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Outline

of this thesis

Human identification is needed in situations such as mass disasters, terrorist attacks, missing person cases and forensic investigations. The identification can be based on body characteristics (for instance facial recognition, dactyloscopy or odontology) or on DNA-based evidence. Especially the latter method has proven to be useful when bodies are beyond recognition or incomplete.

In order to make a DNA profile of a person, the DNA has to be of sufficient quality and quantity. However, when the time between death and discovery of the body is long and/or the body has been exposed to harsh conditions (fire, submerged, warm humid air, acidic soil, chemical agents, etc.) the DNA may be severely degraded and/or available in minute amounts only.

The research described in this thesis has been directed towards method development for cases in which the DNA samples are compromised (i.e. low in quality and/or quantity). The aim of the research is to provide additional or alternative methods to extract information from a person's DNA when standard DNA typing methodology is not sufficient for human identification.

DNA profiles used for human identification are usually based on short tandem repeats (STRs) in the DNA sequence. The number of repeats per allele (depicted as a peak in the DNA profile) differs between persons, and a combination of alleles for several markers makes a DNA profile that is (virtually) unique per person when enough markers are used. To obtain a DNA profile of the best possible quality, it is of utmost importance that samples are excised, handled and stored under the most optimal conditions achievable, until their analysis in a specialised DNA laboratory. Especially in mass disaster conditions, bodies (and samples taken from those) are easily contaminated by DNA from other victims. **Chapter 1** describes a standard operating procedure for sample excision, contamination prevention and optimum sample storage conditions in a mass disaster environment. Sampling instructions are given for femur, rib and tooth (or molar) samples. In addition, practical advice is given on inexpensive and simple solutions for excision tools, decontamination fluid preparation and preservation of the samples.

In order to generate a DNA profile, specific marker regions within the DNA are amplified (by a polymerase chain reaction (PCR)). When DNA is low in quantity, DNA profiles have lower peak heights and may suffer from stochastic amplification effects, such as peak height imbalances, allele and/or locus drop-outs (which result in incomplete DNA profiles), allele drop-ins and elevated stutter peaks (which are artefacts that resemble real peaks). These effects impede DNA profile interpretation and may prevent the identification of a person. In order to obtain more information from the DNA donor, a technique was developed to sensitise the DNA detection method (i.e. capillary electrophoresis), which is described in **Chapter 2**. This technique is based on increased injection settings during electrophoresis to obtain higher peak

heights and more genotyping information from single donor and (unequally) mixed DNA samples. It can be used irrespective of the DNA marker system to amplify the DNA. Since this method makes use of the remaining portion of the PCR product mixture (that otherwise would be discarded after standard DNA analysis) no additional use of DNA extract is needed. Nevertheless, it is regarded as a low template DNA technique and it is therefore recommended to perform replicate analyses.

Amplicons (i.e. the DNA fragments that are multiplied during amplification) of the STR kits used in the first part of this thesis varied in size from 100 to around 400 nucleotides. When the quality of the DNA is low, due to (severe) degradation, the DNA fragments may become shorter than some of the STR amplicons. In these cases, the peaks that represent the longer STRs have low peak heights or are even absent from the DNA profile. Another type of DNA marker is a single nucleotide polymorphism (SNP). SNPs used in human identification typically have amplicons of 55 to around 115 nucleotides, which make them interesting for the analysis of degraded DNA. In **Chapter 3**, we analysed tri-allelic SNPs, a special class of SNPs that exhibits three different alleles (instead of the usual two). This characteristic makes them specifically interesting for use in human identification and forensic analyses, as the detection of mixtures (recognised by a third allele on a locus) is much easier for tri-allelic than for bi-allelic SNPs (for which only peak height differences can be used), thus diminishing the chance on incorrect genotyping results. We developed multiplex genotyping assays and determined allele frequencies for Dutch and Netherlands Antilles populations in order to analyse degraded DNA samples and to assess the power of discrimination of the tri-allelic SNPs.

Subsequently, these assays and several alternatives were evaluated for the analysis of degraded DNA in a comparative study, as described in **Chapter 4**. Next to standard DNA typing, which was performed with SGM Plus™ at that time, we tested DNA repair enzyme cocktails (PreCR™ and Restorase™) that aim to repair the DNA prior to amplification. In addition, an STR kit designed to carry all amplicons in small size (mini-STRs (MiniFiler)) was included, as well as bi-allelic SNPs (a semi-commercial system denoted as GenPlex™) and the tri-allelic SNP set described in Chapter 3. For each of these methods we determined the percentage detected alleles, and showed the required DNA input in perspective to the random match probability that could be obtained.

After the decision by the European Council to add five additional STR markers to the European standard set of STRs, new STR kits were developed by several companies containing 15 STR loci or more. In these kits, mini-STRs (with amplicon sizes from 70 base pairs and up) are incorporated as much as possible to obtain more information from degraded DNA, and the sensitivity is enhanced by using optimised buffers and (an) additional amplification cycle(s). The NFI decided to work with the AmpF*l*STR® NGM™ kit, which was subsequently validated in-house. Specific aspects

of this validation are described in **Chapter 5**. We determined the stochastic threshold (below which alleles are prone to drop out due to low template amplification effects), together with the stutter ratio filters and the optimal strategy to sensitise genotyping of low template DNA. These aspects will assist in the optimal interpretation of unequal mixtures and low template DNA samples.

Sometimes, the 15 STR loci in the current generation STR kits do not provide enough discrimination power. This can occur when the DNA is degraded to the extent of locus drop-out, but also in complex kinship analyses, for example. A relatively new kit to the forensic market (HDplex™) contains 9 STRs that are additional to the commonly used markers, and additional genotyping with this kit can increase the power of discrimination. In **Chapter 6** we assessed whether the 30 STRs present in NGM™, HDplex™ and Identifiler™ can be regarded as independent, which is particularly relevant for the markers that are present on the same chromosome (an occurrence that is inevitable when the number of markers increases). In addition, we calculated the (combined) match probabilities (under the assumption of independence) for the supplementary genotyping results of the three kits. This information will point out which markers can be combined within one calculation to assist complex kinship or degraded DNA analyses.

Information about the longer loci in an STR profile can be missing due to degradation of the DNA or low template amounts of the minor contributor in an unequal mixture. Sensitising the reactions (e.g. by increased capillary electrophoresis injection settings as described in Chapter 2) may result in over-amplified or overloaded DNA profiles for the shorter loci and/or the major component in the mixture. In **Chapter 7** we explored whether DNA profiling of such samples can be improved by preferential capturing of the longer amplified fragments. We developed a size-selective post-PCR purification protocol (based on AMPure™ XP beads) and compared it to an unselective post-PCR purification system (DTR gel filtration) and no purification of the PCR products. These methods have the advantage that the remaining portion of the PCR products is used (likewise the method described in Chapter 2), without usurping additional DNA extract.

In **Chapter 8**, different aspects of DNA-based human identification are discussed with emphasis on low quality and/or quantity of the DNA. In addition, some recent developments and future possibilities are considered that may aid human identification and forensic analyses.