SNaPshot® Multiplex Systems for Reconstructing Hair, Iris, and Skin Pigmentation from Single Samples of Degraded DNA

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Introduction

DNA phenotyping is a biomolecular technique that involves analysing an individual's physical appearance based on genetic marker variation (Kayser et al. 2023), which is relevant in forensics but also in provenance research and museum contexts. Combining single-base extension with capillary electrophoresis, SNaPshot® enables straightforward sequencing of single nucleotide polymorphisms (SNPs) required for the analysis of phenotypic traits (Fondevila et al. 2017). The SNaPshot® method offers an accessible approach for DNA phenotyping, particularly for laboratories processing small sample numbers (Mehta et al. 2016), making high-throughput techniques such as the Dynamic Array™ IFC by Fluidigm® less suitable. In contrast, previous research has explored genome-wide SNP typing of ancient DNA (aDNA) using the Fluidigm® system, highlighting its potential for high-throughput applications (Schmidt et al. 2020).

My master's thesis focused on revising, optimising, and implementing three SNaPshot® multiplex systems suitable for aDNA analyses (IrisPlex, HairPlex, SkinPlex) that were initially developed at the Department of Historical Anthropology and Human Ecology in Göttingen. Additionally, the analysis of SNP genotyping results using different phenotyping webtools was assessed.

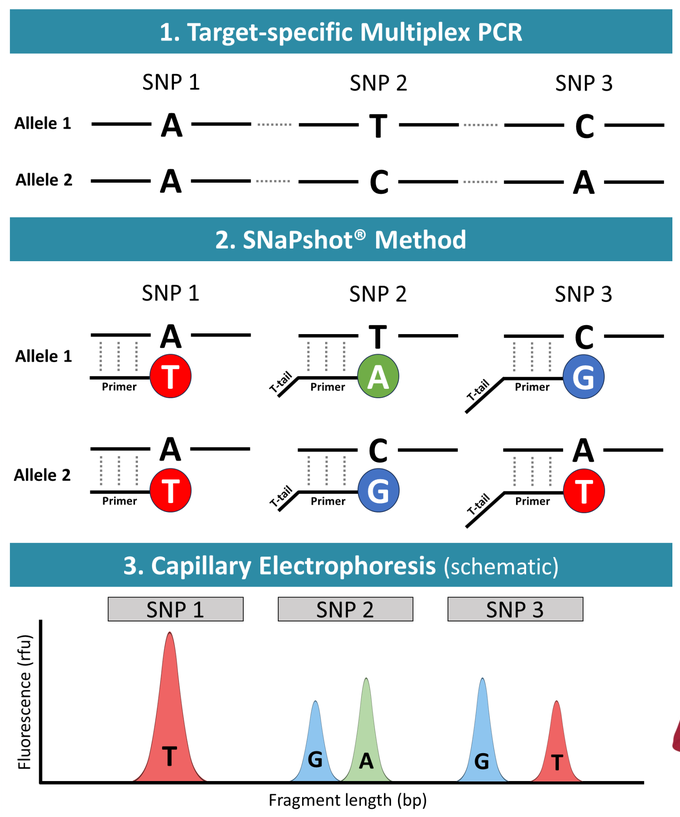


Fig. 1: The three main steps of a SNaPshot® analysis (based on Fondevila et al. 2017).

The SNaPshot® Method

There are three main steps to a SNaPshot® analysis (Fig. 1):

1. Target-specific Multiplex PCR

The target-specific multiplex PCR is performed to amplify the DNA regions around the SNP loci of interest. The SNP variations (alleles) may be either homozygous or heterozygous.

2. SNaPshot® Reaction

The amplicons of the target-specific multiplex PCR are used as templates in a single-base extension step. Per SNP, only one primer is used that needs to be directly located beside the respective SNP. Solely the desired SNP base is extended through the elongation of only one fluorescent-labelled dideoxynucleotide triphosphate (ddNTP), leading to an immediate strand termination. Specific SNaPshot® primers were designed with a non-binding 5'-end sequence tail (T-tail) to ensure amplicon differentiation based on fragment length differences. This allows for a multiplex approach.

3. Capillary Electrophoresis

The SNaPshot® products are analysed through capillary electrophoresis. The final electropherogram depicts the specific base variation of each SNP allele according to the extended fluorescent-labelled ddNTP. Each SNP locus can be identified via its fragment length.

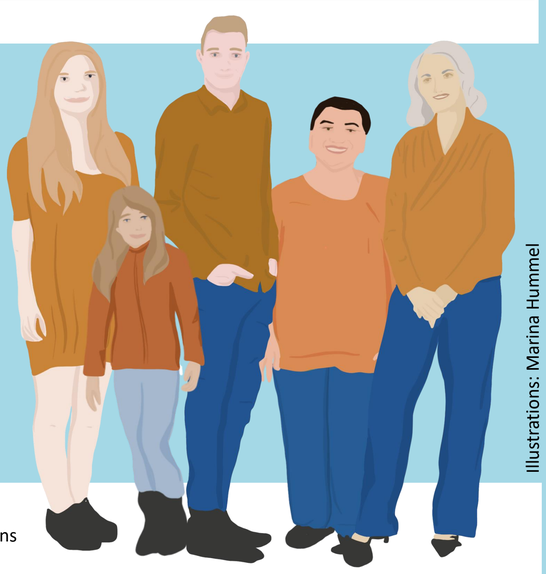
Genotyping & Phenotyping

All three SNaPshot® multiplex systems (IrisPlex, HairPlex, SkinPlex) for the analysis of iris, hair, and skin pigmentation proved to be suitable for processing recent and aDNA samples on the same level of accuracy as high-throughput Fluidigm® analyses. The systems each comprise 6-8 highly informative SNPs (Fig. 2). Recent DNA samples (n = 57) representing diverse phenotypes were used to verify the systems' functionality. Subsequently, the systems were successfully tested with aDNA samples from the Lichtenstein Cave and Boilstädt. Only minor artefact formation was observed for all three systems, which was evaluated and reported in order to facilitate the evaluation of future analyses. Guidelines for SNaPshot® analyses were established, emphasising the influence of webtools (*HIrisPlex-S*; *Snipper*) in translating SNP genotypes into phenotypes. *HIrisPlex-S* proved most suitable for eye and hair colour prediction, while for skin pigmentation algorithm 2 and 3 from *Snipper* were favourable (Tab. 1).

Fig. 2: Electropherograms of the IrisPlex, HairPlex, and SkinPlex from samples of recent DNA.

Tab. 1: Phenotype matching rate for phenotyping with the *Snipper* and *HIrisPlex-S* webtools based on the analysis of recent DNA samples.

SNaPshot® system	*Snipper*				*HIrisPlex-S*
	Algorithm 1: Naive Bayes	**Algorithm 2**: Genetic dist. (allele freq.)	**Algorithm 3**: Genetic dist. (genotype freq.)	**Algorithm 4**: Multinomial logistic regr.	
IrisPlex	91.2 %	89.5 %	89.5 %	82.5 %	96.5 %
HairPlex	86.0 %	87.7 %	84.2 %	80.7 %	96.5 %
SkinPlex	84.2 %	91.2 %	91.2 %	82.5 %	not applicable



Illustrations: Marina Hummel

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Literature

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