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Research article

# Automatic data processing of reference DNA-profiles from FTA and non-FTA samples

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**Abstract**

The new Swedish DNA legislation resulted in a huge increase in reference samples. In 2006 approximately 25,000 reference samples were received compared to 5000 in 2005. To meet this increase the reference samples had to be handled in a more automatic process than previously. A new module in the LIMS system automatically compares duplicate results and creates confirmed results if the DNA profiles meet the set requirements. Profiles without automatically confirmed results need to be manually investigated. Certain rules and settings in the LIMS sort these samples. Evaluators are able to combine the results to form confirmed results or chose to reanalyse the samples. At a rough estimate, 80% of all FTA samples are automatically assigned confirmed results, without any manual handling. Only 0.09% of the reference samples was terminated without results in every marker.


*Keywords:* LIMS; FTA; Reference sample

## 1. Introduction

The new Swedish DNA legislation, Act on Police registers [1,2] valid from 1st of January 2006, resulted in a huge increase in reference samples.

In 2006 approximately 25,000 reference samples were received compared to 5000 in 2005. In addition all reference samples were now to be processed as duplicates. To meet this increase the reference samples had to be handled in a more automatic process than previously used. A new module in the LIMS system (Ida Infront AB) automatically compares these duplicate results and creates confirmed results if the DNA profiles meet the set requirements.

## 2. Method

FTA reference samples are punched in sets of pairs. If the first set fails another punch round is performed. If both sets fail Chelex extraction is performed on pieces of the FTA card. The DNA extract is quantified (ABI 7300 Real Time PCR System, Quantifiler™ Human DNA Quantification Kit; Applied Biosystems) before the amplification (GeneAmp® PCR System 9700, AmpF*l*STR® SGM Plus® PCR Amplification Kit; Applied Biosystems). If the result still cannot be confirmed a purification (Centricon®-100; Millipore) is made, also followed by quantification (Fig. 1).

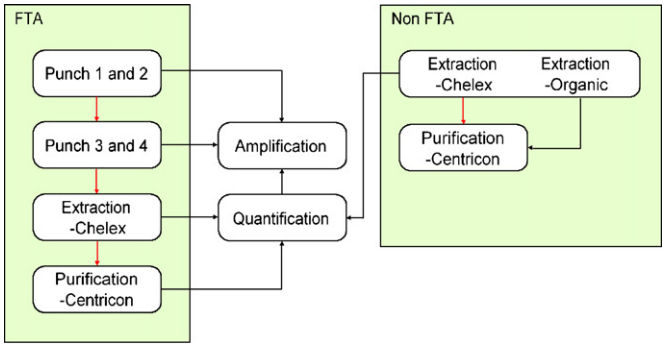


Fig. 1. The laboratory flow of reference samples. The red arrows symbolise the flow if results could not be confirmed. The analysis of FTA cards starts with punching them twice to obtain duplicates. If the two profiles cannot be confirmed, a second pair is punched. PCR is carried out directly on the punches. Chelex extraction is performed on the card if the results still do not meet the criteria for creating confirmed results. When needed a purification is also made. The extraction or the purification is followed by quantification before PCR. Non-FTA analyses (mostly blood and muscle) are always quantified before amplification. Blood is extracted with Chelex and when needed also purified. Organic extraction is performed on muscles where a purification is obligatory.

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| Help Class | Status | Max unbalance | Max peaks | Nb of markers containing peaks | | | | | Nb of markers that have to be higher than a given percentage of the peak height threshold | | | | | | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | OK | <= 0 | <= 2 | > 9 | AND | < 11 | >= 10 | <= 10 | markers | >= | 100% | | | | |
| 2 | OK | <= 0 | <= 2 | > 9 | AND | < 11 | >= 10 | <= 10 | markers | >= | 50% | | | | |
| 3 | OK | <= 0 | <= 2 | > 9 | AND | < 11 | >= 7 | <= 10 | markers | >= | 50% | | | | |
| 4 | OK | <= 0 | <= 2 | > 9 | AND | < 11 | >= 0 | <= 6 | markers | >= | 50% | | | | |
| 5 | OK | <= 0 | <= 2 | > 4 | AND | < 10 | >= 1 | <= 9 | markers | >= | 50% | | | | |

Fig. 2. The classification system. The expert system assigns a class to each profile depending on its characteristics.

| Active | Class | Alleles | Code | Result Status | Result Type | Remark |
|---|---|---|---|---|---|---|
| Rule 1 | 0 7 | 7 | PG | Partially accepted | No change | Decision taken in GeneMapper. |
| Rule 2 | 0 7 | 7 | T | Under further investigation | No change | Decision taken in GeneMapper. |
| Rule 3 | 9 7 | 7 | | No change | No peaks | Blank sample. |
| Rule 4 | 0 7 | 7 | U | Not accepted | No change | Decision taken in GeneMapper. |
| Rule 5 | 0 7 | 3 | | No change | Alleles | Mutation or mixed profile. |
| Rule 6 | 0 7 | 7 | ROX | Not accepted | No peaks | Internal standard is missing. |
| Rule 7 | 0 7 | 7 | | No change | No change | |

Fig. 3. The rule system. The expert system assigns result status and result type to each profile according to fixed rules and codes received from GeneMapper™*ID*.

Blood reference samples are extracted with a Chelex based method and muscle samples with an organic extraction. The Chelex extraction can be followed by a purification if the analysis fails. The purification is obligatory after the organic extraction. Both extraction methods are followed by quantification (Fig. 1).

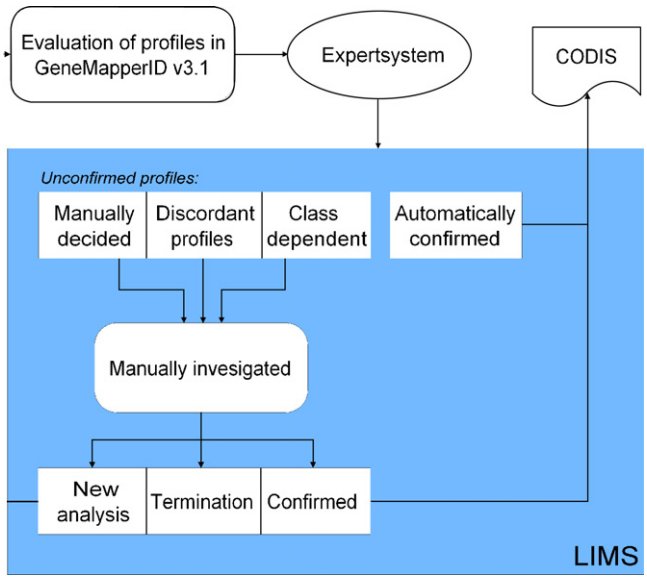


Fig. 4. The data flow and handling inside and outside the LIMS system. All reference samples are analysed as duplicates. The data produced in Applied Biosystems 3130*xl* Genetic analyzer is first processed in GeneMapper™*ID*. The investigator is able to override the requirements for confirming results if there is a risk of the LIMS wrongly interpreting bad or corrupted profiles. Data tables are exported from GeneMapper™*ID* to the expert system. Duplicates are confirmed and profiles are sent to CODIS if they meet the set requirements. The profiles are further investigated in the LIMS system if confirmed results not are created. A new analysis is requested if confirmed results cannot be formed manually. The analysis of the sample is terminated if the analysis fails after several attempts.

After the amplification, samples are analysed with Applied Biosystems 3130*xl* Genetic analyzer. Raw data is imported to clients where the data is processed by GeneMapper™*ID* software, version 3.1, (Applied Biosystems). All profiles are manually checked to clear them from artefacts, OL-alleles etc. (Fig. 4).

After analysis in GeneMapper™*ID* the sample- and genotype tables are exported to an expert system, a custom-made Excel application (Fig. 4). This system assesses the profile's quality and automatically assigns a class and result status and type to each profile (Figs. 2 and 3). Factors considered when measuring quality are peak height, number of alleles in each marker and if there is an unbalance within a marker. A poor class is assigned to the profile if the quality is unsatisfactory. During the processing in GeneMapper™*ID*, it is possible to assign codes which the expert system later uses to change the result status and type of the profile. This prevents the LIMS from confirming results automatically and allows a manual and closer examination of all results for related samples in the LIMS system (Fig. 4).

A confirmed result is automatically formed if a set of reference profiles fulfil certain requirements (Fig. 4).

However, the requirements for automatically confirming results can be overruled when there is a possibility that the LIMS could interpret a poor or corrupted result as an accepted profile (Fig. 4). This prevents the LIMS from creating confirmed results and sending the results to CODIS, even if the profiles fulfil all requirements set for confirming results.

When profiles are checked manually in the LIMS, the investigator chooses to either confirm results, select a new analysis or to terminate the analysis.

To confirm results manually a full profile (class 1 or 2) needs to be confirmed by another profile in at least four markers. Weaker profiles need to confirm each other in every marker and

the peak height has be at least 50% (higher if homozygot) of ruling thresholds.

The analysis of FTA reference samples is terminated when two sets of punches and a Chelex extraction followed by purification have failed. A new sample is then requested from the police by e-mail.

## 3. Results

Of the 25,000 reference samples handled in 2006, 85.5% were confirmed in the first analysis attempt (punch 1 and 2).

Almost all samples, 97.7% were confirmed after punching remaining FTA cards four times.

After the last few samples were analysed with a Chelex extraction (and sometimes followed by a purification) only 0.09% were terminated and new samples requested from the police.

At a rough estimate 80% of all FTA samples are automatically assigned confirmed results, without any manual handling.

## Conflict of interest

None.

## References

[1] Polisdatalagen (Police Data Act), SFS 1998:622.
[2] Lag om ändring av Polisdatalagen (Amendment Act Concerning Police Data Act) SFS 2005:878.