DNA: Profile Quality

Quality of the DNA profile

The quality of the DNA profile developed for each sample should be evaluated for

- off-scale data
- elevated baseline
- artifactual peaks
- stutter peaks
- non-template addition
- degradation
- inhibition
- stochastic effects

Off-scale data

When too much sample DNA is added to the PCR reaction, the PCR products may exceed the linear dynamic range for detection by the instrument. This is referred to as "off-scale data." Samples with off-scale peaks may exhibit elevated baselines, excessive pull-up, or high stutter.

When necessary, samples that have off-scale data can either be re-amplified with less DNA or diluted to bring the data on-scale.

Elevated baseline

An elevated baseline occurs when the baseline is higher than usual, typically observed at around 10-12 RFU. Occasionally the baseline is high enough that artifacts exceed the detection threshold and may interfere with the interpretation of the sample.

The analyst may choose to rerun the sample after replacing the polymer and/or performing a spectral calibration.

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Artifactual peaks

Artifactual peaks result from technology used to resolve fluorescently labeled PCR products or the presence of species-specific, non-human DNA. The analyst should note artifactual peaks by labeling them on the electropherogram.

If they interfere with the recognition of true alleles, the sample can be re-injected or diluted and re-run.

- **Pull-up:** Small artifactual peaks can appear in other colors under true allelic peaks. This phenomenon is termed "pull-up." Pull-up is a result of spectral overlap between the dyes, which is normally corrected by the spectral calibration. If a pull-up peak is above the detection threshold, it will be sized at the same base pair location as the true peak.
  - Application of a sub-optimal spectral calibration can cause pull-up. If necessary, a new spectral calibration plate can be run and applied.
  - Amplification using excess input DNA can lead to pull-up peaks (see *Off-scale data* above). The spectral calibration may not perform properly with off-scale data.
- **Spikes:** Spiked peaks with no known cause may occur and may be observed in the scanning regions of an electropherogram. They will commonly be observed in most, if not all, of the dye colors of the sample at approximately the same base pair location, though they can be observed in only a single dye color. They are typically not reproducible in multiple injections.
- **Non-human DNA:** a 103 base pair peak has been observed at the amelogenin locus in samples containing horse, pig, dog, cow, and sheep DNA.

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Stutter peaks

In addition to an allele's primary peak, artifactual minor "stutter" peaks can occur at different base pair intervals depending on the number of bases in the repeating unit.

In general, the most common stutter peak observed in all loci is one repeating unit smaller (n-4) than the primary peak. It is also possible to see additional peaks that are one or more repeating units larger or smaller (e.g., n+4 or n-8). Sequence microvariants can also affect the amount of stutter.

Stutter peaks are evaluated by examining the ratio of the stutter peak height to the height of the appropriate adjacent allele, expressed as a percentage. The height of the stutter peaks can vary by locus- longer alleles within a locus generally have a higher percentage of stutter.

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Stutter peak percentages

Maximum expected percentages of n-4 stutter for Identifiler Plus are listed in the following table:

Locus	Stutter threshold	Locus	Stutter Threshold
D8S1179	10.32%	D2S1338	12.44%
D21S11	10.67%	D19S433	11.21%
D7S820	9.69%	vWA	12.45%
CSF1PO	9.2%	TPOX	6.84%
D3S1358	12.27%	D18S51	13.68%
THØ1	4.49%	D5S818	10.06%
D13S317	9.93%	FGA	13.03%
D16S539	10.39%		

Maximum expected percentages of stutter for Yfiler are listed in the following table:

Locus	Stutter location	Stutter threshold
DYS456	n–4	13.21%
DYS389I	n–4	11.79%
DYS390	n–4	10.40%
DYS389II	n–4	13.85%
DYS458	n–4	12.20%
DYS19	n–2	10.21%
DYS19	n–4	11.04%
DYS385	n–4	13.90%
DYS393	n–4	12.58%
DYS391	n–4	11.62%
DYS439	n–4	11.18%
DYS635	n–4	10.75%
DYS392	n–3	16.22%
DYS392	n+3	7.90%
Y GATA H4	n–4	11.08%
DYS437	n–4	8.59%
DYS438	n–5	4.28%
DYS448	n–6	4.96%

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Elevated stutter peaks

Stutter peak heights may be elevated above the expected thresholds. Peaks in stutter positions that exceed the above stated values are often present in mixtures. However, stutter peaks have also been observed in single-source profiles and could be due to the following:

- The CCD camera may be saturated resulting in peak heights "off-scale." As a result, the observed percent stutter will be inaccurately high. If the stutter peak is greater than the maximum allowed and the primary peak is above 3000 RFU or has been labeled off-scale, the analyst should interpret the results with caution. The sample may be re-injected with diluted amplified product to help resolve this issue.
- For alleles differing by two repeats, the stutter peak from the larger alleles may overlap the trailing shoulder of the smaller allele and therefore exhibit an increased stutter percentage.
- Somatic mutations can result in elevated stutter.
- An allelic peak in a mixture may co-migrate with the stutter peak of another allelic peak, resulting in an apparent stutter peak height greater than that typically observed for stutter in a single-source sample.

If a peak consistent with high stutter is observed, the analyst may manually cross-out the allele designation and re-label it as high stutter.

Non-template addition

Amplification conditions have been set to maximize the non-template addition of a 3′ terminal nucleotide by AmpliTaq Gold DNA polymerase. Failure to attain complete terminal nucleotide addition results in "peak splitting" visualized as two peaks one base apart. This is most often seen when an excessive amount of DNA is amplified or amplification is performed under sub-optimal PCR conditions. Excepting microvariants, the presence of peaks differing by one base pair is characteristic of this event.

Degradation

Profiles exhibiting degradation result from exposure to factors (environmental or chemical) that compromise a sample's quality by cleaving the DNA molecule into smaller fragments. Degraded samples are typically partial profiles where alleles at the largest loci have significantly decreased RFU values or completely fail to type.

"Differential degradation" refers to mixture profiles in which the relative contributions of the contributors change across the profile.

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Inhibition

Profiles exhibiting inhibition arise from the presence of components (e.g., humic acid, heme, dyes, bleach) that co-extract with the sample and decrease the efficiency of or suppress PCR amplification.

Samples containing inhibitors may result in partial profiles containing fewer loci than tested or in the complete failure of PCR amplification.

Stochastic effects

Stochastic effects lead to the uneven amplification of two alleles in an individual who is heterozygous at a given locus. After PCR amplification, one or both alleles may fail to amplify (allele dropout) or may have imbalanced peak height ratios.

Samples containing small amounts of template DNA, typically less than 200 pg of input DNA, are subject to this phenomenon. These samples are often:

- contact (touch) DNA samples
- samples with low amounts of biological material (ex: few sperm in the sperm fraction)