UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
DEPARTAMENTO DE BIOLOGIA ANIMAL

# Transfer and persistence of DNA on drug wrappings

Sara Isabel Pereira de Faria

**Mestrado em Biologia Humana e Ambiente**

Dissertação orientada por:
Prof. Maria Teresa Rebelo
Dr. Ane Elida Fonneløp

2021

# Dedication

I dedicate this dissertation to my parents, Fernanda and Eduardo Faria.

Who always support and encourage me to follow my dreams.

# Acknowledgments

The realization of this thesis would not be possible without the collaboration of people and institutions.

To Faculty of Sciences of the University of Lisbon, for the opportunity of going on Erasmus.

To my supervisor Prof. Maria Teresa Rebelo, who was by my side from the beginning of the thesis and was always available to answer my questions.

To the Forensic Genetics Research group of the Oslo University Hospital, for receiving me so well.

To my supervisor, Dr. Ane Elida Fonneløp, thank you for the opportunity, I learned so much there. You, Helen, Maria and Chiara made me feel at home for the ten months I was in Oslo.

# Resumo

A área da genética é responsável pelo estudo da transmissão e expressão dos genes. Na população humana, existe uma imensa diversidade genética. Esta variabilidade genética permite o uso da técnica de análise de perfis de DNA por geneticistas forenses.

Um perfil de DNA consiste no conjunto de genótipos de múltiplos loci. A análise de perfis de DNA é utilizada na identificação de indivíduos. O perfil de DNA pode ligar um individuo específico a uma amostra recuperada numa cena de crime. Dos diversos marcadores de DNA existentes, o mais indicado para se utilizar na análise de perfis de DNA são os STR, Short Tandem Repeat. Estes correspondem às características necessárias para este tipo de análise: são extremamente polimórficos, são de fácil caraterização e permitem uma leitura simples dos perfis de DNA.

A análise de amostras forenses é conhecida pelos seus desafios. Este tipo de amostras é suscetível a alterações analíticas devido a diversos fatores, desde a sua natureza biológica, substrato e contaminação. Estes fatores poderão afetar a qualidade do DNA e o resultado final da análise. Quando se realiza as análises a perfis de DNA existe uma elevada probabilidade de deteção de misturas de DNA. Estes perfis de DNA possuem mais de um contribuidor de DNA, possuindo múltiplos genótipos. Estas misturas de DNA acarretam uma dificuldade maior na análise deste tipo de perfil de DNA. EuroForMix é um programa de interpretação de misturas de DNA que facilita a análise de perfis de DNA, e fornece informação como o valor de Likelihood Ratio (LR) – medida que compara dois cenários e indica qual o mais provável.

O DNA recolhido numa cena de crime deve ser analisado em diferentes níveis. Nesta dissertação estamos a estudar o nível de atividade, que diz respeito às questões de como e quando o vestígio foi depositado na cena de crime.

Os mecanismos de DNA, transferência, persistência, prevalência e recuperação (DNA-TPPR) possuem um grande impacto na qualidade e quantidade de DNA.

O DNA pode ser transferido, principalmente, por dois modos, transferência direta (primária) e por transferência indireta (secundária). O modo como o DNA foi transferido para a cena de crime possui consequências na interpretação do perfil de DNA. É importante ter em consideração a existência de ‘Background DNA’ e ‘Shedder Status’. ‘Background DNA’ corresponde a DNA que não está relacionado com o ato criminal, mas que está presente antes de o crime ocorrer. Este é inevitável e afeta a transferência de DNA. ‘Shedder Status’ corresponde à quantidade de DNA que nós depositamos quando tocamos num objeto, este fator é influenciado pelo género, idade, suor, doenças de pele e hábitos de cada pessoa. Determinados fatores possuem impacto na transferência DNA: o tipo de substrato do objeto, a origem do material biológico e o tipo de contacto entre as superfícies.

A persistência do material biológico é afetada por fatores ambientais que podem acelerar o processo de degradação, pelo tipo de substrato do objeto e se o objeto é um objeto pessoal ou partilhado por diversas pessoas.

A prevalência do DNA pode ser perturbada por diversas questões anteriormente mencionadas, como o ‘Background DNA’ e se o objeto é um objeto pessoal ou partilhado.

A recuperação do DNA pode influenciar a qualidade dos resultados das análises futuras. A escolha do método mais adequado de recuperação é um fator importante. Os métodos mais utilizados são os esfregaços e a fita.

Atualmente, em Portugal temos vindo a assistir um aumento dos números de casos relacionados com narcóticos, com apreensões cada vez maiores de estupefacientes. Este aumento também é uma realidade na Noruega, onde as taxas de casos de narcóticos têm vindo a aumentar nos últimos anos. Uma grande parte das amostras que vêm para os laboratórios forenses são embalagens de drogas para analisar e conectar a um potencial suspeito.

O DNA tem sido contestado mais frequentemente em tribunal. Especificamente a atividade ou a altura dos acontecimentos que resultaram na transferência do DNA. Em muitos casos, a defesa dos suspeitos tenta dar uma explicação alternativa sobre como o DNA do suspeito foi transferido. Pois um perfil de DNA pode apenas fornecer informações sobre a identidade do dador, não diz como e quando ocorreu a deposição deste.

O principal objetivo desta dissertação é obter um maior conhecimento sobre os mecanismos de transferência, persistência e recuperação de DNA, de modo que no futuro seja mais fácil para os cientistas forenses interpretar questões relacionadas com a atividade em casos relacionados com drogas.

Foram realizados dois tipos de experiência: simulação de manuseamento de embalagens de droga e 'Shedder status'. A simulação de manuseamento de embalagens de droga englobou três experiências diferentes: simulação de transferência direta (em que os participantes manusearam múltiplas vezes uma embalagem de droga); simulação de transferência indireta (participantes colocaram uma embalagem de droga numa mala previamente usada) e por último simulação do empacotamento de uma embalagem de droga (esta experiência realizou-se aos pares, em que o primeiro participantes manuseou o rolo de fita cola, e o segundo participante embalou um pedaço de cartão com o rolo de fita cola). Na experiência de determinação do 'Shedder status', os participantes manusearam três tubos de plástico em diferentes alturas.

Os métodos utilizados para análise são os métodos padrão usados em laboratórios forenses. Após a recolhas das amostras através de esfregaços, foi utilizada a extração de DNA Chelex® 100 (Laboratórios Bio-Rad), seguido por quantificação através de PowerQuant® (Promega) e amplificação com Promega Fusion®6C Kit. Por último, a análise de fragmentos foi realizada no sistema de eletroforese capilar (CE). Na experiência de determinação do 'Shedder status' optou-se por realizar uma amplificação direta com Promega Fusion®6C Kit, em vez de se realizar a extração de DNA. A amplificação foi seguida pela realização de eletroforese capilar.

Na primeira experiência em que os participantes manusearam diretamente as embalagens de droga, foi obtida a maioria dos perfis de DNA dos participantes. Na experiência de transferência secundária através da mala, foram obtidos perfis de DNA parciais e perfis nulos. Na experiência de simulação do empacotamento de uma embalagem de droga obteve-se misturas de perfis de DNA. Com base nos resultados da experiência de 'Shedder status', foi possível classificar os participantes em baixo, médio e alto 'shedders'. Tendo em conta a classificação atribuída aos participantes, os resultados das experiências anteriores foram analisados. Na primeira experiência foi observada uma correlação entre os valores de LR e RFU (Relative Fluorescence Unit) obtidos e a classificação dos participantes, tal como na terceira experiência, empacotamento de uma embalagem de droga. Na segunda experiência, transferência secundária através da mala, não foi observada uma correlação entre os valores de LR e RFU e a classificação atribuída aos participantes.

Em suma, observou-se uma maior deteção de DNA após transferência direta do que por transferência indireta. Foi mais frequentemente detetado o DNA do participante que realizou o empacotamento da droga do que o DNA do participante que manuseou o rolo de fita cola. Com base nos resultados obtidos foi possível classificar os participantes em baixo, médio e alto 'shedders', tendo sido, a maioria dos participantes, classificados como médio 'shedders'. Os dados que foram obtidos nesta dissertação fornecem informação que pode ajudar os cientistas forenses na avaliação de questões relativas ao nível de atividade. Tendo em conta a base de dados, criada com os resultados obtidos, foi formulado um caso fictício, de forma a comprovar se a base de dados criada pode ajudar na análise de questões ao nível de atividade do vestígio depositado na cena de crime. Com este caso demonstramos que o valor de LR referente ao nível de atividade de um caso, pode ser calculado através da nossa base de dados.

Palavras-Chave: transferência, persistência, DNA, embalagens de droga

# Abstract

DNA profiling is an instrumental aid in the investigation of illegal activities. Nowadays, the explanation on how DNA evidence was deposited in the crime scene is particularly important in court. The quantity and quality of DNA traces are affected by the mechanisms: transfer, persistence, prevalence and recovery (DNA-TPPR). DNA traces can be transferred directly during the illegal activity or indirectly, where an intermediary has transferred DNA. The existence of high levels of background DNA on the surface may decrease the detection of transferred DNA. The persistence of DNA can be affected by environmental conditions the object is exposed to in that period of time, as well by the nature of the substrate of the object. Hands are a well-known vector when considering transfer of DNA. All these possible sources of DNA in a sample can produce a mixed profile when analysing the results. A large portion of the samples that come to the Norwegian forensic laboratories are drug wrappings to analyse and link it to a person of interest. We simulated drug wrappings in different scenarios, from direct transfer, indirect transfer from inside of a pre used bag and the packing of drug wrappings (one participant handled the tape, a second participant packed the drug wrap). We also determined de shedder status of the participants. It was observed a higher detection of DNA after direct transfer in comparison with the indirect transfer results. The DNA from the participant that packed the drug wrap was more frequently detected than the DNA from the participant that previously handled the tape. Based on a tube holding experiment we were able to categorise the participants as high, low and medium shedders. The majority of the participants was classified as medium shedders.

**Key words:** transfer, persistence, DNA, drug wrappings

# Table of contents

# Index of figures

# Index of tables

# Index of equations

# List of abbreviations

µl – microliter

A – adenine

AdjRFU – Adjusted RFU

Alleles C1 – alleles matching contributor 1

Alleles C2 – alleles matching contributor 2

bp – base pairs

C - cytosine

CCD – Charged coupled Device

CE – Capillary Electrophoresis

Chr – Chromosome

CODIS - Combined DNA Index System

Ct – Threshold cycle

DNA - Deoxyribonucleic acid

DNA-TPPR – DNA mechanisms: transfer, persistence, prevalence, recovery

DNAq – DNA quantity (ng/ul)

dNTPs – deoxynucleotide triphosphates

E – evidence

ENFSI - European Network of Forensic Science Institutes

ESS – European Standard Set

FBI - Federal Bureau of Investigation

Fwd – Forward

G - guanine

Hd – defence hypothesis

Hp – prosecution hypothesis

IPC - Internal PCR control

ISFG - International Society for Forensic Genetics

kb – kilobyte

kV- kilovolt

LOD – Limit of detection

LR – Likelihood Ratio

MGB - minor groove binder

Mix C1 – mixture proportion contributor 1

Mix C2 – mixture proportion contributor 2

Mix C3 – mixture proportion contributor 3

NCBI - The National Center for Biotechnology Information

NDIS - National DNA Index System

ng – nanogram

NOK – Norwegian krone

PCR - Polymerase Chain Reaction

POI - Person of interest

Pr – Probability

Q1 - quartile 1 (25% percentile)

Q2 - quartile 2 (50% percentile)

Q3 - quartile 3 (75\5 percentile)

RFLP - restriction fragment length polymorphism

RFU – Relative Fluorescence Unit

rpm – Rotations per minute

RT-qPCR – Real Time quantitative Polymerase Chain Reaction

Rvs – Reverse

SR – Stutter Ratio

SSM – Slipped Strand Mispairing

STR - Short Tandem Repeats

T – thymine

TaqMan - 5’ Nuclease Assay

Tm – Melting Temperature

U – unknown

UV – ultraviolet

VNTR - variable number of tandem repeats

ϕA - Area/height of the allelic peak

ϕS - Area/height of the stutter peak

# 1 Introduction

Currently, the activity level evaluation of the DNA evidence has a vast significance in court. In many cases questions are raised about how the DNA evidence was deposited; during the criminal activity, by direct or secondary transfer, or if does not relate do the illicit event.

Forensic scientists should provide an unbiased opinion regarding the activity level of the evidence, DNA evidence is evaluated according to different propositions, from the defence and prosecution.

Due to this, the study of the mechanisms transfer, and persistence of DNA is exceptionally important.

## 1.1 Basic Principles

The basic unit of life is the cell, which we have trillions of. Within the nucleus of our cells, we can encounter nuclear DNA, deoxyribonucleic acid. DNA contains the informational code for duplicating the cell and building the necessary proteins. Nuclear DNA is divided into chromosomes (longer DNA molecules, that store the information required for cellular development, proliferation and function), and protection proteins called histones. The human genome consists of 22 matched pairs of autosomal chromosomes and 2 sex determining chromosomes, males are termed XY and female XX [1, 2].

DNA is composed by nucleotide units that consist in three parts, a nucleobase or base, a five-carbon sugar and phosphate. The base inputs the variation in each nucleotide unit, while the phosphate and sugar parts form the structure of the DNA molecule. DNA uses four bases, purines, adenine (A) and guanosine (G) and the pyrimidines, cytosine (C) and thymine (T) [1, 3].

Each DNA molecule is composed of two strands of four different nucleotides, by hybridization. Individual nucleotides pair up with their complementary base, as followed, A hybridize to T and C hybridize to G. Directionality of the DNA sequence is provided by defining the five-prime (5') end and the three-prime (3') end. The two strands of the DNA molecule are anti-parallel, one is in the 5' to 3' orientation and the other in the 3' to 5' [1, 3].

The genome can be divided into different categories of DNA based on the structure and function of the sequence The DNA material in chromosomes is composed of coding and non-coding regions. The coding regions are known as genes, nucleotide sequences required to make a protein or RNA product. A gene can range from a few thousands to tens of thousands of base pairs in size. Genes consist of exons (protein coding portions) and introns (intervening sequences). Genes only make for approximately 5% of our chromosomal material [1, 4].

Individuals differ throughout the non-coding regions of the human genome, there are variable polymorphic markers between individuals. The position of a gene or DNA marker in a non-coding region is referred as locus or loci (plural). The possible variants for a locus are termed alleles, if the two alleles on homologous are different they are termed heterozygous, if the alleles are identical in a specific locus they are termed homozygous [1].

Most of the genome, approximately 75%, is extragenic. Around 20% of the genome is single copy DNA, which in most cases does not have any known function. The majority of the 75% corresponds to repetitive DNA (54%), where 45% of the repetitive DNA is interspersed and only 9% corresponds to tandemly repeated DNA, which can be divided into satellite DNA, microsatellite and minisatellite. Tandemly repeated DNA have major varieties of polymorphisms, which is helpful in forensic science, allowing to differentiate different people (Figure 1.1) [5, 6].

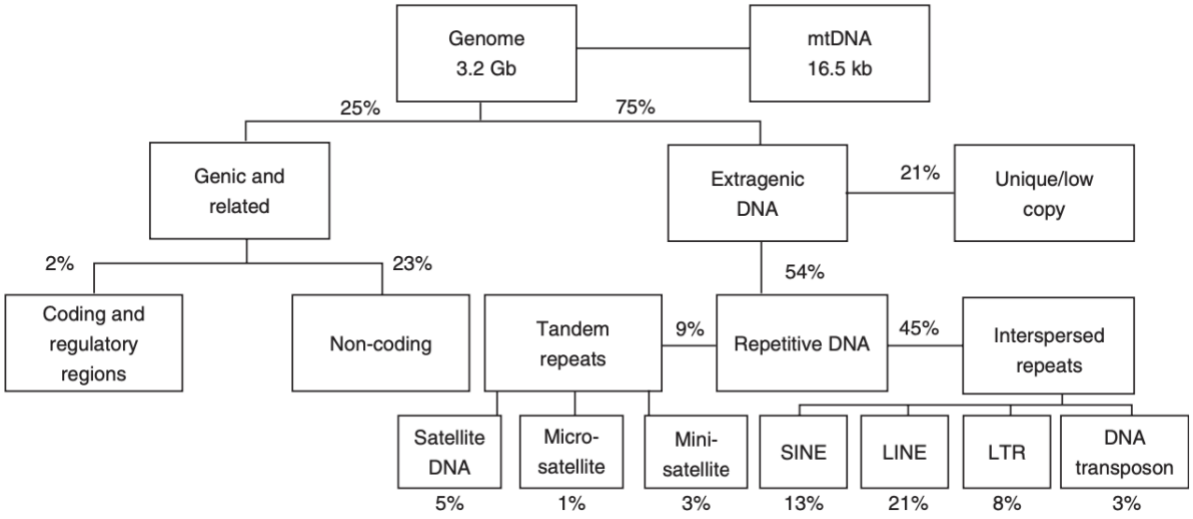


Figure 1.1: The human genome can be divided into different types of DNA based on its function and structure. From: [5]

## 1.2 DNA profiling

In the begin of the 20th century, Karl Landsteiner, gave the first step in forensic haemogenetics, by describing the ABO blood grouping. This allowed developments of molecular biology that enabled scientists to examine DNA sequences. In 1985, Dr. Alec Jeffreys showed the potential that DNA has in forensics by describing 'DNA profiling' or 'DNA typing' [5, 7].

DNA profiling is a technique employed by forensic geneticists to assist in the identification of individuals or samples by their respective DNA profiles, so it is the process of determining the genotype present at specific locations along the DNA molecule. A DNA profile is the combination of genotypes obtained for multiple loci [1, 8].

The first use of DNA profiling occurred in the 80's. Two young girls were sexually assaulted and then brutally murdered in 1983 and 1986. Both murders occurred in Leicestershire, England, with similar features, leading the police to suspect that the same man had committed both crimes. Under public pressure, police obtained a confession from a local man to killing one of the girls. His blood was compared to semen recovered from the crime scenes, but the DNA did not match DNA evidence from either crime. A mass screening to collect blood for DNA testing from all adult men in three local villages was conducted in an exhaustive search for the serial killer. More than 4000 men were tested, without a positive result. About a year later a woman at a bakery overheard Ian Kelly bragging about how he had given a blood sample for a friend named Colin Pitchfork. The police interviewed Pitchfork and collected a blood sample from him and found that his DNA profile matched semen from both murder scenes. He was subsequently convicted and sentenced to life in prison [7, 9].

The technique employed in the Pitchfork case was developed by Dr. Alec Jeffreys. The method examines the length variation of DNA repeat sequences, known as variable number of tandem repeats (VNTRs). The technique is called restriction fragment length polymorphism (RFLP), it involves the use of a restriction enzyme to cut the regions of DNA surrounding the VNTRs. RFLP has a high power of discrimination, however, requires a great amount of DNA, labor, time, and expertise to produce and interpret a DNA profile.

In the last decades the techniques have gained increased sensitivity and are easier to perform, which produces results with a higher level of confidence and faster. This improvement are due to the evolution of chemistry in the kits applied (e.g., PowerPlex® Fusion 6C System from Promega) and the increased sensitivity of the machines (e.g., Applied Biosystems® 3500xL Genetic Analyzer) [7].

So, we can say that DNA profiling detects and record polymorphisms in the genomes of individuals. This produces strong evidence of individual identity, evidence that is extremely valuable in paternity and forensic cases [10].

## 1.3 Short Tandem Repeats

The optimal DNA markers to be used for forensic genetics should have some key properties; highly polymorphic, easy and cheap to characterize, give profiles that are simple to interpret and easy to compare between laboratories, not be under any selective pressure and have a low mutation rate [5].

Short Tandem Repeats, STR, or microsatellites fit in the description previously mentioned.

STR consists of an array of tandemly repeated, normally identical, multibase motifs that vary in number. These microsatellites occur approximately once every 2 kb and are composed of 2-7 bp. STRs are common in all eukaryotic species and share the main characteristics of being abundant, short in length and highly polymorphic, so they provide an ideal means to identify [10-13].

STR are dispersed throughout the genome and typically occur every 10.000 nucleotides. Not only do they vary in the length of the repeat unit (Figure 1.2) but also STR differ in the consistency in which they conform to an incremental repeat pattern (Figure 1.3) [12].

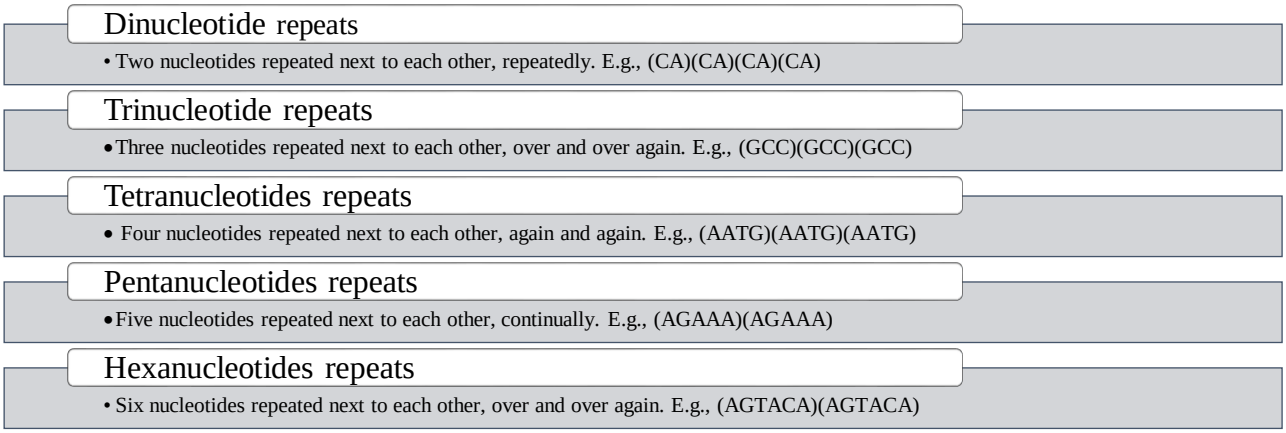


Figure 1.2: Type of STR markers. STR repeats sequences are titled by the length of the repeat unit. Adapted from: [12]

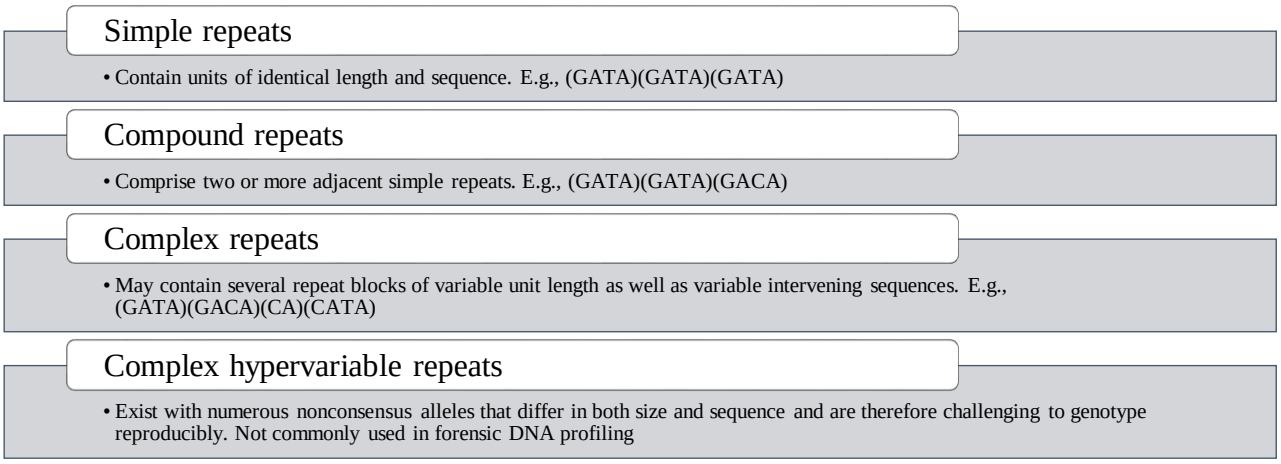


Figure 1.3: Repeat pattern of STR. Microsatellites are distributed into categories according to the repeat pattern. Adapted from: [12]

Due to all the characteristics of microsatellites, the STR markers have become one of the most important tools for human identity testing [14].

### 1.3.1 STR Loci

For DNA typing markers to be effective across a wide number of jurisdictions, it is necessary to have a common set of standardized markers. European forensic laboratories utilize many of the same STR loci as used in the United States of America [12].

The Combined DNA Index System (CODIS), began as an FBI pilot software project in 1990, serving 14 states and local laboratories. The DNA Identification Act of 1994 formalized the FBI's authority to establish a National DNA Index System, NDIS. NDIS contains the DNA profiles contributed by federal, state, and local participating forensic laboratories, it was implemented in October 1998 [15].

The European Network of Forensic Science Institutes (ENFSI) was founded in 1995 with the purpose of improving the mutual exchange of information in the field of forensic science, and one of the main purpose is to facilitate comparisons of DNA profiles between laboratories [16].

The STR loci used by forensic laboratories should have some characteristics, such as: no known association with medical conditions or defects, low mutation rate (preferably less than 0,30%), high level of independence and high level of discrimination [17].

Common sets of STR marker are required for entry of DNA genotype into national or international databases used to link serial crimes and offenders, these are normally called core loci, and permit equivalent genetic information to be shared and compared (Table 1.1) [18].

Table 1.1: Core STR Used in Human Identity Testing. Adapted from: [16]

| *Country* | *Loci* |
|---|---|
| *U.S. Core Loci* | 13 Core loci: CSF1PO, FGA, THO1, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11 and Amelogenin<br><br>7 extra loci (since 2017): D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, D22S1045 |
| *European Standard Set (ESS)* | Core loci: FGA, TH01, VWA, D1S1656, D2S441, D3S1358, D8S1179, D10S1248, D18S51, D21S11, D22S1045<br><br>Additional Loci (part of European STR Kits): D2S1338, D16S539, D19S433, SE33 and Amelogenin |

### 1.3.2 STR Genotyping

Forensic samples are known for their challenges, where the quality and quantity are the major issues of these samples [19].

In forensic casework the samples are always susceptible to analytical challenges due to their nature, the substrate that they were deposited on, the probability of contamination, the possibility of mixture with PCR inhibitors and exposure to environmental conditions. All of which can lead to the decrease of the quantity and quality of the DNA and will affect the analysis and the end result [19].

*Technical artefact peaks and analytical challenges*

Current forensic DNA analysis uses PCR of STR loci as the standard technique, due to is sensitivity, allowing the analysis of small and degraded samples. During the amplification of the STR loci the formation of a nonallelic product or an anomaly of the detection process can occur, these are called STR artifacts [20, 21].

#### I. Allelic Dropout

Allelic dropout occurs during amplification by PCR, when a microsatellite locus is not replicated by the DNA polymerase, making heterozygote alleles appear as homozygotes at the affected loci or even no alleles are detected [14].

This can result from various factors: primer binding site mutations, stochastic effects when small amounts of DNA are present in the sample, DNA degradation or even due to technical imperfections of analysis methods [12, 14, 22].

#### II. Stutter

Stutter products corresponds to additional peaks that appear in the electropherogram of an STR sample. This occurs when STRs are being amplified, due to a slipped strand mispairing (SSM), resulting in the insertion or deletion of one repeat unit on the new strand [14, 20, 23].

A back stutter corresponds to a smaller peak one repeat unit shorter than the corresponding true allele peak. A forward stutter corresponds to a smaller peak with an insertion of one repeat unit. There exists a process to evaluate if a peak corresponds to a stutter, by using stutter ratio (SR) (Equation 1.1) [20, 23, 24].

Equation 1.1

$$SR = \frac{\phi S}{\phi A}$$

ϕS: area/height of the stutter peak

ϕA: area/height of the allelic peak

This type of artifact is usually less than 15% the size of the major allele. There is a correlation between stutter size and the allele length, with the stutter ratio increasing and with the increase of the repeat number (dinucleotides > trinucleotides > tetranucleotides > pentanucleotides) [20, 23, 25].

### III. Incomplete 3' nucleotide addition

An incomplete 3' nucleotide addition occurs when the Taq DNA polymerase, used in the amplification of the STR loci, catalyses the addition of an extra nucleotide, most often an "A", adenine. This happens on the 3' end of the double-stranded PCR products. When an incomplete 3' nucleotide addition takes place the result is split peaks, the allele of interest will be represent by two peaks, 1 base pair apart, this is referred as N+1 peaks [23, 25].

### IV. Tri-Allele Patterns

Triallelic patterns are observed when an extra peak is detected as three peaks in a single genome, as opposed to the expected homozygous or heterozygous peak. This results from extra chromosomal fragments being present in a sample or the DNA sequence where the primers anneal being duplicated on one of the chromosomes [23, 25].

There exist two types of tri-alleles patterns. Type I happens when the three peaks have balanced peak heights and type II occurs when the sum of the heights of two of the peaks is equal to the height of the third allele [23, 25, 26].

### V. Pull-up

Pull-up occurs because of the inability of the detection instrument to properly resolve the dye colours used to label STR amplicons, meaning that a peak of another colour is pulled up as a result of exceeding the linear range of detection for the instrument. Pull-ups are most frequently observed from high amounts of DNA in the sample. This artifact is visualized by the presence of false bands in the excessively amplified fragment size range in differently coloured markers [14, 25].

### VI. Dye blobs

Left-over fluorescent dye molecules, that are not removed by post-synthesis purification can be carried through the PCR amplification step and injected onto the capillary, producing dye blobs in the electropherograms. This is created due to the incomplete attachment of the fluorescent dye, at 5' end of the primer, during oligonucleotide primers synthesis, that happens from a 3'-5' direction [23, 25].

### VII. Drop-in

Drop-in occur from random fragments of DNA present in the PCR, creating additional alleles. This type of artifact is usually not reproducible upon repeated amplification [27, 28].

### VIII. Contamination

DNA samples can be contaminated, unintentionally, with exogenous DNA causing mixtures, but also the samples can be contaminated with non-DNA contaminants. These contaminants that fluoresce in the visible region of the spectrum (~500 nm), can interfere with DNA typing when using fluorescence detection capillary electrophoresis by appearing as identifiable peaks in electropherogram [25].

## IX. Degradation

Many samples in forensic casework may have been exposed to chemicals or non-optimal environmental conditions for a period. This can cause the degradation of the DNA. The environmental conditions, e.g., humidity, extreme heat, and the presence of bacteria that speed up the degradation process. This will result in the breakdown of the DNA molecule into smaller fragments. The effect of the degradation can result in amplification failure, resulting in a partial profile or be severe enough that nothing is amplified in PCR, due to the loss of the alleles in the locus of the forensic markers.[5, 14].

## X. Inhibition

When performing DNA extraction, if this procedure does not remove certain chemicals, inhibition can occur in the amplification phase. These chemicals can interfere with the capacity of the DNA polymerase enzyme to make new DNA, either by interfering with the enzyme or by binding to the DNA. Known inhibitors are organic components of soil, a component of red blood cell, heme, humic acids, indigo dyes, and denim fabric [5, 14].

### *Mixtures*

When analysing DNA profiles, sometimes we obtain biological material from more than one contributor, which implies that there will be multiple genotypes mingled in the sample [24, 25].

The interpretation of DNA mixtures is an intricate problem and, unfortunately, it is very common for them to appear in the field of forensic genetics.

The Clayton guideline was the first protocol established to evaluate a mixture, in 1998 by Tim Clayton. This guideline consists of a series of steps (Figure 1.4) [29]:

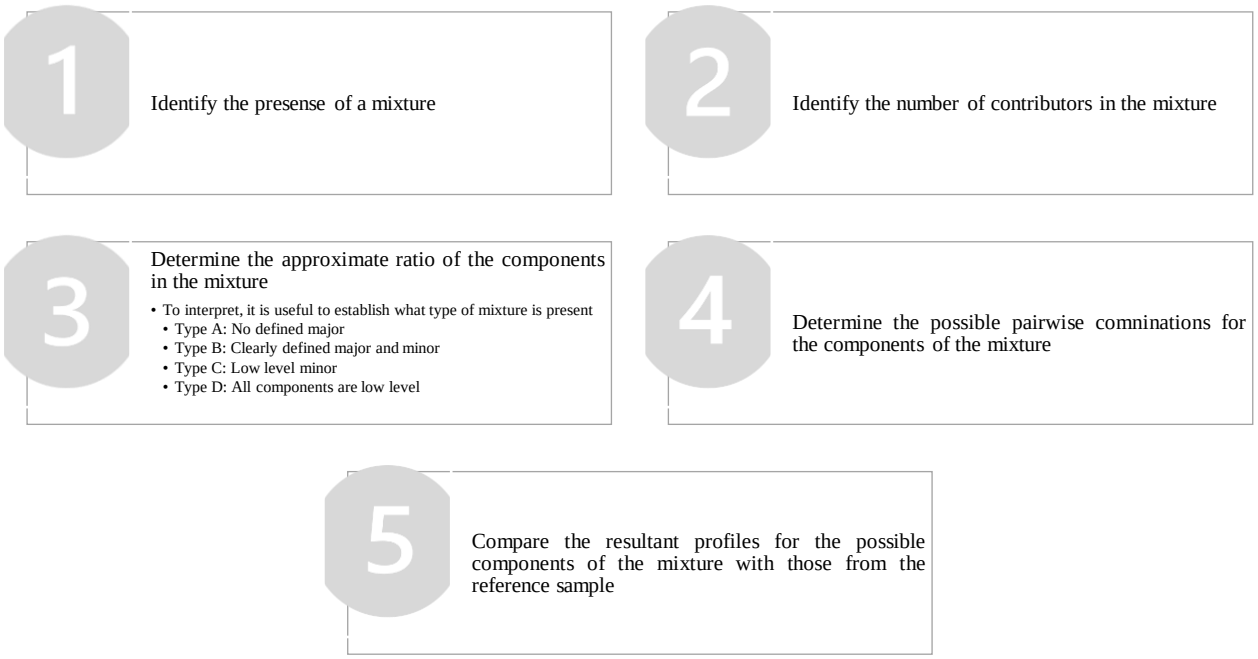


Figure 1.4: Clayton Guideline. Interpretation of a mixture according to Clayton guideline follows 5 steps and will help to evaluate the presence of a mixture in a DNA profile. Adapted from: [29]

According to the International Society for Forensic Genetics (ISFG), the value of DNA and biological results should be given by assigning a likelihood ratio. This implies the formulation of two mutually exclusive propositions, prosecution, and alternate defence, they should be formulated according to the circumstances of the case [30].

Likelihood ratio (LR) is a measure of weight of evidence, that compares evidence given propositions and gives an indication of which one is most probably (Equation 1.2).

How LR works in forensic genetics is given an observed data, the evidence (DNA results), two hypotheses are formulated [24]:

- Pr(E|Hp) - the probability of the evidence if the trace sample originated from the suspect (prosecution hypothesis)
- Pr(E|Hd) - the probability of the evidence if the trace sample originated from an unknown individual, not related to the suspect (defence hypothesis)

Equation 1.2

$$LR = \frac{Pr(E|Hp)}{Pr(E|Hd)}$$

LR evaluates how many times more likely it is to observe the evidence, given that Hp is true in comparison to the alternative Hd is true [24].

If the LR=1this means that the data support neither of the hypothesis; if LR<1 this means that the data supports Hd; and if LR>1 this LR supports Hp. In the case of a full DNA profile matching the suspect, the probability of the evidence given Hp=1. The probability of the defence hypothesis can be calculated from the genotype frequency of the particular DNA profile.

An example of the likelihood ratio for STR D13S317 alleles 11 and 14 is calculated in the Equation 1.3. Where p is the allele frequency of 11 (0.3394) and q the allele frequency of 14 (0.04801). In this example the STR typing result is heterozygous. The allele frequencies were calculated based on the U.S Caucasian allele frequencies [31, 32].

Equation 1.3

$$LR = \frac{Pr(E|Hp)}{Pr(E|Hd)} = \frac{Hp}{Hd} = \frac{1}{2pq} = \frac{1}{(2(0.33940)(0.04801)} = \frac{1}{0.03259} = 30.7$$

In the example above the LR for STR D13S317 is 30.7. Because the LR value is higher than 1, it provides support to the prosecution hypothesis [31].

For more complex profiles as mixtures or partial profiles a model that can account for artefacts and peak heights is necessary. Several statistical software for forensic samples has been developed, like EuroForMix [33, 34].

## EuroForMix

In mixture interpretation it is helpful to use a software due to the challenges that STR DNA profile analyses presents. Very often trace samples are complicated to analyze due to the presence of artifacts (stutters) and the presence of a mixture of various contributors.

EuroForMix was launched in 2015 and is a freely open-source continuous model, different amount of DNA (RFU – peak heights) deposited by the different contributors can be used to separate contributions. EuroForMix is included in the R-package euroformix and is accessible at the site www.euroformix.com [33]. It is a quantitative model, which means the contribution from each individual (peak heights) also are considered in the LR calculations. The program can account for artefacts as stutters, drop in and degradation (Figure 1.5) [33].

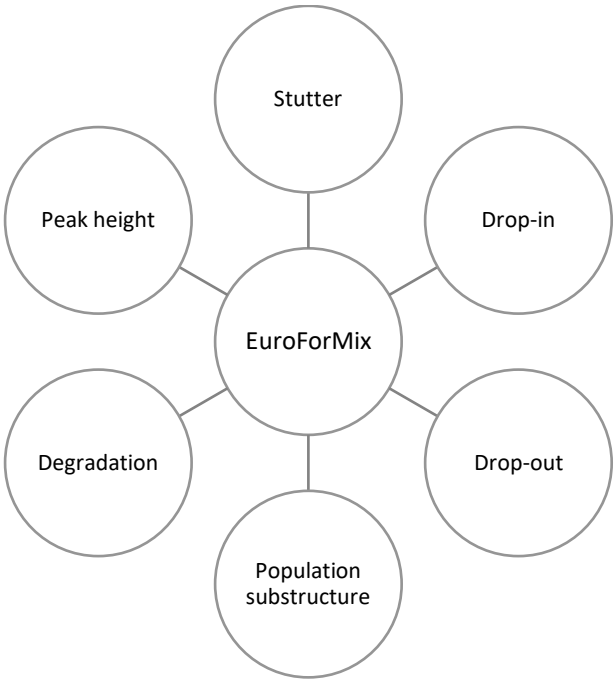


Figure 1.5: EuroForMix program. EuroForMix accommodates peak heights, drop-in, drop-out, stutter, degradation and population substructure on the continuous model. EuroForMix assumes peak heights to be gamma distributed with mixture proportions, stutter proportion and peak height proportion mean and variation as unknown parameters. Also includes models for allele drop-in, degradation and sub-population structure. Adapted from: [33]

To quantify the weight-of-evidence that a specific candidate is a contributor to the evidence is based on the following Hp and Hd:

- Hp: The DNA is from A
- Hd: The DNA is from an unknown individual

In this method, the fit between the hypothesis and the trace sample are quantified by the corresponding likelihood functions, and the likelihood functions are compared through the LR statistic.

### 1.3.3 Methodology

*Chelex*

To isolate and purify DNA it is necessary to perform a DNA extraction. To do this we need to consider that the sample contains different substances besides DNA, and that proteins that pack and protect DNA in the environment of the cell, can inhibit the analysis of the DNA, such as PCR inhibitors. Care must be taken to avoid any further DNA degradation [35-37].

Consequently, the purposes of the DNA extraction are to lyse cells to release the DNA molecules; separate the DNA molecules from other cellular material and finally to isolate DNA, removing degrading enzymes. The methods for DNA extraction can be divided into three different groups, organic, solid-phase DNA, and ionic chelating resins. We used the last one, more specifically Chelex [35-37].

Chelex is a DNA extraction method, that denatures double-stranded DNA and is frequently used in the forensic community due to the simplicity, low-priced and absence of harmful reagents of the method. This method is quick, allowing less opportunities for contamination of the sample. It is also criticized because several PCR inhibitors are not removed [14, 35, 36].

The Chelex procedure (Figure 1.6) is a combination of the heating steps and the Chelex beads. Chelex is a resin constituted of styrene divinylbenzene copolymers containing paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions such as magnesium (Mg2+). The metal ions are drawn in and bound by the resin. By chelation of the metal ions, DNA-destroying nuclease enzymes are inactivated, and the DNA molecules are protected minimizing cleavage [14, 35-37].

Chelex beads in suspension are added to the samples, and after incubation, the DNA is released from the cells by heating. The alkalinity of the solution and the high temperature cause the cell membranes to rupture, freeing the DNA [14].

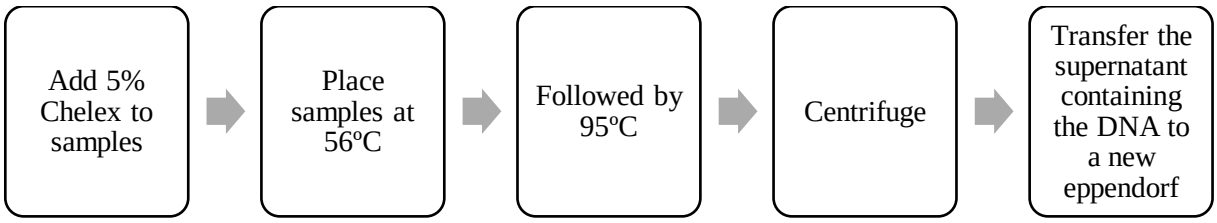


Figure 1.6: Flowchart of Chelex extraction. In most protocols, biological samples are added to a 5% Chelex suspension and boiled for several minutes to break open the cells and release the DNA. The exposure to 56ºC for 30 minutes and 95°C for 8 minutes denatures the DNA as well as disrupting the cell membranes and destroying the cell proteins. Adapted from: [14, 35-37]

## *RT-qPCR*

Determination of the quantity of DNA in a sample is essential for most PCR based analyses, as the main goal is to determine the amount of amplifiable DNA. A narrow concentration works best with multiplex STR, nowadays 0,5-2ng is optimal with commercial STR kits. Too much DNA results in exaggerated electropherograms, making interpretation more difficult, too little DNA can result in loss of alleles due to stochastic amplification [38].

Real-time quantitative Polymerase Chain Reaction (RT-qPCR) is a method for providing the amount of a target sequence that is present in a sample. RT-qPCR allows the monitoring of the progress of the PCR as it occurs, meaning that reactions are characterized by the point in time during cycling when amplification of a target is first detected [39-41].

RT-qPCR can also be called kinetic analysis, since it analyses the cycle-to-cycle change in fluorescence signal that results from amplification of a target sequence during PCR [42].

There are multiple approaches to performing RT-qPCR, the most common are the 5' Nuclease Assay. The 5' Nuclease Assay, also known as TaqMan, uses a fluorogenic probe to allow the detection of an amplicon as it accumulates during PCR cycles. In the 5' nuclease assay, the TaqMan probe, an oligonucleotide, is added to the PCR reagent master mix, the TaqMan is intended to anneal to a specific sequence of template between the forward and reverse primers [40, 42].

The 5' Nuclease Assay occurs in three phases (Figure 1.7). In the first stage of the RT-qPCR reaction, the temperature is increased to denature the double-stranded DNA. In this phase, the signal from the fluorescent dye on the 5′ end of the TaqMan probe is quenched by the minor groove binder (MGB), nonfluorescent quencher on the 3′ end of the probe (Figure 1.7 b). In the second phase, the reaction temperature is diminished permitting the primers and probe to anneal to their precise target sequences (Figure 1.7 b). Finally, Taq polymerase synthesizes a complementary DNA strand using the unlabeled primers and template. When the polymerase reaches the TaqMan probe, the 5′ exonuclease activity cleaves the probe, separating the dye from the quencher (Figure 1.7 c). With every cycle of PCR, additional dye molecules are released, consequently a rise in fluorescence intensity proportional to the quantity of amplicon synthesized occurs [43].

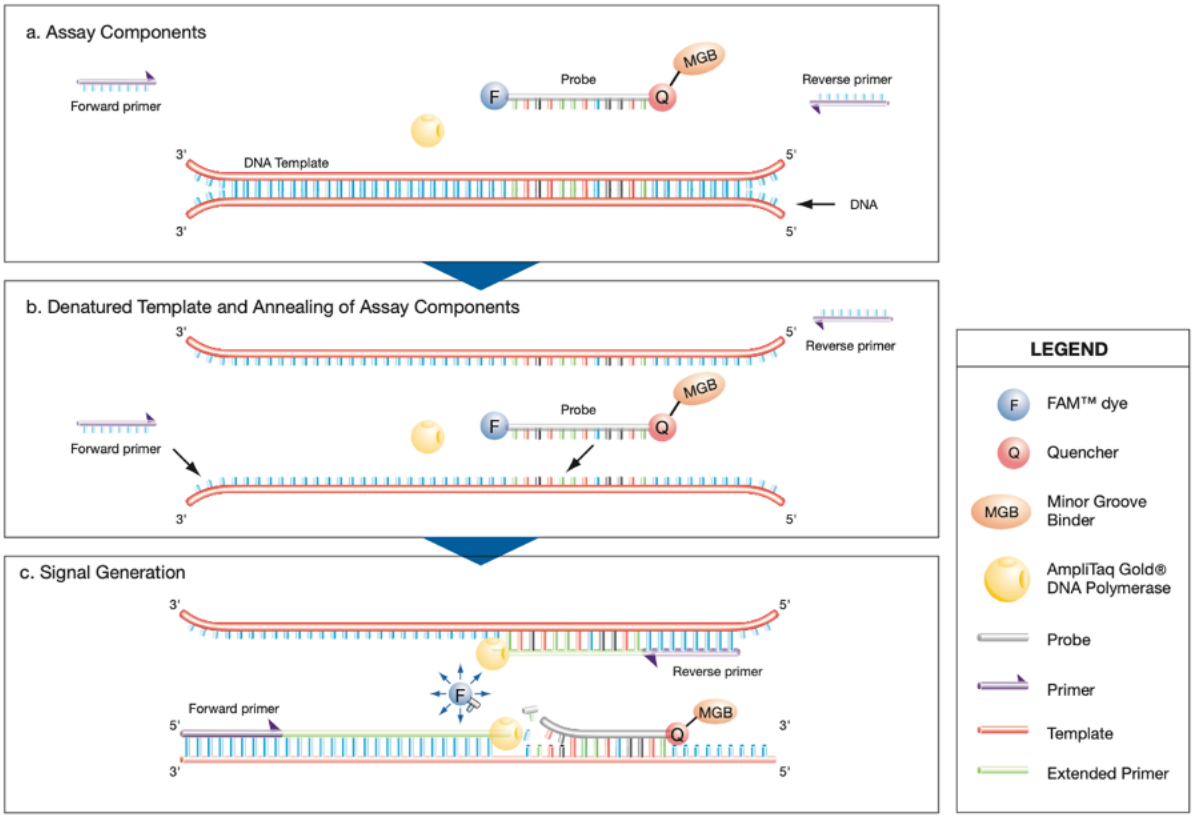


Figure 1.7: TaqMan Gene Expression Assay. The 5' Nuclease Assay occurs in three phases: a. Assay components; b. Denatured Template and Annealing of Assay Components – where the temperature is raised allowing the denature of the DNA, followed by the decrease of the temperature that will allow the anneal of the primers and probe to the target sequences; c. Signal Generation - in this phase occurs the separation of the dye from the quencher. Adapted from: [43]

RT-qPCR determines the quantity of amplifiable DNA segments that are present in a sample, using a standard curve. The goal of this method is to monitor the quantity of accumulated amplified product at every cycle by detecting the signal emitted by a fluorescent reporter dye [19].

The cycle threshold (CT) value is the cycle at which the fluorescent signal crossed the threshold at the beginning of the exponential phase of the amplification curve (Figure 1.8 (B)), in this phase the amount of product and the input DNA is more likely to be consistent [19, 37]. The cycle threshold is an arbitrary value that is based on the variability of the baseline data at the initial cycles of amplification. The lower the CT value, the higher the amount of DNA present in the sample. A linear relationship between the CT values and the quantity of DNA is observed. The well-to-well variation in the signal strength is reduced by normalizing the fluorescence of the reporter dye, Rn, to a passive reference dye present in each well [19, 38].

For each run a set of DNA standards, purified DNA with known concertation, is included. With this a standard curve is generated by plotting the CT values against the log concentration of DNA. The concentration of DNA present in the sample is computed by measuring the CT value and comparing it with the standard curve (Figure 1.9) [19].

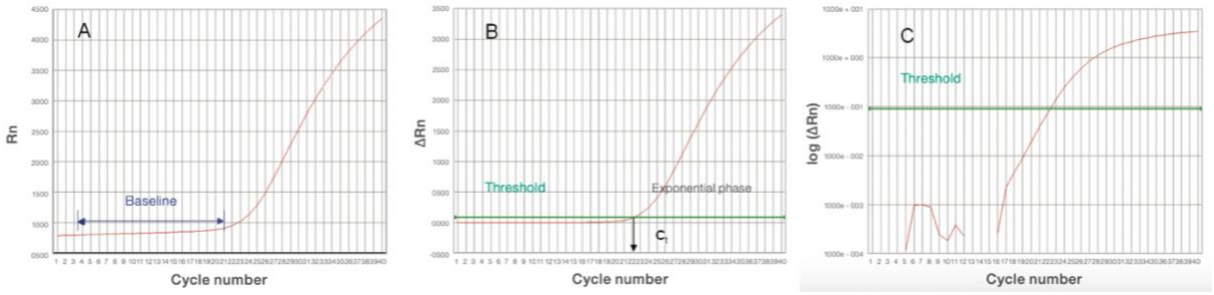


Figure 1.8: Graphical representation of real-time PCR data. Rn is the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye; Rn is the reporter signal normalized to the fluorescence signal of the reporter dye. (A) Rn is plotted against PCR cycle number ($C_T$); (B) ΔRn is Rn minus the baseline; ΔRn is plotted against PCR cycle number; (C) An amplification plot shows the variation of log (ΔRn) with PCR cycle number. Adapted from: [39]

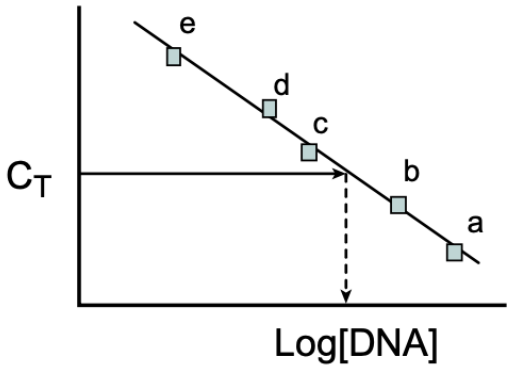


Figure 1.9: Standard curve, generated by plotting the $C_T$ values against the log concentration of DNA. Using this standard curve, concentrations of DNA of unknown samples can be deduced based on where they fall along the standard curve. Adapted from: [38]

## *PCR*

Forensic samples are known to be difficult to examine as they often consist of low amounts of DNA and can be degraded. Without the ability to make more copies of DNA, these samples would be impossible to analyze [44].

Polymerase chain reaction, PCR, is an enzymatic process in which a specific region of DNA is replicated multiple times. PCR allowed the increasing of the sensitivity of STR typing [11, 45].

During a PCR reaction, a solution is set up (Figure 1.10 (a)) that includes DNA template, a set of forward and reverse primers, DNA polymerase, dNTPs, and a buffer. During the denaturation step (Figure 1.10 (b)), the reaction is brought up to 95 °C at which temperature the DNA melts. Allowing the annealing step (Figure 1.10 (c)) to occur when the temperature is dropped so that the primers can anneal to the complementary target sequences on the DNA. During the extension step (Figure 1.10 (d)), the temperature is raised to 72 °C permitting the DNA polymerases to synthesize a new complementary DNA strand at the locations of the annealed primers using the dNTPs. This describes one cycle of PCR and will be repeated 20–40 times (Figure 1.10 (e)). During the second cycle, both the DNA template and the partial DNA template synthesized from the previous cycle will be bound by primers and extended. The primers extended off the partial DNA template generating a sequence that begins at the location of the forward primer and ends at the location of the reverse primer. This complete sequence will describe the final product, which will be created for the first time after three complete cycles. From

there, the final product will be amplified exponentially and outcompete the DNA template and partial DNA templates for dNTPs, creating a solution composed mostly of amplified product [44, 46].

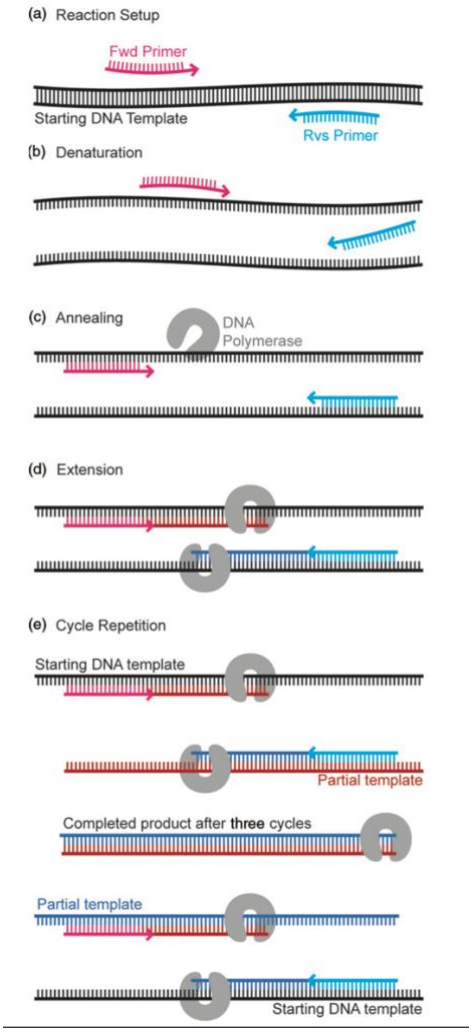


Figure 1.10: PCR process. PCR occurs in a thermal cycling typically that involves 3 different temperatures that are repeated over and over again, 25 to 35 times. At 95°C, the DNA strands separate, or 'denature'. At 60°C, primers bind or 'anneal' to the DNA template and target the region to be amplified. At 72°C, the DNA polymerase extends the primers by copying the target region using the deoxynucleotide triphosphate building blocks. Each cycle on the PCR takes 5 minutes on conventional thermal cyclers: 1 minute each at 94°C, 60°C, and 72°C and about 2 minutes ramping between the 3 temperatures. This process allows the amplification of the DNA Adapted from: [46]

Multiplex PCR refers to the simultaneous amplification of two or more regions of DNA. Multiplex is composed of different length of amplicons and fluorescent colours so that several markers can be runed and separated simultaneously [45].

*Capillary Electrophoresis*

Fragment analysis is done by separating the DNA by size, applying capillary electrophoresis (CE). Currently, capillary electrophoresis is the principal methodology used for separating and detecting STRs in forensic laboratories.

In the CE, DNA separations occurs in thin – diameter 50μm – fused silica capillaries filled with a sieving polymer. These capillaries have the benefit of great capacity to dissipate heat, allowing high electric field strengths, which permits quick and efficient separations. Detection of the sample is performed via fluorescence, measuring the time span from sample injection to sample detection, a laser passes through a window, burned into the capillary [47-49].

When preparing the sample for injection it is necessary to use formamide in the dilution of the sample, this will allow the DNA molecules to denature and reduce the ionic strength of the PCR mixture; afterwards the sample is heated at 95ºC and then placed in an ice bath to generate denatured fragments prepared for electrophoresis [47-49].

Subsequently, the injection of the sample occurs. During this process the labelled DNA fragments of different size enter the capillary as a result of an electrokinetic injection where a high voltage, typically 3-15 kV, is applied across the capillary varying from 1 to 24 seconds. A laser excites a fluorophore, dye, that is connected to the end of the DNA fragment, then the fluorophore emits a light at a lower energy. The DNA fragments are separated by size based on their total charge; short DNA fragments migrate faster than the long fragments [47-51].

To accurately determine the size of the DNA fragments in the sample a size standard containing fragments of known size is added to ach samples. The size of the fragments is determined through a comparison of migration speeds during CE. The fluorescent dye labelling enables the analyze of multiple independent markers, loci, in the same injection by using different dye colors allowing distinction between markers [50].

Before reaching the positive electrode, the fluorescently labelled DNA fragments, move across the path of a laser beam. This beam causes the dyes attached to the fragments to fluoresce. The dye signals are separated by a diffraction system and are projected onto a CCD (charged coupled device) camera in a predictably spaced pattern. By using several dyes, we can include fragments of similar lengths for different markers. Each dye emits light at different wavelength when excited by a laser. This signal is then converted into an electronical signal producing the results in peaks that are measured in relative fluorescence unites (RFUs) and are seen in a capillary electropherogram. RFU will give the strength of the DNA itself. Genotyping occurs in a software provided by the instrument manufacturer [47-50].

The run conditions in the capillary electrophoresis can affect the electrophoretic mobility of the sample. The conditions of the run are the type of buffer, concentration, pH, temperature, the quantity of voltage applied, and the type of polymer used [50].

This technique presents diverse advantages, being the primary ones: the steps injection, separation and detection can be entirely automated, which allows multiple samples to be run simultaneous; CE only requires minor amounts of sample, leaving enough sample for a retest; the higher voltages of this technique allow a quick separation. [47, 48, 52, 53].

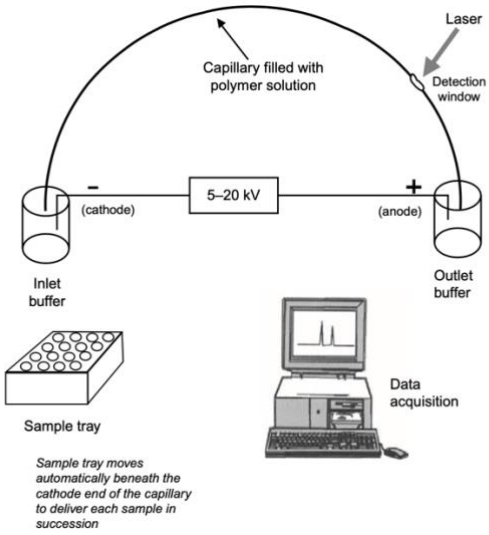


Figure 1.11: Schematic of capillary electrophoresis instruments. These instruments are used in the separation of the DNA by size, by means of the capillary electrophoresis. Adapted from: [47, 48]

## 1.4 DNA Evidence

Nowadays, with all the advances that are constantly happening in forensic genetics, DNA profiling is an invaluable tool in the investigations of illegal activities. A DNA profile can link a specific person to a sample retrieved from the crime scene.

According to Gill P., "Trace DNA is defined as any sample where there is uncertainty that it may be associated with the crime event itself-so that it is possible that the transfer may have occurred before the crime event (innocent transfer) or after the crime event (investigator mediated)" [54].

DNA evidence should be evaluated at different levels. These levels provide a hierarchy where the probative value of the evidence increases with the escalation of the levels. This grading of propositions differentiates between the sub-source level, the source level, the activity level, and the offense level [54, 55]:

1. Sub-source level: relates to the donor of DNA.
2. Source level: hypothesis are built about the cellular origin of the DNA and the association with the DNA donor.
3. Activity level: relates to how or when a trace was deposited.
4. Offense level: questions concern if a crime has been committed and who is guilty.

More often, it is not the source of 'Trace DNA' found at a crime scene that is disputed, but the activity or timing of events that resulted in their transfer. Because it has been demonstrated that detection of a DNA profile upon a surface is not always due to direct deposition [55-57].

Almost a century ago, Locard raised a notion about 'Trace DNA'. He stated that during a criminal act, an offender will both leave trace evidence at a scene and take it away on their person or clothes [57].

It is important to consider the DNA mechanisms transfer, persistence, prevalence, and recovery (DNA-TPPR). DNA-TPPR affect the quantity and quality of DNA traces [57, 58].

### 1.4.1 DNA mechanism: Transfer

Transfer mechanisms relate to the routes by which DNA may be transferred to a crime scene. There are three different mechanisms whereby DNA may be transferred, primary or direct, secondary or indirect and aerosol. Direct or primary transfer is where DNA is transferred directly from a person to an object or to another person, and indirect or secondary transfer is where an intermediary has transferred DNA, either from an object, or from another person (Figure 1.12). Aerosol transfer is achieved without an intermediary to a person, a surface, or an object [54, 57, 59].

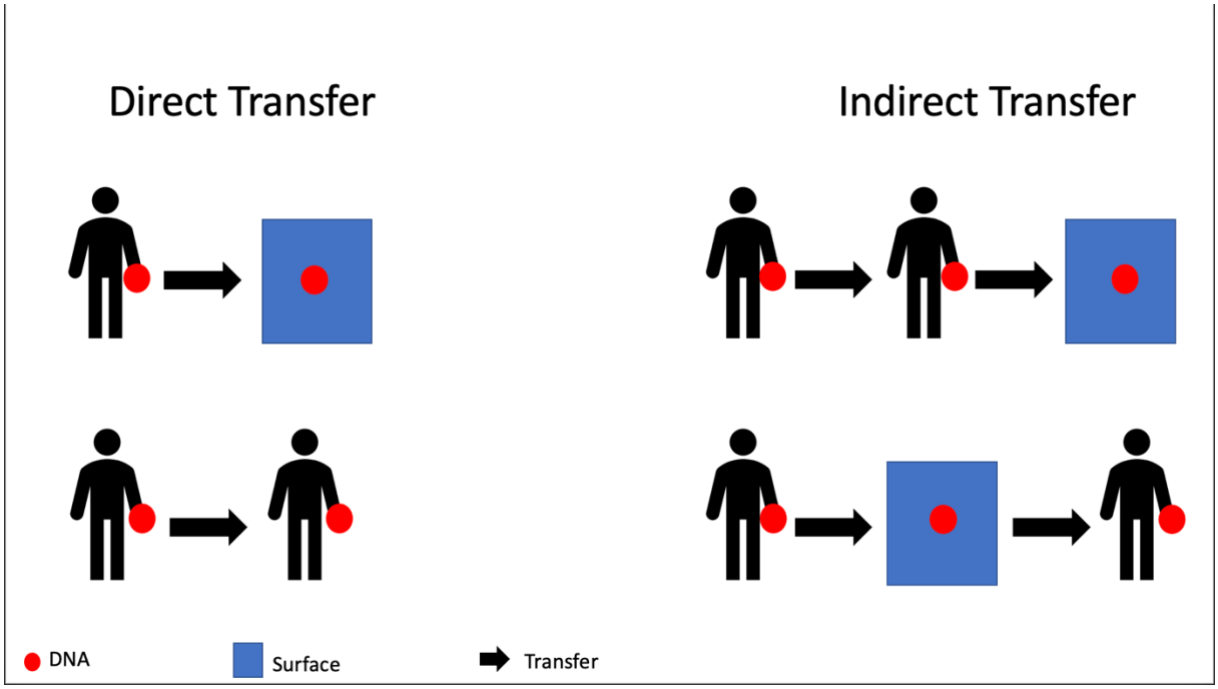


Figure 1.12: Different routes by which DNA may be transferred to a crime scene, Direct transfer from an individual to an object or person and indirect transfer where an intermediate person or surface is involved.

The way of transfer by which DNA came into the crime scene has great consequences on the interpretation of the DNA profiling results and the way they are considered in light of questioned activities [58].

Regarding transfer of DNA, it is important to consider the pre-existence of 'Background DNA'. Background DNA refers to DNA that is not related to the illegal action, however it is present at a crime scene prior to the crime. This type of DNA can originate from known and unknown individuals and can be disseminated either by direct or indirect transfer. It is important to consider Background DNA because increased levels of this type of DNA on the surface may decrease the chance of the detection of any transferred DNA. Or be mistaken as relevant to the crime. Background DNA is unavoidable and should always be considered [54, 57, 59].

Some important factors need to be considered regarding the transfer of DNA. The type of substrate of the object, the nature of the biological material, the manner of contact between the contacting surfaces and shedder status of the involved individuals [57].

The type of substrate of the object is a very important factor. If a substrate is porous (e.g., cotton) or non-porous (e.g., plastic) affects the transfer of DNA. Less DNA will be transferred from a porous primary substrate than a non-porous primary substrate, while a porous secondary substrate will facilitate transfer from the primary substrate [57, 60, 61].

The source of the biological material is also significant and affects the quality and quantity of DNA. Wet or liquid biological material are transferred more easily than dried biological material. Blood, saliva, and semen are easily transferred, especially if stains are fresh. DNA deposits of skin cells, transfer at a different rate than liquid biological material. Unlike the liquid biological material, drying has a small impact on transfer of skin cells but the quantity of DNA is not always comparable to liquid DNA. Skin cells are not a good source of DNA, the deposition of self-cellular components is affected by shedder status (further explained in Section 1.5) [57, 62].

The manner of contact is an important factor, especially considering the type of substrate and the moisture content of the biological material. Goray et al. [60] described three different types of contact, passive, pressure, and friction. Passive contact was defined as a placing two substrates together for one minute, pressure was described as placing two substrates together for one minute with an addiction of one kilogram of weight, and lastly friction explained as placing two substrates together with one kilogram of weight and moving the weight around for one minute. If pressure and friction are applied on the biological material, the amount of DNA transferred increases, considering that the primary substrate is non-porous [57, 60, 61].

### 1.4.2 DNA mechanism: Persistence

The persistence of biological material is affected by a range of environmental factors that can speed up the degradation progress, such as: temperature, UV exposure, humidity, wind, and presence of microorganisms on the surface. These environmental factors affect the quality and quantity of the DNA trace [57].

The type of substrate of the object is also an important factor to consider regarding the persistence of trace DNA. For instance, more DNA will be removed by contact with a sleek surface (e.g., glass) than by contact with a porous surface (e.g., cotton). Additionally, we need to consider that the persistence of DNA can be affected by the use of the object by a second person. Different studies have demonstrated that DNA on a personal object derived from the original user can persist after the use of the item by a second individual. Nonetheless, it is important to consider the possibility of non-personal object (object shared by multiple people, e.g., office supplies), because this type of object is handled my multiple people, the expected result of an analysis is a mixed DNA profile [57, 63].

Former studies have indicated that DNA from a person that previously handled an object can be detected for a long period of time after the object has been handed to a second user. Nonetheless, it is expected that DNA from the new user of the object will be detected and that the new users DNA will become the major contributor over time [57]. van Oorschot et al. [63] observed an average 50% drop of the DNA from the previous user immediately after handling by a new user when the surface was hard and non-porous.

All these factors are influenced by the extent of time that that trace DNA is on the object [63].

### 1.4.3 DNA mechanism: Prevalence

As referred previously, 'Background DNA' can affect the detection of any transferred DNA, which means that, correspondingly, affects the prevalence of DNA. This type of DNA can be derived from one person or personal objects and from multiple people, or shared objects. For instance, we expect to find DNA from regular occupants of an office in samples collected from different surfaces at that office. When several persons occupy an area or touch a surface this may create a mixed profile when analysing the results [57].

Background cellular material may be found on any type of public or private item. We need to consider that the strongest profile obtained from the analysis is not always that of the person who last held the object but is dependent on the individual shedder capacities [64, 65].

### 1.4.4 DNA mechanism: Recovery

Different methods can be used to collect DNA from identical objects, different laboratories use distinctive techniques. Efficient sampling is crucial for optimal DNA analysis [57, 66].

To collect DNA from a sample there is a variety of methodologies, from collection devices to direct collection from the substrate the sample is on. These different methods have different ranges of efficiency. The principal methodologies for recovery are swabbing and tape recovery [57].

Swabs are an important device for collecting DNA. There exist different techniques to collect sample with swabs, dry swab, wet swab, and double swab, where first swabbing is performed with a wet swab and then a dry swab. Double swabbing is the most common method for optimal DNA sampling [66].

Cotton swabs have been used for a long time, but alternative swabs have appeared. Foam swabs are better for absorbing surfaces as wood, due to their large swab size. Due to the ability of the foam swab to retain the wetting agent during sampling and possibly the open structure of this type of swab. Nylon flocked swab are reported to have an increased DNA recovery when sampling pure DNA, however other studies have reported no improvements collecting pure DNA with this type of swab. Cotton swabs continue to be the preference in a numerous of surfaces, smoot/non absorbing surfaces as well when sampling pure DNA [66, 67].

Another recovery method is the tape. This method is effective for recovering cellular material from clothing. The tape is pressed over the area in the fabric, where cellular material may be, and then is placed directly into the extraction tube [68].

Is important to have in mind what is going to be sampled, targeting (sampling the correct area) is extremely important, and it allow us to gain the best profile in the end. Poor targeting can affect the quality of results obtained [57].

## 1.5 Shedder Status

There is a variation in the quantity of DNA that individuals transfer to an object during direct contact. One explanation to why this occurs is due to the different shedder statuses between individuals. Shedder Status relates to the capacity of an individual to deposit DNA directly onto an object. [59, 69].

We can categorize individuals in terms of their shedder statuses. It was described, in 2002, that there exists two types of individual shedder statuses, good shedders and poor shedders [70]. This means that there are people that consistently deposit more or less DNA, than others, respectively. The existence of a constant shedder state for individuals has been debated. However, most of the studies on shedder status have shown that an individual shedder status is maintained during long periods of time, maybe even years [59, 69-71].

Shedder status is influenced by inter and intra-individual differences. Gender, age, sweat, skin diseases and specific habits, such as touching the face, can influence the propensity to deposit DNA. However, the cause why some people deposit more DNA than others is still unclear [62, 69].

Being able to categorize people as good or poor shedders can be helpful during a criminal investigation. Knowing if the POI is a good or poor shedder, impacts the expectations regarding the presence of DNA on any surface, both for direct and indirect transfer [69].

There is a need to acquire more knowledge about the quantity of self and non-self-DNA deposited after direct contact with an individual's hands[72].

## 1.6 Statistics

In this dissertation statistical analyses were utilized for interpretation of the results obtained.

For all the samples obtained, the number of alleles were counted, LR was calculated using the EuroForMix software. RFU was determined by summing the peak heights. Peak heights correspond to the relative fluorescence intensity of a peak, reflecting the quantity of the PCR product being measured [25]. Average RFU was calculated by summing the peak heights and the result was divided by 23 (23 full loci, excluding Amelogenin).

In case of a sample with more than one contributor, mixture, the RFU was multiplied with the mixture proportion, provided in EuroForMix.

## 1.7 Bayesian network

Since 1990, forensic scientists have shown an interest in the application of Bayesian Network in judicial contexts. Bayesian Network are a graphical approach of displaying and conducting complex probabilistic evaluations [73, 74].

Bayesian Network are a combination of graph theory, that provides a qualitative model structure, and probability theory, used to characterize the nature and strength of the associations that occurs within a model [75].

A Bayesian network is a probabilistic graphical model that consists of nodes, arcs and probability assignments. The network uses Bayesian inference for probabilistic assessments. The networks models conditional dependences represented by the arcs in the network.

Many examples now exist on how Bayesian networks can be applied to include the relevant variables in a case and make evaluations at activity level by calculating a likelihood ratio of two opposing hypothesis. The probability tables of each node needs to be informed with relevant probabilities. Hence, research on transfer and persistence under different circumstances are essential to perform the calculations [73].

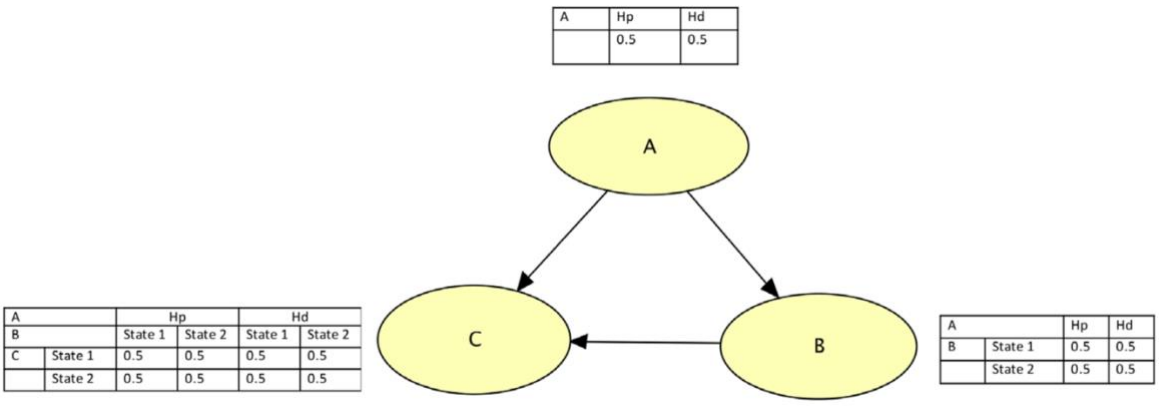


Figure 1.13: Example of a Bayesian network with three nodes A, B, and C with their associated probability tables. Note: This example has "0.5" for every probability; in a real case, probabilities would be based on experimental data or observations. From: [76].

## 1.8 Court

Nowadays, in court, the matching of 'Trace DNA' to a suspect is not the decisive matter in concluding guilt. Inquiries regarding the activity level, how Trace DNA came into the crime scene, has been gaining increased attention as it has been demonstrated that detection of a DNA profile upon a surface is not always due to direct deposition [55, 56, 77].

Due to the multiple sources of DNA, that can be in a sample collected from a crime scene, the analysis can generate a mixed profile, which means that DNA from multiple contributing individuals is represented together in one profile. Because of this, and due to the difficulty to determinate when or how the trace was deposited, the role of the forensic scientist in the assessment of the Trace DNA results is more important today than in the past years [57, 77].

## 1.9 Drug cases

### 1.9.1 Norway

Norway is known for the low crime rates in major crimes, and due to this is one of the safest countries in the world [78].

Nevertheless, in the last decade, the drug related crimes have been more frequent, with rates comparable to other Western European nations.

The total extent of drug crime is difficult to calculate because the degree of detection is considered to be low. However, there is a high number of narcotic cases (Table 1.2) [79].

Table 1.2: Number of narcotics offenses reported in Norway in the period, 2015-2019. And the percentage changes between 2018-2019 and 2015-2019. The data was retrieved from Kripos. Adapted from: [79].]

| Type of crime / Year | 2015 | 2016 | 2017 | 2018 | 2019 | % Change 2018-2019 | % Change 2015-2019 |
|---|---|---|---|---|---|---|---|
| **Narcotics** | 44708 | 39728 | 36785 | 35296 | 31320 | -11.3% | -29,9% |

In the first half of the past year, 2020, it was registered a decrease of 16.7% in the drug cases sent to Kripos (Figure 1.13). However, the values may have been impacted by the coronavirus pandemic [79, 80].

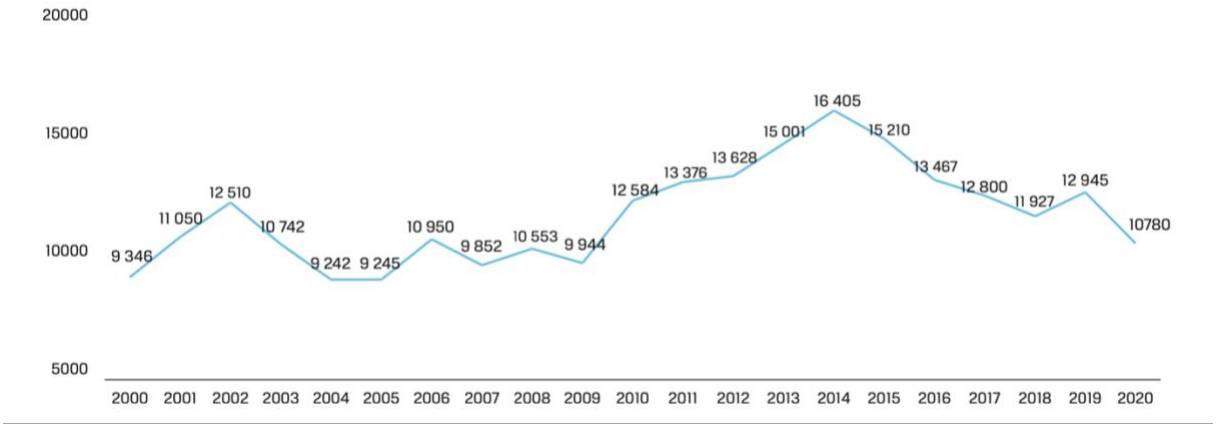


Figure 1.14: Number of narcotic cases in the first half of the years, reported in Norway, from 2000 up to 2020. The data was retrieved from Kripos. Adapted from: [80]

Although a decrease in the number of drug cases has been happening since 2015, the existent number is still a high figure for Norway.

In the last years, the proportion of drug cases has been stable in the forensic laboratories (Table 1.3).

Table 1.3: Percentage of drug cases forwarded for DNA investigations in the last four years in the Department of Forensic Medicine, Section of Forensic Biology, Oslo University Hospital

| Year | Total Cases | Drug Cases | Percentage of drug cases (%) |
|---|---|---|---|
| 2020 | 5427 | 691 | 12,73 |
| 2019 | 5707 | 685 | 12,00 |
| 2018 | 6014 | 731 | 12,15 |
| 2017 | 7236 | 895 | 12,37 |

### 1.9.2 Portugal

Portugal constitutes the final destination of different varieties of drugs, but also serves as an entry point to the rest of Europe. Nonetheless of the tight control by the Portuguese authorities, criminal organizations still use the connections between Portugal and Latin America to bring cocaine to Europe. Due to this, cocaine became the most trafficked drug with nearly 75% increase in apprehensions [81].

Besides the high numbers of drug apprehensions and an increase in drug related crimes in the last decade, 2019 displays a decrease of drug related crimes in Portugal, by almost 17% (Figure 1.15) [81].

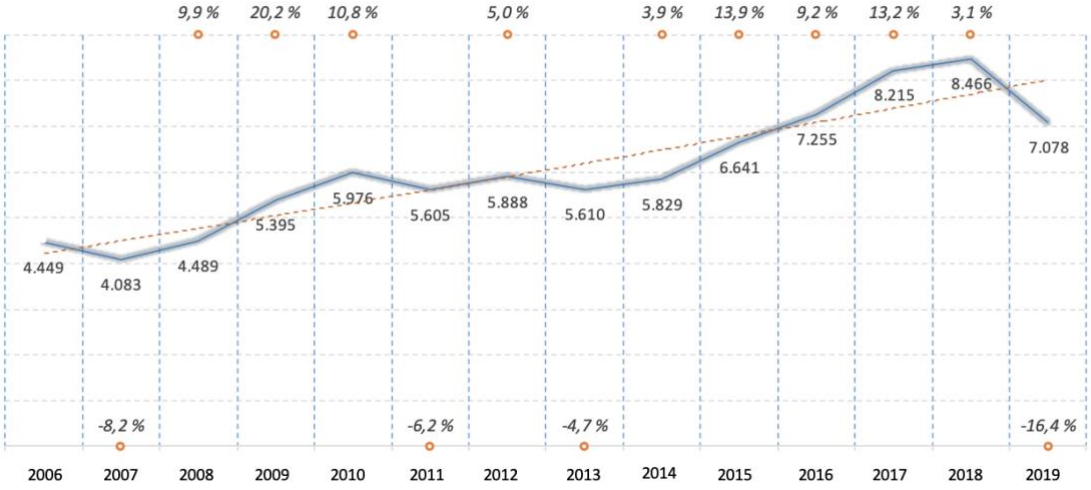


Figure 1.15: Numbers of drug related cases, reported in Portugal since 2006. Aldo it is visible a rapid increased in cases since 2010, in 2019 occur a decreased of 1388 drug related cases (-16.4%). $R^2$=0.84. The data was retrieved from the Portuguese government. Adapted from [81].

We can see the that according to the official numbers, Norway had more narcotic reported offences in the first half of 2019, than Portugal in the entire year of 2019 [80, 81].

# 2 Objectives

## 2.1 Aim

The aim of this study is to gain increased knowledge on DNA transfer, persistence and recovery that will aid forensic scientists in interpretation of activity related questions in drug related cases.

## 2.2 Objectives

The aim of this dissertation would be achieved through the following objectives:

1. Study DNA transfer, persistence and recovery on simulated drug wrappings at different scenarios.

2. Construct a dataset of direct and indirect transfer at different scenarios.

3. To detect individual differences in the amount of DNA deposited onto a touched item (shedder status), and study how this influences transfer, persistence and recovery of DNA.

## 2.3 Hypotheses

This dissertation would help forensic scientists on the following hypotheses:

1. To increase the understanding of DNA transfer, persistence and recovery in different scenarios, would provide insights on the effect of these mechanisms in drug cases.

2. A dataset on direct and indirect transfer in different scenarios would aid interpretation of evidence at activity level in drug related cases.

3. Improved comprehension on shedder status would allow us to understand the impact that this has on transfer rates.

# 3 Methods

The dissertation was approved by the data protection officer at Oslo University Hospital, case number 20/22532.

## 3.1 Participants

Our study recruited twenty participants, 5 males and 15 females. They were asked to participate in the four experiments planed in this dissertation: direct transfer, indirect transfer, transfer and persistence by handling of the tape and determination of the shedder status. All the participants signed an informed consent.

## 3.2 Preparation of the kits

The preparation of the kits was done in a "DNA-free" laboratory. To enter the laboratory, it was required to use individual protection equipment, a laboratory coat, a hair net, a face mask, and gloves. In addition, all surfaces were cleaned with 1% hypochlorite wipes before and after use.

For each participant, three kits were prepared, the first kit contained one zip lock bag, the second kit a zip lock bag with a plastic tube filled with water and the third kit a new role of tape and a piece of cardboard. The zip-lock bags (16,7x10 cm) were exposed to UV light for 10 minutes in each side, the cardboards and the tape rolls were exposed for 20 minutes on each side and the 15 mL plastic tubes (vwrTM, Centrifuge tube high Performance) were cleaned with 70% ethanol wipes and left to dry for at least one minute before packing them. To control that the material was DNA free, negative control samples were collected from the zip-lock bags, tape and cardboard.

## 3.3 Experiments

### 3.3.1 Direct Transfer

In this study participants were asked to open and close a zip-lock bag three times, with at least one hour interval between handlings. We also asked the participants to wait no less than one hour after washing the hands and/or using antibacterial, before handling the zip lock bag.

### 3.3.2 Indirect Transfer

In the second experiment, we wanted to simulate a drug wrapping that was found inside a pre used personal bag.

We asked the participants to put the zip lock bag (the zip lock bag had a plastic tube filled with water to create weight) inside a pre used personal bag, leave it there for twenty-four hours and to create some movement in the bag, such as taking the bag to work. The participants were asked to place and to take the zip lock bag while wearing gloves, so they never directly touch the zip lock bag.

The participants were asked to give information about the personal bags that they used. They were asked what type of pre used bag they used, if it was a backpack, a purse or other; and also, if they previously had used the bag every day, frequently or rarely.

### 3.3.3 Transfer and persistence by handling of tape

In this experiment the participants were divided randomly in pairs where each participant had a different assignment.

The first participant, handler (C2), was asked to handle a tape roll for 1 minute, then to place the tape inside an envelope provided and give this envelope to us. The second participant, wrapper (C1), received the tape from us and was asked to wrap a drug wrap, wrap the tape around the cardboard. All participants participated as both C1 and C2 once, making a total of 20 samples. Due to covid restrictions, at the time of the experiment the rolls of tape were stored in the envelope for a minimum of 10 days before given to C1.

### 3.3.4 Shedder Status

The participants were asked to hold a plastic tube (15 mL vwrTM, Centrifuge tube high Performance) for 10 seconds in their dominant hand. The experiment was repeated three times, with a new clean tube. The participants were asked to wait at least one hour after washing hands before performing the experiment and to wait at least three hours between each experiment.

## 3.4 Methodology

### 3.4.1 Sample collection

All DNA samples were collected through swabbing the surface, of the zip-lock bag, the drug wrap and the plastic tube.

For the first two experiments, direct and indirect transfer, the DNA sample was collected by using a moistened cotton swab (mwe, Tubed Sterile DryswabTM, MW1041) on the top 3 cm of the zip lock bag, inside and out. In the second experiment the plastic tube was discarded carefully before collecting the samples (Figure 3.1 and Figure 3.2).

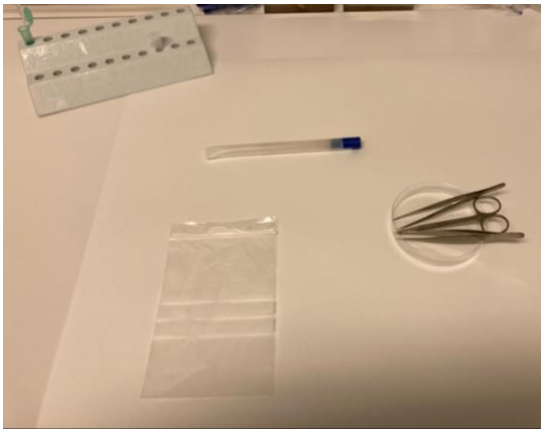

Figure 3.1: Sampling procedure of experiment 1, direct transfer. In this figure we can see a zip lock bag and the equipment used for sampling in experiment 1 (tweezers, scissors, swab and the eppendorf tube).

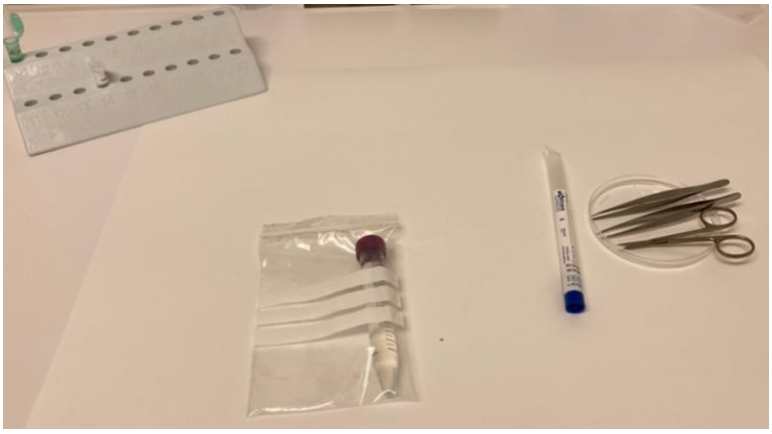

Figure 3.2: Sampling procedure of experiment 2, indirect transfer. In this figure we can see a zip lock bag containing a tube filled with water (the tube is discarded) and the equipment used for sampling in experiment 2 (tweezers, scissors, swab and the eppendorf tube).

In the third experiment, transfer and persistence by handling of tape, the DNA sample was collected by using a moistened cotton swab (mwe, Tubed Sterile DryswabTM, MW1041), and swabbing the entire surface of the “drug wrap” (Figure 3.3).

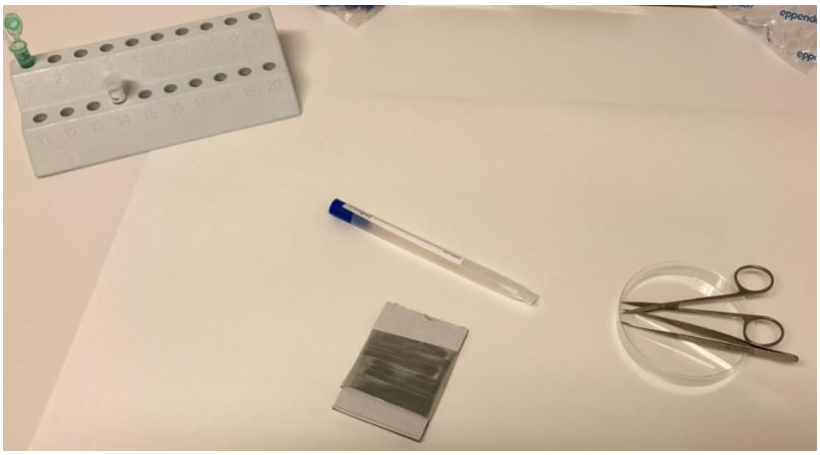

Figure 3.3: Sampling procedure of experiment 3, transfer and persistence by handling of tape. In this figure we can see the “drug wrap” (cardboard wrapped with tape) and the equipment used for sampling in experiment 3 (tweezer, scissors, swab and the eppendorf tube).

After the swabbing, the tip of the swab was cut with sterile DNA free scissors and placed inside a labelled eppendorf tube and stored in temperature freezer (-20 C) until the next phase of the analysis, to avoid degradation of DNA.

For the last experiment, shedder status, using the same method as before the entire body of the plastic tube (vwrTM, Centrifuge tube high Performance) was swabbed with the exception of the lid. Immediately after the swabbing, the tips of the swabs were cut and placed into 0.2 mL PCR tubes.

### 3.4.2 DNA extraction

To each sample tube 200µl of 5% Chelex® 100 (Bio-Rad Laboratories) was added. One negative control (only reagents) was included in each extraction. The samples were incubated (eppendorf Thermomixer comfort) at first, at 56ºC for 30 minutes at 600 rpm and in the last-minute the speed was increased to 1400 rpm. The samples were moved to a second incubator at 95ºC for 8 minutes and in the last-minute the speed was increased to 1400 rpm. Afterwards, the samples were centrifuged (eppendorf Centrifuge 5430) for 3 minutes at 13100 rpm. The procedure was finished by transferring the supernatant to a new eppendorf, correspondingly.

The new eppendorfs were stored in the fridge until the next phase of the DNA analysis.

### 3.4.3 Quantification

The samples where quantitated with the PowerQuant® (Promega) according to the manufacture's instruction. The PowerQuant® System is a five-dye, four-target hydrolysis probe-based qPCR multiplex. This system amplifies multicopy targets to quantify the total human, human male DNA present in a sample and assess the degree of DNA degradation. This amplification system includes an internal PCR control (IPC) to detect inhibitors in an amplification reaction. The PowerQuant® 20X Primer/Probe/IPC Mix includes a passive reference dye primers and probes for the: autosomal DNA target, Y-chromosomal target, degradation target and IPC [82].

A master mix was prepared, as described in table 3.1 and 18µl of the master mix and 2µl of sample were added to the each well of the MicroAmp® Optical 96-Well Reaction Plate. In addition, four premade standards with the concentrations, 25, 2, 0,08 and 0,0032 ng/µl, were added to the first and last row of the plate, to achieve the standard curve. Two negative controls were also included in the analysis.

Table 3.1: Preparation of Reaction Mix for DNA Quantification using the PowerQuant® System. Adapted from:[82]

| Component | Volume per reaction (µl) |
|---|---|
| Water | 7 |
| PowerQuant® 2X Master Mix | 10 |
| PowerQuant® 20X Primer/Probe/IPC Mix | 1 |
| Final volume | 18 |

The reaction was run on the Applied Biosystems™ 7500 Fast Real-Time PCR System with the software Applied Biosystems® 7500, according to the manufacturers recommendations (Figure 3.4) [82].

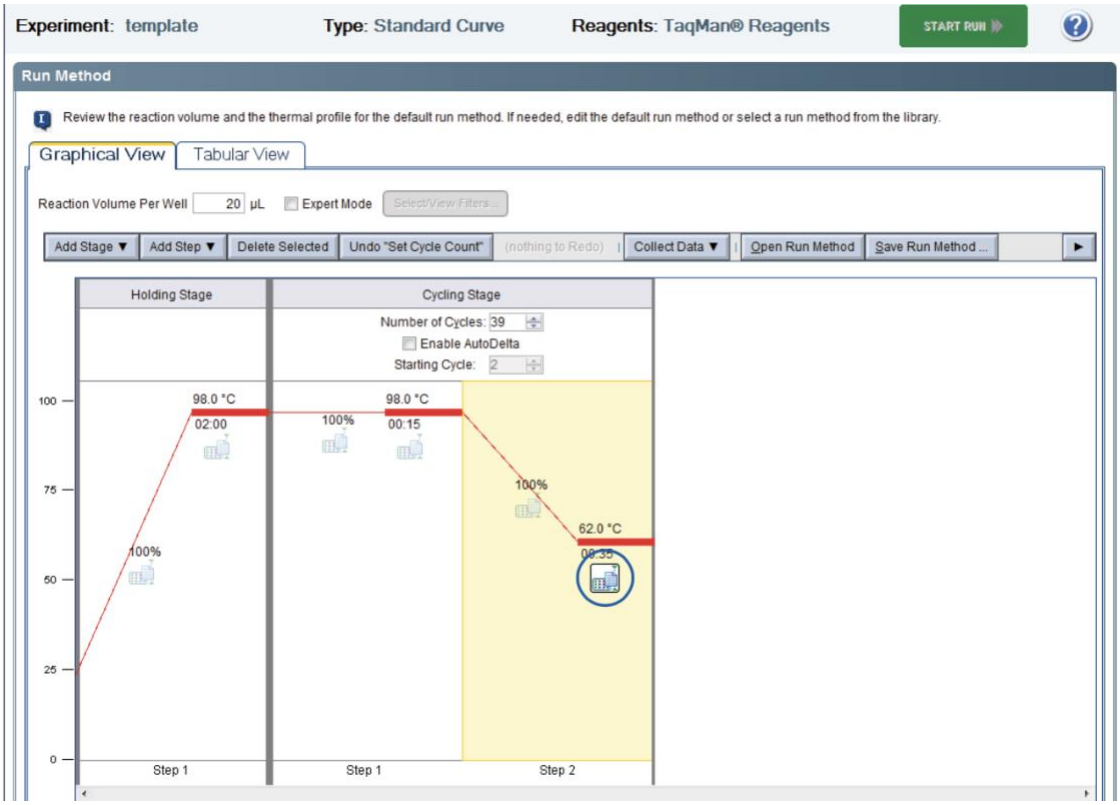


Figure 3.4: PowerQuant® System run method on the Applied Biosystems™ 7500 Fast Real-Time PCR System with the software Applied Biosystems® 7500. The holding stage occurs for 2 minutes at 98ºC. The cycling stage has 39 cycles, with step 1 occurring for 15 seconds at 98ºC and step 2 at 62ºCfor 35 seconds. From: [82]

The DNA concentration in each sample was calculated based on the achieved CT value in the HiD version 1.2 software.

### 3.4.4 Amplification

*Transfer experiments*

Amplification was performed using PowerPlex® Fusion 6C System. PowerPlex® Fusion 6C System is a 27-locus multiplex for human identification applications. It is a six-colour system that allows co-amplification and fluorescent detection of the 18 autosomal loci in the expanded CODIS core loci (CSF1PO, FGA, TH01, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433 and D21S11), also includes Amelogenin and DYS391 for gender determination. The Penta D, Penta E, D22S1045, TPOX and SE33 loci are included to improve.This system also  contain two rapidly mutating Y-STR loci, DYS570 and DYS576 [51].

PCR reaction Mix for DNA amplification was prepared according to the manufacturer's instructions, by combining the components, water, PowerPlex® 5X Master Mix, PowerPlex® 5X Primer Pair Mix. Sufficient amount of reaction Mix was prepared based on the number of reactions to be set up. The quantity of reagents was calculated according to the volume per reaction present in the Table 3.2. After the master mix was prepared and vortexed it was added 1 ng of template DNA to a final reaction volume of 25 µL. Samples that had lower concentrations than the recommended template amount were amplified with the maximum template volume of 15 µL. DNA. Additionally, the plate had a positive amplification control (a tube of 2800M Control DNA was placed on a vortex, and then dilute an aliquot to 1.0 ng in the desired template DNA volume) and a negative amplification control (PCR Amplification Mix plus Amplification-Grade Water or TE–4 buffer instead of template DNA). In the end the plate was sealed with adhesive film and centrifuged (eppendorf centrifuge 5804). The thermal cycler (Veriti® 96-Well Thermal Cycler, Applied Biosystems®) was set up according to the conditions present in the Table 3.3 for 29 amplification cycles. Afterwards the amplified samples were stored at -20ºC [51].

Table 3.2: Preparation of PCR Amplification Mix for DNA Amplification using PowerPlex® Fusion 6C System from Promega. Adapted from:[51]

| Component | Volume per reaction (µl) |
|---|---|
| Water | To a final volume of 25 |
| PowerPlex® Fusion 6C 5X Master Mix | 5 |
| PowerPlex® Fusion 6C 5X Primer Pair Mix | 5 |
| Final volume | 25 |

Table 3.3: Thermal cycling protocol for the GeneAmp® PCR System 9700, Veriti® 96-Well Thermal Cycle. Adapted from:[51]

| Cycle | Temperature | Time |
|---|---|---|
| 1x | 96ºC | 1 minute |
| 29x | 96ºC | 5 seconds |
| | 60ºC | 1 minute |
| 1x | 60ºC | 10 minutes |
| Hold | 4ºC | ∞ |

*Shedder status experiment*

Samples from the shedder status experiment were amplified directly without extraction, Direct PCR, using the PowerPlex® Fusion 6C System (Promega). Following the recommendations of the manufacturer, the tips of the swabs were cut directly into the PCR tubes, afterwards the PCR Amplification Mix was added to the tubes. The thermal cycler (Veriti® 96-Well Thermal Cycler, Applied Biosystems®) was set up according to the conditions previously referred on Table 3.3 [51, 62].

### 3.4.5 Capillary Electrophoresis

The last process in the analysis, for all experiments, is the capillary electrophoresis (CE). After centrifuging and vortexing the WEN Internal Lane Standard 500 (Wen ILS 500) the amount necessary of WEN ILS 500 and Hi-DiTM formamide was calculated according to Table 3.4. Loading mix was prepared as described in Table 3.4. Ten µl of the loading mix and 1µL sample was added to each well. The pipetting was performed by eppendorf epMotion® P5073. The plate was covered with adhesive film and centrifuged.

Subsequently, the samples were denatured at 95ºC for 3 minutes, then immediately chilled on ice bath for 3 minutes. Succeeding, the plate was placed on Applied Biosystems® 3500xL Genetic Analyzer for Human Identification and the run on the capillary electrophoresis at 1.2 kV for 24 seconds [51].

Table 3.4: Loading Mix of the Capillary electrophoresis. Adapted from:[51]

| Component | Volume per reaction (µl) |
|---|---|
| WEN Internal Lane Standard 500 | 0,5 |
| Hi-Di$^{TM}$ formamide | 9,5 |

### 3.4.6 Data analysis

The data analysis was done using GeneMapper® ID software, version 1.6 (Applied Biosystems®). A limit of detection (LOD) for alleles was set to 100 RFU, and the DNA profiles obtained were compared to the reference profiles of each participant. Homozygote alleles were counted as two, and the Y-chromosome STRs were omitted from the experiments. A stutter filter was applied in GeneMapper, stutters were filtered according to internal guideline.

Adjusted RFU was obtained by dividing the RFU value (sum of the peak heights) by 23.

LR Calculations and data analysis were performed in R (www.r-project.org) using the software EuroForMix v3.0.4 [33].

Mixtures were identified when more than one contributor was detected in the DNA profile. Identification of a mixture occurred when examining the DNA profiles and observing the number of alleles in each locus, or when the peak heights were imbalanced . The value of the adjusted RFU was multiplied by the mixture proportion, obtained in EuroForMix. In the third experiment, transfer and persistence by handling of tape, the DNA profiles obtained were compared to the reference sample of contributor 1 and contributor 2.

On EuroForMix, defence (Hd) and prosecution (Hp) hypothesis were used for each experiment.

- Hp: The DNA is from A
- Hd: The DNA is from an unknown individual

## 3.5 Shedder status category

The participants were classified into high, low and medium shedders based on the quality of DNA results obtained in the experiment. The classification was based on the quality of the DNA profiles obtained as described by H. Johannesen et al. [62].

# 4 Results

The results from the negative controls taken from the zip lock bags, tape and cardboard, all came back negative.

## 4.1 Experiment 1: Direct Transfer to zip lock bag

The samples collected from the directly handled zip lock bags provided varied results between the 20 participants.

The measured DNA concentration has an average of 0.005 ng/µl, the number of detected alleles matching the participant ranged from 0 to 46 (without amelogenin), with an average of 17 alleles. In total 4 samples had no results, 4 samples had full profiles and 12 had partial profiles.

The logarithmic of likelihood ratio had an average of 8.8. The average RFU from the donor was 454.1, this value was calculated by dividing the total RFU (sum of the height of the alleles) divided by 23 (23 pairs of alleles, excluding amelogenin), results can be found in Attachment 1.

## 4.2 Experiment 2: Indirect transfer to zip lock bag

The second experiment, indirect transfer from the personal bag presented differences between the participants. The average DNA concentration was 0.003ng/µl and the average number of matching alleles was 11, the range varied between 0 and 46 alleles (without amelogenin).

In total 10 samples had no results, 3 samples had full profiles and 7 had partial profiles.

The logarithmic of likelihood ratio had an average of 5.6. The average RFU from the donor was 592.0, it was calculated by dividing the total RFU divided by 23 (23 pairs of alleles, excluding amelogenin), results can be found in Attachment 1.

In this experiment we asked the participants what type of personal bag they used and also if they use the bag every day, frequently or rarely.

Regarding the type of use of the bag, we obtained a positive result from 3 out of 8 participants that used a backpack every day, 1 out of 2 participants that used a backpack frequently but a negative result from the participant that utilized the backpack rarely. Regarding the PC bag, the only participant that used this type of bag reported to use the computer bag everyday however the result was negative. Considering the results from the purse/handbag, a positive result was obtained from 1 out of 2 participants that used this type of bag every day, positive result was obtained from the three participants that used the purse/handbag frequently and the participant that only used the purse/handbag rarely got a positive result. From the 2 participants that used the shopping bag frequently 1 positive result was obtained (Table 4.1, Figure 4.1).

Table 4.1: Total number of participants using the different types of bags distributed by the categories of amount of use, everyday, frequently and rarely,

| Type of bag | Positive samples / total number of samples | | | Total Number Participants |
|---|---|---|---|---|
| | Everyday | Frequently | Rarely | |
| Backpack | 3/8 | 1/2 | 0/1 | 11 |
| PC bag | 0/1 | | | 1 |
| Purse/handbag | 1/2 | 3/3 | 1/1 | 6 |
| Shopping bag | | 1/2 | | 2 |
| Total Number of positive samples | 4 | 5 | 1 | 10 positive samples in 20 participants |

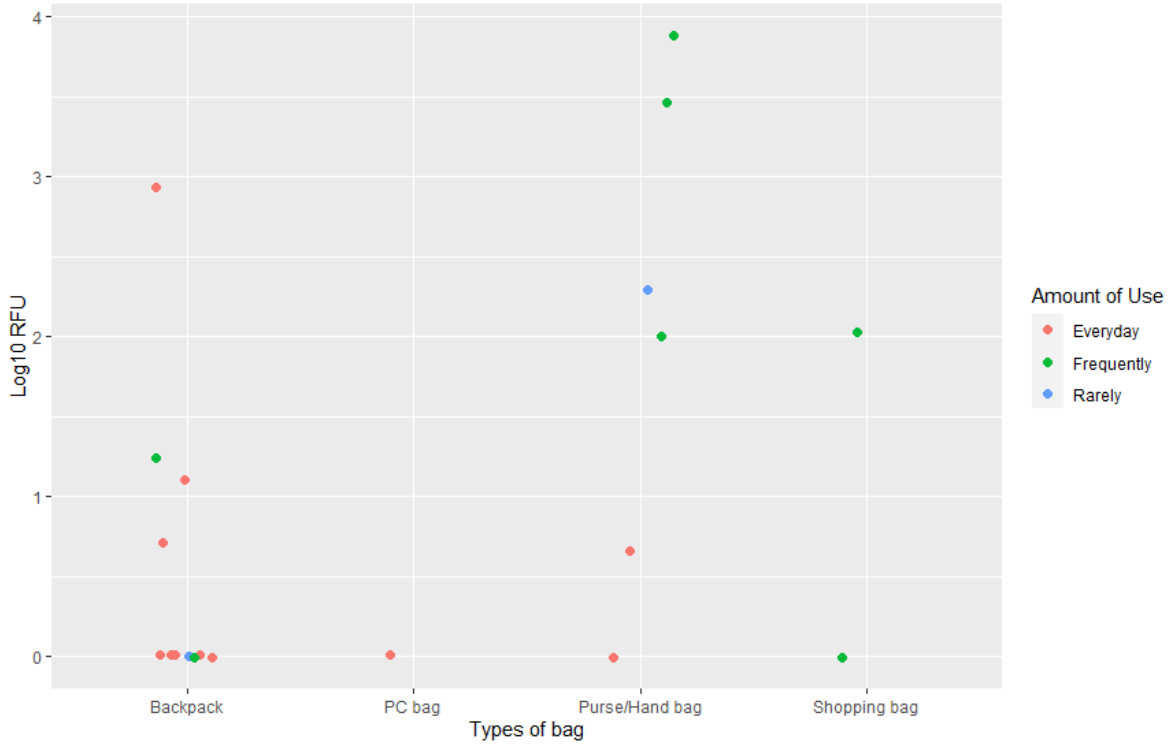


Figure 4.1: Point plot of the type of personal bag used by the participants according to the $Log_{10}$ RFU and the amount of use in experiment 2. The different colours show the different amount of use, every day, frequently and rarely are represent by red, green and blue, respectively.

In the sample from participant B, 21 alleles from an unknown person were detected.

In the sample from participant I, 9 alleles from an unknown person were detected. These 9 alleles seemed to belong to the child of the participant I. We tested this hypothesis in EuroForMix and used the following hypothesis:

- Hp: The DNA is from A and an unknown individual
- Hd: The DNA is from an unknown individual and the child from A

Equation 4.1

$$LR_{rel} = \frac{\Pr\,(E|H_p\colon A + U)}{\Pr\,(E|H_d\colon U + A_{child})}$$

The LR obtained was 0.00043, supporting the defence hypothesis, that the DNA from participant I child is present in the sample.

## 4.3 Direct transfer to zip lock bag compared to indirect transfer to zip lock bag

The differences between experiment 1 and experiment 2 was studied by comparing the average $Log_{10}$ RFU and $Log_{10}$ LR values.

Typically, higher quantities of DNA and better-quality profiles were detected after direct contact with the zip lock bag (Experiment 1) in comparison with indirect transfer (Experiment 2), but some exceptions were observed (Figure 4.2).

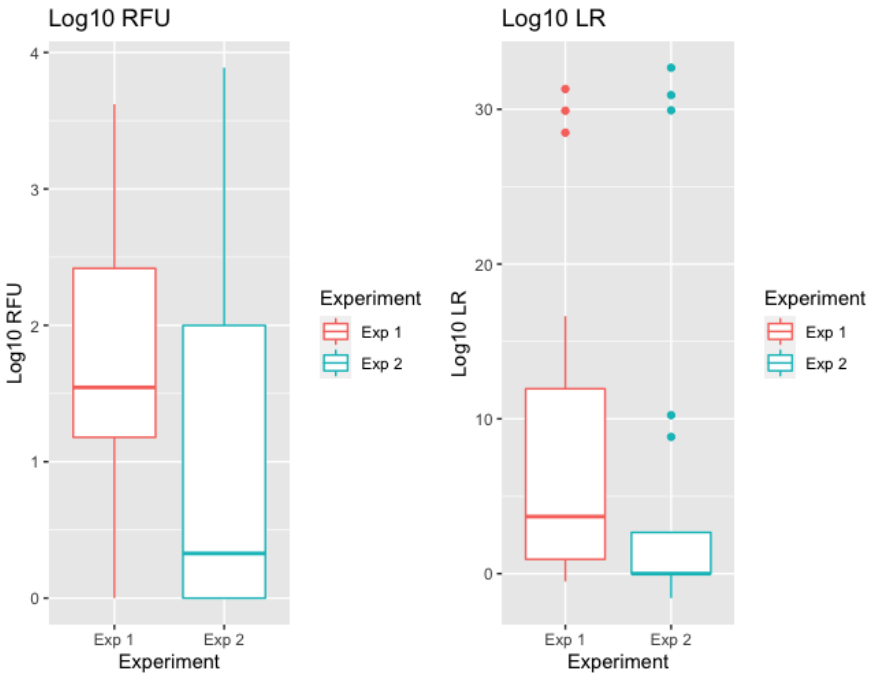


Figure 4.2: Comparison between $log_{10}$ RFU and $Log_{10}$ LR values of Experiment 1 (direct transfer to zip lock bag) and Experiment 2 (indirect transfer to zip lock bag). Experiment 1 (Exp 1) and Experiment 2 (Exp 2) are represented by the red and blue box plot, respectively.

The probability of detecting DNA from a POI was investigated using a cut of LR, above $10^4$. As the LR is influenced by the rarity of the profile, the average RFU was also used to find a cut off.

The correlation between the $Log_{10}$ RFU and $Log_{10}$ LR values from Experiment 1 and 2 was investigated by linear regression. A linear correlation was observed between these two parameters (Figure 4.3).

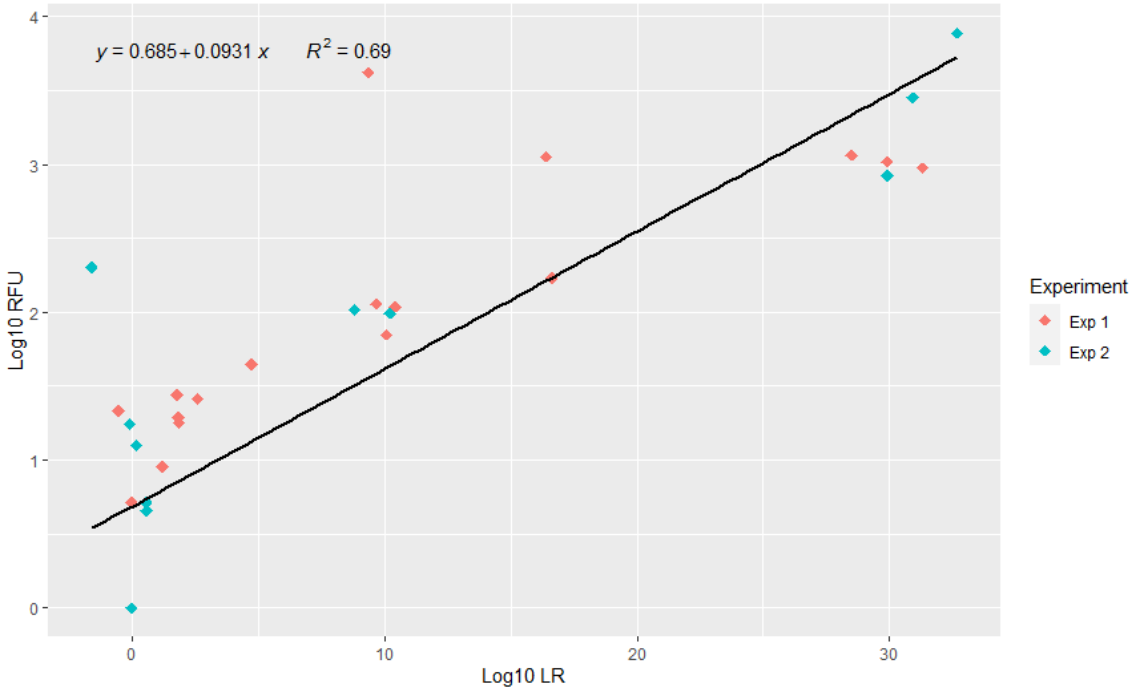


Figure 4.3: Linear regression of the $Log_{10}$ LR and $Log_{10}$ RFU. This correlation got a score of 0.83, and a p-value=3.132e-11, which means it is statistically significant. Experiment 1 (Exp 1) and Experiment 2 (Exp 2) are represented by the red and blue colours, respectively.

The correlation indicates that average RFU also can be used as a parameter in activity level evaluations as suggested by Gill et al. [83].

## 4.4 Transfer and persistence by handling of tape

The third experiment, handling of the tape showed differences between participants and also between contributors in the same samples, results can be found in Attachment 2.

The average DNA concentration for each contributor as calculated by multiplying the DNA quantity and the mixture proportion. The average DNA concentration for contributor 1 was 0.002 ng/µl, the average number of alleles matching C1 was 21.2. The average of the adjusted RFU for C1 was 209.7.

The average DNA concentration for contributor 2 corresponded to 0.0007 ng/µl. The average number of alleles matching C2 was 4.5. The average of the adjusted RFU for C2 was 73.1.

The probabilities of transferring DNA when handling the tape (C2) or packing the “drug wrap” (C1) was investigated with the same approach as in the previous experiments (Figure 4.4).

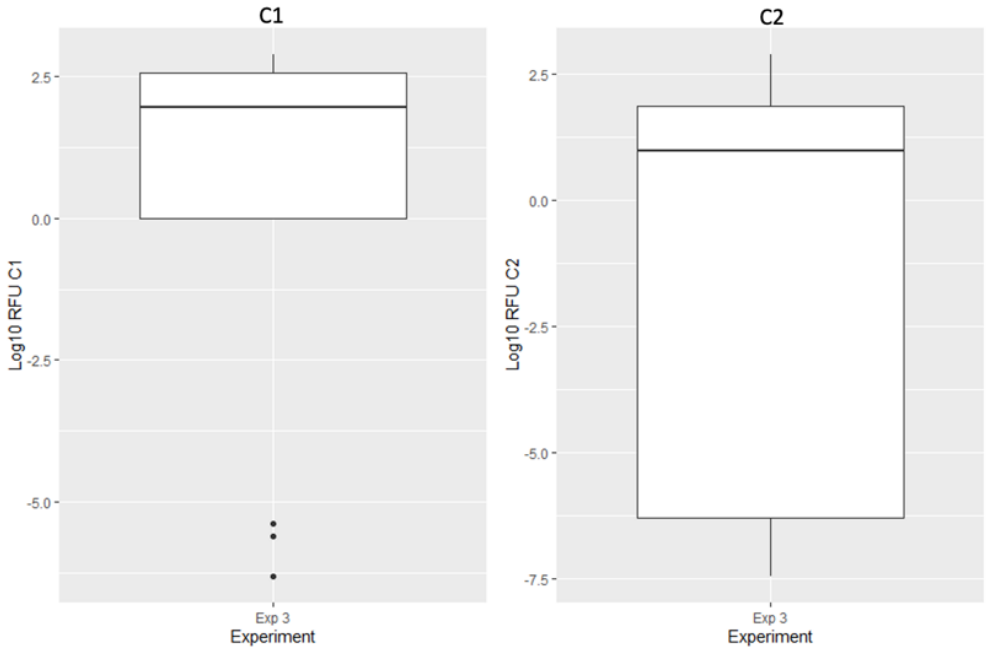


Figure 4.4: Box plot comparing the profile quality of Contributor 1 (C1) and Contributor 2 (C2), displayed by the $\log_{10}$ RFU obtained in Experiment 3 (Exp 3). Contributor 1 has better quality profiles than Contributor 2.

## 4.5 Shedder Status

A fourth experiment was realized to determine the shedder status of the participants.

The total RFU values for each sample was calculated by summing the RFU of all alleles and multiplying with the mixture proportion, in the case of the sample being a mixture. The average for the adjusted RFU is approximately 62520.

A difference between the participants was observed in the adjusted RFU (Figure 4.5).

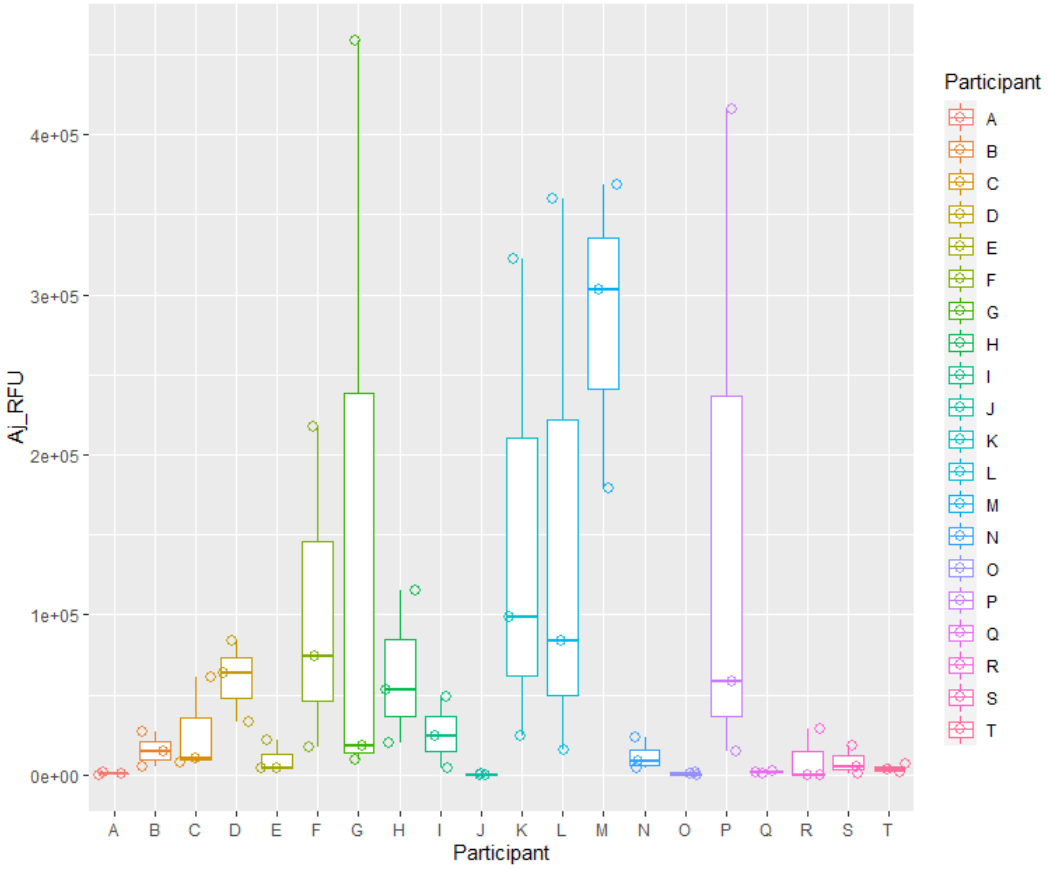


Figure 4.5: Box plot of the adjusted RFU (Aj_RFU) per participant in Shedder Status experiment, demonstrating the difference between the three samples collected of each participant.

Participants were classified as low shedders when results from all 3 samples presented partial or null profiles and the RFU was situated below the 25% percentile (2585.5), high shedder class was attributed to participants when 2 out of 3 samples were above the average RFU (62520), and 2 out of 3 samples had 20 out of 24 full loci (counting amelogenin). The remaining group was classified as medium shedders (Figure 4.6).

Out of the 20 participants, 20% are low shedders (4 participants), 60% are medium shedders (12 participants) and 20% are high shedders (4 participants) (Table 4.2).

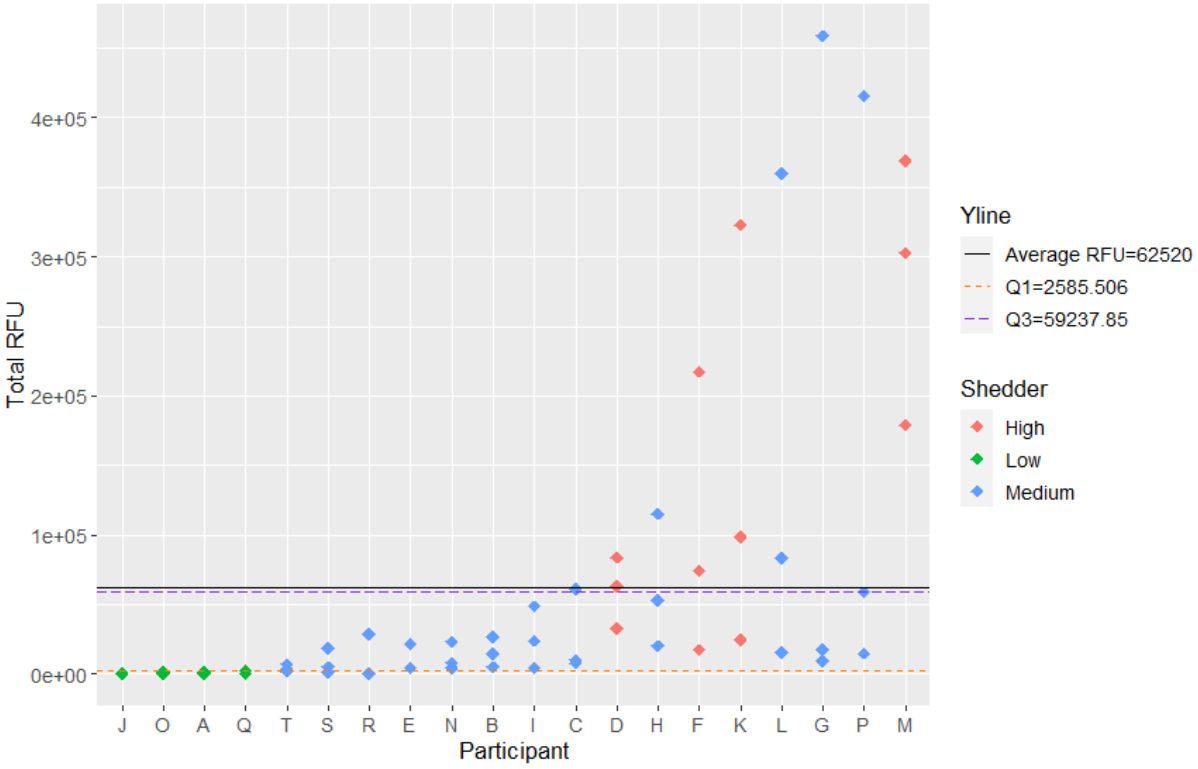


Figure 4.6: Total RFU of the participants in Experiment 4, and the division of the participants in low, medium and high shedders. According to the shedder type obtained there are 4 low shedders, 12 medium shedders and 4 high shedders. Red, green and blue colour represents the high, medium and low shedders, respectively. The first Y line, starting from 0, orange dotted line (Q1), corresponds to the 25% percentile (59237). The second line, purple dotted line (Q3) corresponds to the 75% percentile (2585) and the third line, solid black corresponds to the average RFU (62520).

Table 4.2: Participant with the information about the gender (amelogenin) and the shedder status they were attributed according to the results obtained in Experiment 4.

| Participant | Amelogenin | Shedder Type |
|---|---|---|
| A | XX | Low |
| B | XX | Medium |
| C | XX | Medium |
| D | XX | High |
| E | XX | Medium |
| F | XX | High |
| G | XX | Medium |
| H | XX | Medium |
| I | XX | Medium |
| J | XX | Low |
| K | XY | High |
| L | XY | Medium |
| M | XX | High |
| N | XX | Medium |
| O | XY | Low |
| P | XX | Medium |
| Q | XY | Low |
| R | XY | Medium |
| S | XX | Medium |
| T | XX | Medium |

## 4.6 Correlation between shedder status and results in transfer experiments

A correlation between the participants shedder status and the DNA results was observed in Experiment 1, direct handling to zip-lock bag. This was true both when considering $log_{10}$ LR and $log_{10}$RFU, better quality profiles were observed from high shedders followed by medium and the lowest quality was from the low shedders (Figure 4.7).

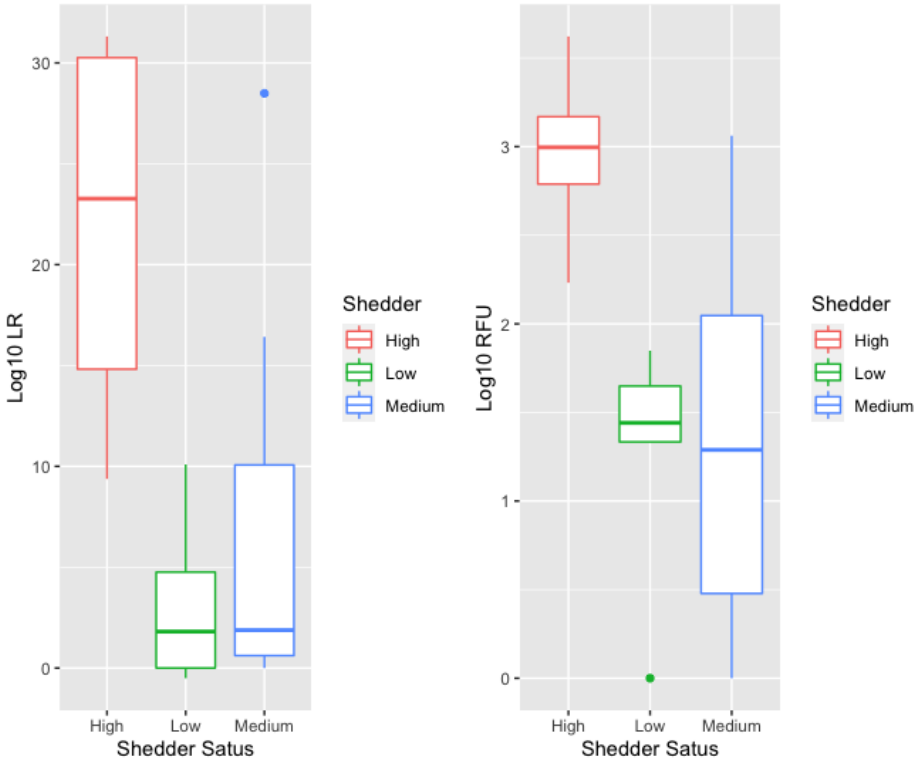


Figure 4.7: Box plot displaying the $Log_{10}$ LR and $Log_{10}$ RFU according to the Shedder type of the participants in Experiment 1 (direct transfer). Red, green and blue box plot correspond to high, low and medium shedders respectively.

Considering the DNA results from Experiment 2, indirect transfer to zip-lock bag from personal bag and the participants shedder status the results are more variable (Figure 4.8). In fact, the best quality profile was detected after storage of the zip-lock bag in the personal bag of a low shedder.

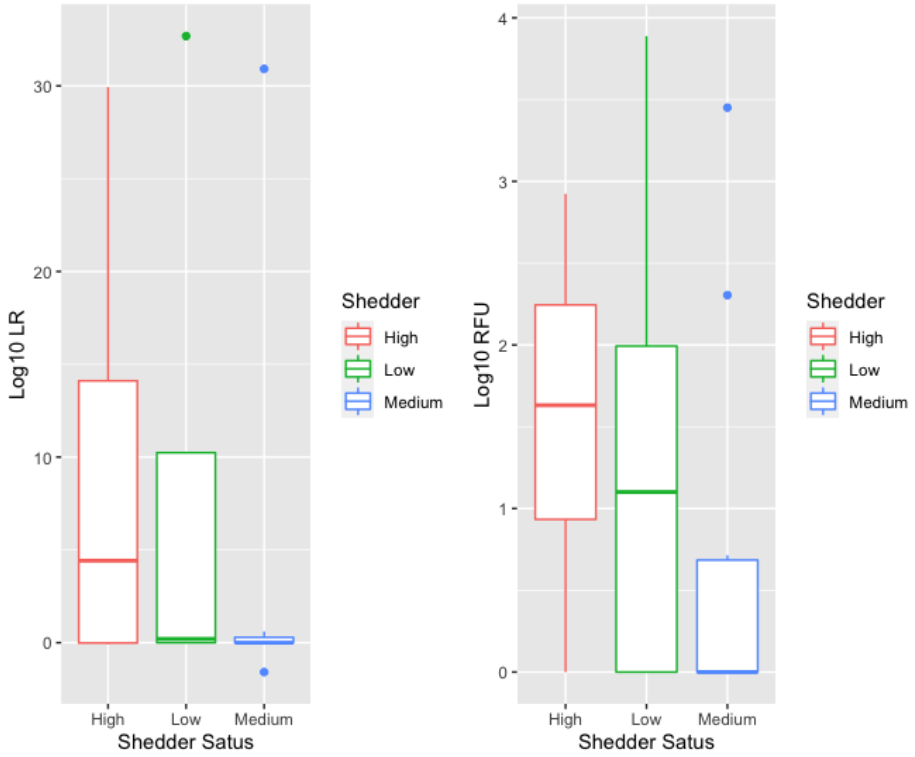


Figure 4.8: Box plot displaying the $Log_{10}$ LR and $Log_{10}$ RFU according to the shedder type of the participants of Experiment 2 (indirect transfer). Red, green and blue box plot correspond to high, low and medium shedders respectively.

The shedder status of the participants corresponded well with the DNA results from the wrapper (last handler) in experiment 3 (Figure 4.9). Higher RFU values were detected in the samples from the high shedders, followed by the medium and the lowest from the low shedders. The correlation was also observed for the DNA results corresponding the previous toucher (C2). However, the C2 result were of lower quality and more variation was seen.

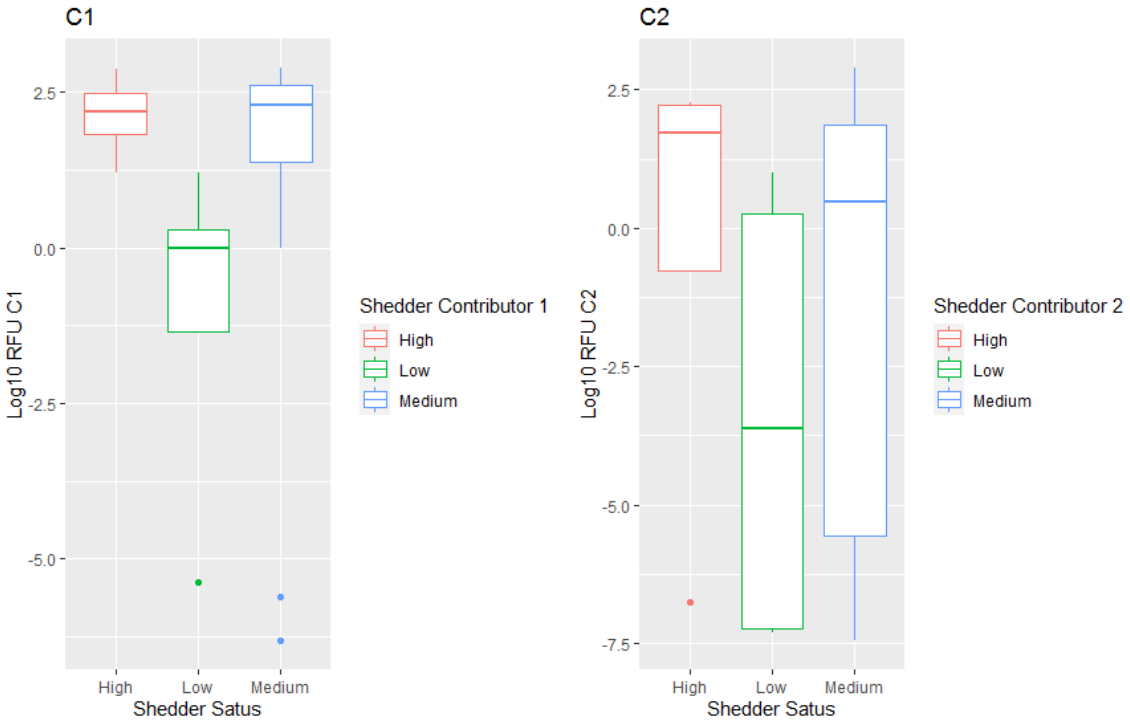


Figure 4.9: Box plot displaying the Log $_{10}$RFU and Shedder type of contributor 1 (wrapper) and contributor 2 (handler) of Experiment 3 (transfer and persistence by handling of tape). Red, green and blue box plot correspond to high, low and medium shedders respectively.

To further assess the probabilities of transfer, the frequency of observations was recorded both considering LR, RFU for the different shedder groups (Table 4.3-4.5). Increasing the LR limit to 10^6 decreased only the probabilities of medium shedders. Including also an RFU limit highly decreased the probability for low shedders, increasing the RFU limit to 1000 decreased the probability for all categories. For further calculations the limit of 100 RFU was chosen.

Table 4.3: Probability of transfer of DNA according to the shedder type of the participants and the thresholds for LR and RFU in Experiment 1 (direct transfer).

| Experiment 1 | Criteria | Low Shedder (4) | Medium shedder (12) | High shedder (4) |
|---|---|---|---|---|
| LR | LR>10.000 | 0,25 | 0,41667 | 1 |
| | LR>$10^6$ | 0,25 | 0,333 | 1 |
| | | | | |
| RFU | RFU>100 (LR>10.000) | 0 | 0,333 | 1 |
| | RFU>100 (LR>$10^6$) | 0 | 0,333 | 1 |
| | RFU>1000 (LR>10.000) | 0 | 0,1667 | 0,5 |
| | RFU>1000 (LR>$10^6$) | 0 | 0,1667 | 0,5 |

Table 4.4: Probability of transferring DNA according to the shedder type of the participants and the thresholds for LR and RFU in Experiment 2 (indirect transfer).

| Experiment 2 | Criteria | Low Shedder (4) | Medium shedder (12) | High shedder (4) |
|---|---|---|---|---|
| LR | LR>10.000 | 0,5 | 0,0833 | 0,5 |
| | LR>$10^6$ | 0,5 | 0,0833 | 0,5 |
| | | | | |
| RFU | RFU>100 (LR>10.000) | 0,25 | 0,0833 | 0,5 |
| | RFU>100 (LR>$10^6$) | 0,25 | 0,0833 | 0,5 |
| | RFU>1000 (LR>10.000) | 0,25 | 0,0833 | 0 |
| | RFU>1000 (LR>$10^6$) | 0,25 | 0,0833 | 0 |

Table 4.5: Probability of transferring DNA according to the shedder type of contributor C1 (wrapper) and contributor C2 (handler) and the thresholds for LR and RFU in Experiment 3 (transfer and persistence by handling of tape).

| Experiment 3 | Criteria | Low Shedder (4) | | Medium shedder (12) | | High shedder (4) | |
|---|---|---|---|---|---|---|---|
| Contributor | | *C1* | *C2* | *C1* | *C2* | *C1* | *C2* |
| RFU | RFU>100 | 0 | 0 | 0,5833 | 0,0833 | 0,75 | 0,5 |
| | RFU>450 | 0 | 0 | 0,25 | 0,0833 | 0,25 | 0 |
| | RFU<100 (Above 50) | 0 | 0 | 0,1667 | 0,333 | 0 | 0 |

## 4.7 Case example

To illustrate the use of the dataset obtained in this study we have created a fictive case example, based on previous experiences in casework. A large depot of drugs is found at a hideout, the drugs were packed in Ziplock bags and hidden in a black gym bag. DNA samples are collected from the outside and opening of the Ziplock bag, and the result obtained was a full DNA profile. A database search was conducted and a match in DNA database to person A was obtained.

However, person A claims to have no knowledge of the drugs. But he recognizes the gym bag and claims this used to belong to him but has been lost for some time.

The prosecution and defence hypothesis are the following in this scenario:

- Hp: The suspect packed the drugs, he has not previously used the bag.
- Hd: The suspect has no relation to the drugs but previously used the bag.

A Bayesian network was adapted from Gill et al. [84] and the nodes were updated to fit the described scenario in the case examples and probability tables based on the results from the experiments as given in Table 4.3-4.5.

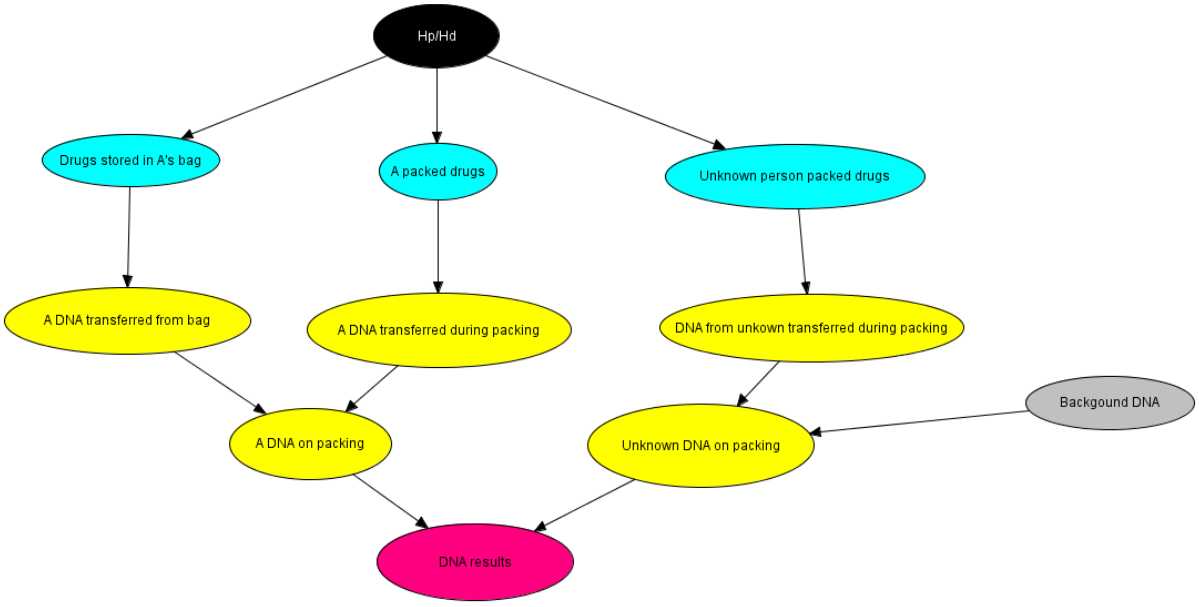


Figure 4.10: Bayesian Network of the case example. The BN was adapted from the example from Gill et al. [84], the nodes were updated to match the scenario of the case example. The black node refer to de hypothesis node, the blue nodes relate to the activity level, the yellow nodes refer to the transfer, the grey node to the existence of background DNA and the pink node to the final DNA results.

Hypothesis from the Bayesian Network of the case example are the following:

- Both propositions are set with equal probabilities

Table 4.6: Probabilities from Bayesian Network, when both propositions, Hp and Hd are set with equal probabilities.

| | |
|---|---|
| Hp | 0.5 |
| Hd | 0.5 |

- Drugs are stored in A's bag
    - This is true under Hd and false under Hp.

Table 4.7: Probabilities from Bayesian Network, when drugs are stored in A´s bag.

| | Hp | Hd |
|---|---|---|
| Yes | 0 | 1 |
| No | 1 | 0 |

- A packed the drugs
    - This is true under Hp and false under Hd.

Table 4.8: Probabilities from Bayesian Network, when A packed the drugs.

| | Hp | Hd |
|---|---|---|
| Yes | 1 | 0 |
| No | 0 | 1 |

- Unknown person packed the drugs
    - This is true under Hd and false under Hp.

Table 4.9: Probabilities from Bayesian Network, when an unknown person packed the drugs

| | Hp | Hd |
|---|---|---|
| Yes | 0 | 1 |
| No | 1 | 0 |

- A´s DNA was transferred from bag
    - The probability of secondary transfer of A´s DNA from bag (s) is found from experiment 2, for this example we have chosen to classify a positive finding based on the adjusted RFU in the sample and set the limit to AdjRFU>100.

Table 4.10: Probabilities from Bayesian Network, when A´s DNA was transferred from the bag

| | Drugs stored in A´s bag | |
|---|---|---|
| A DNA transferred from bag | Yes | No |
| Yes | 0.2 | 0 |
| No | 0.8 | 1 |

- A DNA transferred during packing
    - The probability of transfer from A during pacing (t) is found from experiment 1, for this example we have chosen to classify a positive finding based on the adjusted RFU in the sample and set the limit to AdjRFU>100.

Table 4.11: Probabilities from Bayesian Network, when A´s DNA was transferred during packing.

| | A packed the drugs | |
|---|---|---|
| A DNA transferred during packing | Yes | No |
| Yes | 0.4 | 0 |
| No | 0.6 | 1 |

- DNA from unknown person transferred during packing
    - Transfer from an unknown offender during packing (t').

Table 4.12: Probabilities from Bayesian Network, when DNA from an unknown person was transferred during packing.

| | Unknown packed drugs | |
|---|---|---|
| Unknown DNA transferred during packing | Yes | No |
| Yes | 0.4 | 0 |
| No | 0.6 | 1 |

- A DNA on packing
    - Boolean node will be yes when true and no when false.

Table 4.13: Probabilities from Bayesian Network, A DNA on packing, Boolean node.

| A DNA transfer from bag | Yes | | No | |
|---|---|---|---|---|
| A DNA transferred during packing | Yes | No | Yes | No |
| Yes | 1 | 1 | 1 | 0 |
| No | 0 | 0 | 0 | 1 |

- Background DNA
    - The probability of background DNA (b) is found from the occurrences of unknown DNA in the samples collected in both experiments (3/40).

Table 4.14: Probabilities from Bayesian Network, Background DNA

| | |
|---|---|
| Yes | 0.075 |
| No | 0.925 |

- Unknown DNA on packing
    - Boolean node will be yes when true and no when false.

Table 4.15: Probabilities from Bayesian Network, when unknown DNA is transferred on packing, Boolean Node.

| Background DNA | Yes | | No | |
|---|---|---|---|---|
| U DNA transferred during packing | Yes | No | Yes | No |
| Yes | 1 | 1 | 1 | 0 |
| No | 0 | 0 | 0 | 1 |

- DNA results

Table 4.16:Probability from Bayesian Network, DNA results.

| Unknown DNA on packing | Yes | | No | |
|---|---|---|---|---|
| A DNA on packing | Yes | No | Yes | No |
| A DNA | 0 | 0 | 1 | 0 |
| A and U DNA | 1 | 0 | 0 | 0 |
| U DNA | 0 | 1 | 0 | 0 |
| No DNA | 0 | 0 | 0 | 1 |

The likelihood ratio was calculated with the Bayesian network (Equation 4.2):

Equation 4.2

$$LR = \frac{Pr\ (E|Hp)}{Pr(E|Hd)} = 3.33$$

It is 3.33 times more likely to observe the evidence if Hp was true than if Hd was true.

The calculations can also be shown by applying the formula S6 and S1 in the supplementary 1 of Gill et al. [84].

The list of variables that have to be considered in this case are the following:

- t is the probability of transfer, persistence and recovery of DNA from the POI under Hp
- s is the probability of transfer, persistence and recovery of DNA on the drug wrapping transferred from the gym bag
- b is the probability of background DNA.
- Pr(Ud) is the combined probability of recovering unknown DNA.

To calculate the likelihood ratio based on the activity level the following formula is used (Equation 4.3).

Equation 4.3

$$\mathrm{LR} = \frac{\Pr(\mathrm{E}|\mathrm{Hp})}{\Pr(\mathrm{E}|\mathrm{Hd})} = \frac{\mathrm{t\ x\ (1-b)}}{\mathrm{s\ x(1-Pr(Ud))}}$$

Probabilities for t, s and b were found from experiment 1 and 2 according to the criteria of a good profile a RFU higher than 100 and a LR above 10.000 and 1 million (not considering shedder status). The combined probability of recovering unknown DNA was calculated with a formula from Gill et al. (Equation 4.4) [84].

Equation 4.4

$$\Pr(\mathrm{Ud}) = \mathrm{t'} + \mathrm{b}(1-\mathrm{t'}) = 0.4 + 0.075(1-0.4) = 0.445$$

Equation 4.5

$$LR = \frac{\mathrm{t\ x\ (1-b)}}{\mathrm{s\ x(1-Pr(Ud))}} = \frac{0.4\ \mathrm{x}(1-0.075)}{0.2\ \mathrm{x}(1-0.445)} = 3.33$$

With the calculation of the LR in this scenario we can say that the evidence is 3 times more likely given Hp, the suspect packed the drugs, than given Hd, the suspect has no relation to the drugs but previously used the bag (Equation 4.5).

# 5 Discussion

In our study we used Chelex to extract DNA. We could have used a different extraction method, and obtained different results, for instance it is possible to concentrate the samples and use a smaller elution volume. However, loss of DNA have been observed when using for instance magnetic beads extractions [85]. Internal validations at the institute has shown that Chelex provides comparable results to QIAamp (Qiagen) [86]. Chelex was the method used in casework in the laboratory at the time of the experiment, and consequently gives relevant results. We used two different methods in the experiments, Direct PCR, in the shedder status experiments, versus extraction (Chelex) with amplification (PCR) in the other three experiments. In the shedder status experiments higher peaks were observed in the DNA profiles obtained, with a higher average of RFU that other experiments. Direct PCR has previously been demonstrated to provide better results from some samples. Templeton et al. [87] described a protocol for direct PCR for low template DNA, deposited in different subtracts, adhering to swab fibers. They observed that direct PCR has the ability to amplify low quantities of DNA recovered from different subtracts. It could have been favourable to also perform direct PCR on the other samples collected in this study since the amounts of DNA detected were low level and no dilutions prior to PCR was needed. To determine if the direct PCR would have an impact on the other experiments a new study could be performed.

The mechanisms that affect the DNA transfer, persistence, prevalence and recovery (DNA-TPPR) (e.g., a person's shedder status) have a huge impact on the quantity and quality of a DNA profile obtained from a crime scene stain. This is further demonstrated by the findings in this study where we have seen large variations in the results, between the experiments, participants and different sampling mechanisms.

## 5.1 Transfer and persistence of DNA

In this study the direct transfer of DNA to a simulated drug wrapping was investigated. The zip lock bags used in this experiment are made of plastic and considered to be a non-porous surface. Previous studies has shown that less DNA was transferred to a plastic and glass (non-porous substrate) than to wood and cotton (porous substrate) [61].

The results that we achieved corresponded well with our anticipations; the majority of the profiles that we obtained were partial profiles, with an average of 17 alleles. However, we also obtained a few full profiles. Zamir et al. [88], conducted a study where they examined if DNA could be extracted and typed from burnt edges of a plastic drug wrap. They concluded that it was possible to obtained DNA from the burned edges but that is also was possible to retrieve DNA from the plastic bag. Our results correlate with the findings of Zamir et al. [88].

All the zip lock bags used in our study were exposed to UV light, so that all DNA previously present on the bag was eliminated. Assuming that the zip lock bags were DNA free, all DNA profiles obtained in our study, were considered to be a direct self-DNA deposit made by each participant. Nevertheless, non-self-DNA may also be detected, probably due to indirect transfer by the participants [57, 59, 61].

Secondary transfer can occur in different ways. In this dissertation we studied the hypothesis of DNA transfer occurring by placing a zip lock poly bag in a pre-used personal bag, investigating whether

indirect transfer of DNA could occur from the participant's personal bag and onto the zip lock bag. The type of bags varied between backpacks, handbags, computer bags and shopping bags.

M. Goray et al. [60, 61] stated that transfer rates are influenced by the combination of the substrates in question and the manner of contact. Secondary transfer of DNA from porous primary substrate (e.g., cotton on the inside of a bag) to a non-porous secondary substrate (e.g., plastic) is not expected to carry a great percentage of DNA, especially if the manner of contact is passive. Our results correlate with these expectations. The majority of the samples gave no DNA results, while 3 samples presented low number of alleles, the average for this experiment was 11 alleles. However, two exceptions happened, participant J and K obtained a full DNA profile (46 alleles).

van den Berge et al. [64] collected a set of private samples (e.g., neck, fingernails, clothing, etc), and found prevalence of non-self-DNA. In our experiment we achieved similar results with samples from two participants, the samples presented mixtures, where a major and minor contributor were observed. One of the samples contained a mixture where the POI was the major contributor and in addition an unknown minor contributor was detected. The other samples contained alleles corresponding to a child of the participant, this was confirmed by LR calculations in EuroForMix.

In contrast to other studies [72] we did not detect many mixtures in our transfer experiments, only 2 mixtures were detected in total. This could be due to the limitations of our study. The Covid-19 pandemic was the main one. The Covid-19 pandemic had a huge effect on our study, especially with the number of mixtures detected. It was expected to observe secondary transfer of non-self-DNA from the participants, but with the safety restrictions in place (e.g., use of antibacterial, social distancing and the use of masks) secondary transfer may not have occurred in the same scale as pre pandemic.

Interestingly a larger proportion of mixtures were detected in the samples collected in the shedder status experiment. This could be due to increased sensitivity when using direct PCR, but also due to a different type of contact – the whole hand was in contact with the tube in the shedder study, while mostly fingertips represent the contact when opening a zip-lock bag or wrapping with tape.

To study the probability of transfer from the inside of a previously used bag it would be of interest also to quantify the amount of DNA present before and after placing an item there. This DNA represents the potential of transfer to an item placed inside the bag. We did not sample the inside of the bags in this study. However, several studies on the quantity present on primary and secondary surfaces already exist [60, 61, 89] and can be used to infer transfer probabilities related to quantities present on an item. The present study is a case study and provides probabilities in a general situation where not much information is available. This is often the case in real casework, where items involved in transfer might not be available for sampling. We do however recommend the sampling and preservation of relevant items in casework to better be able to answer questions related to transfer and persistence in real casework.

In the third experiment we studied the possibility of DNA transfer from a tape roll handled by one person and onto a simulated drug wrap when person two used the tape to wrap the "drugs". Both transfer and persistence of DNA from the handler (C2) and the wrapper (C1) were studied.

L. Mayuoni-Kirshenbaum et al. [56] investigated a technique commonly used in burglaries where "foil impressioning" of aluminium foil are inserted into the lock. When this type of items is presented in court, questions related to when and how the DNA was deposited are often raised (e.g., does DNA belong to the person that cut the foil or to the person that inserted the foil into the lock?). The authors conducted a study to determine the probabilities of detecting DNA from the person that pick the lock and cut the foil. They concluded that there is a higher chance of finding DNA of the person that pick the

lock than the person that cut the aluminium foil. Similar observations were made in our study: the wrapper (C1) was more frequently detected (average of 21 alleles) in the samples, and the wrapper was detected as the major contributor in the majority of samples.

The surface of the tape constitutes a non-porous surface. M. Goray et al. [61] and R.A.H. van Oorschot et al. [63] concluded from their studies that when there is a transfer of DNA from two different users to the same object, the second user's DNA will over time replace the DNA from the first user.

Our results support this statement except for two DNA profiles that only correspond to the handler (C2). The tape roll has a rougher surface on the side, where more DNA is expected to be transferred upon the initial contact [59]. Due to this characteristic, it is expected that some DNA, deposited on the side, could be transferred to the second user hands (the wrapper), and further to the packing.

Assuming the participants followed the instructions, no other items should have been touched between handling the tape and preforming the wrapping, hence there is no opportunity of loss of C2 DNA to other surfaces. The wrapping procedure was not supervised and the manner of contact during the procedure could influence the result. Nonetheless, this type of information will hardly be known in casework, hence will be challenging to account for.

In the shedder status experiment, the participants were classified into low, high and medium shedders based on the results obtained with the tube holding experiment, repeated three times. Categorization of the participants according to their shedder status was based on previous studies. H. Johannesen et al. [62] categorized the participants into high, low and medium shedders based on the handheld tube method. High shedders were classified when minimum of two of the three profiles had a total RFU value above the average of all the participants and they provided a high-quality profile, low shedders were categorized if all three samples had RFU values below 10,000 RFU and gave partial or negative profiles. And by cell count method according to the mean value of detected cells in the two fingerprints. Fonneløp et al. [59] categorized the participants into high and low shedders based on the handheld tube method, high shedders had to have at least two of the three samples, where DNA quantity had to be above the average concentration in deposits made by all participants and at least 2 profiles had to be high quality with 12 or more full loci. The remaining of the participants were defined as low shedders. M. Goray and R.A.H. van Oorschot [69] categorized the participants into high, intermediate and low shedders based on the amount of DNA and value of RFU. Where high shedders deposited a bigger amount of self-DNA that was then reflected in the profile presentation, with increased donor allele detection and higher allele RFU.

Based on our categorization we found that 20% of the participants were low shedders, 20% were high shedders and 60% were medium shedders. Our results are similar to the results obtained by H. Johannesen et al. [62] where 25% of participants were defined as high and low shedders, and the remaining 50 % was defines as medium shedders.

Amongst the five high shedders in our study there was one male and four female participants. These results do not match the results from previous studies, that men are more often categorized as high shedders than females [69, 72]. However, this could be explained by the small dataset and only 5 participants were male while 15 of the participants were females.

Comparing the shedder groups, low and high shedders were consistently depositing low and high amounts of DNA, respectively. The status of medium shedders was attributed to the participants that did not fit in the other categories. As expected, these participants showed varying results, fluctuating between the low and high shedders, supporting the results from other studies [62, 69].

## 5.2 Transfer and persistence of DNA in correlation to shedder status

Based on the results of the shedder status experiment, the data from the previous experiments were analysed to investigate to what extent the shedder status may have on the different transfer scenarios.

There was a correlation between the shedder groups and the quality of DNA profiles of the direct transfer experiment, both when considering the Log10 LR and Log10 RFU, e.g., high shedders deposit more self-DNA and presented a high number of alleles and RFU. This is in concurrence with a study by M. Goray and R.A.H van Oorschot [69], where they observed that high shedders deposit a higher quantity of self-DNA, with increased donor allele detection and higher allele RFU.

Regarding the second experiment, indirect transfer from inside a used bag, there is no perceptible correlation between the shedder status of the participants and the quality of the obtained profiles. A good example is participant J, that is categorized as a low shedder, but obtained a full DNA profile from the handbag/purse used. This may indicate that the quality of the profile is more impacted by the type of bag and the manner of which the bag is used. This agrees with the findings by M. Goray and R.A.H van Oorschot [69], where shedder status categorization showed significant correlation only to DNA results collected from hand touched surfaces or items but not to DNA results originated from secondary transfer.

For the last experiment, the tape roll experiment, the correlation between the quality of the DNA profiles and the shedder status of the participants is observable. As expected, the wrapper, C1, showed better quality profiles than the handler, C2. It could also be that there is a correlation between the combination of shedder groups in this experiment (e.g., C1 high shedder C2 low shedder), but as the data material was not of sufficient size when divided into three shedder groups this was not further investigated in the present study.

Additionally, the size of our dataset was a limitation. Our study was constituted by twenty participants being enough to study transfer and persistence of DNA. However, when we analyse taking in consideration the shedder status of the participants, the number of participants in each group (high, low and medium) becomes small.

## 5.3 Case example

The main purpose of this study was to create a dataset to provide information on the DNA results that can be expected after different activities that are related and not related to the crime in question. With the case example, we illustrate how the data can be applied in a specific (fictive) case. The examples can be expanded to take into account more factors, as shedder status and we propose this for a further study.

We used a threshold for observing a positive result at RFU>100. These values were chosen based on the quality of the results present in our dataset, that were obtained in the direct and indirect experiments. But we acknowledge that other threshold could be used by other laboratories or that a continues model could be tested in the future.

Based on the results of the probabilities, the LR was calculated for the case example. A value of 3.33 was achieved. This LR value is low, meaning that, with the limitations of our study, it is only 3 times more likely to observe this evidence if the person of interest packed the drugs rather than if the person of interest has no relation to the drugs but previously used the bag. This reflects the findings in our study. Although we observed more and better-quality profiles after direct transfer, we have seen that it is possible to detect a good quality profile from a POI on a bag that has not been touched, but instead has been stored in it previously used bag. Hence, a strong statement supporting the prosecution hypothesis is not possible.

All the results of the case example are limited to the datasets obtained on the experiments and the circumstances of how they were conducted.

# 6 Conclusion

This study has the goal of increasing the knowledge of transfer and persistence of DNA.

Detection of DNA after direct transfer has a higher proportion of positive results in comparison with the indirect transfer results.

DNA from the participant that packed the drug wrap was more frequently detected than the DNA from the participant that previously handled the tape.

We were able to classify the participants as high, low and medium shedders based on a tube holding experiment. The preponderance of the participants was classified as medium shedders.

A correlation between transfer rates of DNA and the shedder status of the participants was observed in all experiments with the exception of experiment 2, indirect transfer. Based on the results obtained from the second experiment, indirect transfer of DNA, it seems that the transfer rates of DNA correspond more with the amount of use and the type of the bag used. Still, more data is needed to take a proper conclusion.

Our data provide valuable information that can aid forensic scientists when evaluation activity related propositions. We have shown that the likelihood ratio at activity level for a case example can be calculated based on our dataset. The likelihood ratios obtained are generally low, since in our dataset direct and indirect transfer have similar probabilities. A different LR value could be obtained if our dataset was bigger, and more parameters were added to the Bayesian Network.

The dataset provides a foundation for building further models for instance by implementing shedder status or other related scenarios.

# References


[1] J. M. Butler, "Chapter 2 - Basics of DNA Biology and Genetics," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 19-41.

[2] T. D. Pollard, W. C. Earnshaw, J. Lippincott-Schwartz, and G. T. Johnson, "Chapter 1 - Introduction to Cells," in *Cell Biology (Third Edition)*: Elsevier, 2017, pp. 3-14.

[3] T. D. Pollard, W. C. Earnshaw, J. Lippincott-Schwartz, and G. T. Johnson, "Chapter 3 - Molecules: Structures and Dynamics," in *Cell Biology (Third Edition)*: Elsevier, 2017, pp. 31-52.

[4] T. D. Pollard, W. C. Earnshaw, J. Lippincott-Schwartz, and G. T. Johnson, "Chapter 6 - Research Strategies," in *Cell Biology (Third Edition)*: Elsevier, 2017, pp. 75-101.

[5] W. D. Goodwin, A. Linacre, and S. Hadi, *An introduction to forensic genetics*, 2nd ed. Oxford: Wiley-Blackwell, 2011, p. 216.

[6] B. R. Korf and A. Sathienkijkanchai, "Chapter 19 - Introduction to Human Genetics," in *Clinical and Translational Science*, D. Robertson and G. H. Williams Eds. San Diego: Academic Press, 2009, pp. 265-287.

[7] J. M. Butler, "Chapter 1 - Overview and History of DNA Typing," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 1-18.

[8] E. Giardina, "DNA Fingerprinting," in *Brenner's Encyclopedia of Genetics (Second Edition)*, S. Maloy and K. Hughes Eds. San Diego: Academic Press, 2013, pp. 356-359.

[9] J. D. Aronson, "DNA fingerprinting on trial: the dramatic early history of a new forensic technique," *Endeavour*, vol. 29, no. 3, pp. 126-131, 2005/09/01/ 2005, doi: https://doi.org/10.1016/j.endeavour.2005.04.006.

[10] D. P. Snustad and M. J. Simmons, *Principles of Genetics*, 6 ed. John Wiley & Sons, 2011, p. 821.

[11] M. Lareu, "Short Tandem Repeats," in *Encyclopedia of Forensic Sciences (Second Edition)*, J. A. Siegel, P. J. Saukko, and M. M. Houck Eds. Waltham: Academic Press, 2013, pp. 219-226.

[12] J. M. Butler, "Chapter 8 - Short Tandem Repeat Markers," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 147-173.

[13] K. Tamaki and A. J. Jeffreys, "Human tandem repeat sequences in forensic DNA typing," *Legal Medicine*, vol. 7, no. 4, pp. 244-250, 2005/07/01/ 2005, doi: https://doi.org/10.1016/j.legalmed.2005.02.002.

[14] A. Jamieson and S. Bader, *A guide to forensic DNA profiling*, Chichester, West Sussex, UK: John Wiley & Sons,, 2016, p. 1 online resource. [Online]. Available: Cover image http://catalogimages.wiley.com/images/db/jimages/9781118751527.jpg.

[15] FBI. "CODIS and NDIS Fact Sheet." FBI. https://www.fbi.gov/services/laboratory/biometric-analysis/codis/codis-and-ndis-fact-sheet (accessed 02/12/2020, 2020).

[16] ENFSI. "The European Network of Forensic Science Institutes." https://enfsi.eu (accessed 02/12/2020, 2020).

[17] D. R. Hares, "Expanding the CODIS core loci in the United States," in *Forensic Sci Int Genet*, vol. 6, no. 1). Netherlands, 2012, pp. e52-4.

[18] NIST. "Core STR Loci Used in Human Identity Testing." https://strbase.nist.gov/coreSTRs.htm (accessed 02/12/2020, 2020).

[19] R. H. Liu, & Shewale, J.G. (Eds.). *Forensic DNA Analysis: Current Practices and Emerging Technologies*, 1st ed. Boca Raton: CRC Press, 2013, p. 445.

[20] C. Brookes, J.-A. Bright, S. Harbison, and J. Buckleton, "Characterising stutter in forensic STR multiplexes," *Forensic Science International: Genetics*, vol. 6, no. 1, pp. 58-63, 2012/01/01/ 2012, doi: https://doi.org/10.1016/j.fsigen.2011.02.001.

[21] P. S. Walsh, N. J. Fildes, and R. Reynolds, "Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA," *Nucleic Acids Res*, vol. 24, no. 14, pp. 2807-2812, Jul 15 1996, doi: https://doi.org/10.1093/nar/24.14.2807.

[22] F. Van Nieuwerburgh, E. Goetghebeur, M. Vandewoestyne, and D. Deforce, "Impact of allelic dropout on evidential value of forensic DNA profiles using RMNE," *Bioinformatics (Oxford, England)*, vol. 25, no. 2, pp. 225-229, 2009, doi: https://doi.org/10.1093/bioinformatics/btn608.

[23] M. Coble and B. Hill, "STR Biology and Artifacts," in *Advanced Topics in STR DNA Analysis*, The Copenhagen Forensic Genetic Summer School, 2012: NIST.

[24] P. Gill, Ø. Bleka, O. Hansson, C. Benschop, and H. Haned, *Forensic Practitioner's Guide to the Interpretation of Complex DNA Profiles*, 1. ed. San Diego: Academic Press is an Imprint of Elsevier, 2020, p. 530.

[25] J. M. Butler, "Chapter 10 - STR Genotyping and Data Interpretation," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 205-227.

[26] J. M. Butler and D. J. Reeder. "Tri-Alellic Patterns." https://strbase.nist.gov/tri_tab.htm (accessed 15/02/2021, 2021).

[27] O. Hansson and P. Gill, "Characterisation of artefacts and drop-in events using STR-validator and single-cell analysis," *Forensic Science International: Genetics*, vol. 30, pp. 57-65, 2017/09/01/ 2017, doi: https://doi.org/10.1016/j.fsigen.2017.04.015.

[28] J. M. Butler, "Chapter 14 - Forensic Challenges: Degraded DNA, Mixtures, and LCN," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 315-339.

[29] T. M. Clayton, J. P. Whitaker, R. Sparkes, and P. Gill, "Analysis and interpretation of mixed forensic stains using DNA STR profiling," *Forensic Science International*, vol. 91, no. 1, pp. 55-70, 1998/01/09/ 1998, doi: https://doi.org/10.1016/S0379-0738(97)00175-8.

[30] P. Gill *et al.*, "DNA commission of the International society for forensic genetics: Assessing the value of forensic biological evidence - Guidelines highlighting the importance of propositions: Part I: evaluation of DNA profiling comparisons given (sub-) source propositions," *Forensic Science International: Genetics*, vol. 36, pp. 189-202, 2018/09/01/ 2018, doi: https://doi.org/10.1016/j.fsigen.2018.07.003.

[31] J. M. Butler, "Chapter 11 - Statistical Interpretation: Evaluating the Strength of Forensic DNA Evidence," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 229-258.

[32] J. M. Butler, R. Schoske, P. M. Vallone, J. W. Redman, and M. C. Kline, "Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations," *Journal of Forensic Science*, vol. 48, no. 4, pp. 908-911, Jul 2003.

[33] Ø. Bleka, G. Storvik, and P. Gill, "EuroForMix: An open source software based on a continuous model to evaluate STR DNA profiles from a mixture of contributors with artefacts," *Forensic Science International: Genetics*, vol. 21, pp. 35-44, 2016/03/01/ 2016, doi: https://doi.org/10.1016/j.fsigen.2015.11.008.

[34] J.-A. Bright *et al.*, "Developmental validation of STRmix™, expert software for the interpretation of forensic DNA profiles," *Forensic Science International: Genetics*, vol. 23, pp. 226-239, 2016/07/01/ 2016, doi: https://doi.org/10.1016/j.fsigen.2016.05.007.

[35] J. M. Butler, "Chapter 2 - DNA Extraction Methods," in *Advanced Topics in Forensic DNA Typing: Methodology*, J. M. Butler Ed. San Diego: Academic Press, 2012, pp. 29-47.

[36] J. M. Butler, "Chapter 5 - DNA Extraction," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 99-109.

[37] A. Alonso, "DNA Extraction and Quantification," in *Encyclopedia of Forensic Sciences (Second Edition)*, J. A. Siegel, P. J. Saukko, and M. M. Houck Eds. Waltham: Academic Press, 2013, pp. 214-218.

[38] J. M. Butler, "Chapter 6 - DNA Quantitation," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 111-124.

[39] T. Scientific. "Real-time PCR: understanding $C_t$ | Thermo Fisher Scientific." https://www.thermofisher.com/no/en/home/life-science/pcr/real-time-pcr/real-time-pcr-learning-center/real-time-pcr-basics/real-time-pcr-understanding-ct.html (accessed 22/02/2021, 2021).

[40] *Essentials of Real-Time PCR*, A. Biosystems, Thermo Fisher Scientific. [Online]. Available: https://www.thermofisher.com/no/en/home/life-science/pcr/real-time-pcr/real-time-pcr-learning-center/real-time-pcr-basics/essentials-real-time-pcr.html

[41] S. Maddocks and R. Jenkins, "Chapter 4 - Quantitative PCR: Things to Consider," in *Understanding PCR*, S. Maddocks and R. Jenkins Eds. Boston: Academic Press, 2017, pp. 45-52.

[42] J. M. Butler, "Chapter 3 - DNA Quantitation," in *Advanced Topics in Forensic DNA Typing: Methodology*, J. M. Butler Ed. San Diego: Academic Press, 2012, pp. 49-67.

[43] *TaqMan Gene Expression Assay solutions*, T. F. Scientific, www.gene-quantification.de, 2018. [Online]. Available: http://assets.thermofisher.com/TFS-Assets/LSG/Application-Notes/taqman-gex-brochure.pdf

[44] J. M. Butler, "Chapter 7 - DNA Amplification (The Polymerase Chain Reaction)," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 125-146.

[45] J. M. Butler, "Chapter 4 - PCR Amplification: Capabilities and Cautions," in *Advanced Topics in Forensic DNA Typing: Methodology*, J. M. Butler Ed. San Diego: Academic Press, 2012, pp. 69-97.

[46] D. Caetano-Anollés, "Polymerase Chain Reaction," 2013, pp. 392-395.

[47] J. M. Butler, "Chapter 6 - Capillary Electrophoresis: Principles and Instrumentation," in *Advanced Topics in Forensic DNA Typing: Methodology*, J. M. Butler Ed. San Diego: Academic Press, 2012, pp. 141-165.

[48] J. M. Butler, "Chapter 9 - Fundamentals of DNA Separation and Detection," in *Fundamentals of Forensic DNA Typing*, J. M. Butler Ed. San Diego: Academic Press, 2010, pp. 175-203.

[49] B. R. McCord and E. Buel, "Capillary Electrophoresis in Forensic Genetics," in *Encyclopedia of Forensic Sciences (Second Edition)*, J. A. Siegel, P. J. Saukko, and M. M. Houck Eds. Waltham: Academic Press, 2013, pp. 394-401.

[50] T. F. Scientific, "DNA Fragment Analysis by Capillary Electrophoresis User Guide," Revision B ed. Applied Biosystems: life technologies™, 2014, p. 220.

[51] *Technical Manual PowerPlex® Fusion 6C System for Use on the Applied Biosystems® Genetic Analyzers Instructions for Use of Products DC2705, DC2720 and DC2780*, P. Corporation, www.promega.com/resources/protocols/, 2018. [Online]. Available: https://www.promega.com/-/media/files/resources/protocols/technical-manuals/101/powerplex-fusion-6c-system-protocol.pdf

[52] J. M. Butler, *Forensic DNA typing : biology, technology, and genetics of STR markers*, 2nd ed. Amsterdam ; Boston: Elsevier Academic Press, 2005, p. 660.

[53] M. Coble and B. Hill, "Fundamentals of Capillary Electrophoresis (CE)," in *Advanced Topics in STR DNA Analysis*, The Copenhagen Forensic Genetic Summer School, 2012: NIST.

[54] P. Gill, "Chapter 1 - Definitions: Contamination and Interpretation," in *Misleading DNA Evidence*, P. Gill Ed. San Diego: Academic Press, 2014, pp. 1-20.

[55] B. Kokshoorn, B. J. Blankers, J. de Zoete, and C. E. H. Berger, "Activity level DNA evidence evaluation: On propositions addressing the actor or the activity," *Forensic Science International*, vol. 278, pp. 115-124, 2017/09/01/ 2017, doi: https://doi.org/10.1016/j.forsciint.2017.06.029.

[56] L. Mayuoni-Kirshenbaum, O. Waiskopf, N. Finkelstein, and Z. Pasternak, "How did the DNA of a suspect get to the crime scene? A practical study in DNA transfer during lock-picking," *Australian Journal of Forensic Sciences*, pp. 1-11, 2020, doi: https://doi.org/10.1080/00450618.2020.1793384.

[57] R. A. H. van Oorschot, B. Szkuta, G. E. Meakin, B. Kokshoorn, and M. Goray, "DNA transfer in forensic science: A review," *Forensic Science International: Genetics*, vol. 38, pp. 140-166, 2019/01/01/ 2019, doi: https://doi.org/10.1016/j.fsigen.2018.10.014.

[58] D. Taylor, A. Biedermann, L. Samie, K.-M. Pun, T. Hicks, and C. Champod, "Helping to distinguish primary from secondary transfer events for trace DNA," *Forensic Science International: Genetics*, vol. 28, pp. 155-177, 2017/05/01/ 2017, doi: https://doi.org/10.1016/j.fsigen.2017.02.008.

[59] A. E. Fonneløp, M. Ramse, T. Egeland, and P. Gill, "The implications of shedder status and background DNA on direct and secondary transfer in an attack scenario," *Forensic Science International: Genetics*, vol. 29, pp. 48-60, 2017/07/01/ 2017, doi: https://doi.org/10.1016/j.fsigen.2017.03.019.

[60] M. Goray, E. Eken, R. J. Mitchell, and R. A. H. van Oorschot, "Secondary DNA transfer of biological substances under varying test conditions," *Forensic Science International: Genetics*, vol. 4, no. 2, pp. 62-67, 2010/02/01/ 2010, doi: https://doi.org/10.1016/j.fsigen.2009.05.001.

[61] M. Goray, R. J. Mitchell, and R. A. H. v. Oorschot, "Investigation of secondary DNA transfer of skin cells under controlled test conditions," *Legal Medicine*, vol. 12, no. 3, pp. 117-120, 2010/05/01/ 2010, doi: https://doi.org/10.1016/j.legalmed.2010.01.003.

[62] H. Johannessen, P. Gill, A. Roseth, and A. E. Fonneløp, "Determination of shedder status: A comparison of two methods involving cell counting in fingerprints and the DNA analysis of handheld tubes," *Forensic Science International: Genetics*, vol. 53, p. 102541, 2021/07/01/ 2021, doi: https://doi.org/10.1016/j.fsigen.2021.102541.

[63] R. A. H. van Oorschot, G. Glavich, and R. J. Mitchell, "Persistence of DNA deposited by the original user on objects after subsequent use by a second person," *Forensic Science International: Genetics*, vol. 8, no. 1, pp. 219-225, 2014/01/01/ 2014, doi: https://doi.org/10.1016/j.fsigen.2013.10.005.

[64] M. van den Berge, G. Ozcanhan, S. Zijlstra, A. Lindenbergh, and T. Sijen, "Prevalence of human cell material: DNA and RNA profiling of public and private objects and after activity scenarios," *Forensic Science International: Genetics*, vol. 21, pp. 81-89, Mar 2016, doi: https://doi.org/10.1016/j.fsigen.2015.12.012.

[65] R. A. H. van Oorschot and M. K. Jones, "DNA fingerprints from fingerprints," *Nature*, vol. 387, no. 6635, pp. 767-767, 1997/06/01 1997, doi: https://doi.org/10.1038/42838.

[66] J. Hedman *et al.*, "Enhanced forensic DNA recovery with appropriate swabs and optimized swabbing technique," *Forensic Science International: Genetics*, vol. 53, p. 102491, 2021/07/01/ 2021, doi: https://doi.org/10.1016/j.fsigen.2021.102491.

[67] S. Hartless, L. Walton-Williams, and G. Williams, "Critical evaluation of touch DNA recovery methods for forensic purposes," *Forensic Science International: Genetics Supplement Series*, vol. 7, no. 1, pp. 379-380, 2019/12/01/ 2019, doi: https://doi.org/10.1016/j.fsigss.2019.10.020.

[68] J. M. Butler, "Chapter 1 - Sample Collection, Storage, and Characterization," in *Advanced Topics in Forensic DNA Typing: Methodology*, J. M. Butler Ed. San Diego: Academic Press, 2012, pp. 1-27.

[69] M. Goray and R. A. H. van Oorschot, "Shedder status: Exploring means of determination," *Science & Justice*, vol. 61, no. 4, pp. 391-400, 2021/07/01/ 2021, doi: https://doi.org/10.1016/j.scijus.2021.03.004.

[70] A. Lowe, C. Murray, J. Whitaker, G. Tully, and P. Gill, "The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces," *Forensic Science International*, vol. 129, no. 1, pp. 25-34, 2002/09/10/ 2002, doi: https://doi.org/10.1016/S0379-0738(02)00207-4.

[71] M. Phipps and S. Petricevic, "The tendency of individuals to transfer DNA to handled items," *Forensic Science International*, vol. 168, no. 2, pp. 162-168, 2007/05/24/ 2007, doi: https://doi.org/10.1016/j.forsciint.2006.07.010.

[72] M. Goray, S. Fowler, B. Szkuta, and R. A. H. van Oorschot, "Shedder status—An analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time," *Forensic Science International: Genetics*, vol. 23, pp. 190-196, 2016/07/01/ 2016, doi: https://doi.org/10.1016/j.fsigen.2016.05.005.

[73] D. Taylor, A. Biedermann, T. Hicks, and C. Champod, "A template for constructing Bayesian networks in forensic biology cases when considering activity level propositions," *Forensic Science International: Genetics*, vol. 33, pp. 136-146, 2018/03/01/ 2018, doi: https://doi.org/10.1016/j.fsigen.2017.12.006.

[74] F. Taroni, *Bayesian networks for probabilistic inference and decision analysis in forensic science*, Second edition. ed. Chichester, West Sussex ; Hoboken, NJ: Wiley,, 2014, p. 1 online resource. [Online]. Available: Cover image http://catalogimages.wiley.com/images/db/jimages/9780470979730.jpg.

[75] J. A. Siegel, P. J. Saukko, and M. M. Houck, *Encyclopedia of forensic sciences*, Second edition. ed. London, UK ; Waltham, MA, USA: Elsevier, Academic Press, 2013, p. 4 volumes.

[76] T. Schaapveld, S. Opperman, and S. Harbison, "Bayesian networks for the interpretation of biological evidence," *Wiley Interdisciplinary Reviews: Forensic Science*, vol. 1, 01/28 2019, doi: https://doi.org/10.1002/wfs2.1325.

[77] D. Taylor, B. Kokshoorn, and A. Biedermann, "Evaluation of forensic genetics findings given activity level propositions: A review," *Forensic Science International: Genetics*, vol. 36, pp. 34-49, 2018/09/01/ 2018, doi: https://doi.org/10.1016/j.fsigen.2018.06.001.

[78] insurly. "Classement 2020 des pays les plus sûrs." https://insurly.fr/indice-risque-pays (accessed 30/09/2020, 2020).

[79] Politiet, "STRASAK-rapporten Anmeldt kriminalitet og politiets straffesaksbehandling," Politiet, 2020. [Online]. Available: https://www.politiet.no/globalassets/04-aktuelt-tall-og-fakta/strasak/2019/strasak-2019.pdf

[80] Politiet and Kripos, "Narkotikastatistikk 1. Halvår 2020," Politiet, 2020. [Online]. Available: https://www.politiet.no/globalassets/04-aktuelt-tall-og-fakta/narkotika/narkotikastatistikk_kripos_1_halvar-2020.pdf

[81] S. d. S. Interna, "Relatório Anual de Segurança Interna - Ano 2019," Sistema de Segurança Interna Gabinete do Secretário-Geral, Governo da República Portuguesa, 2020. [Online]. Available: https://www.portugal.gov.pt/download-

ficheiros/ficheiro.aspx?v=%3D%3DBQAAAB%2BLCAAAAAAABAAzNDA0sAAAQJ%2BleAUAAAA%3D

[82] *Technical Manual PowerQuant® System Instructions for Use of Products PQ5002 and PQ5008*, P. Corporation, www.promega.com/resources/protocols/, 2015. [Online]. Available: https://www.promega.com/-/media/files/resources/protocols/technical-manuals/101/powerquant-system-technical-manual.pdf

[83] P. Gill, Ø. Bleka, and A. E. Fonneløp, "RFU derived LRs for activity level assignments using Bayesian Networks," *Forensic Science International: Genetics*, p. 102608, 2021/10/21/ 2021, doi: https://doi.org/10.1016/j.fsigen.2021.102608.

[84] P. Gill, Ø. Bleka, A. Roseth, and A. E. Fonneløp, "An LR framework incorporating sensitivity analysis to model multiple direct and secondary transfer events on skin surface," *Forensic Science International: Genetics*, vol. 53, p. 102509, 2021/07/01/ 2021, doi: https://doi.org/10.1016/j.fsigen.2021.102509.

[85] S. Berensmeier, "Magnetic particles for the separation and purification of nucleic acids," *Applied Microbiology and Biotechnology*, vol. 73, no. 3, pp. 495-504, 2006/12/01 2006, doi: https://doi.org/10.1007/s00253-006-0675-0.

[86] M. Ramse, "Uttesting av ny sporsikringsmetode for epitelceller fra tekstiler -Examination of a new method to recover epithelial cells from textile surfaces," Master Thesis, Norwegian University Of Life Sciences, 2014. [Online]. Available: http://hdl.handle.net/11250/216545

[87] J. E. L. Templeton, D. Taylor, O. Handt, P. Skuza, and A. Linacre, "Direct PCR Improves the Recovery of DNA from Various Substrates," *Journal of Forensic Sciences*, vol. 60, no. 6, pp. 1558-1562, 2015, doi: https://doi.org/10.1111/1556-4029.12843.

[88] A. Zamir, Y. Cohen, and M. Azoury, "DNA profiling from heroin street dose packages," *Journal Forensic Science*, vol. 52, no. 2, pp. 389-392, Mar 2007, doi: https://doi.org/10.1111/j.1556-4029.2006.00360.x.

[89] A. E. Fonneløp, T. Egeland, and P. Gill, "Secondary and subsequent DNA transfer during criminal investigation," *Forensic Science International: Genetics*, vol. 17, pp. 155-162, 2015/07/01/ 2015, doi: https://doi.org/10.1016/j.fsigen.2015.05.009.

# Attachments

| Participant | Experiment | Amelogenin | DNA quantity | Total number of alleles | Alleles matching participant | Number of unknown alleles | LR | RFU |
|---|---|---|---|---|---|---|---|---|
| A | Exp 1 | XX | 0,0002 | 0 | 0 | 0 | 0,00E+00 | 0 |
| A | Exp 2 | XX | 0,0009 | 20 | 20 | 0 | 1,73E+10 | 98,39 |
| B | Exp 1 | XX | 0,0016 | 3 | 3 | 0 | 7,34E+01 | 19,43478261 |
| B | Exp 2 | XX | 0,0222 | 67 | 46 | 21 | 8,23E+30 | 2823,35 |
| C | Exp 1 | XX | 0,0108 | 46 | 46 | 0 | 3,10E+28 | 1152,913043 |
| C | Exp 2 | XX | 0 | 0 | 0 | 0 | 0,00E+00 | 0,00 |
| D | Exp 1 | XX | 0,0086 | 46 | 46 | 0 | 2,05E+31 | 942 |
| D | Exp 2 | XX | 0,0013 | 22 | 22 | 0 | 6,84E+08 | 104,22 |
| E | Exp 1 | XX | 0,0002 | 0 | 0 | 0 | 0,00E+00 | 0,00 |
| E | Exp 2 | XX | 0 | 0 | 0 | 0 | 0,00E+00 | 0,00 |
| F | Exp 1 | XX | 0,0025 | 25 | 25 | 0 | 4,27E+16 | 170,5652174 |
| F | Exp 2 | XX | 0,0002 | 0 | 0 | 0 | 0,00E+00 | 0,00 |
| G | Exp 1 | XX | 0,001 | 4 | 4 | 0 | 7,57E+01 | 17,91304348 |
| G | Exp 2 | XX | 0 | 0 | 0 | 0 | 0,00E+00 | 0,00 |
| H | Exp 1 | XX | 0,002 | 5 | 5 | 0 | 4,17E+02 | 26 |
| H | Exp 2 | XX | 0,0001 | 0 | 0 | 0 | 0,00E+00 | 0,00 |
| I | Exp 1 | XX | 0,0006 | 0 | 0 | 0 | 0,00E+00 | 5,17 |
| I | Exp 2 | XX | 0,0008 | 33 | 24 | 9 | 2,62E-02 | 201,57 |
| J | Exp 1 | XX | 0,0015 | 14 | 14 | 0 | 1,24E+10 | 70,52173913 |
| J | Exp 2 | XX | 0,0333 | 46 | 46 | 0 | 4,82E+32 | 7733,00 |
| K | Exp 1 | XY | 0,0081 | 46 | 46 | 0 | 8,11E+29 | 1042,173913 |
| K | Exp 2 | XY | 0,0067 | 46 | 46 | 0 | 8,53E+29 | 839,09 |
| L | Exp 1 | XY | 0,0096 | 45 | 45 | 0 | 2,61E+16 | 1130,217391 |
| L | Exp 2 | XY | 0,0003 | 2 | 2 | 0 | 4,01E+00 | 4,52 |
| M | Exp 1 | XX | 0,0428 | 46 | 46 | 0 | 2,37E+09 | 4184,73913 |
| M | Exp 2 | XX | 0,0008 | 5 | 5 | 0 | 8,53E-01 | 17,57 |
| N | Exp 1 | XX | 0,0015 | 19 | 19 | 0 | 2,84E+10 | 108,826087 |
| N | Exp 2 | XX | 0 | 0 | 0 | 0 | 0,00E+00 | 0 |
| O | Exp 1 | XY | 0,0012 | 6 | 6 | 0 | 6,41E+01 | 27,60869565 |
| O | Exp 2 | XY | 0 | 0 | 0 | 0 | 0,00E+00 | 0 |
| P | Exp 1 | XX | 0,0012 | 3 | 3 | 0 | 1,71E+01 | 9,043478261 |
| P | Exp 2 | XX | 0,0003 | 1 | 1 | 0 | 3,81E+00 | 5,17 |
| Q | Exp 1 | XY | 0,0006 | 4 | 2 | 2 | 3,12E-01 | 21,56521739 |
| Q | Exp 2 | XY | 0,0009 | 2 | 2 | 0 | 1,59E+00 | 12,61 |
| R | Exp 1 | XY | 0,0003 | 0 | 0 | 0 | 0,00E+00 | 0 |
| R | Exp 2 | XY | 0,0004 | 0 | 0 | 0 | 0,00E+00 | 0 |
| S | Exp 1 | XX | 0,0018 | 15 | 15 | 0 | 4,79E+09 | 113,8695652 |
| S | Exp 2 | XX | 0,0001 | 0 | 0 | 0 | 0,00E+00 | 0 |
| T | Exp 1 | XX | 0,0012 | 10 | 10 | 0 | 5,67E+04 | 44,52173913 |
| T | Exp 2 | XX | 0 | 0 | 0 | 0 | 0,00E+00 | 0 |

Attachment 1: Dataset from direct and secondary transfer experiments, Experiment 1 and Experiment 2.

| Participant | Total number of alleles | Alleles C1 | Alleles C2 | Mix C1 | Mix C2 | RFU | DNA quantity | RFU x Mix C1 | RFU Mix C2 | DNAq x Mix C1 | DNAq x Mix C2 | LR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0 | 0 | 0 | 0 | 0,00E+00 | 0 | 0,0014 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 0,00E+00 |
| B | 17 | 16 | 0 | 1 | 6,47E-10 | 79,81818182 | 0,0022 | 7,98E+01 | 5,16E-08 | 2,20E-03 | 1,42E-12 | 1,19E+00 |
| C | 38 | 38 | 0 | 1 | 5,43E-10 | 341,6363636 | 0,003 | 3,42E+02 | 1,85E-07 | 3,00E-03 | 1,63E-12 | 1,00E+00 |
| D | 4 | 4 | 0 | 1 | 2,12E-09 | 17 | 0,0008 | 1,70E+01 | 3,61E-08 | 8,00E-04 | 1,70E-12 | 1,00E+00 |
| E | 52 | 46 | 6 | 0,90137 | 9,86E-02 | 915,1363636 | 0,003 | 8,25E+02 | 9,03E+01 | 2,70E-03 | 2,96E-04 | 2,07E+05 |
| F | 21 | 21 | 0 | 1 | 6,43E-09 | 111,6818182 | 0,0024 | 1,12E+02 | 7,18E-07 | 2,40E-03 | 1,54E-11 | 1,00E+00 |
| G | 59 | 43 | 16 | 0,68671 | 3,13E-01 | 609,1363636 | 0,0058 | 4,18E+02 | 1,91E+02 | 3,98E-03 | 1,82E-03 | 7,76E+12 |
| H | 71 | 45 | 26 | 0,38219 | 6,18E-01 | 1345,681818 | 0,0067 | 5,14E+02 | 8,31E+02 | 2,56E-03 | 4,14E-03 | 1,94E+15 |
| I | 53 | 46 | 7 | 0,86529 | 0,13471 | 576,6818182 | 0,0056 | 4,99E+02 | 7,77E+01 | 4,85E-03 | 7,54E-04 | 5,80E+05 |
| J | 4 | 3 | 1 | 2,45E-07 | 0,9999998 | 17,72727273 | 0,001 | 4,34E-06 | 1,77E+01 | 2,45E-10 | 1,00E-03 | 2,25E+01 |
| K | 26 | 26 | 0 | 1 | 2,26E-09 | 238,2727273 | 0,0049 | 2,38E+02 | 5,39E-07 | 4,90E-03 | 1,11E-11 | 1,00E+00 |
| L | 36 | 36 | 0 | 0,97475 | 2,53E-02 | 381,7727273 | 0,0044 | 3,72E+02 | 9,64E+00 | 4,29E-03 | 1,11E-04 | 8,72E-01 |
| M | 46 | 46 | 0 | 1 | 5,97E-09 | 757,6363636 | 0,0089 | 7,58E+02 | 4,52E-06 | 8,90E-03 | 5,32E-11 | 1,00E+00 |
| N | 18 | 16 | 2 | 0,92127 | 7,87E-02 | 130,1818182 | 0,0026 | 1,20E+02 | 1,02E+01 | 2,40E-03 | 2,05E-04 | 2,82E+00 |
| O | 0 | 0 | 0 | 0 | 0,00E+00 | 0 | 0,0002 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 0,00E+00 |
| P | 0 | 0 | 0 | 0 | 0,00E+00 | 0 | 0 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 0,00E+00 | 0,00E+00 |
| Q | 4 | 4 | 0 | 1 | 3,71E-09 | 16,59090909 | 0,0016 | 1,66E+01 | 6,15E-08 | 1,60E-03 | 5,93E-12 | 1,00E+00 |
| R | 36 | 22 | 14 | 0,31153 | 6,88E-01 | 238,6363636 | 0,0038 | 7,43E+01 | 1,64E+02 | 1,18E-03 | 2,62E-03 | 2,74E+11 |
| S | 12 | 4 | 8 | 0,9999997 | 2,59E-07 | 76,45454545 | 0,0017 | 7,65E+01 | 1,98E-05 | 1,70E-03 | 4,40E-10 | 1,70E+10 |
| T | 14 | 8 | 6 | 4,26E-08 | 1,00E+00 | 60,45454545 | 0,0014 | 2,58E-06 | 6,05E+01 | 5,97E-11 | 1,40E-03 | 2,67E+04 |

Attachment 2: Dataset from Experiment 3 transfer and persistence by handling of tape.

| Participant | Experiment | Total RFU | Average RFU | Total number of alleles | Alleles C1 | Alleles C2 | Number of Contributors | Mix C1 | Mix C2 | Mix C3 | LR | RFU x Mix C1 | RFU x Mix C2 | RFU x Mix C3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Exp 41 | 607 | 26,39130435 | 5 | 5 | 0 | 1 | | | | | | | |
| A | Exp 42 | 1362 | 59,2173913 | 7 | 6 | 0 | 1 | | | | | | | |
| A | Exp 43 | 0 | 0 | 0 | 0 | 0 | 1 | | | | | | | |
| B | Exp 41 | 6112 | 265,7391304 | 32 | 26 | 6 | 2 | 0,81351 | 0,18649 | | 1,98E+11 | 4972,17312 | 1139,82688 | |
| B | Exp 42 | 28909 | 1256,913043 | 50 | 43 | 8 | 2 | 0,91428 | 0,08572 | | 2,27E+28 | 26430,92052 | 2478,07948 | |
| B | Exp 43 | 16408 | 713,3913043 | 46 | 38 | 8 | 2 | 0,8727 | 0,1273 | | 6,96E+23 | 14319,2616 | 2088,7384 | |
| C | Exp 41 | 8835 | 384,1304348 | 35 | 31 | 4 | 2 | 0,88344 | 0,11656 | | 4,29E+15 | 7805,1924 | 1029,8076 | |
| C | Exp 42 | 11929 | 518,6521739 | 50 | 42 | 8 | 2 | 0,85499 | 0,14501 | | 4,98E+23 | 10199,17571 | 1729,82429 | |
| C | Exp 43 | 81062 | 3524,434783 | 74 | 46 | 28 | 3 | 0,7546 | 0,22842 | 0,01703 | 1,41E+30 | 61169,3852 | 18516,18204 | 1380,48586 |
| D | Exp 41 | 84834 | 3688,434783 | 50 | 46 | 4 | 2 | 0,986871 | 0,013129 | | 2,05E+31 | 83720,21441 | 1113,785586 | |
| D | Exp 42 | 32840 | 1427,826087 | 46 | 46 | 0 | 1 | | | | | | | |
| D | Exp 43 | 63216 | 2748,521739 | 46 | 46 | 0 | 1 | | | | | | | |
| E | Exp 41 | 21581 | 938,3043478 | 43 | 43 | 0 | 1 | | | | | | | |
| E | Exp 42 | 5022 | 218,3478261 | 25 | 23 | 2 | 2 | 0,8989 | 0,1011 | | 9,80E+11 | 4514,2758 | 507,7242 | |
| E | Exp 43 | 4194 | 182,3478261 | 26 | 26 | 0 | 1 | | | | | | | |
| F | Exp 41 | 17736 | 771,1304348 | 42 | 40 | 2 | 2 | 0,9685 | 0,03149 | | 3,84E+25 | 17177,316 | 558,50664 | |
| F | Exp 42 | 219532 | 9544,869565 | 53 | 46 | 7 | 2 | 0,990098 | 0,009902 | | 9,45E+29 | 217358,1941 | 2173,805864 | |
| F | Exp 43 | 96567 | 4198,565217 | 40 | 32 | 8 | 2 | 0,76995 | 0,23005 | | 2,10E+16 | 74351,76165 | 22215,23835 | |
| G | Exp 41 | 458705 | 19943,69565 | 46 | 46 | 0 | 1 | | | | | | | |
| G | Exp 42 | 17714 | 770,173913 | 44 | 44 | 0 | 1 | | | | | | | |
| G | Exp 43 | 9290 | 403,9130435 | 33 | 33 | 0 | 1 | | | | | | | |
| H | Exp 41 | 20776 | 903,3043478 | 40 | 38 | 2 | 2 | 0,97752 | 0,02248 | | 1,19E+28 | 20308,95552 | 467,04448 | |
| H | Exp 42 | 119116 | 5178,956522 | 55 | 46 | 9 | 2 | 0,96757 | 0,03243 | | 4,60E+35 | 115253,0681 | 3862,93188 | |
| H | Exp 43 | 56753 | 2467,521739 | 55 | 45 | 10 | 2 | 0,93791 | 0,06209 | | 3,46E+34 | 53229,20623 | 3523,79377 | |
| I | Exp 41 | 48686 | 2116,782609 | 44 | 43 | 1 | 2 | 1,00E+00 | 1,00E-08 | | 5,72E+25 | 48686 | 0,00048686 | |
| I | Exp 42 | 24746 | 1075,913043 | 41 | 39 | 2 | 2 | 0,97298 | 0,02702 | | 4,53E+24 | 24077,36308 | 668,63692 | |
| I | Exp 43 | 4354 | 189,3043478 | 20 | 20 | 0 | 1 | | | | | | | |
| J | Exp 41 | 0 | 0 | 0 | 0 | 0 | 1 | | | | | | | |
| J | Exp 42 | 21968 | 955,1304348 | 46 | 0 | 46 | 1 | 8,36E-09 | 1,00E+00 | | 1,00E+00 | 1,84E-04 | 2,20E+04 | |
| J | Exp 43 | 636 | 27,65217391 | 3 | 2 | 1 | 2 | 1,00E+00 | 7,64E-10 | | 1,23E+01 | 6,36E+02 | 4,86E-07 | |
| K | Exp 41 | 322782 | 14034 | 46 | 46 | 0 | 1 | | | | | | | |
| K | Exp 42 | 24616 | 1070,26087 | 38 | 38 | 0 | 1 | | | | | | | |
| K | Exp 43 | 98792 | 4295,304348 | 46 | 46 | 0 | 1 | | | | | | | |
| L | Exp 41 | 360099 | 15656,47826 | 43 | 43 | 0 | 1 | | | | | | | |
| L | Exp 42 | 83544 | 3632,347826 | 40 | 39 | 1 | 2 | 0,998105 | 0,001895 | | 2,87E+29 | 83385,68412 | 158,31588 | |
| L | Exp 43 | 17017 | 739,8695652 | 30 | 27 | 3 | 2 | 0,9227 | 0,0773 | | 4,59E+19 | 15701,5859 | 1315,4141 | |
| M | Exp 41 | 368904 | 16039,30435 | 46 | 46 | 0 | 1 | | | | | | | |
| M | Exp 42 | 302857 | 13167,69565 | 46 | 46 | 0 | 1 | | | | | | | |
| M | Exp 43 | 178998 | 7782,521739 | 46 | 46 | 0 | 1 | | | | | | | |
| N | Exp 41 | 5494 | 238,8695652 | 21 | 16 | 5 | 2 | 0,81416 | 0,18584 | | 5,69E+09 | 4472,99504 | 1021,00496 | |
| N | Exp 42 | 24791 | 1077,869565 | 43 | 38 | 5 | 2 | 0,9338 | 0,0662 | | 2,85E+25 | 23149,8358 | 1641,1642 | |
| N | Exp 43 | 8103 | 352,3043478 | 29 | 29 | 0 | 1 | | | | | | | |
| O | Exp 41 | 0 | 0 | 0 | 0 | 0 | 1 | | | | | | | |
| O | Exp 42 | 1384 | 60,17391304 | 12 | 12 | 0 | 1 | | | | | | | |
| O | Exp 43 | 2296 | 99,82608696 | 14 | 9 | 5 | 2 | 0,1257 | 0,8743 | | 1,26E+00 | 288,6072 | 2007,3928 | |
| P | Exp 41 | 415646 | 18071,56522 | 46 | 46 | 0 | 1 | | | | | | | |
| P | Exp 42 | 58594 | 2547,565217 | 43 | 43 | 3 | 1 | | | | | | | |
| P | Exp 43 | 15114 | 657,1304348 | 36 | 35 | 1 | 2 | 0,97756 | 0,02244 | | 3,45E+23 | 14774,84184 | 339,15816 | |
| Q | Exp 41 | 7962 | 346,173913 | 35 | 16 | 19 | 2 | 0,244 | 0,756 | | 1,18E+01 | 1942,728 | 6019,272 | |
| Q | Exp 42 | 223 | 9,695652174 | 2 | 2 | 0 | 1 | | | | | | | |
| Q | Exp 43 | 4771 | 207,4347826 | 23 | 15 | 10 | 2 | 0,58683 | 0,41317 | | 6,01E+02 | 2799,76593 | 1971,23407 | |
| R | Exp 41 | 28709 | 1248,217391 | 36 | 36 | 0 | 1 | | | | | | | |
| R | Exp 42 | 0 | 0 | 0 | 0 | 0 | 1 | | | | | | | |
| R | Exp 43 | 105 | 4,565217391 | 1 | 1 | 0 | 1 | | | | | | | |
| S | Exp 41 | 1034 | 44,95652174 | 7 | 7 | 0 | 1 | | | | | | | |
| S | Exp 42 | 18998 | 826 | 43 | 41 | 2 | 2 | 0,97635 | 0,02365 | | 2,83E+29 | 18548,6973 | 449,3027 | |
| S | Exp 43 | 5369 | 233,4347826 | 21 | 19 | 2 | 2 | 0,91168 | 0,08832 | | 5,01E+12 | 4894,80992 | 474,19008 | |
| T | Exp 41 | 3885 | 168,9130435 | 21 | 20 | 1 | 2 | 0,93896 | 0,06104 | | 2,62E+11 | 3647,8596 | 237,1404 | |
| T | Exp 42 | 15237 | 662,4782609 | 65 | 39 | 26 | 2 | 0,46356 | 0,53644 | | 4,65E+12 | 7063,26372 | 8173,73628 | |
| T | Exp 43 | 1596 | 69,39130435 | 14 | 14 | 0 | 1 | | | | | | | |

Attachment 3: Dataset from Experiment 4 Shedder Status.