Polymorphisms and commercial amplification kits in forensic genetics

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Introduction

Forensic science is a specialization that aims to help judges and juries solve legal issues, not only in criminal law but also in civil cases. The field is very diverse, crossing the boundaries between lots of disciplines.

DNA analysis has evolved to become an indispensable and routinely part of modern forensic framework, because as all living things contain DNA, and all DNA exhibits variability both among and within species, any biological material associated with a legal case carries in it information about its source.[1]

Extremely sensitive PCR-based techniques are used to analyse the samples, in order to provide unbiased information. The most widely used form of DNA testing involve the genetic variants called short tandem repeats, and there are a large variety of robust commercial kits available for its amplification.[2] But we must also consider that genetic profiles also have interpretation challenges, either to problems inherent to the samples or the procedures.

Genotyping workflow

To obtain a reference DNA profile[3]

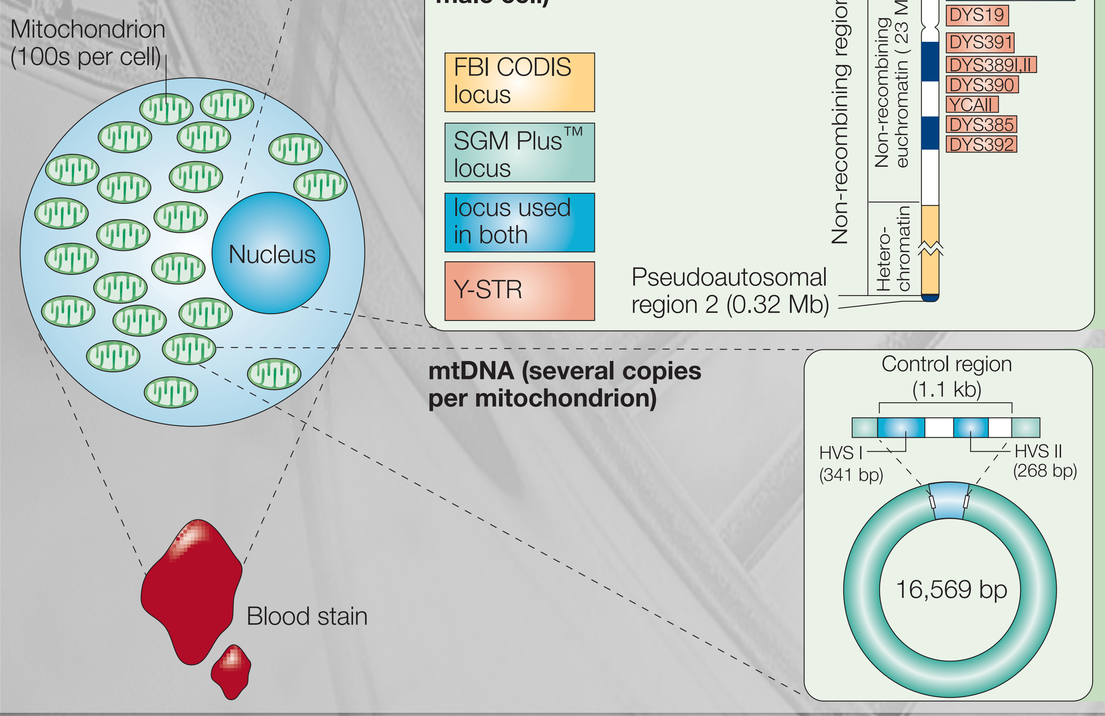
Criminal offense / Humanitarian work → sample analysis → Undoubted (reference) / Dubious (forensic)

Recovery → Extraction → Quantification → Normalization → Amplification → Identification

- Recovery: Orientated and verifying test
- Quantification: Real Time PCR
- Amplification: Multiplex PCR + Capillary electrophoresis → STR allele detection → Determination of genetic profile
- Identification: Comparison of genetic profile with:
  - Other samples
  - Population databases
  - Population genetics Probabilistic calculations → Expert report (CODIS)

Molecular markers: polymorphisms

Figure 1 | **Sources of human genetic variation used in forensic analysis**[1]



Marker types	Source of variation	Advantages	Disadvantages
STRs	Independent chromosomal assortment; recombination; mutation	Extremely high discrimination power	Very degraded DNA difficult to type
SNPs	Independent chromosomal assortment; recombination; mutation - but low rate	Usable on very degraded DNA	Mostly biallelic, so relatively low discrimination power; mixtures difficult to resolve
STRs	Mutation only	Male-specificity; useful in male-female mixtures	Relatively low discrimination power; sharing within patriline; possible population structure problems
SNPs, usually in control region	Mutation only	High copy number, therefore good survival in old/damaged samples	Heteroplasmy; low discrimination power; sharing within matriline; possible population structure problems

Commercially available STR kits[4]

Applied Biosystems (18)	Promega Corporation (17)
~~AmpFlSTR Blue (1996)~~	PowerPlex 1.1 (1997)
~~AmpFlSTR Green I (1997)~~	PowerPlex 1.2 (1998)
Profiler (1997)	PowerPlex 2.1 (1999)
Profiler Plus (1997)	PowerPlex 16 (2000)
COfiler (1998)	PowerPlex ES (2002)
SGM Plus (1999)	PowerPlex Y (2003)
Identifiler (2001)	PowerPlex S5 (2007)
Profiler Plus ID (2001)	PowerPlex 16 HS (2009)
SEfiler (2002)	PowerPlex ESX 16 (2009)
Yfiler (2004)	PowerPlex ESX 17 (2009)
MiniFiler (2007)	PowerPlex ESI 16 (2009)
SEfiler Plus (2007)	PowerPlex ESI 17 (2009)
Sinofiler (2008) - China	PowerPlex CS7 (2009)
Identifiler Direct (2009)	PowerPlex 18D (2011)
NGM (2009)	PowerPlex Y23 (2012)
Identifiler Plus (2010)	PowerPlex 21 (2012)
NGM SElect (2010)	
GlobalFiler (2012)	**PowerPlex Fusion (2012)**

Qiagen (10)
Kits in 2010 primarily selling in Europe.
Due to patent restrictions cannot sell in U.S

Investigator kits
ESSplex
ESSplex SE
Decaplex SE
IDplex
Nonaplex ESS
Hexaplex ESS • HDplex
Triplex AFS QS
Triplex DSF
Argus X-12

The newest ones[5,6]

The 24-locus multiplex system allows co-amplification and fluorescent detection of 23 STR loci and Amelogenin in less than 2 hours, enabling up to 9 orders of magnitude more discrimination power than previous generation kits. They include the STR loci in CODIS and the ESS, as well as five additional loci commonly used in commercial kits.

The difference between the kits PowerPlex Fusion and GlobalFiler is that GlobalFiler utilize a new 6-Dye Matrix Standard, allowing the expansion of available space and the incorporation of additional markers. But in order to make possible the analysis, sequencers should be changed in labs.

Sources of ambiguity in STR interpretation

Problems caused by the sample → Mixtures, inhibitions, contaminations, degradations

Artifacts from PCR amplification

DNA quantification is important prior to multiplex amplification → (Generally 0.5 – 2.0 ng of template DNA is optimal for STR kits) →

Too much DNA
- Off-scale peaks
- Split peaks (+/-A)
- Locus-to-locus imbalance

Too little DNA
- Heterozygote peak imbalance
- Allele drop-out
- Locus-to-locus imbalance

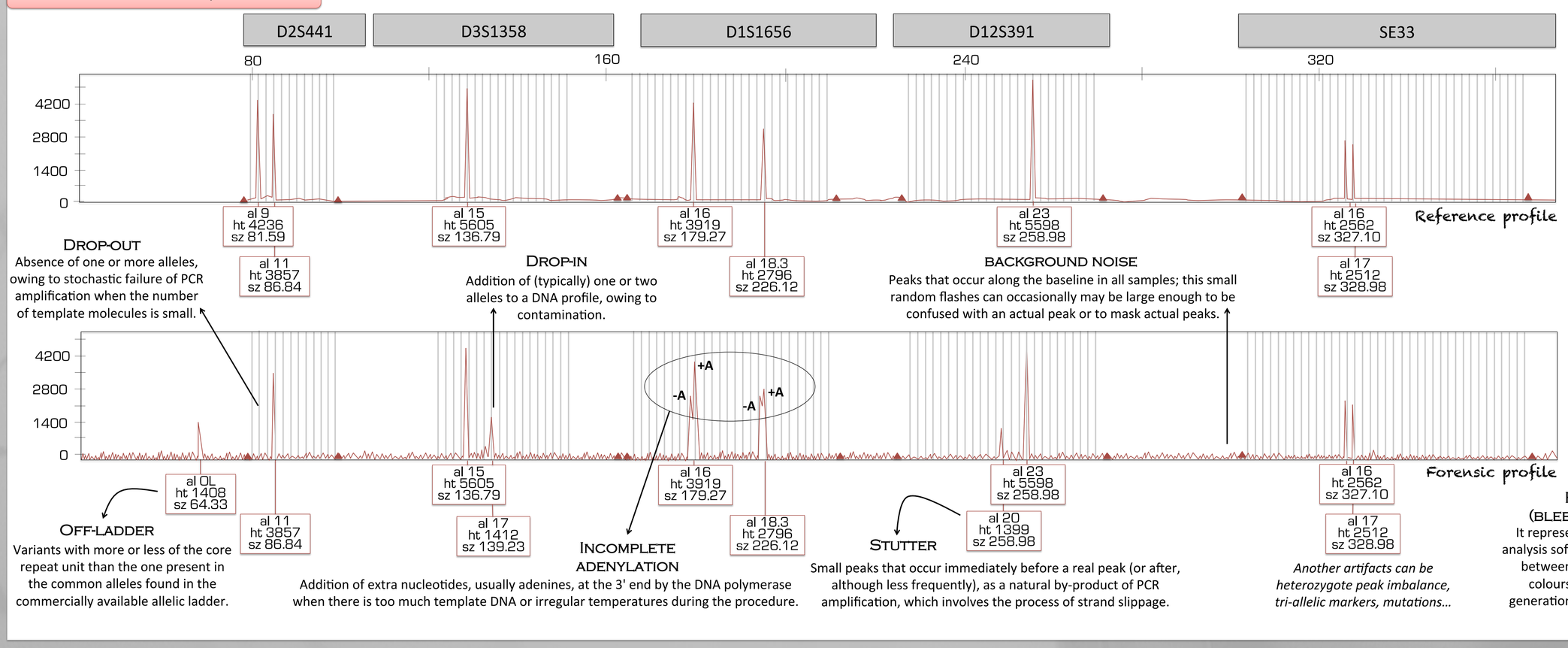


Figure 2 | **Comparison of electropherograms illustrating autosomal STR profiles with abnormalities**

Conclusions

Variability in DNA allows for precise determination of the source, thus analysis of chromosomal and mitochondrial DNA and polymorphisms in the X/Y chromosomes are invaluable to solving cases of dubious paternity and criminal identification in forensics. The availability and robustness of STR commercial kits makes them key aspects of amplification at a forensic scale. However, more studies are needed to implement other methods such as multiplex analysis of SNP. Besides the statistical analysis of data obtained through genetic profiling, it is mandatory to acknowledge and discriminate those problems inherent to samples and the procedure in order to have a precise and coherent result. Thus, the presence of controls in each step of the analysis is vital.

References cited

[1] Jobling, M. a & Gill, P. Encoded evidence: DNA in forensic analysis. *Nat. Rev. Genet.* 5, 739–51 (2004). **[2]** Butler, J. M. Genetics and genomics of core short tandem repeat loci used in human identity testing. *J. Forensic Sci.* 51, 253–65 (2006). **[3]** Solla, L. P. Estudio de polimorfismos de ADN en restos humanos antiguos y muestras forenses críticas: valoración de estrategias y resultados. 218 (2002). at <http://eprints.ucm.es/4594/> **[4]** Hill, B. STR Loci and Multiplex Kits. in 27 (2012). **[5]** Guo, F., Shen, H., Tian, H., Jin, P. & Jiang, X. Development of a 24-locus multiplex system to incorporate the core loci in the Combined DNA Index System (CODIS) and the European Standard Set (ESS). *Forensic Sci. Int. Genet.* 8, 44–54 (2014). **[6]** GlobalFiler. at http://www.lifetechnologies.com/es/en/home/industrial/human-identification/globalfiler-str-kit.html **[7]** William C. Thompson, Simon Ford, Doom, T., Raymer, M. & Krane, D. Evaluating forensic DNA Evidence. *Natl. Assoc. Crim. Def. Lawyers* 16–25 (2003). **[8]** Mccord, B. R. Capillary Electrophoresis in DNA Analysis Steps in STR Genotyping Process. in 14 (2004).