VALIDATION OF A NEXT GENERATION SEQUENCING BASED HLA TYPING SYSTEM

Jonathan Downing, Rebecca Lopes, Aous Al-Ibousi, Heather Dunckley

New Zealand Blood Service National Tissue Typing Laboratory

ABSTRACT

We have validated and implemented the Illumina TruSight HLAv2 kit for typing of HLA-A, -B, -C, -DRB, -DQ and -DP for solid organ and haematopoietic stem cell transplantation. In addition we have validated the use of this kit on the Eppendorf epMotion® 5075 liquid handling robot.

OBJECTIVES

Very high resolution typing of HLA genes by Next Generation Sequencing (NGS) methods is becoming mainstream in histocompatibility laboratories for the workup of haematopoietic stem cell and solid organ transplant patients and donors. Determining the full sequence for class I and extended exon coverage for class II genes produces HLA types with far fewer ambiguities than traditional Sanger sequencing based typing (SBT) and other lower resolution typing methods. In addition NGS brings benefits of higher throughput and lower cost.

This poster describes the validation of the Illumina TruSight HLAv2 HLA typing kit using the Illumina MiSeq instrument and the Eppendorf epMotion® 5075 liquid handling robot.

METHODS

The Illumina TruSight HLAv2 kit was used to type HLA-A, -B, -C, -DRB, -DQ and -DP. Six runs of 24 samples and one run of 12 samples were performed comprising a total of 147 samples. The kit manufacturer's protocol was followed and all library preparation was performed manually. Samples comprised the following: 73 local DNA; 54 UCLA reference DNA; and 20 blinded samples kindly provided by the Transplant Immunology Laboratory at Baylor Scott and White Hospital, Dallas, USA. The local and UCLA DNA samples were selected to cover a range of HLA genotypes commonly seen in the New Zealand laboratory, as well as some rarer alleles.

NGS was performed using the Illumina MiSeq platform and results were analysed using TruSight Assign, (supplied with the Illumina TruSight kit). NGSengine (GenDx) was also used for data analysis, as a comparison.

Concordance with the known genotype to the three field level was assessed. In addition, library yield, cluster density, % clusters passing filter, % reads>Q30 and average read depth were examined.

Subsequent to the main validation the library preparation procedures were transferred to the epMotion® 5075 with the aid of Eppendorf and Illumina technical support. The epMotion® 5075 was programmed to process 12 samples at a time for the PCR clean up, normalization, tagmentation and clean up steps. Just prior to the library amplification step two batches of 12 samples were pooled, so the final library contained 24 samples for NGS. Table 1 shows the library preparation timeline using the epMotion®5075. Four runs of 24 samples were performed using the epMotion®5075, using samples previously typed with the manual library preparation procedure.

Table 1: Library preparation on the epMotion®5075.

Method Step	Time (mins)
PCR clean up	90 (x2)
Normalization, tagmentation, clean up	140 (x2)
Library amplification	20
Clean up	50

Table 2: Average read depth and % genotype ambiguity by locus using the manual method.

Locus	A	B	C	DRB1	DRB3	DRB4	DRB5	DQA1	DQB1	DPA1	DPB1
Average read depth	251	260	245	239	238	215	232	237	218	203	214
% Genotype ambiguity	0.00	0.00	0.00	3.40	0.00	0.00	0.00	0.00	0.00	0.00	21.09

Table 3: Concordance between NGS and known genotype using the manual method.

% Concordance	A	B	C	DRB1	DRB345	DQA1	DQB1	DPA1	DPB1
Local samples	100	100	98.6	98.6	98.6	100	98.6	100	100
UCLA samples	100	98.1	98.1	92.6	100	96.3	100	100	92.6
Blinded samples	100	100	100	100	100	100	100	100	100

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DISCLAIMER

The authors have no conflicts of interest to declare.

RESULTS

With the manual library preparation method average library yield was 1.18 ng/µL (0.22-2.42); average cluster density was 946 K/mm^2 (242-1529); average clusters passing filter was 94.3% and average reads > Q30 was 95.4%. Average read depth using the manual method is shown in Table 2 and was greater than 200x for all loci.

As shown in Table 2 no genotype ambiguities were observed except for the HLA-DRB1 and –DPB1 loci. For DRB1 this was due to DRB1*12:01:01 and DRB1*12:10:01 which differ in exon 1, which is not covered with the Illumina kit. For DPB1 there were a number of ambiguities which could not be resolved, due to polymorphism outside the NGS amplicon or lack of phase continuity.

Concordance between the observed and expected genotype to the three field level as shown in Table 3 was excellent. The lack of concordance for local samples was due to: C* NGS = C*04:82 but ref type = C*04:01 (exon 5 difference); DRB1 NGS giving DRB1*07, *12 but ref type = DRB1*12, 13 (this repeated as ref type); DRB345 NGS giving DRB4/DRB3 whereas the reference type was DRB3 only (this repeated as the reference type); DQB1 allele dropout of DQB1*04. Lack of concordance with the UCLA samples was mostly due to the reference typing being pre-NGS. There was 100% concordance of NGS with the genotypes of the blinded samples.

For the local samples, genotype failure rate (ie. no results obtained) varied between zero (HLA-A, -B, -C, -DQB1, -DPA1 and – DPB1) and 2.8% (DQA1). Failure rate in the UCLA samples was higher (0-7.4%) possibly due to poorer quality DNA. There were no failures with the blinded samples.

During the validation the library preparations performed on the epMotion®5075 gave much higher yields than the manual method (average: 13.8ng/µL), however the cluster density was lower (average: 541.3 K/mm^2), average read depth was lower and failure rate was higher. These findings led to the use of an increased amount (2.5x) of library product for the sequencing procedure, with this change the NGS results with the epMotion®5075 have been as good as the manual method. Use of the epMotion®5075 allows up to four runs of 22 samples per week to be typed by NGS.

CONCLUSIONS

The Illumina TruSight HLAv2 next generation sequencing kit was shown to be highly effective at performing high resolution HLA typing for transplant patients and donors as well as other clinical needs. Typing is achieved at a three field resolution or better in most cases without the need for further time consuming and costly testing. The use of the liquid handling robot reduces variability between technicians, minimizes the risk of errors and alleviates the issue of extreme manual handling stress.