



Approaching variation: an attempt



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Neutral genetic variation among individuals forms the substrate of forensic DNA profiling, a discipline where microsatellites are of major interest. Additionally, the rapid advances in the elucidation of the structure and sequence of the human genome revealed a plethora of novel markers such as polymorphisms at single nucleotide positions (SNPs) or deletions/insertions, which gained significance in forensic science and beyond. Established applications, e.g. genotyping of Y-chromosomal SNPs and sequencing of the mitochondrial control region or parts thereof, fill vital niches, and novel approaches revealing bio-geographic ancestry or physical traits attract growing attention in the forensic scene. However, none of the available genotyping methods perfectly meets all of the diverse needs in everyday genetic testing.

Here, we set out to provide an overview regarding the pros and cons of a number of homogeneous or non-homogeneous SNP typing methods relying on allele-specific or non allele-specific PCR. These assays can be run on instrumentation that is available in most molecular genetic laboratories and either facilitate variant scanning or pinpoint particular SNP sites. Other assays we're lacking hands-on experience (e.g. digital PCR) are not addressed here for this sole reason. ESI-TOF-MS is not dealt with, because of its limited availability in forensic laboratories.

A number of questions shape the decision for or against a particular approach:

- How many samples are to be analyzed, in which time, on what budget?
- How much sample do I have?
- How good/bad is the anticipated DNA quality?
- Do I expect mixed stains?
- Do I need to detect 100% of the variation or can I focus on (a) specific site(s)?
- Will I need multiplexed analyses?
- Which level of experimental complexity is tolerable?
- Which instrumentation is available?

Sanger Sequencing

- still the gold standard
- long cycles
- gel electrophoresis
- short read PCR multiplexing
- expensive DNA
- wide dynamic range
- optimized libraries
- simple design
- no multiplexing
- can be automated
- multiple readouts
- manual interpretation

Next Generation Sequencing (NGS)

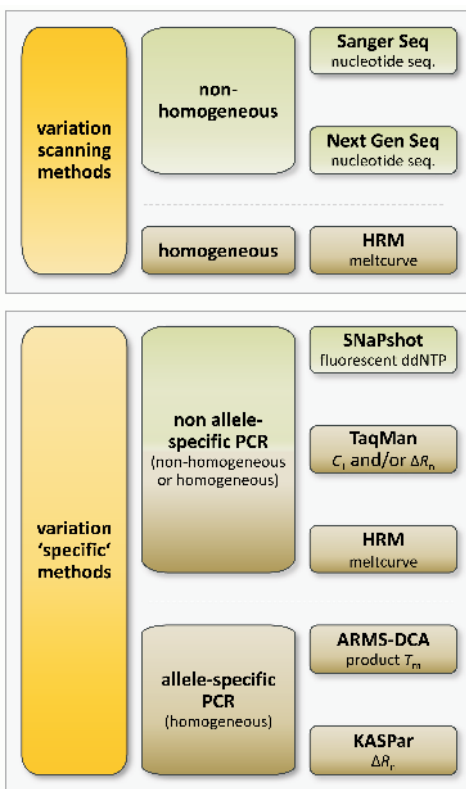
- more, probably the future
- gel electrophoresis
- short/long cycles
- multiplexing
- multiplexing multiple sites
- sample pooling
- variant calling
- detection of rare variants
- detection of rare variants
- detection of rare variants

Single Nucleotide Primer Extension (SNaPshot)

- "missed errors" (primer set)
- gel electrophoresis
- short/long cycles
- multiplexing
- detection of rare variants
- detection of rare variants
- detection of rare variants

5'-3' Exonuclease Hydrolysis of TaqMan Probes

- well established
- TaqMan
- detection of rare variants
- detection of rare variants
- detection of rare variants



High Resolution Melting (HRM) of PCR Amplicons

- low cost method (primers only)
- flexible variant scanning
- simple design
- no multiplexing
- can detect all four SNP classes
- direct molecular haplotyping

Allele-Specific PCR - Dissociation Curve Analysis (ARMS-DCA) (1)

- low cost method (primers only)
- simple design
- can detect all four SNP classes
- direct molecular haplotyping

Allele-Specific PCR - Dissociation Curve Analysis (ARMS-DCA) (2)

- detection of rare variants
- detection of rare variants
- detection of rare variants

KASPar (Kistacence, Huddersdon, UK)

- detection of rare variants
- detection of rare variants
- detection of rare variants

sample quality & quantity	sample number	scanning needed?	multiple sites?	I'm rolling in cash	I'm rather poor and/or lazy
high [DNA]	high	yes	no	Sanger Seq, NGS	HRM (confirmatory Sanger seq)
uniform [DNA]	low	no	yes	NGS, SNaPshot, TaqMan (array card)	ARMS-DCA, HRM, KASPar
no degradation	high	no	no	TaqMan	ARMS-DCA, KASPar, HRM
no mixtures	low	yes	no	Sanger Seq	Sanger Seq
	low	no	yes	any variation specific assay	any variation specific assay
	low	no	no	Sanger Seq	Sanger Seq

sample quality & quantity	sample number	scanning needed?	multiple sites?	I'm rolling in cash	I'm rather poor and/or lazy
low [DNA]	high	yes	no	short amp Sanger Seq (confirming) NGS	(HRM)
wide range of [DNA]	low	no	yes	SNaPshot, NGS, TaqMan (Card)	ARMS-DCA
degradation	high	no	no	short amp Sanger Seq (confirming) NGS	ARMS-DCA
mixed stains	low	yes	no	short amp Sanger Seq (confirming) NGS	(HRM)
	low	no	yes	short amp Sanger Seq (confirming) SNaPshot (if assay is good)	ARMS-DCA
	low	no	no	short amp Sanger Seq (confirming) NGS	ARMS-DCA

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