Approaching variation: an attempt

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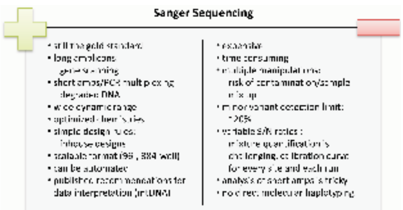
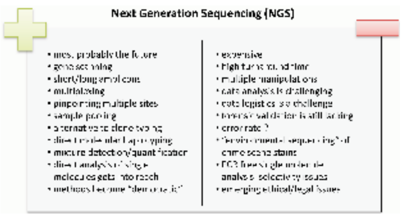
Neutral genetic variation among individuals forms the substrate of forensic DNA profiling, a discipline where microsatellites are of major interest. Additionally, the rapid advances in the elucidation of the structure and sequence of the human genome revealed a plethora of novel markers such as polymorphisms 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.

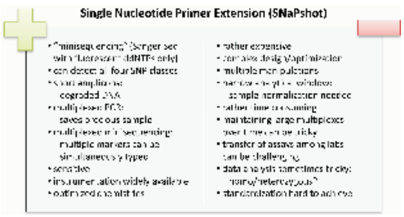
A number of questions shape the decision for or against a particular approach:

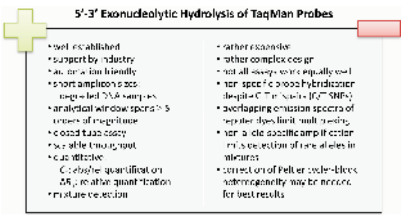
- How many samples are to be analyzed, in which time, on what budget?
- How much sample do I have?
- How good/bad is the anticipated DNA quality?
- Do I expect mixed stains?
- Do I need to detect 100% of the variation or can I focus on (a) specific site(s)?
- Will I need multiplexed analyses?
- Which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

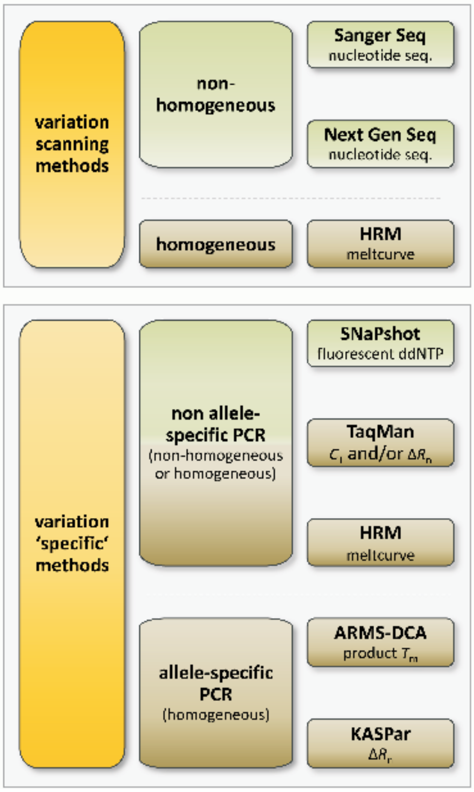
Here, we set out to provide an overview regarding the pros and cons of a number of homogeneous or non-homogeneous SNP typing methods relying on allele-specific or non allele-specific PCR. These assays can be run on instrumentation that is availbale in most molecular genetic laboratories and either facilitate 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.

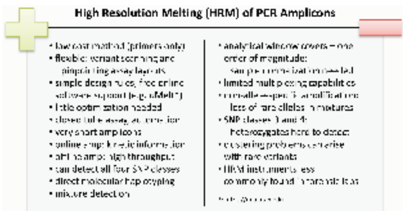


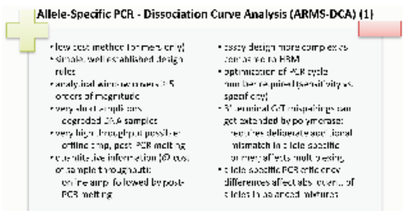


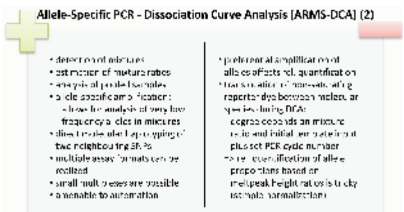


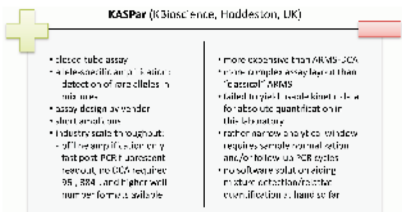












sample quality & quantity	sample number	scanning needed?	multiple sites?	I'm rolling in cash	I'm rather poor and/or lazy
high [DNA] uniform [DNA] no degradation no mixtures	high	yes		Sanger Seq, NGS	HRM (+ confirmatory Sanger Seq)
		no	yes	NGS, SNaPShot TaqMan (Array Card)	ARMS-DCA, HRM, KASPar
		no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

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

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