

## THE NEXT GENERATION SEQUENCING PANEL FOR DNA TYPING OF CHALLENGING SAMPLES

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Next Generation Sequencing (NGS) has opened new possibilities in human individual identification. However, forensic analysis using NGS technology is challenging, as the DNA is often present in low copy number, highly degraded and contaminated. These features limit the quality and quantity of the usable DNA, and will thus require a highly accurate, reproducible, and robust NGS assay. Moreover, as mixtures are commonly seen in forensic analysis, it would be beneficial if determination as well as quantification of mixture components can be performed. Therefore, we have designed and evaluated a new NGS panel for analyses of severely degraded or mixed DNA samples. The final goal is to optimise next generation sequencing for human identification and to achieve as much genetic information as possible from challenging forensic samples.

Our NGS panel allows simultaneous analysis of over 600 markers including autosomal STRs, InDels, SNPs and the entire mtDNA genome. The included SNPs were selected for identification purpose but also to provide information about externally visible characteristic (ECV) as eye, hair and skin colour, as well as ancestry (ancestry informative markers - AIMs). Some SNPs are, moreover, located on X and/or Y chromosome in order to use the panel in sexual assault investigations. Tests of sensitivity and discrimination power of the panel were performed on high-quality DNA, as well as degraded and mixed DNA samples. Aged and degraded DNA was extracted from skeletal remains of Copernicus, Carin Göring, and teeth from Viking-age burials. Moreover, to simulate sexual assault samples, DNA from sperms and female epithelial cells were mixed in a ratio 1:5. The customized panel is based on a target selection with Agilent's HaloPlex system, and the sequencing is performed using a MiSeq system (Illumina).

In cases without a suspect, the panel markers that provide a possibility to perform a prediction of eye, hair and skin colour will be helpful. In our study it was possible to obtain information regarding externally phenotypic characteristic, and ancestry for many of the tested individuals. Overall, a majority of the samples could be analysed, although the success rate for the entire panel was highly correlated with the quality of the DNA samples, but at a minimum mtDNA data was obtained for all samples. Our NGS system has with these preliminary data revealed information useful for identification, phenotype prediction and even interpretation of mixtures despite challenges.