	Intermountain Forensics	SOP #	IAC-203
		Revision #	02
Forensic DNA Technical Leader Approval		Issue Date	
			4/14/2021

Data Assessment and Rework

1. Purpose

To describe the procedure for assessing data quality and assessment of rework.

2. Summary

The laboratory is committed to quality. This procedure defines the baseline for quality for our DNA profiles and reasonable guidelines for if, when and to what extent rework is required or recommended to maintain quality results.

3. Procedure

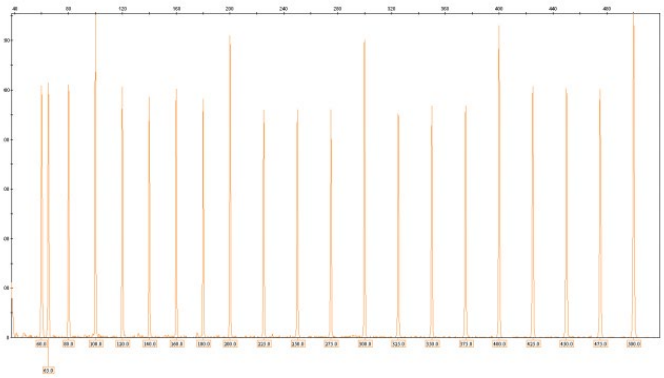
Evaluating Data Quality

1. Re-work is at the discretion of the analyst after assessing the quality on the sample.

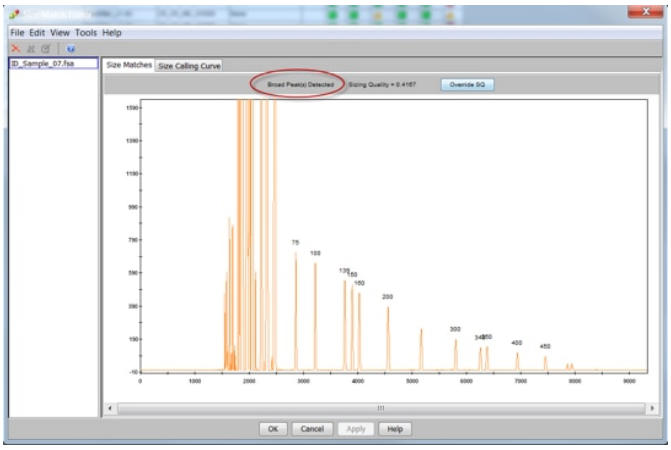
CE

2. Check that the ILS peaks are consistent with the expected kit specific ILS sizes.

3. Check ILS peak resolution.
    a. ILS peak resolution affects the resolution of the DNA peaks. Wide or tailing peaks can possibly hide true DNA peaks. Peak heights may also be affected.
        i. Regular ILS



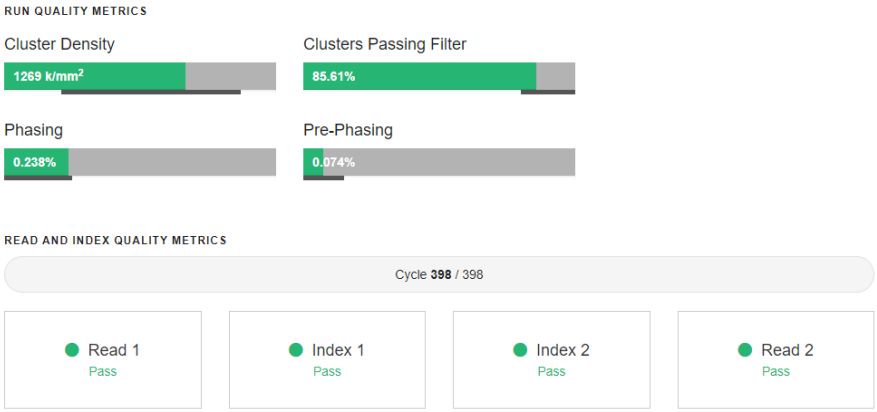
ii. Failing ILS



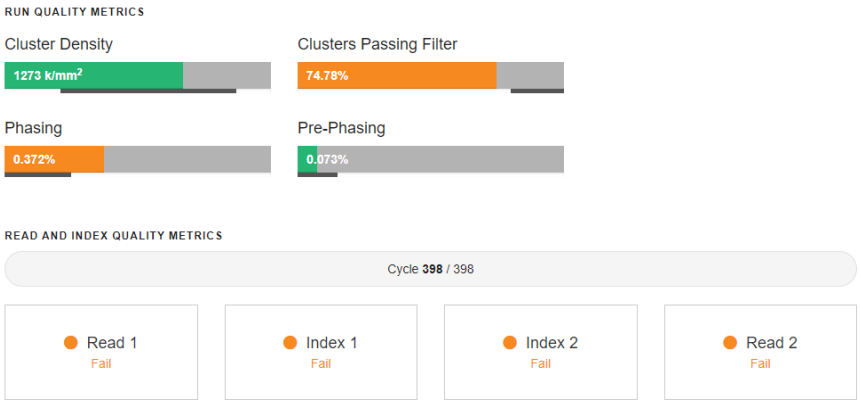
iii. Failed ILS

MPS on MiSeq FGx

4. Evaluate Run Metrics
   a. Good Quality



   b. Lower Quality



i. Data with lower quality metrics may still be interpretable and reportable. Individual samples and controls should be assessed as per procedure listed below. Assessment should be made as to whether obtained results match expectations based on input DNA amount and if higher read counts may affect the interpretation.

Artifacts:

5. Any called peak determined to be an artifact will be called off during interpretation and documented on the electropherogram or allele bar chart. Artifacts that are unable to be confirmed may be called inconclusive.
    a. Stutter
    b. -A or single nucleotide deletions
    c. Sequencing artifacts (MPS only)
        i. Sequence artifacts occur when the incorrect base is added during sequencing. Identify by:
            1. Low read counts (Below the Stochastic Threshold)
            2. Checking if the allele is in the NIST database
            3. Checking if the SNP location is in a known SNP location
                a. Gettings et al 2019 Supplementary
    d. Pull up (CE Only)
    e. Dye blob (CE Only)
    f. Spike (CE Only)

Evaluation of Controls

Positive Control:

6. The positive control is intended to be used to assess whether the amplification process was successful. A positive control should be complete and above the stochastic threshold.
    a. If the positive control profile is below stochastic threshold, data can be interpreted per the general guidelines provided all expected alleles are obtained. The positive control reagent tube used should be discarded if observed across multiple plates.
    b. A partial positive control profile may indicate issues with the amplification process or analyzer/sequencer. If rework does not result in all expected alleles, the positive control reagent tube should be discarded.
        i. Rework is at the discretion of the analyst.
            1. If rework is directed
                a. The positive control may be re-prepped for run on the instrument and included with a subsequent sample run (MPS flow cell prep, CE load)
                b. If reamplification is required, the associated samples must also be reamplified (MPS: PCR1/2, CE: amp setup)
        ii. Loci where the positive control does not obtain all expected alleles will not be interpreted in the associated samples and should be documented within the case file.
            1. This documentation should be approved by the DNA Technical Leader.

7. If true DNA peaks are detected in the amplification positive control that are not consistent with the predicted genotype, the Technical Leader is to be notified and the positive control reagent tube used should be discarded. Increased caution should be used for sample interpretation.
    a. If contamination is suspected in the samples as well then rework should be performed.

Negative Control and Reagent Blanks:

8. Contamination is defined as any indication of amplified DNA present above the analytical threshold and can vary in severity.
    a. If there are no peaks present that are visually distinguishable from baseline, the casework samples may be interpreted per the general guidelines.
    b. If there are peaks present that are visually distinguishable from baseline but below the analytical threshold AND have the general appearance of a DNA profile, use caution in sample evaluation. Possible indications of contamination in a negative control or reagent blank may be present in a higher level in the associated samples.
    c. If there are one or two allele peaks above the analytical threshold this is an indication of drop-in. Drop-in peaks are typically low level (at or near the analytical threshold, but below the stochastic threshold), homozygote in nature, and are not associated with a pattern indicative of a DNA profile. Up to two drop-in events can be classified as artifacts. All associated samples and loci may be interpreted per the general guidelines.
    d. If there are more than two peaks above analytical threshold or only one to two peaks above the analytical threshold and a pattern indicative of a DNA profile is observed below the analytical threshold, then rework must be performed. The affected control as well as the associated samples must be re-injected, a new load plate made, or re-amplified in an attempt to confirm the peaks. If the peaks are not reproducible, then the results may

be reported out. If peaks are reproducible, the Technical Leader must be notified. Interpretation of associated samples may be allowed under specific circumstances determined by the Technical Leader.

i. If rework is required, the RB/Negative control may be re-prepped for run on the instrument and included with a subsequent sample run (MPS flow cell prep, CE load)
ii. If reamplification is required, the associated samples must also be reamplified (MPS: PCR1/2, CE: amp setup)
iii. Any data associated with this negative control/reagent blank that is deemed interpretable must be documented with sufficient reasoning and explanation.
    1. Loci where the negative control/RB obtains non-artifact alleles will not be interpreted in the associated samples and should be documented within the case file.
    2. This documentation should be approved by the DNA Technical Leader.

Allelic Ladders: (CE Only)

9. At least one passing ladder is needed per plate.

10. A passing ladder will have a green quality symbol in the Gene Mapper™ ID-X software samples screen.

11. Failed plates should have a new load plate made.

Evaluation of Samples

Unresolved Peaks (CE)

12. If the unresolved peak is able to be confirmed in another sample, the peak may be called manually and used for comparison.

13. If the unresolved peak is not able to be confirmed in another sample, rework may resolve the peak.

14. If the peak is not able to be resolved then it is treated as an inconclusive peak.

Tri-Alleles:

15. Statistical calculations are not available for tri-allelic loci. Suspected tri-allelic loci will only be used for exclusions.

Off Ladder Alleles (CE):

16. Off ladder Alleles must be confirmed in order to be used for comparison.

17. Confirmation can be from the same allele being observed in another sample/fraction, through re-injection, a new load plate, or re-amplification.
    a. A reference can only be used for confirmation if the item(s) tested are intimate to that individual.

Mixture Tool Evaluation:

18. Evaluate for possible sample to sample contamination within the run.
    a. Gene Mapper™ ID-X software Mixture Tool
    b. ArmedXpert™ Match and Comparison tab

Evaluation for Re-work:

19. Sample re-work is intended to improve the quality of data obtained, confirm Off Ladder Alleles or unresolved peaks, or to address any issues which may have happened during processing.
    a. Documentation of rework decision, direction and reasons should be included in case documentation.

Evaluation of Re-work:

20. Ensure that any re-work is concordant with previously generated data.
    a. Non-concordance should be documented in case documentation and re-worked if necessary.

4. References

SWGDAM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

5. Definitions

Clarify any terms used within the document

Allelic ladder: The allelic ladders contain amplified PCR products of the same repeat lengths for nearly all known allele sizes for each specific locus.
Allele drop-in: Spurious alleles observed in a sample or control not associated with contamination.
Allele drop-out: The failure of an allele to amplify during the PCR reaction or the failure to detect an allele within a sample as the result of stochastic effects or primer binding issues.
Analytical Threshold: The minimum height requirement at and above which detected peaks can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles. PBSO's analytical threshold is defined as 10x the standard deviation of the average signal-to-noise ratio on the 3500xl.
Artifact: A peak that is not attributable to the DNA sample but may also result from the PCR. Examples include stutter, pull-up, dye blob, spurious peak, spike, and noise/background.
Baseline/Background/Noise: Any signal generated by the 3500xl not directly attributed to the light transmitted from defined fluorophores at the required wavelength for peak detection.
CE: Capillary Electrophoresis
Cluster Density: Describes the density of sequencing clusters are on the flow cell.
Clusters Passing Filter: The percentage of clusters that pass the software filter and can be distinguished by the camera.
Composite Profile: A DNA profile generated by combining typing results from different loci obtained from multiple injections of the same amplified sample.
Dye-blob: Occur when fluorescent dyes come off their respective primers and migrate independently through the capillary. These peaks are fairly broad and possess the spectrum of one of the dyes used for genotyping.
Electropherogram: the graphic representation of the separation of DNA fragments by electrophoresis in which data appear as peaks along a line.
Exclusion: Comparative conclusion when profiles do not match.
Heterozygote: Presence of different alleles at a locus.
Homozygote: Presence of identical alleles at a locus.
Inconclusive: "Inconclusive" may be used to describe a specific allele, locus, or entire DNA result.
- **Inconclusive Allele:** Unable to determine whether allele is real DNA or artifact and will only be used in determining number of contributors.
- **Inconclusive Locus:** Locus will not be used for comparison, typically due to incomplete results in a positive control.
- **Inconclusive Interpretation Result**: DNA results do not meet the minimum requirements for interpretation.

Internal Lane Standard (ILS): The size standard containing fluorescently tagged DNA used to aid in sizing amplified alleles and account for capillary to capillary migration variation.
Microvariant: Variants (microvariants/microheterogeneity) have been reported in the literature and have been observed through practical experience in the laboratory. The microvariants are described as intense peaks which are one, two, three, or four nucleotides shorter than the nominal allele size thus causing the amplified allele to migrate below that standard allele in the AL. An example of this is the common TH01 9.3 allele.
Off Ladder (OL): A rare variant that migrates outside of the 0.5 base pair bin of the ladder. Off ladder alleles can be extrapolated using the base pair size of the allele compared to the AL.
Peak Resolution: The condition of the shape of the peak. Can be normal, wide, or have tailing.
Phasing: Shows the percentage of molecules in a cluster that fall behind the current cycle in Read 1.
Pre-Phasing: Shows the percentage of molecules in a cluster that jump ahead of the current cycle in Read 1.
Pull-up: Is the result of the inability of the 3500xl to properly resolve the dye colors in an STR amplicon. This phenomenon is due to spectral overlap resulting in a peak of one dye spectrum present in another (bleed through) as a result of exceeding the linear range of detection for the 3500xl (i.e. sample overloading).
Reads: The number of times a specific result is counted on the flow cell.
RFU: (relative fluorescent units): measurement of signal intensity.
Spike: Can give rise to a false peak in the 3500. These peaks are usually sharp and usually appear equally intense in all four colors (Sometimes will occur in only one spectrum). These peaks are not reproducible and should not be located in the same position if the sample is re-injected into the capillary.
Split Peak: When there is mixture of both +A and –A.
Stochastic Threshold: The peak height value above which it is reasonable to assume that, at a given locus, allelic drop-out of a sister allele has not occurred.
Stutter: Stutter peaks may accompany major peaks (i.e. the most intense signals). These peaks are obviously weaker in signal intensity and typically are one allele repeat smaller or larger than the major peak. Each locus has been well defined for stutter and the expected amount of stutter has been validated and recorded.
Tri-allele: Result from extra chromosomal fragments being present in a sample or DNA sequence where the primer's allele is duplicated on one chromosome thus resulting in an extra peak at a single locus.
Unresolved Peak: A true DNA peak that is not called by the Gene Mapper™ ID-X software due to peak resolution. This typically occurs when there is a drastic peak height difference between two peaks that are one base pair apart.