

## ADVANCED DNA TECHNOLOGIES TRAINING OBJECTIVES

Following the *Introductory Capillary Electrophoresis* and *Forensic DNA Analysis* lectures the student shall:

1. Recognize tandem repeats and the mitochondrial D loop as significant polymorphic DNA sequences in forensic biology.
2. Recall the mechanism of the polymerase chain reaction.
3. Examine various amplification chemistries used in forensic PCR applications.
4. Identify multiple methods of analyzing STR products generated by PCR.
5. Compare and contrast ABI capillary electrophoresis equipment.
6. Comprehend basic capillary electrophoresis concepts such as:
  - a. Equipment component parts
  - b. Electrokinetic injection
  - c. Migration of STR fragments from electrode to cathode
  - d. Capillary window alignment with laser and detection window
  - e. Spectral overlap and matrix correction
  - f. Co-electrophoresis internal size standard
  - g. Multi-colored electropherogram
  - h. Off-scale data
  - i. Allelic ladder

During the *Advanced Capillary Electrophoresis* lecture the student shall:

1. Identify compounds that can compete with DNA fragments during electrokinetic injection and how to overcome.
2. Recognize factors that directly impact DNA separations.
3. Recall fundamental sieving polymer concepts.
4. Identify important chemical components in the polymer and buffer which affect denaturation of STR fragments.
5. Understand the negative effects created by charges on the capillary column internal surface.
6. Appreciate negative factors inherent in amplification such as peak height imbalances, stochastic effects, stutter and  $n-1$  (-a) peaks.

Following the *Functional Uses of GeneMapper™ ID* lecture the student shall:

1. Be familiar with GeneMapper™ *ID* vocabulary.
2. Identify the primary user interface window.
3. Identify the Samples and Genotypes tabs as well as common functions performed from each.
4. Appreciate the significance of the GeneMapper Manager.
5. Comprehend the five GeneMapper software algorithms.
  - a. Peak detection
  - b. Size matching and calling
  - c. Binning
  - d. Allele calling
  - e. Quality Value Determination
6. Outline the basic process of software algorithm flow from peak detection to allele call.
7. Differentiate when to use the advanced or classic modes of analysis.
8. Grasp the importance of determining analysis ranges.
9. Value the difference of the Local Southern Size Calling Method.
10. Appreciate the process of baselining.

During the **GeneMapper™ ID Tutorial** exercise the student shall:

1. Follow trainer demonstration to navigate through GeneMapper ID software.
2. Become familiar with GeneMapper primary interface and successive analysis windows.
3. Use basic software analysis functions.
4. Explore user-driven analysis parameters.

During the **Quantifiler™ Quantitation of DNA Extracts** exercise the student shall:

1. Develop and demonstrate appropriate pipetting procedures imperative to a successful standard curve.
2. Appropriately use kit components following written procedure.
3. Use new instrument and associated software to perform quantification of sample extracts.
4. Become familiar with sequence detection system (SDS) software to operate.
5. Observe automated liquid handler dispensing of real-time PCR reagents into 96 well tray.

During the **3100 & 3130 Hands-On Exercises** exercise the student shall:

1. Recognize significant 3100 & 3130 component parts and relate lecture information regarding function of these parts.
2. Identify appropriate 3100 & 3130 components for removal and cleaning.
3. Develop and demonstrate skills using 3100 & 3130 components and reagents.
4. Follow trainer demonstration to proceed through 3100 & 3130 cleaning and set-up.
5. Comprehend appropriate use of calibration procedures and execute.
6. Become associated with Data Analysis v2.2 and v3.0 software, which operates the 3100 & 3130 instrumentation.
7. Follow written procedures and use appropriate laboratory techniques to prepare 3100/3130 spectral calibration and amplified STR fragment run plates.
8. Follow written procedures to run and assess spectral and spatial calibrations.
9. Follow written procedures to run 3100 & 3130 amplified STR fragment sample plates.

During the **Real-Time PCR Lecture** lecture the student shall:

1. Compare and contrast traditional end point quantification and real-time PCR quantification.
2. Recall the phases of PCR.
3. Identify the appropriate phase of PCR amplification of interest in real-time applications.
4. Differentiate between the two sequence detection chemistry systems.
5. Distinguish the difference between absolute and relative quantification using real-time PCR.
6. Examine the mechanism of the 5'-Nuclease Assay.
7. Appreciate the significance of the 5'-exonuclease activity of the Amplitaq Gold DNA Polymerase in the 5'-Nuclease Assay.
8. Recognize the fundamental FRET principal central to the 5'-Nuclease Assay.
9. Explore Taqman® probe characteristics and function.
10. Discuss the mechanism of the SYBR Green 1 Assay.
11. Comprehend how the fluorescent signal is acquired and used to obtain quantification information.
12. Become familiar with real-time PCR vocabulary and metrics.
13. Ascertain the target genes associated with the Quantifiler™ total human kit and Quantifiler™ male kit.
14. Acknowledge the quality metrics obtained from the standard curve and appreciate their importance.

During the **Quantifiler™ Data Analysis** exercise the student shall:

1. Follow trainer demonstration to navigate through 7500 sequence detection software (SDS).
2. Become familiar with SDS analysis windows and use basic software functions.
3. Explore quality metrics obtained from the standard curve.
4. Observe characteristics of quality data and poor data.
5. Examine internal positive control data and determine how it is properly used.

During the **CE Artifact I.D** lecture and demonstration the student shall:

1. Differentiate biological and technological artifacts.
2. Become familiar with characteristics of individual artifacts.
3. Recognize and distinguish between various C.E. artifacts.

During the **Advanced Use of GeneMapper™ ID Functions** exercise the student shall:

4. Become familiar with process component-based quality values.
5. Discuss how GeneMapper™ ID can be used as an expert system.
6. Recognize the significance of genotype quality (GQ) and sizing quality (SQ).
7. Examine how to quickly access poor quality samples and review.
8. Explore concordance check against internal positive control or kit positive control.

During the **GeneMapper™ ID Practical Exam** the student shall:

1. Demonstrate the ability to navigate through GeneMapper™ ID software.
2. Create a GeneMapper ID project and analyze raw data files (.fsa).
3. Appropriately identify C.E. artifacts such as spikes and spectral pull-up in samples.
4. Recognize microvariants and follow procedure to determine correct allele call.
5. Produce concordant allele calls following analysis demonstrating skill level with software.
6. Gain experience and confidence in their skills and abilities with the software.