Application of a novel rapid extraction system for the generation of template libraries sequence-able on the Roche 454 NGS platform.

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INTRODUCTION

Next Generation Sequencing (NGS) technologies seem to be gaining momentum in routine clinical diagnostic, although a simplification of the inherent workflow will be needed before their application in high-throughput diagnostics becomes a reality.

Fragmentation of DNA is of key importance for preparation of template libraries for NGS and the purification of the template is a bottleneck in the workflow, and a reason why uptake in clinical situations has been limited to date.

In this study we evaluated the purification of target DNA from blood using a novel, rapid, two-step process and assessed performance through random enzymatic digestion of long-range PCR products sequenced via NGS. This method produced a good-quality sequencing library for the 454 platform used to test the single nucleotide polymorphisms (SNPs) located in chromosome 1 [Chr 1 (hg19)]. The method showed good performance in terms of overall sequence quality (PHRED) and read length.

RESULTS

In total, **42,200** reads passed the internal GS Junior quality filter, the reads comprised approximately **13,000,000** bp of DNA sequence.

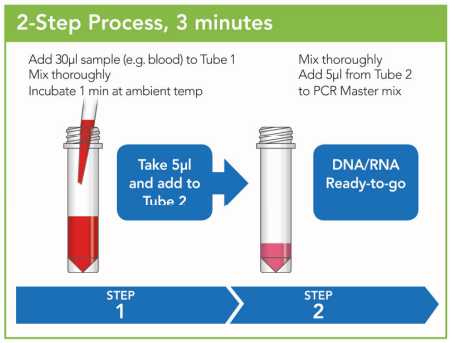

Figure 1. Ultra rapid sample prep process

Eight overlapping fragments ranging between 1,000 and 9,000 bp, completed the hRNAse-P gene and were amplified from a single extract of genomic DNA.
Fragments were titrated and pooled to generate the sequencing library introducing the labelled amplicons (Roche Multiplex Identifier, MID) and sequenced in Roche GS Junior device

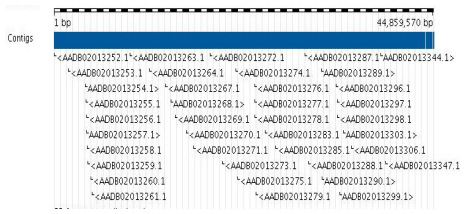

Figure 2. Contigs aligned to reference sequence. Lineage: Eukaryota, Metazoa, Chordata, Craniata, Vertebrata, Euteleostomi, Mammalia, Eutheria, Euarchontoglires, Primates, Haplorrhini, Catarrhini, Hominidae, Homo

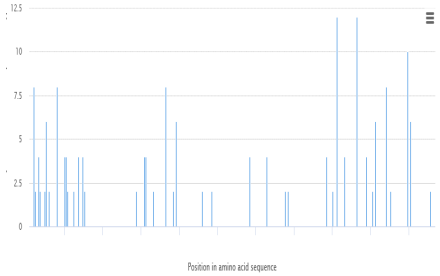

Figure 3. SNP position vs frequency across the curated sequences in %

METHODS

Genomic DNA was isolated from peripheral human blood cells by the ARCIS 3-minute extraction process [Arcis DNA Sample Prep Kit, REF:UFL002]. Oligonucleotide primers for long-range PCR were designed using and ordered from IDT. Eight overlapping fragments ranging between 1,000 and 9,000 bp, covering the entire hRNAse-P gene, were amplified from a single extract of genomic DNA. PCR was performed with a long-range protocol using the Expand Long-Range kit (Roche) for consistent amplification of extra-long templates. The resulting purified amplicons were quantified using a NanoDrop™ photometer before being pooled in equimolar amounts.

The generation of sequencing libraries was performed according to Roche standard protocols, introducing MIDs and subsequent sequencing on the Roche GS Junior Sequencer.

CONCLUSIONS

This initial piece of work shows compliance of the ARCIS rapid DNA extraction process with NGS in the system described. The sample prep protocol was completed in less than 3 minutes. Further comparison of this approach with the standards currently available in the market will define the window of applicability for ARCIS extraction technology to the different approaches of NGS in routine clinical diagnostics.

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