FORENSIC VALIDATION OF THE PRECISION ID mtDNA WHOLE GENOME PANEL USING THE ION CHEF AND THE ION S5 SYSTEM

Abstract: 170050

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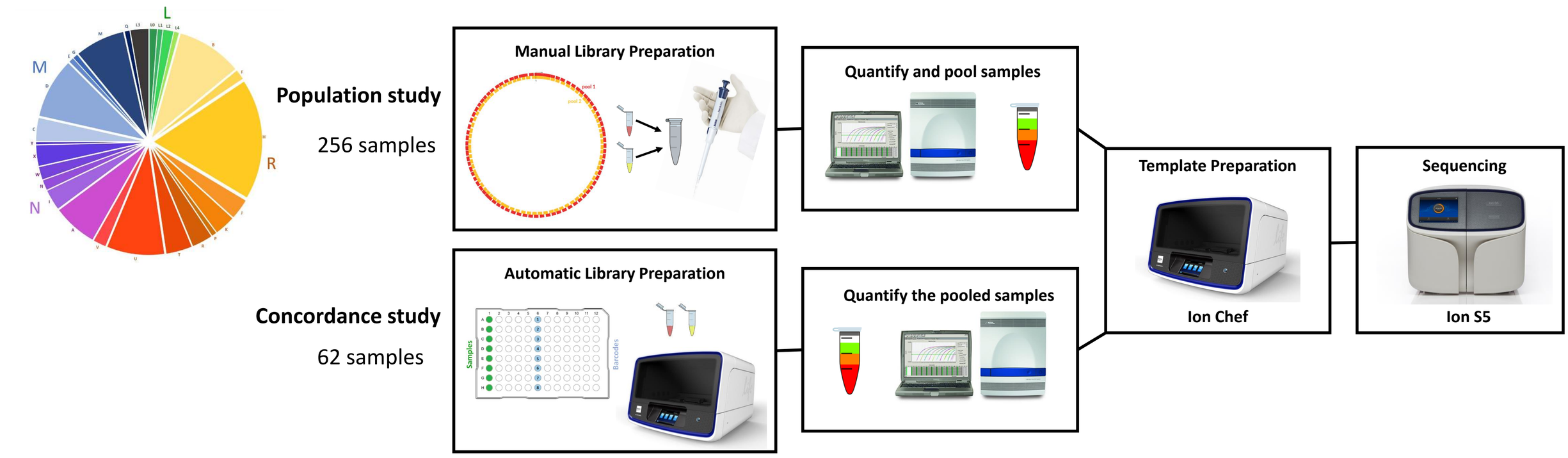
Introduction

In challenging cases where nuclear DNA markers fail to give adequate results, mitochondrial DNA can provide useful information especially when mitogenome sequencing is performed. Such degraded samples require the use of short amplicons, which can be achieved using the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific, CA). This kit allows sequencing the entire mitogenome using 162 amplicons with a maximum length of 175bp. The following study presents initial results from the developmental validation of the Precision ID kit processed with the Ion Chef Instrument and the Ion S5 System according to the manufacturer's recommended protocol.

Materials and Methods

Population study

A total of 256 known samples were selected according to specific mutation patterns defining as many mtDNA haplogroups as possible. The aim was to determine if all haplogroup specific mutations could be called correctly by the Precision ID kit. First, the samples were sequenced in sets of 32 on a 530 Chip, followed by doubling the sample input to 64 to test the effect of increased sample number per chip. Data analysis for the population and concordance studies was done with the Ion Torrent software, Torrent Suite 5.2.1, using the variant caller (v5.2.1.38).

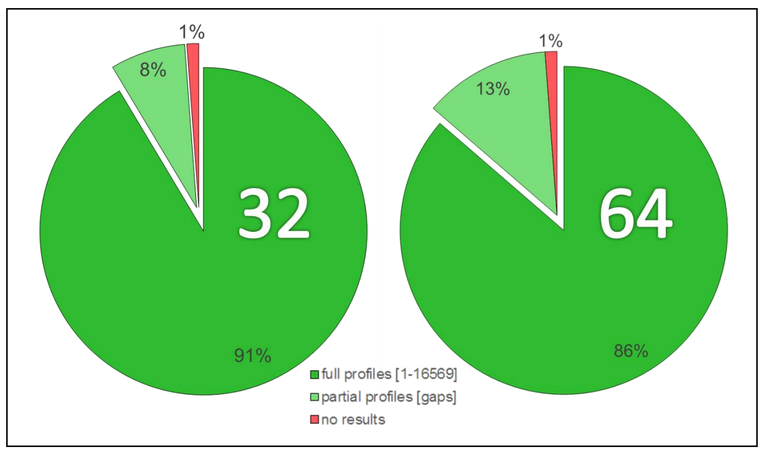


Concordance study

For the concordance study 62 additional samples were used of which 56 mitogenomes were sequenced with Sanger and six with Illumina MiSeq. These results were compared to the ones generated with the Ion S5 System. Length heteroplasmy in poly-C stretches were excluded.

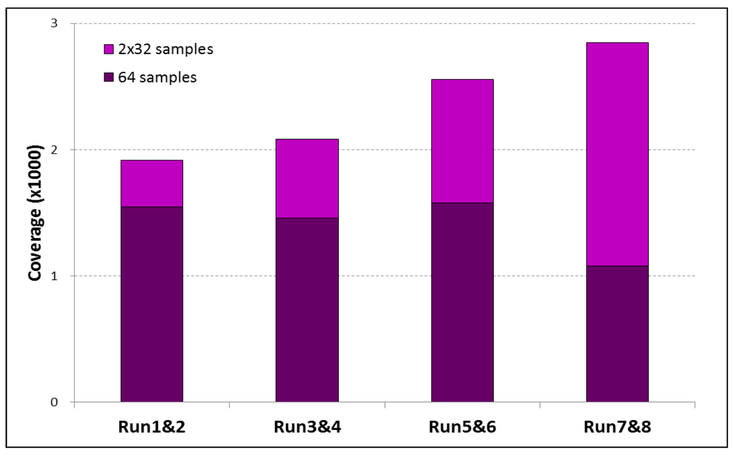
Results

Population study



Out of 256 samples, profiles were obtained for 253 samples with 234 yielding the entire mitogenome. Increasing the number of samples from 32 to 64 per chip resulted in a slight decrease in the number of full profiles obtained.

Depending on the run, the mean coverage value decreased by 19% to 62% when the number of samples per chip was doubled.



Concordance study

The results from all samples matched the ones previously obtained. From a total of 13 point heteroplasmies, three (189R, 6345Y, 9389R) were newly detected in three different samples using the Ion S5 System. These heteroplasmies could not be initially confirmed by Sanger Type Sequencing (STS) due to the sensitivity limitations of the technique.

Discussion

This study presents the initial results from the developmental validation study of the Precision ID mtDNA Whole Genome Panel on the Ion S5 instrument. The population study demonstrated that the kit and the selection of primers amplified and sequenced representatives of all haplogroups. The effect of doubling the samples per run from 32 to 64 resulted in a mild reduction of mean coverage values. The results from the concordance study showed that this highly sensitive method can be useful to detect point heteroplasmies which would be missed with traditional STS. Additional experiments will be performed on this kit to provide a comprehensive overview of its performances.

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