HLA SEQUENCING USING SMRT TECHNOLOGY – HIGH RESOLUTION AND HIGH THROUGHPUT HLA GENOTYPING IN A CLINICAL SETTING

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CONCLUSIONS

- SMRT sequencing of full length/long HLA sequences allows for high resolution allele specific HLA typing
- SMRT sequencing enables complete haplotype phasing and determination of ambiguous genotypes
- SMRT sequencing of Class I and II HLA genes can be readily multiplexed, allowing for a quick turn around time
- Data from Pacific Biosciences analysis pipelines can be readily input into Gendx *NGS*engine software to generate genotype calls

INTRODUCTION

Sequence based typing (SBT) is considered the gold standard method for HLA typing. Current SBT methods are rather laborious and are prone to phase ambiguity problems and genotyping uncertainties. As a result, the NGS community is rapidly seeking to remedy these challenges, to produce high resolution and high throughput HLA sequencing conducive to a clinical setting.

Today, second generation NGS technologies are limited in their ability to yield full length HLA sequences required for adequate phasing and identification of novel alleles. Here we present the use of single molecule real time (SMRT®) sequencing as a means of determining full length/long HLA sequences. Moreover we reveal the scalability of this method through multiplexing approches and determine HLA genotyping calls through the use of third party Gendx NGSengine® software.

OBJECTIVES

- To generate long HLA sequences for high resolution HLA genotyping and phasing for 12 previously SBT typed patients
- To sequence full length Class I loci and the relevant exons of Class II (DQB1,DRB1) through multiplexing on one SMRT cell
- To evaluate the use of GenDx NGS-go® primers & GenDx *NGS*engine® software for HLA genotyping

METHODS

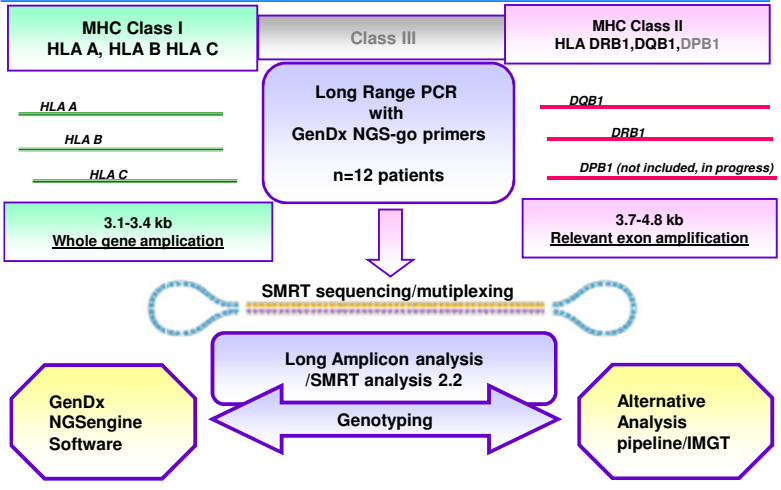

Fig.1. Flow diagram of HLA genotyping. The LAA pipeline in SMRT analysis 2.2 generated clusters and consensus sequences for each HLA allele. Such sequences were then input to GenDx software or analysed via an alternative in-house pacific biosciences pipeline and the genotyping confirmed by comparison with the IMGT-HLA data base.

STUDY DESIGN

SMRT Sequencing

TRIAL 1 Single amplicon Sequencing
- 12 patients
- Class I
- Class II
- 1 amplicon/cell

TRIAL 2 Multiplexing of Class I
- 8 patients
- HLA A, B,C
- 24 amplicons/cell
- Pooled & barcoded

TRIAL 3 Multiplexing of Class I and II
- 2 patients
- HLA A,B,C,
- HLA DRB1,DQB1
- 10 amplicons/cell
- Pooled & barcoded

TRIAL 4 *On-going*
- Extensive Multiplexing
- Full-length Class II loci Multiplexing
- Trials with alternative primer sets

RESULTS

- SMRT sequencing provided full length/long sequences allowing for high-resolution HLA allele level genotyping

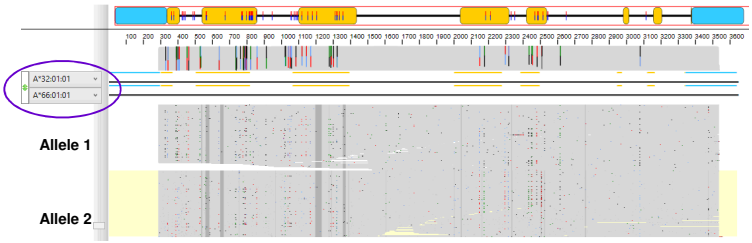

Fig.2. Full length gene sequencing output from GenDx NGSengine software. 5' & 3'ends are denoted in blue, repective exons coloured in yellow and introns denoted by the black line. A 6 digit level of genotyping was called for both alleles (circled).

- High resolution: 6 to 8 digit level typing
- 100% concordance to 4 digit SBT results
- Novel intronic sequences detected
- Ambiguous SBT genotypes resolved
- Full phasing of SNPs
- Full length Class I HLA genes & relevant Class II exons can be easily sequenced on a single SMRT cell

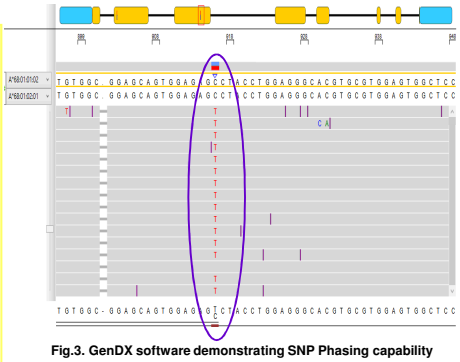

Fig.3. GenDX software demonstrating SNP Phasing capability

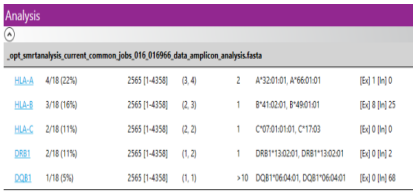

Fig.4. GenDx genotyping results of both Class I and Class II Loci derived from a single SMRT cell

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Poster courtesy of Uppsala University.