

## DNA: Evaluation of DNA Typing Results

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**Introduction** The evaluation of DNA results in casework is a matter of professional judgment and expertise. Not every situation can or should be covered by a pre-set rule; however, it is important that the laboratory develops and adheres to minimum criteria for evaluation of analytical results. These criteria are based on validation studies, scientific literature references, casework experience, SWGDAM documents, the FBI's QAS document, and input from the forensic community at large.

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**Purpose** The purpose of these guidelines is to establish a general framework and outline minimum standards to ensure that conclusions in casework reports are scientifically supported by the analytical data, including data obtained from appropriate standards and controls.

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**Terminology** A glossary defining DNA terms is available within the manual. See [DNA: Glossary](#).

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**Evaluation of STRs** The steps for the evaluation of amplified STR data are listed in the table below.

Step	Action
1	Evaluate the run.
2	Evaluate the sample.
3	Assess the quality of the profile.

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## DNA: Evaluation of DNA Typing Results, Continued

### Data analysis

Data analysis is accomplished using GeneMapper® ID-X. All projects should include at least one allelic ladder, one positive control, one negative control, and all applicable case samples.

GeneMapper® ID-X projects containing all applicable case samples will be analyzed with stutter peaks filtered. Additional GeneMapper® ID-X projects containing probative evidence samples that will be interpreted with STRmix™ will be analyzed with +/- one repeat stutter peaks not filtered. All projects should be analyzed using the following parameters:

	<b>Fusion 6C</b>	<b>Yfiler</b>
Analysis Method	<i>PowerPlex Fusion 6C</i>	<i>Yfiler Analysis Method</i>
Panel	<i>PPF6C_STUTTER</i>	<i>Yfiler_v2</i>
	<i>PPF6C_NO STUTTER</i>	
Size Standard	<i>PowerPlex Fusion 6C</i>	<i>Yfiler Size Standard</i>
Analytical Threshold	100 RFU	75 RFU

NOTE: An alternate Analysis Method, *PowerPlex Fusion 6C PR*, can be used if an analyst experiences sizing issues using the default method. *PowerPlex Fusion 6C PR* types at partial range and the range can be adjusted as needed.

### Evaluate the run

Evaluate the following items in the run:

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

### Formamide blanks

Evaluate the electropherogram data of all formamide blanks for artifacts, spikes, and/or injection abnormalities (e.g., carryover). Select one formamide blank to print. If carryover is observed, the electropherogram with carryover should be printed.

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**Allelic ladders** The allelic ladder(s) used for the project should be 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® ID-X to assign these allelic peaks as off-ladder (OL) 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 added to 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.

**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 sample 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 Fusion 6C are listed in the following table:

Fusion 6C - Control DNA 2800M					
Locus	Genotype	Locus	Genotype	Locus	Genotype
Amel	X,Y	D2S1338	22,25	D8S1179	14,15
D3S1358	17,18	CSF1PO	12,12	D12S391	18,23
D1S1656	12,13	Penta D	12,13	D19S433	13,14
D2S441	10,14	TH01	6,9.3	SE33	15,16
D10S1248	13,15	vWA	16,19	D22S1045	16,16
D13S317	9,11	D21S11	29,31.2	DYS391	10
Penta E	7,14	D7S820	8,11	FGA	20,23
D16S539	9,13	D5S818	12,12	DYS576	18
D18S51	16,18	TPOX	11,11	DYS570	17

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## DNA: Evaluation of DNA Typing Results, Continued

**Positive control (continued)** The haplotypes for the positive amplification control for Yfiler are listed in the following table:

Yfiler - AmpF $\phi$ STR <sup>®</sup> 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

**Negative control** If typeable non-artifactual 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(s)** If typeable non-artifactual signals occur in a reagent blank, the reagent blank should 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.

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**Evaluate each sample** Prior to the interpretation of DNA data, each peak identified by GeneMapper® ID-X software must be verified as an allele, a stutter peak, or an artifact.

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**Threshold values** **Analytical Threshold:** The level of detection, 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:

Fusion 6C- 100 RFUs  
Yfiler®- 75 RFUs

**Stochastic Threshold:** The region in which uneven PCR amplification can lead to allele dropout. Because STRmix™ is a fully-continuous probabilistic genotyping system, the probability of dropout will be modeled within STRmix™. Therefore, a Fusion 6C stochastic threshold of 450 RFUs will only be applicable for assessing homozygous genotypes for reference samples or, in some cases, an evidence sample being used as a reference sample.

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**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 determined 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 3, 4, or 5-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. When a tri-allelic pattern is observed, the results will be noted, but the locus will not be included in the interpretation of the sample.

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### Null alleles

A null allele is an allele that is either not detected or exhibits extreme heterozygote peak height imbalance due to a deletion or a source of genetic variation at or near the primer binding site.

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

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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.

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### 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.

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### 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 and edited electronically. The off-ladder allele designation should be confirmed using at least two sample injections or be present in multiple samples.

When a non-artifactual off-ladder allele occurs between two loci and one of the loci to the left or the right contains two peaks and the other contains only one, the allele will be considered to belong to the locus containing the single peak. Based on the base pair value of the allele, when possible, an allele designation will be assigned. If it cannot be determined which locus the allele belongs to, the allele will be designated as > or < the closest allele in the allelic ladder. For STRmix™ purposes, both loci on either side of the ambiguous allele will be ignored.

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### 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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### 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

Peaks determined to be alleles or +/- one repeat stutter are the only peaks that can be entered into STRmix™. All other artifactual peaks should be labeled appropriately and may be electronically deleted from the profile. After the quality of the DNA profiles have been assessed, the GeneMapper® ID-X project will be evaluated using the Profile Comparison tool.

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**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, high stutter, or other artifacts. Samples may be interpreted in STRmix™ regardless of whether they contain off-scale data, provided that the camera saturation does not interfere with calls at other loci. When the sample is a mixture, STRmix™ interpretation should be limited to samples having no more than two off-scale STR loci. If three or more off-scale STR loci are detected, the analyst should attempt to re-amplify the sample to bring the data on-scale unless there is clear documentation indicating the reason to proceed.

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**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.

If these peaks interfere with the recognition of true alleles, the sample can be re-injected and rerun.

- **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 approximately 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 the casework plate can be rerun.
    - 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.
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### Artifactual peaks (continued)

- **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:** Artifactual peaks not from pull-up or spikes may result from the amplification of non-human DNA. The observation of detected peaks from non-human sources is described in the Fusion 6C developmental validation. See [DNA: References](#).
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### Stutter peaks

A stutter peak is a well-characterized PCR artifact that refers to the appearance of a minor peak one repeat unit smaller (back stutter) or one repeat larger (forward stutter) than the major STR product. Less frequently, stutter peaks can also occur half a repeat smaller, half a repeat larger, or two repeats smaller than the major STR product. Most of the loci in the Fusion 6C kit are tetranucleotide repeat units. However, D22S1045 is a trinucleotide locus, and Penta D and Penta E are pentanucleotide loci.

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. Average stutter percentages vary by locus. Longer alleles within a locus generally have a higher percentage of stutter.

During the internal validation study for the Fusion 6C kit, expected stutter percentages for each locus were calculated. Expected back stutter (minus one repeat) percentages for each allele at each locus were also calculated. These percentages are used by STRmix™ to assess peaks in evidence samples as being allelic or stutter.

When it is appropriate to filter stutter, maximum stutter thresholds have been calculated for every locus.

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### Stutter ratio thresholds

The following table lists the maximum stutter thresholds that will be applied when filtering stutter in GeneMapper® ID-X.

Locus	-1 repeat	+1 repeat	-1/2 repeat	+1/2 repeat
D3S1358	0.1667	0.0304	0.0101	-
D1S1656	0.1462	0.0377	0.0325	-
D2S441	0.1052	0.0230	-	-
D10S1248	0.1626	0.0136	-	-
D13S317	0.1156	0.0385	0.0101	-
Penta E	0.0869	0.0169	0.0101	-
D16S539	0.1266	0.0652	0.0101	-
D18S51	0.1445	0.0774	0.0101	-
D2S1338	0.1686	0.0354	-	-
CSF1PO	0.1288	0.0313	-	-
Penta D	0.0415	0.0164	-	-
TH01	0.0533	0.0404	0.0101	-
vWA	0.1498	0.0273	0.0101	-
D21S11	0.1567	0.0348	-	-
D7S820	0.1055	0.0342	0.0278	-
D5S818	0.1154	0.0307	0.0169	-
TPOX	0.0634	0.0166	-	-
D8S1179	0.1200	0.0536	0.0334	-
D12S391	0.2099	0.0194	0.0101	-
D19S433	0.1274	0.0247	0.0101	-
SE33	0.1686	0.0373	0.0683	0.0305
D22S1045	0.2119	0.0930	-	-
DYS391	0.1039	0.0312	-	-
FGA	0.1219	0.0234	0.0101	-
DYS576	0.1514	0.0217	0.0101	-
DYS570	0.1437	0.0236	-	-

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**Stutter  
 threshold ratios**  
 (continued)

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** When the stutter filters are used, minor peaks in stutter positions above the maximum threshold will not be filtered in GeneMapper® ID-X, but should be further evaluated. These peaks may be called elevated stutter at the analyst's discretion based on specific circumstances. The following circumstances may lead to elevated stutter:

- The CCD camera may be saturated resulting in “off-scale” peak heights. 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 30,000 RFU for the 3500 xL (7000 RFU for the 3130xL), the analyst should interpret the results with caution. The sample may be re-amplified to help resolve this issue.
- For alleles differing by two repeats, the back stutter peak of the larger allele may overlap the forward stutter peak of the smaller allele and therefore exhibit an increased stutter percentage for the peak that falls between the two alleles.
- Samples with stochastic effects due to very low input DNA may have stutter peaks that are elevated.
- Larger molecular weight alleles may have a higher stutter ratio than smaller molecular weight alleles.
- Alleles with multiple, wide-spread Longest Uninterrupted Sequence (LUS) values may exhibit higher or lower stutter ratios than expected.
- Somatic mutations can result in elevated stutter.

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**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. With the exception of microvariants, the presence of peaks differing by one base pair is characteristic of this event.

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**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.

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**Stochastic effects** Random PCR effects can lead to the uneven amplification of two alleles in an individual who is heterozygous at a given locus. One or both alleles may fail to amplify (allele dropout) or may have imbalanced peak heights.

Samples containing small amounts of template DNA, typically less than 250 pg of input DNA, are subject to this phenomenon. These samples are often contact (touch) DNA samples or samples with low amounts of biological material (ex: few sperm in the sperm fraction)

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**Profile Comparison tool** The Profile Comparison tool within GeneMapper® ID-X software is used to evaluate the run by automatically performing multiple quality checks. Profiles within a project are compared to one another and to designated controls. Profile Comparison results can be used to evaluate quality, detect possible contamination and sample-switch events, and identify sample associations by allowing the analyst to:

- evaluate reagent blanks, negative controls, and formamide blanks as a group
  - compare samples in a project to one another using a defined match percent
  - assign a threshold to evaluate sample concordance and allele matching
  - compare samples in a project to laboratory reference profiles
  - evaluate concordance of all designated controls
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**Using the Profile Comparison tool**

This is the procedure for using the Profile Comparison tool.

Step	Action
1	Ensure that allele labels have been edited as needed so that no OL (off ladder) allele labels are present in the project. Samples containing OL peaks are not considered in comparisons.
2	In the project window toolbar, select <b>Tools</b> and go to <b>Profile Comparison</b> .
3	Click the <b>Sample Concordance</b> tab. This tab displays groups of samples with 100% concordance. To review the results, expand the profiles listed within each group by hovering with the mouse. Evaluate the single-source group containing “blank” samples (reagent blanks, formamide blanks, and negative controls) for expected results. Evaluate the results of each additional Single Source or Mixed Source group.
4	Click the <b>Sample Comparison</b> tab and verify that the Percent Match Threshold is set to 80%. Click <b>Compare Profiles</b> and review the results of pairwise Single-Single, Single-Mixed, and Mixed-Mixed sample comparisons within the project. To review the results, expand the profiles listed within each group by hovering with the mouse.
5	Click the <b>Lab Reference</b> tab and verify that the Percent Match Threshold is set to 80%. Click <b>Compare Profiles</b> and review the results of comparisons between samples within the project and known laboratory reference samples.
6	If needed, the analyst may adjust the Percent Match Threshold to adjust the stringency and re-evaluate the results.

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## DNA: Evaluation of DNA Typing Results, Continued

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### Evaluating Profile Comparison results

To aid in the detection of possible contamination or sample-switch events, the analyst should identify and evaluate:

- blank samples observed in groups that contain detected alleles
- evidence samples expected to contain no DNA results or very partial DNA
- samples from different or unrelated cases existing within the same group
- associations between samples (other than QC samples) and known laboratory reference samples

The analyst should use caution when evaluating the significance of profiles with high percentages of allele matches. The following are general considerations when evaluating Profile Comparison results:

- When partial profiles are compared to other single-source or mixture profiles, high percentage allele matches may be observed. In general, the fewer alleles in the partial profile, the higher the tendency to generate up to 100% allele matches to other profiles or to laboratory reference samples.
- As the number of contributors to a mixture profile increases, more alleles will be detected. Therefore, there may be a greater tendency to generate high percentage allele matches to other profiles within the project or to laboratory reference samples.
- The analyst may adjust the Percent Match Threshold, as necessary when performing Profile Comparison, to adjust the stringency of the comparison.
- When Profile Comparison results indicate that possible contamination or sample switching has occurred, the analyst should evaluate the electropherograms to aid in the assessment.

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### Electronic data access and security

Authorized access to electronic DNA quantitation files and run folder data on the Genetic Analyzers is limited to those who have pre-programmed access cards for the Biology Unit.

GeneMapper® ID-X data analysis software is password-protected and limited to DNA analysts and the Biology Unit supervisor. All electronic data files associated with a DNA request are stored on a DVD as evidence.

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**Electronic data storage** The DVD containing the electronic data is stored as evidence. The data should consist of:

- any photographs taken during examination
  - quantitation file(s)
  - run folder(s)
  - GeneMapper<sup>®</sup> ID-X project(s)
  - STRmix<sup>™</sup> results folder(s)
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**Second analyst review** A second analyst will review the DNA data and initial and date the sample sheet/injection list page(s).

Refer to [DNA: Data Review by Second Analyst](#) for additional information.

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