DNA: Sample Evaluation

Evaluate each sample

Prior to the interpretation of DNA data, analysts should evaluate each sample to determine the overall suitability of the sample for interpretation. In particular, analysts should evaluate

- allele designations
- peak height ratios exhibited by the alleles using Identifiler® Plus
- quality of the DNA profile

Threshold values

Detection Threshold: The detection threshold, determined by internal validation studies, defines the minimum peak height where a true allelic peak can confidently be distinguished from baseline noise or artifacts. Peaks that are above the threshold may be designated as an allele; peaks that are below the threshold will not be interpreted.

The following thresholds have been set within GeneMapper® ID-X:

- Identifiler® Plus- 50 RFUs
- Yfiler®- 75 RFUs

Stochastic Threshold: The stochastic region, determined by internal validation studies, is the region in which uneven PCR amplification can lead to allele dropout. The stochastic threshold for Identifiler® Plus will be set at 225.5 RFUs and peaks in the range between 50 and 225.5 RFUs are within the stochastic region.

- The analyst should assume that peaks within this range have heterozygote partner peaks that may have dropped out.
- The minimum peak height to designate a single peak as a homozygous genotype is 225.5 RFUs.

Allele designations

The allelic ladder provided in the PCR Amplification Kit contains the majority of alleles for each locus. The alleles contained in the allelic ladder are named by the kit manufacturer. Allele designations are in accordance with the recommendations of the DNA Commission of the ISFH (DNA Recommendation, 1994; Bar et al., 1997). The number of complete 4-bp repeat units observed is designated by an integer.

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Tri-allelic patterns

Tri-allelic patterns are occasionally observed at a single locus in a multiplex STR profile. The three peaks are not the result of a mixture but are reproducible alleles in the sample due to extra chromosomal occurrences or mutations. The three peaks seen at a particular locus may or may not be equal in intensity. The tri-allelic designation should be confirmed using at least two sample injections or be present in multiple samples.

Null alleles

A null allele is an allele that

- is not detected
- exhibits extreme heterozygote peak height imbalance due to a source of genetic variation at or near the primer binding site, or
- has significantly reduced peak height ratio due to a mutation in the primer-annealing region or a deletion

Null alleles can be observed in one or both of the alleles at a locus.

Software allele assignment

Genemapper® ID-X software uses virtual bins to predict the locations of alleles. Therefore, the software may assign alleles not present, both inside and outside of the range of the allelic ladders. An analyst may report the allele designated by the software even if it is not in the allelic ladder.

Alleles smaller than the lowest virtual bin (A) in the allelic ladder for that locus will be designated as "<A." Alleles larger than the largest virtual bin (B) in the allelic ladder for that locus will be designated as ">B."

Variant alleles

Non-artifactual variant alleles will generally conform to the same characteristics as other alleles. The analyst may choose to re-amplify and type any sample containing a peak not properly interpreted as an allele by the software (i.e. an "OL" or off-ladder allele), especially if it is not appropriately balanced with an associated allele or at a height expected for a homozygote.

When a non-artifactual off-ladder allele occurs within the ladder region, the allele designation will be determined by interpolation. The off-ladder allele designation should be confirmed using at least two sample injections or be present in multiple samples.

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Alleles containing partial repeat

Some peaks may represent variant alleles containing a partial repeat.

Variant alleles that contain a partial repeat are designated by a decimal point followed by the number of bases in the partial repeat. For example, an FGA 26.2 allele contained 26 complete repeat units and a partial repeat of 2 bp.

Variants in Yfiler®

It is possible to observe variants when typing Y-STR profiles that include but are not limited to

- deletion of portions of the Y chromosome that may affect one or more loci
- duplication of a locus
- deletion of a single base (for example, microvariant)
- transversion of a base that can affect mobility
- triplication or quadruplication

For current information regarding observed genetic anomalies, see the Y Chromosome Haplotype Reference Database website at http://yhrd.org/or the National Institute of Standards and Technology website at http://cstl.nist.gov/biotech/strbase/.

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Identifiler® Plus peak height ratios

The peak height ratio of heterozygote alleles for Identifiler® Plus is defined as the ratio of the shorter peak's height to the taller peak's height, expressed as a percentage.

On average, the quality of the peak height ratio is relative to the height of the observed alleles. For example, two alleles with peak heights greater than 1000 RFUs will generally have a more balanced (closer to 100%) peak height ratio than two alleles with peak heights less than 200 RFUs. The following table outlines the expected peak height ratios for varying peak heights based on the laboratory's internal validation studies.

Taller peak > 700 RFUs	Expected minimum 55% PHR
Taller peak ≤ 700 RFUs and > 400 RFUs	Expected minimum 35% PHR
Taller peak ≤ 400 RFUs and > 225 RFUs	Expected minimum 25% PHR
Taller peak ≤ 225 RFUs	No expected minimum (second allele could have dropped out)

When a peak height ratio is observed below the percentages listed in the table above, it is either an indication of a mixture or it is a non-mixed sample which can occasionally exhibit peak height ratio imbalance. Peak height ratios for a non-mixed sample vary between the different PCR amplification kits.

Imbalances in heterozygote peak height ratios may be due to

- degraded DNA
- inhibited DNA
- widely–spread alleles
- stochastic effects
- the presence of an allele containing a rare primer binding site variation that does not amplify as efficiently as the other allele

Quality of the DNA profile

The quality of the DNA profile developed for each sample should be evaluated. The guidelines are found in *DNA: Profile Quality*.