President's DNA Initiative – DNA Analyst Training
Laboratory Training Manual

Subject 6: STR Data Analysis and Interpretation

Colorado Bureau of Investigation 2015

Laboratory Training Manual

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Purpose
To instruct the trainee to analyze, interpret, and troubleshoot forensic DNA data, utilizing the appropriate software programs and SOPs.

Objectives
Upon successful completion of these exercises, the trainee will be able to:

- Operate relevant instrument software
- Create, apply, and evaluate parameters to include:
  - Matrices
  - Analysis parameters
  - Sizing standard (defining)
- Evaluate previously analyzed data
- Demonstrate the ability to recognize acceptable and unacceptable data per laboratory SOPs
- Identify and troubleshoot artifacts or anomalies
- Evaluate and interpret single source and mixed samples per the laboratory's SOPs
- Evaluate and interpret data from training samples and mock case(s).

Preparation for Exercises

Trainer Responsibilities
1. Assign samples, data sets, and/or mock cases to be evaluated, analyzed and interpreted, as outlined in the Individual Training Plan.
2. Demonstrate the use of the relevant software.
3. Observe the trainee using the software.
4. Provide the trainee with data sets that include the artifacts and anomalies outlined in Exercise 2.
5. Determine the assessment criteria.
6. Review, verify, and document exercise completion.

Trainee Responsibilities
1. Observe the use of the software.
2. Perform the assigned exercises.
3. Document and submit exercise completion, as required by the trainer.

Literature-see link in portal

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Exercise 1: Analysis Parameters

Purpose

To establish analysis parameters to include creation and application of a matrix file and definition of a size standard.

Tasks

- View Applied Biosystems Operator Training CD/DVD for GeneMapperID (obtain from Technical Leader)
- Create and apply a matrix file to sample data
- Define and apply a size standard
- Compare analysis parameters, as outlined in the SOPs, with those defined in the software program

Resources

Laboratory Manuals

User Manuals: Applied Biosystems

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Exercise 2: Practice Data

Purpose

To perform data evaluation and troubleshooting on provided practice data, to include the identification of artifacts and anomalies.

Tasks

Evaluate data
- Identify artifacts and/or anomalies:
    - Spurious peaks (spikes, dye blobs, noise)
    - Artifacts (3'-A nucleotide addition, stutter, pull-up)
    - Microvariants and off ladder alleles
    - Allele drop-out
    - Imbalanced alleles (stochastic/preferential amplification)
    - Background noise – raw and analyzed data
    - Off scale data – raw and analyzed data
- Identify other factors:
    - Mislabeled peaks due to incorrectly labeled internal size standard
    - Controls (determine if they meet the criteria established in the laboratory's SOPs)
    - Degradation
    - Balance of internal size standard
    - Identification of single source and mixed samples

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Exercise 3: Data Evaluation

Purpose

To evaluate and troubleshoot data obtained from previous exercises to determine if it is suitable for interpretation as defined by the laboratory's SOPs.

Tasks

Evaluate the following:
- Internal size standards from each sample
- Allelic ladders from each run
- All controls to ensure the expected results are obtained
- Each sample for the presence of extraneous peaks
- Maximum and/or minimum threshold for each sample
- Heterozygote peak height percentage
- Single source versus mixed sample

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Exercise 4: Data Interpretation

Purpose

To interpret data obtained from previous exercises as outlined in the laboratory's SOPs.

Tasks

Identify and interpret single source samples:
- Confirm allele calls
- Determine suitability for CODIS upload or application of statistics, if appropriate

Identify and interpret mixed samples:
- Determine minimum number of donors
- Determine the appropriate category:
    - Mixture with major and minor component(s)
    - Mixture with known contributor(s)
    - Mixture with indistinguishable contributor(s)
- Assess percent contribution and peak height percentages if necessary
- Determine suitability for CODIS upload or application of statistics, if appropriate

Note: Statistics is covered in a separate section of the Laboratory Training Manual.

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Exercise 5: Conclusions

Purpose

To provide conclusions based on the analyzed and interpreted data following the laboratory's SOPs.

Tasks

Provide conclusions from data sets in previous activities following the laboratory's SOPs to include:

- Inclusion/match
- Exclusion/non-match
- Inconclusive/uninterpretable
- No results

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Subject Review

After completion of the laboratory manual exercises and having previously completed the corresponding theory modules, the trainee should be able to answer the following questions:

- What is a matrix file? What is its purpose?
- What is multicomponent analysis?
- What are the names of the dyes used for each PCR system used in the laboratory?
- What is the purpose of a size standard? What are the indications of an improperly applied size standard?
- What sizing method is used in the laboratory?
- What causes a raised baseline? How is it corrected?
- What is the benefit(s) of evaluating raw data?
- What steps are taken to verify that each tube or well contains a sample?
- What causes spikes? How are they identified?
- Why is off-scale data problematic?
- What is stutter? How is it identified?
- What factors contribute to increased noise?
- What is an acceptable signal-to-noise ratio?
- How does the software make the allele assignment(s)? Can this be accomplished without software?
- What is the purpose of the allelic ladder?
- What is a microvariant?
- What is a virtual allele?
- How is a mixture identified?
- What causes peak height imbalance?
- How is degradation detected?
- What are the mixture categories and how are they determined?
- What dye is used for an internal size standard? What pattern of peaks is exhibited by the internal size standard?
- What is pull-up? How is it recognized and resolved?
- What are some examples of alleles that fall outside the allelic ladder?
- What are the characteristics of nonspecific amplification?
- What is a heteroduplex?
- What is non-template nucleotide addition? How can this be corrected?
- What is the profile of the known controls at each locus?
- What is the size range for each locus?
- What are the laboratory's SOPs for minimum and maximum threshold?
- What are the criteria for accepting data from controls per the laboratory's SOPs?
- How are peak height percentage and percent contribution used in data interpretation? Why?
- What is the laboratory's SOP for peak height percentage in a single source sample? Mixed sample?
- What is the laboratory's SOP for dealing with anomalies or artifacts in sample data?
- How is a mixture determined?

- How many non-matching loci are required to declare an exclusion per the laboratory SOPs?
- What are the SOPs for declaring the following:
    - Inconclusive/uninterpretable results
    - No results