DNA: Run Evaluation

Evaluate the run

After the run is finished, the analyst must evaluate the following items:

- allelic ladder(s)
- internal size standards
- positive and negative controls
- reagent blanks

Allelic ladders

The allelic ladder used for the project is reviewed.

Significant room temperature fluctuation may result in size variation between injections such that the allelic ladder peaks differ by > 0.5 bp from allelic peaks in other injections. This will cause GeneMapper® to assign these alleles as off-ladder alleles.

Genotyping with a different injection of the allelic ladder or averaging multiple injections of the ladder may alleviate this problem. If desired, the sample(s) and an allelic ladder may be re-injected to confirm the typing.

Internal size standards

DNA fragments represented by peaks in an electropherogram can be sized relative to an internal size standard that is mixed with the DNA samples prior to capillary electrophoresis.

Analysts should confirm that all peaks in the internal size standard are above the analytical threshold and that they are accurately designated by the software.

The 250 and 340 bps peaks are typically omitted from sizing due to their sensitivity to temperature fluctuation

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DNA: Run Evaluation, Continued

Positive control

The positive PCR control sample ensures that the amplification and typing process is working properly. If the positive control fails to yield a typeable signal (after rerunning on the instrument), the results may be reported as long as the QC stain control gives the correct genotype. If the positive control fails to give the correct results, the analysis must be repeated.

- The genotypes for the positive amplification control for Identifiler Plus are listed in the following table:

Identifiler Plus - AmpFℓSTR® Control DNA 9947A			
Locus	**Genotype**	**Locus**	**Genotype**
D8S1179	13,13	D2S1338	19,23
D21S11	30,30	D19S433	14,15
D7S820	10,11	vWA	17,18
CSF1PO	10,12	TPOX	8,8
D3S1358	14,15	D18S51	15,19
THØ1	8,9.3	Amelogenin	X,X
D13S317	11,11	D5S818	11,11
D16S539	11,12	FGA	23,24

- The haplotypes for the positive amplification control for Yfiler are listed in the following table:

Yfiler - AmpFℓSTR® Control DNA 007			
Locus	**Haplotype**	**Locus**	**Haplotype**
DYS456	15	DYS391	11
DYS389I	13	DYS439	12
DYS390	24	DYS635	24
DYS389II	29	DYS392	13
DYS458	17	Y GATA H4	13
DYS19	15	DYS437	15
DYS385 a/b	11,14	DYS438	12
DYS393	13	DYS448	19

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DNA: Run Evaluation, Continued

Negative control

If typeable signals occur in the negative control samples (negative amplification control or, for Yfiler, female DNA 9947A), the negative control should be rerun on the instrument.

If after rerunning, typeable signals still appear in the negative control, the analyst will attempt to determine, based on their experience, the source of the peak(s). If it is suspected that the peaks result from contamination, the results of the associated DNA samples may be considered inconclusive. The analyst must re-amplify those samples associated with the contaminated negative control.

In the case of a limited sample, the results must be discussed with the DNA Technical Lead who will determine the appropriate action.

Reagent blank

If typeable signals occur in the reagent blank, the reagent blank can be rerun on the instrument. If after rerunning, typeable signals still appear, the results must be discussed with the DNA Technical Lead who will, in turn, determine the appropriate action.