

DNA Analyst Training Laboratory Training Manual

Protocol 6.02
PCR: Interpretation



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S
DNA
INITIATIVE



INTRODUCTION

Interpretation is used to define genotypes for comparison purposes. Analysts must determine whether a profile from a specific individual matches or does not match another profile, or that a specific profile can be excluded or cannot be excluded from a mixture of DNA profiles. All probative inclusionary statements must be accompanied by a statistical evaluation of the match.

SAFETY CONSIDERATIONS

Standard Laboratory Practices

PREPARATIONS

Not required

INSTRUMENT SPECIFICATIONS

No instrumentation required

COMPUTER SOFTWARE

GeneScan Analysis Software, version 2.1 or higher
Genotyper Analysis Software, version 2.0 or higher
CODIS, Popstats

MINIMUM STANDARDS AND CONTROLS

1. A successful electrophoresis run must contain a usable allelic ladder, usable ROX internal sizing standard, and a correctly typed positive amplification control. If an electrophoresis run is deemed unusable and no data is analyzed, documentation in the case file must include the date of the run, a list of the samples, and reason the run was not used for interpretation. The entire run must be permanently archived on CD.
2. If the allelic ladder demonstrates low RFUs, a 10 second injection can be conducted.
3. Negative amplification controls and manipulation blanks must not exhibit reproducible peaks greater than 150 RFU in any of the dyes within the size ranges covered by any of the loci for each amplification.
4. For each amplification and electrophoresis run, the positive amplification control must type correctly at all loci (see Profiler Plus and COfiler controls). If multiple

injections are made of a control sample in a specific electrophoresis run, only a copy of the injection used to validate the run or amplification needs to be retained in the case file.

PROCEDURE

Inclusions can be reported as “match” or as “cannot be excluded.” All probative inclusionary statements must be accompanied by a statistical evaluation of the match. Statistics are calculated using random match probability on genotypes from loci with alleles over 150 RFU.

Exclusions can be reported as “does not match” or “can be excluded.” Exclusions can be determined using data below the interpretation threshold of 150 RFU for peak intensities above 50 RFU. Lowering the threshold from 150 RFU to 50 RFU should be considered to aid in the exclusion of an individual when necessary. Data in the range of 50 RFU to 150 RFU will only be used for exclusionary purposes.

Match: For a single source profile: A single interpretable genotype(s) is present in the unknown at the loci being used for interpretation. The genotypes of the standard are the same as the genotypes of the unknown at each of these loci.

For a mixed DNA profile: A single interpretable genotype (major and/or minor) is differentiated from a mixture at the loci being used for interpretation. The genotypes of the standard are the same as the genotypes of this differentiated unknown at each of these loci.

Does not match: Failure to meet the above criteria

Cannot be excluded: More than one possible genotype is present in the unknown above the 150 RFU threshold at the loci being used for interpretation. The genotypes of the standard are contained in the possible genotypes at each of these loci. (This includes loci with allele, inc calls).

Can be excluded: More than one possible genotype is present in the unknown at the loci being used for interpretation. The alleles of the standard are not contained in these genotypes. The standard possesses alleles that are foreign to the unknown that cannot be explained by allelic dropout; however, the number of contributors to a mixed sample will be taken into consideration when excluding based on allelic dropout.

Inconclusive: Alleles of the standard are present in the unknown, but no statistical evaluation of this match can be made due to the possibility of allelic dropout.

ASSESSMENT OF DATA

1. Interpret with caution any DNA profile which exhibits the following, since incomplete genotype results may be present for some loci:

Degradation: A marked decrease in peak heights from smaller to larger basepair loci (left to right) within a given dye.

Inhibition: Locus specific peak height diminishment and/or allele (locus) dropout.

Preferential amplification: Uneven amplification of alleles within a locus attributable to large differences in base pair size.

2. Each sample will be examined for artifacts that fall within the analytical range and may be present at a given base pair size in two or more dyes. A second injection is only required if an artifact is sized as an allele (either "OL allele" or a numeric designation) at a locus.
3. Each sample will be examined for fluorescent pull up. Pull up is defined as a peak caused by under compensation of the matrix, and present in one or more dyes within 0.5 basepair of a large allele from another dye. Peaks determined to be fluorescent pull up must be noted and do not require another injection. If they appear often, a new matrix should be developed.
4. It may be necessary to interpret data across more than one electropherogram.
5. Each analyst is required to search all open profiles with at least six loci against the Forensic Biology DNA Analyst/Technician/Intern DNA Database. If a potential hit is made, the analyst will notify the Statewide Technical Leader.

ASSESSMENT OF A PEAK

1. Alleles are defined as peaks greater than 150 RFU with an allelic designation by Genotyper. Off-ladder microvariants are included in this definition.
2. Interpretation of off scale data and peaks less than 150 RFU will be conducted with caution.
3. Peaks may be considered stutter in Profiler Plus if their peak height percentages to the larger peaks are equal to or less than 12% at the D8S1179, D5S818, D13S317 and D7S820 loci; equal to or less than 15% for the

D3S1358, D21S11, vWA and FGA loci and equal to or less than 18% at the D18S51 locus given on scale data for the primary allele.

Peaks may be considered stutter in COfiler if their peak height percentages to the larger peaks are equal to or less than 10% at the CSF1PO locus, equal to or less than 12% at the D7S820 locus, equal to or less than 15% for the D3S1358 and D16S539 loci, and equal to or less than 7% at the TH01 and TPOX loci given on scale data for the primary allele.

4. Peaks located at a position one base pair smaller than a major peak will be interpreted as incomplete A nucleotide addition and noted as such on the electropherogram.
5. Peaks representing true alleles that have been designated as "OL- allele" by Genotyper will be evaluated against the allelic ladder and an allelic assignment made. The allelic ladder used to provide the allelic assignment must be included in the case file. When making allelic assignments, analysts must consider potential variations in migration due to the time span between the ladder run and the sample run. This allelic assignment will be listed on the Summary Worksheet and the Summary of DNA Analytical Results table.

Alleles outside the ladder range will need to be entered into CODIS and Popstats as < the smallest allele or > the largest allele for that locus.

SINGLE PROFILES

1. Single source profiles exhibit no more than two alleles at a given locus unless it is considered tri-allelic.
2. Caution must be exercised when alleles are present at or below 214 RFU (224 RFU for D18S51 and 234 RFU for FGA). In such cases, a sister allele of a heterozygote pair could fall below the threshold for calling an allele. Thus, the sister allele would go unlabeled.

MIXTURES INVOLVING TWO CONTRIBUTORS

1. Heterozygote Model
 - A. The heterozygote model asserts that the peak height of heterozygote alleles generally fall within 70% of each other at all loci with the exception of the D18S51 and the FGA loci. At the D18S51 locus, heterozygote alleles generally fall within 67% of each other, and at the FGA locus, generally within 64%.
 - B. The heterozygote model will be applied when attempting to differentiate mixtures consisting of two contributors.
 - C. The heterozygote model will be applied with caution when attempting to differentiate mixtures exhibiting off-scale data, degradation, inhibition or low levels of DNA.
2. Known Contributors to Mixtures

The assumption of a profile within a mixture should be used with caution. This would include, but not limited to, the following:

 - A. An individual's profile on a swabbing from his/her body.
 - B. An owner's profile on articles of clothing known to have been worn by the owner at the time of the incident.
 - C. An individual's profile on an article which that individual has had a verifiable intimate and timely association with. These may include such items as towels, facial wipes, mouths of bottles, etc.
3. Differential Extractions Exhibiting Carry-Over
 - A. Any two person mixture which may be present in an F1 or F3 need not be differentiated, so long as a single source profile is identified or deduced in the F2 fraction, and the alleles in the F1 or F3 are consistent with a mixture of the victim and the F2 profile. Such mixtures can be reported as single source male profiles.
(Refer to pdi_lab_pro_8.02.doc, DNA Report Wording.)
 - B. Any mixed F2 fraction which contains a single source profile and low levels of additional alleles that are consistent with the F1 profile, can be interpreted as the single source male profile. Such mixtures can be reported as single source male profiles.
(Refer to [pdi lab pro 8.02](#), DNA Report Wording)

- C. Any three person mixture which may be present in an F1 or F3 need not be differentiated, so long as a mixed F2 fraction is interpreted as two male profiles, and the alleles in the F1 or F3 are consistent with a mixture of the victim and the two male profiles.

4. Determination of Minor Genotypes

Caution must be used when determining a minor genotype when only one minor allele is detected above threshold. The following situations must be taken into consideration when determining the presence of a possible sister allele:

A. Sister allele falling below threshold

A single allele whose peak height is below 214 RFU (D18S51: 224 RFU, FGA: 234 RFU) may have a sister allele which falls below the interpretation threshold of 150 RFU. In such cases, a 10 second injection may be used to bring a possible sister allele into the interpretable range. If no such allele is detected, then the minor genotype will be interpreted with caution. It may be necessary to lower the GeneScan and Genotyper thresholds to 50 RFU to aid in interpretation for exclusionary purposes only.

B. Sister allele sharing with a major allele

A sister allele may be masked beneath a major allele present at a given locus. Utilization of the heterozygote model when evaluating that locus may help discern its presence or absence and assist in determining the possible genotypes for that locus.

C. Sister allele in a stutter position

A sister allele may be present in a stutter position and filtered as stutter. All peaks filtered as stutter should be evaluated both in terms of peak height percentage to the observed minor allele present at the locus and in terms of peak height percentage to its associated major peak.

- The lower the height of the stutter peak in relation to the observed minor allele, the less likely it is that a real allele is present but masked in the stutter position.
- The higher the height of the stutter peak in relation to the observed minor allele, the more likely it is that a real allele is present but masked in the stutter position.

MIXTURES INVOLVING GREATER THAN TWO CONTRIBUTORS

These mixtures will be evaluated to determine whether a given profile (individual) can or cannot be excluded as a potential contributor, and to assign a statistical weight to those profiles (individuals) which cannot be excluded as potential contributors.

1. A major contributor(s) can be separated from a three person mixture provided it is a readily apparent major. The minor contributors will be evaluated using all of the alleles (major and minor) present in the mixture using the guidelines below.
2. If a major contributor cannot be separated from a mixture, the mixture will be evaluated using peaks above 150 RFU at the core loci and between 50 - 150 RFU at the remaining loci. The core loci for Profiler Plus are defined as D3S1358, vWA, D8S1179, and D5S818. The core loci for COfiler are defined as D3S1358, TH01, and TPOX.

If any of these alleles above 150 RFU were not called by Genotyper due to stutter filtering, the peak height ratio in relation to the adjacent peak will be evaluated to determine if it represents a true allele. If it is determined that it represents a true allele, it will be listed on the Summary of Analytical Results table in parenthesis as an allele which may be present.

INCLUSIONS

A given profile (individual) will be included as a potential contributor to a mixture, if:

- A. all of its alleles are present at or above 150 RFU at the four Profiler Plus core loci or (when available) at the three COfiler core loci.

- AND -

- B. all of its alleles are present at or above 50 RFU at the remaining loci.

Note: For samples exhibiting degradation, consideration must be given for the possibility of allele/locus dropout.

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