Rapid HLA typing using ONT MinION sequencing

Pascal van der Weele, Sjoerd Creutzburg, Bart Valkenburg, Maarten Penning

GenDx, Utrecht, The Netherlands

Introduction

Rapid HLA yping of deceased donors for solid organ transplantation is crucial to ensure optimal matching of donor and recipient and minimizing graft rejection post-transplant.
Current methods are able to rapidly characterize 11 loci (HLA-A, B, C, DRB1, and DRB3/4/5, DQB1, DQA1, DPB1, and DPA1) via qPCR, but the typing resolution is low to intermediate. To improve matching of donor materials, unambiguous second field characterization of all 11 HLA genes is desired. Next generation sequencing (NGS) of HLA genes is able to produce high resolution typing results, but is currently restricted to recipient typing due to an unacceptable turn-around time. Here we demonstrate development of a rapid HLA typing method based on Oxford Nanopore Technologies (ONT) MinION sequencing.

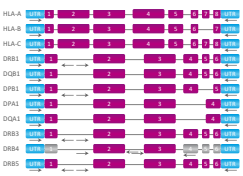
Methods

Existing NGSgo®-MX11-3 and NGSgo®-AmpX v2 primer assays (GenDx, the Netherlands) for 11 HLA loci were used on DNA isolated from cell lines (Coriell Institute, USA) to generate amplicons covering all exons (Figure 1) using a redesigned amplification protocol. Amplicons were subjected to a customized workflow to prepare libraries. The workflow prepares amplicons for ligation of an ONT adapter. After library preparation, Flongle flow cells (R9.4.1) were run for 60 minutes on a MinION Mk1C. Basecalling was performed on the fly with the MinKNOW high accuracy basecalling algorithm. Resulting FastQ files were analyzed in NGSengine 2.25 for base variation plots. HLA typing results were obtained with a concept typing tool for ONT HLA data.

Results

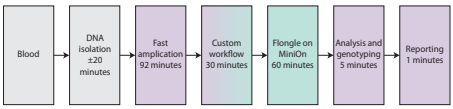
Amplicons were produced in 92 minutes. The workflow produced sequence-ready libraries in 30 minutes. After sequencing, HLA typing results were obtained in less than 5 minutes, resulting in a complete protocol duration of 3 hours and 10 minutes (Figure 2), starting from isolated DNA. MinION sequencing generates higher noise than other NGS techniques, with class II loci being especially challenging due to homopolymers and repetitive sequence stretches (Figure 3). Despite this, good typing results were generally obtained with the concept typing tool (Table 1).

Figure 1.



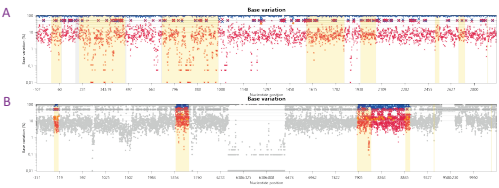
Schematic representation of amplicons. Arrows indicate primer locations. Purple and grey boxes indicate amplified and non-amplified exon regions respectively.

Figure 2.



Turn-around time of the fast HLA typing concept. Third party solutions are shown in grey, GenDx products in development in purple, and required products from ONT in green.

Figure 3.



Base variation plot examples of good (A: HLA-C) and poor (B: HLA-DRB1) quality sequence results. The y-axis shows base variation (%) on a logarithmic scale. Large red crosses indicate heterozygous positions, while small red crosses indicate noise at homozygous positions. Circles indicate uncertain calls (question mark positions) by the NGSengine algorithm.

Table 1.
HLA typing results. Green typing results are concordant with pretypes, red typing results are discordant. The single discordant typing result observed is a missed heterozygous typing in HLA-A. ESA indicates estimated second allele %, with 50 being a perfect heterozygous result.

Locus	NA10830 Reads	ESA	Allele A	Allele B
HLA-A	1724	46,4	A*01:01	A*02:01
HLA-B	1500	48,9	B*07:02	B*08:01
HLA-C	1250	47,3	C*07:01	C*07:02
DRB1	1306	47,4	DRB1*03:01	DRB1*12:01
DPB1	1466	49,7	DPB1*04:01	DPB1*04:02
DQB1	1251	48,0	DQB1*02:01	DQB1*03:01
DRB3	1498	43,4	DRB3*01:01	DRB3*02:02

Locus	NA07020 Reads	ESA	Allele A	Allele B
HLA-A	1665	-	A*11:01	A*11:01
HLA-B	1375	-	B*38:02	B*38:02
HLA-C	1205	-	C*07:02	C*07:02
DRB1	1379	44,4	DRB1*08:03	DRB1*12:02
DPB1	1374	-	DPB1*02:02	DPB1*02:02
DQB1	1955	49,3	DQB1*03:01	DQB1*06:01
DRB3	792	-	DRB3*03:01	DRB3*03:01

Locus	NA17221 Reads	ESA	Allele A	Allele B
HLA-A	1172	47,8	A*24:02	A*29:02
HLA-B	821	41,2	B*15:01	B*44:03
HLA-C	823	49,3	C*16:01	C*16:01
DRB1	790	46,3	DRB1*12:01	DRB1*15:01
DPB1	888	-	DPB1*02:01	DPB1*02:01
DQB1	896	48,8	DQB1*03:01	DQB1*06:02
DRB3	458	-	DRB3*02:02	DRB3*02:02

Locus	NA17219 Reads	ESA	Allele A	Allele B
HLA-A	1188	44,5	A*02:01	A*33:01
HLA-B	815	49,2	B*07:06	B*14:01
HLA-C	711	48,9	C*08:02	C*15:05
DRB1	999	25,8	DRB1*04:05	DRB1*07:01
DPB1	871	49,1	DPB1*45:01	DPB1*124:01
DQB1	718	46,0	DQB1*02:02	DQB1*03:02

Locus	NA17440 Reads	ESA	Allele A	Allele B
HLA-A	1382	-	A*02:01	A*02:01
HLA-B	918	43,3	B*15:15	B*15:23
HLA-C	1061	49,4	C*01:02	C*04:01
DRB1	1248	-	DRB1*08:02	DRB1*08:02
DPB1	1099	-	DPB1*04:01	DPB1*04:01
DQB1	1006	-	DQB1*04:02	DQB1*04:02

Green = Two field concordance
ESA >40 = Green
ESA < 40 = Yellow
ESA <30 = Red

Conclusion

The present method demonstrates feasibility of generating HLA sequence data in a time frame suitable for solid organ transplantations. The method can produce correct second field HLA typing results in less than 3.5 hours, including third party DNA isolation. The increased resolution compared to existing techniques may improve donor organ matching. Sequence data quality remains a challenge (also seen on Poster 89) and may interfere with robust results, especially for Class II loci. However, as the Flongle flow cells use R9.4.1 chemistry, it is expected that the MinION R10.4 flow cells produce higher quality data and therefore potentially more reliable HLA typing results in the near future.

GENDX