Quality Analysis of Next-Generation Sequencing in Generating HLA Genotypes in a Clinical Laboratory

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INTRODUCTION

- High-resolution HLA genotypes provide explicit information about HLA proteins.
- These data are becoming increasingly important in the field of transplantation, especially for eplet-matching to improve graft outcomes.

HLA-B*44 vs. HLA-B*44:01

Fig 1. An example of a low-resolution genotype on the left, and a high-resolution genotype on the right.

- High-resolution HLA genotyping has proven difficult with issues such as phasing ambiguities that would require additional testing[1]
- Next-generation sequencing (NGS) has been proposed as a high-throughput, efficient, and reliable method to generate these genotypes
- NGS has been implemented in a leading Canadian clinical HLA laboratory (Vancouver General Hospital Provincial Immunology Laboratory) and is now being extended to other laboratories across the country.

OBJECTIVES

To determine NGS performance in a clinical transplantation laboratory in a formal quality assurance project.

MATERIALS AND METHODS

A **retrospective analysis** was performed on the genotypes generated by NGS from the VGH laboratory. Patients and donors being worked up for a solid organ or bone marrow transplant are routinely sequenced in the lab.

Sequencing

- DNA was extracted from blood or saliva.
- Omixon HLA Holotype Kit version 3 was used to prepare libraries of the 11 HLA genes: HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1-, -DQB1, -DPA1, -DPB1
- Libraries were sequenced on the Illumina MiSeq.
- Sequencing data were analyzed on HLA Twin to check QC metrics and generate genotypes.

Data Retrieval

From the HistoTrac database, the following data were retrieved:

- List of samples sequenced by NGS with run data.
- Genotypes of 11 HLA genes from NGS.
- Additional testing information.

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RESULTS

Samples

1054 samples were sequenced and included in analysis.

Sequencing occurred during October 2018 – March 2019.

Table 1. Categories sequenced samples.

Category	Samples Sequenced
Kidney	242
Bone Marrow	150
Heart	27
Liver	37
Lung	55
Pancreas	6
Bone Marrow Donor	283
Cadaver Donor	74
Living Donor	159
Quality Control	13
Research	5
Other	3

Confirmatory Testing

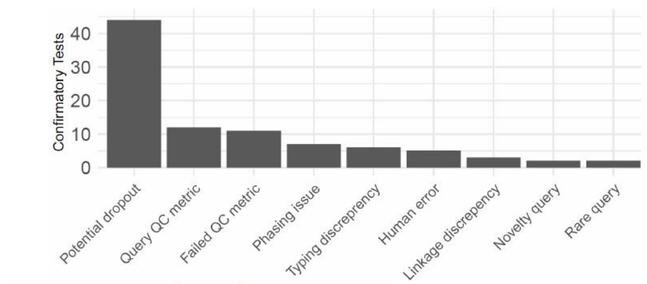
A number of samples required an additional test to clarify sequencing ambiguities. These tests were called confirmatory tests.

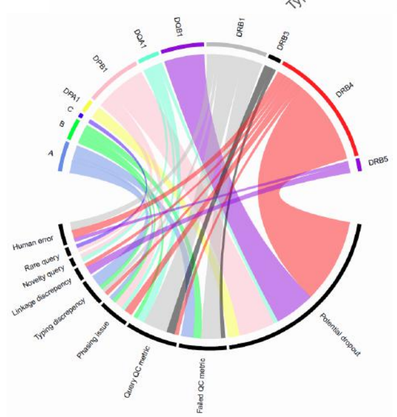
8% of samples sequenced underwent confirmatory testing.

97 confirmatory tests were performed.

Discerning the Rationale for Confirmatory Testing

Each confirmatory test was analyzed to identify the rationale for additional testing.





- All genes had at least one confirmatory test.
- Genes that required the most confirmatory testing in descending order: DRB4, DPB1, DRB1, DQB1, A, B, DQA1, DPA1, DRB3, DRB5, C.

Fig 2. Top: The rationale for confirmatory testing was investigated for each test.

Bottom: The relationship between the number of confirmatory tests by HLA gene and the 9 categories for confirmatory testing.

RESULTS

Dropouts

A dropout is defined when there is PCR amplification bias for one allele over another, where one allele is masked from detection at the point of allele detection. The result is either "No Data" or a homozygous typing.

As a proactive quality control initiative, all samples that were suspected of a potential dropout underwent confirmatory typing. The confirmed dropout alleles were DQB1*03:01, DQB1*03:02, or DRB4*01:01. There was an overall dropout rate of 0.9%.

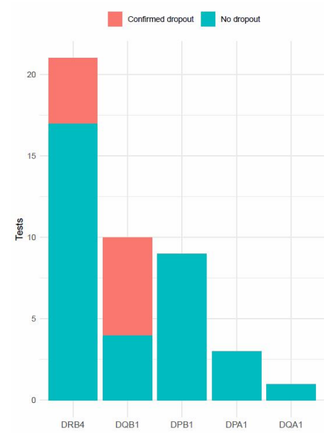
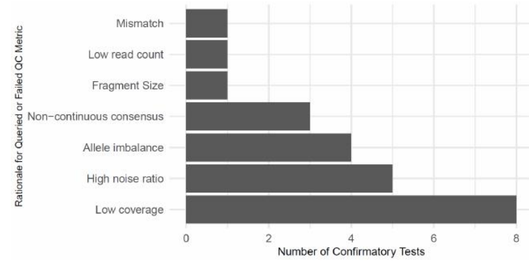


Fig 3. The genes that underwent confirmatory testing for a potential dropout.

Queried and Failed QC Metrics

Multiple parameters were considered a QC metric (Fig 4 top) and many QC flags can be attributed to an inherent genetic property of the HLA alleles (Fig 4 bottom).



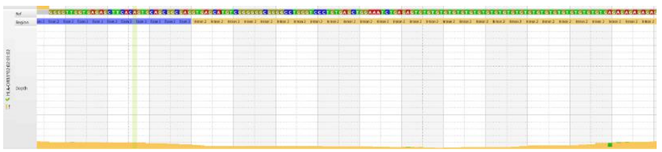


Fig 4. Top: Reasons for queried/failed QC metric. Bottom: Screenshot of HLA Twin genome viewer of intron 2 of DRB3 that is difficult to in sequence due to a homopolymer rich region .

CONCLUSION

- This data supports NGS as a clinical tool to produce desired genotypes with a minimal and predictable error rate.
- Further advancements include multiplex PCR that will reduce risk of error, facilitate a streamlined workflow, and reduce turn around times which will enable significant improvement in HLA genotyping.

REFERENCES

1. Weimer ET, Montgomery M, Petraroia R, Crawford J, Schmitz JL. Performance Characteristics and Validation of Next-Generation Sequencing for Human Leucocyte Antigen Typing. J Mol Diagnostics. American Society for Investigative Pathology and the Association for Molecular Pathology; 2016;18: 668–675. doi:10.1016/j.jmoldx.2016.03.009