DNA-DATABASE MANAGEMENT
REVIEW AND RECOMMENDATIONS

ENFSI DNA Working Group
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1 Introduction

This document discusses the different aspects of forensic DNA-database management and makes recommendations where this is deemed useful. Questions, remarks and additions in relation to this document can be sent to the chair of the DNA-database & Legislation subgroup of the ENFSI DNA Working Group Dr. Ir. C.P.(Kees) van der Beek MBA (k.v.d.beek@nfi.minvenj.nl) who has compiled this document with the help of the members of the ENFSI DNA Working Group and other experts. The first (2008) version of this document was approved at the 28th ENFSI DNA Working Group meeting which was held on 23rd - 24th April 2008 in Prague. Every year an updated version of the document is presented at the ENFSI DNA Working Group meeting and republished on the ENFSI website after the approval of the group.

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2 Establishing a forensic DNA-database

The power of a forensic DNA-database is that it can assist in the investigation of crimes by linking DNA-profiles from crime-related biological trace material to each other and to the possible donors (or their relatives) of that biological trace material. Over the past 10 years forensic DNA-databases have proven to be very powerful in this respect. In spite of this success not all ENFSI member countries have a DNA-database yet.

The Council of the European Union invited Member States to consider establishing DNA Databases[1] back in 1997. In 2001 a European Standard Set (ESS) of loci was established to enable the comparison of DNA profiles from different countries[2] and in 2009 the ESS was expanded with 5 extra loci[3]. In June 2008, the Council of the European Union converted the Treaty of Prüm into EU legislation (The EU-Prüm-Decision). The new EU-legislation requires every EU member state to establish a forensic DNA-database and to make this database available for automated searches by other EU member states. As DNA-profiles are regarded as personal data, national privacy legislation derived from the European Data Protection Directive 95/46 also applies to forensic DNA-databases. This has certain consequences, which will be explained in chapter 14. It is therefore preferable to have specific DNA-database legislation.

The DNA-Working Group of the ENFSI strongly feels that every European country should have a forensic DNA-database to enhance:
the possibility to solve crimes
the number of crimes that are solved
the speed with which crimes are solved
the time the police can spend on other work
the possibility to link unsolved crimes
the possibility to identify false identities

The purpose of a National DNA database is usually defined in the legislation (e.g. intelligence tool, evidence provider, combat volume crime, combat serious crime, identify donors of stains, link crime scenes etc.). This defined scope determines which categories of individuals should be included in the National DNA database.

ENFSI-recommendation 1
Every EU/ENFSI-country should establish a forensic DNA-database and specific legislation for its implementation and management.

[1] EU-Council Decision of 9 June 1997 on the exchange of DNA analysis results
[2] EU-Council Resolution 9192/01
[3] EU-Council Resolution 2009/C 296/01

3 Inclusion criteria

Several criteria determine whether a DNA-profile can/will be included in a DNA-database. In the paragraphs below, these criteria are discussed.

3.1 Source of the DNA-profiles

In most countries with a DNA-database, specific DNA-legislation regulates which DNA-profiles can or should be included in that DNA-database. Some countries require the additional specific authorization of a magistrate. Because the purpose of a DNA-database is to find matches between crime-related stains and persons, these two types of DNA profiles are almost always both present in a DNA-database.

Crime-related stains

These are the DNA-profiles which are assumed to originate from the perpetrators of crimes. It is the responsibility of the police to collect crime-related items. When the origin of the trace is unclear, reference samples (e.g. from the victim or from witnesses) should be collected and their DNA-profiles should be compared to those of the crime related specimens to prevent DNA-profiles from innocent people being included in the DNA-database. DNA-testing in high-volume-crime (burglaries etc.) is often very standardized and automated, to increase the number of traces analyzed and to decrease the throughput time from crime scene to inclusion in the DNA-database. Specimens taken at these types of crime scenes should be chosen in such a way that the possibility that they originate from a perpetrator is maximized. Examples of such "safe" traces are: bloodstains (e.g. on broken windows), saliva stains (e.g. on tins, cups, bottles), cigarette butts and chewing gum, of which the people who live in house which has been burgled can testify that they did not produce those samples.

Usually the types of crime from which the stains originate correspond with the types of crime for which persons can be forced to provide a DNA sample. However, in some countries there are no limitations with regards to the types of crime from which stains can be included in the DNA-database. In practice, stains related to minor crimes are not collected due to the priority given to more serious crimes, but the absence of limitations on crime scene stains opens up the possibility of solving minor crimes (like littering or damaging public or private property) if the person corresponding to the stain has already been included in the DNA-database for a more serious crime. Moreover, linking minor to more serious crimes may yield additional investigative information which may speed up the investigation into the more serious crime.

ENFSI-recommendation 2
The type of crime-related stain DNA-profiles which can be included in a DNA-database should not be restricted.

Persons

Several categories of persons may be included in a DNA-database.

- Convicted persons, persons who have been found guilty of a crime by a court of law and may (or may not) be (conditionally) convicted to imprisonment, a penalty, labor, hospitalization or a combination of these. A conviction can be overturned by a successful appeal to a higher court. In some countries it is

possible to include persons in the National DNA-database who have been convicted in the past and who have already completed their imprisonment. This is called retrospective sampling.

- Suspects, persons who have not yet been found guilty but are officially the subject of investigation and/or prosecution.
- Arrestees, persons who have been taken into custody by the police but are not (yet) a suspect as defined above.
- Volunteers, persons outside the abovementioned categories who have agreed to give a DNA-sample for investigative purposes. In some countries volunteers can also be included in the national DNA-database with their consent.

The legal criteria for the inclusion of convicts, suspects and arrestees in a national DNA-database are usually either specific types of crime or the maximum punishment that the law allows for a crime.

Obtaining a DNA-sample from convicted persons, suspects and arrestees may involve several steps.

- A person may first be asked to give a sample on a voluntary basis,
- An official police or judicial order may be given to provide a sample, either directly or upon refusal to give the sample on a voluntarily basis
- Various actions are possible in different countries upon refusal to provide a sample: conviction for the refusal, physical force to obtain a sample or taking a sample from an object with cell material from the person. A conviction for the refusal does not result in the production of a DNA-profile (and the inclusion of the DNA-profile in the National DNA-database) and hence is not a logical measure in DNA database legislation.

Since the identification of the donor of a depends on the presence of the donor in the DNA-database, more donors can be identified if more relevant persons are included in the DNA-database. Moreover, the persons included in the DNA-database should fit in the scope of the DNA-database. For instance including high volume crime scene stains but only persons convicted of sexual and capital crimes will not produce many matches.

ENFSI-recommendation 3
To increase the chance of identifying the donors of stains, the number of persons in a DNA-database who are likely to be the donors of those stains should be as high as legally (and financially) possible.

Apart from nationally collected DNA-profiles, DNA-profiles originating from international legal comparison requests may also be included to enable repeated comparisons against newly added DNA-profiles. See also chapter 20.

Victims

Some countries allow the inclusion of DNA-profiles of dead victims of unsolved crimes in their DNA-database. The purpose of this is to find matches which may help to solve the crime. If for instance the DNA-profile of a dead victim who was stabbed to death later on matches a blood stain on a knife, then the owner of the knife may become a murder or a manslaughter suspect. There are two types of victims. Identified and unidentified victims. Unidentified persons who apparently are not a victim of a crime usually are included in a missing persons DNA-database but may be compared with the criminal DNA-database in an attempt to identi-

fy them (see chapter 22). The “risk” of including victims is getting matches with other unsolved crimes in which case the victim becomes a suspect. Therefore, victims who are still alive, like other volunteers, should be informed and asked to give their consent.

Missing persons
Some countries allow the inclusion of DNA-profiles of missing persons if there is a suspicion that a crime is involved in their missing. The purpose of this is the same as for the inclusion of victims namely to find matches which may help to solve the crime.

Elimination profiles
The inclusion of DNA-profiles in the DNA-database for contamination detection purposes is dealt with in § 4.5.

3.2 Choice of loci

Most countries use commercially available kits to produce DNA-profiles for inclusion in their DNA-databases. Table 1 shows the contents of the different commercially available kits as well as the composition of the different standard sets discussed below. Some kits are included which are no longer sold commercially (e.g. QUAD, SGM). Historically these kits were used in relation to the first DNA-databases but their discriminating power is insufficient to generate meaningful matches in relation to the millions of DNA-profiles available for comparison today.

		Applied Biosystems														Promega													Qiagen												Biotype	Serac			Historic kits			Standard sets		
Chromosome	Locus	SGM+	Profiler	Profiler+	Cofiler	SE-filer	Blue-kit	Identifiler	Minifiler	NGM	NGM Select	Sinofiler	Globalfiler	Verifiler	Powerplex 1.1 or 1.2	Powerplex 2.1	Powerplex 16	Powerplex ES	Powerplex ESX 16 (Fast)	Powerplex ESX 17 (Fast)	Powerplex ESI 16 (Fast)	Powerplex ESI 17 (Fast)	Powerplex 18D	Powerplex CS7	Powerplex S5	Powerplex 21	Powerplex Fusion	Investigator ESSplex Plus Kit	Investigator ESSplex SE Plus Kit	Investigator Decaplex SE Kit	Investigator IDplex Plus Kit	Investigator Nonaplex ESS Kit	Investigator Hexaplex ESS Kit	Investigator TriplexDSF Kit	Investigator Triplex AFS QS Kit	Investigator HDplex Kit	Mentype Nonaplex I or QS	Mentype Pentaplex ESS	MPX II or MPX II-LF	Locally prepared kit Austria	Locally prepared kit Belgium	FFFL	QUAD	SGM	ENFSI/European Union (ESS)	Interpol (acceptable)	USA (CODIS core-loci)
1	F13B																							+																			+					
	D1S1656									+	+		+	+					+	+	+	+				+	+	+	+			+	+								+			+	+	
2	D2S1338	+				+		+	+	+	+	+	+	+					+	+	+	+	+				+	+	+	+	+	+											+		
	TPOX		+		+			+					+		+	+	+						+			+	+				+													+	+	
	D2S441									+	+		+	+					+	+	+	+					+	+	+				+	+										+	+	
	D2S1360																																			+												
3	D3S1358	+	+	+	+	+	+	+		+	+	+	+			+	+	+	+	+	+	+	+				+	+	+	+	+	+	+		+		+		+	+				+	+	+
	D3S1744																																			+												
4	FIBRA (FGA)	+	+	+		+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+			+		+	+			+	+	+	+
	GABA																																															
	D4S2366																																			+												
5	D5S818		+	+				+				+	+		+		+							+			+	+				+												+	+	
	CSF1P0		+		+			+	+			+	+		+		+							+			+	+				+												+	+	
	D5S2500																																			+												
6	F13A																							+																			+	+				
	ACTBP2 (SE33)					+					+		+					+		+		+							+	+		+			+		+				+				+		
	D6S1043											+		+												+																						
	D6S474																																			+												
7	D7S820		+	+	+			+	+			+	+		+		+							+			+	+				+												+	+	
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8	D8S1179	+		+		+		+		+	+	+	+			+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+					+		+	+			+	+	+	+
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10	D10S1248									+	+		+	+					+	+	+	+					+	+	+			+	+								+			+	+	
	D10S2325																																			+												
11	TH01	+	+		+	+		+		+	+		+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+				+		+	+			+	+	+	+
12	vWA	+	+	+		+	+	+		+	+	+	+		+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+				+		+	+			+	+	+	+
	CD4																																										+					
	D12S391									+	+	+	+	+					+	+	+	+				+	+	+	+			+	+				+							+	+	
13	D13S317		+	+				+	+			+	+		+		+						+			+	+				+													+	+	
14	No loci																																																
15	FES/FPS																							+																			+	+				
	Penta E															+	+							+	+		+	+																	+		
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17	No loci																																																
18	D18S51	+		+		+		+	+	+	+	+	+			+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+					+	+			+	+	+	+
19	D19S433	+				+		+		+	+	+	+	+					+	+	+	+	+			+	+	+	+	+	+												+		
20	No loci																																																
21	D21S11	+		+		+		+	+	+	+	+	+			+	+	+	+	+	+	+	+			+	+	+	+	+	+	+					+		+	+			+	+	+	+
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22	D22S1045									+	+		+	+					+	+	+	+					+	+	+			+	+											+	+	
X/Y	Amelogenin	+	+	+	+	+		+		+	+		+				+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+		+		+		+				+	+	+
Y	DYS391												+														+																					
Y	Y Indel												+																																				

Table 1: Commonly used loci and kits for DNA-databases

The EU-Council resolutions 2001/C 187/01 and 2009/C 296/01 call upon European countries to use the European Standard Set (ESS) as a minimum to enable international comparison of DNA-profiles. In the USA 13 loci are presently required for the inclusion of a reference profile in the National DNA-database of the USA (CODIS). The INTERPOL Standard Set of Loci (ISSOL) is equal to the European Standard Set plus the Amelogenin locus. Until December 2009, the European Standard Set of Loci contained only 7 loci. This was enough for occasional exchanges of DNA-profiles between countries. However, when massive exchanges of DNA-profiles are undertaken, as has been made possible by the INTERPOL DNA-database and the EU-Prüm-Decision, 7 loci are generally not enough because the chance of getting adventitious matches becomes significant and makes a routine process inefficient. In addition, each DNA-database contains a significant

portion of partial profiles with a much higher probability of matching randomly. This is why ENFSI has recommended that the European Standard Set of Loci should be extended by 5 additional loci and the Council of the European Union adopted this recommendation on 30 November 2009. In the meantime, commercial companies have produced kits which contain these new loci to enable the implementation of the new ESS loci. Also the FBI has recently published an intended expansion of the CODIS core loci set.[4]

3.3 Number of loci

For the comparison of DNA-profiles between EU-countries, they must comply with the Prüm inclusion rules. For comparison of DNA-profiles in a single country, however, other criteria may apply. DNA-profiles from crime-scene stains may not contain all the loci present in the kit(s) used in a country to produce DNA-profiles. These partial DNA-profiles may be included in national DNA-databases provided they have a high enough evidential value and/or the chance of producing adventitious matches is not too high (see chapter 6). Two criteria commonly used for the inclusion of partial profiles are 1) minimum number of loci and 2) maximum random match probability. The second criterion is better because a DNA-profile containing only 4 or 5 loci may have a lower random match probability than a DNA-profile containing 6 loci if (some of) the alleles in the former are rare.

A simulation study has been published in which the influence of including DNA-profiles with lower numbers of loci on the number of genuine and adventitious matches generated in a simulated Swiss DNA-database is shown[5].

ENFSI-recommendation 4
Managers of national DNA-databases should establish (together with other stakeholders) criteria for the inclusion of partial DNA-profiles to obtain an acceptable balance between the minimum allowable level of evidential value (maximum random match probability) of a DNA-profile and maximum number of adventitious matches a partial DNA-profile is expected to generate.

Sometimes an unsolved crime is so serious that a DNA-profile which does not meet the minimum criteria for inclusion in the National DNA-database is still searched against a National DNA-database, accepting the fact that many of the matches which are found are adventitious matches. Tactical police work is then necessary to find out if one of the matches leads to a potential suspect. When no potential suspect is found by the police, the search action may be repeated after some time or at regular intervals, because new people will have been added to the National DNA-database. The CODIS-autosearcher-mode produces only the new matches in these types of search actions, which saves work in sorting out the old and the new matches.

For historic reasons the countries who started early with their DNA-databases (like the UK and the Netherlands) still have DNA-profiles in their DNA-databases which

[4] D.R. Hares (2012) FSI Genetics 6(1), E52-54. Expanding the CODIS core loci in the United States
D.R. Hares (2012) FSI Genetics 6(5), E135. Addendum to expanding the CODIS core loci in the United States

[5] T. Hicks et al (2010) FSI Genetics 4(4) 232-238. Use of DNA profiles for investigation using a simulated national DNA database: Part I. Partial SGM Plus® profiles

were produced by the older commercial kits like QUAD (4 loci) and SGM (6 loci + Amelogenin). For economic reasons these DNA-profiles are often only upgraded when they produce a match. This also implies, however, that these profiles often do not fulfill the criteria for international comparison, which is a missed chance to solve the case from which the DNA-profile originates. An upgrade of a DNA-profile is of course only possible if the cell material or the DNA-extract is still available for further testing.

ENFSI-recommendation 5
DNA-profiles produced by older commercial kits should be upgraded (if possible) after a match in the National DNA-database to increase the evidential value of the match and to decrease the possibility of an adventitious match and also to fulfill the criteria for international comparison if a country wants to include DNA-profiles produced by older commercial kits in international search actions.

The number of loci in reference samples should be the maximum of the number of loci present in the kit(s) used for the production of the DNA-profiles of the reference samples, to enhance the chance of finding relevant matches with partial DNA-profiles

ENFSI-recommendation 6
To enhance the chance of finding relevant matches with partial crime stain DNA profiles, reference samples profiles should only be loaded to a database where a complete profile is obtained using the PCR chemistry of choice.

3.4 Supplier of profiles

It goes without saying that the reliability of the matches produced in a DNA-database is dependent on the reliability of the DNA-profiles participating in the match. A wrongly called allele may prevent a match and a sample mix-up may produce a false match. That is why labs producing DNA-profiles for DNA-databases should objectively be able to show that they produce DNA-profiles with quality-driven processes, meaning for example, that there must be arrangements in place whereby the laboratory can demonstrate:

- The validation of its analytical processes
- Arrangements for continuous monitoring of data quality and consistency
- Arrangements for error identification, error handling and incorporation of corrective and preventative actions

Council Framework Decision 2009/905/JHA of 30 November 2009 on accreditation of forensic service providers carrying out laboratory activities in fact requires ISO 17025 accreditation of DNA laboratories

ENFSI-recommendation 7
Labs producing DNA-profiles for a DNA-database should, as a minimum, be ISO-17025 (and/or nationally equivalent) accredited and should participate in challenging proficiency tests.

3.5 DNA-profiles produced from low levels of DNA

DNA-profiles produced from low levels of DNA, either by the standard number or an enhanced number of PCR-cycles or by signal enhancing techniques like in-

creased injection settings or post-PCR clean-up, can contain allele drop-ins and allele drop-outs even if a consensus profile is produced from repeated determinations[6]. Hence they may never cause matches when included in a DNA-database if all alleles are required to match. So if DNA-profiles produced from low levels of DNA are included in a DNA-database, they should be recognizable and/or a dedicated match strategy (allowing one or more mismatches) should be used for them to detect possible allelic drop-ins and drop-outs (as will be discussed in § 5.4). For a discussion on mixed profiles from low levels of DNA see § 3.9

ENFSI-recommendation 8
When DNA-profiles produced from low levels of DNA are included in a DNA-database they should be recognizable and/or a dedicated (near) match strategy should be used for them.

3.6 Composite DNA-profiles

The smaller PCR-products of DNA-profiles from stains regularly show higher peak heights than the larger PCR-products. This is due to partial breakdown of the DNA. It can even happen that the larger PCR-products disappear below the detection threshold, while the smaller PCR-products still give good peaks. Sometimes the peak heights of the larger PCR-products can be improved by increasing the input of the PCR-reaction but this then often results in overloaded peaks of the smaller PCR-fragments. By using a low as well as a high input during the PCR-reaction, two DNA-profiles may be obtained in one of which the peaks of the smaller fragments are OK and in the other one the peaks of the larger fragments are OK. These can then be combined into a composite DNA-profile. This should, however, only be done with DNA-profiles obtained from the same DNA-extract and not with DNA-profiles obtained from different DNA-extracts, because it can not be excluded that different samples contain DNA-from different persons.

ENFSI-recommendation 9
Composite DNA-profiles should only be created from DNA-profiles generated from the same DNA-extract because it cannot be excluded that different samples contain DNA-from different persons.

3.7 Rare alleles/chromosomal anomalies

For each commercial kit, the known alleles of each locus and their relative frequency (in several different populations) is described in the manual of the kit. From time to time new alleles are observed in DNA-profiles and the question is whether these new alleles should be included in the DNA-database and which frequency they should get in order to assign the probability of the DNA-profile in the population of interest (i.e., the so-called random match probability). When a new allele is observed, its appearance should of course be confirmed by repeated DNA-isolation, PCR, Capillary Electrophoresis and allele calling. Before including the new allele in the DNA-database, a literature search may be conducted to see whether the new allele has been observed and/or sequenced before. A good source for this is the DNA-database of NIST

[6] C.C.G. Benschop et al. Forensic Science International Genetics 2011 5 (4), pp. 316-328. Low template STR typing: Effect of replicate number and consensus method on genotyping reliability and DNA database search results.

(http://www.cstl.nist.gov/biotech/strbase/index.htm). If a new allele has not been sequenced yet it can be sent to NIST for sequencing. Only new alleles of which the size can be accurately determined using the internal DNA-size-standard should be included in the DNA-database. An additional criterion for including a new allele in the DNA-database is the number of internal or/and external observations of the new allele.

The relative frequency attributed to a new allele may be one divided by the size of the reference database used to estimate allelic proportions, a predetermined (low) relative frequency or a proportion calculated according to alternative statistical estimation procedures. Allelic relative frequencies can be estimated using methods like the Balding[7] size correction formula (i.e., a Bayesian estimator).

ENFSI-recommendation 10
When a new allele is observed in a DNA-profile, its presence should be confirmed by repeated DNA-isolation, PCR, Capillary Electrophoresis and allele calling of the DNA-profile. Only new alleles of which the size can be accurately determined using the internal DNA-size-standard should be included in the DNA-database.

Sometimes chromosomal anomalies are observed in DNA-profiles. As a result, a locus may show more than 2 peaks. As these chromosomal anomalies are rare and hence contribute to the evidential value of the DNA-profile, it would be logical to recommend that they should be included in the DNA-database. However, extra peaks can also be caused by somatic mutations which may only appear in certain tissues/body fluids. This means that DNA-profiles from different sample types (e.g. buccal scrape and blood) may not fully match. They can of course contribute to the evidential value after the match has been found in the DNA-database. An inventory of tri-allelic loci observations can be found at:
http://www.cstl.nist.gov/biotech/strbase/tri_tab.htm

ENFSI-recommendation 11
Alleles from loci with chromosomal anomalies should not be included in a DNA-database as they may be caused by somatic mutations which may only occur in certain tissues/body fluids.

The inclusion rules for DNA-profiles which are compared on the basis of the EU-Prüm Council Decisions say that a tri-allelic locus should be converted into the first allelic value plus a wildcard. This is in contrast with recommendation 11 but cannot be changed at this moment because Council Decision 2008/616/JHA which contains the inclusion rules, will not be changed until all EU-countries are operational.

3.8 Wildcards

If there is uncertainty about the presence or absence of an allele in a DNA-profile, a so-called “wildcard” may be included in the DNA-profile. This may be the case with low peaks of which the DNA-analyst is not sure whether it is a homozygote peak or a locus of which one allele has dropped out.
In some countries a wildcard is used to replace a rare allele which is not in the ladder-range of the DNA-kit used. In this case the wildcard represents a designated allele which can be used to verify a match with a DNA-profile containing such a

[7] Balding, DJ (1995) Estimating products in forensic identification. J. Am. Stat. Assoc. 90:839-844.

wildcard. Searching with wildcards means that any allele is accepted as a match for the wildcard-allele. Different countries use different designations for their wild-cards. For the international comparison these national designations have to be converted into mutual designations. Countries that exchange DNA-profiles under the terms of the EU-Prüm-decision presently use an "*" for a wildcard. There has been a proposal to use the "*" for a wildcard which represents a designated allele and to use a "B" for wildcard which represents an unknown allele, but this proposal has not yet been implemented.

3.9 Mixed profiles

Mixed profiles can occur when two or more persons have left cell-material on the same object (e.g., smoking from the same cigarette or drinking from the same bot-tle) or when e.g., cells of a perpetrator are mixed with cells of a victim (which often occurs in rape cases). If possible, mixed DNA-profiles should be interpreted and designated into their contributing DNA-profiles. Mixed profiles from (known) victims and (unknown) donors sometimes can be resolved because the alleles of the DNA-profile of the victim can be subtracted from the mixed profile. The remaining alleles must belong to the unknown donor. Mixed DNA-profiles from two donors, however, can often only be completely designated into separate contributors if there is a significant difference in contribution between the two donors (Major-Minor-situation). A working group of the IFSG has produced a document with guidelines for the analysis of mixed profiles. Several software tools, both commer-cial and open-source, have become available that can deconvolute mixtures and produce possible combinations of donor profiles (see website of the ISFG http://www.isfg.org/ for open source software). Such tools may also be used pro-vided they are properly validated.

ENFSI-recommendation 12
The guidelines in the document of the ISFG-working group on the analysis of mixed profiles should be used for the analysis of mixed profiles. Software tools may also be used provided they are properly validated.

In some DNA-databases (like CODIS) mixed DNA-profiles can be included and searched against. This is very useful when a mixed DNA-profile cannot be reliably resolved in its contributing components. In CODIS it is even possible to designate remaining alleles as "required" if one of the participants of a mixed DNA-profile has been identified. Matches with reference samples will only be shown if these re-quired alleles are present in the reference sample DNA-profile. A numerical match between a reference sample and a mixed profile must always be checked against the electropherograms of the DNA-profile because a numerical match may not be a real match, as shown in figure 1. For this reason, mixed profiles cannot be used at this moment for the automated international comparison of DNA-profiles, such as the comparisons which are performed under the terms of the EU Prüm Council Decision and comparisons in the INTERPOL DNA-database

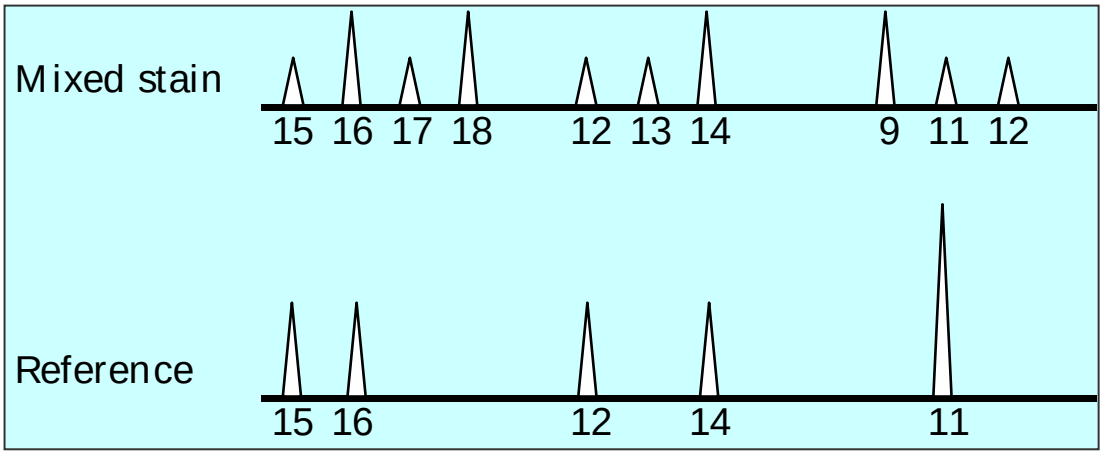

Figure 1: Three loci of a mixed stain and a reference sample which match on a numerical basis but are an unlikely combination when peak heights are taken into account

ENFSI-recommendation 13
A numerical match between a reference sample and a mixed profile must always be checked against the electropherogram of the mixed profile.

Mixed profiles of more than 2 persons should not systematically be included in a DNA-database because they generally will produce too many adventitious matches. Manual searches with this type of profiles may, however, be useful.

ENFSI-recommendation 14
Mixed profiles of more than 2 persons should not systematically be included in a DNA-database because they generally will produce too many adventitious matches.

Special software exists to deconvolute mixed DNA-profiles into possible contributors (see above). These possible contributors can then be searched against the national DNA-database of a country. Some people have expressed their concern that this will lead to an increase of false positive matches. Compared to the situation where mixed profiles themselves are included in a DNA-database (which can for instance be done by countries using CODIS), searching with possible contributors of a mixed DNA-profile will not lead to more false positive matches.

In rare cases a mixed profile can be obtained from one person. This can happen when a buccal swab is taken from a person who has had a bone-marrow transplantation. The blood has the DNA-profile of the donor and the rest of the body still has the original profile. When taking a buccal swab very small superficial blood vessels may be damaged causing a mixed profile.

Mixed profiles obtained from low levels of DNA can contain allelic drop-ins and drop-outs and are even more difficult to analyse than single profiles obtained from low levels of DNA. The use of consensus and pool profiles may assist in the analysis and

interpretation of these profiles[8]. Special software has been developed to compare these profiles to reference samples resulting in a likelihood ratio expressing the ratio of the probability of the results given that the trace came from the person at the source of the reference profile and an unknown person, and the probability of the results given that the trace came from two unknown persons[9]. Attempts are being made to link this software to a DNA-database to be able to compare complex mixed profiles to all reference profiles in the DNA-database[10]. This will result in a list of likelihood ratios for each reference profile in the DNA-database. The names of the persons associated with the reference profiles with the highest likelihood ratios can then be used by the police as an investigative tool. Additional DNA-testing may be necessary to confirm/infirm that a candidate obtained in this way could be a real contributor to the mixed profile.

3.10 Sequence variation between STR alleles of similar size

The present designation of STR-alleles is based on their number of repeats as determined by their size in capillary electrophoresis. More sensitive analyses using ion-pair reversed-phase high-performance liquid chromatography electrospray-ionization quadrupole time-of-flight mass spectrometry (ICEMS)[11,12] or next generation sequencing have shown, however, that STR-alleles in general display considerable sequence variability, which results in additional discrimination for alleles with identical sizes. These findings have significant consequences for forensic DNA-typing:

- Alleles determined as similar by capillary electrophoresis will be differentiated due to sequence variability
- Match probabilities will be lower than presently calculated because allelic proportions will be smaller resulting in an enhanced discrimination power of DNA typing which is especially important for mixtures and partial DNA profiles.
- The established DNA databases can still be used but the nomenclature of the alleles will have to be adjusted to deal with different alleles of similar size.

3.11 Non autosomal STR-markers

In the previous paragraphs only autosomal STR-markers were discussed. However also the X- and Y-chromosome contain STR-markers. Especially the Y-chromosomal markers are important and frequently used in forensic DNA-testing because they can be used to reveal the presence of male DNA amongst an excess of female DNA and to help establish male familial relationships because it inherits unchanged from a father to his sons (provided there is no mutation). X- and Y-chromosomal markers can easily be stored in DNA-databases like CODIS. The difference with other STR-markers is of course that most Y-chromosomal STR-markers contain only one allele due to their haploid nature. Searching with Y-chromosomal STR-markers is also possible but this implies a familial search which may need special permission of the competent authorities.

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[8] C. Benschop et al (2013) Consensus and pool profiles to assist in the analysis and interpretation of complex low template DNA mixtures. Int. J. Legal Med. 127, 11-23.

[9] http://forensim.r-forge.r-project.org/

[10] http://dnadatabank.forensischinstituut.nl/Images/hinda-haned-interpol-nov2013_tcm127-491037.pdf

[11] Oberacher et al. Human Mutation 29(2008) 3: 427-32. Increased forensic efficiency of DNA fingerprints through simultaneous resolution of length and nucleotide variability by high-performance mass spectrometry

[12] Oberacher et al. Electrophoresis 29(2008) 23: 4739-50. The next generation of DNA profiling - STR typing by multiplexed PCR - ion-pair RP LC-ESI time-of-flight MS

With rapidly mutating Y-STR's, males of the same male lineage may still be distinguished from each other[13]

3.11.1 Y-chromosomal STR-markers

Y-chromosomal markers belong to the lineage (or haploid) markers. Due to the lack of recombination and linear mode of inheritance both the sampling strategy and the reporting of frequencies differs to autosomal DNA markers but follows the same principles based on population genetics theory and laws of probability. Because of full linkage of markers within a Y-STR profile (haplotype) the product rule cannot be applied and large haplotype reference databases are mandatory instead to perform calculations. The YHRD (Y Chromosome Haplotype Reference database) is the largest, annotated, tightly curated and quality controlled forensic database.[14] It is designed to store haplotypes from hundreds of population samples from around the globe and to rapidly disseminate haplotype frequency data via the internet to forensic analysts. The databases also include several tools to analyze population substructure effects, to interpret matches between Y-STR profiles, to attach likelihood ratios in mixture analyses and to formulate valid forensic testimonies.YHRD is built by direct submissions of population data from individual certified laboratories. Upon receipt of a submission, the YHRD staff examines the originality of the data and assigns an accession number to the population sample and performs quality assurance checks. The submissions are then released to the public database, where the entries are retrievable by search for haplotypes, populations, contributors or accession numbers. Currently the YHRD presents about 130,000 haplotypes in 900 different populations. All population data published in forensic journals as FSI Genetics or International Journal of Legal Medicine are required to be validated by the YHRD custodians and are subsequently included in the YHRD[15].

3.11.2 X-chromosomal STR-markers

X-chromosomal STR markers can be useful in analyzing specific kinship cases. Like Y-chromosomal markers they can be stored and searched in DNA-databases like CODIS but also here familial search restrictions may apply.

3.12 Amelogenin

Most commercial kits contain the amelogenin marker which is present on both the X- and Y-chromosome. The amelogenin gene on the X-chromosome contains a 6 basepair deletion which results in different PCR fragment lengths and the ability to distinguish male and female DNA-profiles. In rare cases a mutation or a deletion in the amelogenin gene can result in the inability to produce a PCR-fragment which then gives a wrong impression about the gender of the DNA-profile donor[16]. Because the amelogenin marker does not give full-proof results some companies are now also adding other Y-chromosomal markers to their kits (e.g. Globalfiler, Powerplex Fusion).

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[13] K.N. Ballantyne et al. A new future of forensic Y-chromosome analysis: rapidly mutating Y-STRs for differentiating male relatives and paternal lineages. Forensic Sci Int Genet. 2012 6: 208-18

[14] Willuweit S, Roewer L (2007) Y-chromosome haplotype reference database (YHRD): update. FSIGEN 1(2):83-7

[15] Carracedo et al. (2014) Update of the guidelines for the publication of genetic population data. FSI Genetics 10, A1-A2.

[16] For more information about amelogenin anomalies see: http://www.cstl.nist.gov/biotech/strbase/Amelogenin.htm

3.13 Mitochondrial DNA (mt-DNA) information

Also mt-DNA-information is frequently used in forensic DNA-testing. In contrast to autosomal DNA, of which only two copies are present in each cell, mt-DNA is present in many hundreds of copies. For that reason traces, which fail to give an autosomal DNA-result may still give an mt-DNA-result. Just like Y-chromosomal DNA-results can be used to help establish male familial relationships, mt-DNA-results can be used to help confirming (or not) relationships in the female lineage as it inherits unchanged from a mother to her (male and female) children (provided there are no mutations). It is common practice in forensic genetics to determine the rarity of an mt-DNA haplotype by searching the profile in question in dedicated mt-DNA databases. The largest and highest quality freely available mt-DNA database is the EDNAP Mitochondrial DNA Population Database EMPOP (www.empop.org) that offers the following features to forensics:

a) EMPOP offers tools and help for quality control of population datasets and individual sequences deriving from evidentiary samples. EMPOP is performing quality control for scientific studies on mt-DNA for the leading forensic genetic journals as a requirement before manuscript submission actually takes place.
b) EMPOP is using alignment-free haplotype searches to guarantee that matches are found in the database regardless of the alignment used.
c) In its new version (Vs. 3) EMPOP is designed to offer haplogroup determination of mt-DNA sequences based on a maximum likelihood concept.

Mt-DNA-information is included in DNA-databases as differences between the investigated DNA-profile and the Revised Cambridge Reference Sequence. As with Y-chromosomal markers searching is also possible but as this implies a familial search, special permission of the competent authorities may be needed. Depending on the legislation, storing such data may not be permissible.

ENFSI-recommendation 15
When non-autosomal STR profiles or mitochondrial profiles are added to criminal DNA-databases, specific operating procedures must be in place to avoid unintended familial searches

4 Deletion criteria

In this chapter the reasons for deleting DNA-profiles from DNA-databases are discussed. Regardless of the reason for deletion, the deletion of a DNA-profile should always be recorded in a verifiable way, including the reason for deletion. Deleting a DNA-profile from the DNA-database may also require the destruction of the cell material and hard copies of the DNA-profiles and their electropherograms. Deletion of DNA-profiles from back-ups or analytical data files is usually more difficult to do.

4.1 End of maximum storage time

In most countries there is a maximum time during which DNA-profiles are stored. Below is a list of criteria which are used by different countries for reference samples:

- Fixed time after inclusion
- Variable time after inclusion depending on the type of crime
- Variable time after inclusion depending on repeated convictions
- Until the death of a person
- Fixed time after the death of a person
- Variable time after the death of a person depending on the type of crime
- Fixed time after the completion of a person's punishment
- Variable time after the completion of a person's punishment depending on the type of punishment or punishment history
- Until no longer relevant (criterion from data-protection legislation)

In all but the first two situations, the custodian of the DNA-database is dependent on external information for the determination of the deletion date of a DNA-profile. In these cases the custodian should have access to this information preferably by means of automated messages after an event which influences the deletion date of a DNA-profile.

ENFSI-recommendation 16
If the removal of a DNA-profile from the DNA-database is dependent on external information, a process should be in place to give the custodian of the DNA-database access to this information preferably by means of an automated message after an event which influences the deletion date of a DNA-profile.

For DNA-profiles of stains which do not match, the storage time is usually fixed or variable depending on the type of crime or the statue of limitation of the crime. For DNA-profiles of stains which do match see § 4.3.

4.2 Non-conviction of a person

Suspects, arrestees and convicted persons who have successfully appealed against their conviction may have to be removed from the DNA-database if they are not convicted. If the law prescribes this, the manager of the DNA-database is dependent on information about the conviction or acquittal of these persons. Experiences in several countries have shown that this kind of information is not always provided in time by the courts or the public prosecution service. This has resulted in matches with persons who should have been removed from the DNA-database and courts have ruled that these matches are inadmissible as evidence. The

ENFSI-recommendation in the previous paragraph is equally applicable to this removal condition.

4.3 Match of stain with person

When a reference DNA-profile has matched a DNA-profile from a crime-scene-stain in the DNA-database and the match has been dealt with by the judicial authorities, the latter may be removed from the DNA-database because it has fulfilled its purpose. If the match occurs within the same case this is called a "benchwork-match". In some countries (like the Netherlands) a crime-scene-DNA-profile cannot be removed from the DNA-database until the custodian of the DNA-database has received a message that either the suspect has been convicted or that the prosecution has decided not to use the DNA-evidence. The ENFSI-recommendation in paragraph 4.1 is equally applicable to this removal condition. For various reasons countries may retain crime-scene stain profiles in their DNA-database even after they have shown a match with a person. The Nuffield Council for Bioethics even recommends this in their 2007 Bioethics report to verify possible future doubts about a match.[17]

4.4 Duplication

Sometimes persons are sampled repeatedly for inclusion in the DNA-database. As this is a waste of resources there should be a system which can be consulted by those responsible for sampling persons to see whether a person is already present in the DNA-database.

ENFSI-recommendation 17
There should be a system that can be consulted by those responsible for sampling persons to see whether a person is already present in the DNA-database.

Sometimes people use a false identity and for this reason duplication of sampling is not always avoidable. Therefore, a rapid biometric identification system like fingerprints should be linked to the system indicating whether a person is already present in the DNA-database.

ENFSI-recommendation 18
The system which can be consulted by those responsible for sampling persons to see whether a person is already present in the DNA-database should be combined with a rapid biometric identification system like fingerprints to verify whether a person is already present in the DNA-database.

The analysis of unintentional and (low level) intentional duplicates however is a useful quality control instrument. When removing a duplicate, the sample with the least chance of being removed in the future should be kept (if legally possible). Duplicates produced with partially non-overlapping sets of loci are of course also useful to keep (e.g., Powerplex 16 and Identifiler).

[17] http://www.nuffieldbioethics.org/fileLibrary/pdf/The_forensic_use_of_bioinformation_-_ethical_issues.pdf

4.5 Match with elimination database

Any DNA-database should have a so-called elimination DNA-database (or databases) associated with it which contains the DNA-profiles of persons that may have caused cross-contamination of the investigated traces. Such elimination databases should include of course anybody working on the DNA-samples in the DNA-lab and also people cleaning the labs or performing any other kind of maintenance. Also people earlier in the chain of custody such as the police and other persons present at the scene of crime should be included. In addition, unidentified DNA-profiles found in negative controls which may come from people involved in manufacturing disposables and/or chemicals should be included and shared with other ENFSI countries. When a DNA-profile in the DNA-database matches a DNA-profile from the elimination DNA-database, it should of course be deleted because it is not meant to be included. However, this should not be done before the contamination incident has been analyzed to confirm the presumed cause of the match (contamination) and actions have been formulated to prevent this (and similar) accidents happening again. Laboratories supplying DNA-profiles to the DNA-database may have their own elimination databases to exclude their own employees as a possible source of contamination.

ENFSI-recommendation 19
Any DNA-database should have an associated elimination DNA-database (or databases). This should include laboratory staff of all categories as well as visiting maintenance personnel. Profiles from those with access to traces (e.g. police) should also be included in addition to unidentified DNA-profiles found in negative controls which may originate in manufacturing disposables and/or chemicals. The latter category of DNA-profiles should be shared with other ENFSI-countries.

Manufacturers of disposables and/or chemicals should follow the joint recommendations of ENFSI, SWGDAM and SMANZL[18] to prevent contamination of their products.

ICMP[19] has developed a manufacturers' elimination database application that was devised in concert with the forensic DNA community, based on ICMP's independent status and data protection capabilities. The application has been successfully tested and is intended provide forensic DNA laboratories with the ability to query a database of DNA profiles of individuals from participating companies involved in the forensic DNA supply chain, to avoid the inadvertent inclusion of manufacturers' staff profiles in either forensic DNA databases or investigations.

4.6 New information demonstrating that the DNA-profile should not have been included

Sometimes during a police investigation new information becomes available showing that a trace, which was thought to be relevant to the crime, has another origin. Also a person may accidently have been asked or ordered to give a buccal swab

[18] Manufacturer contamination of disposable plastic-ware and other reagents—An agreed position statement by ENFSI, SWGDAM and BSAG. Forensic Science International: Genetics, Volume 4, Issue 4, July 2010, Pages 269-270.

[19] See paragraph 22.7.1 for more information about ICMP

related to a crime for which the law does not allow that. If such a DNA-profile has already been included in the DNA-database, it has to be removed as soon as possible to prevent unauthorized DNA-profiles to be present in the DNA-database.

ENFSI-recommendation 20
Policies and procedures should be in place to ensure that non relevant DNA-profiles are deleted immediately after their irrelevance has become clear

5 Matching rules

This chapter describes the criteria which are used to define the resemblance between DNA-profiles as a match.

5.1 Match/hit definition

The words “match” and “hit” are sometimes used in different ways. The Dutch police use the word match if DNA-profiles of crime related stains are similar and the word hit if a DNA-profile of a crime related stain is similar to a DNA-profile of a reference sample. In the USA the word “match” is used if two DNA-profiles in the CODIS DNA-database correspond to each other and the word “hit” is used if a match is confirmed by a DNA-expert. In this document we use the ENFSI definition[20], which does not differentiate between a hit and a match:

Hit/Match: A confirmed match between DNA profiles discovered by a database search at a single instant in time. It can be stain to stain or stain to person.

In this document the word ‘match’ will be used.

5.2 Search modes

DNA-profiles can be compared in different ways. In CODIS these are called search-stringencies:

- High-stringency means that all alleles of the loci which are present in both DNA-profiles must be equal
- Moderate-stringency means that of two DNA-profiles the alleles of a locus with the least number of alleles must be present in the corresponding locus of the other DNA-profile. This stringency is used when comparing mixed DNA-profiles with single DNA-profiles. Because in CODIS homozygotes are designated by a single allele value, searching at moderate stringency with single DNA-profiles also detects an allele drop-out in one of both DNA-profiles (e.g. 12/13 will also match the apparent homozygotes 12/ or 13/)
- Low-stringency means that in each locus which is compared between two DNA-profiles at least one allele of that locus must be present in the other DNA-profile. This stringency is used to find parent-child-relationships.

[20] http://www.enfsi.org/ewg/dnawg/db/exfile.2004-09-20.3012308078/attach/ENFSI%20DNA%20WG%20Terms%20and%20Abbreviations.pdf

The table below gives some examples of match results when a target[21] profile (15,16) is searched against different candidate profiles in CODIS.

Target profile	15,16	Match stringency		
		High	**Moderate**	**Low**
Candidate profile	15,16	Match	Match	Match
	15[22]	No match	Match	Match
	15,17	No match	No match	Match
	17,18	No match	No match	No match
	15,16,17	No match	Match	Match
	15,16,17,18	No match	Match	Match

Table 2: Examples of match results when a target profile (15,16) is searched against different candidate profiles in CODIS

In some countries a search strategy called "familial searching" is allowed. This means that apart from searching for full matches it is also allowed to search for matches with possible relatives of the donor of a crime scene associated DNA-profile.

This search strategy uses the above mentioned "low stringency" search mode to find possible parent-child relationships and may also search for profiles which:

1. share higher than the average number of alleles in random unrelated DNA-profiles (which may indicate a possible sibling)
2. contain rare alleles (which may indicate a possible family member)
3. have a high likelihood ratio and therefore provide for example very strong support for the proposition that the persons are related (e.g., are siblings) rather than for the proposition that the persons are unrelated..

From a statistical point of view the 3rd approach is the preferred strategy[23]. The programs mentioned in §22.6 may be used to perform a familial search against a national DNA-database. This requires however that all DNA-profiles of persons from a national DNA-database are present in or can be exported to this program. The outcome of the search is a starting point to find the real owner of the crime-scene-stain by tactical police-work. This police-work may be preceded or accompanied by Y-chromosomal and/or mitochondrial DNA testing to decrease the number of candidates and/or their priority order. Simulation studies have been published showing how many candidates a familial search in a DNA-database may yield[24,25,26,27]

[21] In CODIS, the profile with which the search is conducted, is called the target profile

[22] In CODIS a homozygote is designated with a single allele value.

[23] D.J. Balding et al (2013) Decision-making in familial database searching: KI alone or not alone? Forensic Science International Genetics 7, 52-54.

[24] Hicks et al. Forensic Science International: Genetics 2010 4 (5), pp. 316-322. Use of DNA profiles for investigation using a simulated national DNA database: Part II. Statistical and ethical considerations on familial searching.

[25] C. van Kooten et al. (2010) Poster nr 9 presented at the 21st International Symposium on Human Identification. It's all relative(s): Familial Searching in the Netherlands. (http://dnadatabank.forensischinstituut.nl/Images/lr-52634-poster-a0-verwantschapsonderzoek_tcm127-464088.pdf).

[26] Ge et al (2011) Journal of Forensic Sciences Volume 56, Issue 6, pages 1448–1456, November 2011 Comparisons of familial DNA database searching strategies.

[27] K. Slooten en R. Meester (2014). "Probabilistic strategies for familial DNA searching." *Journal of the Royal Statistical Society: Series C (Applied Statistics)*

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The outcome of the search may point in the wrong direction in the same way as a match may turn out to be an adventitious match. The search results should therefore be reported with a warning similar to the warning mentioned in recommendation 21 and 22. An extensive review on the ethical aspects of familial searching has been written by Professor Sonia Suter.[28]

5.3 Number of matching loci/match probability

The number of matching loci depends on the number of loci of the DNA-profiles which can be compared. The lower the number of loci, the higher the match probability of the DNA-profile, the higher the chance of an adventitious match, especially with large DNA-databases. For this reason DNA-profiles which are included in the DNA-database on a permanent basis should have a minimum number of loci or even better a maximum random match probability as indicated in § 3.3. For reference samples, the number of loci is usually 10 or higher to increase the chance of finding a match with a (partial) DNA-profile of crime-related biological material. At a national level, a lower number is also possible but then the DNA-profile should have a low match probability. This is the case in Germany, which uses the 7 old ESS loci plus the highly discriminating locus SE33. The matching rules of the EU-Prüm implementation decision require a minimum number of 6 fully matching loci.

5.4 Near matches

Several situations may lead to a near match (one locus does not match) between two DNA-profiles of the same person:

- A human error made during the production of one of the profiles. This may for instance happen when an allele is incorrectly called, a mixed profile is incorrectly split up into one or more of its contributors or a typographical error is made when a DNA-profile is entered manually into the DNA-database. When setting up a new DNA-database, the allele calling and the DNA-database import process should be automated as much as possible to avoid this problem. Manually entering DNA-profiles into a DNA-database has been shown to be the greatest source of errors, hence this should be done by a process which detects typographical errors such as the double blind method (entering a DNA-profile twice without seeing the first one and the database software checking if both entries are equal).

ENFSI-recommendation 21
The occurrence of errors in DNA-databases as a result of human mistakes associated with data entry should be avoided as much as possible by automating the allele calling and the DNA-database import process. When DNA-profiles are entered manually into the DNA-database this should be done by a process which detects typing errors, for example by double (blind) entry of data.

- An allele drop-in or drop-out due to low level DNA-profiling of one of the DNA-profiles (see §3.5)
- The occurrence of so-called “null-alleles”. These are alleles which are not amplified in the PCR-reaction due to a mutation in the primer binding-site region. When 2 kits use different primers for the same heterozygous locus and the DNA of a person contains a mutation in the primer region used in one kit but does not contain a mutation in the primer region which is used in the other kit,

[28] http://jolt.law.harvard.edu/articles/pdf/v23.2/23HarvJLTech309.pdf

the former kit will detect only one allele (apparent homozygote) and the latter will detect two alleles (heterozygote). The presence of a null-allele may be detected by the unexpected low peak height of the apparent homozygote but this requires an attentive DNA-analyst or intelligent allele-calling software. More information about the occurrence of null-alleles can be found at: http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm. A special case of a null-allele is the disappearance of the Y-amelogenin allele which makes the DNA-profile of a male look female.

- The occurrence of a shifted allele in one of the DNA-profiles. When 2 kits use different primers for the same heterozygous locus and the DNA of a person contains a deletion or an insertion after the primer region used in one kit but before the primer region which is used in the other kit, there will be a shift in the size of an allele in one DNA-profile compared to the other profile.

Given the abovementioned phenomena only searching a DNA-database for full matches (high stringency) may lead to missed matches (false negative matches) To find false negative matches a "less stringent" search strategy must be used permanently or occasionally. Countries, such as Switzerland, the Netherlands and the UK, already regularly perform this kind of quality control check by searching for near matches, which are then checked for possible mistakes. Searching for near matches may lead to matches with close relatives, hence the pros and cons of this strategy should be evaluated in advance (see also chapter 9). The software which is used for the international comparison of DNA-profiles under the terms of the EU-Prüm-decision also allows for one mismatch to detect near matches[29]. After finding such a match both countries contact each other to check the original data and the way they were processed. Near matches involving 8 loci or less loci often prove to be adventitious (false positive) matches but it may be worth to investigate them further if this can help investigating a serious case (see also chapter 9).

ENFSI-recommendation 22
To prevent false exclusions DNA-profiles should also be compared allowing at least one mismatch. The DNA-profiles involved in such near matches should be checked for possible mistakes during their production and processing.

5.5 Match validation

There are several reasons why a DNA-database match may need to be validated:

- Confirmation of the original DNA-test
  - Some countries require a new sample to be taken from the suspect and have that new sample re-analyzed.
  - Some countries perform a second analysis on a duplicate sample previously taken from the involved person but not yet analyzed.
  - Some countries require a new sample and re-analysis because a database match may influence a jury in court (because this is an indication of earlier convictions).

29 Forensic DNA Profiles Crossing Borders in Europe (Implementation of the Treaty of Prüm). Profiles in DNA 2011 (http://www.promega.com/resources/articles/profiles-in-dna/2011/forensic-dna-profiles-crossing-borders-in-europe/).

  - Some countries do an independent duplicate analysis for all their reference samples, avoiding any match validation needs.
  - The requirement for a duplicate analysis may be linked to a suspect making a plea of not-guilty, and contesting the DNA evidence.
- Possibility of an adventitious match
  In this case more loci should be analyzed to decrease the possibility of an adventitious match.
- Near match (one allele does not match)
  In this case the original data of both DNA-profiles should be checked to eliminate the possibility of a typing or an allele calling error.
- Match with a mixed DNA-profile
  A DNA-database match based on numbers of a single DNA-profile with a mixed DNA-profile is not necessarily a real match (see § 3.8). A DNA-expert should indicate whether this type of match can be a real match or not.

5.6 Dispositioning

After finding a candidate match in the DNA-database, this match has to be confirmed. When a match is found between two full DNA-profiles, this confirmation could be done by specially qualified DNA-database personnel or in an automated way. However, matches with partial and/or mixed profiles have to be examined and given a final disposition by a DNA-expert. The final disposition of a match can also usually be registered in the DNA-database to prevent the same match from being reported again after a new search action.

5.7 Match counting

One of the parameters to determine the efficiency of a DNA-database is the number of matches it generates. The counting of matches between two DNA-profiles is easy. In serial crimes committed over a period of time, however, different approaches are possible. Table 3 shows the number of matches that will be found when a single (unknown) individual commits a series of 7 crimes over time and leaves his DNA at all these crime scenes.

DNA-profile	Nr of matches	Description of the matches
A	-	
B	1	B -> A
C	2	C -> A&B
D	3	D -> A&B&C
E	4	E -> A&B&C&D
F	5	F -> A&B&C&D&E
G	6	G -> A&B&C&D&E&F
H	7	H -> A&B&C&D&E&F&G
Total	28	

Table 3. Number of matches that will be found when a single (unknown) individual commits a series of 7 crimes over time and leaves his DNA at all 7 crime scenes.

For a series of X crimes the number of matches is (X-1)X/2. For high volume crime cases this way of counting leads to match counts which are not representative as compared to the number of cases involved. This is why the ENFSI counts matches

in serial crimes in a different way. The following definition is taken from the document: "ENFSI DNA Working Group Terms and Abbreviations"[30]

For statistical purposes hits/matches with multiple identical profiles from the same case will be counted as one hit/match, but as separate hits/matches if they originate from different cases. In serial crimes, the total number of hits/matches is N-1 to the number of matching profiles (e.g.: a series of 8 identical stain profiles from different crimes yields 7 stain to stain hits/matches). If subsequently the DNA profile of a person matches the series, it yields 8 stain to person hits/matches. The number of stain to stain matches should then be removed from statistics.

An expression that is also used in match counting is "the number of investigations aided". This equals the number of DNA-profiles involved in matches. In the example above dealing with a series of 8 identical DNA-profiles there are 7 matches and 8 investigations aided.

A series of matching DNA-profiles may be given a unique identification code to indicate that they are similar. In the Netherlands this is called the DNA-cluster-number which has proved to be very useful for the investigators in order to designate the series.

5.8 Output/efficiency measurement

The output of a DNA-database is the number of matches it generates. Simon Walsh et al[31] have published a formula which describes the output of a DNA-database:

$$H = \frac{\alpha N}{M} \times \omega C$$

Where…
H = number of hits/matches
N = number of persons on 'offender' database
M = active criminal population
C = number of crimes on 'forensic' database
α = quality factor (person sampling)
ω = quality factor (crime/exhibit sampling)

The two quality parameters in the formula determine the efficiency of a DNA-database. If H, N, M and C are known, the product of the two quality factors can be determined by transforming their formula into: $\alpha\omega = HM/NC$.

Walsh et al propose an efficiency measurement parameter, the return index (RI) RI=H/NC. As this parameter is inversely proportional to the size of the database, it wrongly suggests that big DNA-databases are less efficient than smaller DNA-databases. The ENFSI DNA WG proposes the use of two different DNA-database performance parameters which express two different types of efficiencies:

H/C: the number of stain-to-person-matches relative to the number of stains included in the DNA-database. This parameter expresses the chance that a stain profile which is included in the DNA-database will match a subject profile. This is a very

[30] http://www.enfsi.eu/get_doc.php?uid=242

[31] S.J. Walsh et al (2010) J. For. Sci. 55(5) 1174-1183

important parameter because it shows the crime solving capacity of the DNA-database and the fact that the right items were collected by the police. It is self-evident that more stains will match a person as more persons of the (criminal) population are included in the DNA-database. So as the size of the DNA-database increases H/C will increase.

H/N: the number of stain-to-person-matches relative to the number of persons included in the DNA-database. This parameter indicates whether you have the right people in your DNA-database. This parameter is also important because it does not make sense to put effort in including people in the DNA-database who never will cause matches (although it is a one time effort).

Van der Beek has compared the efficiencies of the DNA-databases of the ENFSI member states in the Annual Report 2006 of the DNA-database of the Netherlands using the DNA-database performance parameter H/N. The last column of Table 4 shows this parameter for the June 2013 version of the semi-annual ENFSI DNA-data-base overview.

Country	Population size	Persons				Stains	Matches						Stain-person matches per person
		A	S	CO	T		Person/Stain			Stain/Stain	Total	Date	
							S	CO	T				
Austria	8.100.000				173.338	61.115			17.804	7.856	25.660	aug-13	0,11
Belgium	10.400.000		n/a	26.467	26.467	32.625	346	2.062	2.408	4.144	6.552	jun-13	0,09
Bulgaria	7.900.000				17.618	1.147			377	122	499	jul-09	0,02
Croatia	4.600.000				29.293	5.180			3.752	1.519	5.271	feb-10	0,13
Cyprus	772.000	n/a	n/a	305	305	10.628	n/a	78	78	212	290	jun-13	0,26
Czech Republic	10.515.000		2.436	110.760	113.196	18.727			7.703	2.896	10.599	jun-13	0,07
Denmark	5.500.000				92.206	44.740			20.798	4.284	25.082	jun-13	0,23
Estonia	1.286.540				34.012	10.475			3.957	932	4.889	dec-12	0,12
Finland	5.402.145				136.963	15.633			17.496		17.496	jun-13	0,13
France	64.300.000	128.312	1.886.876	427.649	2.314.525	202.427	66.315	19.642	85.957	11.260	97.217	jul-13	0,04
Georgia	4.700.000												
Germany	80.200.000				793.628	234.205			123.845	32.482	156.327	jun-13	0,16
Greece	10.600.000					8.112				520	520	jun-13	
Hungary	9.982.000		92.614	8.441	101.055	4.454			451	226	677	jun-13	0,00
Ireland	4.200.000												
Italy	58.000.000												
Kosovo	1.800.000												
Latvia	2.400.000		36.421	9.627	46.048	3.263			1.267	223	1.490	jun-13	0,03
Lithuania	2.960.000				66.566	3.786			1.810	378	2.188	jun-13	0,03
Luxembourg	500.000		85	1.229	1.314	1.186	197	262	459	2.448	2.907	jun-13	0,35
Former Yugoslavian Republic of Macedonia	2.000.000				7.996	3.145			898	133	1.031	Jun.13	0,11
Malta	400.000												
Montenegro	650.000												
Netherlands	16.100.000				170.788	55.482			38.762	4.868	43.630	jun-13	0,23
Northern Ireland	1.685.000												
Norway	5.000.000		9.577	35.043	54.506	9.078			8.967	2.588	11.555	nov-13	0,16
Poland	38.200.000				32.624	3.600			225	164	389	jun-13	0,01
Portugal	10.300.000	0	0	1.134	1.134	409	0	4	4	30	34	jun-13	0,00
Romania	22.000.000		930	17.229	18.159	757			3.159	42	3.201	jun-13	0,17
Russia	143.800.000												
Scotland	5.500.000		174.219	136.888	311.107	18.725			31.249	2.556	33.805	apr-13	0,10
Serbia	7.335.000												
Slovakia	5.500.000				38.559	8.181			4.029	1.391	5.420	jun-13	0,10
Slovenia	2.000.000				26.548	6.226			3.975	549	4.524	jun-13	0,14
Spain	44.800.000				244.243	70.380			28.475	34.537	63.012	jun-13	0,12
Sweden	9.000.000		13.979	114.039	128.018	26.698	25.674	13.243	38.917	15.617	54.534	jun-13	0,30
Switzerland	7.779.000				152.913	45.796			40.130	9.716	49.846	jun-13	0,26
Turkey	66.800.000												
UK (England & Wales)	53.700.000				4.795.615	414.982			1.905.436	362.252	2.267.688	jun-13	0,40
Ukraine	47.600.000												
Total	784.266.685				9.928.744	1.321.162			2.392.388	503.945	2.896.333		

Table 4. Semi annual ENFSI DNA-database overview.

This parameter can be followed over time or it can be applied to subgroups of persons in the DNA-database. In the Netherlands, for example, this ratio was 0.52 for suspects (in 2005) and 0.06 for convicted persons (in 2006).

Because the policies for keeping or removing DNA-profiles from stains from DNA-databases are different in different countries the other DNA-database performance parameter H/C cannot be determined reliably from table 4.

The number of stain-to-stain-matches can either be expressed as the number (or percentage) of stains involved in matches (investigations aided) or as the number (or percentage) of profiles giving a match at inclusion, which is lower because the first profile of a cluster does not result in a match (see table 2 in § 5.7).

As a national DNA-database is regularly subject to attention from the public, politicians and the media, a DNA-database manager should consider establishing performance parameters and making these publicly available.

ENFSI-recommendation 23
As a national DNA-database is regularly subject to attention from the public, politicians and the media, a DNA-database manager should consider establishing performance parameters and making these publicly available.

The abovementioned performance parameters only apply to the performance of the DNA-database itself. The efficiency of a DNA-database as a tool to investigate and solve crimes of course also depends on many other factors which have been reviewed by Bieber.[32]

[32] F.R. Bieber (2006) Journal of Law, Medicine & Ethics 34(2) p 222-233. Turning base hits into earned runs: Improving the effectiveness of forensic databank programs.

6 Adventitious matches

As DNA-databases become larger, the chance of finding adventitious matches also increases, especially with partial and mixed profiles and DNA-profiles of relatives, which have higher random match probabilities. If a crime stain DNA-profile has a random match probability of 1 in 1 million and a DNA-database contains 3 million DNA-profiles, a mean of three matches can be expected and none of them may be the actual originator of the crime stain DNA-profile. Therefore, every DNA-database manager should be able to determine the chance of finding adventitious matches in his/her DNA-database. Table 5 may help in this respect. This table gives the expected number of adventitious matches when a DNA-database of a given size is searched with a DNA-profile with a given match probability.

		Size of the DNA-database			
		10,000	**100,000**	**1,000,000**	**10,000,000**
RandomMatch Probability (1:X)	**10,000**	1	10	100	1,000
	100,000	0.1	1	10	100
	1,000,000	0.01	0.1	1	10
	10,000,000	0.001	0.01	0.1	1
	100,000,000	0.0001	0.001	0.01	0.1
	1,000,000,000	0.00001	0.0001	0.001	0.01
	10,000,000,000	0.000001	0.00001	0.0001	0.001

Table 5: Expected number of adventitious matches when searching a DNA-database of a given size with a DNA-profile with a given random match probability

The expected numbers of adventitious matches in table 4 are the expected numbers for one search with a DNA-profile with a given random match probability in a DNA-database with a given size. On an annual basis the number of searches is usually much higher than one. Hence on an annual basis the expected number of adventitious matches is the expected number of adventitious matches of one search times the annual number of those searches. So a DNA-database to which many crime scene DNA-profiles are compared can expect more adventitious matches on an annual basis than a DNA-database of similar size to which much less crime scene DNA-profiles are compared per year. An estimation of the annual expected number of adventitious matches can be made by splitting up the crime related DNA-profiles in match probability classes and estimating how many of each class are compared to the reference samples in the DNA-database.

Table 6 gives a theoretical example of a DNA-database which contains 4 million reference DNA-profiles to which 70,000 crime related DNA-profiles of different random match probabilities (RMP) are compared on an annual basis and calculates the expected number of adventitious matches from those figures (but there may be more or less than the expected number).

DNA-database size	RMP crime related stain	Number of searches	Expected Number of Adventitious Matches
4.000.000	1 : 10.000.000.000	50.000	20
	1 : 1.000.000.000	10.000	40
	1 : 100.000.000	5000	200
	1 : 10.000.000	3000	1200
	1 : 1.000.000	2000	8000
Total		70.000	

Table 6: Theoretical example of a DNA-database which contains 4 million reference DNA-profiles to which 70,000 crime related DNA-profiles of different random match probabilities are compared

Another factor which influences the expected number of adventitious matches is the presence of relatives in the DNA-database. This results from the fact that the match probabilities between relatives are higher than the random match probability. Table 7 lists the mean theoretically calculated approximate match probabilities between different kinds of relatives as compared to a random match probability of 1 in 10^{12}

Relationship	**Match Probability**
No relationship (random match probability)	1 in 10^{12}
First cousin	1 in 10^{11}
Half-sib or uncle/nephew	1 in 10^{10}
Parent or child	1 in 10^{8}
Full-sib	1 in 10^{5}

Table 7: Approximate match probabilities between different kinds of relatives as compared to a random match probability of 1 in 10^{12} [33]

Identical twins of course have the same DNA-profile.

The exact expected number of adventitious matches due to the presence of relatives in a DNA-database is impossible to calculate without knowing the numbers and types of relatives present.
The impact of the presence of relatives in a DNA-database on the expected number of adventitious matches seems limited, however, as shown in the next example: If 50,000 full SGM+ DNA-profiles from crime related stains are searched against a DNA-database of 4,000,000 reference profiles and 10% of the crime related stain donors has a brother in the DNA-database, 5,000 DNA-profiles will have a match probability of 1:10,000 instead of 1:1,000,000,000 The extra expected number of adventitious matches caused by the DNA-profiles of these 5,000 persons who have a brother in the DNA-database is 5,000 x 1/10,000 = 0.5. This is only a small extra number as compared to the 20 adventitious matches which are expected anyway by searching a DNA-database of 4,000,000 reference profiles with 50,000 DNA-profiles from crime related stains of persons who are unrelated. The effect of relatives on the expected number of adventitious matches will increase over time as more persons

[33] A.J. Hopwood et al (2012) Science and Justice 52, 185-190. Consideration of the probative value of single donor 15-plex STR profiles in UK populations and its presentation in UK courts.

related to each other in some way will be included in the DNA-database. At this moment we are only dealing with one generation of relatives but in 10 years a next generation of relatives may also be present

Because the risk of adventitious DNA-database matches cannot be neglected, a warning should be included indicating the factors that increase the possibility of finding an adventitious match (size of the database, number of searches, mixed and partial profiles/random match probability, presence of family members) when reporting a DNA-database match. An example of such a warning can be found in annex 3.

ENFSI-recommendation 24
DNA-database managers should be aware of the possibility of adventitious matches and be able to calculate their expected numbers for the matches they report. When reporting a DNA-database match, a warning should be included indicating the factors that increase the possibility of finding an adventitious match (size of the database, number of searches, mixed and partial profiles/random match probability, presence of family members).

To compare theoretical numbers of adventitious matches with actual ones, a DNA-database manager should record adventitious matches and the conditions under which they were found (size of the database, number of searches, etc) for future analysis like Tvedebrink et al have done[46].

Special attention for the occurrence of false positive matches is needed when performing large scale international comparisons of DNA-profiles such those based on the EU-Prüm-decision[34]

[34] Forensic DNA Profiles Crossing Borders in Europe (Implementation of the Treaty of Prüm). Profiles in DNA 2011.

7 Reporting results

Matches in DNA-databases are often so-called “cold hits”, meaning that there was no prior evidence suggesting that the match would occur. Also, in cases where there is prior evidence, this usually is not known to the DNA-database-manager. This means that reporting should be done in such a way that it does not create misconception in the mind of the person receiving the match report.

Apart from reporting a match between two DNA-profiles (which may contain different loci) as a fact, the match probability or the likelihood ratio of the corresponding loci/alleles should be reported to give the person receiving the report an idea about the evidential value of the match. Because the present kits produce DNA-profiles with random match probabilities which are difficult to comprehend for lay people, Hopwood et al[31] have recommended to use maximum likelihood ratio’s for reporting the weight of the evidence for a full matching 15-plex DNA-profile which are shown in the table below

Relationship	**Likelihood ratio**
No relationship (random match probability)	1 in 10^9
First cousin	1 in 10^9
Half-sib or uncle/nephew	1 in 10^9
Parent or child	1 in 10^7
Full-sib	1 in 10^5

Table 8: Proposed maximum likelihood ratio’s for reporting the weight of the evidence for a full matching 15-plex DNA-profile

The evidential value of matches with mixed profiles should be reported as a likelihood ratio, that is the ratio of the probability of the results given two alternative propositions e.g. 1) the crime stain contains DNA from the suspect and an unknown unrelated person and 2) the crime stain contains DNA from two unknown unrelated persons (see also § 3.9 and recommendation 12). The reported LR is only valid for the evaluated propositions and should be recalculated if alternative propositions are put forward.

There has been discussion in the literature and in courts about the appropriate way of reporting the evidential value of DNA database search results[35,36,37]. In essence, the difference between the evidential value of a DNA match obtained through a DNA database search and through comparison with a single suspect lies in the other evidence that is available in the case: with a “cold hit” other incriminating evidence against the matching person may be completely missing, whereas if a single suspect is compared, this is necessarily based on other incriminating evidence. As argued in Sjerps and Meester[38], the report should therefore contain a warning concerning the possibility of adventitious matches as mentioned in recommendation 21.

[35] Kaye, DH. (2009) Rounding up the usual suspects: a legal and logical analysis of DNA database trawls, North Carolina Law Review 87: 425-503.

[36] Gittelson S. et al. (2012) The database search problem: A question of rational decision making. Forensic Science International 222, 186-199

[37] Biedermann A. et al. (2012) A Bayesian network approach to the database search problem in criminal proceedings. Investigative Genetics 3, 16

[38] Sjerps, M. & R. Meester (2009). Selection effects and database screening in forensic science. Forensic Science International 192 (2009), 56–61.

This warning should make clear that adventitious matches are possible, and that this possibility should be taken into account especially in situations where other incriminating evidence is missing or weak. Meester and Sjerps[39] have suggested including a table in the match report which describes the relation between the prior probability and the posterior probability given the match probability of the match to help jurors to determine the evidential value of the match. An alternative option which is currently used for example by the Netherlands Forensic Institute is to include a special textbox in the match report which explains the possibility of adventitious matches (see Annex 3).

ENFSI-recommendation 25
A DNA-database match report of a crime scene related DNA-profile with a person should be informative and apart from the usual indication of the evidential value of the match (LR/RMP) it should also contain a warning indicating the possibility of finding adventitious matches (as mentioned in recommendation 21) and its implication that the match should be considered together with other information.

[39] Meester, R. and Sjerps, M. (2004) Law, Probability and Risk, 3, 51-62.

8 DNA-database software[40]

Software programs which have been designed for at least the storage and comparison of DNA-profiles are referred to as DNA-database software. Some programs can also do other things. DNA-database software can either be internally developed by a country to meet its own specific needs or it can be obtained from a producer which provides it without cost or offers it on a commercial basis. Examples of DNA-database programs which can be obtained without cost are:

- CODIS, which has been developed by the FBI for the USA but which is also available for non-USA-governmental organizations. A private company, SAIC, which has developed the original program, provides training courses and runs a well-organized and skillful helpdesk. CODIS has three levels of storing and comparing DNA-profiles: local, state and national, which can be used to combine data if there is more than one DNA-database in a country (e.g. Spain).
- STR-lab, a program developed in South-Africa which can be downloaded from: http://strlab.co.za/

Programs which are or have been commercially available are:

- FSS-iD™ of the Forensic Science Service in the UK
- Dimensions of the Austrian company Ysselbach Security Systems
- eQMS::DNA of the Croatian company Pardus (www.Pardus.hr)
- *f*DMS-STRdb distributed by the Czech Republic company Forensic DNA Service (http://dna.com.cz/files/file/fdms-strdb.pdf)
- *Rapid**DNA*** of the Australian company Forensics International (http://www.rapiddna.biz)

DNA-database programs should comply with national personal data-protection guidelines, especially those dealing with data-quality, integrity and security.

One company has launched a cloud-based DNA-database specifically for local law enforcement agencies to easily archive, search and reference DNA-profiles from crime scene samples[41]. It remains to be seen if this storage method will be acceptable to the authorities responsible for DNA-testing and or the data protection authorities.

Table 9 shows which DNA-database programs are used by different European countries and some international organizations

[40] The mentioning of trade names does not mean that ENFSI recommends these programs. ENFSI's aim is just to give an overview of what is available on the market.

[41] http://www.sorensonforensics.com/forensics-lab-forensic-dna-testing/dna-forensics-lab-news-forensic-lab-development/sorenson-forensics-launches-new-cloud-based-database-to-simplify-crime-scene-case-management-archival-of-dna-profiles?category=press+releases

Country	DNA-database program
Albania	?
Armenia	No DNA-database yet
Austria	Self-developed program
Belgium	CODIS
Bosnia & Herzegovina	CODIS
Bulgaria	Self-developed program
Croatia	CODIS
Cyprus	Self-developed program
Czech Republic	CODIS
Denmark	Self-developed program + CODIS
Estonia	CODIS
Finland	CODIS
Former Yugoslavian Republic of Macedonia	eQMS::DNA
France	CODIS + Self-developed program
Germany	Self-developed program
Georgia	CODIS
Greece	CODIS
Hungary	CODIS
Iceland	CODIS
Ireland	CODIS
Italy	CODIS
Kosovo	CODIS
Latvia	CODIS
Liechtenstein	Included in the Swiss DNA-database
Lithuania	CODIS
Luxembourg	Self-developed program
Malta	CODIS
Montenegro	CODIS
Netherlands	CODIS
Northern Ireland	Self-developed program
Norway	CODIS
Poland	CODIS
Portugal	CODIS
Romania	CODIS
Russia	No DNA-database yet
Scotland	Self-developed program
Serbia	CODIS
Slovakia	CODIS
Slovenia	Self-developed program
Spain	CODIS
Sweden	CODIS
Switzerland	CODIS
Turkey	No DNA-database yet
Ukraine	Self-developed program
UK (England, Wales, Scotland, North Ireland)[42]	Self-developed program
INTERPOL	Self-developed program
Prüm-Treaty-countries (exchange-database)	Self-developed program or CODIS 7

Table 9: DNA-database programs used by different European countries and some international organizations.

[42] Northern Ireland and Scotland have their own DNA-databases, even though their profiles are also loaded to the UK National DNA Database.

9 Data integrity control measures

For forensic reasons, but also required by personal data protection legislation, DNA-profiles and their associated information should be entered and stored correctly. For this reason, the manual entry of DNA-profiles should be avoided. If this is not possible, DNA-profiles should be entered using the double blind method.[43] A reliable professional database program should be used with proper logging of all actions and secure ways of importing the DNA-profiles as indicated in § 4.3. Access to the DNA-database should be limited by physical and organizational methods to those persons who need to have access. Regular back-ups should be made, stored in a safe place and put back at regular intervals to simulate recovery from a disaster. If the DNA-profiles and/or DNA-profile associated information are also registered in another system like a LIMS or a judicial or police system, the contents of these systems should regularly be compared to check whether the systems are still properly synchronized.
Official recognition of compliance with personal data protection legislation may be sought by submitting the organization and its working processes to an independent external audit.

ENFSI-recommendation 26

- DNA-profiles should be entered into a database in a way that guarantees their correct import.
- Access to the DNA-database should be limited to those persons who need to have access, by physical and organizational measures.
- Regular back-ups should be made, stored in a safe place, and put back at regular intervals to simulate recovery from a disaster.
- When DNA-profiles and their associated information are present in different systems, these systems should be regularly compared to check whether they are still properly synchronized.

The abovementioned recommendations are to prevent errors as much as possible. It has been shown, however, that despite all of these measures, DNA-profiles may occasionally contain errors as a result of:

- Allele drop-ins or drop-outs
- Allele calling errors (of long DNA-fragments)
- Primer mutation differences between commercial kits
- Mixture interpretation errors by DNA-analysts

When searching at moderate stringency (see §5.2), DNA-profiles containing allele drop-outs and primer mutation differences will be found as a match between a heterozygote and an apparent homozygote, but DNA-profiles containing other types of errors will not match their correct counterparts. To detect these false negative matches or false exclusions (e.g. matches which should be found but are not found because one of the DNA-profiles contains an error) regular full DNA-database searches allowing one or more mismatches should be performed, as indicated and recommended in §5.2. The software which is used by countries exchanging DNA-profiles under the terms of the EU Prüm Decision also allows for one mismatch.

[43] The double blind method is also used for changing passwords. A new password is entered twice while only asterisks are shown. The computer compares the two blind entries and only accepts it if both entries are equal.

When a match between two DNA-profiles contains a mismatch in one of the loci, the original data of both DNA-profiles should be checked to see if one of the DNA-profiles contains an error.

ENFSI-recommendation 27

- To detect false exclusions (e.g. matches which should be found but are not found because one of the DNA-profiles contains an error) regular full DNA-database searches allowing one or more mismatches should be performed.
- When a match between two DNA-profiles contains a mismatch in one of the loci, the original data of both DNA-profiles should be checked to see if one of the DNA-profiles contains an error.

If no error is found in either profile the conclusion must be that the mismatch is a true mismatch. During the international exchange of DNA-profiles based on the EU-Prüm-Council-decisions many 6 and 7 locus matches plus a mismatch are found. Nearly all of these mismatches have proven to be true mismatches and statistical calculations also show that these high numbers of 6 and 7 locus matches plus a mismatch can be expected Some countries therefore have chosen to ignore these matches except for those which may assist in solving serious cases in their country.

10 Inclusion of case information and personal data

In some countries, the DNA-database program also contains case and personal information, but in other countries this is strictly separated for legislative or other reasons. The DNA-database program CODIS has only been developed to store and compare DNA-profiles, so CODIS-using countries always need a second system to store other DNA-profile associated information. As indicated in the previous paragraph, regular comparisons of the systems are then required to check whether they are still properly synchronized and if the DNA-profiles are correctly linked to their associated personal and/or case information.

Whether or not the DNA-profiles are kept separated from personal data, the identity of persons should be properly verified when they are sampled to avoid matches with wrong or non-existing persons.

11 Interaction with other databases

It can be very useful for investigative reasons to combine DNA-information with other technical or tactical forensic information. If, for example, a series of crimes has been linked by the presence of a DNA-profile of an unknown person and on one of the crime scenes a fingerprint matching a known person has also been found, the combined information may solve the whole series of cases. Countries like the United Kingdom[44], Switzerland[45] and the Netherlands[46] are working on systems to combine the contents of different forensic databases and to visualize the links between different cases and different persons which are the result of that combination. Figure 2 shows an example of the visualization of such cluster of crimes and persons derived from DNA and fingerprint information.

[44] http://webarchive.nationalarchives.gov.uk/+/http://www.homeoffice.gov.uk/docs2/resconf2002/richardlearyroleenimflints.pdf

[45] Ribaux et al. (2010) Intelligence-led crime scene processing. Part II: Intelligence and crime scene examination. Forensic Science International 199 (1-3) 63-71

[46] See English summary of: http://www.wodc.nl/images/1203_volledige%20tekst_tcm44-58753.pdf

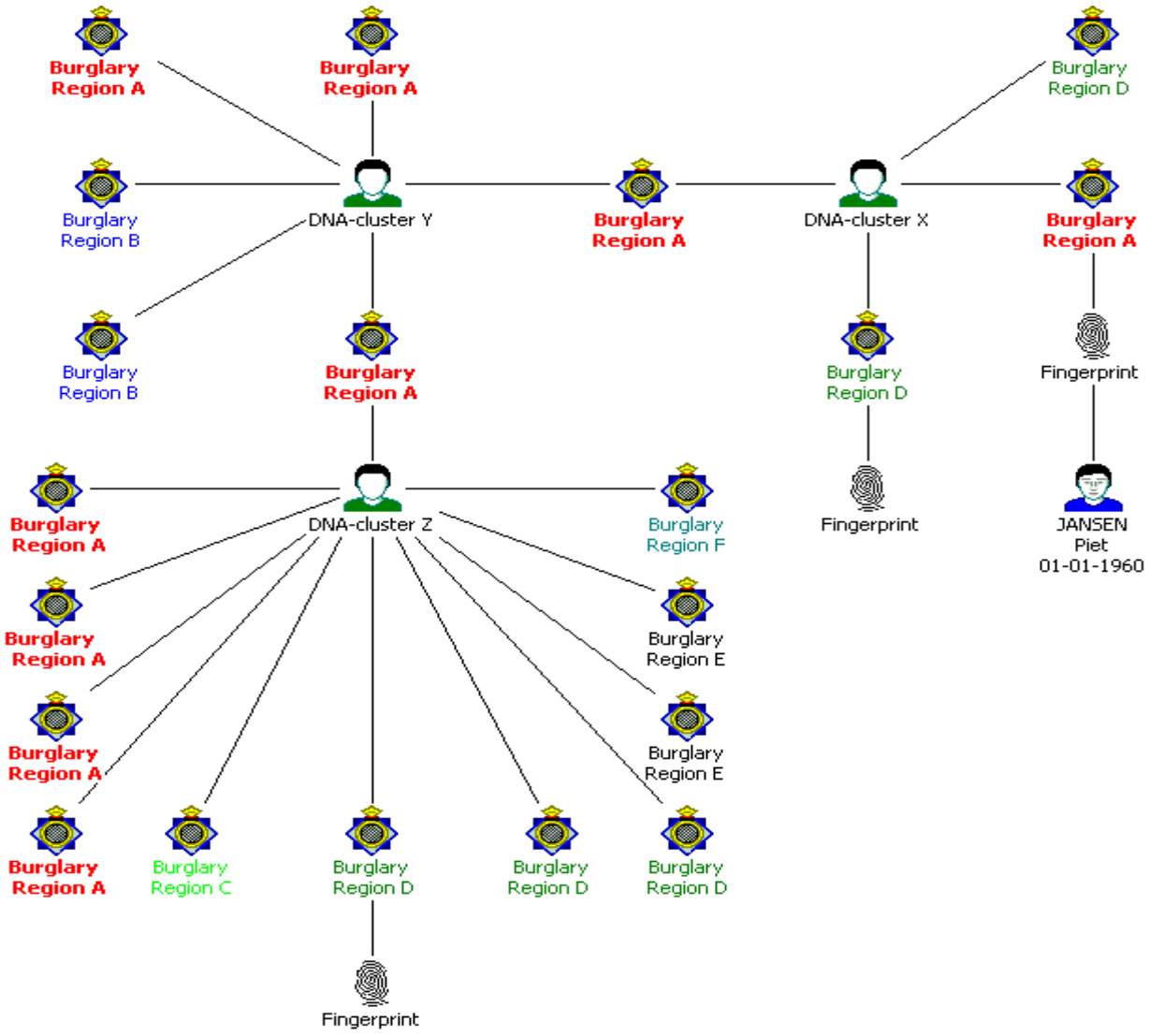

Figure 2: Three DNA-clusters (X, Y, Z) linked by 2 crime scenes where DNA-profiles from 2 clusters were found (X+Y and Y+Z) combined with two unidentified and one identified fingermarkst.

ENFSI-recommendation 26
Information from a National DNA-database should be combined with other types of evidence to increase the number of crimes for which a lead can be identified.

12 Automation of working processes

Automation of DNA-database working processes can take place at different levels:

- Import of DNA-profiles as already discussed in § 4.3
- Comparison of DNA-profiles using saved sets of matching rules
- Comparison of DNA-profiles at scheduled points in time (e.g. overnight)
- Reporting unambiguous results
- Sending out the unambiguous results

As automated processes reduce the possibility of human errors, they should be introduced for those processes that are straightforward, like the production of DNA-profiles from reference samples.

ENFSI-recommendation 29
As automated processes reduce the possibility of human errors, they should be introduced for those processes that are straightforward.

As already stated in § 2.7, candidate matches with mixed profiles should always be checked by a DNA-specialist to determine whether the numerical match could be a real match. This is also the reason why mixed DNA-profiles are not included in the automated DNA-comparisons between countries operating under the terms of the EU-Prüm-Decision.

In the past year so-called rapid DNA-machines have come on the market for the fully automated production of DNA-profiles from reference samples in less than 2 hours. Once these machines have proven themselves, they may alter the DNA-testing landscape because they would enable DNA-testing to be quickly done at police stations on arrestees or suspects to verify on line if the person matches the DNA-database before he/she has to be released.

13 Storage of cell material

The cell material of crime scene stains from which a DNA-profile has been generated is usually stored. With regards to the storage of cell material of reference samples, however, countries have different policies. Some countries allow the storage of the reference samples for later reuse if this becomes technically or legally necessary and in other countries the reference samples have to be destroyed as soon as the DNA-profile has been generated and included in the DNA-database. In two examples it will be shown that from a forensic point of view it is better to store the cell material.

Example 1

In the recent past several improved DNA-typing technologies have been developed. Multiplex kits with more loci to get a higher evidential value, as well as mini STR-kits and SNP-kits to obtain DNA-profiles from degraded DNA are good examples. It has become possible to re-examine stains from (c)old cases which could not be examined in the past. But if the stain has been retyped with a new technology, the reference sample must also be retyped to enable comparison between the two. If the reference sample has been destroyed, the police or the judiciary have to obtain a new reference sample from the suspect which may not always be possible.

Example 2

A Prüm-treaty-member-country sends an SGM+ DNA-profile of a crime scene stain to another Prüm-treaty-member-country. A match with a reference DNA-profile is reported on 7 loci due to the fact that the other country uses a different kit. To exclude the possibility of an adventitious match, the SGM+ country first tries to improve its own DNA-profile, but if this is not possible it then asks the other country to upgrade its reference DNA-profile. If the reference sample has been destroyed this upgrade is not possible without obtaining a new reference sample from the person involved, which may not always be possible.

Example 3

Countries which are allowed to perform familial searching in their DNA-database usually get many possible candidates after an initial DNA-database search. False positives can be eliminated from this possible candidate list by additional Y-chromosomal or mitochondrial DNA-testing. However this is only possible if the samples from which the DNA profiles were generated are still available

The ENFSI DNA-working group realizes that the storage of cell material from reference samples is a politically very delicate subject. Although the European personal data-protection directive clearly states that personal data (which includes DNA-profiles and the cell material from which the DNA-profiles were derived) can only be used for the purpose for which they were obtained, there are people who fear that they could be misused in the future and hence choose the “better safe than sorry” principle.

On the other hand one could also argue that keeping the samples is privacy enhancing because there is no need for resampling if additional DNA-testing is necessary to investigate if a possible false positive match is a real match or not.

ENFSI-recommendation 30
From a forensic point of view the cell material of reference samples should be stored as long as their corresponding DNA-profiles.

14 Legislative matters

As the compulsory taking of a DNA-sample is a breach of someone's privacy and bodily integrity, article 8 of the European Convention on Human Rights demands a justification and legislation. For arrestees and suspects, the justification can be found in the fact that DNA-testing can help to solve the case by either finding a match (resulting in possible incriminating evidence) or an exclusion (resulting in possible exonerating evidence) with a DNA-profile from a crime scene which is thought to be left behind by the culprit of the crime. This means, however, that crime scene DNA must be present for this type of justification. The inclusion of someone's DNA in a DNA-database is justified by the fact that it can help solving old and future crimes committed by the same person and that it may prevent new crimes because the person involved may fear being detected when they commit new crimes. The indefinite retention in a DNA-database of persons who have not been prosecuted or convicted has been condemned by the European Court of Human Rights[47]. The court explicitly approved the retention of DNA of innocent people in appropriate circumstances by praising the Scottish retention system. Also in the Netherlands suspects can be kept in the DNA-database until their case has been dealt with by the public prosecution office.

Every EU-country is supposed to have data protection legislation derived from the European Data Protection Directive 95/46. Because DNA-profiles and the cell-material from which they are derived are also regarded as personal data, they fall under the umbrella of this legislation unless the data protection legislation is overruled by specific DNA-legislation containing other provisions. (Lex Specialis precludes Lex Generalis). Some examples are given below to illustrate why it is useful to have specific DNA-legislation in addition to data protection legislation:

- According to the data protection legislation personal data must not be stored longer than necessary for the purpose for which it was collected. It is practically impossible to determine this necessity for all the DNA-profiles in a DNA-database at regular intervals. So the DNA-legislation should say something about storage times (see also: § 3.1).
- According to the data protection legislation, individuals have certain rights with regard to their own data (access/modification/removal). For investigative reasons, this is usually not desirable. So the DNA-legislation should say something about who has access to information present in and generated by the DNA-database.
- In some countries, the data protection legislation states that genetic information can only be used in relation to the person from whom this information is derived. If such a country wants to allow familial searching in the DNA-database, there should be rules for this in the DNA-legislation.

DNA-profiles are not only very specific for an individual but they also contain information about relatives of that individual. That means that when people voluntarily give their DNA-profile (e.g. in a mass screen), they should be informed that this may possibly also incriminate a relative. In this way a person can decide whether they will make use of their right not to testify against relatives.

[47] http://www.bailii.org/eu/cases/ECHR/2008/1581.html

Most countries also allow the inclusion of DNA-profiles of minors in their DNA-database. The legitimacy of this is being questioned in some countries, amongst others, by referring to the international convention on the rights of the child. Several appeal court cases are ongoing to develop jurisprudence on this. The Supreme Court of the Netherlands has ruled that there are no reasons to differentiate between minors and adults. Also the European Court of Human Rights does not regard minority as a reason to exclude a person from the Dutch DNA-database.[48]

[48] European Court on Human Rights 20689/08.

15 Financing

In most countries the costs of establishing and maintaining a National DNA-database are financed by a dedicated annual budget of the Ministry of Justice or the Ministry of the Interior. In the UK, however, (parts of) the budget are managed by the police, who pay for the production and the storage of the DNA-profiles.

16 Personnel requirements

It goes without saying that persons working on a DNA-database should be properly trained to use the DNA-database software. If the program is self-developed, this will be in-house training. If the DNA-database software is commercially obtained, the company selling the software will usually also offer training in the use of the software. CODIS can be obtained from the FBI by law enforcement organizations and both an instructor led teaching as well as a computer based training are available. Apart from being properly trained, DNA-database personnel must at least have the following personal skills:

- Being able to work very conscientiously
- Being able to keep confidential information confidential
- Being able to accept to be checked by colleagues
- Being able to report own mistakes to enable further process improvement

Apart from the abovementioned requirements, a “proof of good conduct” may be required or even a positive outcome of an investigation by the police or the secret service into somebody’s reliability.

17 Governance

When a DNA-database is established in a country, its custody is either assigned to an existing organization or to a newly established organization. In some countries (like the UK), a special supervisory board has been established with representatives of different stake-holders. Also in the UK, a special ethics group has been established[49] to provide independent advice on the ethical aspects of DNA-database management. If there is no dedicated supervisory board, the data-protection authority of a country usually has the power to audit the organization managing the DNA-database to check its compliance with the data-protection legislation of that country.

[49] See: http://police.homeoffice.gov.uk/operational-policing/forensic-science-regulator/ndnad-ethics-group/

18 Research and Development

Studies on the statistics, performance and different search strategies of DNA-databases are usually done using simulated DNA-databases (e.g. footnotes 5 and 24). Some scientists however have asked for disclosure of the real DNA-profiles contained in DNA-databases to allow them to evaluate some of the population genetic assumptions underlying DNA-testing.[50] Of course this should be done under strict conditions and removing any links to the identity of the owner of the DNA-profile. Some countries do already allow this in the interest of quality assurance and/or process improvement[51,52,53]

A big problem for DNA-database managers is that they cannot distinguish matches with monozygotic twins. Both epigenetic[54] as well as next generation sequencing[55] research is going on but the amounts of DNA which are necessary for analysis need to be reduced to enable the analysis of forensic traces containing low amounts of DNA.

[50] Krane et al. (2009) Science 326,1631-1632. Time for DNA-disclosure.

[51] Sjerps et al (2009) Oral presentation at the European Academy of Forensic Science (EAFS) 2009 conference in Glasgow. Observed and expected numbers of (partially) randomly matching profiles in the Dutch DNA-database and in international searches.
http://www.forensischinstituut.nl/Images/EAFS%202009%20Glasgow%20presentatie%20Marjan%20Sjerps_tcm68-418718.ppt

[52] Tvedebrink et al (2012) Forensic Science International: Genetics Volume 6, Issue 3 , Pages 387-392, May 2012. Analysis of matches and partial matches in a Danish STR data set.

[53] Hedell et al. (2011) Forensic Science International: Genetics Supplement Series Volume 3, Issue 1, Pages e135-e136, December 2011.

[54] Li et al (2011) Forensic Science International: Genetics Supplement Series Volume 3, Issue 1 , Pages e337-e338, December 2011. Identical but not the same: The value of DNA methylation profiling in forensic discrimination within monozygotic twins.

[55] J. Weber-Lehmann et al (2014) Forensic Science International: Genetics 9, 42-46. Finding the needle in the haystack: Differentiating “identical” twins in paternity testing and forensics by ultra-deep next generation sequencing

19 External Communication

Because DNA-databases are usually publicly funded, politicians, the public and the media have a right to know how the DNA-database is managed and what results are obtained.

19.1 Annual report

A good way to do so is to produce an official annual report. Such a report can either be part of an annual report of the organization which is responsible for the management of the National DNA-database or it can be a separate annual report dedicated just to the DNA-database. In Europe, the United Kingdom and the Netherlands have already produced such dedicated annual reports. Outside Europe, the Royal Canadian Mounted Police also produces an annual report about their DNA-database. Below are the locations where the most recent issues of these annual reports can be downloaded:

- United Kingdom: http://www.npia.police.uk/en/14189.htm
- Netherlands: http://www.dnasporen.nl/docs/literatuur/jaarverslag%20DNA%202010%20met%20aangepaste%20voetnoten.pdf
- Canada: http://www.nddb-bndg.org/an_report_e.htm

19.2 Internet site

Whereas annual reports are milestones in a written form, websites provide a continuous way of providing information to interested parties. Below is a list of internet sites devoted to DNA-databases or containing information about DNA-databases:

<u>Europe</u>

- United Kingdom: https://www.gov.uk/government/collections/dna-database-documents
- Germany: https://www.bka.de/nn_205980/DE/ThemenABisZ/DnaAnalyse/dna.html#doc205380bodyText5
- Ireland: http://www.lawreform.ie/_fileupload/Reports/Report%20DNA%20Database.pdf (comprehensive thoughts on setting up a DNA-database in Ireland)
- Netherlands: http://dnadatabank.forensischinstituut.nl/
- Switzerland: http://www.fedpol.admin.ch/content/fedpol/en/home/themen/sicherheit/personenidentifikation/dna-profile.html

<u>Rest of the world</u>

- USA (CODIS) http://www.fbi.gov/about-us/lab/codis/codis
- USA (Florida): http://www.fdle.state.fl.us/Content/getdoc/6835b26c-ae3f-49c5-845e-0c697bb86001/DNA_Investigative.aspx
- USA (NewYork): http://criminaljustice.state.ny.us/forensic/dnabrochure.htm
- USA (Legislation): http://www.dnaresource.com/
- Canada: http://www.nddb-bndg.org/main_e.htm
- Australia: http://www.crimtrac.gov.au/our_services/BiometricServices.html
- New Zealand: http://www.esr.cri.nz/competencies/forensicscience/dna/Pages/DNAdatabank.aspx

- Hong Kong: http://www.govtlab.gov.hk/english/abt_fsd_dds.htm
- South Africa: http://strlab.co.za/

ENFSI-recommendation 31
Because DNA-databases have a very important but also very delicate role in society, the custodian of a DNA-database should develop tools to make information about the DNA-database available to politicians, the public and the media.

20 International overviews

Several documents have been published in the past which contain overviews per country of different aspects of DNA-database legislation and DNA-database management. However these documents are all older than 5 years now.

The only recent per country overview presently known to the author can be found at the website of the Council of responsible genetics:
http://www.councilforresponsiblegenetics.org/dnadata/world_map.html

21 International comparison of DNA-profiles

As crimes committed in one country may be committed by a person from another country, it is very useful to have means for international comparisons of DNA-profiles. Chapter 2 described how a European Standard Set of Loci has been agreed upon to enable such comparisons. In addition to common loci, DNA-profile exchanging countries should, of course, also use the same quality standards for the production of their DNA-profiles as described in § 3.5.

There are different channels through which the international comparison of DNA-profiles can take place:

- Individual legal assistance requests on paper
  Until recently, this was the most commonly used channel. Depending on the legal embedding of the DNA-legislation in a country, either police channels or judicial channels are used for this method of exchanging DNA-information. Before the advent of XML to communicate DNA profiles, Interpol developed a special form to standardize and facilitate this way of exchanging DNA-information. A great disadvantage of this way of information exchange is that it is very time consuming.

- INTERPOL DNA-database and DNA-gateway
  INTERPOL has a central DNA database, in which DNA profiles and their sample codes can be included by its member states for comparison. The database is an autonomous database and does not keep any nominal data linking a DNA profile to any individual. Member states retain the ownership of their profile data and control its submission, access by other countries and destruction in accordance with their national laws. As soon as a match is found a message is sent to the countries contributing to the match. This message contains the basic case information that was provided and can optionally provide the sample codes. Member countries then decide if they wish to pursue this forensic intelligence link. A central DNA-database is most effective when all participating countries submit all their crime-scene DNA-profiles and all their reference sample DNA-profiles. Some countries have already done so and others have indicated that they will do so. The DNA Gateway provides for the transfer of DNA-profiles between two or more countries and for the management of a country's own DNA-profiles in the central DNA-database. Access to the DNA-gateway is provided directly to a country via the INTERPOL National Central Bureaus (NCBs), using INTERPOL's secure communications system, I-24/7. For more information about the DNA-gateway of INTERPOL see:
  http://www.interpol.int/Public/ICPO/FactSheets/FS01.pdf.
  Interpol has also recently published a second (2009) edition of the Interpol Handbook on DNA Data Exchange:
  http://www.interpol.int/Public/ICPO/Publications/HandbookPublic2009.pdf

  In addition to the regular DNA-gateway facilities, INTERPOL also offers the possibility for dedicated bilateral comparisons

  INTERPOL's secure communications system I-24/7 is available for the exchange of DNA profile comparison requests between some of the G8-countries (USA, Japan, Canada and the UK). A special G8-request form has been developed which can be forwarded directly to a person associated with the national

DNA database, who will carry out the comparison and will return the result back to the requesting country.
(http://www.interpol.int/Public/ICPO/PressReleases/PR2007/PR200729.asp)

- Europol
  Europol is authorised to process DNA profiles within the "Analysis Work Files" (Council Decision 2009/9343/JHA). In this context, DNA profiles are used together with other intelligence for criminal analysis purposes in order to fight serious international crime. Europol can also process DNA-profiles in the Europol Information System (EIS) based on Council Decision 2009/371/JHA. Europol National Units can directly insert or search data, including DNA-profiles, in the EIS. Non EU states which have signed a cooperation agreement with Europol can also provide DNA-profiles to Europol for insertion into the EIS

- The EU-Prüm-Decision (derived from the Treaty of Prüm)
  The EU-Prüm-Decision deals with the exchange of judicial and police information between the EU-member states. Some associated countries (Norway, Switzerland, Liechtenstein and Iceland) are also allowed to join this undertaking. With regards to DNA, countries can search each others' DNA-databases in an automated way. To enable this, each country creates a copy of its DNA-database with a standardized table structure, which can be accessed by common data-exchange and DNA-comparison software, which is present in each country. The DNA data exchange and matching system used by the EU member states is similar to the DNA data exchange and matching system of the INTERPOL DNA Gateway. When this ENFSI-document was approved, the following countries were already exchanging DNA-profiles on a day-to-day basis with one or more of the other countries, under the terms of the EU-Prüm-Decision: Austria, Germany, Spain, Luxembourg, Slovenia, Finland, France, Bulgaria, Slovakia, Latvia, Lithuania, Romania, Hungary, Poland, Cyprus and the Netherlands. As of 15 March 2013 these countries have updated their exchange software to also enable the exchange of the 5 new ESS-loci.

  The EU-Prüm-Decision (2008/615/JHA) and the EU-Prüm-implementation-Decision (2008/616/JHA) can be found at the following internet locations:

  http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:210:0001:0011:EN:PDF
  http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:210:0012:0072:EN:PDF

Chapter 2 of the Annex to the EU-Prüm-implementation-decision contains the DNA-inclusion, matching and reporting rules. Like the INTERPOL DNA-database, the Prüm DNA-profile exchange system is a match/no-match-system, meaning that only DNA-profiles are compared. After finding a match, countries can obtain the personal and/or case information associated with the DNA-profile via existing police or judicial channels. The minimum number of matching loci under the terms of the Prüm system is six. However it can be calculated and it has been shown in daily practice[56] that six and seven locus matches have a non negligible chance of being false positive. Therefore it is recommended to analyze these matches further by additional DNA-testing before any legal action is undertaken against a matching person.

[56] Forensic DNA Profiles Crossing Borders in Europe (Implementation of the Treaty of Prüm). Profiles in DNA 2011 (http://www.promega.com/resources/articles/profiles-in-dna/2011/forensic-dna-profiles-crossing-borders-in-europe/).

ENFSI-recommendation 32
Six and seven locus international matches obtained under the terms of the Prüm system should be further analyzed by additional DNA-testing before requesting information from another country. If a Prüm related information request is received from another country, the quality of the corresponding match should be checked before providing the requested information to the other country.

To be able to improve the evidential value of a match by additional DNA-testing one needs to know which loci are present in the DNA-profile of the other country. Therefore also loci that are not used by the receiving country should be configured in the DNA-database of the receiving country. If this is not done, those loci will not be visible in the DNA-profile received from the other country.

ENFSI-recommendation 33
All regularly used loci (also the ones not used by the receiving country) should be configured in the DNA-databases of countries participating in the international exchange of DNA-profiles under the terms of the Prüm system to be able to see the full composition of the DNA-profile of the sending country.

national DNA-database always contains DNA-profiles from national crime-related stains. However, as already mentioned in § 3.1, if the national law of both countries allows it, crime-related stains from other countries may also be included if an international legal request for comparison from another country has not resulted in a match. By including the DNA-profile from the other country, there is no need for a regular repeat of the often lengthy legal request. This is of course not necessary for countries who can already search each others' DNA-databases under the terms of the EU-Prüm-Decision, because they can repeat the request as frequently as they wish.

22 Missing persons

22.1 Introduction

The main purpose of a DNA-database for missing persons is to see whether DNA-profiles of unidentified human remains can be linked to DNA-profiles of missing persons or their family members. In this way, family members of missing persons can become aware of the fact that their missing relative is no longer alive and can start coming to terms with their loss. A second purpose is to link body parts of the same person which may be found in different places (e.g. two feet washed ashore at different places and at different times) or in situations where more than one person has been killed and unidentified body parts cannot reliably be attributed to one person (e.g. airplane crash or secondary mass grave). Missing persons DNA-databases are usually operated together with or as part of a system where other important attributes of missing persons and unidentified bodies/body parts can also be included and compared (e.g. dental records, fingerprints, externally visible traits, medical data, etc.). Samples obtained from personal items of missing persons or samples obtained from their family members are indicated as *ante mortem* samples and a sample from an unidentified body(part) is indicated as a *post mortem* sample.

22.2 Different missing person situations

A person can either become a missing person as an individual or as part of a mass fatality. Mass fatalities may either have a natural cause (e.g. tsunami, earthquake) or an unnatural cause (war situation, terrorist attack). In mass fatalities a distinction can also be made between closed systems where the number, names and mutual relationships of the missing persons are known (e.g. airplane crash) and open systems where the number of missing persons cannot be properly accessed (e.g. tsunami or earthquake).

22.3 Different types of matches

In the DNA databases of missing persons, a distinction can be made between direct and indirect comparisons.

- A direct match is a full match between the DNA-profile of a missing person and a DNA-profile of an unidentified body(part). This is the most reliable type of match but it requires that the DNA-profile of the missing person is available because it could be obtained, for instance, from a personal item or a medical specimen of the missing person. When a DNA-profile is obtained from a personal item of the missing person, care should be taken to ensure that the personal item was only used by the missing person. To check this, DNA-profiles could also be obtained from parents or children of the missing person for comparison with the DNA-profile obtained from the personal item of the missing person. The strength of a direct match is usually expressed as the random match probability of the matching loci between the DNA-profile of the missing person and the DNA-profile of the unidentified body(part) or as a likelihood ratio expressing the probability of the results given the following two propositions: either the DNA is from the missing person Mr X or it comes from an unknown person.
- An indirect comparison is a comparison between the DNA-profiles of persons that are possibly related. For instance, to investigate if an unidentified body(part)

is a particular missing person, the DNA profile can be compared to the DNA profiles of the missing person's relatives. This approach is used when the DNA-profile of the missing person is not available for direct comparison. In this case, the strength of the match is usually expressed as a likelihood ratio (e.g. assuming that X is indeed the biological child of Ms Y and Mr Z, the result of the DNA-analysis is x times more likely if the unidentified body(part) is X than if it is a random unrelated person. Specialized software is available to perform these calculations (see § 21.5). The use of prior odds for missing persons identifications has recently been discussed by Budowle et al.[57] and Thompson et al. [58]

22.4 Markers

A comparison between DNA-profiles in a missing persons DNA-database usually starts with 10-15 autosomal STR-markers. In the case of a direct match, the evidential value of the match will usually be sufficient the decision maker to identify the person, but in the case of an indirect match, additional autosomal markers may have to be determined and/or Y-STR-markers and/or Mt-DNA to verify or falsify the match.

22.5 Relationship between DNA-databases for missing persons and DNA-databases for solving crimes

In some countries, DNA-profiles of missing persons (and/or their relatives) and unidentified human remains are kept in the same DNA-database as the DNA-profiles which are used for solving crimes, while in other countries a separate DNA-database is used for missing persons (and/or their relatives) and unidentified human remains. There may be several reasons for this:

- Data protection considerations. By keeping DNA-profiles of missing persons and their relatives separate from the DNA-profiles in the criminal DNA-database, they cannot accidentally be compared with profiles with which they should not be compared
- Both DNA-databases may be managed by different organizations (e.g. Ministry of Justice versus the Police)
- Specialized software is needed to find and evaluate matches between unidentified human remains and multiple relatives in pedigrees of missing persons

If two separate DNA-databases are used it must be kept in mind that it can be useful to compare DNA-profiles of unidentified human remains with the DNA-profiles of the criminal DNA-database:

- DNA-profiles of unidentified human remains found in one location may match with stains found at a crime scene at another location, indicating that the unidentified person may have been the victim of a crime (if this was not yet obvious) and has been transported to another location
- DNA-profiles of unidentified human remains may match with a reference sample, which may assist an identification. This comparison needs to be done only once, as the unidentified person is dead and hence cannot be added to the DNA-database as a reference sample in the future.

[57] Budowle et al (2011) Investigative Genetics 2: 15. Use of prior odds for missing persons Identifications

[58] Thompson et al. (2013) Frontiers in Statistical Genetics and Methodology 4(220), pp. e1-e3, 10-2013. The role of prior probability in forensic assessments.

22.6 Software

Specialized software is available to search for relatedness between (a series of) DNA-profiles and/or to calculate the likelihood ratio of the relatedness of a person and their putative family member(s). This type of software is also used in forensic and civilian cases to verify or falsify the biological relationship between known persons. Table 10 lists the different programs that are known to the author[59].

Program name	Producer	Reference
Anonymous	Petr Linhart	http://www.fsigenetics.com/article/S1872-4973(08)00182-8/abstract
Bloodhound	Ananomous	http://www.ananomouse.com/products/bloodhound.asp
Bonaparte	SNN/Smart Research	www.bonaparte-dvi.com
CODIS 7.0	FBI	http://www.fsigenetics.com/article/S1872-4973(10)00105-5/abstract
DNAStat	Jaroslaw Berent	http://www.ncbi.nlm.nih.gov/pubmed/17907628
DNAView	Charles Brenner	http://dna-view.com/
EasyDNA	Wing Kam Fung	http://www.hku.hk/statistics/EasyDNA/
EasyPat	Michael Krawczak	http://www.uni-kiel.de/medinfo/mitarbeiter/krawczak/download/
Familias	Petter Mostad	http://www.math.chalmers.se/~mostad/familias/
FINEX	R.G. Cowell	http://www.ncbi.nlm.nih.gov/pubmed/12850417
Genemarker HID	SoftGenetics	http://www.softgenetics.com/GeneMarkerHID.html
fDMS	ICMP	http://www.ic-mp.org/activities/?page_id=551
FSS DNA Lineage	FSS	http://www.promega.com/geneticidproc/ussymp21proc/abstracts/poster_89.pdf
GenomiCalc	Genomic	http://www.genomicalc.com.br
Genoproof	Qualitype	http://qualitype.de/genoproof/
Genotype	Kvant s.r.o.	http://www.dip.sk/typo3/dip.sk/index.php?id=9&no_cache=1&L=1
Grape	DNA-soft	http://www.DNA-soft.com
Hugin	Hugin	http://www.hugin.com/productsservices/demo/hugin-lite
KinCALc	California DOJ/Steven Myers	Steven.Myers@doj.ca.gov
KINGROUP	Dmitry A. Konovalov	http://www.kingroup.org/
LISA	Future Technologies, Inc.	http://www.ftechi.com/dna_biometric.shtml
M-FISys	Gene Codes Forensics	www.genecodesforensics.com
MPKin	Institute of Investigative Genetics	www.investigativegenetics.com/content/1/1/8
PatCan	Jose Antonio Riancho	Forensic Science International Volume 135, Issue 3, 27 August 2003, Pages 232-234
Patern	Michael Krawczak	http://www.uni-kiel.de/medinfo/mitarbeiter/krawczak/download/
Paternity Index	Michel Jung	FSI Genetics Volume 3, Issue 2, March 2009, Pages 112-118
PatPCR	Juan Antonio Luque	http://www.fsigenetics.com/article/S1872-4973(08)00182-8/abstract
PedExpert	Sérgio Danilo Junho Pena	spena@dcc.ufmg.br
Program for Biostatistical Paternity and Relationship	Max Baur, Rolf Fimmers, W. Spitz	
RELPAIR	William L. Duren, Michael Epstein, Mingyao Li, and Michael Boehnke	http://csg.sph.umich.edu/boehnke/relpair.php

Table 10: Software programs to search for relatedness between (a series of) DNA-profiles and/or to calculate the likelihood ratio of the relatedness of a person and their putative family member(s).

[59] The mentioning of trade names does not mean that ENFSI recommends these programs. ENFSI's aim is just to give an overview of what is available on the market.

Depending on the required application of the software, different program properties will be more or less important to have. Table 11 lists some program parameters which may be considered when choosing (buying) a missing persons software program.

Parameter category	Parameter
Data which can be compared	Autosomal STRs
	Y-STRs
	MT-DNAs
	SNPs
	Metadata
Search strategies	UHR against UHRs
	UHRs against UHRs to find relations
	UHR against MPs
	UHR against pedigrees of MP
	UHRs against pedigrees of MP
	Familial searching (shared alleles)
	Familial searching (LR-ranking)
Calculations	Pedigree likelihood ratio calculation
	Fst correction
	Size bias correction
	Mutation correction[60]
	Allele drop out correction
	Multiple allele relative frequency tables
	Minimum allele relative frequency substitution for rare alleles
	Datafilters
	Resultfilters
Other features	Graphical pedigree manager
	Combining DNA-profiles of the same person
	Incestuous relationships
	Reporting module
	Simulations module
	Import module
	Replacing MP by IP in pedigree

Table 11: Program parameters which may be considered when choosing (buying) a missing persons software program (UHR: Unidentified Human Remains; MP: Missing Person; IP: Identified Person; LR: Likelihood Ratio)

Because software programs are continuously adapted and improved, interested persons should refer to the producer of the program to find out the latest properties.

[60] A recent discussion about different mutation models can be found in: Chakraborty et al (2011) Investigative Genetics 2:8. Response to: DNA identification by pedigree likelihood ratio accommodating population substructure and mutations- authors' reply.

22.7 International Organisations

22.7.1 International Commission on Missing Persons (ICMP)[61]

The International Commission on Missing Persons was established at the initiative of U.S. President Clinton in 1996 at the G-7 Summit in Lyon, France. Its primary role is to ensure the cooperation of governments in locating and identifying those who have disappeared during armed conflict or as a result of human rights violations. ICMP also supports the work of other organizations, encourages public involvement in its activities and contributes to the development of appropriate expressions of commemoration and tribute to the missing.

The organization was established to support the Dayton Peace Agreement, which ended the conflicts in Bosnia and Herzegovina. ICMP is currently headquartered in Sarajevo. In addition to its work in the countries of former Yugoslavia, ICMP is now actively involved in helping governments and other institutions in various parts of the world address social and political issues related to missing persons and establish effective identification systems in the wake of conflict or natural disaster.

Since November 2001, ICMP has led the way in using DNA as a first step in the identification of large numbers of persons missing from armed conflict. ICMP has developed a DNA database of over 90,000 relatives of 29,500 missing people, and more than 54,000 bone samples taken from mortal remains exhumed from clandestine graves in the countries of former Yugoslavia. By kinship matching of STR profiles from bone samples and family references, ICMP has been able to identify over 17,000 people who were missing from the conflicts and whose mortal remains were found in hidden graves. Additionally, ICMP provides extensive assistance in similar post conflict or human rights missing persons efforts in Latin America, Africa, the Mediterranean and the Middle East.

ICMP has been involved in a number of large-scale DVI efforts, including the 2004 SE Asian tsunami, 2006 Hurricane Katrina and 2008 Typhoon Frank in the Philippines. ICMP has established agreements to efficiently partner with INTERPOL in particular DVI operations to provide DNA testing and matching capabilities, and to participate in INTERPOL's Incident Response Teams (IRTs) that are often deployed upon invitation by relevant national authorities, to assess and help guide DVI response activities.

ICMP has developed a comprehensive Forensic Data Management System (fDMS) that permits data tracking and analysis for an integrated identification system that includes forensic archaeology, forensic anthropology, missing persons and relatives, DNA matching, and reporting. The fDMS can be flexibly modified to various contexts, and includes a web-based Online Inquiry Center that aids in the establishment of missing persons databases and provides an information link for data to both the general public and partnering forensic authorities, as deemed appropriate for the context and particular roles. Central to ICMP's role in maintaining large databases of potentially sensitive information are

[61] The text of this paragraph was supplied by ICMP

ICMP's data protection policies and recognized privileges and immunities that ensure data protection both in policy and practice.

22.7.2 INTERPOL

INTERPOL member countries can call for assistance in disaster victim identification (DVI).
The services offered by INTERPOL include:

- A downloadable DVI guide with with Ante Mortem and Post Mortem report forms.forms on the INTERPOL public website
- Assistance from the Command and Co-ordination Centre at the INTERPOL General Secretariat in Lyon, France, to send messages between National Central Bureaus 24 hours a day in Arabic, English, French or Spanish
- An Incident Response Team to provide further assistance upon request, such as on-site investigative support or connection to INTERPOL's databases

After the 2004 Tsunami in Thailand, it was decided to establish an international 'Missing Persons/Unidentified Dead Bodies' database at the INTERPOL General Secretariat in Lyon. This database will also contain a DNA-module. A three year EU-funded project started in April 2010 to establish the database. More information about the project can be found at: http://www.interpol.int/Public/FASTID/
In 2013 INTERPOL also decided to explore the use of the Bonaparte software for the comparison of DNA-profiles of unidentified persons and family members of missing persons[62].

INTERPOL also has a central DNA-database at its General Secretariat in Lyon, which is described in chapter 20 of this document. In this DNA-database, DNA-profiles of missing persons and unidentified bodies can also be included and compared. The following countries have submitted missing persons profiles and/or unidentified human remains profiles to the INTERPOL DNA Database:

Algeria	Germany	Romania
Australia	Greece	Russian Federation
Azerbaijan	Hong Kong	Slovakia
Belarus	Hungary	Slovenia
Belgium	Ireland	South Africa
Bosnia & Herzegovina	Israel	Spain
Bulgaria	Italy	Sweden
Canada	Japan	Switzerland
Chile	Jordan	Syrian Arab Republic
Costa Rica	Latvia	Thailand
Croatia	Liechtenstein	The Netherlands
Cyprus	Lithuania	Turkey
Czech Republic	Mexico	Ukraine
Denmark	Montenegro	United Arab Emirates
Estonia	Norway	United Kingdom
Finland	Panama	United States
France	Poland	
FYR of Macedonia	Portugal	

[62] http://www.interpol.int/News-and-media/News/2013/PR141

22.8 European missing persons DNA-databases

The table below which is based on the INTERPOL Global DNA-profiling 2008 Survey and on directly obtained information contains an inventory of countries in Europe which have DNA-profiles of missing persons and unidentified human remains in their DNA-databases.

Country	Missing Persons and Unidentified Human Remains	Separated from (S) or included (I) in the criminal DNA-database	Software
Albania	?	?	?
Austria	Yes	?	?
Belgium	No	n/a	n/a
Bosnia & Herzegovina	?	?	?
Bulgaria	No	n/a	n/a
Croatia	?	?	?
Cyprus	Yes	?	?
Czech Republic	Yes	?	?
Denmark	Yes	S	Plasdata
Estonia	Yes	?	?
Finland	No	n/a	n/a
Former Yugoslavian Republic of Macedonia	No	n/a	n/a
France	Yes	?	?
Germany	Yes	S	Self developed program
Georgia	?	?	?
Greece	?	?	?
Hungary	No	n/a	n/a
Iceland	?	?	?
Ireland	?	?	?
Italy	Yes	?	?
Latvia	Yes	?	?
Liechtenstein	Yes	?	?
Lithuania	Yes	?	?
Luxembourg	No	n/a	n/a
Malta	?	?	?
Montenegro	?	?	?
Netherlands	Yes	S	Bonaparte; CODIS; DNAView
Northern Ireland	?	?	?
Norway	No	S	CODIS

Poland	No	n/a	n/a
Portugal	No	n/a	n/a
Romania	No	n/a	n/a
Russia	?	?	?
Scotland	?	?	?
Serbia	?	?	?
Slovakia	Yes	I	CODIS
Slovenia	Yes	?	?
Spain	Yes	S	CODIS
Sweden	No	n/a	n/a
Switzerland	Yes	I	CODIS
Turkey	No	n/a	n/a
Ukraine	No	n/a	n/a
UK (England + Wales)	No	n/a	n/a

Table 12: Missing persons DNA-databases in Europe (? = not known to the author; n/a = not applicable)

Annex 1: Summary of ENFSI-recommendations on DNA-database management

1) Every EU/ENFSI-country should establish a forensic DNA-database and specific legislation for its implementation and management.
2) The type of crime-related stain DNA-profiles which can be included in a DNA-database should not be restricted.
3) To increase the chance of identifying the donors of stains, the number of persons in a DNA-database who are likely to be donors of those stains should be as high as legally (and financially) possible.
4) Managers of national DNA-databases should establish (together with other stakeholders) criteria for the inclusion of partial DNA-profiles to obtain an acceptable balance between the minimum allowable level of evidential value (maximum random match probability) of a DNA-profile and maximum number of adventitious matches a partial DNA-profile is expected to generate.
5) DNA-profiles produced by older commercial kits should be upgraded (if possible) after a match in the National DNA-database to increase the evidential value of the match and to decrease the possibility of an adventitious match and also to fulfill the criteria for international comparison if a country wants to include DNA-profiles produced by older commercial kits in international search actions.
6) To enhance the chance of finding relevant matches with partial crime stain DNA profiles, reference samples profiles should only be loaded to a database where a complete profile is obtained using the PCR Chemistry of choice.
7) Labs producing DNA-profiles for a DNA-database should, as a minimum, be ISO-17025 (and/or nationally equivalent) accredited and should participate in challenging proficiency tests.
8) When DNA-profiles produced from low levels of DNA are included in a DNA-database they should be recognizable and/or a dedicated (near) match strategy should be used for them.
9) Composite DNA-profiles should only be created from DNA-profiles generated from the same DNA-extract because it cannot be excluded that different samples contain DNA-from different persons.
10) When a new allele is observed in a DNA-profile, its presence should be confirmed by repeated DNA-isolation, PCR, Capillary Electrophoresis and allele calling of the DNA-profile. Only new alleles of which the size can be accurately determined using the internal DNA-size-standard, should be included in the DNA-database.
11) Alleles from loci with chromosomal anomalies should not be included in a DNA-database as they may be caused by somatic mutations which may only occur in certain tissues/body fluids.
12) The guidelines in the document of the ISFG-working group on the analysis of mixed profiles should be used for the analysis of mixed profiles. Software tools may also be used provided they are properly validated.
13) A numerical match between a reference sample and a mixed profile must always be checked against the electropherogram of the mixed profile.
14) Mixed profiles of more than 2 persons should not systematically be included in a DNA-database because they generally will produce many adventitious matches.
15) When non-autosomal STR profiles or mitochondrial profiles are added to criminal DNA-databases, specific operating procedures must be in place to avoid unintended familial searches.
16) If the removal of a DNA-profile from the DNA-database is dependent on external information, a process should be in place to give the custodian of the DNA-database access to this information preferably by means of an automated message after an event which influences the deletion date of a DNA-profile.

17) There should be a system that can be consulted by those responsible for sampling persons to see whether a person is already present in the DNA-database.

18) The system which can be consulted by those responsible for sampling persons to see whether a person is already present in the DNA-database should be combined with a rapid biometric identification system like fingerprints to verify whether a person is already present in the DNA-database.

19) Any DNA-database should have an associated elimination DNA-database (or databases). This should include laboratory staff of all categories as well as visiting maintenance personnel. Profiles from those with access to traces (e.g., police) should also be included in addition to unidentified DNA-profiles found in negative controls which may originate in manufacturing disposables and/or chemicals. The latter category of DNA-profiles should be shared with other ENFSI-countries.

20) Policies and procedures should be in place to ensure that non relevant DNA-profiles are deleted immediately after their irrelevance has become clear

21) The occurrence of errors in DNA-profiles as a result of human mistakes associated with data entry should be avoided as much as possible by automating the allele calling and the DNA-database import process. When DNA-profiles are entered manually into the DNA-database this should be done by a process which detects typing errors, for example by double (blind) entry of data.

22) To prevent false exclusions DNA-profiles should also be compared allowing at least one mismatch. The DNA-profiles involved in such near matches should be checked for possible mistakes during their production and processing.

23) As a national DNA-database is regularly subject to attention from the public, politicians and the media, a DNA-database manager should consider establishing performance parameters and making these publicly available.

24) DNA-database managers should be aware of the possibility of adventitious matches and be able to calculate their expected numbers for the matches they report. When reporting a DNA-database match, a warning should be included indicating the factors that increase the possibility of finding an adventitious match (size of the database, number of searches, mixed and partial profiles/random match probability, presence of family members).

25) A DNA-database match report of a crime scene related DNA-profile with a person should be informative and apart from the usual indication of the evidential value of the match (RMP/LR) it should also contain a warning indicating the possibility of finding adventitious matches (as mentioned in recommendation 22) and its implication that the match should be considered together with other information.

26) DNA-profiles should be entered into a database in a way that guarantees their correct import. Access to the DNA-database should be limited to those persons who need to have access, by physical and organizational measures. Regular back-ups should be made, stored in a safe place, and put back at regular intervals to simulate recovery from a disaster. When DNA-profiles and their associated information are present in different systems, these systems should be regularly compared to check whether they are still properly synchronized.

27) To detect false exclusions (e.g. matches which should be found but are not found because one of the DNA-profiles contains an error) regular full DNA-database searches allowing one or more mismatches should be performed. When a match between two DNA-profiles contains a mismatch in one of the loci, the original data of both DNA-profiles should be checked to see if one of the DNA-profiles contains an error.

28) Information from a National DNA-database should be combined with other types of evidence to increase the number of crimes for which a lead can be identified.

29) As automated processes reduce the possibility of human errors, they should be introduced for those processes that are straightforward.

30) From a forensic point of view the cell material of reference samples should be stored as long as their corresponding DNA-profiles.

31) Because DNA-databases have a very important but also very delicate role in society, the custodian of a DNA-database should develop tools to make objective information about the DNA-database available to politicians, the public and the media.

32) Six and seven locus international matches obtained under the terms of the Prüm system should be further analyzed by additional DNA-testing before requesting information from another country. If a Prüm related information request is received from another country, the quality of the corresponding match should be checked before providing the requested information to the other country.

33) All regularly used loci (also the ones not used by the receiving country) should be configured in the DNA-databases of countries participating in the international exchange of DNA-profiles under the terms of the Prüm system to be able to see the full composition of the DNA-profile of the sending country

Annex 2: ENFSI guidelines for auditing DNA-databases

This annex document aims to provide practical guidelines for teams auditing a DNA-database to verify compliance with the ENFSI DNA Working Group recommendations. The document also aims to provide a reporting format for the auditing team which can be filled out at the auditing site and can be presented to the person(s) who requested the audit. The recommendations of the ENFSI DNA Working Group as listed in annex 1 have been taken as a basis for the auditing operation and the opinion of the auditor can be added to each item.

In 2008, the Council of the European Union agreed on converting major parts of the Treaty of Prüm into two EU-council decisions (2008/615/JHA and 2008/616/JHA). These decisions contain the obligation for EU member states to establish a DNA-database and to make it available for automated searches by other member states.
Any member state that wishes to start exchanging data after 13 October 2009 also has to pass an evaluation procedure (6661/2/09 Rev 2) consisting of:

- Filling out a questionnaire on data protection (6661/1/09 Rev 1 Add 1 Rev 1)
- Filling out a questionnaire on the exchange of DNA-profiles (6661/1/09 Rev 1 Add 2 Rev 1)
- A pilot run to test and validate the IT-environment
- An evaluation visit by an external evaluation team to verify all the information that was provided
- The approval of the EU Council based on the report of the evaluation team

The EU Working Party on Data Protection and Information Exchange (DAPIX) has developed guidelines and a reporting format for the evaluation teams. Although there is some overlap between the guidelines of the EU and ENFSI, their focus is quite different. The ENFSI guidelines focus on the proper functioning and management of a DNA-database in a national environment and the EU guidelines focus on the interaction of a DNA-database with other DNA-databases and on compliance with the contents of the two EU-council decisions (2008/615/JHA and 2008/616/JHA). Together they offer an instrument to determine the proper management in a national as well as in an international environment

It should be noted that ENFSI has established its recommendations based on forensic optimization criteria. Sometimes the national legislation is in contradiction with the ENFSI recommendations. In such cases, the auditor can indicate that there is a Noncompliance with the ENFSI guidelines but that this Noncompliance is acceptable because the national law supersedes the ENFSI guidelines.

GENERAL INFORMATION

Country	
Audit date(s)	
Audit requested by	
Hierarchy of the database	national/sub-national
Organizational position of the database	
Auditing persons (function)	
Database manager(s)	
Database user(s)	
Database IT-personnel	
Sources of DNA-profiles	

AUDIT QUESTIONS

ENFSI recommendation 1	Every EU/ENFSI-country should establish a forensic DNA-database and specific legislation for its implementation and management
Audit question(s)	Are copies of the legislation available (or internet sources where they can be found)?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 2	The types of crime stain-related DNA-profiles which can be included in a DNA-database should not be restricted
Audit question(s)	What are the criteria for the inclusion of stains?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 3	To increase the chance of identifying the donors of stains, the number of persons in a DNA-database who are likely to be donors of those stains should be as high as legally (and financially) possible
Audit question(s)	What are the criteria for the inclusion of individuals?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 4	Managers of national DNA-databases should establish (together with other stakeholders) criteria for the inclusion of partial DNA-profiles to obtain an acceptable balance between the minimum allowable level of evidential value (maximum random match probability) of a DNA-profile and maximum number of adventitious matches a partial DNA-profile is expected to generate.
Audit question(s)	What are the criteria for the inclusion of partial profiles?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 5	DNA-profiles produced by older commercial kits should be upgraded (if possible) after a match in the National DNA-database to increase the evidential value of the match and to decrease the possibility of an adventitious match and also to fulfill the criteria for international comparison if a country wants to include DNA-profiles produced by older commercial kits in international search actions
Audit question(s)	Is there a written procedure for the upgrading of older/partial profiles?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 6	To enhance the chance of finding relevant matches with partial crime stain DNA profiles, reference samples profiles should only be loaded to a database where a complete profile is obtained using the PCR Chemistry of choice.
Audit question(s)	What are the criteria for the inclusion of reference samples?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 7	Labs producing DNA-profiles for a DNA-database should, as a minimum, be ISO-17025 (and/or nationally equivalent) accredited and should participate in challenging proficiency tests
Audit question(s)	Which labs produce DNA-profiles for the DNA-database and are they (getting) accredited?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 8	When DNA-profiles produced from low levels of DNA are included in a DNA-database they should be recognizable and/or a dedicated (near) match strategy should be used for them.
Audit question(s)	Is there a written procedure for the processing of low level DNA-profiles (both in the lab and in the DNA-database)?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 9	Composite DNA-profiles should only be created from DNA-profiles generated from the same DNA-extract because it can not be excluded that different samples contain DNA from different individuals.
Audit question(s)	Does the DNA-database contain composite DNA-profiles and if so how were they created?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 10	When a new allele is observed in a DNA-profile, its presence should be confirmed by repeated DNA-isolation, PCR, Capillary Electrophoresis and allele calling of the DNA-profile. Only new alleles of which the size can be accurately determined using the internal DNA-size-standard, should be included in the DNA-database.
Audit question(s)	Is there a written procedure for the inclusion of new/rare alleles?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 11	Alleles from loci with chromosomal anomalies should not be included in a DNA-database as they may be caused by somatic mutations which may only occur in certain tissues/body fluids.
Audit question(s)	Is there a written procedure for the handling of chromosomal anomalies?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 12	The guidelines in the document of the ISFG-working group on the analysis of mixed profiles should be used for the analysis of mixed profiles. Software tools may also be used provided they are properly validated.
Audit question(s)	Is there a written procedure for the processing of mixed DNA-profiles (both in the lab and in the DNA-database)?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 13	A numerical match between a reference sample and a mixed profile must always be checked against the electropherogram of the mixed profile.
Audit question(s)	See the question associated with recommendation 12.
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 14	Mixed profiles of more than 2 persons should not systematically be included in a DNA-database because they generally will produce many adventitious matches.
Audit question(s)	See the question associated with recommendation 12.
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 15	When non-autosomal STR profiles or mitochondrial profiles are added toe criminal DNA-databases, specific operating procedures must be in place to avoid unintended familial searches.
Audit question(s)	Are non-autosomal STR profiles or mitochondrial profiles added to the criminal DNA-database? If yes, are specific operating procedures in place to avoid unintended familial searches.
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 16	If the removal of a DNA-profile from the DNA-database is dependent on external information, a process should be in place to give the custodian of the DNA-database access to this information preferably by means of an automated message after an event which influences the deletion date of a DNA-profile.
Audit question(s)	What are the rules and procedures for the removal of DNA-profiles from the DNA-database?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 17	There should be a system that can be consulted by those responsible for sampling persons, to see whether a person is already present in the DNA-database.
Audit question(s)	Is there a system that can be consulted by those responsible for sampling persons to see whether a person is already present in the DNA-database?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 18	The system which can be consulted by those responsible for sampling persons to see whether a person is already present in the DNA-database should be combined with a rapid biometric identification system like fingerprints to verify whether a person is already present in the DNA-database.
Audit question(s)	How is a person identified before they are sampled for the DNA-database?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 19	Any DNA-database should have an associated elimination DNA-database (or databases). This should include laboratory staff of all categories as well as visiting maintenance personnel. Profiles from those with access to traces (e.g. police) should also be included in addition to unidentified DNA-profiles found in negative controls, which may originate in manufacturing disposables and/or chemicals. The latter category of DNA-profiles should be shared with other ENFSI-countries.
Audit question(s)	Is there an elimination DNA-database and what kind of profiles are included? Are profiles found in negative controls shared with other countries?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 20	Policies and procedures should be in place to ensure that non relevant DNA-profiles are deleted immediately after their irrelevance has become clear
Audit question(s)	Are there policies and procedures in place to ensure that non relevant DNA-profiles are deleted immediately after their irrelevance has become clear?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 21	The occurrence of errors in DNA-profiles as a result of human mistakes associated with data entry should be avoided as much as possible by automating the allele calling and the DNA-database import process. When DNA-profiles are entered manually into the DNA-database this should be done by a process which detects typing errors, for example by double (blind) entry of data.
Audit question(s)	Describe the allele calling and DNA-database inclusion process. If not fully automated, which measures have been put in place to avoid human errors?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 22	To prevent false exclusions DNA-profiles should also be compared allowing at least one mismatch. The DNA-profiles involved in such near matches should be checked for possible mistakes during their production and processing.
Audit question(s)	Are DNA-profiles checked for mistakes using a near match approach?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 23	As a national DNA-database is regularly subject to attention from the public, politicians and the media, a DNA-database manager should consider establishing performance parameters and making these publicly available.
Audit question(s)	How is the performance of the DNA-database monitored and reported and to whom?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 24	DNA-database managers should be aware of the possibility of adventitious matches and be able to calculate their expected numbers for the matches they report. When reporting a DNA-database match, a warning should be included indicating the factors that increase the possibility of finding an adventitious match (size of the database, number of searches, mixed and partial profiles/random match probability, presence of family members).
Audit question(s)	How are DNA-database matches reported?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 25	A DNA-database match report of a crime scene related DNA-profile with a person should be informative and apart from the usual indication of the evidential value of the match (RMP/LR) it should also contain a warning indicating the possibility of finding adventitious matches (as mentioned in recommendation 22) and its implication that the match should be considered together with other information.
Audit question(s)	See the question associated with recommendation 21.
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 26	DNA-profiles should be entered into a database in a way that guarantees their correct import. Access to the DNA-database should be limited to those persons who need to have access, by physical and organizational measures. Regular back-ups should be made, stored in a safe place, and put back at regular intervals to simulate recovery from a disaster. When DNA-profiles and their associated information are present in different systems, these systems should be regularly compared to check whether they are still properly synchronized.
Audit question(s)	See the question associated with recommendation 19 and include the above mentioned issues.
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 27	To detect false exclusions (e.g. matches which should be found but are not found because one of the DNA-profiles contains an error) regular full DNA-database searches allowing one or more mismatches should be performed. When a match between two DNA-profiles contains a mismatch in one of the loci, the original data of both DNA-profiles should be checked to see if one of the DNA-profiles contains an error.
Audit question(s)	Describe the procedure to detect false negative matches.
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 28	Information from a National DNA-database should be combined with other types of evidence to increase the number of crimes for which a lead can be identified.
Audit question(s)	Is DNA-information combined with other types of information and if yes, how?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 29	As automated processes reduce the possibility of human errors, they should be introduced for those processes that are straightforward.
Audit question(s)	See the question associated with recommendation 19.
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 30	From a forensic point of view, the cell material of reference samples should be stored as long as their corresponding DNA-profiles.
Audit question(s)	What are the rules and procedures for the destruction of reference samples?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 31	Because DNA-databases have a very important but also very delicate role in society, the custodian of a DNA-database should develop tools to make information about the DNA-database available to politicians, the public and the media.
Audit question(s)	Which information about the DNA-database is communicated and how?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 32	Six and seven locus international matches obtained under the terms of the Prüm system should be further analyzed by additional DNA-testing before requesting information from another country. If a Prüm related information request is received from another country, the quality of the corresponding match should be checked before providing the requested information to the other country.
Audit question(s)	Which procedures are in place to prevent the reporting of adventitious matches and the transfer of case and/or personal information to other countries based on adventitious matches?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

ENFSI recommendation 33	All regularly used loci (also the ones not used by the receiving country) should be configured in the DNA-databases of countries participating in the internation-al exchange of DNA-profiles under the terms of the Prüm system to be able to see the full composition of the DNA-profile of the sending country.
Audit question(s)	Can you show which loci have been configured in your DNA-database?
Audit result	
Compliance? If no, why not?	Yes / No
Noncompliance acceptable? If yes, why?	Yes / No
Remark(s)/recommendation(s)	

Annex 3: English translation of the textbox included in Dutch match reports

POINT OF ATTENTION WITH REGARDS TO A DNA-DATABASE MATCH

DNA-databases contain large numbers of DNA-profiles of known persons and of biological traces related to unsolved crimes.

When the number of DNA-profiles in a DNA-database increases, so does the chance of getting an adventitious match with a person who is not the actual donor of the trace.

This is especially true for partial DNA-profiles and mixed DNA-profiles because the chance that they would match with a randomly chosen person is greater than the chance that a full single DNA-profile would match a randomly chosen person.

If there are doubts if the matching person is the donor of the trace, for instance because there is no other tactical or technical evidence which links the person to the crime, the possibility to do additional DNA-testing can be considered.

This point of attention particularly applies to matches which are found as a result of the large scale international DNA-profile comparisons based on the EU-Prüm decisions.

Annex 4: Changes in the 2014 document relative to the 2013 document

- The Verifiler kit was added to table 1
- The word “sample” was replaced by “item” or “trace” on several places in the document
- Table 4 (ENFSI DNA-database overview) was replaced by a more recent version
- A sentence was added to paragraph 3.7 saying that the Prüm inclusion rule on the treatment of triallelic loci is in contrast with recommendation 11 and should be changed as soon as the Prüm inclusion rules can be changed (when all Prüm countries are operational)
- In paragraph 3.11 a recommendation was added saying that “When non-autosomal STR profiles or mitochondrial profiles are added to criminal DNA-databases, specific operating procedures must be in place to avoid unintended familial searches”.
- The development of a manufacturers’ elimination database application by ICMP was added to paragraph 4.5
- Paragraph 4.6 was made more general and a recommendation was added saying that “Policies and procedures should be in place to ensure that non relevant DNA-profiles are deleted immediately after their irrelevance has become clear”
- The use of software tools to analyze mixed profiles has been added to paragraph 3.9 and recommendation 12
- Paragraph 3.10 (Sequence variation between STR alleles of similar size) has been updated
- New paragraphs on non-autosomal STR-markers, amelogenin and mitochondrial DNA have been added to chapter 3
- An explanation of the expression “target profile” was added to paragraph 5.2
- Table 9 (DNA-database programs used by countries) has been updatated
- Chapter 16 has been updated with respect to the training of people working with CODIS
- Next generation sequencing was added as an approach to distinguish identical twins in chapter 18
- Several dead links were replaced in paragraph 19.2
- Chapter 20 (International overviews) was updated
- The possibility for dedicated bilateral comparisons has been added to the INTERPOL paragraph of chapter 21
- The Europol paragraph in chapter 21 has been updated by Europol
- Paragraph 22.7.1 on ICMP has been updated by ICMP and their software program fDMS has been added to table 10 (as well as some other kinship testing programs)
- The 2013 Interpol decision to explore the use of the Bonaparte software for the comparison of DNA-profiles of unidentified persons and family members of missing persons was added to paragraph 22.7.2
- Several textual changes were made to improve the readability and/or correctness of the document
- The annexes which contain changes relative to the versions 2009-2012 have been deleted. Only the changes relative to the previous version will be included from now on
- Because two new recommendations were added, they were renumbered and the new recommendations were added to the auditing guidelines