. Also ensure the plate has no dust particles on it, as this can cause false readings.** You may blow canned air onto the bottom side of the plate to ensure there are no particles present.

6. Centrifuge the plate.
7. To run the plate on the instrument refer to the FBU Policy Manual and/or QuantStudio 5 User Manual.

19.3.7.8.4 When the standard curve is generated it produces an R^2, slope, and Y-intercept values that are associated with the regression analysis. These values are quality indicators.

19.3.7.8.4.1 The R^2 value measures the closeness of fit between the standard curve regression line and the individual data points. The values that are possible are between 0 and 1, with 1 being a perfect fit. The R^2 value should be ≥ 0.98.

If the R^2 value is <0.98 check the following:

- Quantity values entered for quantification standards in the Plate Setup-Assign Target to the selected wells during experiment setup
- Making of serial dilutions of quantification standards
- Loading of reactions for quantification standards
- Failure of reactions containing quantification of reactions

19.3.7.8.4.2 The slope is an indicator of amplification efficiency, where a slope value of -3.3 corresponds to an optimal 100% efficiency. The table below is the typical ranges for the slope of the targets:

Range and average of standard curve slope values

Quantifiler® HP/Trio Targets	Typical Slope (range)	Average Slope
Small Autosomal (SA)	-3.0 to -3.6	-3.3
Large Autosomal (LA)	-3.1 to -3.7	-3.4
Y Target (Y)	-3.0 to -3.6	-3.3

If the slope varies beyond the typical range indicated above, check the assay setup, software setup, reagents, and instrument.

19.3.7.8.4.3 The Y-intercept is an indicator of the expected C_T value, from the standard curve regression line, for a sample with an input DNA quantity of 1ng/µL.

In addition to variations that can be caused by pipetting of standards or minor lot-to-lot variations in the quantitation kits, Y-intercept can also be affected by target-to-target variation and instrument-to-instrument variation.

Target-to-target variation: The Y-intercept for the large autosomal target is typically lower than the Y-intercept for the small autosomal target or the Y target. This is because the higher copy number of the large autosomal target relative to the copy number of the small autosomal and Y targets.
Instrument-to-instrument variation: Differences between QuantStudio 5 instruments result in small differences in Y-intercept values for each of the targets. Minor differences do not affect assay performance or quantification accuracy.

Y-intercepts for the small autosomal target in OSBI internal validation ranged from 26.532 to 26.816, for the large autosomal target ranged from 24.792 to 25.09, and for the Y-chromosome target ranged from 25.654 to 26.054.

19.3.7.8.5 The Degradation Index (DI) refers to the data observed when a sample displays a decrease in measured amount for large DNA fragments. It is used a general indicator to determine whether large DNA fragments may perform more poorly relative to small DNA fragments. The DI is calculated as the ratio of the small autosomal target concentration to the large autosomal target concentration.
Lower large autosomal target concentrations may indicate the sample is inhibited.
The DI can also be affected by PCR inhibitors, which may negatively affect amplification of the large autosomal target more than the small autosomal target.
When the large autosomal target is undetermined, this can be an indication of significant degradation and/or inhibition affecting a sample.

19.3.7.8.5.1 The Degradation Index is automatically calculated by the HID software using the following formula:

Small autosomal target DNA conc. (ng/µL)

Large autosomal target DNA conc. (ng/µL)

19.3.7.8.5.2 The Degradation Index value will be displayed in the well table view in any of the analysis screens.

19.3.7.8.5.3 The Degradation Index can be affected by:

- Degree of degradation of large autosomal target DNA
- Presence of PCR inhibitors

19.3.7.8.5.4 To determine the Quality Index, evaluate the Degradation Index in conjunction with the IPC C_T to assess the potential presence of PCR inhibitors and degradation that may have an impact on downstream sample processing. The Quality Index can help you determine what the next processing steps are best for the sample. The table below is guidance on interpreting the quality index.

IPCCT flag triggered?	Degradation Index	Quality Index interpretation†
No	<1	Typically indicates that DNA is not degraded or inhibited.
	1 to 10	Typically indicates that DNA is slightly to moderately degraded. PCR inhibition is also possible, however not enough to significantly suppress IPC amplification.
	>10 or blank (no value)	Typically indicates that DNA is significantly degraded. PCR inhibition is also possible, however not enough to significantly suppress IPC amplification. Highly degraded samples that cannot be recovered by STR can be analyzed with HID-Ion AmpliSeq™ Panels and the Ion Personal Genome® (PGM™) System (see "Degraded sample studies: GlobalFiler® STR Kit and HID-Ion AmpliSeq™ Identity and Ancestry Panel" on page 105).
Yes	<1	Although theroretically possible, this result is unlikely because PCR inhibitors in sufficient concentration to trigger the IPCCT flag typically would affect the large autosomal target as well.
	>1 or blank (no value)	Typically indicates that the DNA is affected by degradation and/or PCR inhibition.

† These are general guidelines that may not apply to all samples depending on the inhibitors present, the varying quantity of contributor DNA in mixed samples and the STR kit used. (STR kits are For Forensic or Paternity Use Only.)

19.3.7.8.6 The thermal cycler parameters that the Quantifiler® Trio kit and AB QuantStudio 5 instrument uses are Stage 1: 95˚C for 2 minutes for 1 cycle and Stage 2: 95˚C for 9 seconds followed by 60˚C for30 seconds for 40 cycles.

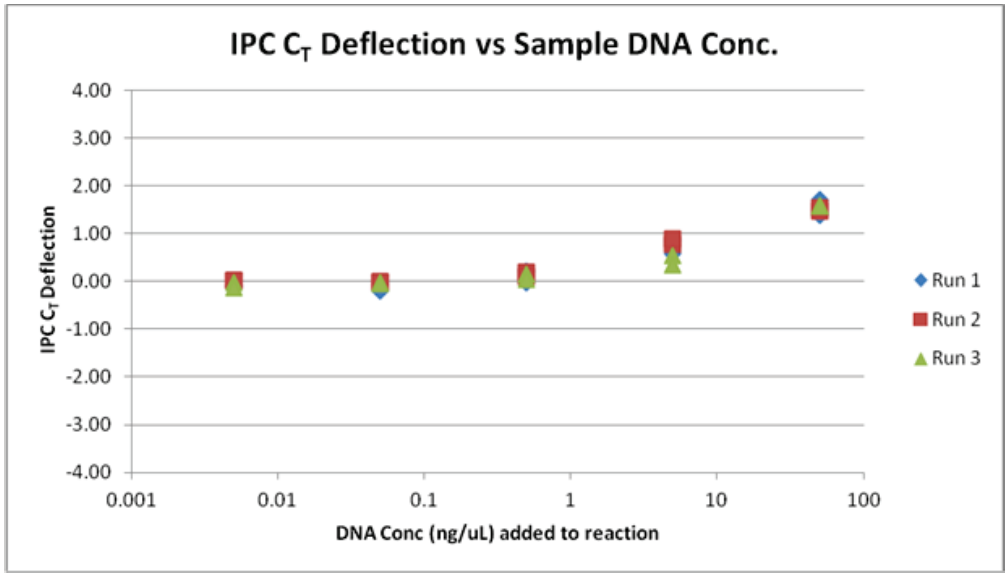
19.3.7.8.7 The fluorescence is detected on the QuantStudio 5 instrument with the use of a bright white LED excitation source that directs light to each well on the reaction plate. The light passes through the Optical Adhesive Cover and excites the fluorescent dyes in each well of the plate. A system of lenses,

filters, and mirrors focuses the fluorescence emission. A Complementary Metal Oxide Semiconductor (CMOS) detects the fluorescence emission. The data collection software obtains the fluorescence emission data and uses information from the pure-dye calibration to de-convolute the raw spectra-signal data for each PCR cycle during the run. The instrument also includes the following technologies:

- OptiFlex™ technology: provides enhanced fluorescence detection enabling accurate and sensitive data analysis
- VeriFlex™ Blocks: provide the option for independent temperature zones for precise temperature control during qPCR optimization

19.3.7.8.8 Typically the C_T value for the IPC results should be between 20 and 30. If there is a high level of human DNA (≥ 10 ng/µL), the competition between the human and IPC reactions may suppress the IPC amplification for a particular sample.

To assess C_T values for the Internal PCR Control (IPC), view the JUN® dye signal in the amplification plots for the quantification standards. Typical reactions are expected to show relatively consistent IPC amplification for standards with concentrations ≤ 5 ng/µL. With higher concentrations of human genomic DNA, competition between the human and/or male-specific and IPC PCR reactions may suppress IPC amplification. Observed IPC C_T values begin to increase at concentrations > 5 ng/µL, and a greater magnitude of increase at concentrations >50 ng/µL. The figure below displays an example of how the IPC C_T values may deflect upwards with increasing DNA concentrations.



Note: This is an example and the magnitude of deflection may vary.

19.3.7.8.9 The IPC system template DNA is present at a consistent concentration across all reactions on the plate. The IPC (JUN® dye) C_T should be relatively constant in typical reactions. However, the presence of PCR inhibitors and/or higher concentrations of DNA can increase the IPC C_T relative to the average IPC C_T of the quantification standards on the same plate. In the amplification plot window of the HID software, amplification of the assay targets can be observed.

An IPC value of ≥ 30 can indicate that the sample is inhibited. If at all possible, when the sample is inhibited it should be attempted to be cleaned up. Take care in concentrating samples that show an indication of being inhibited or are close to indicating being inhibited, as drying the sample down will concentrate the inhibitor as well.

19.3.7.8.10 If there is a quantitation value greater than 50 ng/µL the sample should be diluted and re-quantitated, as it is outside the maximum value of the standard curve and the results are not reliable. If necessary, Technical Manager approval may be obtained.

19.3.7.9 The DNA Quantitation procedure is covered under FBU Policy Manual DNA_4 DNA Quantification.

NOTE: Based on the validation data alone, there is not sufficient data to establish a "cut-off" or value at which to cease further analysis (amplification) or a Male:Female ratio cutoff / recommendation other than the generalities outlined above.

19.3.7.10 Quality Control (QC_13 QuantStudio 5 Real-Time PCR Systems)

19.3.7.10.1 On a monthly basis an instrument self-verification test and background calibration should be done.

19.3.7.10.2 Every 12 months an ROI/Uniformity calibration, background calibration, and dye calibration for system dyes and custom dyes should be done. This is generally completed as part of the annual Planned Maintenance performed by a service technician.

19.3.7.10.3 The ROI/Uniformity calibration maps the position of the wells on the sample block so that the software can associate increases in fluorescence during a run with specific wells of the plate and evaluate well-to-well consistency of the signals. Because the instrument uses a set of optic filters to distinguish the fluorescence emissions gathered during runs, a calibration image must be generated for each individual filter to account for minor differences in the optical path.

19.3.7.10.4 The background calibration measures the level of background fluorescence in the instrument. The software captures background images for each optical filter in the absence of sample and reagent, and it checks that the fluorescence from each well is below a fluorescence threshold. The software

uses calibration data to remove background fluorescence during a run.
NOTE: This can also be used to determine if contamination is related to the sample block or the plate.

19.3.7.10.5 The dye calibration is a collection of spectral data from a series of system and custom dye standards so that the QuantStudio 5 instrument software can characterize and distinguish the individual contribution of each dye in the total fluorescence signals collected by the instrument.

19.3.7.10.6 After the completion of the annual PM visit from the Life Technologies technician, the values for the threshold should be checked to ensure they are as follows:
T.IPC = 0.1; T.LA, T. SA, T.Y = 0.2

19.4 Polymerase Chain Reaction (PCR) Amplification

19.4.1 Polymerase chain reaction (PCR) is an enzymatic process in which a specific region of DNA is replicated over and over again to yield many copies of a particular sequence.

19.4.2 This molecular “Xeroxing” process involves heating and cooling samples in a precise thermal cycling pattern over ~30 cycles. During each cycle, a copy of the target DNA sequence is generated for every molecule containing the target sequence. The boundaries of the amplified product are defined by oligonucleotide primers that are complementary to the 3’ ends of the sequence of interest.

19.4.3 In the ideal reaction with 100% amplification efficiency, approximately 1 billion copies of the target region on the DNA template have been generated after 32 cycles. However, a reduction in amplification efficiency through PCR inhibition or poor primer annealing leads to lower quantities of PCR product being produced. The equation for the number of target molecules produced, which incorporates the amplification efficiency is $X_n = X_o (1+E)^{(N-2)}$; where X_n is the predicted number of target molecules created, X_o is the number of starting molecules and E is the efficiency of the reaction (between 0% and 100% or 0 to 1), and N is the number of cycles. The N-2 takes into account that for the first two cycles the specific double stranded target is not yet created. At 100% efficiency, there is a doubling of target molecules with each cycle after the third cycle, where the ends of the PCR product are fully defined by the forward and reverse primers.

19.4.4 Following amplification, the PCR product or amplicon is in sufficient quantity that it can be easily measured by a variety of techniques, the most common of which is fluorescent detection.

19.4.5 For this reason, commercial short tandem repeat (STR) typing assays label one primer in every primer pair with a fluorescent dye. The amplicon is now fluorescently labeled and can be detected when the dye label is excited by light of the appropriate wavelength.

19.4.6 Using dye labels that can be distinguished from one another based on their fluorescent emission characteristics (dye color), the number of regions of DNA that can be analyzed simultaneously is increased. Thus, the primer positions within a sequence and their spacing from one another define the overall PCR product length while the fluorescent dye establishes the amplicons detection characteristics.

19.4.7 The PCR amplification process has a dual purpose: (1) to increase the number of molecules representing a specific target site, and (2) to attach a label, most often a fluorescent dye, that enables detection of the amplicons produced.

19.4.8 PCR has been simplified with the availability of commercial reagent kits that allow a forensic DNA laboratory to simply add a DNA template to a pre-made PCR mix containing all necessary components for the amplification reaction. These kits are optimized through extensive research efforts on the part of commercial manufacturers. The amplification kits that the OSBI FBU uses are the GlobalFiler and Yfiler Plus amplification kits.

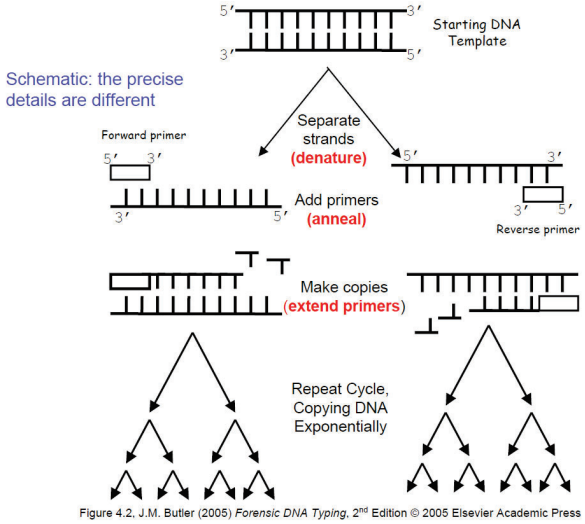


Figure 4.2, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

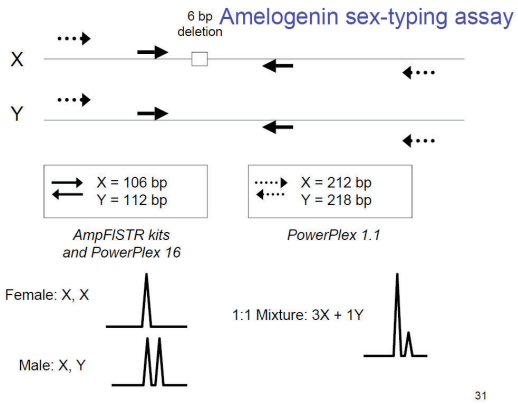


Figure 5.11, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

19.4.9 Thermal Cyclers

19.4.9.1 A thermal cycler is the instrument that heats and cools DNA samples in order to perform the PCR reaction.

19.4.9.2 Accurate and consistent sample heating and cooling is crucial to PCR in order to guarantee consistent results.

19.4.9.3 The OSBI FBU currently uses the ProFlex Thermal Cyclers from Applied Biosystems.

19.4.9.4 Thermal cycling typically involves 3 different temperatures that are repeated over and over again 25 to 30 times, depending on the kit used and the validation of the kit. GlobalFiler is validated in the OSBI FBU for 29 cycles, Yfiler Plus uses 30 cycles.

19.4.9.5 At ~94˚C the DNA strands separate or denature and at ~59˚C the primers bind or anneal to the DNA template and target the region to be amplified. At ~60˚C the DNA polymerase extends the primers by copying the target region using the deoxynucleotide triphosphate (dNTP) building blocks. The specific temperatures and duration of the thermal cycling process is dependent upon the particular amplification kit that is being used.

19.4.9.6 The thermal cycling parameters for the GlobalFiler amplification kit are as follows:

19.4.9.6.1 95˚C for 1 minute (activate the Taq Polymerase)

94˚C for 10 seconds (denature)
59˚C for 90 seconds (anneal/extend)) 29 cycles

60˚C for 10 minutes (-A nucleotide addition (adenylation))
4˚C hold until samples are removed from instrument

19.4.10 The most important components of a PCR sample are the two primers, which are short DNA sequences that precede or "flank" the region to be copied. A primer acts to identify or "target" the portion of the DNA template to be copied. It is a chemically synthesized oligonucleotide that is added in high concentration relative to the DNA template to drive the PCR sample. Considerable knowledge of the DNA sequence to be copied is required in order to select the appropriate primer sequences.

19.4.11 The other component of a PCR sample consists of the DNA template that will be copied, deoxyribonucleotide triphosphate (dNTPs) building blocks that supply each of the four nucleotides, and a DNA polymerase that adds the building blocks in the proper order based on the template DNA sequence.

19.4.12 The most commonly used thermally stable polymerase is *Taq* which comes from bacterium named *Thermus aquaticus* that inhabits hot springs.

19.4.13 A PCR master mix is prepared from the primer mix and master mix that can be dispensed in proper quantities to each PCR tube. This procedure helps to insure relative homogeneity among samples.

19.4.14 Hot Start PCR

19.4.14.1 Regular DNA polymerase exhibits some activity below their optimal temperature, which is 72^0 C for *Taq* polymerase. The primers would then be able to anneal non-specifically to the template DNA at room temperature when PCR reactions are being setup and non-specific products may result.

19.4.14.2 It is also possible at a low temperature for the primers to bind to each other creating products called primer dimers. These are a particular problem because of their small size relative to the PCR products means that they will be preferentially amplified.

19.4.14.3 Once the low-temperature, non-specific priming occurs, these undesirable products will be efficiently amplified throughout the remaining PCR cycles. Because the polymerase is busy amplifying these competing products, the target DNA region will be amplified less efficiently. If this happens, you will get less of what you are looking for and you may not have enough specific DNA to run your other tests.

19.4.14.4 This is avoided by initiating PCR at an elevated temperature, a process that is referred to as "Hot Start" PCR. This is accomplished through using a modified form of *Taq* polymerase which requires thermal activation and thus enables a closed tube hot start PCR. This enzyme, named AmpliTaq Gold, has greatly benefited the specificity of PCR amplifications.

19.4.14.5 AmpliTaq Gold DNA Polymerase

19.4.14.5.1 It is a chemically modified enzyme that is rendered inactive until heated. An extended pre-incubation of 95˚C for 1 minute is used to activate the AmpliTaq Gold.

19.4.14.5.2 The chemical modification involves a derivatization of the epsilon-amino groups of the lysine residues. At a pH below 7.0, the chemical moieties fall off and the activity of the polymerase is restored.

19.4.14.5.3 The pH of the Tris Buffer in the PCR reaction varies with temperature; higher temperatures cause the solution pH to go down by approximately 0.02 pH units with every 1˚C. A Tris buffer with pH of 8.3 at 25˚C will go down to pH of ~6.9 at 95˚C. Thus, not only is the template DNA well denatured but the polymerase is activated just when it is needed.

19.4.15 Short Tandem Repeats (STRs)

19.4.15.1 It is estimated that over 99.7% of the human genome is the same from individual to individual and regions that differ are in the remaining 0.3%. This allows us to tell people apart at the genetic level.

19.4.15.2 DNA regions that are 2 bp to 7 bp in length are called microsatellites, simple sequence repeats (SSRs), or most commonly in forensic DNA short tandem repeats (STRs).

19.4.15.3 STRs are popular DNA repeat markers because they are easily amplified by the PCR reaction without the problems of differential amplification. This is because both alleles from heterozygous individuals are similar in size since the repeat size is small.

19.4.15.4 The number of repeats in STR markers is highly variable among individuals, which makes these STRs effective for human identification purposes.

19.4.15.5 Microsatellites account for approximately 3% of the total human genome. STR markers are scattered throughout the genome and occur on average every 10,000 nucleotides. Not all STR loci exhibit variability between individuals.

19.4.15.6 STR repeat sequences are named by the length of the repeat unit. Dinucleotide repeats have two nucleotides repeated next to each other over and over again. Trinuleotides have three nucleotides in the repeat unit, tetranucleotides have four, pentanucleotides have five, and hexanucleotides have six nucleotides in the core repeat.

19.4.15.7 STRs are often divided into several categories based on the repeat pattern.

19.4.15.7.1 Simple repeats contain units of identical length and sequence.

19.4.15.7.2 Compound repeats comprise two or more adjacent simple repeats.

19.4.15.7.3 Complex repeats may contain several repeat blocks of variable unit length as well as variable intervening sequences.

19.4.15.7.4 Complex hypervariable repeats also exist with numerous non-consensus alleles that differ in both size and sequence and are therefore challenging to genotype reproducibility.

19.4.15.8 Not all alleles for an STR locus contain complete repeat units; even simple repeats can contain non-consensus alleles that fall in between alleles with full repeat units.

19.4.15.9 Microvariants are alleles that contain incomplete repeat units. An example of this is allele 9.3 at TH01 locus, which contains 9 tetranucleotide repeats and one incomplete repeat of three nucleotides.

19.4.15.10 For human identification purposes it is important to have DNA markers that exhibit the highest possible variation or a number of less polymorphic markers that can be combined in order to obtain the ability to discriminate samples.

19.4.15.11 The small size of STR alleles (~60bp to 460bp) compared to VNTR alleles (~400bp to 1000bp) make STR markers better candidates for use in forensic application where degraded DNA is common.

19.4.15.12 Tetranucleotide repeats have become more popular then di- or trinucleotides; penta- and hexanucleotides repeats are less common in the human genome but are being examined.

19.4.15.13 Stutter is a biological phenomenon and results when STR alleles are PCR amplified. Stutter products are amplicons that are typically one or more repeat units less in size than the true allele and arise during PCR because of strand slippage. Stutter can also be one or more repeat unit larger than the true allele.

19.4.15.14 Stutter products vary depending on the STR locus and even the length of the allele within the locus, but are usually less than 15% of the allele product quantity with tetranucleotide repeats. The di- and trinucleotides the stutter percentage is much higher (30% or more) and makes it difficult for interpretation. The tetranucleotides are much better in regards to stutter production predictability.

19.4.15.15 The advantages of using STR loci in forensic DNA typing over VNTRs are:

19.4.15.15.1 A narrow allele size range that permits multiplexing.

19.4.15.15.2 A narrow allele size range that reduces allelic dropout from preferential amplification of smaller alleles.

19.4.15.15.3 The capability of generating small PCR product sizes that benefit the recovery of information from degraded DNA.

19.4.15.15.4 Reduced stutter product formation with tri- and tetranucleotide repeats compared to dinucleotide repeats that benefit interpretation.

19.4.15.16 The selection criteria for good STR loci in human identification applications include the following characteristics:

19.4.15.16.1 High discriminating power with observed heterozygosity of 70%.

19.4.15.16.2 Separate (or widely spaced) chromosomal locations to ensure that closely linked loci are not chosen. This is done to be able to use product rule and to avoid any problems with linkage between the markers.

19.4.15.16.3 Robustness and reproducibility of results when multiplexed with other markers.

19.4.15.16.4 Low stutter characteristics.

19.4.15.16.5 Low mutation rate.

19.4.15.16.6 Predicted length of alleles that fall in the range of 90bp to 500bp with smaller sizes better suited for analysis of degraded DNA samples.

19.4.15.17 The FBI sponsored a community wide forensic science effort, in 1996, to establish core STR loci for inclusion within the National DNA database known as Combined DNA Index System (CODIS). In 1997, they chose 13 core CODIS Loci: CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11. In 2017, the FBI expanded the CODIS core loci to include: D13S317, D7S820, D5S818, CSF1PO, D1S1656, D12S391, D2S441, D10S1248, D18S51, FGA, D21S11, D8S1179, vWA, D16S539, TH01, D3S1358, D2S1338, D19S433, TPOX, and D22S1045.

19.4.15.17.1 Using the previously described classification scheme for categorizing STR repeat motifs, the CODIS Core Loci are divided into four categories.

19.4.15.17.1.1 Simple repeats consisting of one repeating sequence: TPOX, CSF1PO, D5S818, D13S317, D16S539
Simple repeats with non-consensus alleles: TH01, D18S51, D7S820
Compound repeats with non-consensus alleles: vWA, FGA, D3S1358, D8S1179
Complex Repeats: D21S11

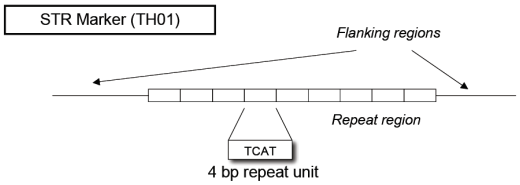


Figure 5.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

19.4.16 Multiplex PCR

19.4.16.1 The polymerase chain reaction (PCR) permits more than one region to be copied simultaneously by simply adding more than one primer set to the reaction mixture.

19.4.16.2 The simultaneous amplification of two or more regions of DNA is commonly known as multiplexing or multiplex PCR.

19.4.16.3 For a multiplex reaction to work properly the primer pairs need to be compatible.

19.4.16.3.1 The primer annealing temperatures should be similar.

19.4.16.3.2 Excessive regions of complement between the primers should be avoided to prevent the formation of primer dimers that will cause the primers to bind to one another instead of the template DNA.

Schematic for multiplex PCR

(A) Simultaneous amplification of three locations on a DNA template

(B) Resolution of PCR products with a single size-based separation method

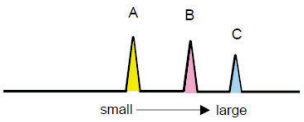


Figure 4.3, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

19.4.16.4 General Guidelines for PCR Primer Design

Parameter	Optimal Values
Primer length	18-30 bases
Primer T_m (melting temperature)	55-72°C
Percentage GC content	40-60%

No self-complementarity (hairpin structure)	≤ 3 contiguous bases
No complementarity to other primer (primer-dimer)	3 contiguous bases (especially at the 3'-ends)
Distance between two primers on target sequence	<2000 bases apart
Unique oligonucleotide sequence	Best match in BLAST[a] search
T_m difference between forward and reverse primers in pair	≤ 5°C
No long runs with the same base	< 4 contiguous bases

19.4.17 PCR Inhibition

19.4.17.1 Inhibitors can (1) interfere with cell lysis necessary for DNA extraction, (2) interfere by nucleic acid degradation or capture, and (3) inhibit polymerase activity thus preventing enzymatic amplification of the target DNA.

19.4.17.2 Occasionally substances such as textile dyes from clothing or hemoglobin from red blood cells can remain with the DNA throughout the sample preparation process and interfere with the polymerase to prevent successful PCR amplification.

19.4.17.3 The result of amplifying a DNA sample containing an inhibitor, such as hematin, is a loss of alleles from the larger sized STR loci or even complete failure of all loci.

19.4.17.4 Samples containing PCR inhibitors often produce partial profile results that look similar to a degraded DNA sample, therefore failure to amplify the larger STR loci can be either due to degraded DNA where there are not enough intact copies of the DNA template or due to the presence of sufficient levels of PCR inhibitor that reduce the activity of the polymerase.

19.4.17.5 Solutions to PCR Inhibition

19.4.17.5.1 PCR inhibitors can be removed or effects reduced by:

19.4.17.5.1.1 The genomic DNA template may be diluted, which will dilute the PCR inhibitor, and re-amplified in the presence of less inhibitor.

19.4.17.5.1.2 More DNA polymerase can be added to overcome the inhibitor.

19.4.17.5.2 Additives to the PCR reaction, such as bovine serum albumin (BSA), have been shown to prevent or minimize the inhibition of PCR.

19.4.17.5.3 A sodium hydroxide treatment of DNA has also been shown to neutralize inhibitors of *Taq* polymerase.

19.4.17.5.4 Solutions that can be done to overcome inhibition in the forensic laboratory included: additional purification of the extract (Microcon washes), diluting the inhibitor, amplifying the maximum amount of Neat extract (without concentrating) and a dilution of the extract

19.4.17.5.5 Inhibited samples should not be concentrated and consultation with the Technical Manager can be done to see how to proceed with your analysis

19.4.18 Stochastic Effects

19.4.18.1 Forensic DNA samples often possess low levels of DNA. When amplifying very low levels of DNA template, a phenomenon known as stochastic fluctuation can occur.

19.4.18.2 Stochastic effects, which are unequal sampling of two alleles present from a heterozygous individual result when only a few DNA molecules are used to initiate PCR. Under conditions of limited template, the PCR primers used to amplify a specific region may not consistently find and hybridize to the entire set of DNA molecules present in the PCR amplification reaction.

19.4.18.3 With a heterozygous locus, where two alleles are present, the unequal sampling of the alleles can result in failure to detect one or both of the alleles. Loss of a single allele is referred to as “allele drop-out” while the loss of both alleles is termed as “locus drop-out.”

19.4.18.4 Stochastic (random) variation is a fundamental physical law of PCR amplification process when examining low amounts of DNA. Stochastic effects manifest as a fluctuation of results between replicate analyses. In other words, PCR amplifying the same DNA extract twice can result in different alleles being detected at a locus.

19.4.18.5 Stochastic effects are evaluated during the internal validation process. The FBU established a stochastic threshold based on the internal validation process of 950 RFU with the GlobalFiler amplification kit using a 3500 Genetic Analyzer. This level is used when interpreting the genetic profile and using the appropriate statistics when making comparisons. For Yfiler Plus amplifications, an RFU level of 450 is used when interpreting mixtures of males DNA.

19.4.18.6 Use caution when interpreting profiles with total inputs of less than 100 pg of DNA (0.100 ng). Generally, samples with less than 100 pg of DNA (fewer than 16 diploid cells) are low-level and caution should be used when interpreting due to the potential for stochastic effects such as drop-out, drop-in, allele imbalance, and loss of Y at Amelogenin.

19.4.19 Null (Silent) Alleles

19.4.19.1 When amplifying DNA fragments that contain STR repeat regions, it is possible to have a phenomenon known as allele drop-out. Sequence polymorphisms are known to occur within or around STR repeat regions.

19.4.19.2 These variations can occur in three locations, relative to the primer binding sites. They can occur (1) within the repeat region, (2) in the flanking region, or (3) in the primer binding region.

19.4.19.3 If a base pair change occurs in the DNA template at the PCR primer binding region, the hybridization of the primer can be disrupted resulting in a failure to amplify, and therefore failure to detect an allele that exists in the template DNA.

19.4.19.4 Simply, the DNA template exists for a particular allele but fails to amplify during PCR due to primer hybridization problems.

19.4.19.5 Null alleles are rather rare because the flanking sequence around STR repeats is fairly stable and consistent between samples.

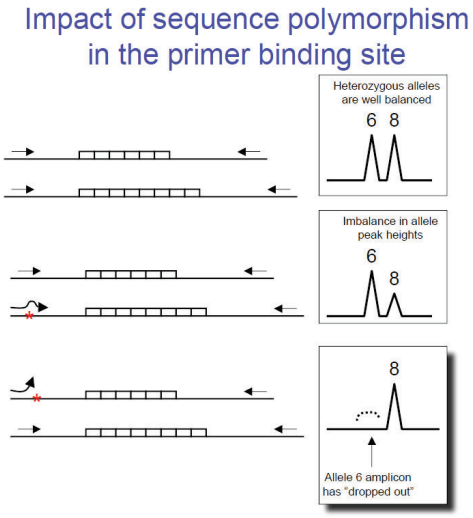


Figure 6.9, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

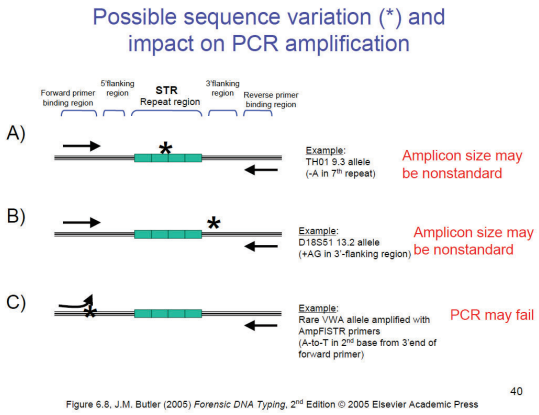


Figure 6.8, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

19.4.20 Applied Biosystems GlobalFiler (GF)

19.4.20.1 The following are the 24 loci that the GlobalFiler kit amplifies, with the associated dye color:

- 6-FAM (blue)
  - D3S1358, vWA, D16S539, CSF1PO, TPOX
- VIC (green)
  - Y Indel, Amelogenin, D8S1179, D21S11, D18S51, DYS391
- NED (yellow)
  - D2S441, D19S433, TH01, FGA
- TAZ (red)
  - D22S1045, D5S818, D13S317, D7S820, SE33
- SID (purple)
  - D10S1248, D1S1656, D12S391, D2S1338
- LIZ (orange) (Size Standard)
  - GS-600 v1.2

Additional gender markers were added to the GlobalFiler kit compared to previous kits on the long arm of the Y chromosome in order to

minimize the risk of a double deletion. DYS391 is a stable locus with a relatively narrow allele range. Y Indel is very short and therefore more likely to perform even if DYS391 drops out in degraded samples.

A “1” result at Y Indel means there is a deletion while a “2” result means there is an insertion. According to Life Technologies staff, a “1” result is rare, while a “2” is generally the result.

19.4.20.2 Components of Amplification Kits

The GlobalFiler amplification kit consists of the GlobalFiler Master Mix, GlobalFiler Primer Mix, Control DNA 007 along with the GlobalFiler Allelic Ladder.

The *Master Mix* is composed of five (5) different components:

- Salt ($MgCl_2$)
  - $MgCl_2$ provides free Magnesium, which acts as a cofactor for Taq polymerase; hence, Taq requires free Mg^{2+} to function properly.
- dNTPs
  - dNTPs include the bases adenine, guanine, cytosine, and thymine, which are the building blocks of DNA; these bases are necessary for extension of the newly synthesized DNA products.
- Bovine Serum Albumin (BSA)
  - BSA enhances the efficiency of the PCR reaction and also helps to prevent inhibition.
- Sodium Azide
  - Sodium azide is important during the annealing steps of the PCR reaction because it provides positively charged molecules to help mitigate the electrostatic repulsion between the annealing DNA strands, which is caused by the negatively charged phosphate backbones. It is also a preservative.
- Enzyme (Taq Polymerase)
  - Performs DNA replication of the loci of interest by extending the primers; also adds an extra “A” to the 3’ end PCR product during extension.

Primer Mix

- Contains forward and reverse primers to amplify human DNA targets.

007 DNA

- (0.1ng/µL human male genomic DNA from cell line in 0.05% Sodium Azide (NaN_3) in buffer and salt)
- Serves as an internal positive control to ensure that amplification was satisfactory.

GlobalFiler Allelic Ladder

- Contains amplified alleles.

The Yfiler Plus amplification kit consists of the Yfiler Plus Master Mix, Yfiler Plus Primer Set, Control DNA 007, along with the Yfiler Plus Allelic Ladder.

The *Master Mix* is composed of five (5) different components:

Salt ($MgCl_2$)

$MgCl_2$ provides free Magnesium, which acts as a cofactor for Taq polymerase; hence, Taq requires free Mg^{2+} to function properly.

dNTPs

dNTPs include the bases adenine, guanine, cytosine, and thymine, which are the building blocks of DNA; these bases are necessary for extension of the newly synthesized DNA products.

Bovine Serum Albumin (BSA)

BSA enhances the efficiency of the PCR reaction and also helps to prevent inhibition.

Sodium Azide

Sodium azide is important during the annealing steps of the PCR reaction because it provides positively charged molecules to help mitigate the electrostatic repulsion between the annealing DNA strands, which is caused by the negatively charged phosphate backbones. It is also a preservative.

Enzyme (Taq Polymerase)

Performs DNA replication of the loci of interest by extending the primers; also adds an extra "A" to the 3' end PCR product during extension.

Primer Set

Contains forward and reverse primers to amplify human DNA targets.

007 DNA

(2.0ng/µL human male genomic DNA in 0.05% Sodium Azide (NaN_3) in buffer and salt)
Serves as an internal positive control to ensure that amplification was satisfactory.

Yfiler Plus Allelic Ladder

Contains amplified alleles.

19.4.21 The PCR procedures for GlobalFiler and Yfiler Plus are covered in the FBU Policy Manual DNA_6 DNA Amplification Using GlobalFiler and DNA_7 DNA Amplification Using Yfiler Plus.

19.4.22 Quality Control

19.4.22.1 Controls use to monitor PCR

19.4.22.1.1 Controls are used to monitor the effectiveness of the chosen experimental conditions and/or the technique of the experimenter.

19.4.22.1.2 The Negative Control (NC) is the entire PCR reaction mixture without any DNA template. The negative control usually contains water or buffer (TE^{-4}) of the same volume as the DNA template and is useful to assess whether or not any of the PCR components have been contaminated by DNA.

An extraction “blank” is also useful to verify that the reagents used for DNA extraction are free from any extraneous DNA templates.

19.4.22.1.3 The Positive Control (PC) is a valuable indicator of whether or not any of the PCR components have failed or were not added during the reaction setup phase of experiments conducted. A standard DNA template of known sequence with good-quality DNA should be used for the positive control (007 male [0.1ng/µL (GlobalFiler) and 2.0ng/µL (Yfiler Plus) male] provided in kits). The DNA template should be amplified with the same PCR primers as used on the rest of the samples in the batch that is being amplified. The purpose of a positive control is to ensure confidence that the reaction components and thermal cycling parameters are working for amplifying a specific region of DNA.

19.4.22.2 Precautions against Contamination

19.4.22.2.1 The sensitivity of PCR necessitates constant vigilance on the part of the analysts to ensure that contamination does not affect DNA typing results.

19.4.22.2.2 Some tips for avoiding contamination with PCR reactions in a laboratory setting include:

19.4.22.2.2.1 Pre- and post-PCR sample processing areas should be physically separated. Usually, a separate room or a containment cabinet is used for setting up the PCR amplification reactions. Reactions may also be setup in a laminar flow hood, if available.

19.4.22.2.2.2 Equipment such as pipettors and reagents for setting up PCR should be kept separate from other laboratory supplies, especially those used for analysis of PCR products.

19.4.22.2.2.3 Disposable gloves should be worn and changed frequently.

19.4.22.2.2.4 Aerosol-resistant pipette tips should be used and changed between each sample to prevent cross contamination during liquid transfers. Pipette tips should never be reused.

19.4.22.2.2.5 Reagents should be carefully prepared to avoid the presence of any contaminating DNA or nucleases.

19.4.22.2.2.6 Ultraviolet irradiation of laboratory PCR setup space when the area is not is use and cleaning workspaces and instruments with isopropanol and/or 10% bleach solutions

help ensure that extraneous DNA molecules are destroyed prior to DNA extraction or PCR setup.

19.4.22.2.2.7 Wear appropriate PPE (mask, gloves, lab coats, etc.).

19.4.22.2.2.8 Use tube de-cappers to open tubes.

19.4.22.3 Quality Control of ProFlex Thermal Cyclers (FBU Policy Manual QC_11 ProFlex)

19.4.22.3.1 An annual performance check of all ProFlex thermal cyclers will be performed by a Life Technologies technician or in-house using a Temperature Verification System (TVS).

19.4.22.3.2 A performance check will be completed using a TVS after any repair, service, or calibration before the thermal cycler is returned for use in casework.

19.4.22.4 Quality Control of PCR Amplification Kits

19.4.22.4.1 The PCR amplification kits will be quality controlled checked by amplifying and profiling two NIST traceable known DNA samples.

19.4.22.4.2 For the kit to pass the QC process, the correct profile for the two samples must be obtained. The negative control must not show any indication of contamination and the positive control must yield the correct allele calls.

19.5 Direct Amplification

19.5.1 The same basic theory of STR amplification (GF) applies to GlobalFiler Express Direct Amplification (GFE). However, a different commercially produced Applied Biosystems Amplification Kit is utilized for GFE direct amplification.

19.5.2 Components of the GlobalFiler Express Amplification Kit

19.5.2.1 Master Mix: The components necessary to carry out the reaction (enzyme, buffer, Sodium Azide, $MgCl_2$, BSA, dNTPs).

19.5.2.2 Master Mix Additive: Reagent to be added to the Master Mix following first time thawed

19.5.2.3 Primers: The forward and reverse primers to amplify the human male DNA targets.

19.5.2.4 Allelic Ladder: Ladder for GlobalFiler Express

19.5.2.5 Positive Control: 007 (2ng/ul male positive control)

19.5.3 The thermal cycling parameters for the Globalfiler Express Direct Amplification Kit is as follows:

19.5.3.1 95˚ C for 1 minute (activate the Taq Polymerase)

94˚ C for 3 seconds (denature)
60˚ C for 30 seconds (anneal/extend)] 27 cycles

60˚ C for 8 min (-A nucleotide addition (adenylation))
4˚ C for infinite amount of time

19.5.4 The FBU policy that covers PCR direct amplification using Globalfiler Express Amplification Kit is FBU Policy Manual DNA 8 DNA Amplification Using Globalfiler Express.

19.6 Capillary Electrophoresis

19.6.1 Capillary Electrophoresis (CE) is the primary methodology used for separating and detecting short tandem repeat (STR) alleles in forensic DNA laboratories.

19.6.2 To achieve reliable STR typing the following conditions must be met.

19.6.2.1 Spatial resolution is needed to separate STR alleles that may differ in size by a single nucleotide.

19.6.2.2 Spectral resolution is needed to separate fluorescent dye colors from one another so the PCR products from loci labeled with different dyes can be resolved.

19.6.2.3 DNA sizing precision from run to run must be consistent enough so that samples can be related to allelic ladders that are fun for calibration purposes.

19.6.3 Principles and Components of Capillary Electrophoresis

19.6.3.1 The primary elements of a basic CE instrument include a narrow glass capillary, buffer vials, and two electrodes connected to a high-voltage power supply.

19.6.3.2 CE systems also contain a laser excitation source, a fluorescence detector, an auto sampler to hold the sample tubes or tray, and a computer to control the sample injection and detection.

19.6.3.3 CE capillaries are made of glass and typically have an internal diameter of 50µm. Capillary length for Human Identification (HID) work is 36cm in length.

19.6.3.4 Instead of a gel matrix, like in gel electrophoresis, the DNA molecules pass a viscous polymer solution that serves as the sieving medium. The linear, flexible polymer chains act as obstacles that must be navigated by the negatively charged DNA fragments on their way to the positive electrode.

19.6.3.5 The larger DNA molecules move more slowly through the capillary than the smaller, more agile DNA fragments, which allows the molecules to be separated based on their size.

19.6.3.6 Prior to injecting each sample, a new gel is “poured” by filling the capillary with a fresh aliquot for the polymer solution. The CE can be thought of as one lane in a gel that is only wide enough for one sample at a time.

19.6.3.7 A constant set of known size DNA fragments are part of the master mix that are injected with each sample that serve as internal size standards (ILS) in order to compare the results from run to run. An important difference between CE and gels is that the electric fields are on the order of 10 to 100 times stronger with CE which results in much faster run times for CE.

19.6.3.8 Detection of the sample is performed automatically by the CE instrument by measuring the time span from sample injection to sample detection with a laser placed near the end of the capillary.

19.6.3.9 DNA fragments are excited (illuminated) as they pass by this window.

19.6.3.10 The smaller DNA molecules arrive at the detection point first followed by the larger molecules in order of their migration speed, which correlates with length or the number of base pairs.

19.6.3.11 Data from CE separations are plotted as a function of the relative fluorescence intensity observed from fluorescence emission of dyes passing the detector. The florescent emission signals from dyes attached to the DNA molecules can then be used to detect and quantify the DNA molecules passing the detector.

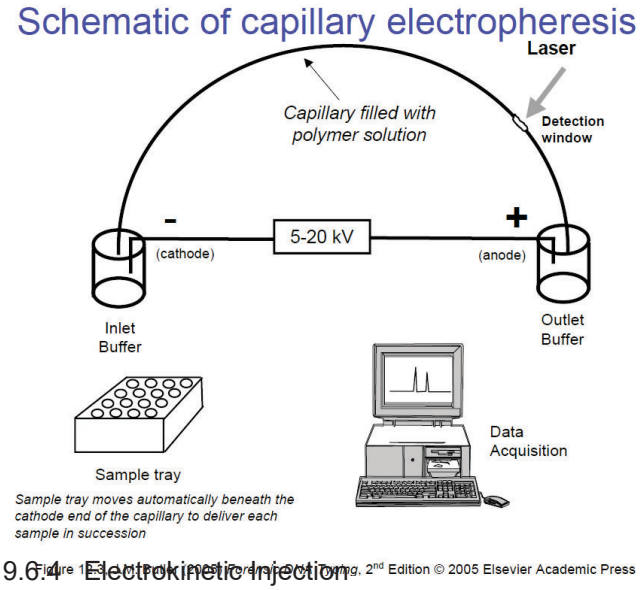


Figure 15-1 Lehninger Principles of Biochemistry, 2nd Edition © 2005 Elsevier Academic Press

19.6.4 Electrokinetic Injection

19.6.4.1 The most common method used to introduce DNA samples into a capillary is a process called electrokinetic injection, where a voltage is applied to a liquid sample immersed in one end of a capillary for a defined time.

19.6.4.2 As DNA molecules are negatively charged in a neutral pH environment, a positive voltage draws the DNA molecules into the capillary.

19.6.4.3 Electrokinetic injections produce narrow injection zones that permit high-resolution DNA separations to occur in a relatively short separation distance.

19.6.4.4 In order to get the DNA molecules onto the CE capillary, an electric voltage is applied while the end of the capillary is immersed into the liquid DNA sample (plate). An electrode (cathode) is enclosed with the capillary in a platinum electrode.

19.6.4.5 The flow of current generated by the voltage applied and the resistance experienced pulls the negatively charged DNA molecules into the end of the capillary. CE injections of DNA are highly sensitive to the sample matrix-specifically the ionic strength of small negative ions, such as chloride from the PCR solution, provide competition for larger, slower DNA molecules in entering the end of the capillary.

19.6.4.6 The quantity of DNA injected into a CE column is dependent on several variables. Some of the variables include voltage or electric field applied, the injection time, the concentration of DNA in the sample, area of the capillary opening, and the ionic strength of the sample versus the buffer.

19.6.4.7 Sample preparation

19.6.4.7.1 Sample preparation can impact electrokinetic injection. The amplified DNA is typically diluted to levels of 1 to 10 with deionized formamide, i.e.,, 1µL of amp product with 9µL of formamide. This is both to help denature the double-stranded DNA molecules and help reduce the salt levels and aid the electrokinetic injection process.

19.6.4.7.2 Formamide is a strong denaturant and is used in the preparation of single stranded DNA samples for CE. Merely placing the sample in formamide is sufficient to denature it; however, heating a sample to 95˚C for several minutes (3 minutes) followed by rapid cooling to around 0˚C (3 minutes) will ensure the two complementary strands are denatured.

19.6.4.7.3 Use of high quality formamide with low conductivity is important. As formamide degrades it produces ionic decomposition products including formic acid, which are negatively charged at neutral pH and will be preferentially injected into the capillary. The formamide by-products can cause problems in both sensitivity and resolution. The Hi-Di formamide is commercially prepared and purchased; it generally works well.

19.6.5 DNA Separation

19.6.5.1 The DNA molecules separate out along the capillary once a voltage is applied. The smaller molecules move more quickly and are detected earlier than the larger ones. The time at which the DNA molecules cross the detection window is converted to base pair size through the use of internal size standards.

19.6.5.2 Several components impact DNA separations within CE systems: the polymer used for enabling the separation, the capillary, the electrophoresis buffer, and the voltage applied or electric field strength.

19.6.5.3 STR allelic ladders are useful tools for monitoring system resolution, since there are multiple PCR products that are 4 bp or less difference in size.

19.6.5.4 DNA moves with electrophoretic flow from the negative electrode (cathode) towards the positive electrode (anode). Strands of entangled polymer form transient pores that serve as obstacles to inhibit progress of DNA molecules based on their size. Smaller DNA molecules are able to move more easily through the obstructions.

19.6.5.5 The capillaries are made of glass or fused silica (silicon dioxide, SiO_2) but have hydroxyl groups (silanol, SiOH) along the inner walls and are negatively charged above a pH of 5. The electrophoresis buffer used is typically around pH of 8; positive ions from the buffer solution will line up with the negative siloxy (SiO^-) ions along the walls creating what is known as a double layer.

19.6.5.6 Under the electric field applied during CE separation, the positive ions will migrate toward the negative electrode. The movement of these positive ions will create a bulk flow of the solution within the capillary is known as electro-osmotic flow or EOF. EOF can be reduced or eliminated by coating the inner capillary wall to prevent exposure of negative charges along the wall and buildup of positive ions from solution.

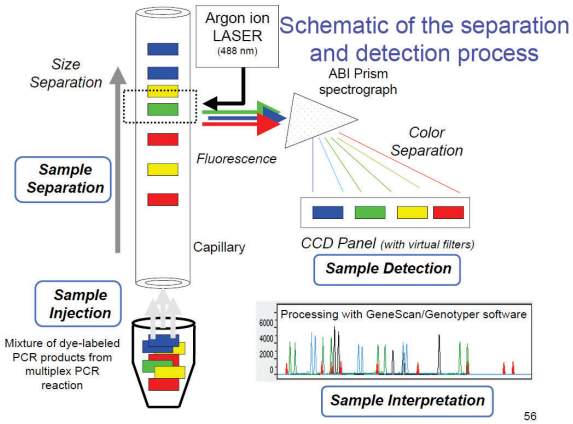


Figure 13.8, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

19.6.5.7 Polymer Solution

19.6.5.7.1 The polymer that is used for forensic HID applications is POP-4. POP stands for Performance Optimized Polymer, with a 4% concentration of linear uncross-linked dimethyl polyacrylamide. POP-4 consists of 4% linear dimethylacrylamide, 8 mil/L urea, 5% 2-pyrrolidinone.

19.6.5.7.2 A high concentration of urea is also present in the polymer solution to help create an environment in the capillary that will keep the DNA molecules denatured.

19.6.5.7.3 Entangled polymers are characterized by a rapid increase in viscosity as the polymer concentration reaches a certain

threshold value. The viscosity of this material is also dependent on the polymer's relative molecular mass. The ideal polymer should have at least the same separation properties as classical polyacrylamide gels, combined with a low viscosity that allows easy replacement before each run is conducted on the CE instrument.

19.6.5.8 Buffer

19.6.5.8.1 The buffer that is used to dissolve the polymer in CE systems is important as it stabilizes and solubilizes the DNA and provides charge carriers for the electrophoretic current.

19.6.5.8.2 If the buffer concentration and associated conductivity are too high, then the column will overheat resulting in a loss of resolution.

19.6.5.8.3 In the process of electrophoresis, the composition of the anode and cathode buffers may change due to electrolysis and migration of buffer ions. To avoid problems with poor size calibration over time, it is good to regularly replace the CE buffers with fresh solution.

19.6.5.8.4 Capillary run temperatures of 60˚C are commonly employed to help reduce secondary structures in DNA. In addition, the high concentrations of urea in the buffer and the elevated temperatures are used to keep various STR alleles uniformly denatured, since the mobility of DNA fragments can be affected by its conformation.

19.6.5.8.5 The CE instruments need a stable ambient temperature, as temperature variations can have profound effects on allele migration.

19.6.5.9 Capillary Array

19.6.5.9.1 The capillary array is central to the separation capabilities of CE. In uncoated capillary array columns, residual changes on the silica surface induces a flow of the bulk solution toward the negative electrode. This EOF creates problems for reproducible DNA separations because the velocity of the DNA molecules can change from run to run. The siloxy groups on the capillary walls can be chemically modified or dynamically coated to prevent EOF separations.

19.6.5.9.2 The commercially available POP-4 polymers are successfully used in DNA genotyping because they provide a sieving matrix for the separation of single stranded DNA and at the same time suppress the EOF.

19.6.5.9.3 Through effective monitoring of sample resolution, capillaries can be replaced when resolution declines.

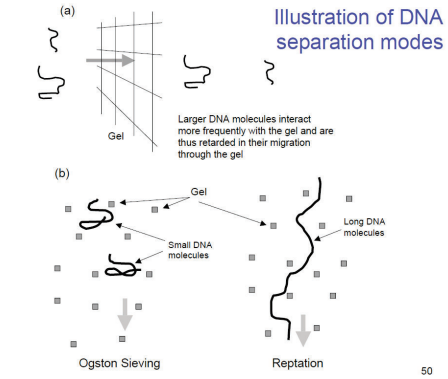


Figure 12.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

19.6.5.9.3.1 In the data, when there is an excessive amount of broadening of the peaks, it is an indication that the capillary needs to be changed.

19.6.6 Fluorescence Detection

19.6.6.1 Fluorescence-based detection assays are widely used in forensic laboratories due to their capabilities for multicolor analysis and their rapid and easy to use formats.

19.6.6.2 The fluorescent dye is attached to a PCR primer that is incorporated into the amplified target region of DNA.

19.6.6.3 A significant advantage of fluorescent labeling over other methods is the ability to record two or more fluorophores separately using optical filters. The signal produced has to be spectrally resolved. This is accomplished using a fluorophore color separation algorithm known as a spectral calibration.

19.6.6.4 A fluorescence detector is a photosensitive device that measures the light intensity emitted from a fluorophore. Detection of low intensity light may be accomplished with a photomultiplier tube (PMT) or a charge coupled device (CCD). The action of a photon striking the detector is converted to an electric signal. The strength of the resultant current is proportional to the intensity of the emitted light. This light intensity is typically reported in arbitrary units, such as relative fluorescent units (RFU).

19.6.6.5 The different dyes are covalently bound onto the 5' (nonreactive) end of each primer or set of primers. The dyes are all excited by a single argon ion laser turned to 488 nm, but fluoresce in different regions of the spectra.

19.6.6.6 Lasers are an effective excitation source because the light they emit is very intense and mostly at one or a few wavelengths. The older (310 and 3130 GAs) used an argon ion gas laser which produced a blue light with dominant wavelengths of 488 nm and 524.5 nm. The 3500 instrument uses a 505 nm diode laser.

19.6.6.7 The intensity of the light emitted by a fluorophore is directly dependent on the amount of light that the dye has absorbed. The excitation source is very important in the behavior of a fluorophore. Other important instrument parameters to be considered include optical filters used for signal discrimination and the sensitivity and spectral response of the detector.

19.6.6.8 The CCD camera is used to determine which dye is present, based on emission of each fragment as it passes the detector window. This technique permits the analysis of fragments of DNA that overlap in size as long as they are labeled with different dyes, which fluoresce at different wavelengths.

19.6.6.9 The following is an example of the path of the laser light for detection in the AB 310 Genetic Analyzer. Light from the argon ion laser passes through the laser filter, which removes low-intensity sidebands and other broadband spontaneous emission. Light is then focused by the diverging lens onto the di-chroic mirror. The di-chroic mirror is used to separate excitation and emission light paths. The beam then passes through the microscope objective to the sample. The emitted light is then reflected by the di-chroic mirror onto a reimaging lens after it passes through a long-pass filter. The long pass filter prevents light from the argon ion laser from interfering with the detection of the dye signals. Light is then directed onto a spectrograph where a diffraction grating disperses the light by wavelength and focuses the resulting spectrum onto a CCD array.

19.6.6.10 With capillary array systems, the laser light must illuminate multiple capillaries. The most common method Applied Biosystems uses is called on-capillary side irradiation.

19.6.6.11 The dye-labeled PCR primers label only a single strand of the PCR product. This simplifies data interpretation because the complementary DNA strand in not visible to the detector. In addition, dye labeled primers enable multiple PCR products to be labeled simultaneously in an independent fashion thus increasing throughput capabilities because amplicons of overlapping size can be distinguished from one another by their dye label.

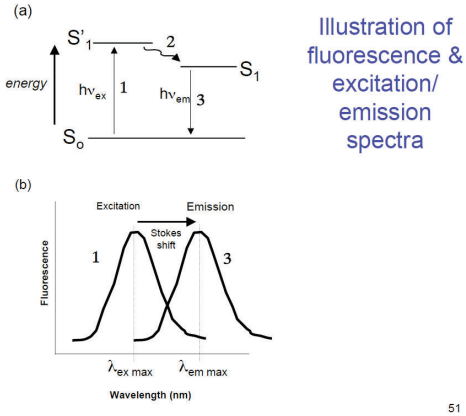


Illustration of fluorescence & excitation/ emission spectra

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Figure 13.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

19.6.6.12 Spectral Calibration

19.5.6.12.1 In order for the computer software that is used for data analysis to know with what dye color a detected DNA fragment is labeled, the instrument detector and data collection software need to be calibrated.

19.6.6.12.2 A multi-component spectral calibration is performed by testing a standard set of DNA fragments labeled with each individual dye (DS-36 [J6] for six dye systems).

19.6.6.12.3 The computer software then analyzes the data from each of the dyes and creates a spectral calibration file to reflect the color overlap between the various fluorescent dyes.

19.6.6.12.4 The spectral calibration files are a summary or template of how much overlap one should except to see just by virtue of the dyes themselves given a particular instrument and environmental conditions.

19.6.6.12.5 A spectral calibration should be performed any time a new laser or detector is installed. With the 3500 instrument a spectral calibration will be done each time a capillary array is installed.

19.6.6.12.6 If the color deconvolution does not work properly to separate spectral overlap of the dyes, then the baseline analytical signal from the instrument can be uneven or a phenomenon known as “pull up” can occur.

19.6.6.12.7 Pull up is the result of color bleeding from one spectral channel into another, usually because of off-scale peaks. Alleles at the same data point can saturate the pixels and add up to more information for the camera and saturate even if a single peak is not above 30,000 RFU.

19.6.6.12.8 When there is an excessive amount of pull up seen in the data, it is an indication that a new spectral calibration needs to be performed.

19.6.7 Instrumentation (AB 3500 Genetic Analyzer)

19.6.7.1 CE systems are easy to use with automated data collection and reduce labor compared to the early gel electrophoresis systems.

19.6.7.2 The AB 3500 instrument is available in 8 capillary or 24 capillary formats. The OSBI FBU uses the 8 capillary instruments.

19.6.7.3 The AB 3500 instrument has a smaller area for the array with better seals for more consistent temperature control, an improved mechanical pump for polymer filling of the capillaries with less waste, new laser technology (110 V instead of 220 V), and 6-dye detection capability.

19.6.7.4 Radio frequency identification (RFID) tags are used to track consumables which are made available in ready-to-use formats to enable easier installation of reagents.

19.6.7.5 The data collection software includes quality control features for rapid identification and re-injection of failed samples.

19.6.7.6 With the use of a specific internal size standard (LIZ 600 v2.0), peak height normalization is possible, leading to improved peak height uniformity across capillaries, runs, and instruments within the laboratory.

19.6.7.7 The laser on the 3500 instrument is a single excitation line, solid state laser that has a wavelength of 505nm and output power of 20mW. The laser has minimum heat output and uses a standard voltage plug (110v).

19.6.7.8 The laser light is directed into the plane of the capillaries from both the bottom and top (dual illumination). The light passes through a transmission grating and is projected onto a scientific grade CCD array.

19.6.7.9 Status lights of the 3500 instrument

19.6.7.9.1 All lights off indicates the instrument is off.

19.6.7.9.2 A solid green light is an indication that the instrument is on and operational and awaiting a run.

19.6.7.9.3 A blinking green light indicates that the instrument is in the middle of a run.

19.6.7.9.4 A blinking amber light indicates the instrument is powering up, a self-test is in progress, a run was paused, a door is open, or a run failure that does not a require a restart of the instrument has occurred.

19.6.7.9.5 A steady amber light indicates the instrument is in standby mode.

19.6.7.9.6 A red light indicates that the self-test failed, the instrument failed, or the instrument and computer require a restart.

19.6.7.10 Normalization

19.6.7.10.1 Normalization normalizes the signal variations associated with instrument, capillary array, sample salt load, and injection variability among capillaries and instruments.

19.6.7.10.2 Normalization is applied when the data is collected on the instrument, it can be turned on or off in the analysis of the data through GMID-X. The OSBI FBU unit currently collects and analyzes data with normalization off. The OSBI internal validation for the expanded CODIS core loci kits established that each 3500 Genetic Analyzer within the OSBI FBU system is within ~20% of one another; therefore, no normalization using the software or differing injection times is necessary.

19.6.8 The 3500 Genetic Analysis procedure is covered under FBU Policy Manual DNA_10 3500 Series Genetic Analyzer Analysis

19.6.9 Quality Control of the 3500 Instruments (FBU Policy Manual QC_11 3500 Series Genetic Analyzer)

19.6.9.1 Spatial Calibration determines the position of the image from each capillary on the CCD array.

19.6.9.1.1 Spatial is required when you:

19.6.9.1.1.1 Remove or replace the capillary array

19.6.9.1.1.2 Open the detector door or move the detection cell

19.6.9.1.1.3 Move the instrument

19.6.9.2 Spectral calibration is an examination of the contribution of overlap in the emission spectrum for fluorescent dyes used for a specific DNA test on the GA. It permits the color deconvolution necessary of multicolor STR typing to be performed.

19.6.9.2.1 Spectral is required when you:

19.6.9.2.1.1 Use a dye set that you have not previously calibrated (uncommon)

19.6.9.2.1.2 Change the capillary array

19.6.9.2.1.3 Change the polymer type (uncommon)

19.6.9.2.1.4 Have a service engineer perform an optical service procedure, such as realigning or replacing the laser or CCD camera or mirrors on the instrument.

19.6.9.2.1.5 See a decrease in spectral separation (pull-up peaks) in the raw or analyzed data.

19.6.9.2.1.6 A new spectral will not be required to be performed yearly; since it is required when installing a new array, it will already be done.

19.6.9.3 Performance Checks ensure the instrument is functioning properly and is run when procedures are changed that affect the sizing.

19.6.9.3.1 Performance Checks (HID Install Standards) required when:

19.6.9.3.1.1 The instrument was installed

19.6.9.3.1.2 As needed per Technical Manager

19.6.9.3.2 The observed number of alleles and size standard peaks should be the same as the expected number, if they are then the results are accepted and the performance check has passed. If they are not, then the performance check has failed, and you should proceed to the troubleshooting section of the 3500 User's Manual.

19.6.9.4 Maintenance Requirements

19.6.9.4.1 Weekly

19.6.9.4.1.1 Record number of runs

19.6.9.4.1.2 Check storage condition of used arrays to ensure array tip is covered in reservoir.

19.6.9.4.1.3 Run wash pump and channels wizard (Water Wash).

19.6.9.4.2 Bi-Weekly

19.6.9.4.2.1 Replace Anode and Cathode Buffer containers (ABC and CBC).

19.6.9.4.2.2 Replace septa on reservoirs

19.6.9.4.2.3 Replace POP-4 pouch

19.6.9.4.3 Monthly

19.6.9.4.3.1 Flush the pump trap; there is no wizard for this, the procedure is in the 3500 User's Manual.

19.6.9.4.3.2 Empty the condensation container and water trap waste container.

19.6.9.4.4 As Needed

19.6.9.4.4.1 Restart instrument and computer

19.6.9.4.4.2 Install new capillary

19.6.9.4.4.3 Perform spatial calibration

19.6.9.4.4.4 Perform spectral calibration

19.6.9.4.4.5 Defragment the computer hard drive

19.6.9.4.4.6 Run HID Install Standard

19.6.9.4.4.7 Archive the Library

19.7 DNA Interpretation

19.7.1 The computer program that analyzes the data generated from the AB 3500 Genetic Analyzer is called GeneMapper ID-X.

19.7.2 GeneMapper ID-X Sizing

19.7.2.1 Actions performed by the software during sizing:

19.7.2.1.1 Performs size matching of the internal size standard in all samples against the size standard definition selected in the software.

19.7.2.1.2 Generates a sizing calling curve.

19.7.2.1.3 Sizes a DNA sample peaks.

19.7.2.1.4 Assesses the sizing quality.

19.7.2.2 During size matching, the software matches the size standard fragments from the electropherogram to the list of fragment sizes in the size standard definition specified in the software (GS-600 LIZ v2.0). GS-600 LIZ v2.0 contains 36 DNA fragments ranging in size from 20 to 600 bp.

19.7.2.3 Size matching uses ratio matching based on relative height and distance of neighboring peaks. It then derives quality values statistically by examining the similarity between the theoretical (from the size standard definition) and actual (observed) fragment patterns.

19.7.2.4 The software constructs a best-fit curve using the data points of each size standard fragment detected. A comparison between the sizes calculated from the best-fit curve and the matched peaks from the size standard definition file using the array of numbers is performed. Size-matching and subsequent size calling fails if significant difference in peak patterns is found, if no match can be made based on the expected patterns, or if all peaks are not found.

19.7.3 Size Calling Curve Generation and Size Calling in GeneMapper ID-X

19.7.3.1 To generate the size calling curve, the software plots the actual data points of the size standard against the expected size of each size standard peak. The size calling method determines how the size calling curve is generated and used to size each sample.

19.7.3.2 During size matching and size calling:

19.7.3.2.1 Two size calling curves are generated for each sample

19.7.3.2.1.1 Black: A best fit second order curve, regardless of the size calling method that is selected.

19.7.3.2.1.2 Red: A curve based on the size calling method that is selected in the analysis method.

19.7.3.2.2 The data points of non size standard peaks are plotted against the size calling curve.

19.7.3.2.3 Peaks are sized according to the size calling method that is selected in the analysis method.

19.7.3.3 Size Calling – Local Southern Method

19.7.3.3.1 The local southern method determines the sizes of fragments by using the reciprocal relationship between fragment length and mobility, as described by E.M. Southern (1979).

19.7.3.3.2 This method uses the four fragments closest in size to the unknown fragment to determine the best-fit line value. Only the region of the size standard near the fragment of unknown length is analyzed.

19.7.3.3.2.1 The fitting constraints of the curve are calculated for each group of three neighboring points on the standard.

19.7.3.3.2.2 A curve is then created using three standard points (two points below and one point above the fragment); then a fragment size is determined.

19.7.3.3.2.3 Another curve is created using an additional set of three points (one point below and two points above the fragment) to assign another value.

19.7.3.3.2.4 The two curves are averaged to determine the unknown fragment length.

19.7.4 After the sizing quality is determined, the following occur: allele matching, allelic ladder quality assessment, and bin offsetting.

19.7.5 Allele Matching

19.7.5.1 During allele matching, the software assigns allele labels to allelic ladder peaks by matching peaks with the bins that are specified in the panel. The matching algorithm uses ratio matching, based on relative distance and height of neighboring peaks.

19.7.6 Allelic Ladder Quality Assessment

19.7.6.1 After allele matching, allelic ladders are evaluated to determine whether they are used to create bin offsets.

19.7.7 Bin Offsetting

19.7.7.1 Bin offsetting evaluates the alleles found in allelic ladder samples and adjusts bins before allele calling. Marker ranges are also adjusted based on the bin offsets. The software calculates the bin offsets and is explained in the Reference Guide of the GeneMapper ID-X software.

19.7.7.2 The allelic ladder for each kit contains the most common alleles for each locus and is used to generate the reference sizes in the bin sets provided with the software.

19.7.8 Physical and Virtual bins

19.7.8.1 The bin set provides reference allele sizes for alleles physically present in the allelic ladder (physical bins) and alleles that are not present in the allelic ladder (virtual bins), but have either been reported in STRbase or discovered during developmental validation of a particular chemistry at Applied Biosystems and can be genotyped by reference to the alleles present in the

ladder. In the GMID-X software, the virtual bins will be pink and the physical bins will be grey.

19.7.9 Sample Genotyping

19.7.9.1 After allelic ladders are detected and sized, sizing quality is determined; then allelic ladders are genotyped.

19.7.9.2 After allelic ladders are genotyped, samples are detected and sized, sizing quality is determined, and samples are genotyped.

19.7.9.3 During genotyping of samples, the GeneMapper ID-X Software:

19.7.9.3.1 Performs allele calling and filtering

19.7.9.3.2 Assesses genotype quality

19.7.9.3.3 Assesses sample quality

19.7.9.4 Allele calling and filtering of samples

19.7.9.4.1 During allele calling of samples, the software

19.7.9.4.1.1 Labels allele peaks in unknown samples:

- Compares the sizes of the unknown peaks to the offset bins
- Assigns the relevant allele label if a peak falls within one of the defined bins.
- Assigns an OL (Off-Ladder) label if a peak falls outside of the defined bins

19.7.9.4.1.2 Applies filters to eliminate peaks from consideration as allele peaks:

- Allele tab analysis method settings (Minus A, Stutter, Amelogenin cutoff, and range filters)
- Marker Specific stutter ratios, if specified in the Analysis method and defined in the panel

19.7.9.4.2 If a peak falls outside one of the defined bins, the software labels OL.

19.7.9.4.2.1 OL alleles may be caused by (1) the presence of a microvariant allele at a particular locus or (2) sample migration anomalies and artifacts.

19.7.9.4.3 Allele names are assigned based on number of complete (i.e., four base pair repeat units) and partial repeat units:

19.7.9.4.3.1 Complete base pair repeat units are designated by an integer.

19.7.9.4.3.2 Variant alleles that contain a partial repeat are designated by a decimal followed by the number of bases in the particular repeat (i.e., FGA 26.2 allele contain 26 complete repeat units and a partial repeat unit of two base pairs).

19.7.9.5 Genotype and Sample Quality Assessment

19.7.9.5.1 To determine sample quality, the software applies user-defined and software-defined thresholds to generate marker level quality value flags and sample level quality flags. It also applies genotyping quality (GQ) weighting from marker level quality value flags to determine GQ. It uses GQ to determine the sample level composite genotyping quality (CGQ) assessment.

19.7.10 An STR genotype is the allele, in the case of a homozygote, or alleles in the case of a heterozygote, present in a sample for a particular locus and is normally reported as the number of repeats present in the allele.

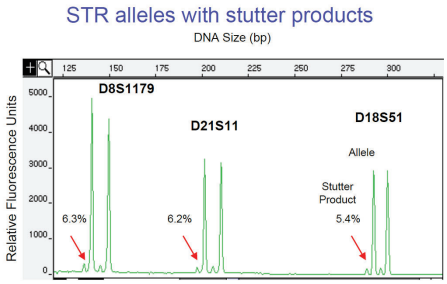
19.7.11 A full sample genotype or STR profile is produced by the combination of all of the locus genotypes into a single series of numbers. This profile is what is reported or used for DNA database comparisons.

19.7.12 During the internal validation of the kits and instruments there were several thresholds that were determined. The baseline threshold is the “noise” of the instrument. An analytical threshold is the point at which any peaks below this level is considered unreliable. The stochastic threshold is the point above which there is a low probability that the second allele in a truly heterozygous sample has dropped out. All the threshold levels are defined in the OSBI FBU Policy Manual in DNA_11 GMID-X Data Analysis and CASE_5 DNA Interpretation & Comparison Guidelines.

19.7.13 Once the data is sized, the peaks in each sample are converted from size to STR allele calls through the use of allelic ladders. The data analysis software enables a conversion of all peaks in samples being processed from size to repeat number. A common size range for the genotyping allele bins is +/- 0.5 bp around each allele. The size range enables PCR products that are 1bp different from one another to be differentiated. Due to slight changes in instrument environmental conditions over time, allelic ladders are run regularly in order to keep the size-to-allele conversion process well calibrated.

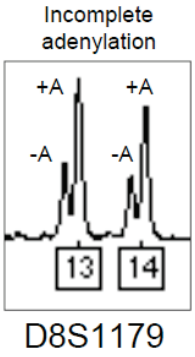
19.7.14 Biology-related Artifact Peaks

19.7.14.1 Stutter Products: These are the most common artifacts that will be encountered. A minor product peak shorter than the corresponding main allele peak is observed; in tetranucleotide repeats it is usually four bases (n-4) shorter than the main peak. Stutter occurs due to strand slippage during primer extension. Stutter products can also be in the forward position (n+4, n+3, n+2) as well, but occur less frequently. Stutter percentage usually increases with allele length. This trend is demonstrated best at D18S51 and vWA. Generally, plus stutter is generally 2% or below the height of the parent peak, as evidenced in the internal validation.



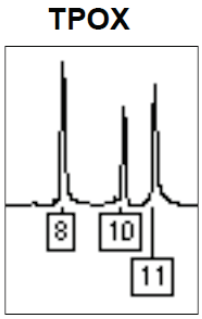
Additive Stutter: If minus and plus stutter combine, and the combined stutter percentage falls at or below the additive value of the plus stutter and the minus stutter filter, the peak may be labeled as “additive stutter”. This will be documented in the technical notes and the technical reviewer must agree.

19.7.14.2 Incomplete 3’ (A) Nucleotide Addition (Commonly referred to as Minus A (-A)): This results with amplifications containing too much DNA template or thermal cycling conditions that affect the optimization of the PCR reaction. This does not allow the *Taq* polymerase to catalyze the addition of an extra nucleotide, usually A (adenine) on the 3’ end of double-stranded PCR products. The target allele will be represented by two peaks one base pair apart.



19.7.14.3 Tri-Allelic Patterns: This pattern results from extra chromosomal fragments being present in a sample or the DNA sequence where the primers anneal being duplicated on one of the chromosomes. These rare abnormalities are detected by an extra peak at a single locus, as opposed to multiple loci as would likely indicate a mixture. The three peaks can either be of approximate equal intensity or possess peak heights such that two of the peaks sum up to approximately the height of the third allele.

(A)



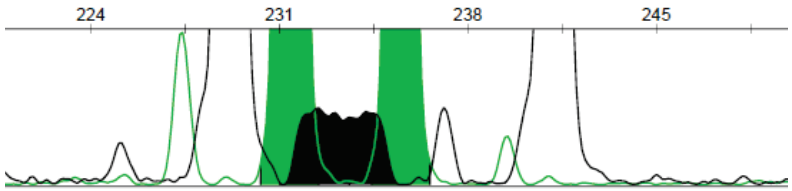
19.7.14.4 Other Commonly Observed Artifacts: TH01 (yellow dye) n-10 to n-12, D5S818 (red dye) ~180 basepairs, and SE33 (red dye) ~394 basepairs called as allele 26.

19.7.15 Technology Related Artifacts

19.7.15.1 Pull-Up: It is the result of the inability of the detection instrument to properly resolve the dye colors used to label STR amplicons due to spectral overlap.

A peak in another color is pulled-up as a result of exceeding the linear range of detection for the instrument (excessive amount of DNA). Some samples may indicate saturation even if a peak is not at 30,000 RFU. This is due to alleles in some data points in differing dyes that compound to saturate the pixels of the camera. A spectral failure is observed as a peak beneath a peak or as an elevation of the baselines for any color. A new spectral calibration may need to be performed to correct this problem. In the GlobalFiler internal validation, most pull-up peaks were below 3% and all pull-up peaks were below 5%.

19.7.15.2 Complex Pull Up: Occurs when a locus is heterozygous and the two peaks are within one repeat unit of each other. This can cause a single bridge-like peak to be observed in another color channel.



19.7.15.3 Dye blobs: These occur when fluorescent dyes come off of their respective primers and migrate independently through the capillary. Most dye artifacts, which are created through incomplete attachment of the fluorescent dye during primer synthesis, are typically removed or significantly reduced through primer purification performed by the STR kit manufacturer. Dye blob peaks are fairly broad and possess the spectrum of one of the dyes used for genotyping and will migrate through the capillary at characteristically consistent sizes.

19.7.15.4 Spikes: These can give rise to a false peak and are usually sharp and appear equally intense throughout all four colors. These peaks are not reproducible and should not appear in the same position if the sample is re-injected.

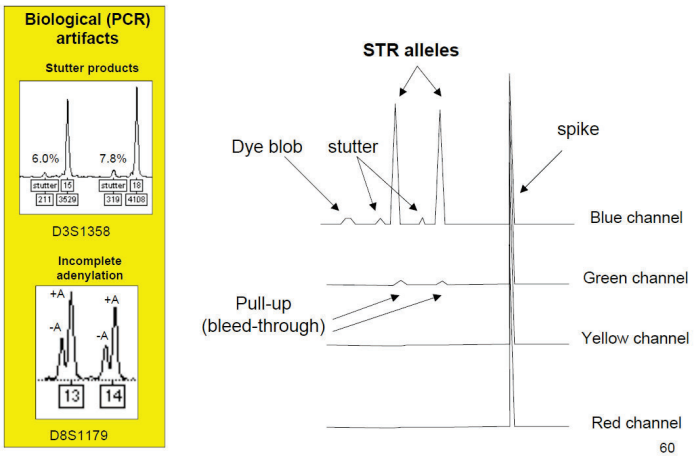


Figure 15.4, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

19.7.16 Common Artifacts Associated with the GlobalFiler Amplification Kit

19.7.16.1 In late 2017, Life Technologies released a Technical Note titled “Artifacts Identified Post-Developmental Validation: GlobalFiler PCR Amplification Kit”. These artifacts include bacterial, fungal, and environmental artifacts. In communication with Life Technologies, they communicated this will be a “living document” and will be updated and provided to the forensic DNA community periodically. Analysts observing the artifacts described in this Technical Note (and in future revisions of the Technical Note) will reference the Life Technologies Technical Note in their case file technical notes and rename the artifact “ART” on the electropherogram.

19.7.17 The interpretation guidelines to follow when interpreting data is covered under FBUs’ Policy Manual CASE_5 DNA Interpretation & Comparison Guidelines.

19.7.18 The procedure for using the GMID-X software is covered under FBUs’ Policy Manual DNA_11 GMID-X Data Analysis.

19.8 DNA Statistics

19.8.1 Since no one has access to DNA profiles of everyone living on the planet, smaller population data sets must be used to extrapolate the possibility of a random match.

19.8.2 To estimate this match probability, allele frequencies are collected from various ethnic/racial samples sets and based on their allele frequencies from validated databases, population genetics principles are applied to infer how reasonable it is that a random, unrelated individual could have contributed the DNA profile in question.

19.8.3 It is important to distinguish between unrelated and related individuals in assumptions being made for the calculations. Related individuals have DNA profiles that are more similar than unrelated individuals who are compared. In these sections, it will be assumed that will be using unrelated individuals.

19.8.4 There are three possible outcomes when making comparisons from questioned samples to known profiles. They are exclusion, inconclusive, and inclusion. The only outcome that requires statistics is the last one, inclusion. If inconclusive results are obtained, reasoning must be included as to why the result was inconclusive.

19.8.5 Statistics attempt to provide meaning to the match. The match statistics are usually provided in the form of an estimate of the random match probability, in other words, the frequency for the particular genotype (DNA profile) in a population.

19.8.6 Population Data

19.8.6.1 To assess how common or rare a particular allele combination is, data is gathered from representative groups of individuals.

19.8.6.2 It is possible to run a small subset of the population and reliably predict allele and genotype frequencies in the entire population. The key is collecting information from enough individuals to reliably estimate the frequency of the major alleles for a genetic locus.

19.8.6.3 The primary goal of generating a population database is to find all common alleles and sample theses alleles multiple times in order to reliably estimate

the frequency of alleles present in the population under consideration. Some alleles, especially variant alleles, have only been observed a few times and are therefore rare.

19.8.6.4 After the samples have been determined, they are genotyped and the information is converted into allele frequencies by counting the number of times each allele is observed.

19.8.6.5 Allele frequency information allows for more compact data storage and enables Hardy-Weinberg equilibrium testing.

19.8.7 Samples sizes used for allele frequency estimation

19.8.7.1 Most published population data include on the order of 100 to 200 STR types per locus per population examined.

19.8.7.2 From studies it was concluded that 100 to 150 individuals per population could provide an adequate sampling for a genetic locus provided that the allele frequencies below 1% were not used for forensic calculations.

19.8.7.3 Collecting information from more samples usually only improves the accuracy of frequency estimates for rare alleles. Comparisons of data collected with typical population sizes versus thousands of individuals show similar allele frequency results.

19.8.8 Minimum Allele Frequency

19.8.8.1 To obtain a reliable estimate of an allele frequency, it is important to collect more than one data point for that allele.

19.8.8.2 A conservative minimum allele frequency is used to ensure that an allele has been sampled sufficiently to be used reliably in statistical tests.

19.8.8.3 The NRC report states that an estimate of an allele frequency can be very inaccurate if the allele is so rare that it is represented only once or a few times in a database, and some rare alleles might not be represented at all. Thus, it is recommended that each allele be observed at least five times to be included in reliable statistical calculations.

19.8.8.4 The minimum allele frequency is therefore 5/(2N); where N is the number of individuals sampled from a population pairs due to inheritance of one allele from one's mother and one from one's father.

19.8.8.5 When an observed allele frequency falls below the minimum allele frequency, the minimum allele frequency will be used instead.

19.8.9 Hardy-Weinberg Equilibrium

19.8.9.1 STR alleles are inherited by an individual from his or her mother and father in a Mendelian fashion and frequencies of occurrence follow a predictable pattern of probability.

19.8.9.2 If two alleles "A" and "a" occur with frequencies p and q in the population, then the genotypes "AA" and "aa" (homozygotes) should occur p^2 and the genotype "Aa" (heterozygous) should occur with frequency 2pq.

19.8.9.3 Allele frequencies are used to generate expected genotype frequencies that are then compared to the observed genotype frequencies. If observed and expected values are similar, then it is assumed that alleles within the genetic locus are stable, or in equilibrium.

19.8.9.4 Hardy-Weinberg equilibrium (HWE) predicts the stability of allele and genotype frequencies from one generation to the next.

19.8.9.5 The primary purpose in testing for HWE is to determine if alleles within a locus are independent of each other.

19.8.9.6 Frequencies should not change over the course of many generations if the locus is generically stable. However, natural populations usually violate HWE to some degree and thereby cause allele frequencies to change over time.

19.8.9.7 HWE Assumptions

19.8.9.7.1 Large population: Lots of possible allele combinations.

19.8.9.7.2 No natural selection: No restriction on mating so all alleles have equal chance of becoming part of next generation.

19.8.9.7.3 No mutation: No new alleles being introduced.

19.8.9.7.4 No immigration/emigration: No new alleles being introduced or leaving.

19.8.9.7.5 Random mating: Any allele combination is possible.

19.8.9.8 HWE assumes a random mating population of infinite size with no migration or mutation to introduce new alleles, which of course does not exist in real human populations.

19.8.9.9 If minor departures are seen from HWE, there is generally no major cause for concern with using a particular database.

19.8.9.10 Most people agree that random mating does not occur. Individuals living in close proximity to each other are more likely to mate than individuals living states or nations apart. In addition, people may also choose their mates according to other physical traits. For forensic DNA statistics, we must think of random mating in terms of genetic markers (STR) we are analyzing. A person is not going to choose a mate because he or she has a particular STR profile.

19.8.9.11 HWE and linkage equilibrium (LE) tests are done on population databases to assess independence of alleles and loci. Linkage equilibrium is when STR loci or any DNA sequence is transferred independently of another DNA segment during meiosis. The inheritance of alleles at a given locus is completely independent of the inheritance of alleles at another locus.

19.8.9.12 With the assumptions of independence, it then becomes possible to equate the overall match probability with the product of locus-specific match

probabilities. This combination of locus-specific match probabilities is referred to as the product rule.

19.8.9.13 Genetic mixing of alleles is not completely random because parents often share some common ancestry. The consequence of this non-random mating is that there is usually a decrease in heterozygotes and an increase in homozygotes. This population substructure can be adjusted for with the use of a correction factor referred to as theta (θ). Typically θ is 0.01 for at large populations and 0.03 with smaller, isolated, and more inbred groups of people. A number of studies have demonstrated that 0.01 is a reliable and conservative estimate of population substructure with extensive population data.

19.8.9.14 Following are the equations that the OSBI FBU uses for statistical calculations.

19.8.9.14.1 Single Source above Peak Stochastic Threshold

$f = p^2 + p(1-p)\theta$ for a single source homozygote
$f = 2pq$ for a single source heterozygote
"p" and "q" are values that reflect the relative frequencies of each allele in the population. Theta (θ) is generally employed to account for the effects of population subdivision and is estimated to be 0.01. However, sometimes it may be appropriate to use a theta value of 0.03 for potentially "isolated populations".
The probability of the entire single source profile is calculated by multiplying the products of all the individual locus probabilities (product rule).

19.8.9.14.2 Single source below Peak Stochastic Threshold (assuming drop-out)

For single peaks with a peak height below the stochastic threshold, another equation must be utilized that accounts for drop out. The equation is:
$f=2p-p^2$

19.8.9.14.3 Mixtures without Drop-Out

For mixtures where all genetic information appears to be present and not peaks in the mixture are below the stochastic threshold at any of the loci that will be used in statistical calculation, then the following calculation will be used at all loci:
Probability of Inclusion (f_{mix}) = $(p_1 + p_2 + p_3\ldots)^2$

19.8.9.14.4 Mixtures with Possible Drop-Out

For mixtures where some genetic information may be missing due to potential drop-out at any one of the locis to be used in statiscal cacluations, the following equation will be used at all loci:

$$Probability = 2\sum_{i=1}^{n} p_i + \sum_{i=1}^{n} p_i^2 + 2\sum_{m=1}^{n-1} p_i \sum_{s=m+1}^{n} p_s$$

When expanded, this equation for a three allele profile would look like:

$$Probability = (2p_1 + p_1^2) \perp (2p_2 \perp p_2^2) \perp (2p_3 \perp p_3^2) - 2p_1p_2 - 2p_1p_3 - 2p_2p_3$$

19.8.9.14.5 Major/Minor Mixtures

The major component of the major/minor mixture is calculated in the same manner as a single source sample.

The minor component of the major/minor mixture is calculated as an indistinguishable mixture using all the alleles for both major and minor alleles if it is assumed that the minor component is a mixture of more than one person. If it is assumed that the minor component is a single source, then the single source with drop out calculation is used with just the loci that have the minor component present and just the minor component alleles.

19.8.9.14.6 Because all loci being analyzed are assumed to be in linkage equilibrium, the product rule can be utilized to calculate an overall estimate of the profile frequency within the general population. For calculating the frequency of the entire profile, the individual calculations for each locus included in statistical calculations are multiplied together, and the inverse of this calculation is taken to provide the overall frequency as described below:

$$Overall\ Profile\ Frequency = {}^{1}\Big/{}_{\left(\prod_{i=1}^{n} P_i\right)}$$

or RMP=1/$f_{requency\ of\ profile}$

19.8.9.15 The OSBI FBU uses an Excel spread sheet that is titled OSBI STATS, to calculate the statistics for forensic questioned profiles. The allele frequencies used for the calculations in OSBI STATS version 2.2 are obtained from the STRbase website using the NIST-US1036, 2017 revised database. Previous versions of OSBI STATS utilized the FBI's PopStats allele frequency database for loci used except D2S1338 and D19S433 (which used the NIST database allele frequencies).

19.8.9.16 Taylor v State of Oklahoma (1995 OK CR 10) is a ruling of 1995 that established the admissibility of RFLP testing and established the necessity of including DNA statistic probabilities in order for juries to assess the significance of match evidence.

19.8.9.17 Wood v State of Oklahoma (1998 OK CR 19) is a ruling of 1998 that established the admissibility of PCR testing with a reiteration that DNA statistic probabilities are necessary to the admission of DNA evidence.

19.8.9.18 The OSBI FBU calculates statistics for Caucasians, African Americans, and Southwest Hispanics. These groups represent those largest racial groups/populations in the State of Oklahoma. Only the most conservative

statistics of these three populations will be reported. The race of the defendant has no bearing on the reported statistics. In fact, by definition "random match probability" is determining the chance of randomly selecting an unrelated individual from a population having the same profile. The statistical number is independent of and secondary to the match statement. It is independent of the match statement because the purpose of the statistics calculation is to add meaning, value, weight, or significance to the match that has already occurred. It is secondary to the match statement because it only happens after a match has occurred.

19.8.10 Statistical calculations are covered in FBU Policy Manual CASE_6 DNA Statistics.

19.9 Tasks

19.9.1 Observe a qualified Analyst perform all phases of the DNA analysis process (extraction, quantitation, amplification, genetic analysis, data interpretation and statistical calculations). The trainee may observe as many analysts as they feel necessary to become comfortable with the processes.

19.9.2 Complete Assignment 6 DNA Analysis of Single Source samples, Assignment 7 Globalfiler Express Analysis, and Assignment 8 DNA Analysis of Mixture Samples. ** Technicians complete Technician Assignment 3 DNA Quantitation Assistance and/or Technician Assignment 5 DNA Analysis Assistance, as applicable.*

19.9.3 Take the associated quizzes for the DNA analysis processes.

19.9.4 Review the above sections.

19.10 Evaluation

19.10.1 Successfully pass all quizzes.

19.10.2 The successful completion of the above tasks.

19.10.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

19.10.4 **Technician Mock Trial Assignment*

19.10.4.1 **Following the successfully completion of the technician competency test for a given sample set (in conjunction with a qualified analyst), the trainee will complete an informal mock trial evaluation with the assigned trainer and/or Technical Manager. The technician will be released for technician duties in casework by Memorandum from the Technical Manager after successful completion of this assignment.*

20 Mixture Interpretation

20.1 Goals

20.1.1 To allow the trainee to merge the concepts of mixture interpretation and statistical analysis into a report format.

20.1.2 To ensure the trainee understands how to interpret and report different types of mixtures routinely encountered in casework.

20.2 Tasks

20.2.1 The trainee will be provided with ten example DNA mixture electropherograms that they will be required to interpret according to the interpretation guidelines in FBU Policy Manual CASE_5 DNA Interpretation & Comparison Guidelines. The trainee will compile the data into a table and report the results in a semi-narrative format with the appropriate statistics.

20.2.2 This will give the trainee experience in interpreting simple and complex mixtures that will be routinely encountered in casework.

20.2.3 Complete DNA Assignment 9 DNA Interpretations, Comparisons, and Statistics.

20.2.4 Take the quiz covering interpretation and statistics.

20.3 Evaluation

20.3.1 Successfully pass the quiz.

20.3.2 The successful completion of the above tasks.

20.3.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

21 Y-STR (Male) DNA Analysis

21.1 Goals

21.1.1 To introduce the trainee to the theory behind Y-STR DNA analysis.

21.1.2 To introduce the trainee to the techniques utilized in the OSBI Forensic Biology Unit to successfully recover, isolate, quantitate, amplify, analyze (capillary electrophoresis (CE)), and interpret Y-STR DNA profiles.

21.1.3 To ensure the trainee understands the criteria dictating whether or not Y-STR analysis may be beneficial to a specific case.

21.1.4 To ensure the trainee understands the limitations of Y-STR analysis.

21.2 Introduction

21.2.1 Autosomal DNA markers such as STRs are shuffled with each generation because half of an individual's genetic information comes from his/her father and half from his/her mother.

21.2.2 The Y chromosome and mitochondrial DNA (mtDNA) markers represent lineage markers. These are passed down from generation to generation without changing, except for mutational events.

21.2.3 Maternal lineages can be traced with mtDNA sequence information, while paternal lineages can be followed with Y chromosome markers.

21.2.4 With lineage markers, the genetic information from each marker is referred to as a haplotype rather than a genotype because there is usually only a single allele per individual.

21.2.5 Because Y chromosome markers are linked on the same chromosome and are not shuffled with each generation, the statistical calculations for a RMP cannot involve the product rule.

21.2.6 Haplotypes from lineage markers can never be as effective in differentiating between two individuals as genotypes from autosomal markers that are unlinked and segregate separately from generation to generation.

21.2.7 Y chromosome DNA testing is important for several different applications of human genetics including forensic evidence examination, paternity testing, historical investigations, studying human migration patterns throughout history, and genealogical research.

21.2.8 The primary value of Y chromosome testing in forensic DNA is that it is found only in males. The SRY (sex-determining region of the Y) gene determines maleness. This can be very useful in sexual assault cases where the vast majority of the cases involve males.

21.2.9 Benefits of performing Y-STR testing:

21.2.9.1 Interpretable results can be obtained in some cases where autosomal tests are limited by the evidence, such as high levels of female DNA in the presence of minor amounts of male DNA.

21.2.9.2 These situations include sexual assault evidence from azoospermic or vasectomized males and blood-blood or saliva-blood mixtures where the absence of sperm prevents a successful differential extraction for isolation of male DNA.

21.2.9.3 Can be used to determine the number of individuals in a sexual assault with multiple assailants since it may be easier to decipher Y chromosome results than with highly complicated autosomal STR mixtures. It can be helpful in determining the number of male assailants.

21.2.9.4 It can improve the chances of detecting low levels of perpetrator's DNA in a high background of female victim's DNA.

21.2.9.5 The Y chromosome test can be used to verify Amelogenin Y-deficient males.

21.2.10 Limitations of Y-STR testing:

21.2.10.1 A majority of the Y chromosome is transferred directly from father to son without recombination to shuffle its genes and provide greater genetic variety to future generations. Random mutations are the only mechanisms for variation over time between paternally related males.

21.2.10.2 Therefore, while exclusions in Y chromosome DNA testing results can aid forensic investigations, a match between a suspect and evidence only means that the individual in question could have contributed the forensic stain-as could a brother, father, son, paternal uncle, paternal cousin, or even a distant cousin from his paternal lineage.

21.2.10.3 Needless, to say, inclusions with Y chromosome testing are not as meaningful as autosomal matches from a random match probability point-of-view.

21.2.11 The number of relatives sharing the same paternal lineage expands the number of possible reference samples in missing persons investigations and mass disaster victim identification efforts.

21.2.12 The Y chromosome is a popular tool for tracing historical human migration patterns through male lineages. Anthropological, historical, and genealogical questions can be answered through the Y chromosome results.

21.2.13 The Y Chromosome Structure

21.2.13.1 The Y chromosome is the third smallest human chromosome – only slightly larger than chromosome 21 and 22.

21.2.13.2 The tips of the Y chromosome called pseudoautosomal regions (PAR) recombine with their sister sex X chromosome homologous regions.

21.2.13.3 The remainder of the Y chromosome is known as the non-recombining portion of the Y chromosome (NRY). The NRY remains the same from father to son unless a mutation occurs.

21.2.13.4 Due to duplicated, palindromic regions of the Y chromosome, some Y-STR loci occur more than once and, when amplified with a locus-specific set of primers, produce more than one PCR product.

21.2.13.5 This duplication can lead to some confusion in terms of counting the number of loci present in a haplotype. A single set of primers can produce two amplicons, which may be thought of as "two loci" for a Y chromosome haplotype.

21.2.13.6 Y-STR Yfiler Plus loci DYS385 and DYF387S1 are present in two regions along the long arm of the Y chromosome. For DYS385, these duplicated regions are located about 40,000 bp apart and can generate two different alleles when amplified with a single set of primers. The two alleles are typically labeled "a" and "b" with the "a" designation going to the smaller-sized allele. It is also possible to have both "a" and "b" alleles be the same size in which case only a single peak would appear in an electropherogram.

21.2.13.7 Two PCR products can also be generated at the DYS389 locus with a single set of primers. However, in this case the DYS389I PCR product is a subset of the DYS389II amplicon because the forward PCR primer binds to the flanking region of two different repeat regions that are approximately 120 bp apart.

21.3 Applied Biosystems Yfiler Plus PCR Amplification Kit

21.3.1 The same basic theory of STR analysis applies to Yfiler Plus Y-STR analysis.

21.3.2 Components of the Yfiler Plus Amplification Kit

21.3.2.1 Master Mix: The components necessary to carry out the reaction (buffer, Sodium Azide, $MgCl_2$, BSA, dNTPs).

21.3.2.2 Primers: The forward and reverse primers to amplify the human male DNA target.

21.3.2.3 Allelic Ladder: Ladder for Yfiler Plus

21.3.2.4 Positive Control: 007 (male positive control)

21.3.2.4.1 For YFP PCR Amplification of the Positive Control (007), the stock Positive Control (2ng/µl) must be prepared in a 1:20 dilution

21.3.3 The loci that are amplified in the Yfiler Plus PCR Amplification Kit with the associated dye colors.

6-FAM (Blue): DYS576, DYS389I, DYS635, DYS389II, DYS627
VIC (Green): DYS460, DYS458, DYS19, YGATAH4, DYS448, DYS391
NED (Yellow): DYS456, DYS390, DYS438, DYS392, DYS518
TAZ (Red): DYS570, DYS437, DYS385, DYS449
SID (Purple): DYS393, DYS439, DYS481, DYF387S1, DYS533
LIZ (Orange): GS-600 v2.0 ILS

21.3.4 The base pair repeats of the loci for the Yfiler Plus Amplification Kit

DYS392 and DYS481 are tri-nucleotide repeats (3 base pair repeats)
DYS576, DYS389I, DYS635, DYS389I/II, DYS627, DYS460, DYS458, DYS19, Y_GATA_H4, DYS391, DYS456, DYS390, DYS518, DYS570, DYS437, DYS385 a/b, DYS449, DYS393, DYS439, DYF387S1, and DYS533 are tetra-nucleotide repeats (4 base pairs)
DYS348 is a penta-nucleotide repeat (5 base pair repeats)
DYS448 is a hexa-nucleotide repeat (6 base pair repeats)

21.3.5 There are eleven loci that are eligible for the CODIS database
DYS393, DYS19, DYS391, DYS439, DYS389 I/II, DYS438, DYS385 a/b, DYS390, DYS392

21.3.6 The thermal cycling parameters for the Yfiler Plus Y-STR Amplification Kit is as follows:

21.3.6.1 95˚ C for 1 minute (activate the Taq Polymerase)

94˚ C for 4 seconds (denature)
61.5˚ C for 1 minute (anneal/extend)] 30 cycles

60˚ C for 22 min (-A nucleotide addition (adenylation))
4˚ C for infinite amount of time

21.3.7 The FBU policy that covers Y-STR PCR amplification is FBU Policy Manual DNA_7 DNA Amplification Using Yfiler Plus.

21.4 Yfiler Plus Direct Mode (YFPdm) PCR Amplification Kit

21.4.1 The same basic theory of STR direct amplification (GFE) applies to Yfiler Plus Direct Mode (YFPdm) Y-STR analysis. The same commercially produced Applied Biosystems Yfiler Plus Amplification Kit is utilized for YFPdm amplification.

21.4.2 Components of the Yfiler Plus Amplification Kit

21.4.2.1 Master Mix: The components necessary to carry out the reaction (buffer, Sodium Azide, $MgCl_2$, BSA, dNTPs).

21.4.2.2 Primers: The forward and reverse primers to amplify the human male DNA target.

21.4.2.3 Allelic Ladder: Ladder for Yfiler Plus

21.4.2.4 Positive Control: 007 (male positive control)

21.3.2.4.1 For YFP PCR Amplification of the Positive Control (007), the stock Positive Control (2ng/µL) must be prepared in a 1:20 dilution

21.4.3 The thermal cycling parameters for direct amplification using the Yfiler Plus Y-STR Amplification Kit are as follows:

21.4.3.1 95˚ C for 1 minute (activate the Taq Polymerase)

94˚ C for 4 seconds (denature)
61.5˚ C for 1 min (anneal/extend)] 28 cycles

60˚ C for 22 min (-A nucleotide addition (adenylation))
4˚ C for infinite amount of time

21.4.4 The FBU policy that covers Y-STR PCR direct amplification is FBU Policy Manual DNA_9 DNA Amplification Using Yfiler Plus in Direct Mode.

21.5 Y-STR Interpretation

21.5.1 There are three possible interpretations resulting from comparing Y-STR haplotypes:

21.5.1.1 Excluded: The Y-STR profiles from the Q and K do not match.

21.5.1.2 Inconclusive: There is insufficient data to render an interpretation or ambiguous results were obtained (i.e., indistinguishable mixture).

21.5.1.3 Matches: The Y-STR profiles from the Q and K are the same and could have originated from the same source. This means that the source of the sample and all of their paternal male relatives cannot be excluded from the sample.

21.5.1.4 Generally samples with less than 100 pg of DNA (0.100 ng) are low-level and caution should be used when interpreting due to the potential for stochastic effects, such as drop-out, drop-in, and allele imbalance.

21.5.2 Common Artifacts Associated with the Yfiler Plus Amplification Kit

21.5.2.1 Peak in the TAZ (red) dye at ~412-413 bp when an excess of female DNA is present in the sample.

21.5.2.2 Stutter: The common n-4 stutter is seen, but there is also an n-2 stutter at DYS19, DYS481, DYS533, and DYS627. DYS392 and DYS481 are trinucleotides where n-3 and n+3 stutter is commonly seen. There is also more plus stutter observed in this kit than in the GlobalFiler kit.

21.5.2.3 At DYS391, a reproducible artifact is present at -10bp – it is generally ~1-1.5% of the main allele peak. An artifact at -5bp of the main allele peak is also possible. Additionally, in the OSBI internal validation for Yfiler Plus, DYS392 had artifacts at +6bp, DYS437 had two artifacts at -5bp and -8bp, and DYF387S1 had artifacts at +4bp.

In the Positive Control (007), in the blue dye (FAM), there is sometimes an artifact that is called as a “17” peak.

There are some artifacts that are observed in the non-calling regions: In blue at approximately 90-91bp, in green at approximately 70bp and 234-235bp, in purple at approximately 213-214bp, and in yellow at approximately 91-96bp, 229-230bp, and 234-235bp.

21.5.2.4 The typical artifacts that are seen in the GlobalFiler kit are also seen in this kit including pull-up, minus A, spikes, etc.

21.5.2.4.1 Minus A was observed at DYS19, DYS389I, DYS438, and DYS439 in the internal validation when greater than 0.5 ng total input DNA was amplified.

21.5.2.5 In 2020, Life Technologies released a Technical Note titled “Artifacts Identified Post-Developmental Validation: Yfiler Plus PCR Amplification Kit”. In communication with Life Technologies, they communicated this will be a “living document” and will be updated and provided to the forensic DNA community periodically. Analysts observing the artifacts described in this Technical Note (and in future revisions of the Technical Note) will reference the Life Technologies Technical Note in their case file technical notes and rename the artifact “ART” on the electropherogram

21.5.3 The FBU policy that covers interpretation of Y-STR profiles is FBU Policy Manual CASE_5 DNA Interpretation & Comparison Guidelines.

21.6 Y-STR Statistics

21.6.1 To calculate Y-STR statistics the counting method is utilized. The profile is entered into the US Y-STR Database and displays how many times the profile has been observed out of how many samples are in the database.

21.6.2 The database is located at the following location: www.yhrd.org

21.6.3 The statistic reported is how many times the haplotype was observed for the total number of individuals in the database (i.e., 1 of 32594).

21.6.4 The FBU policy that covers Y-STR statistics is FBU Policy Manual CASE_6 DNA Statistics.

21.7 Tasks

21.7.1 Complete DNA Assignment 10 Male Specific Y-STR DNA Analysis and Assignment 11 Yfiler Plus Direct Mode DNA Analysis.
**Technicians complete Technician Assignment 5, as applicable.*

21.7.2 Take the quiz on male specific Y-STR analysis.

21.7.3 Review the above sections.

21.8 Evaluation

21.8.1 Successfully pass the quiz.

21.8.2 The successfµcompletion of the above tasks.

21.8.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

22 Family Relatedness Comparisons and Statistics

22.1 Goals

22.1.1 To introduce the trainee to family relatedness comparisons.

22.1.2 To introduce the trainee to family relatedness statistics.

22.1.3 To introduce the trainee to the theory behind the family relatedness statistics and how they are performed at the OSBI FBU.

22.1.4 To give the trainee a brief introduction to missing persons and mass disaster identifications.

22.2 General Information

22.2.1 Typical paternity cases involve the mother, the child, and one or more alleged fathers.

22.2.2 The determination of parentage is made based on whether or not alleles are shared between the child and the alleged father when a number of genetic markers are examined. Thus, the outcome of parentage testing is simply inclusion or exclusion.

22.2.3 The same amplification kits are used and the source of non-maternal or “obligate paternal allele” at each genetic locus is under investigation.

22.2.4 The basis of paternity comes down to the fact that, in the absence of a mutation, a child receives one allele matching each parent at every genetic locus.

22.2.5 The most commonly applied statistical test is the paternity index. The paternity index (PI) is the ratio of two conditional probabilities where the numerator assumes paternity and the denominator assumes a random man was the father.

22.2.6 The numerator is the probability of observed genotypes, given the tested man is the father, while the denominator is the probability of the observed genotypes, given that a random man is the father.

22.2.7 The paternity index then is the likelihood ratio of two probabilities conditional upon different competing hypotheses.

22.2.8 The likelihood ratio reflects how many times more likely it is to see the evidence (i.e., a particular set of alleles) under the first hypothesis compared to the second hypothesis.

22.2.9 When mating is random, the probability that the untested alternative father will transmit a specific allele to his child is equal to the allele frequency of the specific allele in consideration.

22.2.10 The PI is generally represented in the formula X/Y, where X is the chance that the alleged father (AF) could transmit the obligate allele and Y is the chance that some other man could have transmitted the allele. Typically X is assigned the value of 1 if the AF is homozygous for the allele of interest and 0.5 if the AF is heterozygous.

22.2.11 The PI is calculated for each locus and then individual PI values are multiplied together to obtain the combined paternity index (CPI) for the entire set of genetic loci examined. The generally accepted minimum standard for an inclusion of paternity is a PI of 100 or

greater. A PI of 100 correlates to the probability that the alleged father has a 99 to 1 chance of being the father compared to a random man.

22.2.12 The OSBI FBU uses the OSBI STATS spreadsheet to calculate the paternity statistics. The FBU procedure for Family Relatedness Statistics is covered under FBU Policy Manual CASE_6 DNA Statistics.

22.2.13 The following is an example of Paternity Calculation:

22.14.1 Genotypes present at the STR locus D13S317
M: 8, 12 C: 12, 14 AF: 11, 14
Allele Frequencies: 8=0.113, 12= 0.248, 14=0.048, 11= 0.339
$PI=1/2f_{14}$ ⟶ 1/(2x0.048)=10.4

Because the child and AF share allele 14, the frequency of this allele is used to determine the PI calculations for this STR marker. The results of the DNA testing using a single STR locus are 10.4 times more likely it the tested man is the biological father of the child than if the biological father is another man, unrelated to the tested man.
By multiplying the various PI values for each STR maker together, the CPI might be greater than 100,000 with 14 or 15 STRs examined. The probability of parentage is calculated by using the CPI values: [CPI/(CPI+1)] x 100%; therefore with a CPI of 100,000 the probability of paternity would be 99.9999%.

22.3 Impact of mutational events

22.3.1 Since parentage and kinship testing involve measuring genetic relationships across generations, mutations that may occur must be taken into account.

22.3.2 These mutations are germ-line mutation in that they occur in either the father's sperm or mother's egg cell and are passed on during zygote formation. Somatic mutations occur within different cells or tissues coming from the same individual, such as with cancerous tumors.

22.3.3 It is important because an exact match cannot be made when a mutation is present. Mutation rates for genetic markers are typically measured through analysis of many parent-offspring allele comparisons.

22.3.4 In paternity testing, a single mutation event is allowable and the OSBI STATS spreadsheet allows for two mutations. The entry of two mutational events will be flagged and may only be reported with the approval of the Technical Manager.

22.3.5 The equation for a mutation event is $LR = \mu/P_{ex}$; where μ is the mutation frequency and P_{ex} is the probability of exclusion.

22.4 Reverse Parentage Testing

22.4.1 In some cases the question under consideration may be whether or not a child belongs to the mother and father tested or other biological references tested.

22.4.2 This is essentially the opposite question as that asked in parentage testing; namely, given a child's genotype, who are the parents?

22.4.3 Sometimes it is possible to have the alleged mother, alleged father, and child references available, but typically a single parent or sibling samples are the only ones available, which make the reverse parentage analysis more challenging.

22.4.4 An example of this in casework would be if a bone was found and the agency is trying to identify the bone to an alleged mother or father.

22.5 Mass Disaster and Missing Persons Investigations

22.5.1 Generally military causalities are identified by the Defense Forensic Science Center (DFSC) with its subordinate unit being the U.S. Army Criminal Investigation Laboratory (USACIL) – formerly the Armed Forces DNA Identification Laboratory (AFDIL) – and airplane crashes are examined by National Traffic Safety Board (NTSB), which often contracts with USACIL to identify the air crash victims through DNA testing as part of the investigation.

22.5.2 Mass disaster DNA involves the identification of the individual pieces recovered at the scene and performing DNA analysis on these pieces and then comparing them to known reference samples (either secondary reference samples (i.e., toothbrush, hairs, etc.) or kinship comparisons).

22.5.3 There are three categories of samples associated with missing persons cases: direct reference samples, family reference samples, and unidentified human remains.

22.5.4 The unidentified human remains are generally skeletal remains, teeth, or tissue. The direct reference samples are medical samples from the missing individual such as a biopsy sample. Family reference samples can be buccal swabs from close biological relatives, such as parents, children, or siblings of the missing individual.

22.5.5 DNA databases can play important role in helping identify missing individuals over time. When a family member goes missing, DNA samples can be obtained from direct reference samples or biological relatives. DNA profiles from these samples would then be uploaded to the database and searched against DNA profiles from unidentified human remains in an effort to make an association to a missing individual.

22.5.6 The NDIS database contains a Missing Persons Index.

22.5.7 In an effort to help make connections between family members and their missing relatives, in 2007 a National Missing and Unidentified Persons System (NamUs) web site was developed.

22.6 Example calculations for paternity testing:

22.6.1 Assume that a 50% prior probability and calculate the LR for each locus and the CPI, and the posterior probability of parentage.

Locus	M	C	AF	Allele Frequencies
D8S1179	12, 13	12, 15	13, 15	15=0.1097
D21S11	29	29, 31	30, 31	31= 0.0714
D7S820	8	8	8	8=0.1626
D16S539	10, 12	10, 12	11, 12	10= 0.0668; 12=0.3391

L.R. D8 = (0.5_{M12}) * (0.5_{AF15}) / (0.1097) (0.5_{M12}) = 4.55

L.R. D7 = (1.0_{M8}) * (1.0_{AF8}) / (1.0_{M8}) (0.1626) = 6.15

L.R. D21 = (1.0_{M29}) * (0.5_{AF31}) / (1.0_{M29}) (0.0714) = 7.00

L.R. D16 = (0.5_{M10}) * (0.5_{AF12}) / 0.5 $(0.0668_{RM10} + 0.3391_{RM12})$ = 1.23

Combined L.R. = (4.55) * (6.15) * (7.00) * (1.23) = 240.9293

Prob Post = $(Prob_{Prior})$ * (Combined L.R.) / $(Prob_{Prior}$ (Combined L.R.) + $(Prob_{Prior})$

= (0.5) (240.9293)/((0.5)(240.9293) + 0.5) = 0.99586 x 100% = 99.59%

22.6.2 There was an inconsistent result encountered at D8S1179 locus. If the combined L.R. for all the non-excluding systems is 1,000,000 what is the likelihood ratio when the inconsistent result is included? The mutation frequency for D8S1179 is 0.002 and the probability of exclusion is 0.55.

Locus	M	C	AF	Allele Frequencies
D8S1179	12, 13	12, 15	13, 15	15=0.1097

L.R= μ/P_{ex} ⟶ 0.002/0.55 = 0.00364

$LR_{Combined}$ = 1,000,000 x 0.00364 = 3636.34

$Prob_{Post}$ = (0.5) (3636.34) / ((0.5) (3636.34) + 0.5) = 1818.17/1818.67 = 0.99973 x 100% = 99.97%

22.7 Tasks

22.7.1 Complete DNA Assignment 12 Family Relatedness Comparisons and Statistics.

22.7.2 Complete the exercise on Family Relatedness Calculations.

22.7.3 Take the quiz on Family Relatedness Comparisons and Statistics.

22.7.4 Review the above sections.

22.8 Evaluation

22.8.1 Successfully pass the quiz.

22.8.2 The successful completion of the above tasks.

22.8.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

23 Casework Approach and Documentation for DNA Case Files

23.1 Goals

23.1.1 To become familiar with documentation of DNA cases in the Analysts' notes.

23.1.2 To become familiar with the format and report wording of Analysts within the Forensic Biology Units.

23.2 Digital Photography

23.2.1 In several cases it may be necessary to take photos of the item of evidence to help describe the evidence or document certain conditions of the evidence. In these instances, the photo is not considered part of the evidence and does not need to be handled in any specific way.

23.2.2 Typically these types of photos that document the evidence are either included in the case record by inserting the picture into the BEAST Image Vault. If the Analyst wishes to put the picture in their notes, they can add it to the BEAST worksheet as well. Photos added to the BEAST Image Vault must bear the case number and Item number in the file name.

23.2.3 In cases where the evidence is being consumed, it is required to photograph the evidence before consumption. For example, if consuming a swab, a photo will be taken of the swab prior to taking the cutting, ensuring that the swab staining can be seen in the photograph. In this case, at minimum, the case number, item number, and description of the evidence should be in the file name. These photos should be available in the BEAST Image Vault.

23.2.4 Every attempt should be made when photographing evidence to include the case number, item number, date, analyst's initials, and a scale in the photo. Some exceptions to this requirement are when taking photos of hairs on the microscope at magnification and close up pictures of particular staining, etc.

23.3 General Guidelines for Documentation of Evidence in DNA Notes

23.3.1 All of the information in the serology section (section 13) applies to the DNA notes and the following are the specific requirements for DNA case notes.

23.3.2 A description of the approximate amount of material that is used for DNA testing needs to be noted, so that one can extrapolate the remaining amount of sample from the notes.

23.3.3 The following DNA worksheets should be included in the case file if they are used: DNA extraction sheet, DNA lot number sheet, DNA quantitation sheet, DNA amplification setup sheet(s), and the 3500 setup sheet.

23.3.4 All of the injections need to be accounted for as well as a verification of all parameters, size standards, and ladders. This can be documented on a "Technical Notes" worksheet or documented in another location in the case file. The technical notes should be used to explain any deviations from protocol, any occurrences resulting in re-injections, or any additional analysis and to document reasons for decisions about interpretations or reasons why any further analysis was/was not performed.

23.3.5 The common known artifacts (pull up, spikes, stutter, etc.) do not require any further documentation other than noting them in the technical notes, but if there are any

uncommon artifacts, a “GeneScan” view (zoomed in) of the electropherogram should be included in the case file.

23.3.6 The GMID-X projects are to contain all injections of samples and controls for each batch/case. The raw data is available for each injection so it is retained with each sample/control in the GMID-X project. At a minimum all the electropherograms that are being reported and the associated controls should be “printed” (electronically using Adobe) and included in the case file.

23.3.7 If a sample needs to be re-injected, re-setup, re-amplified, or re-extracted it should be noted on the electropherogram so that the reviewer can clearly determine where the sample came from and in what order it was generated.

23.3.8 The QuantStudio 5 quantitation data, 3500 run folder, and GeneMapper ID-X project should be available for technical review. This data is stored on the “DNA-Server” on the network under the analyst’s initials. It is at the following location: \\pm-fsc16273s\DNA_Data

23.3.9 A DNA results table must be included in the case file and the DNA profiles can be reported as phenotypes or genotypes depending on the sample. In the table, the homozygote alleles that are below the stochastic threshold need to be documented in some way (i.e., bolding and changing the font color to red for alleles that are below the stochastic threshold). A key should be included with the table denoting all symbols that are on the table.

23.3.10 The DNA profiles should not be e-mailed outside the OSBI e-mail system. NDIS regulations and requirements state that no DNA profiles will be transmitted outside a secure network. The OSBI’s IT department state that as long as e-mails are within the OSBI e-mail system that the transmissions are secure. DNA profiles are not to be transmitted outside the OSBI’s network for security reasons and NDS requirements.

23.3.11 **If a technician is utilized in casework, the analyst must clearly identify what the technician did and who the technician is; this is accomplished by putting the technician’s initials or name on the appropriate worksheet and having them initial this section - either print off the notes and initial by hand or with the electronic initials in PDF. If an electronic version is used, it should be uploaded to the documents tab in the BEAST.*

23.4 General Guidelines for DNA Report Writing

23.4.1 All the general guidelines from section 13 above apply here as well; the following are specific to DNA reports.

23.4.2 DNA reports will have qualitative or quantitative interpretive statements, technology used (STR, Y-STR), the amplification system or loci used (GlobalFiler, Yfiler Plus, etc.), and a disposition of the evidence (returned to requesting agency, transferred to another unit, etc.).

23.4.3 The DNA report will have a statement that states what kit was used of analysis. Typically, the statement will read something like this “Deoxyribonucleic acid (DNA) was isolated from the following items and characterized through the polymerase chain reaction (PCR) at the short tandem repeat (STR) GlobalFiler [*or male-specific short tandem repeat (Y-STR) Yfiler Plus*] PCR amplification kit loci.”

23.4.4 Results or conclusions will be provided for every sample that DNA was attempted on and all known reference samples will be compared to all forensic samples in the case.

23.4.5 All inclusions will have a statistical statement with them. Statistics are not appropriate for exclusions. If the sample is an intimate sample (i.e., swabs from the body, fingernails, etc.) no statistical statement needs to be provided; however, a statement indicating that due to the intimate nature of the sample it is presumed to be from the person will be included with the inclusion statement.

23.5 Criminalistics Statistics

23.5.1 Every case the Analyst works, will require them to track how many items were submitted, the time it took to work the case, and the number of examinations for the case.

23.5.2 This is recorded in the notes tab of the BEAST.

23.5.3 The Forensic Biology Unit utilizes an Excel spreadsheet that will assist in calculating the number of examinations. This Excel spreadsheet is "Biology_Number_of_Exams_Stats_Calculator_v1.0 and is located at: \\pm-fsc16273s\Biology

23.5.4 The Analyst can maintain their own personal spreadsheet to track the cases they have worked.

23.5.5 In the notes section, the Analyst will indicate what type of case it is by putting a 1 in the block for doing this type of case and a 0 if this type of case was not done in this instance (serology, DNA, Y-STR).

23.6 Report Wording

23.6.1 The report wording is covered in CASE_7 Report Wording of the FBU Policy Manual.

23.6.2 Any testing performed must be reported.

23.6.3 The wording described in the protocol is a guideline for the report wording and does not have to be followed exactly; however, every attempt should be made to keep the wording in the report consistent with other analysts, so as to limit the confusion to the customer when reading reports from multiple analysts.

23.6.4 Every case is different and there is not a set report wording for every circumstance.

23.7 Tasks

23.7.1 The trainee will review at least 5 cases from different analysts to become familiar with how analysts put their DNA case files together. An attempt should be made to review different types of DNA cases, i.e., blood, touch, sexual assault, etc.

23.7.2 Read FBU Policy Manual CWQM_11 Reports, CASE_1 Case Notes, CASE_2 Photography, CASE_4 DNA Analysis, CASE_7 Report Wording, CASE_8 Retention of Samples, and OSBI Quality Manual QP 28 Report Writing and QP 29 Criminalistics Statistics.

23.8 Evaluation

23.8.1 The successful completion of the above tasks.

23.8.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

24 Administrative and Technical Review Training for DNA Analysis

24.1 Goals

24.1.1 To introduce the trainee to the requirements for reviewing a DNA case file.

24.1.2 Allow the trainee to become familiar with the administrative and technical review process.

24.2 General Information

24.2.1 Administrative and Technical Reviews are governed by QP 31 Reviews and CWQM_12 Review.

24.2.2 The review process does not shift the responsibility of the Analyst's findings to the reviewer; each Analyst is responsible for their own work.

24.2.3 Each Analyst should review their own cases thoroughly before submitting the case for review.

24.2.4 When the Analyst has reviewed their case, they will select the sign button in the BEAST, then route the case assignment to the person reviewing the case and put in the appropriate designation code (i.e.,, TR).

24.2.5 Administrative and technical reviews can be done separately by different reviewers or at the same time. Typically, in the FBU, one reviewer will do both the administrative and technical reviews. The list of qualified individuals to perform these reviews is maintained by the Technical Manger and is available on the server.

24.2.6 When the reviewer has completed the review, they will route the case back for any corrections as necessary by using the routing tab and putting the designation as for corrections (C). If there are no corrections, it will be routed for approval by putting in the appropriate designator (RA).

24.2.7 When the necessary corrections are made, the analyst will again sign the report and route it for review; once the reviewing Analyst has verified all corrections are made, they will route it back for approval. At this point the analyst can "approve" the case, which will close the assignment and affix the Analyst's signature to the report.

24.3 Administrative Reviews

24.3.1 An administrative review is an evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness.

24.3.2 General Guidelines for Administrative Reviews

24.3.2.1 Review the report for spelling and grammatical accuracy.

24.3.2.2 Ensure that any hardcopies in the case file have the Analyst's initials and case number on them.

24.3.2.3 Ensure the report has all the necessary information and sections that are required included in the report.

24.3.2.4 Ensure the original RFLE and BEAST information is accurate.

24.3.3 All DNA cases will be 100% administratively and technically reviewed by a qualified Analyst.

24.4 Technical Reviews

24.4.1 A technical review is an evaluation of reports, notes, data, and other documents to ensure there is an appropriate and sufficient basis for the scientific conclusions.

24.4.2 General Guidelines for Technical Reviews

24.4.2.1 Ensure that all lot numbers and expirations dates are properly documented.

24.4.2.2 Ensure that all dates of analysis are documented.

24.4.2.3 Ensure that the conclusions are supported by the data from the analysis of evidence.

24.4.2.4 Ensure that all appropriate testing was done as dictated by case circumstances and what was requested by submitting agency.

24.4.3 Completion of the casework technical review shall be documented and the review should include the following as a minimum:

24.4.3.1 All notes, worksheet, and data supporting the conclusions.

24.4.3.2 All DNA types to verify that they are supported by the raw or analyzed data.

24.4.3.3 All the profiles to verify that the correct inclusions and exclusions were made, as well as to ensure the profile was entered correctly in the OSBI STATS program for inclusions.

24.4.3.4 All controls, internal lane standards, and allelic ladders were verified that the expected results were obtained.

24.4.3.5 The report to verify that the results/conclusions are supported by the data.

24.4.3.6 Verification that all profiles that are going to be entered into CODIS are eligible and have the correct DNA types and specimen categories.

24.4.3.7 The profiles being entered into CODIS will be documented on CODIS Data Entry Form and this should be reviewed to ensure the profile and all the information is correct and accurate.

24.4.3.8 A review of the chain of custody will be conducted by verifying the items' location, submittal dates, and where it has traveled since being submitted to the lab, in the LIMS.

24.5 Tasks

24.5.1 Read QP 31 Reviews and CWQM_12 Review.

24.5.2 The trainer or designee will give the trainee a DNA case they are reviewing from another analyst, after they initially review it and without telling the trainee what corrections were found; the trainee will review it and note any corrections they may notice. This will be done with at least 4 DNA cases, and will be up to the trainer and Technical Manager if more cases should be completed.

24.5.3 Upon being released to perform independent casework in DNA, the trainee will be given cases from other Analysts to review. The trainee will review these cases and will then give the case to the Technical Manger to review, with their comments. The Technical Manager will determine if there are any problems with the review. If, after a number of cases determined by the Technical Manager, there are no problems, the trainee will be released to perform independent reviews. If there are problems, the Technical Manager will develop a plan to remediate the cause of the problem.

24.6 Evaluation

24.6.1 The successful completion of tasks 24.5.1 - 24.5.3.

24.6.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the above task.

25 CODIS Overview

25.1 Goals

25.1.1 To introduce the trainee to the eligibility requirements for the various levels of CODIS.

25.1.2 To introduce the trainee to what a qualifying offense is for the state of Oklahoma to be entered into the CODIS database.

25.1.3 To introduce the trainee to the steps for entering a profile generated from casework into the database.

25.1.4 The trainee will be introduced to what happens to a casework profile once it is entered into the CODIS database.

25.1.5 The trainee will become familiar with filling out a CODIS Data Entry Form and when it is necessary to use the Match Estimator tool.

25.2 Combined DNA Index System (CODIS)

25.2.1 CODIS represents the software used to connect law enforcement laboratories housing U.S. DNA data at local, state, and national level in LDIS, SIDS, and NDIS, respectively.

25.2.2 These U.S. sites are all networked together on the CJIS SEN (Criminal Justice Information Systems Shared Enterprise Network), a standalone law enforcement computer network that operates in similar fashion to the internet. The software is the same at all sites with various configurations that permit different levels of access (LDIS, SIDS, or NDIS).

25.2.3 The CODIS software consists of four main subprograms: Specimen Manager handles the DNA profiles, Match Manager handles the candidate matches, Autosearcher conducts the database searches, and PopStats enables DNA profile probability calculations from population data.

25.2.4 Profiles in CODIS are assigned to a specimen category, and specimen categories are assigned to an index. The index a profile is assigned to determines what other indices the profile will be searched against.

25.2.5 The offender index and forensic index are the two largest groups of data. The offender index contains DNA profiles from individuals arrested for or convicted of a felony or certain misdemeanors. The forensic index DNA profiles come from crime scene evidence. The database helps link serial crimes and aids the investigation of an unsolved case.

25.2.5.1 The “offender index” refers to following indices collectively: convicted offender, detainee, arrestee, legal, multi-allelic offender (and any associated incomplete indices).

25.2.5.2 The “forensic index” refers to the following indices collectively: forensic, forensic mixture, forensic partial, forensic targeted (and any associated incomplete indices).

25.2.6 There are several missing person indices in NDIS that contain DNA profiles from missing persons, unidentified human remains, and biological relatives of missing persons that can be searched against one another to try to find a direct or kinship match.

25.2.7 Thirteen core CODIS loci were established so there would be common search criteria regardless of what amplification kits were used. The original 13 CODIS core loci were: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, CSF1PO. In 2017, the FBI expanded the CODIS core loci, adding D1S1656, D12S391, D2S441, D10S1248, D2S1338, D19S433, and D22S1045 to the original 13 core loci.

25.2.8 Since the thirteen core CODIS loci were selected in November of 1997, they have been required for data entry into the national level of the U.S. DNA database. The original 13 STR markers provide a random match probability (assuming unrelated individuals) of at least approximately 1 in 100 trillion. In 2017, the expanded core loci are required to be attempted for data entry into the national level of the U.S. DNA database. The expanded loci available in the GlobalFiler amplification kit provide a random match probability (assuming unrelated individuals) of at least approximately 1 in 7.12×10^{-26}.

25.3 Specimen Categories and Indices Maintained (from OSBI CODIS Policy Manual, Data Entry and Search Procedures) that a forensic DNA analyst routinely encounters.

25.3.1 Forensic, Unknown (Forensic Index)

25.3.1.1 These are single source DNA profiles with no locus or allelic dropout that are from evidence found at crime scenes. A major component of a DNA mixture may be entered as a Forensic, Unknown if there are no obligate alleles designated.

25.3.2 Forensic Partial (Forensic Partial Index)

25.3.2.1 These are single source DNA profiles that are from evidence found at crime scenes that have locus or allelic dropout at any of the core loci. Forensic partial profiles need to have at least 8 of the original 13 CODIS core loci (excluding Amelogenin). All of the core loci must have been attempted to be amplified. Loci where a partial profile is indicated shall be referenced with a "(p)" on the CODIS Data Entry Form. Casework analysts should keep in mind the reported inclusions and associated statistical values should a match occur to entries in this specimen category. If there would be no inclusion for any profile matching the specimen, the profile should not be entered into the CODIS database.

25.3.2.2 The forensic partial profiles need to satisfy the statistical threshold. The profile shall be reviewed to ensure that it meets a Moderate Match Estimate (MME) value of at least 1×10^7 (10,000,000) calculated without a mismatch.

25.3.3 INC. Forensic STR (Incomplete Forensic Index)

25.3.3.1 These are partial, single source DNA profiles with results at 8 or more of the original 13 CODIS core loci that do not meet the NDIS Moderate Match Estimate (MME) that are from evidence found at crime scenes.

25.3.3.2 INC. Forensic STR profiles shall be reviewed to ensure they satisfy a Moderate Match Estimate (MME) of 5×10^4 (50,000) (SDIS MME) when calculated with no mismatch.

25.3.3.3 The profiles in the Incomplete Forensic Index will be searched routinely against SDIS only and will not be uploaded to NDIS.

25.3.4 Forensic Mixture (Forensic Mixture Index)

25.3.4.1 These are DNA profiles from forensic samples that contain DNA contributed from more than one source. Major components of mixture DNA profiles with dropout or obligate alleles, minor components of mixture DNA profiles, or deduced profiles from an intimate sample are also stored in the forensic mixture index.

25.3.4.2 The samples must have at least 8 of the original 13 CODIS core loci, must not have more than 4 alleles at any locus, and shall meet a Moderate Match Estimate (MME) value of at least 1×10^7 (10,000,000) calculated without a mismatch.

25.3.5 INC. For. Mixture (Incomplete Forensic Mixture Index)

25.3.5.1 These are mixture profiles that have results from at least 8 of the original 13 CODIS core loci and do not meet the NDIS Moderate Match Estimate (MME). These cannot have more than 4 alleles at any one locus, and alleles which are unambiguously attributed to the victim shall not be included.

25.3.5.2 These samples must satisfy the SDIS Moderate Match Estimate (MME) of 5×10^4 (50,000) when calculated with ONE (1) mismatch.

25.3.5.3 The profiles in the Incomplete Forensic Mixture Index will be searched routinely against SDIS only and will not be uploaded to NDIS.

25.3.6 Forensic Targeted (Forensic Targeted Index)

25.3.6.1 These are forensic partial and forensic mixture profiles which do not meet the Moderate Match Estimate (MME) of 1×10^7 and may be NDIS eligible in the Forensic Targeted specimen category.

25.3.6.2 Profiles in the Forensic Targeted specimen are searched “stringency by locus” at NDIS. Loci marked partial and loci with more than 2 alleles will be searched at Moderate and all other loci are searched at High stringency. Profiles in the Forensic Targeted specimen category must have at least 8 of the original CODIS core loci and meet a Match Rarity Estimate (MRE) (stringency by locus) value of 1 in 10 million. Loci with only one allele that should be searched at Moderate Stringency shall be marked (p) on the CODIS Data Entry Form.

25.3.6.3 The Forensic Targeted Index consists of DNA profiles in the Forensic Targeted Specimen Category. Profiles maintained in the Forensic Targeted Index will be forwarded to the FBI for inclusion in NDIS.

25.3.7 SDIS Forensic Targeted (SDIS Targeted Index)

25.3.7.1 Some forensic partial and forensic mixture profiles which do not meet the NDIS Moderate Match Estimate (MME) of 1×10^7, the SDIS MME of 5×10^4, or the NDIS Match Rarity Estimate (MRE) of 1×10^7 may be SDIS eligible in the SDIS Forensic Targeted specimen category.

25.3.7.2 Profiles in the SDIS Forensic Targeted specimen category are searched “stringency by locus” at SDIS. Loci marked partial and loci with more

than 2 alleles will be searched at Moderate and all other loci are searched at High stringency. Profiles in the SDIS Forensic Targeted specimen category must have at least 8 of the original 13 CODIS core loci and meet a Match Rarity Estimate (MRE) (stringency by locus) value of 5×10^4 (50,000). Loci with only one allele that should be searched at Moderate Stringency shall be marked (p) on the CODIS Data Entry Form.

25.3.7.3 The SDIS Targeted Index consists of DNA profiles in the SDIS Forensic Targeted Specimen Category. Profiles maintained in the SDIS Targeted Index will not be forwarded to the FBI for inclusion in NDIS.

25.3.8 Missing Person/Deduced Missing Person (Missing Person Index)

25.3.8.1 A DNA profile from a sample that has been known to be collected from the missing person (medical specimens) is in the missing person specimen category.

25.3.8.2 A DNA profile obtained from an intimate sample that has been reported belonging to the missing person (toothbrush) is in the deduced missing person specimen category.

25.3.8.3 The profiles must be single source and consist of at least 7 of the 20 CODIS core loci **plus** Amelogenin, with a second technology (e.g., Y-STRs or mito) recommended. Any locus with known dropout (an allele visible below threshold) will not be used for entry. These profiles will be forwarded for inclusion into NDIS.

25.3.9 Relatives of Missing Person Index

25.3.9.1 These are DNA profiles from the known reference samples of biological relatives of the missing person.

25.3.9.2 The specimen categories in this index are: Biological Child, Biological Father, Biological Mother, Biological Sibling, Maternal Relative, and Paternal relative. These describe the relationship between the missing person and the relative.

25.3.9.3 The profiles must be single source and consist of all CODIS core loci plus Amelogenin. The profiles will be forwarded to the FBI for inclusion in NDIS.

25.3.10 Unidentified Person (Unidentified Human Remains (UHR) Index)

25.3.10.1 These are DNA profiles obtained from recovered deceased individuals or from an individual who is unidentified.

25.3.10.2 The profiles must be single source and consist of at least 1 of the 20 CODIS core loci **plus** Amelogenin. At least 7 or more of the original 13 CODIS core loci allows for more robust searching at NDIS. Loci with apparent dropout shall not be included in the profile. The profiles will be forwarded to the FBI for inclusion in NDIS.

25.3.11 Legal (Legal Index)

25.3.11.1 These are DNA profiles obtained from known suspects in criminal investigations. These should only be entered if the suspect is not already in the CODIS database.

25.3.11.2 Profiles will be searched routinely and uploaded to NDIS per approval from the NDIS custodian.

25.3.12 INC. Legal (Incomplete Legal Index)

25.3.12.1 These are DNA profiles that contain at least 8 or more of the original CODIS core loci, excluding Amelogenin, obtained from known suspects in criminal investigations.

25.3.12.2 The profiles will be searched routinely against SDIS only and will not be uploaded to NDIS.

25.3.13 Y-STR Forensic Unknown

25.3.13.1 DNA profiles from forensic samples consisting only of alleles derived from Y-STR genetic markers. Profiles must be single source or a full major component profile from a mixture.

25.3.13.2 Profiles must contain at least 24 of the 25 Y-STR loci in the Y-Filer Plus amplification kit.

25.3.13.3 Profiles will not be searched, nor will they be uploaded to NDIS.

25.3.14 Y-STR Legal

25.3.14.1 DNA profiles from known suspects consisting only of alleles derived from Y-STR genetic markers.

25.3.14.2 Profiles must contain results at all 25 Y-STR loci in the Y-Filer Plus amplification kit.

25.3.14.3 Profiles will not be searched, nor will they be uploaded to NDIS.

25.3.14.4 Profiles should only be entered into the Y-STR Legal category if an STR profile is in CODIS from an offender sample, or no STR profile was attempted on the known DNA profile.

25.3.15 For additional specimen categories and Indexes reference the CODIS Data Entry and Search Procedures section in the CODIS Policy Manual.

25.4 Qualifying Offenses

25.4.1 The convicted offender and arrestee qualifying offenses are in Oklahoma Statute Title 74 section 150.27a.

25.5 Determination of Forensic Unknown Samples for the CODIS Database

25.5.1 The determination of what is allowable into the CODIS database is based on case circumstances and is on a case-by-case basis.

25.5.2 Some of the information that you need to know includes:
from where the evidence was collected,
was it taken directly from the suspect,
was there a crime committed,
is there anyone else who could have left the biological evidence (i.e.,, does the victim smoke if there are cigarette butts or for touch DNA,
is it a common area that many people routinely touch), etc.?

25.5.3 Once this information is obtained then you need to follow the "A Guide to Determining What is Allowable in the Forensic Index at NDIS" flow sheet.

25.5.4 On a case-by-case basis, if you are still not sure if the item is admissible to CODIS, the Analyst should ask the CODIS Administrator for guidance on whether or not to submit the profile to CODIS.

25.6 Submission of the profile to the CODIS Unit

25.6.1 Once the determination is made on what sample(s) is/are submitted to CODIS, the CODIS Data Entry Form is filled out. The profile is entered into the form and the Specimen Category, Item Number, Description of Profile entered, etc. are filled in on the form. Once all applicable areas are filled out, the Analyst signs the form and it is submitted with the case file for Administrative and Technical review.

25.6.2 After the reviewer reviews with the case and agrees with the item(s) being entered into CODIS and ensures that the form is filled out properly, the form is signed and returned with the case to the Analyst.

25.6.3 Once the review is completed and prior to closing the case, the CODIS Data Entry Form is submitted the CODIS Unit, where they enter the profile into the CODIS database. After the profile is entered the CODIS Unit sends the form back to the Analyst. At this point the Analyst verifies that the correct profile was entered and signs the CODIS Data Entry Form and enters it into the Documents tab of the BEAST. At this point, the Analyst can "close" the case and reports made available, as appropriate. The Analyst will have stated in their report which profile was entered into the CODIS database.

25.7 CODIS Searches

25.7.1 Primary searches are conducted daily between the offender and forensic indices at both the state (SDIS) and national (NDIS) levels. New crime scene profiles being added to the forensic index are searched against the entire offender index and new offender profiles are searched against the entire forensic index. The new crime scene profiles are searched against the entire forensic index to help connect serial crimes. The arrestee index is treated like the offender index for these searches.

25.7.2 An offender or arrestee hit is produced when a match occurs between a DNA profile in the convicted offender or arrestee index against the crime scene DNA profiles in the forensic index during a search of offender or arrestee DNA profiles.

25.7.3 A forensic hit results from searching DNA profiles in the forensic index against other crime scene DNA profiles – essentially an effective method to look for crimes committed by a serial offender. This type of hit is sometimes referred to as a case-to-case hit.

25.7.4 Search Stringencies Used

25.7.4.1 Search algorithms have to take into account that DNA profiles in the forensic index come from challenging evidentiary material and may be mixtures or partial profiles.

25.7.4.2 In order not to miss the true connection between two DNA samples from the same source, search strategies must have the capability to permit two DNA profiles to "match" without 100% allele agreement.

25.7.4.3 There are three levels of search stringency possible: high, moderate, and low.

25.7.4.4 High stringency means all available alleles match in the two DNA profiles. High stringency is the standard search configuration for Forensic, Unknown profiles at NDIS (with one mismatch allowed).

25.7.4.5 Moderate stringency search requires all available alleles to match, but the two profiles can contain a different number of alleles (i.e., you have 9, 10, 12 from a mixture and the candidate profile has 9,12 these would match). Moderate stringency is the standard search configuration at SDIS for all forensic samples, and at NDIS for all mixture and partial samples.

25.7.4.6 Low stringency match occurs when one or more alleles match at a given locus when the two profiles are compared (i.e., two samples have alleles 8, 11 and 11, 14 at a locus, they would match because 11 is common to the two profiles at that locus). Low stringency is used in missing person related searches where a parent's DNA profile is being used to search for a child or vice versa.

25.7.5 Once a match has been determined to be a hit, the CODIS unit will verify the offender/arrestee sample and write a letter to the submitting agency with the offender information. The letter can be used as probable cause for a search warrant to obtain a known buccal swab from the individual which may be submitted to the lab for direct comparison to the case.

25.7.6 For forensic hits, each agency will be given the contact information for the other case involved in the hit so the agencies can contact each other and exchange information.

25.8 John Doe Warrants

25.8.1 Many states have statute of limitations meaning that, after a certain period of time, a crime cannot be prosecuted. In order to stop the clock on the statute of limitations for commencing a criminal case, "John Doe Warrants" are used.

25.8.2 This action can extend the timeframe for possibly solving a case. If DNA evidence exists from a crime scene yet no suspect has been located to be charged with the crime, a John Doe warrant may be issued based solely on the assailant's genetic profile.

25.8.3 DNA profiles should not be e-mailed outside the OSBI e-mail system. NDIS regulations and requirements state that no DNA profiles will be transmitted outside a secure network. The OSBI's IT department state that as long as e-mails are within the OSBI e-mail system that the transmissions are secure. DNA profiles are not to be transmitted outside the OSBI's network for security reasons and NDIS requirements.

25.9 Tasks

25.9.1 The trainer or designee will go over with the trainee how to properly fill out a CODIS Data Entry Form and use the Match Estimator.

25.9.2 The trainee will read the current version of the NDIS Procedures Manual.

25.9.3 The trainee will meet with the CODIS Administrator and complete the required CODIS Annual Review of DNA Data Accepted at NDIS training.

25.9.4 Complete Assignment 13 Introduction to CODIS.

25.10 Evaluation

25.10.1 The successful completion of the above tasks.

25.10.2 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

26 Mock Case Assignment

26.1 Goals

26.1.1 To become familiar with the case working requirements for forensic DNA analysis according to the policies and procedures of the OSBI Forensic Biology Unit and OSBI Laboratory system.

26.1.2 To ensure the trainee is capable of independent case work analysis and techniques used in DNA.

26.1.3 To ensure the trainee has developed good laboratory practices.

26.1.4 To ensure the trainee can compile a comprehensive, accurate, and straightforward report that meets the report writing requirements.

26.1.5 To ensure the trainee can compile complete case files that are accurate.

26.2 Mock Cases

26.2.1 There will be approximately three to five mock cases that will be prepared and completed; these will generally consist of 1 for standard extraction, one will include differential extractions, and there may also be a batch or batches of mock cases that will contain both standard and differential extractions.

26.2.2 These cases will be completed in a manner that will resemble actual casework analysis, which will include all the required documentation in the LIMS.

26.2.3 The final mock case will include various types of samples and will be completed as the mock case that will be used as the competency and the one for the mock trial.

26.3 Tasks

26.3.1 The mock cases will be completed with the skills that were obtained throughout the serology training.

26.3.2 Complete Assignment 14 DNA Mock Cases

26.4 Evaluation

26.4.1 The successful completion of the above tasks.

26.4.2 The successful completion of final mock case that will serve as the competency test.

26.4.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the above tasks.

26.4.4 **Technician Competency Assignment*

26.4.4.1 **The technician will be assigned a set of competency samples for which the technician must successfully complete the duties of a technician for the sample set working in conjunction with a qualified analyst. Both the technician and the assigned qualified analyst will provide results to the Technical Manager for evaluation. Technicians will be released for*

technician duties in casework by Memorandum from the Technical Manager after successful completion of this assignment.

27 Testimony and Expert Witness Qualification for DNA Analysts

27.1 Goals

27.1.1 To be aware of the etiquette required in the Oklahoma and Federal Court Systems.

27.1.2 To become familiar with the legal terms, procedures, and some of the pertinent legal developments in both federal and state jurisdictions as they apply to forensic DNA.

27.1.3 To become skilled at expressing results and conclusions to a court of law in both jury and technical formats.

27.1.4 To allow the trainee the opportunity to practice testimony prior to the mock trial for DNA.

27.2 Review sections 16.2 to 16.4 above for some general information on testimony and expert witness qualification for the DNA analyst.

27.3 Tasks

27.3.1 The trainee should observe other qualified analysts when they testify in court. The trainee should document any testimony observed on the trainee checklist and/or in the trainee notebook.

27.3.2 The trainee and trainer or designee should have several practice mock court sessions that cover the various aspects of testimony.

27.3.3 The trainee will complete a formal mock trial and oral technical question session over the areas of DNA analysis.

27.3.4 Read the following references, if not already completed during serology training.

27.3.4.1 Appendix 4 and Chapter 18: Legal Aspects of DNA Testing and the Scientific Expert in Court from Advanced Topics in Forensic DNA Typing: Methodology by John Butler.

27.3.4.2 Chapters 1, 3, 4, and 5 from Feder's Succeeding as an Expert Witness 4th Edition

27.3.4.3 Twenty-Five Suggestions to a Court Witness document, Oklahoma District Attorney's Council

27.4 Evaluation

27.4.1 The successful completion of the above tasks.

27.4.2 The successful completion of a mock trial and technical answer question session covering the areas for forensic DNA analysis.

27.4.3 The trainee and trainer will initial and date the trainee checklist documenting completion of the exercises.

Approval

Per OSBI CSD QP 2 (Document Control), technical protocols/procedures, discipline quality manuals, and related attachments and references will be approved by the appropriate technical manager and the OSBI CSD director, or designee.

Joseph Orcutt,
Technical Manager,
Forensic Biology Discipline

Signature

11/28/23
Date

J. Janice Joslin,
Laboratory Director,
OSBI CSD

Signature

11/28/2023
Date

(↑ **Table of Contents**)

Appendix A

Glossary

Regular Black Font – a technical definition
Regular Red Font – a jury definition

A (↑)

ABA card / Seratec card
Prostate specific antigen test designed to qualitatively to detect p30 for the forensic identification of semen.
A commercially prepared test, similar to a pregnancy test that tests for the presence of p30.

Absolute Quantitation
Absolute quantitation is the process that determines the absolute quantity of a single nucleic acid target sequence within an unknown sample. [Source: Absolute Quantification Getting Started Guide, Applied Biosystems]

Accreditation
Accreditation is formal recognition that a DNA laboratory is competent and meets or exceeds a list of standards, including the FBI Director's Quality Assurance Standards, to perform specific tests. The accrediting organization must be a nonprofit professional association of persons actively involved in forensic science that is nationally recognized within the forensic community in accordance with the provisions of the Federal DNA Identification Act or subsequent laws.

Accuracy
Accuracy is the degree of conformity of a measured quantity to its actual (true) value.

Acid Phosphatase (AP)
A phosphatase with optimum functioning at pH 5.4 and present in the prostate gland. A phosphatase (as the phosphomonoesterase from the prostate gland) optimally active in acid medium
A component found in seminal fluid, vaginal fluid, and erythrocytes

Administrative Review
Administrative review is an evaluation of the report and supporting documentation for consistency with laboratory policies and for editorial correctness. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *September 1, 2011*]

Absorption-Inhibition
Two dimensional inhibition takes advantage of the best features of inhibition-titration and titration-inhibition and is shown to be more sensitive than either of them. Results obtained using all the inhibition methods on secretor saliva, semen, urine, urine stain, and perspiration stain specimens show that the new technique is especially powerful in correctly determining the ABH antigens in secretor body fluids having lower concentrations of soluble blood group antigens. A two stage version of the two dimensional procedure that makes it a practical casework method described as well.
Useful in determining body fluids with low concentrations of blood group antigens (Assay used to detect blood group substances).

Agarose (Agar)
A polysaccharide obtained from agar that is used as a supporting medium in gel electrophoresis.
A gel that has a similar consistency to Jell-O.

Agglutination
The clumping together of biologic material, such as red blood cells or bacteria, that is suspended in liquid, usually in response to a particular antibody. A reaction in which particles (as red blood cells or bacteria)

suspended in a liquid collect into clumps and which occurs especially as a serological response to a specific antibody.
Clumping together of materials such as blood.

Allele
An alternate form of a gene or section of DNA at a particular genetic location (locus); typically multiple alleles are possible for each genetic marker. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Allele Frequency
The allele frequency is the number of copies of an allele in a population divided by the total number of all alleles in the population. [Source: An Introduction to Forensic DNA Analysis]

Allelic Ladder
An allelic ladder is an artificial mixture of the common alleles present in the human population for a particular STR marker. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Alternate Light Source
Other forms of lighting used to observe an object (i.e., UV light, Crimescope at different wavelengths)
More powerful and useful lighting used to observe staining on certain objects.

Amelogenin
Amelogenin is the locus responsible for determining the sex of the sample profile.

Amino Acids
Any of a large number of compounds found in living cells that contain carbon, oxygen, hydrogen, and nitrogen, and join together to form proteins. Amino acids contain a basic amino group (NH_2) and an acidic carboxyl group (COOH), both attached to the same carbon atom. Since the carboxyl group has a proton available for binding with the electrons of another atom, and the amino group has electrons available for binding with a proton from another atom, the amino acid behaves as an acid and a base simultaneously. Twenty of the naturally occurring amino acids are the building blocks of proteins, which they form by being connected to each other in chains. Eight of those twenty, called **essential amino acids,** cannot be synthesized in the cells of humans and must be consumed as part of the diet. The remaining twelve are **nonessential amino acids.**
A compound that is found in many living things that joins together to form larger compounds called proteins.

Amplification
Amplification is the cyclical process (denature, anneal, extend) of making copies of specific locations of DNA using an amplification kit, template DNA, and a thermal cycler.

Amplification Plot
An amplification plot is the plot of fluorescence signal versus cycle number. Reactions are characterized by the point in time during cycling when amplification of a PCR product is first detected. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. [Source: Real-Time PCR: Understanding C_T]

Amylase
Any of various enzymes that cause starches to break down into smaller sugars, especially maltose, by hydrolysis. There are two types of amylases, *alpha-amylases* and *beta-amylases.* In humans, an alpha-amylase known as **ptyalin** is present in saliva and is also produced by the pancreas for secretion into the small intestine. Beta-amylases are found in bacteria, molds, yeasts, and the seeds of plants. Any of a group of enzymes that are present in saliva, pancreatic juice, and parts of plants and catalyze the hydrolysis of starch to sugar to produce carbohydrate derivatives.
Digestive fluid in body that turns starches into sugars.

Appendix A

Analysis Method
An analysis method is a set of algorithm parameters that are applied to data during genetic analysis when using GeneMapper™ ID-X. [Source: GeneMapper™ ID Software User Guide]

Analytical Threshold
The relative fluorescence unites (RFU) value above which an observed well-formed peak that is not the result of a diagnosable issue such as stutter, pull-up, spike, minus A, etc. can be assumed to be observed due to the presence of an allele.

Annealing
Annealing is the process by which forward and reverse primers bind locations on separated (denatured) DNA strands.

Anode
The anode or anode electrode provides a positive electrical current for electrophoresis. [Source: ABI PRISM 310 Genetic Analyzer User Guide, Applied Biosystems, 2001] The negatively charged (anions) DNA fragments travel to the anode.

Antibody
Any of numerous proteins produced by **B-lymphocytes** in response to the presence of specific foreign antigens, including microorganisms and toxins. Antibodies consist of two pairs of polypeptide chains, called **heavy chains** and **light chains** that are arranged in a Y-shape. The two tips of the Y are the regions that bind to antigens and deactivate them. Also called *immunoglobulin*. A protein substance produced in the blood or tissues in response to a specific antigen, such as a bacterium or a toxin, which destroys or weakens bacteria and neutralizes organic poisons, thus forming the basis of immunity. An immunoglobulin present in the blood serum or body fluids as a result of antigenic stimulus and interacting only with the antigen that induced it or with an antigen closely related to it.
A substance in the body that is part of the immune system and combines with antigens.

Antigen
A substance that stimulates the production of an antibody when introduced into the body. Antigens include toxins, bacteria, viruses, and other foreign substances.
A substance in the body that combines with antibodies.

Antigen-Antibody Reaction
The binding of an antibody with an antigen of the type that stimulated the formation of the antibody, resulting in agglutination, precipitation, complement fixation, greater susceptibility to ingestion and destruction by phagocytes, or neutralization of an exotoxin.
The reaction that occurs when an antibody connects to an antigen that can be viewed as similar to key fitting a lock.

Antisera/Antiserum
Human or animal serum containing antibodies that are specific for one or more antigens.
A substance that contains antibodies and can be used to detect the presence of specific antigens.

Artifact
An artifact is any biology-related peak (i.e., stutter, minus A) or technology-related peak (i.e., pull-up, spikes) that does not meet the definition of a true allele.

ASCLD-LAB / ANAB ANSI-ASQ National Accreditation Board
American Society Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB); ANSI-ASQ National Accreditation Board (ANAB); American National Standards Institute (ANSI) and the American Society for Quality (ASQ)
The organization that accredits the OSBI Laboratory.

Appendix A

Assay
Qualitative or quantitative analysis of a substance, especially of an ore or a drug, to determine its components. A substance to be so analyzed. The result of such an analysis. An analysis or examination. Examination and determination as to characteristics (as weight, measure, or quality) analysis (as of a drug) to determine the presence, absence, or quantity of one or more components.
A scientific test

Autosome
An autosome is a chromosome that is not directly involved in determining sex, as opposed to the sex chromosomes. [Source: Biology, *Fourth Edition*, Neil Campbell]

Auramine-O Stain
Is a diarylmethane dye used as a fluorescent stain. It has the appearance of yellow needle crystals. It can be used to stain acid-fast bacteria. (i.e., Mycobacterium, where it binds to the mycolic acid in its cell wall) in a way similar to Ziehl-Neelsen stain. It is very soluble in water and soluble in ethanol. It can be used as a fluorescent version of Schiff reagent.

Azoospermia
Absence of spermatozoa from the seminal fluid, Failure to form live spermatozoa, Absence of live spermatozoa in the semen
A semen sample in which no sperm was present

B (↑)

Background Calibration
A background calibration is a measurement of the level of background fluorescence in the instrument. [Source: Absolute Quantification Getting Started Guide, Applied Biosystems]

Background Fluorescence
A composite signal found in all spectral data resulting from background electronic signal, contaminants in the sample block and the plastic consumables (i.e., plates). [Source: Absolute Quantification Getting Started Guide, Applied Biosystems]

Barberio Test
Basis: Detection of Spermine, Procedure: A few drops of Berberio's reagent when added to spermatic fluid produces crystals of sperm in picrate (needle shaped, rhombic & of yellow color). For various valid reasons, like non-specificity and lack of reproducibility, the Florence and Berberio's tests have not been accepted universally.
A presumptive test for the identification of seminal fluid.

Base Pair
A base pair is the complementary binding of two nucleotide bases by hydrogen bonds. Adenine and thymine bind with two hydrogen bonds and guanine and cytosine bind with three hydrogen bonds to connect two strands of DNA in the shape of a double helix.

Baseline
In the initial cycles of PCR there is little change in fluorescence signal. This defines the baseline for the amplification plot. In these cycles we see the fluorescence background of the reaction. This will be subtracted from the results when setting the baseline. [Source: Real-Time PCR: Understanding C_T]

BCIP: -Bromo-4-chloro-3-indolyl phosphate
(BCIP) is a chemical compound used in immunology for sensitive detection of alkaline phosphatase with nitro blue tetrazolium chloride (NBT). NBT serves as the oxidant (and gives also dark blue dye) and BCIP

is the AP-substrate. is a chromogenic substrate for alkaline phosphatase, used in combination with the oxidant NBT (nitro blue tetrazolium) to enhance blue color development
A substrate that can be used to test for Acid Phosphatase.

Bins
Bins are either a fragment size or base pair range and dye color that defines an allele". Bins allow for the averaging of "the minor variations in size that occur from run to run and capillary to capillary. [Source: GeneMapper™ ID Software User Guide] Bins are 1 base pair representations of the locations for each allele that can be called by the software (real or virtual alleles). A +/-0.5 base pair range is defined by the allelic ladder from the center point of an allelic ladder peak. According to the GeneMapper™ ID tutorial, a bin is defined as an expected location for a particular allele within a marker.

Biological Evidence
Biological evidence is evidence recovered from crime scenes in the form of blood, semen, saliva, hair, tissue, bones, teeth, or other bodily fluids/body parts which pertain to life and living things.

Biological Fluids
Biological fluids are fluids pertaining to life and living things. Typically encountered at crime scenes are fluids of human or animal origin such as blood, semen, saliva, mucous, perspiration, vaginal fluid, and urine.

Birefringence
The property or capacity of splitting a beam of light into two beams, each refracted at a different angle, and each polarized at a right angle to the other. Certain crystals such as calcite and quartz have this property. The difference in the index of refraction between two beams passing through a substance that has this property. The resolution or splitting of a light wave into two unequally reflected waves by an optically anisotropic medium such as calcite or quartz.
Splitting of light that creates visual effects such as color change of a crystal when viewed at different angles.

Blank
An item to be tested where the results are expected to be negative and are often used to show that reagents are free of contaminants that would interfere with testing.
A test to provide evidence that the material will not provide a positive test on its own.

Blood
The fluid tissue that circulates through the body of a vertebrate animal by the pumping action of the heart. Blood is the transport medium by which oxygen and nutrients are carried to body cells and waste products are picked up for excretion. Blood consists of plasma in which red blood cells, white blood cells, and platelets are suspended. A fluid that is similar in function in many invertebrate animals.
The substance in the body that transports oxygen throughout the body and is necessary for life functions.

Blood Group Substances
These substances are important because they contain specific sequences of amino acids and carbohydrate which are antigenic.
Various other substances that can be found within the blood

Buccal Swabs
A cotton-tipped applicator or piece of gauze that is wiped on the inside of a person's cheek in order to collect epithelial cells.
A piece of cotton on a stick used in collection of DNA on inside of person's cheek.

Buffer
Used on the genetic analyzers, the electrophoresis buffer supplies the ions for conducting current across the capillary between the cathode and anode.

Appendix A

C (↑)

Calibration
Calibration is a comparison of the laboratory instrument or equipment to an established standard allowing a verification that the laboratory instrument or equipment functions properly.

Capillary Array
More than one capillary joined together in parallel in a single system is referred to as a capillary array electrophoresis system. The use of an array allows multiple samples to be injected and analyzed at the same time (parallel CE separations).

Capillary Electrophoresis
Capillary electrophoresis is an electrophoretic technique for separating DNA molecules by their size based on migration through a narrow glass capillary tube filled with liquid polymer. [Source: Fundamentals of Forensic DNA Typing]

Casework Laboratory
A casework laboratory is the laboratory responsible to NDIS for a DNA profile developed from crime scene evidence.

Carcinogen
A substance or agent that can cause cells to become cancerous by altering their genetic structure so that they multiply continuously and become malignant. Asbestos, DDT, and tobacco smoke are examples of carcinogens.
A substance that can cause cancer.

Cathode
The cathode or cathode electrode provides a negative pole for electric current for electrophoresis [Source: ABI PRISM 310 Genetic Analyzer User Guide, Applied Biosystems, 2001]. The cathode is the site of the electrokinetic injection.

CCD Camera
A charge-coupled device detector used on the ABI 310 and 31xx instruments to collect fluorescence emissions across a visible spectrum from the electrophoretically separated, dye-labeled PCR products. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Cell
A cell is an organism's basic unit of structure and function.
The smallest structural unit of life capable of independent biological reproduction and from which DNA is isolated for forensic DNA analysis.

Chain of Custody
A chain of custody is a record of individuals who have had physical possession of the evidence and the process used to maintain and document the chronological history of the evidence. (Documents can include, but are not limited to- name or initials of the individual collecting the evidence, each person or entity subsequently having physical possession of it, dates the items were collected or transferred, where the item(s) were collected from, agency and case number, victim's or suspect's name (if known), and a brief description of the item.)

Appendix A

Characteristics of Semen
Liquid fluid commonly ejaculated in 3-5 mL volume.

Chemiluminescence
The emission of light by a substance as a result of undergoing a chemical reaction that does not involve an increase in its temperature. Chemiluminescence usually occurs when a highly oxidized molecule, such as peroxide, reacts with another molecule. The bond between the two oxygen atoms in peroxide is relatively weak, and when it breaks the atoms must reorganize themselves, releasing energy in the form of light.
A reaction that produces light as a product of the reaction.

Choline
A natural amine often classed in the vitamin B complex. It is incorporated into the structure of many other biologically important molecules, such as acetylcholine and lecithin. *Chemical formula:* **$C_5H_{15}NO_2$.** A natural amine, $C_5H_{15}NO_2$, often classed in the vitamin B complex and a constituent of many other biologically important molecules, such as acetylcholine and lecithin Choline and its metabolites are needed for three main physiological purposes: structural integrity and signaling roles for cell membranes, cholinergic neurotransmission (acetylcholine synthesis), and as a major source for methyl groups via its metabolite, trimethylglycine (betaine) that participates in the S-adenosylmethionine synthesis pathways. When choline is metabolized by the body, it may form trimethylamine, a compound with a fishy odor. Hence, when large amounts of choline are taken the person may suffer from a fishy body odor. Choline was discovered by Andreas Strecker in 1862 and chemically synthesized in 1866. In 1998 choline was classified as an essential nutrient by the Food and Nutrition Board of the Institute of Medicine (U.S.A.). Choline is an organic compound, classified as an essential nutrient and usually grouped within the Vitamin B complex. This natural amine is found in the lipids that make up cell membranes and in the neurotransmitter acetylcholine. Adequate intakes (AI) for this micronutrient of between 425 to 550 milligrams daily, for adults, have been established by the Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences
A substance that can be found in seminal fluid

Christmas Tree Stain
Differential stain used to microscopically observe semen. Consists of Nuclear Fast Red: stains nuclear material and Picroindigocarmine: stains the tails.
A stain used to observe semen microscopically; Red stain stains the sperm heads and green stain stains the tails.

Chromosome
Chromosomes are the organized thread-like structures or strands composed of DNA and protection proteins (called histones) within the cell nucleus by which hereditary information is physically transmitted from one generation to the next through meiosis or by which cells self-replicate through mitosis.

Clean
Clean refers to the process of removing biological and/or chemical contaminants from tools and/or equipment.

CODIS (COmbined DNA Index System)
The Combined DNA Index System is an electronic database of DNA profiles that was started in 1998. These profiles are generated from convicted offenders and/or from crime scene evidence. State statutes determine which offenses are required to be included in the database. The database also includes a missing persons' index.

CODIS, administered by the FBI, can link DNA evidence obtained from crime scenes, thereby identifying serial criminals. CODIS also compares crime scene evidence to DNA profiles obtained from offenders, thereby providing investigators with the identity of the putative perpetrator. In addition, CODIS contains profiles from missing persons, unidentified human remains and relatives of missing persons. There are

three levels of CODIS: the Local DNA Index System (LDIS), used by individual laboratories; the State DNA Index System (SDIS), used at the state level to serve as a state's DNA database containing DNA profiles from LDIS labs; and the National DNA Index System (NDIS), managed by the FBI as the nation's DNA database containing all DNA profiles uploaded by participating states. [Source: Quality Assurance Standards for DNA Databasing Laboratories, FBI, *effective September 1, 2011*]

CODIS Comparisons
A CODIS comparison is a comparison of one DNA profile to another for the purpose of establishing an association between two specimens.

CODIS Core Loci
The CODIS core loci are the STR loci which are nationally shared among amplification kits and compared during CODIS database searches. The original 13 CODIS core loci were D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, D16S539, TH01, TPOX, and CSF1PO. In 2017, the FBI expanded the 20 CODIS core loci to include: D13S317, D7S820, D5S818, CSF1PO, D1S1656, D12S391, D2S441, D10S1248, D18S51, FGA, D21S11, D8S1179, vWA, D16S539, TH01, D3S1358, D2S1338, D19S433, TPOX, and D22S1045.

CODIS User
A CODIS user is a government employee who: (1) has login access to the CODIS (i.e.,, state or local) system and is authorized to read, add, modify or delete DNA records in CODIS; or (2) is a qualified DNA analyst responsible for producing DNA profiles stored in NDIS.

Collection Software
Collection software is instrument software designed to communicate instructions to the instrument and record data generated by an instrument.

Components of Seminal Fluid
Seminal fluid is comprised of secretions produced by the prostate gland, Cowper's gland and the seminal vesicles. All three combine to produce various types of alkaline fluids. Fluid from the prostate gland account for about 30% of seminal fluid. Because of its alkaline makeup, this fluid helps to neutralize the acids naturally found in the urethra and the vagina. This prevents the sperm from being killed off on contact. The seminal vesicles produce about 60% of the seminal fluids. Also an alkaline fluid, these secretions contain fructose, a type of sugar, which give sperm energy, thereby allowing them to move faster and aid them in their swim up through the uterus. The Cowper's gland along with fluid from the testes contribute the remaining fluid to the semen.
Other substances, other than semen that are in seminal fluid.

Confirmatory Test
An examination that can verify the presence of a specific compound, the nature of the test should be such that it limits most other compounds from detection and gives unique results for the compound to be confirmed. It typically is less sensitive than a presumptive test.
A test that is used to positively identify a substance.

Contamination
The act or process of rendering something harmful or unsuitable. The presence of extraneous, especially infectious, material that renders a substance or preparation impure or harmful. Contamination is the unintentional introduction of exogenous DNA into a DNA sample or PCR reaction [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]
An outside substance that comes in contact with the substance be tested and interferes with the analysis.

Control
A sample that is used as a comparison for testing or for determining materials are working properly or for detecting interferences that produce false negatives. Controls are test samples designed to demonstrate that a procedure worked correctly and performed in parallel with experimental samples.

A sample of known value that verifies the test is functioning properly.

CJIS-SEN (formerly CJIS-WAN)
CJIS-SEN is the FBI's Criminal Justice Information Services Shared Enterprise Network that provides communications network for the United States law enforcement community. Originally designed to support the Integrated Automated Fingerprint Identification System (IAFIS), the FBI is expanding the scope of CJIS-SEN to include all federal, state, and local crime laboratories participating in the National DNA Index System.
The network was formerly called the CJIS-WAN (CJIS-WAN Criminal Justice Information Services Wide Area Network)

Critical Instrument
Critical instruments are laboratory instruments which require calibration or a performance check prior to use and periodically thereafter. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]

Critical Reagent
A critical reagent is a reagent which requires testing on established samples before use on evidentiary samples or casework reference samples. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]

Criminalist
A specialist in the collection and examination of the physical evidence of crime.
A person who analyzes evidence submitted to laboratories and produces a report on the findings of those analyses.

Cross Contamination
Cross contamination is the undesirable transfer of material between two or more sources of physical evidence.

Cross Linker
An instrument used to expose reagents and supplies to ultraviolet light (UV) as a DNA decontamination method.

Cross Reactivity
The reaction between an antigen and an antibody that was generated against a different but similar antigen.
The reaction of an antibody with an antigen that is similar to the antigen for which the antibody was created.

Crystal
A homogenous solid formed by a repeating, three-dimensional pattern of atoms, ions, or molecules and having smooth external surfaces with characteristic angles between them. Crystals can occur in many sizes and shapes. The particular arrangement in space of these atoms, molecules, or ions, and the way, in which they are joined, is called a crystal lattice**.** There are seven crystal groups or systems. Each is defined on the basis of the geometrical arrangement of the crystal lattice.
A solid that has an ordered appearance like a diamond or grain of sugar.

D (↑)

Degradation
Degradation is the deterioration in quality or quantity of a substance.

Appendix A

Delta Rn
ΔRn is the normalization of the Rn obtained by subtracting the baseline (ΔRn = Rn – baseline). [Source: Real-Time PCR: Understanding C_T]

Denaturation
Denaturation is the process of breaking the hydrogen bonds that hold the DNA strands together, allowing the strands to be separated. This process is done either by heating or through the use of chemical. It is a reversible process (re-naturing).

Derivative Evidence
Derivative evidence is any tangible material removed or derived from an evidence item already having an assigned item number such as cuttings, debris collections, latent lifts, and retained stain samples. [Source: OSBI CSD Quality Manual]

Differential Amplification
Differential amplification is the selection of one target region or locus over another during the polymerase chain reaction. Differential amplification can also arise between two alleles within a single locus if one of the alleles has a mutation within a PCR primer-binding site, causing this allele to be copied less efficiently because of the primer-template mismatch. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]

Differential Extraction
Differential extraction is a DNA extraction procedure in which sperm cells are separated from other cells before the DNA is purified. [Source: An Introduction to Forensic DNA Analysis]

Discrete allele
A discrete allele is any allele in a genetic typing system in which the detection method can clearly distinguish between the variants being tested. Short Tandem Repeats (STRs) are discrete alleles in that alleles differing by only one base pair can be distinguished from one another. Restriction Fragment Length Polymorphisms (RFLPs) are non-discrete or continuous alleles in which boundaries between adjacent alleles are ambiguous and an allele is defined by the size class or binning. [Source: An Introduction to Forensic DNA Analysis]

Direct Amplification
Development in forensic DNA typing that allows for PCR amplification without the need for extraction/isolation or quantitation steps [Source: Advanced Topics in DNA Typing: Methodology]

DNA (Deoxyribonucleic acid)
DNA is the molecule that encodes genetic information. DNA is a nucleic acid contained in cells (that contain a nucleus) which determines each person's individual characteristics. An individual's DNA is unique except in cases of identical twins.
The genetic material that is unique between individuals. No two individuals have the same DNA except identical twins.

DNA Advisory Board (DAB)
The DNA advisory board, in existence from March 1995 through December 2000, was established to address DNA quality assurance methods. The Board was appointed by the FBI Director in accordance with the DNA Identification Act of 1994.

DNA Analysis
DNA analysis is the process of testing biological samples to identify DNA patterns or types. In the forensic setting, this testing is used to exclude or include individuals as possible sources of body fluid stains (blood, saliva, semen) and other biological evidence (bones, teeth, hair). This testing can also be used to indicate parentage.

Appendix A

DNA Analyst
A DNA analyst is an individual who independently conducts and/or directs the analysis of samples and independently conducts the interpretation of data and determination of conclusions.

DNA Polymerase
A DNA polymerase is an enzyme that assists in the DNA replication process by catalyzing the synthesis of new DNA strands using existing DNA strands as templates.

DNA Profile
A DNA profile is the result of determining the relative positions of DNA sequences at several locations on the molecule. Each person (except identical twins) has a unique DNA profile when used in the context of the CODIS database, which evaluates specific DNA locations.
A DNA profile consists of a set of DNA identification characteristics which may permit the DNA of one person to be distinguishable from that of another person.

DTT *(dithiothreitol)*
DTT is a differential extraction chemical responsible for breaking the disulfide bonds found in sperm cell membranes.

Double Helix
A double helix is the native form of DNA when single strands are held together by complementary base pairing and twined around each other in the form of a double helix [Source: An Introduction to Forensic DNA Analysis]
Commonly referred to as a "twisted ladder" shape, a double helix is the shape the DNA assumes after it replicates during cell life.

Double Immunodiffusion
A technique for studying reactions between antigen and antibodies by observing precipitates formed by the combination of specific antigens and antibodies that have diffused in a gel in which they have been separately placed.
A test that results in a visible line forming from the reaction of antigens with antibodies that have been placed in separate wells.

Dropout
Also referred to as allelic dropout, dropout is the lack of allelic information above the laboratory-defined peak amplitude threshold at a locus. Various degrees of dropout exist; dropout ranges from complete sister allele dropout (no indication of a peak) to an allele peak just slightly below the peak amplitude threshold.

Dye blob
Dye blobs are artifacts, typically broad and in one color only, which occur when fluorescent dyes come off of their respective primers and migrate independently through the capillary. [Source: Fundamentals of Forensic DNA Typing, John Butler, page 219-220]

Dye Calibrations
Dye calibrations for system dyes and custom dyes (ABY and JUN) are a collection of spectral data from a series of pure dye and custom dye standards so that the QuantStudio Real-Time PCR instrument software can determine the contribution of each fluorescent dye used in the sample by comparing the raw spectra from the sample to the pure spectra file. [Source: Life Technologies, QuantStudio 5 Real-Time PCR Instrument User Guide (for Human Identification), Revision A.0, June 15, 2017]

E (↑)

Appendix A

EDTA
Ethylenediaminetetraacetic acid: a colorless compound, $C_{10}H_{16}N_2O_8$, capable of chelating a variety of divalent metal cations: as a salt used as an anticoagulant, antioxidant, blood cholesterol reducer, food preservative; as a calcium-disodium salt used in the treatment of lead and other heavy-metal poisonings.
A substance that prevents blood from clotting.

Ejaculation
The act of discharging semen from the urethra during orgasm. The act of ejaculating. An abrupt discharge of fluid. The expulsion of seminal fluid from the urethra of the penis during orgasm
The process of releasing semen.

Electropherogram
An electropherogram is the graphical representation of the separation of molecules by electrophoresis or other means of separation.

Electrode
An electrode is a conductor used to established electrical contact with a nonmetallic part of a circuit. [Source: www.merriam-webster.com/medical/Electrode]

Electrokinetic Injection
An electrokinetic injection is the method by which DNA samples are loaded into the capillary. A voltage is applied to the sample to help draw it into the capillary opening.

Electrophoresis
Electrophoresis is a method of separating large molecules (such as DNA fragments) from a mixture of similar molecules. An electric current is passed through a medium at a different rate, depending on its electrical charge and size. Separation of DNA markers is based on these differences.

Elimination/Reference samples
A term used to describe a sample of known source taken for comparison purposes.

A biological sample from a known individual, other than the alleged perpetrator or victim, which is analyzed for purposed of identifying those portions of a forensic DNA profile attributable to the alleged perpetrator.

An elimination sample is one of known source taken from a person who had lawful access to the crime scene (e.g. blood or cheek [buccal] swabs for DNA analysis, fingerprints from occupants, tire tread impressions from police vehicles, footwear impressions from emergency medical personnel) to be used for comparison with evidence of the same type.

A reference sample is material of a verifiable/documented source which, when compared with evidence of an unknown source, shows an association or linkage between an offender, crime scene and/or victim (e.g., a carpet cutting taken from a location suspected as the point of transfer for comparison with the fibers recovered from the suspect's shoes, a sample of paint removed from a suspect's vehicle to be compared with paint found on a victim's vehicle following an accident, or a sample of the suspect's and/or victim's blood submitted for comparison with a bloodstained shirt recovered as evidence).

Employee
An employee is a person: (1) in the service of the applicable federal, state or local government, subject to the terms, conditions and rules of federal/state/local employment and eligible for the federal/state/local benefits of service; or (2) formerly in the service of a federal, state, or local government who returns to service in the agency on a part time or temporary basis. For purposes of a vendor laboratory, an employee is a person in the service of a vendor laboratory and subject to the applicable terms, conditions and rules of employment of the vendor laboratory. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]

Appendix A

Enzyme
Any of numerous proteins produced in living cells that accelerate or catalyze the metabolic processes of an organism. Enzymes are usually very selective in the molecules that they act upon, called substrates, often reacting with only a single substrate. The substrate binds to the enzyme at a location called the active site just before the reaction catalyzed by the enzyme takes place. Enzymes can speed up chemical reactions by up to a million fold, but only function within a narrow temperature and pH range, outside of which they can lose their structure and become denatured. Enzymes are involved in such processes as the breaking down of the large protein, starch, and fat molecules in food into smaller molecules during digestion, the joining together of nucleotides into strands of DNA, and the addition of a phosphate group to ADP to form ATP. The names of enzymes usually end in the suffix -ase.
A protein that accelerates a reaction (catalyst).

Epifluorescence
Epifluorescence microscopy is a method of fluorescence microscopy that is widely used in Life Sciences. In this process, instead of transmitting the excitatory light through the specimen it is passed through the objective onto the specimen. Since only reflected excitatory light filters through, the transmitted light is filtered out, giving a much higher intensity. Fluorescent or fluorochrome stains are applied to the specimen to provide an estimated count. It can be used to find routine direct total counts of bacteria in water samples.
Glowing effect given by certain bacteria and flavins, visible with alternate light source.

Epithelial Fraction
The epithelial fraction is the first portion of the liquid removed during the differential extraction process ideally containing non-sperm cells (primarily consisting of epithelial cells).

Epitope
The site on the surface of an antigen molecule to which an antibody attaches itself. The surface portion of an antigen capable of eliciting an immune response and of combining with the antibody produced to counter that response.
The part of the antigen that activates the body's immune response.

Erythrocyte
A cell in the blood of vertebrates that transports oxygen and carbon dioxide to and from the tissues. In mammals, the red blood cell is disk-shaped and biconcave, contains hemoglobin, and lacks a nucleus. Also called *erythrocyte*, *red cell*, *red corpuscle*.
Red Blood Cell

Evidence
Evidence is something that may help identify the responsible persons, establish an element of crime, reconstruct crime events, or link crimes.
All material submitted for scientific analysis during the course of an official criminal investigation. [Source: OSBI CSD Quality Manual]

Evidence Retention
Evidence retention is the maintaining of evidence for an indefinite period of time by the OSBI or requesting agency in order to preserve the evidence for potential future analysis, court presentation, or as required by statute.

Evidence Transfer
Evidence transfer is the physical transfer of evidence between individuals or locations.

Evidence Return
Evidence return refers to the process of giving evidence back to the original submitting agency. With the exception of evidence samples which require or warrant retention, evidence will be returned to the appropriate submitting or requesting agency.

Exclusion
Exclusion is the failure to match two DNA profiles or an inability for the known sample to have contributed to the questioned sample. In a criminal case, "exclusion" does not necessarily equate to "innocence."

Extension
In PCR, extension refers to the addition of nucleotides to form a new DNA strand from a primed template. [Source: An Introduction to Forensic DNA Analysis]

Extract
To draw or pull out, using force or effort. To obtain from a substance by chemical or mechanical action, as by pressure, distillation, or evaporation. A concentrated preparation of a drug obtained by removing the active constituents of the drug with suitable solvents, evaporating all or nearly all of the solvent, and adjusting the residual mass or powder to the prescribed standard
To withdraw from a liquid or substance.

Extraction
Extraction is a technique for separating components in a mixture that have different solubilities. In forensic DNA analysis, the term “extraction” specifically refers to the separation/removal of the DNA from the other components of the cell.

F (↑)

False Negative
A test result that is incorrect because the test failed to recognize an existing condition or finding. Test result that is read as negative but actually is positive, a test that shows no evidence of a disease when it is actually present
A test that is read as a negative but is actually positive.

False Positive
A test result that is read as positive but actually is negative, a test that shows evidence of a disease when it not present. A test result that is incorrect because the test indicated a condition or finding that does not exist.
A test that is read as positive but is actually negative.

Flavin(s)
Any of a class of yellow water-soluble nitrogenous pigments derived from isoalloxazine and occurring in the form of nucleotides as coenzymes of flavoproteins **:** PLATYHELMINTH; Any of various water-soluble yellow pigments, including riboflavin, found in plant and animal tissue as coenzymes of flavoproteins. A ketone that gives color to various natural yellow pigments.
A component of seminal fluid.

Florence Test
Basis: Choline is detected in this method.
Procedure: A few drops of watery solution of the stain is extracted and taken on a slide and a drop of Florence reagent (8%) W/V solution of Iodine in water containing 5% W/V of Potassium Iodide) is poured & allowed to mix slowly under a cover slip. Dark brown crystals of choline periodide, generally needle shaped, formed with a few minutes. Non-specifics & false negative results are common.
A test used to detect choline a component of seminal fluid.

Forensic
Relating to, used in, or appropriate for courts of law or for public discussion or argumentation. Of, relating to, or used in debate or argument; rhetorical. Relating to the use of science or technology in the investigation and establishment of facts or evidence in a court of law: *a forensic laboratory.*

Using science and scientific reasoning to analyze evidence and establish facts, which can be presented in a court of law.

Forensic Science
The application of science to analyze evidence involved in criminal and civil litigation. In the most general sense, forensic science is the application of science to the law.

Formamide
Formamide is a chemical used in creating a denaturing environment to keep complementary strands of DNA apart from one another during genetic analysis. [Source: Advanced Topics in Forensic DNA Typing: Methodology, John Butler, Chapter 6, page 146]

Forensic Sample
A forensic sample is a biological sample originating from and associated with a crime scene. For example, a sample associated with a crime scene may include a sample that has been carried away from the crime scene. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]

Fragile evidence
Fragile evidence is evidence that will lose its evidentiary value if not preserved and protected, either because of its nature or the conditions at the scene (e.g., blood in the rain).

G (↑)

Gene
A gene is the basic unit of heredity; a sequence of DNA nucleotides on a chromosome. [Source: An Introduction to Forensic DNA Analysis]

Genetics
Genetics is the study of the patterns of inheritance of specific traits.

Genome
The genome is the total genetic makeup of an organism. [Source: An Introduction to Forensic DNA Analysis]

Genotype
A genotype is the genetic makeup of an organism, as distinguished from its physical appearance or phenotype. A genotype may pertain to one locus or many loci. [Source: An Introduction to Forensic DNA Analysis]

In a DNA profile, a genotype is the allele calls for all alleles at a locus. A homozygous locus would be represented by two identical alleles for a genotype.

Globular Proteins
Soluble proteins with a globular (somewhat rounded) shape. One of two main proteins classes, comprising of globelike proteins that are more or less soluble in aqueous solutions, which helps them distinguish them from the fibrous proteins which are insoluble.
One of the two main types of proteins found in the body.

Glycoprotein
Any of a group of cellular macromolecules that are made up of proteins bonded to one or more carbohydrate chains. Any of a group of conjugated proteins that contain a carbohydrate as the non-protein component, any of a group of complex proteins, as mucin, containing a carbohydrate combined with a simple protein.

A group of cellular components in the body.

H (↑)

Haplotype
A haplotype is a group of tightly linked polymorphisms that are inherited as a unit. There is only a single allele per locus.

Hardy-Weinberg Equilibrium
Hardy-Weinberg equilibrium (HWE) predicts the stability of allele and genotype frequencies from one generation to the next. The primary purpose in testing for HWE is to determine if alleles within a locus are independent of each other. Frequencies should not change over the course of many generations if the locus is genetically stable. Five basic assumptions are associated when testing for HWE: 1) large population, 2) no natural selection, 3) no mutation, 4) no immigration/emigration, and 5) random mating.
An observation reported independently in 1908 by Godfrey Hardy and Wilhelm Weinberg that in a large random intrabreeding population, not subjected to excessive selection or mutation, the gene and genotype frequencies will remain constant over time; the sum of $p^2 + 2pq + q^2$ applies at equilibrium for a single allele pair where p is the frequency of allele A, q is the frequency of a, p^2 is the frequency of genotype AA and q^2 is the frequency of aa, and 2pq if the frequency of Aa; permits relating allele frequencies to genotype frequencies. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Hemastix
Commercially prepared blood detection strips used for the presumptive identification of blood.
A presumptive test for the presence of blood.

Heme
The deep red, non-protein, iron-containing component of hemoglobin that carries oxygen. Heme is a porphyrin with an iron atom at its center. One of the free valence electrons of the iron atom of heme is bound to the hemoglobin molecule, while the other is available for binding to an oxygen atom. A hemoglobin molecule contains four hemes. *Chemical formula:* $\mathbf{C_{34}H_{32}FeN_4O_4}$.
The oxygen carrying component of blood and is what makes it red.

Hemochromogen
A colored compound formed from or related to hemoglobin; *especially*: a bright red combination of a nitrogen base (as globin or pyridine) with heme. A body obtained from hemoglobin by the action of reducing agents in the absence of oxygen.
The crystal that is formed when performing the confirmatory test for blood.

Hemoglobin
An iron-containing protein present in the blood of many animals that, in vertebrates, carries oxygen from the lungs to the tissues of the body and carries carbon dioxide from the tissues to the lungs. Hemoglobin is contained in the red blood cells of vertebrates and gives these cells their characteristic color. Hemoglobin is also found in many invertebrates, where it circulates freely in the blood. It consists of four peptide units, each attached to a non-protein compound called **heme** that binds to oxygen.
An individual unit of a heme.

Heredity
Heredity is the transmission of characteristics from one generation to the next.

Heterozygote
Heterozygote means possessing two different forms (alleles) of a particular gene or defined section of DNA.

High Dose Hook Effect (HDHE)
For any ELISA to give accurate results there must be an excess of antibodies, both capture and enzyme conjugated, relative to the analyte being detected. It is only under the conditions of antibody excess that the dose response curve is positively sloped and the assay provides accurate quantitation. As the concentration of analyte begins to exceed the amount of antibody the dose response curve will flatten (plateau) and with further increase may paradoxically become negatively sloped in a phenomenon termed "High Dose Hook Effect". Because the possibility exists that some samples may have analyte concentrations in excess of the antibody it is necessary to validate all sample types by dilutional linearity analysis to establish if they are on the valid, positively sloped region of the curve or on the negatively sloped hook region of the curve. Failure to validate the potential for Hook Effect can result in severe under-estimation of true contaminant concentrations!
A large concentration of p30 that dye occurs for ABA card or Seratec testing and produces a false negative.

Homozygote
Homozygote means possessing two identical forms (alleles) of a particular gene or defined section of DNA.

Hot Start
Hot start refers to a chemical modification applied to a DNA polymerase (i.e., AmpliTaq Gold DNA Polymerase) which prevents undesired PCR products from forming at the beginning of PCR reactions. The DNA polymerase is only activated by increasing the temperature causing the chemical modification to be removed.

Human Serum Proteins
Proteins, such as fibrinogen, globulin, and human serum albumin, which are found in the liquid portion of blood with the clotting factors removed.
Proteins found in the liquid portion of human blood.

I (↥)

Immunoglobulin
Any of a group of large glycoprotein's secreted by plasma cells in vertebrates that function as antibodies in the immune response by binding the specific antigens. A class of proteins produced in lymph tissue in vertebrates and that function as antibodies in the immune response. There are five classes of immunoglobulin: IgA, IgD, IgE, IgG, and IgM.
A substance in the body that behaves like an antibody.

Infertility
The inability to achieve conception after persistent attempts over a given period of time, usually one year in humans.
The inability to conceive a child.

Inhibition
Inhibition is the process by which a chemical or substance interferes with efficient amplification of DNA by either directly interacting with DNA or blocking DNA polymerase activity. [Source: An Introduction to PCR Inhibitors]

Inhibitors
Inhibitors are molecules, chemicals, or substances that decrease or prevent a chemical reaction. Inhibitors are also called negative catalysts.

Injection List
On the 310 Genetic Analyzer, an injection list is an instructional list of samples for the instrument defining when samples will be injected and the parameters of each injection.

Appendix A

Instrument Baseline Threshold
The relative fluorescence units (RFU) value below which a peak is indistinguishable from baseline noise.

Internal PCR Control (IPC)
IPC refers to an assay within each reaction setup on a Quantifiler plate that contains the necessary components to determine whether the real-time PCR amplification occurred as expected and whether the sample may contain inhibitors.

Itemization
Itemization is the process of assigning a unique item number to each item or sub-item of evidence.

J (↑)

K (↑)

L (↑)

Liesgang Phenomenon
The phenomenon of ring or line formation seen in many chemical systems undergoing a precipitation reaction, under certain conditions of concentration or if more than one antigen and antibody being present in an antibody-antigen reaction.
More than one line developing during an ouchterlony test.

Length polymorphism
Length polymorphisms are variations in the population of loci based on the differences in the length of DNA fragments.
In other words, a length polymorphism is a locus that exhibits variation in length when…amplified with PCR primers. [Source: An Introduction to Forensic DNA Analysis]

Likelihood Ratio
A likelihood ratio is the direct comparison of the probabilities of two competing hypotheses. [Source: An Introduction to Forensic DNA Analysis]

Limit of Detection (LOD)
As it pertains to genetic analysis, this is the relative fluorescence units (RFU) value below which a peak is unable to be brought up to a peak height useful for interpretation through increased injection time(s). This may or may not be equivalent to the baseline threshold; however, it cannot be less than the baseline threshold and should be relatively close to the baseline threshold to minimize any data loss.

Lineage Markers
Y chromosome and mitochondrial DNA markers represent lineage markers. They are passed down from generation-to-generation without changing (except for mutational events). Maternal lineages can be traced with mitochondrial DNA sequence information while paternal lineages can be traced with Y chromosome markers. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Linkage Equilibrium
Linkage equilibrium is the inheritance of the alleles at a given locus completely independent of the inheritance of alleles at another locus.

Local Southern Method
The sizing of DNA fragments with internal standards involves using an algorithm known as the Local Southern Method to determine the DNA fragment size. This method uses the size of two peaks on either side of the unknown peak being measured in order to make calculations. The Local Southern method works very well for accurate sizing of DNA fragments over the 100-450bp size range necessary for STR alleles. [Source: Fundamentals of Forensic DNA Typing, John Butler, page 215]

Locus (pl. loci)
A locus is the specific physical location of a gene or marker on a chromosome.

LDIS
The lowest level of the three levels of CODIS, the Local DNA Index System (LDIS) is used by individual laboratories. [Source: Quality Assurance Standards for DNA Databasing Laboratories, FBI, *effective September 1, 2011*]

Low Copy DNA Analysis
Based upon a laboratory's internal validation, any DNA typing results generated from limited quantity and/or quality DNA template using conditions that have demonstrated increased stochastic effects are defined as low template or low copy DNA analyses.

Low Power Microscopy
Use of microscopes to magnify an object up to 630 times the normal size.
The aid of equipment to enhance the view of an item to get a more detailed description than can be seen with the naked eye.

M (↑)

Maintenance
Maintenance is a task designed to keep the laboratory equipment or instrument working properly whether this task is preventive (schedule cleaning, inspecting, testing) or a repair of an identified problem.

Marker
Markers are pieces of DNA sequence of known locations on chromosomes that are used to identify the specific genetic variations an individual possesses.

Master Mix
A mixture of all of the necessary reagents for a reaction minus the DNA sample to ensure a consistent amount of each reagent is dispensed to all samples in the set.

Matrix
A matrix is a mathematical equation that removes spectral overlap resulting from the use of multiple fluorescent dyes.

Microscope
Any of various instruments used to magnify small objects that are difficult or impossible to observe the naked eye.
Optical microscopes use light reflected from or passed through the sample being observed to form a magnified image of the object, refracting the light with an arrangement of lenses and mirrors similar to those found in telescopes.
An instrument used to magnify objects to been seen clearly with the eye.

Microcon
A microcon is a device consisting of a specialized filter which is used in forensic purposes to concentrate and purify a DNA extract.

Minus A (-A)
Minus A is the incomplete 3' (A) nucleotide addition resulting from an amplification containing too much DNA template or thermal cycling conditions that affect the optimization of the PCR reaction preventing the Taq DNA polymerase from catalyzing the addition of an extra nucleotide on the 3' end of double stranded PCR products. The target allele will be represented by two peaks one base pair apart.
The form of a PCR product that does not possess an extra nucleotide at the 3' end; sometimes referred to as n-1. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Mitochondrial DNA
Mitochondrial DNA is a small circular piece of DNA found outside the nucleus in most cells and generally involved in the production of proteins responsible for energy production in the body. It is inherited maternally.

Multiplex PCR
Multiplex refers to the co-amplification of multiple regions of a genome with more than one set of primers; enables information from the different target sequences to be collected simultaneously. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Monoclonal Antibody
Any of a class of highly specific antibodies produced by the clones of a single hybrid cell formed in the laboratory by the fusion of a B cell with a tumor cell and widely used in medical and biological research.
A single type of antibody that is produced to bind to a specific type of antigen.

N (↑)

NDIS (National DNA Index System)
NDIS is one component of CODIS. It is the national and highest level index containing the DNA records contributed from participating federal, state, and local laboratories. NDIS is administered by the FBI in accordance with the provisions of the DNA Identification Act of 1994, as amended.

The highest level of the three levels of CODIS, the National DNA Index System (NDIS) is managed by the FBI as the nation's DNA database containing all DNA profiles uploaded by participating states. [Source: Quality Assurance Standards for DNA Databasing Laboratories, FBI, *effective September 1, 2011*]

Negative Control
Used in scientific testing, this is the control that is used to show how the environment alone effects the reaction that is taking place, and is done by exposing the experiment to a situation which is known to produce no reaction.
A control to show how the object by itself is affected by the test.

Nine-Nine-Four-Seven-A (9947A)
The female positive control DNA used during some PCR reactions to indicate whether the thermal cycler successfully amplified the set of samples.

Normospermia
Contain sperm cell concentrations of approximately 50 $X10^6$ to $150x10^6$ /mL
Normal average sperm count in human males.

Nuclear DNA
Nuclear DNA is DNA found in the nucleus of the cell. It is inherited from both the mother and the father.

Nucleotide
A nucleotide is the basic building block of DNA consisting of a nucleoside (sugar and nitrogenous base) linked to a phosphate group.

Nucleotide Sequence
The ordered listing of nucleotide bases (A, G, C, T) occurring along a strand of DNA.

O (↑)

Objective v. Subjective
Objective is defined as Based on observable phenomena; presented factually. Undistorted by emotion or personal bias; based on observable phenomena. Subjective is defined as of a person's attitude etc.) arising from, or influenced by, his own thoughts and feelings only; not objective or impartial
Objective is an observable fact that is not distorted, where subjective is an opinion and is influenced by thoughts, feeling, own ideas, etc.

Offender Hit
An offender hit is a CODIS match between a crime scene profile and an offender profile.

Offender Sample
An offender sample is a sample collected from an offender who is defined as an individual who is required by statute to submit a sample for DNA analysis and databasing. The term 'offender' includes individuals who are convicted of or arrested for a crime or juveniles adjudicated delinquent for an offense and required by state or federal law to provide a DNA sample for analysis and databasing. [Source: Quality Assurance Standards for DNA Databasing Laboratories, FBI, *effective September 1, 2011*]

Off-Ladder Allele
An off-ladder allele is defined as an allele whose size is not represented within the allelic ladder. An allele that does not fall within the 0.5bp precision window of an allele from the corresponding locus-specific allelic ladder; these alleles are designed as 'off-ladder' (OL) alleles or microvariants. There are two types of OL alleles. The OL allele peak may be larger or smaller than the alleles spanning the allelic ladder range or it may fall in between the rungs on the allelic ladder.

Off-Scale Data
Off-scale data is data which exceeds the linear detection of the instrument.

Oligospermia
Insufficient spermatozoa in the semen.
Having a low sperm count.

Orthophosphoric Monoester Phosphohydrolase
The nonspecific orthophosphoric monoester phosphohydrolase produces a single pentaphosphate, D-*myo*-inositol-1,2,4,5,6-pentaphosphate, whereas the phytase, at both *p*H 2.0 and 5.5, produces a mixture of two pentaphosphates. The major component of this mixture is D-*myo*-inositol-1,2,4,5,6-pentaphosphate and the other is D-*myo*-inositol-1,2,3,4,5-pentaphosphate. Thus, the pathways of dephosphorylation of *myo*-inositol hexaphosphate by these two enzymes differ from that of wheat-bran phytase which forms L-*myo*-inositol-1,2,3,4,5-pentaphosphate.

Oxidation
The chemical combination of a substance with oxygen. A chemical reaction in which an atom or ion loses electrons, thus undergoing an increase in valence. Removing an electron from an iron atom having a valence of +2 changes the valence to +3.

A chemical reaction of a substance that occurs when a substance comes in contact with oxygen.

Oxidation-Reduction Reaction
A chemical reaction in which electrons are removed from one atom and given to another, the reaction must have an oxidizing agent and a reducing agent.
A chemical reaction in which two substances change one another.

Oxidizing Agent (Oxidant)
A substance that readily gives up oxygen or a substance that gains electrons in an oxidation-reduction reaction.
One of the substances required for an oxidation-reduction reaction, characterized by gaining electrons or giving up oxygen.

P (↑)

Passive Reference or Passive Reference Dye
A passive reference is a dye that provides an internal fluorescence reference to which the reporter dye signal can be normalized during data analysis. Normalization is necessary to correct for fluorescence fluctuations caused by change in concentration, volume or sample effects. [Source: Real-Time PCR: Understanding C_T]

Panels
A panel is a set of bin definitions for one or more markers; the grouping of markers in panels is determined by the kit provider. [Source: GeneMapper™ ID Software User Guide]

Patrilineal Inheritance
Patrilineal inheritance refers to the biological path of Y chromosomes in that biological sons are dependent on the Y chromosome of their biological fathers such that all males within the same paternal pedigree tree have the same Y chromosome barring any mutations. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Peak Stochastic Threshold (PST)
The peak stochastic threshold is the laboratory-defined relative fluorescence units level at which it can be assumed that peak "drop-out" does not occur, and any observed allelic peak that has no sister allele present can reasonably be believed to be homozygous.

Peak Height Ratio (PHR)
Peak height ratios are useful for distinguishing major contributors from minor contributors and as a general data evaluation tool since sister alleles typically are within 50-70% of each other.
The peak height ratios are calculated as follows:
Heterozygous Peaks: The peak height of the smaller allele divided by the peak height of the larger allele multiplied by 100, expressed as a percentage.
Major-Minor Peaks: The peak height of the largest minor allele divided by the peak height of the smallest major allele multiplied by 100, expressed as a percentage.

Peroxidase-like Activity
Enzymatic activity that catalyzes the oxidation of a substance by peroxide.
A substance that speeds up an oxidation-reduction reaction without being changed itself.

Personal Protective Equipment
Refers to protective clothing, gloves, goggles, helmets, etc., which are intended to protect the analyst from hazards from chemicals, electricity, heat, etc. which could cause job related injuries or health crisis.
Equipment that prevents wearer from hazards and/or contamination, such as gloves, lab coats, etc.

Appendix A

PCI
A chemical solution consisting of the organic solvents, phenol, chloroform, and isoamyl alcohol in a 25:24:1 mixture. PCI, when mixed with extracted cellular material, separates the heavier proteins and lipids into the organic layer while allowing the nucleic acids to move into the aqueous layer.

Personal Protective Equipment (PPE)
PPE are articles such as disposable (latex) gloves, masks, shoe covers and eye protection that are utilized to provide a barrier to keep biological or chemical hazards from contacting the skin, eyes, and mucous membranes and to avoid contamination of the crime scene.

Phenotype
A phenotype is the physical manifestation of the genetic information. For a DNA profile, the phenotype is the allele call observed on the electropherogram. A homozygous locus phenotype is represented by a single allele call.

Phosphorylcholine
A hapten used medicinally in the form of its chloride $C_5H_{15}ClNO_4P$ to treat hepatobiliary dysfunction, molecule secreted by the seminal vesicle, which has a molecular weight to 184.151
A molecule found in semen.

Plane Polarized Light
Light that has been passed through a filter allowing only light that propagates in a single plane to pass through it.
Light that has been passed through a filter.

Plasma
The clear, yellowish fluid portion of blood, lymph, or intramuscular fluid in which cells are suspended. It differs from serum in that it contains fibrin and other soluble clotting elements.
The fluid portion of blood with clotting elements included.

Plasma Cell
Any of the antibody-secreting cells found in lymphoid tissue and derived from B cells upon lymphokine stimulation and reaction with a specific antigen a cell that develops from a B lymphocyte in reaction to a specific antigen; found in bone marrow and sometimes in the blood
A cell that is formed from a reaction to a specific antigen.

Plate Record
On the Genetic Analyzer, a plate record is an instructional list of samples for the instrument defining when samples will be injected, the parameters of each injection, and information for GeneMapper ID / GeneMapper ID-X analysis.

Point of Identity
The point in species testing using the ouchterlony method of analysis where the precipitation lines of the antigen-antibody reaction connect between a known and questioned sample.
A pattern that must appear for an ouchterlony test to be called positive.

Polyclonal Antibody
A mixture of antibodies of different specificities, as in the serum of a person immunized to various antigens
A single type of antibody that is produced from different cells in the body.

Polymer
Polymer is a separating medium through which the DNA fragment travel in the capillary. For example, many STR applications use POP-4™ (Performance Optimized Polymer) which is as 4% concentration of linear, uncross-linked dimethyl polyacrylamide. As the DNA fragments travel through the polymer-filled capillary

in the presence of an electrical force, the smaller DNA fragments pass through this medium faster than larger DNA fragments.

Polymerase Chain Reaction (PCR)
A duplicating process that yields millions of copies of a desired portion of DNA through repeated cycling of a reaction, using heating/cooling and chemicals. This process enables scientists to obtain genetic information from small or degraded specimens.

In other words, PCR is an enzymatic process by which a specific region of DNA is replicated over and over again to yield many copies of a particular sequence. The PCR product is sometimes called an amplicon.

Polymorphism
Polymorphism is the condition of having or occurring in several different forms and generally occurring in greater than 1% of the population.
Polymorphisms are differences in DNA sequence among individuals; genetic variations occurring in more than 1% of a population would be considered useful polymorphisms for linkage analysis. [Source: An Introduction to Forensic DNA Analysis]

Population
A population is a group of individuals of one species that live in a particular geographic area.

Population Database
A population database is a collection of the allelic frequency data from a set of individuals from a particular population group (i.e., Caucasian, African American, and Hispanic).

Population Genetics
In general, population genetics is the study of naturally occurring genetic differences among organisms. In forensic DNA analysis, the genetic differences (alleles and allele frequencies) of STR loci are analyzed.

Population Substructure
Population substructure is the presence of smaller, isolated populations within a larger population. These isolated groups of people may or may not have fewer alleles at particular loci or may or may not have more homozygote loci than heterozygote loci because mating may not be random.

Porphyrin
Any of various organic pigments containing four pyrrole rings bonded to one another. The rings form the corners of a large flat square, in the middle of which is a cavity that often contains a metal atom. Porphyrins occur universally in protoplasm and function with bound metals such as iron in hemoglobin and magnesium in chlorophyll. Any of various organic compounds containing four pyrrole rings, occurring universally in protoplasm, and functioning as a metal-binding cofactor in hemoglobin, chlorophyll, and certain enzymes
A compound that is found in hemoglobin and is part of the iron binding process.

Positive Control
Used in scientific testing; this is where a reactant that produces a known reaction when applied to another substance.
A test where the result is expected to be positive to verify testing materials are functioning properly.

Post-Zone
Point in Ouchterlony testing where the concentration of the antigen is higher than the concentration of the antibody which inhibits the formation of precipitin bands by individual antigens connecting to both sites on the antibody, therefore not allowing them to form a chain which produces a precipitate; also called Ag-excess zone.
A situation where too much antigen is present in Ouchterlony testing, and a false negative will be observed.

Precipitin
An antibody that under suitable conditions combines with and causes a specific soluble antigen to precipitate.
The product of an antigen-antibody reaction that is insoluble and able to be seen.

Precipitation
The process by which a substance is separated out of a solution as a solid. Precipitation occurs either by the action of gravity or through a chemical reaction that forms an insoluble compound out of two or more soluble compounds.
A process that separates a solid out of a solution.

Presumptive Test
A screening test that is usually less specific but more sensitive than a confirmatory test and gives results that indicate the presence of a substance but not always to the exclusion of all other substances.
A screening test used to indicate the presence of a substance.

Preferential Amplification
Preferential amplification is the unequal sample of the two alleles present in a heterozygous locus primarily due to stochastic (random) fluctuation arising when only a few DNA molecules are used to initiate the polymerase chain reaction. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]

Primate Specific
Higher order primates exclusively, which includes humans and apes.
Anything that relates to humans, apes, chimpanzees, etc. that are in a group of closely related species.

Primer
A primer is an existing DNA chain bound to the template DNA to which nucleotides must be added during DNA synthesis.

Primer-Dimer
A primer-dimer is a product resulting at low temperatures in which primers bind to each other. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Probe
A probe is a segment of DNA or RNA which corresponds to a gene or sequence of interest. The probe is labeled radioactively or with a detectable molecule (for example, biotin) which will allow the probe label gene or sequence to be visualized.

Probability
Probability is the likelihood of the occurrence of any particular form of an event, estimated as a ratio of the number of ways or times that the event may occur in that form to the number of ways it could occur in any form. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Process Quality Value (PQV)
PQVs are quality values assigned to bins based on the success rate of the bins to located allele peaks correctly. [Source: ABI PRISM 310 Genetic Analyzer User Guide, Applied Biosystems, 2001] The PQVs are present in the “Genotypes” tab within a GeneMapper ID-X project.

Product Rule
The product rule is the multiplication of locus-specific match probabilities to generate an overall profile match probability based on locus-specific genotypes being independently inherited.

Appendix A

Proper Seal
An evidence container is “properly sealed” only if its contents cannot readily escape and only if opening the container would result in obvious damage/alteration to the container or its seal. Staples alone cannot provide a sealed condition on evidence packaging. It is acknowledged that not all evidence can be sealed inside a container. A proper seal would constituted tape sealing, heat-sealing, or lock sealing and initialing the seal. A date on the seal is also recommended. [Source: OSBI CSD Quality Manual].

Prosthetic Group
A metal ion or an organic compound (other than an amino acid) that is covalently bound to a protein and is essential to its activity.
A component of a protein that allows it to perform some function.

Prostate Gland
A gland in male mammals located at the base of the bladder. The prostate gland opens into the urethra and secretes a milky fluid that is a major component of semen. a firm partly muscular partly glandular body that is situated about the base of the mammalian male urethra and secretes an alkaline viscid fluid which is a major constituent of the ejaculatory fluid called also *prostate,* A chestnut-shaped body that surrounds the beginning of the male urethra at the base of the bladder, consists of two lobes connected anteriorly by an isthmus and posteriorly by a middle lobe lying above and between the ejaculatory ducts, controls the release of urine from the bladder, and whose milky fluid secretion is discharged into the urethra during semen emission
Organ found in male humans that stores the fluid that comprises up to one third the volume of semen.

Prostate Specific Antigen (p30)
A protein produced by the cells of the prostate gland. PSA is present in small quantities in the serum of normal men, and is often elevated in the presence of prostate cancer and in other prostate disorders. A blood test to measure PSA is the most effective test currently available for the early detection of prostate cancer. Higher than normal levels of PSA are associated with both localized and metastatic prostate cancer (CaP). Prostate specific antigen (PSA), also known as kallikrein III, seminin, semenogelase, γ-seminoprotein and P-30 antigen) is a glycoprotein manufactured almost exclusively by the prostate gland; PSA is produced for the ejaculate where it liquefies the semen and allows sperm to swim freely.[1] It is also believed to be instrumental in dissolving the cervical mucus cap, allowing the entry of sperm.[2] Biochemically it is a serine protease (EC 3.4.21.77) enzyme, the gene of which is located on the nineteenth chromosome (19q13). PSA is normally present in the blood at very low levels; normal PSA levels are defined as between 0-4.0 ng per milliliter.[5] Increased levels of PSA may suggest the presence of prostate cancer. However, prostate cancer can also be present in the complete absence of an elevated PSA level, in which case the test result would be a false negative.[6] PSA levels can be also elevated due to prostate infection, irritation, benign prostatic hypertrophy (enlargement) or hyperplasia (BPH) or recent ejaculation[7], in which case it may give a false positive. [8] It is a myth that digital rectal exam raises PSA. [9] Despite earlier findings,[10] recent research suggests that the rate of increase of PSA (the PSA velocity) is not a more specific marker for prostate cancer.[11] However, the PSA rate of rise may have value in prostate cancer prognosis. Men with prostate cancer whose PSA level increased by more than 2.0 ng per milliliter during the year before the diagnosis of prostate cancer have a higher risk of death from prostate cancer despite undergoing radical prostatectomy.[12] Most PSA in the blood is bound to serum proteins. A small amount is not protein bound and is called free PSA. In men with prostate cancer the ratio of free (unbound) PSA to total PSA is decreased. The risk of cancer increases if the free to total ratio is less than 25%. (See graph at right.) The lower the ratio the greater the probability of prostate cancer. Measuring the ratio of free to total PSA appears to be particularly promising for eliminating unnecessary biopsies in men with PSA levels between 4 and 10 ng/mL.[13] However, both total and free PSA increase immediately after ejaculation, returning slowly to baseline levels within 24 hours.
A substance found almost only in prostate gland

Protein
Any of a large class of complex organic chemical compounds that are essential for life. Proteins play a central role in biological processes and form the basis of living tissues. They consist of long chains of amino acids connected by peptide bonds and have distinct and varied three-dimensional structures, usually containing **alpha helices** and **beta sheets** as well as looping and folded chains. Enzymes, antibodies, and hemoglobin are examples of proteins.

A substance in an organism that helps regulates most biological activities.

Pro-Zone
The phenomenon in which mixtures of specific antigen and antibody do not agglutinate or precipitate visibly because of an excess of either antibody or antigen. In Ouchterlony testing the concentration of the antigen inhibits the formation of the precipitin bands by too few antigens binding with antibodies and remaining in solution therefore not allowing them to form a chain which produces a precipitate, also called Ab-excess zone.
A situation in which too much antibody is present in Ouchterlony testing and a false negative will be observed.

Pull-up
Pull-up is a peak of one color overlapping into another color causing a peak to appear in the second color at the ~same size and ~same data point. Pull-up is a result of the inability of the detection instrument to properly resolve the dye colors used to label STR amplicons due to spectral overlap of the dye colors. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Q (↑)

Qualified DNA Analyst
A qualified DNA analyst is a DNA analyst who has satisfied and continues to satisfy the experience, education, training, proficiency testing, and continuing education requirements of the FBI Director's Quality Assurance Standards (Standards 5 and 13), issued in accordance with the DNA Identification Act of 1994, as well as successful completion of a qualifying test prior to beginning casework or databasing responsibilities.

Quality Assurance
Quality assurance is a program conducted by a laboratory to ensure accuracy and reliability of tests performed. [Source: An Introduction to Forensic DNA Analysis]

Quality Control
Quality control refers to the day to day operational techniques or activities required to consistently provide accurate and reliable analytical results that fulfill the requirements for quality work.

R (↑)

Radial Immunodiffusion
A quantitative test, which involves the antigen-antibody reaction. A questioned sample of antigen (or antibody) is placed in a single well of a gel plate and allowed to diffuse and react with a known amount of antibody (or antigen). This is a single diffusion test and the concentration of antigen (or antibody) is proportional to the diameter of the precipitin ring that forms around the sample well.
A method of estimating the concentration of an antigen (or antibody) in a sample.

Random Match Probability
Random match probability is the probability or chance that a person randomly selected from the population will have an identical STR profile or combination of genotypes as the DNA loci tested. Sometimes, this can be explained by equating it to how many planet earths it would take to expect to see the profile observed once in order to help the jury understand the magnitude of a RMP.

Rape
Unlawful sexual activity and usually sexual intercourse carried out forcibly or under threat of injury against the will usually of a female or with a person who is beneath a certain age or incapable of valid consent because of mental illness, mental deficiency, intoxication, unconsciousness, or deception —see also STATUTORY RAPE NOTE: The common-law crime of rape involved a man having carnal knowledge of a woman not his wife through force and against her will, and required at least slight penetration of the penis into the vagina. While some states maintain essentially this definition of rape, most have broadened its scope esp. in terms of the sex of the persons and the nature of the acts involved. Marital status is usually irrelevant. Moreover, the crime is codified under various names, including first degree sexual assault sexual battery unlawful sexual intercourse, and first degree sexual abuse.
The act of forcing another person to have sexual intercourse against a person's will using violence, torture, or threats

Raw Data
Raw data is unanalyzed data as collected from the instrument which is used to 1) verify the presence of a primer peak, 2) evaluate the start and stop points for analysis, and 3) determine if other injection/sample issues may result in poor allele calling when analyzed.

Reagent Blank
A reagent blank is a type of negative control, consisting of only the chemicals used for a particular procedure. Reagent blanks, extracted concurrently with a set of samples, are used to ensure that there is no DNA contribution from the reagents and to monitor the analysts sample handling techniques.
A test of the chemicals used in a test to verify they are free of contamination

Real-Time PCR
Real-Time PCR is a quantitation method designed to determine the total amount of amplifiable human (and higher primate) DNA present in a sample. Reactions are characterized by the point in time during cycling when the amplification of a target is first detected rather than by the amount of target accumulated at the end of PCR. The method utilizes the amplification of a human specific DNA target.

Reducing Sugar
A sugar (as glucose, maltose, or lactose) that is capable of reducing a mild oxidizing agent, any sugar with an aldehyde or ketone functional group that can undergo oxidation.
A sugar such as glucose, which can be involved in an oxidation-reduction reaction.

Reduction
A chemical reaction in which an atom or ion gains electrons, thus undergoing a decrease in valence. If an iron atom having a valence of +3 gains an electron, the valence decreases to +2.
A chemical reaction that causes a gain of electrons.

Regions of Interest (ROI) Calibration
Regions of Interest (ROI) Calibration is the mapping of the positions of the well on the sample block so that the software can associate increases in fluorescence during a run with specific wells of the plate. Because the instrument uses a set of optic filters to distinguish the fluorescence emissions gathered during runs, you must generate a calibration image for each individual filter to account for minor differences in the optical path. [Source: Absolute Quantification Getting Started Guide, Applied Biosystems]

Relative Fluorescence Unit (RFU)
A RFU is a unit of measurement of light intensity from the fluorescently labeled pieces of DNA analyzed by a genetic analyzer.

Reporter Dye
Reporter dye is the dye attached to the 5' end of the TaqMan® probe. The dye provides a fluorescence signal that indicates specific amplification. [Source: Real-Time PCR: Understanding C_T]

Restriction enzyme
A restriction enzyme is a protein harnessed from bacteria that recognizes specific, short nucleotide sequences and cuts DNA at those sites.

Restriction fragment length polymorphism (RFLP)
RFLP is a molecular method of genetic analysis that allows individuals to be identified based on unique patterns of restriction enzyme cutting specific regions of DNA. The term polymorphism refers to the slight differences between individuals, in base pair sequences of common genes. [Source: http://biotech.about.com/od/glossary/g/RFLPdef.htm]

In other words, RFLP refers to the variation between individuals in DNA fragment sizes cut by specific restriction enzymes; polymorphic sequences that result in RFLPs that are used as markers on both physical maps and genetic linkage maps; RFLPs are usually caused by mutation at a cutting site.

Review
A review is an evaluation of documentation to check for consistency, accuracy, and completeness. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]

R_n
Normalized reporter is the ratio of the fluorescence emission intensity of the reporter dye to the fluorescence emission intensity of the passive reference dye. [Source: Real-Time PCR: Understanding C_T]

Rocket Immunoelectrophoresis
Two-dimensional immunoelectrophoresis, a combination of conventional electrophoretic separation and electroimmunodiffusion; electrophoresis is first carried out, then the electrophoretic strip is placed on a second slide and an antibody-containing agarose solution is allowed to solidify adjacent to it; electrophoresis is then performed at right angles to the original separation. A quantitative method for serum proteins which involves electrophoresis of antigen into a gel containing antibody; the technique is restricted to detection of antigens that move to the positive pole on electrophoresis.
A testing procedure.

S (↑)

Saline
A solution of sodium chloride in sterile water.
A salt-water solution.

Sample Sheet
On the 310 Genetic Analyzer, the sample sheet is a list of samples corresponding to their placement in the 310 tray. The sample sheet is then used by the collection software in the development of the injection list.

Satellite DNA
Satellite DNA is a repeated region of a DNA strand that is typically designated by the length of the core repeat unit as well as the number of repeats in the overall length of the repeat region. Satellite DNA is divided into two categories: mini-satellite and micro-satellite. Mini-satellites are VNTRs where the core repeat is 10-100 base pairs in length whereas the core repeat of micro-satellites (STRs) is 2-6 base pairs.

Saturated
Relating to an organic compound in which all the carbon atoms are joined by single bonds and therefore cannot be combined with any additional atoms or radicals. Propane and cyclopentane are examples of saturated hydrocarbons. Relating to a solution that is unable to dissolve more of a solute. Containing as much water vapor as is possible at a given temperature. Air that is saturated has a relative humidity of 100 percent.
Unable to dissolve any more of a substance.

Appendix A

SDIS
The middle level of the three levels of CODIS, the State DNA Index System (SDIS) is used at the state level to serve as a state's DNA database containing DNA profiles from the LDIS labs. [Source: Quality Assurance Standards for DNA Databasing Laboratories, FBI, *effective September 1, 2011*]

Secretor
An individual of blood group A, B, or AB who secretes the antigen characteristic of these blood groups in bodily fluids (as saliva)
People who secrete their blood types in other bodily fluids

Semen
A viscous whitish secretion of the male reproductive organs, containing spermatozoa and consisting of secretions of the testes, seminal vesicles, prostate, and bulbourethral glands.
Fluid discharged from male reproductive organs that contains sperm.

Semen Standard
A standard of known semen concentration used for testing.
A known semen concentration.

Seminal Acid Phosphatase
Is a phosphatase used to free attached phosphate groups from other molecules during digestion, It is stored in lysomes and functions when these fuse with endosomes, which are acidified while they function, therefore it has an acid pH optimum. Different forms of acid phosphatase are found in different organs, seminal AP is found in the male prostate.
Acid Phosphatase that is found in male seminal fluid.

Seminal Fluid
Semen, especially the fluid part of semen without the spermatozoa.
Semen

Seminal Plasma
The liquid portion of seminal fluid.
The liquid portion of seminal fluid.

Sensitivity
The ability of a test to detect the presence of a substance in decreased amounts or a measure of a test that can be given as a proportion of cases that have a positive test result of all positive cases.
The minimum concentration needed of a substance for a test to give a reliable result of positive (or negative).

Sera
The plural for the word serum. Blood serum extracted from an animal that has immunity to a particular disease. The serum contains antibodies to one or more specific disease antigens, and when injected into humans or other animals, it can transfer immunity to those diseases. The clear yellowish fluid obtained upon separating whole blood into its solid and liquid components after it has been allowed to clot.
The plural of serum.

Serology
The science that deals with the properties and reactions of serums, especially blood serum.
The science of the identification of blood and other body fluids.

Serum
Blood serum extracted from an animal that has immunity to a particular disease. The serum contains antibodies to one or more specific disease antigens, and when injected into humans or other animals, it

can transfer immunity to those diseases. The clear yellowish fluid obtained upon separating whole blood into its solid and liquid components after it has been allowed to clot.
The liquid portion of blood after it separates into its solid and liquid components after it has clotted.

Serum Proteins
Proteins found in the liquid portion of blood that include albumin and globulin proteins that help regulate and provide information about many body functions.
Proteins found in the liquid portion of blood.

SWGDAM (Scientific Working Group of DNA Analysis Methods)
SWGDAM was formerly known as the Technical Working Group of DNA Analysis Methods (TWGDAM). SWGDAM is responsible for making suggestions to the FBI Director with respect to the quality assurance standards.

Sequence Polymorphism
Sequence polymorphisms are variations in the population of loci determined based on the differences in the sequence of DNA fragment.

Sequencing
Sequencing is the determination of the order of base sequences in a DNA molecule.

Short Tandem Repeats (STR)
Short Tandem Repeats are multiple copies of short identical DNA sequences arranged in direct succession in particular regions of chromosomes. STRs typically have repeating units of only 2 to 6 base pairs long and therefore are easily amplified by PCR. The number of repeating units at STR loci can be highly variable among individuals, making STRs effective for human identification purposes.

Size Standard
A size standard is a collection of single-stranded DNA fragments of known lengths with an attached fluorescent dye. A standard curve is created from the size standard and the DNA fragments are sized by the local southern method. The date point from each DNA fragment peak is compared to the size standard curve to generate a DNA fragment size in base pairs. The local southern method involves using two peaks above and two peaks below the size of an unknown DNA fragment; therefore, the size standard must have two fragments of known length greater or less than every possible allele produced using a specific STR kit.

Spatial Calibration
A spatial calibration establishes a relationship between the signal emitted by each capillary and the position when that signal falls on and is detected by the CCD camera. [Source: “Safety”, “Preparing the Instrument”, and “Running the Instrument”

Spectral Calibration
A spectral calibration creates a deconvolution matrix that compensates for dye overlap (reduces raw data from the instrument) in the 4-dye, 5-dye, 6-dye, or AnyDye data stored in each sample file. [Source: Applied Biosystems 3500/3500xL Genetic Analyzer User Guide - revised 06/2010] A matrix is generated for each capillary and the mathematical reduction of overlap is performed as the sample runs on the 3500 and 3130 Genetic Analyzers rather that post-processing as with the 310 Genetic Analyzer. [Source: “Safety”, “Preparing the Instrument”, and “Running the Instrument”]

Specificity
The ability of an enzyme or receptor to discriminate among competing substrates and ligands.
The ability of an enzyme to choose a specific substrate or ligand.

Speed Vac
A Speed Vac is a device which uses vacuum pressure (and heat, as needed) to remove solvent from a solution, thereby increasing the concentration of the overall solution.

Sperm Fraction
During the differential extraction process, the sperm fraction is the remaining portion of the liquid after the epithelial fraction has been removed and the sample has been rinsed with sperm wash buffer. This portion ideally contains sperm cells which can be subjected to DTT to release the DNA.

Sperm Wash Buffer
Sperm wash buffer is a buffer consisting of 10mM Tris-HCl, 50mM NaCl, 10mM EDTA, and 2% SDS that dilutes the remaining epithelial fraction while maintaining a stable environment for the sperm pellet. After the epithelial fraction is removed, the pellet is washed three times with sperm wash buffer in attempt to isolate the sperm cells.

Spermatozoa
A sperm cell produced in the testis of an animal. The mature fertilizing gamete of a male organism, usually consisting of a round or cylindrical nucleated cell, a short neck, and a thin motile tail. Also called *sperm cell*, *zoosperm.*
Produced by male sex organs.

Spermine
A deliquescent crystalline aliphatic tetramine $C_{10}H_{26}N_4$ found in semen in combination with phosphoric acid, in blood serum and body tissues, and in yeast
A component of seminal fluid.

Spike
Air bubbles, urea crystals, or voltage spikes can cause a false peak or "spike" in a genetic analyzer. These peaks are not reproducible and should not appear in the same position if the sample is re-injected onto the capillary. An anomalous peak that can occur in capillary electrophoresis and interfere with data interpretation; this instrumental artifact is typically narrow and produces signal in multiple dye channels. [Source: Fundamentals of Forensic DNA Typing, John Butler] Although typically appearing in all colors, spikes can be present in as little as one color.

Spurring
In Ouchterlony testing is formed when partial fusion of the precipitin lines occurs. It occurs when there is a homologous antigen to the antiserum in one well and a cross reacting antigen in an adjacent well. Is also referred to as partial identity.
A formation in Ouchterlony testing that is referred to as partial identity.

Standard Curve
A standard curve is a plot of known values to compare unknown values in order to determine concentration, size, or another unit of measurement.

Sodium Trimetaphosphate (STMP)
With formula $Na_3P_3O_9$, is a metaphosphate of sodium. It has the empirical formula $NaPO_3$. It is the sodium salt of trimetaphosphoric acid.
A substrate used to test for acid phosphatase.

Stochastic Effects
The term "stochastic effects" represents a variety of issues resulting from the unequal sampling of loci and alleles with loci when an insufficient amount of template DNA is amplified. The main effects observed are extreme sloping, allelic drop-out, and extreme peak height ratio imbalance.

Stutter
Stutter products are amplicons that are typically one or more repeat units less in size than the target allele and arise during PCR because of strand slippage during primer extension. A stutter peak that is one repeat unit

shorter than the main allele is also referred to as n-4, n-2, etc. Stutter product one repeat unit longer (i.e., n+4) occurs more infrequently than n-4, n-2 stutter.

Substrate
The material or substance on which an enzyme acts. See more at enzyme. The surface on or in which plants, algae, or certain animals, such as barnacles or clams, live or grow. A substrate may serve as a source of food for an organism or simply provide support.
The material upon which a substance reacts.

Substrate controls
Substrate controls are cuttings, swabbings, etc. from an unstained adjacent material. A substrate control sample is material of a known source that presumably was uncontaminated during the commission of the crime (e.g., a sample to be used in laboratory testing to ensure that the surface on which the sample is deposited does not interfere with testing. For example, when a bloodstain is collected from a carpet, a segment of unstained carpet would be collected).

T (↑)

Taq Polymerase
Taq polymerase is the enzyme used to copy DNA in the polymerase chain reaction (PCR) technique. [Source: An Introduction to Forensic DNA Analysis]

Technical Review
A technical review is an evaluation of reports, notes, data, and other documents to ensure there is an appropriate and sufficient basis for the scientific conclusions. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, *effective September 1, 2011*]

Technical Reviewer
A technical reviewer is an employee who is a current or previously qualified analyst in the methodology being reviewed that performs a technical review of, and is not an author of, the applicable report or its contents. [Source: Quality Assurance Standards for Forensic DNA Testing Laboratories, FBI, e*ffective September 1, 2011*]

Thermal Cycler
A thermal cycler is an instrument used for the PCR process that has the ability to rapidly and accurately increase or decrease the temperature of the samples.

Theta (θ)
Theta is a correction factor incorporated into forensic DNA calculations to adjust for population substructure. For a normal population 0.01 is typically used while 0.03 is used for isolated populations.

TWGDAM (Technical Working Group of DNA Analysis Methods)
TWGDAM is now known as the Scientific Working Group of DNA Analysis Methods. TWGDAM was the original group responsible for establishing guidelines for DNA analysis quality assurance standards. These guidelines were prepared in a document titled “Guidelines for a Quality Assurance Program for DNA Analysis” Crime Laboratory Digest (vol. 22, No. 2, April 1995).

Template DNA
Template DNA is the extracted DNA that is being subjected to amplification. During the PCR process copies of specific locations of the DNA will be generated by using the genetic information from the “template”.

Temporary Evidence Closure or Seal
A seal consisting of tape across a box, a paper clip on a folded evidence envelope, or some other closure that would not normally constitute a proper seal of evidence. Acceptable when an analyst will be away from the work area for a short period of time or overnight as long as the evidence is secured in a locking drawer or controlled access evidence area. [Source: OSBI CSD Quality Manual]

Threshold (as relevant to quantitation)
Threshold is a level of ΔRn used for the C_T determination in real-time assays. The level is set to be above the baseline and sufficiently low to be within the exponential growth region of the amplification curve. The threshold is the line whose intersection with the amplification plot defines the C_T (threshold cycle). [Source: Real-Time PCR: Understanding C_T]

Threshold Cycle (C_T)
Threshold cycle is the fractional cycle number at which the fluorescence passes the threshold. [Source: Real-Time PCR: Understanding C_T]

Titer
The concentration of a substance in solution or the strength of such a substance as determined by titration. The minimum volume of a solution needed to cause a particular result in titration. The concentration of antibodies present in the highest dilution of a serum sample at which visible clumps with an appropriate antigen are formed.
Concentration of a substance in solution which determines how much of another substance can be added to give a positive reaction.

Trace evidence
Trace evidence is the physical evidence that results from the transfer of small quantities of materials (e.g., hair, textile fibers, paint chips, glass fragments, and gunshot residue particles).

Trisomy
Trisomy is the presence of three copies of a chromosome instead of the normal two copies within a cell.

U (↑)

Ultraviolet Light
Radiation lying in the ultraviolet range; wave lengths shorter than light but longer than X rays, **Ultraviolet** (**UV**) light is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than soft X-rays. It can be subdivided into **near UV** (400–200 nm wavelength; abbrev. NUV), **far** or **vacuum UV** (200–10 nm; abbrev. FUV or VUV), and **extreme or deep UV** (31–1 nm; abbrev. EUV or XUV).
Light that is similar to sunlight

V (↑)

Vaginal Acid Phosphatase
Is a phosphatase used to free attached phosphate groups from other molecules during digestion, It is stored in lysomes and functions when these fuse with endosomes, which are acidified while they function, therefore it has an acid pH optimum. Different forms of acid phosphatase are found in different organs, vaginal AP is found in females.
Acid Phosphatase found in the female vagina area.

Vas Deferens
Either of two ducts through which sperm passes from a testis to the outside of the body. In mammals, the vas deferens connects the testis to the urethra. The main secretory duct of the testicle, through which semen is carried from the epididymis to the prostatic urethra, where it ends as the ejaculatory duct. Also called *deferent duct*, *spermatic duct*, *spermiduct*, a sperm-carrying duct especially of a higher vertebrate that in humans is a small but thick-walled tube about two feet (0.6 meter) long formed by the union of the vasa efferentia, is greatly convoluted in its proximal portion, begins at and is continuous with the tail of the epididymis, runs in the spermatic cord through the inguinal canal, and descends into the pelvis where it joins the duct of the seminal vesicle to form the ejaculatory duct called also *ductus deferens, spermatic duct*
Tubes that connect to the male reproductive organs in order to move sperm.

Variable number of tandem repeats (VNTR)
Repeating units of a DNA sequence, the number of which varies between individuals; sometimes referred to as mini-satellite; typically analyzed by restriction fragment length polymorphism (RFLP) methods; can range in size from approximately 500bp to greater than 20,000bp. [Source: Fundamentals of Forensic DNA Typing, John Butler]

W (↑)

Well
A hole in agar or on a plate where a sample or reagent can be placed for testing.
A hole in a plate or substance in order to put a liquid sample for testing.

X (↑)

Y (↑)

Y chromosome
The Y chromosome is one of the two sex chromosomes. The Y chromosome is located only in males.

Y-Screen Assay
The Y-screen assay, a “Direct to DNA” approach. The Y-screen assay is a confirmatory test that is widely accepted and used throughout the forensic DNA community to screen sexual assault kits for the presence of male DNA, even in the presence of high concentrations of female DNA.
The stop-go determination as to if male DNA is present in a sample, i.e., red-light (stop analysis)/green-light (conduct additional DNA testing).

Y-STR
Y-STRs are STR markers located on the Y chromosome that enable male-specific DNA testing and can be useful in cases involving sexual assault; also used in genetic genealogy to trace male lineages. [Source: Fundamentals of Forensic DNA Typing, John Butler]

Sources: www.dictionary.com, various textbooks including Molecular Cell Biology and Principles of Biochemistry.

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Appendix C

Monthly Training Memorandum Template

Example Monthly Training Memorandum

MEMORANDUM (on official letterhead stationary)

TO: Criminalistics Administrator
THROUGH: Technical Manager
FROM: Trainer
DATE: Date
SUBJECT: Monthly Training Update for (Name)

This report reviews and evaluates the forensic biology training of (Name) for the month of _____.

1. A summary of the progress/training completed during the month

2. An evaluation of the trainee's notebook

3. An evaluation of the progress/training completed during the month, to include:
   a. Problem areas identified during the month
   b. Trainee's strong points
   c. Trainee's weak points and suggested remedies
   d. Statement concerning trainee's overall performance

4. Plans for the upcoming month

cc: Trainee
Trainee's Supervisor
Quality Manager (if different from Criminalistics Administrator reported to)