Human Neutrophil Antigen Genotyping by Next Generation Sequencing

NHS Blood and Transplant

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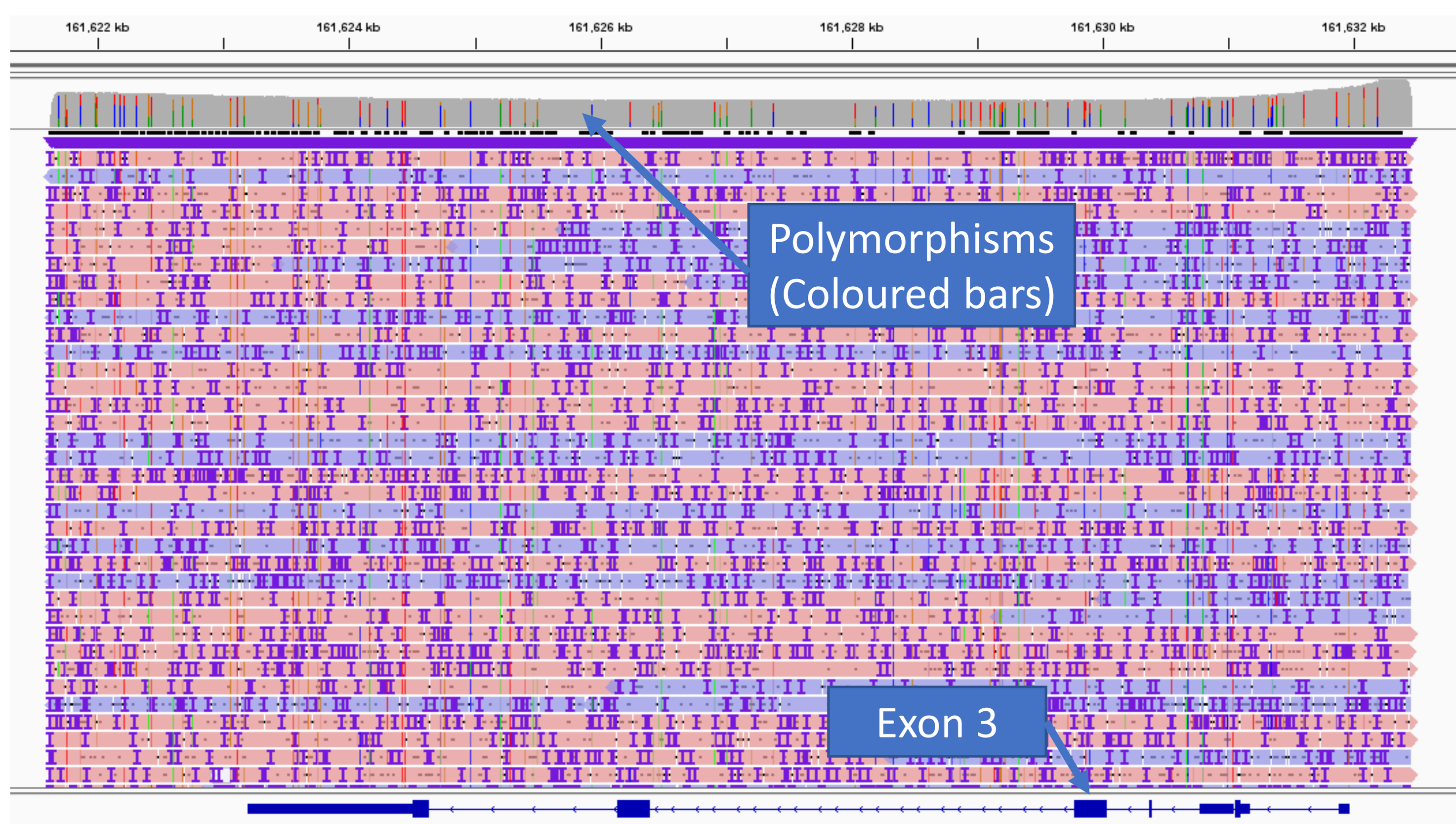
Background

Next generation sequencing (NGS) methods have been designed for high-throughput, massively parallel DNA analysis. An NGS approach can allow more sequencing to be performed and data analysed. Full human neutrophil antigen (HNA) gene analysis would permit greater understanding of the polymorphic nature of the HNA genes allowing the characterisation of known and identification of novel, clinically relevant, polymorphism.

Results

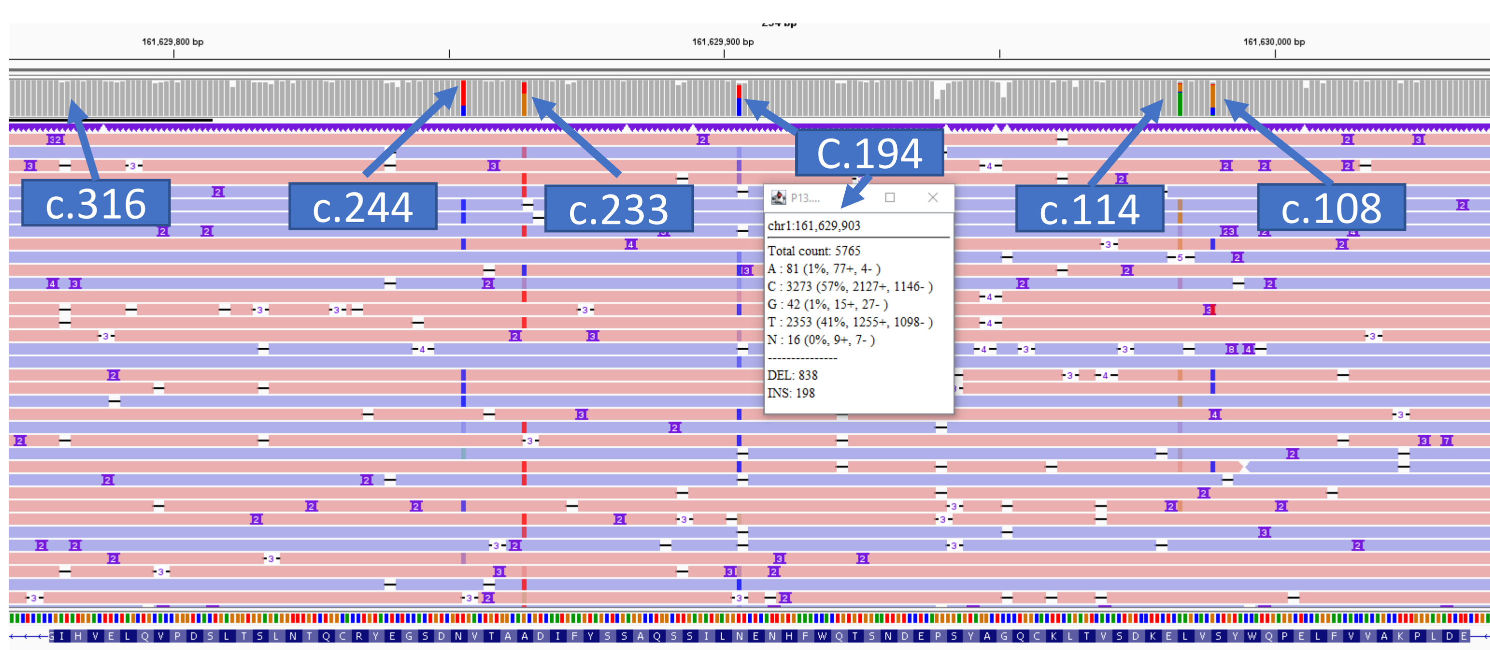
Samples were multiplexed and assigned alleles which corresponded with historic genotypes for HNA-1, -3, -4 and -5 characterised by in -house sanger sequencing based approach. Currently there are no alleles described for HNA-2. An average read depth of >3,000X was obtained for complete HNA-1 and -2 genes (*FCGR3B* and *CD177*, respectively) and the exon of interest for HNA-3, -4 and -5 (*SLC44A2, ITGAM* and *ITGAL*, respectively).

Example of whole FCGR3B gene sequencing



Integrated Genomics Viewer (IGV, Broad Institute, USA) of FCGR3B gene (shown 3'-5' as gene is reverse transcribed). Each read is a single sequenced DNA amplicon ~10.5kb , pink = sense, purple = anti-sense. Read depth ~11,500X across the whole gene.

FCFR3B exon 3 of above sequencing



IGV (Broad Institute, USA) of FCGR3B exon 3. The coloured bars on the top row indicate the polymorphisms shown. In the example highlighted in the text box (c.194) this position is heterozygous C/T (G/A in nomenclature due to sequence orientation). It is possible to remove cis/trans ambiguity as the polymorphisms can be observed on a single DNA fragment.

Example of whole CD177 gene sequencing

IGV (Broad Institute, USA) of CD177 gene. Each read is a single sequenced DNA amplicon ~9kb. Read depth ~12,000X across the whole gene.

Methods

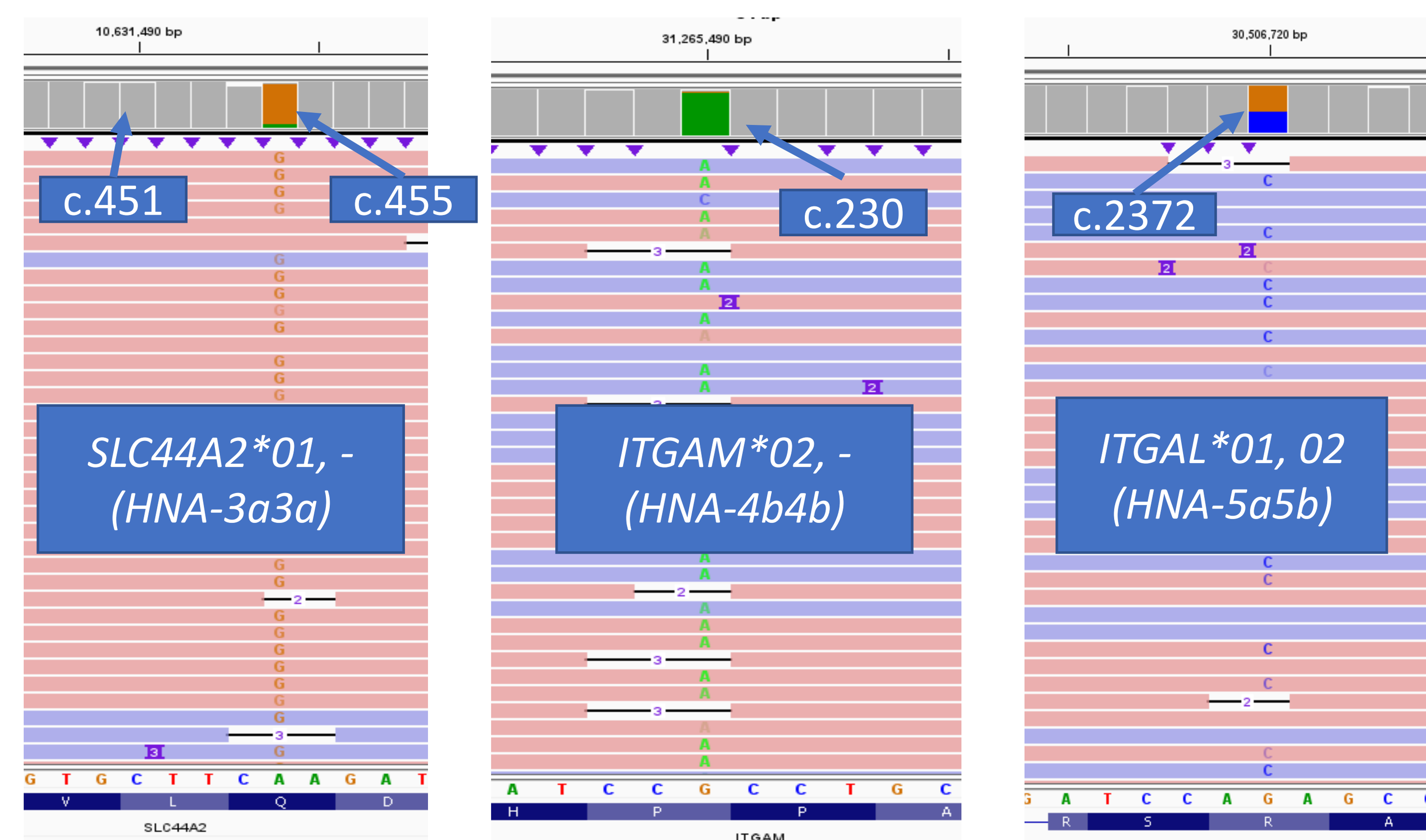
Long range PCR for HNA to obtain complete gene template (HNA-1/*FCRG3B* and -2/*CD177*) and coverage of known clinically relevant exons (HNA-3/*SCL44A2*, -4/*ITGAM* and -5/*ITGAL*) for NGS were performed. PCR amplicons were pooled, a sequencing library was prepared by ligation (LSK-109 and EXP-NBD104, Oxford Nanopore Technologies (ONT), UK) and sequencing performed on an ONT R9.4 MinION flow cell. Base calling was performed by MinKnow (ONT), alignment performed by SAMtools and minimap2, variant calling by Medaka (ONT) and analysed in the Integrated Genomics Viewer (IGV, Broad Institute, USA).

Examples of assigned HNA allelic genotype

Sample	HNA-1 (FCGR3B)	HNA-3 (SLC44A2)	HNA-4 (ITGAM)	HNA-5 (ITGAL)
S13	*02, *03, *04	*01, -	*01, -	*01, *02
S42	*01, *02	*01, *02	*01, -	*01, *02
S46	Null (NS)	*01, -	*01, -	*01, *02
S63	*02, -	*01, *02	*02, -	*01, *02
S79	*01, -	*01, *02	*01, *02	*01, -
S111	*02, *03, *04	*01, *02	*01, -	*01, *02

Allelic assignment of tested samples from NGS sequencing data, in all cases agreed with historic allele assignment. HNA-2 is not included as there are currently no alleles defined in the nomenclature. NS= no sequencing reads obtained. S46 was confirmed CD16b/FCGR3B negative by flow cytometry.

Example of SLC44A2, ITGAM and ITGAL sequencing



IGV (Broad Institute, USA) of SLC44A2 (left), ITGAM (middle) and ITGAL (right) regions of interest to assign alleles based on current genotyping. Sequence reads are 0.8-1kb and read depth >70,000X. Development of whole gene sequencing is in development.

Conclusion

NGS of HNA genes permits both increased sample throughput and complete gene analysis by which known and potentially novel polymorphism may be detected. The method can be performed in less than two working days from DNA extraction to compete analysis. It is scalable and cost is comparable to single exon sanger sequence-based typing ((£49/sample based on 12 samples a run vs £45/sample current, single exon, in-house method). Further work to allow complete gene sequencing for all five HNA genes and further development of the bioinformatics analysis pipeline would make this method suitable for validation for routine laboratory use. This proof of principle could also be applied to other genetic systems e.g. human platelet antigens.