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Verilabs

Client

Loci Forensics
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Project code

62200

Date Report

01-May-2014

Date of entry

17-Apr-2014

Reference number

-

Order number Verilabs

20140221

Forensic DNA Report

DNA-analysis

Authorisation

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Productspecialist
DNA-deskundige NRGD

Project code: 62200
Reference number: -
date of report: 01-may-2014

Verilabs

1. Request for investigation

Make DNA profiles from the supplied stubs, labelled 'single' and 'mix'. Compare these DNA profiles with each other. Do these DNA profiles match?

2. Methods

In the annex methods and techniques used for forensic DNA investigations are described. Our laboratory is accredited according NEN-EN-ISO/IEC 17025 (Accreditation Council AC 666).

3. Material received

The analyses described in this report refer to the evidence material listed below.

Table 1: material received

Identity seal	Material	Sample code	DNA nr.	Date of receipt	Date sample taken	Received from	Notes *
-	DNA-Stub enkel#BC01	4955	2980	17-Apr-2014	-	Loci Forensics	-
-	DNA-Stub mix#BC01	4956	2981	17-Apr-2014	-		-

* If damage or abnormalities are observed concerning the material received, it will be indicated here

4. Analysis

DNA-Stub enkel#BC01
DNA-Stub mix#BC01
The samples were subjected to DNA-analysis using the Powerplex ESI 17 Pro kit.

5. Results and conclusions

Table 2 summary of results

SIN	DNA concentration in ng/µl	DNA may originate from	Calculated random match probability	Notes
DNA-Stub enkel#BC01	0.080	A male person X	Less than 1 in a billion	-
DNA-Stub mix#BC01	0.040	The male person X and at least one other person	Less than 1 in a billion (for major DNA profile)	-

DNA-Stub enkel#BC01
A full DNA profile of a male person X has been obtained from the cell material.

DNA-Stub mix#BC01
A mixed DNA profile of at least two persons, including at least one man, has been obtained from the cell material.
From this mixed DNA profile, a single DNA profile of a male major contributor could be extracted. This major DNA profile matches the DNA profile of the male person X (DNA-stub enkel#BC01). Therefore, the sample contains a relatively large fraction of DNA that can originate from the male person X (DNA-stub enkel#BC01).
From this mixed DNA profile, the possible combinations of DNA-markers of the minor contributor could not be deduced.

6. Recommendation

A replication of the DNA-analysis from the mixed sample is recommended to further investigate the suitability of the minor contributor(s) DNA-markers for DNA comparison.

7. DNA-Database

No DNA profiles were sent to the DNA database, since no such order was received from the prosecutor's office.

8. Deviations concerning the quality of the investigation or samples, remarks, actions taken

There were no deviations regarding the samples and/or our procedures.

9. Enclosures

Annex
Pages included: 4

DNA-profiles
Pages included: 2

1. Introduction

In this annex the methods for the investigation of biological traces and forensic DNA testing that are performed by Verilabs are explained.

2. Investigation of biological traces and forensic DNA testing

The purpose of the analysis is to determine whether biological material is present on a piece of evidence, of what the nature of the cellular material is and to generate a DNA profile from the biological cellular material (DNA analysis).
In general, there are 3 groups of biological traces to distinguish:
Blood, saliva and semen
The presence of blood, saliva or semen is examined on the basis of presumptive testing. The operation of these tests will be explained below.
Hair
Contact traces
Contact Traces contain cells which are transferred during physical contact. Depending on the duration and intensity of the contact much or little cell material is found.

3. Pre-treatment

Visual detection

When a piece of evidence is received, first it should be determined whether and where on the material possible biological traces are situated. As a result of visual examination, certain parts of the material are sampled. Not all types of biological material are easy to observe. This applies for example for saliva or contact traces. A visual detection of contact traces is not possible.

Presumptive testing

If the presence of blood saliva or semen can be presumed on a piece of evidence, the nature of the cell material is examined using a presumptive test method. These tests provide an indication of the presence of blood, saliva or semen in the sample. Using a presumptive test there is always the possibility to get a false positive or false negative result. The researchers take this into account in their conclusion about the nature of the cellular material in the samples.

Tetrabase test for blood

If the presence of blood can be presumed, a tetra base test may be performed. By means of this test a presumptive indication can be obtained if a sample may contain blood or not.
The test is carried out using an extremely small amount of the sample. If the sample contains blood a blue discoloration may occur under the influence of an oxidation reaction. The tetra base test not only responds positively to human blood, but also on the blood of animal origin.

Phosphatesmo test for semen

If the presence of semen can be presumed the Phosphatesmo test may be performed. This test detects the enzyme acid phosphatase, which is present in semen in relatively high concentration.
The test is carried out using a very small amount of the sample. A pink coloration of the test paper provides an indication of the presence of seminal fluid.

Phadebas test for saliva

If the presence of saliva can be presumed, the Phadebas test may be performed. This test detects the enzyme alpha-amylase, which is present in relatively high concentration in saliva.
The test is performed by placing Phadebas test paper on the sample, wetting it and put a weight on the tested area. After some time a blue coloration of the test paper can be observed at the spots as an indication for the presence of saliva.

4. DNA-isolation, quantification and PCR

DNA-isolation

Before the DNA analyses process the DNA has to be isolated from the cells. Two methods for DNA-isolation are being used:

Chemagic Blood250 Special Kit (Chemagen)
Isolation of DNA from reference buccal swab samples is carried out with the aid of the Chemagic Blood250 Special Kit (Chemagen) and the Chemagic Module I, a method which makes use of magnetic beads in order to bind DNA. Due to the charge of the DNA, it will attach to magnetic beads after the cells have been broken. Subsequently, the DNA is by means of a robot removed from the solution.

Qiamp Investigator Kit (Qiagen)

Isolation of the DNA from sample material is carried out with the aid of the Investigator Qiamp Kit (Qiagen); a method in which the DNA binds to columns, followed by centrifugation, the DNA is separated from the other material in the solution.

DNA-quantification

In order to determine the amount of human DNA isolated from the sample quantification is carried out. DNA quantification is carried out with the aid of the Quantifiler Real-Time PCR Amplification Kit (Applied Biosystems). By measuring the concentration dependent fluorescence, the amount of human DNA in the sample is determined.

Polymerase Chain Reaction (PCR)

Before the DNA markers in the sample can be analysed the variable regions (locus, plural loci) of the DNA containing these markers have to be increased. The loci that are examined include STRs (short tandem repeats). The STRs are suitable to show differences between individuals. The amelogenine locus is located on the X and Y chromosomes and is used to determine the gender of the DNA contributor. Verilabs uses several PCR kits.
There is a choice of the following kits:

AmpFℓSTR® Identifiler™ PCR Amplification Kit (Lifetech)
The autosomal PCR analysis is performed equivalent to the method of the kit. This is a 15 STR kit that amplifies variable regions on the human DNA and the Amelogenine gender marker in one reaction.

AmpFℓSTR® NGM™ PCR Amplification Kit (Lifetech)
The autosomal PCR analysis is performed equivalent to the method of the kit. This is a 15 STR kit that amplifies variable regions on the human DNA and the Amelogenine gender marker in one reaction.

AmpFℓSTR® Yfiler PCR Amplification Kit (Lifetech)
The DNA testing of Y-chromosomal DNA is performed with the AmpFℓSTR ® Yfiler ™ PCR Amplification Kit (Kit Yfiler ') and equivalent to the method of this kit. This is a kit which amplifies 16 STRs on the Y-chromosome in one reaction.

Powerplex ESI 17 Pro PCR Amplification Kit (Promega)
The autosomal PCR analysis is performed equivalent to the method of the kit. This is a 16 STR kit that amplifies variable regions on the human DNA and the Amelogenine gender marker in one reaction.

In addition to the 22 autosomal chromosomes, which we examine in the standard DNA analysis, each cell nucleus contains a pair of sex chromosomes. A woman has two X chromosomes and men have one X and one Y. In reproduction both parents pass a sex chromosome on to their child. Of the Y chromosome of the man a copy is transmitted from father to son, therefore, all men related in the same male lineage have the same Y-chromosomal combination of DNA markers (Y-chromosomal haplotype). In addition it is possible that unrelated males by chance have the same Y-chromosomal haplotype. Y-chromosomal haplotypes are therefore not unique. Thus, when Y-chromosome DNA testing results in matching haplotypes of a sample and a person, the probative value of the evidence may be relatively low. However, by Y-chromosomal testing a person may be excluded as a donor of a sample.

Differential lysis
When a sample may comprise both sperm cells and other body cells differential lysis can be carried out. This technique is often applied in sexual assault cases, in order to separate possible sperm cells from other body cells present and results in a sperm cell fraction and a fraction of the other body cells.

Low template DNA analysis (LT-DNA)
If the sample contains very low concentrations of DNA, standard DNA-analysis results in limited genetic information. When analysing LT-DNA samples one has to take into account artefacts such as allelic drop out (loss of DNA markers), increased stutter peaks and drop in. To confirm the presence of a DNA-allele in a LT-DNA sample, the analysis is performed in triplicate and to test reproducibility of the reported DNA markers.

5. Full DNA profiles, partial DNA profiles and mixed DNA profiles

In a full DNA profile all DNA markers are visible at all examined loci. In the DNA profile of a person at each locus one (homozygote) or two (heterozygote), DNA-markers are visible. One of the analysed loci is always the amelogenine which is located on the X-and Y-chromosome. This shows that it is a DNA profile of a male or a female.

In a partial DNA profile not all DNA markers are visible. This may be caused by degradation of the DNA material, or could be due to the small quantity of DNA in the sample. A partial DNA profile is less rare than a full profile, and thus has a lower probative value.

A DNA mixed profile is a profile in which DNA-markers of more than one person are visible. If a DNA sample contains DNA of more than one person, this results in a mixed DNA profile. Mixed DNA profiles can be divided into two groups:

- Mixed DNA profiles of two contributors:

In a mixed DNA profile of two persons, at least at two loci more than two DNA markers are visible and in no locus more than four DNA markers are visible. If the two persons in the mixed DNA profile contributed unequal amounts of DNA, sometimes the DNA profile of the major contributor (of the person who has contributed most DNA) and the DNA profile of the minor contributor can be extracted on the basis of peak height. If there is reference material available, it can be compared with the DNA profile of the major contributor and the DNA profile of the minor contributor.

- mixed DNA profile of more than two persons:

In cases with mixed DNA profiles of more than two persons it is often not possible to determine how many people have contributed DNA. Sometimes it may also be possible to derive a DNA profile of a major contributor from a mixed DNA profile of more than two persons.

6. Statistics

If two DNA profiles match a statistical calculation can indicate the probative value of the evidence. In the case of a single DNA profile the probative value is shown by the rarity of the DNA profile, expressed as a DNA profile frequency or random match probability (RMP). When there is a mixed DNA profile, from which no single DNA profile can be extracted, the inclusion probability (CPI) or the Likelihood Ratio (LR) are calculated to give probative value.

DNA profile frequency (RMP)

The basis for the calculated frequency is a reference database from which the frequencies of the individual DNA-markers are derived. The frequency of a DNA-marker shows how often this DNA marker is found in the population. For each locus, the frequency of the observed DNA markers is estimated using a mathematical model.
By multiplying the frequencies of all DNA markers of all loci in the DNA profile, the frequency of the entire DNA-profile is obtained. This DNA profile frequency is a measure for the probability that the DNA profile is observed in a randomly selected person (the Random Match Probability).

Combined chance of inclusion (CPI)

If a mixed DNA profile is obtained from which no single DNA profiles can be extracted the inclusion probability can be calculated. In the determination of this probability, all combinations of DNA markers that may have contributed to the mixed DNA profile are to be taken into consideration.
A person who has a DNA profile that matches with one of these combinations of DNA markers is a potential donor. The chance of inclusion or CPI (Combined Probability of Inclusion) is a measurement of the probability that the DNA profile of a randomly chosen person matches with the mixed DNA profile.

Likelihood Ratio (LR)

In certain cases it can be assumed that a sample contains cellular material from a person, such as samples taken of the body of a victim in a sexual assault case. In that case, one can expect to observe at least the DNA markers of the victim in the sample. By using the likelihood ratio method, it is possible to include this information in the calculation. The method calculates the probability to obtain the mixed DNA profile, in the framework of two hypotheses. For example: Hypothesis 1: the sample includes cellular material of the victim and the suspect, and Hypothesis 2: the sample contains cellular material of the victim and an unknown unrelated person. The probability of obtaining the mixed DNA profile is calculated, first under the assumption that the first hypothesis is true, and then under the assumption that the second hypothesis is true. By dividing the two conditional probabilities, a likelihood ratio (LR) is calculated. The larger the LR is the more support there is for hypothesis 1: sampling includes cellular material of the victim and the accused.

Match without statistical support

For a statistical calculation, one must be sure that all the DNA markers of all contributors are visible in the (mixed) DNA profile and no artefacts have occurred. This is not always the case. If a person matches such a DNA profile, but there is too little information to perform a reliable statistical calculation, we can in such cases report 'a match without statistical support'.

7. Dutch national DNA database

Submission and registration of DNA profiles and reporting of matches

Verilabs submits DNA profiles of reference material and trace material in the Dutch National DNA database if an order has been given to do so.The DNA profiles that are obtained must be suitable for comparison and/or storage in the DNA database. Verilabs follows the guidelines that the DNA database has prepared herefore. It is also possible to perform a manual search or a periodically repeated search in the database after consultation with the administrator of the DNA database. The storage of DNA profiles and the results of the comparison in the DNA database (eg profile clusters) are included in the report.
For more information about the procedures of the DNA database, see http://dnadatabank.forensischinstituut.nl/.

8. Accreditation and validation

Verilabs is part of Baseclear and is accredited NEN-EN-ISO/IEC 17025 (RvA 457). All methods and techniques are validated through validation studies. The staff is qualified to perform all methods used in the analysis process. If you need more information about the methods for which Baseclear is accredited, you can find it on the site of the Accreditation Council (www.rva.nl).

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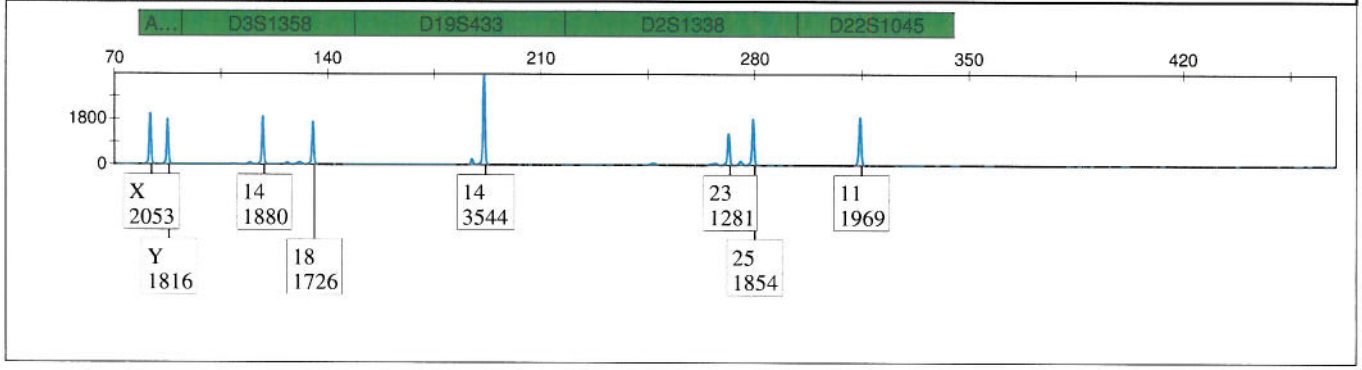
Sample File

P02_62200_DNA-Stub-enkel#BC01_B03_003.fsa



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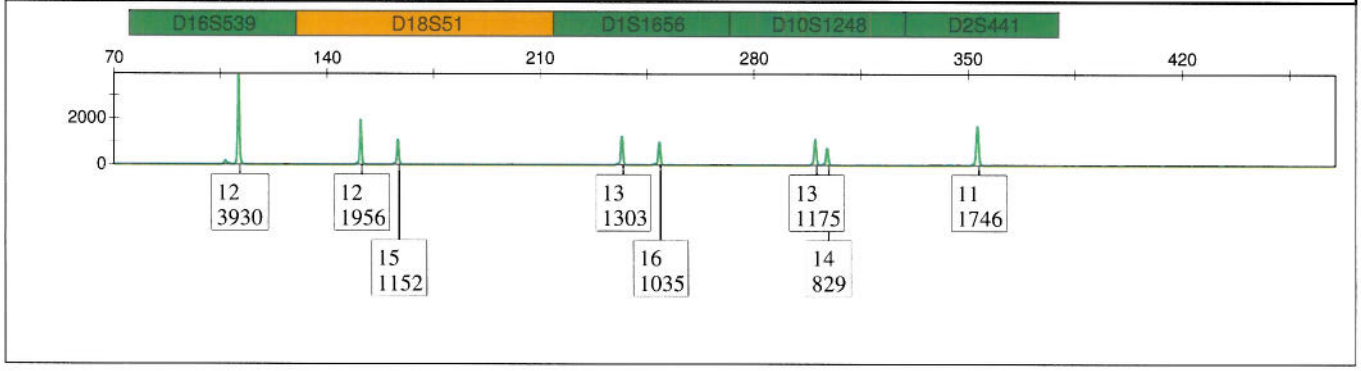

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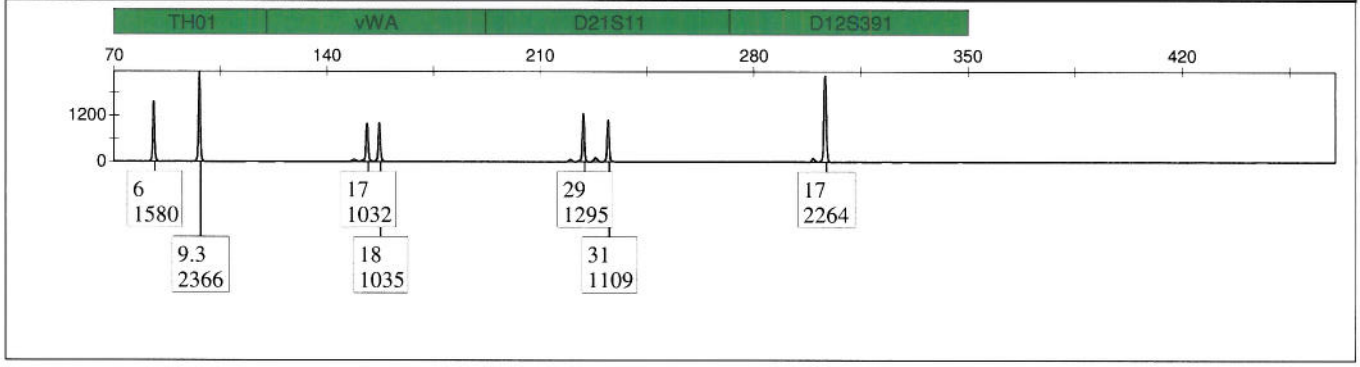

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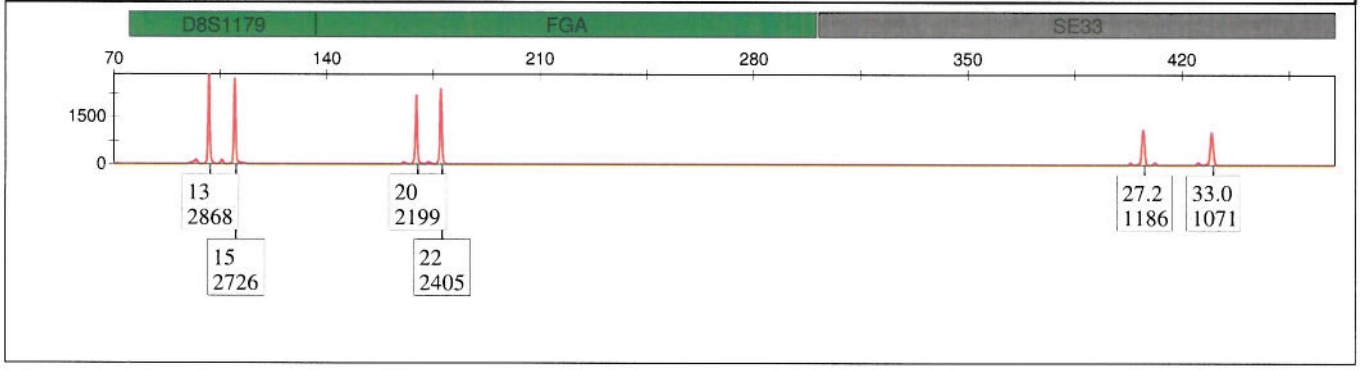

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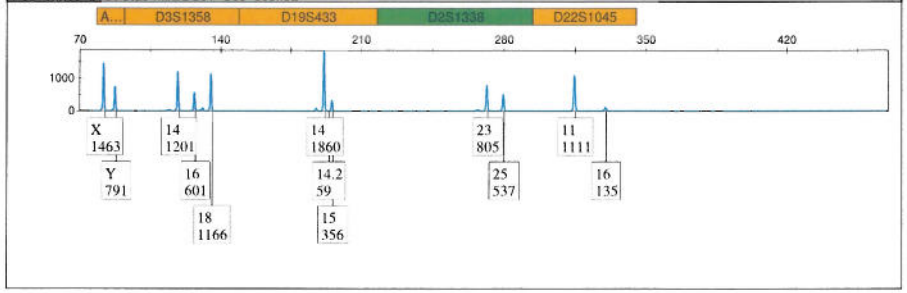

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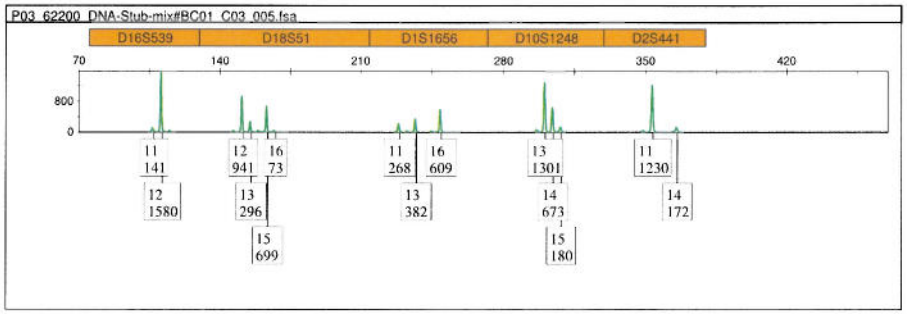

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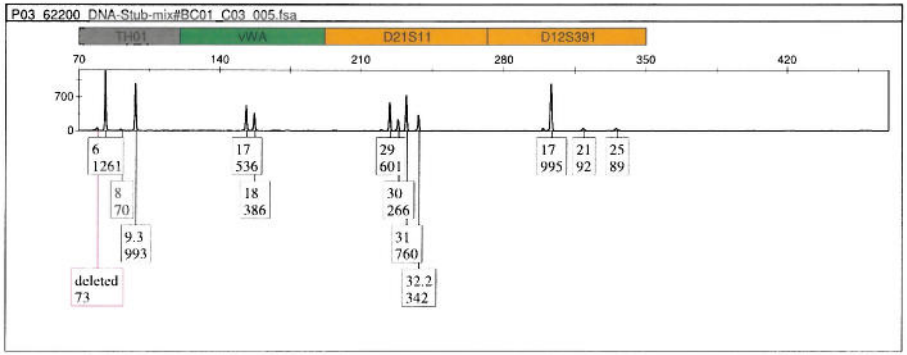

Applied Biosystems
GeneMapper® ID-X 1.2.1

Project: VWS140425A

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