Long-Read HLA Genotyping Resolves DPB1 Ambiguity and Accelerates Testing

Aoife Reynolds[1], Brian Graham[1], Orla Ryan[1], Allison Waters[2] Richard Hagan[1], Diarmaid Ó Donghaile[1]

1. National Histocompatibility and Immunogenetics Reference Laboratory, Molecular Biology and Genetics Department, Irish Blood and Transfusion Service
2.Research and Innovation Department, Irish Blood and Transfusion Service

Introduction

Human Leukocyte Antigen (HLA) typing is critical for matching donors and recipients in hematopoietic stem cell transplantation to enhance patient outcomes and help minimise the chances of Graft-versus-Host Disease (GvHD) or graft rejection. Traditional genetic pre-transplant assessment focuses on 11 HLA genes and the ABO blood group. Short-read HLA typing for large unrelated bone marrow donor registries, patients, and family members can be expensive. Its limited read length can cause allelic ambiguity and phasing issues, leading to incomplete resolution and longer turnaround times.

A new typing method is available, the Oxford Nanopore Technologies (ONT). This study compared long-read and short-read sequencing methods for HLA genotyping of 96 donors from the Irish Unrelated Bone Marrow Registry (IUBMR). Irish bone marrow registry donors were previously typed by AllType™ NGS. An alpha test of One Lambda's LR-PCR LRS HLA typing kit was tested with the ONT platform. Short-read sequencing was performed using the AllType FASTplex™ NGS 11 Loci kit on the Illumina MiSeq™ platform.

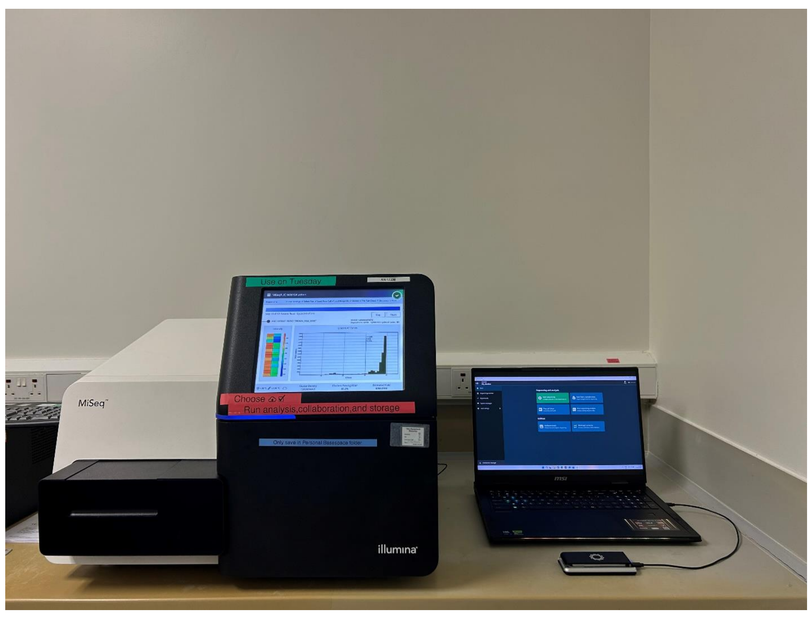


Figure 1. This image illustrates the sequencing equipment used in this study: the Illumina MiSeq™ and the Oxford Nanopore MinION Mk1D connected to a high-performance laptop that managed instrument operation and data processing. It highlights the size and workspace requirements for each platform

Table 1. An example of the HLA-DPB1 ambiguity resolved seen by long-read sequencing within this study

Sample ID	AllType™ NMDP Codes	AllType™ FASTplex™	LR-PCR LRS HLA Typing Kit Assay
19-02397	04:ADCGE, 06:BMSUG	04:01:01/350:01 06:01:01/914:01	04:01:01 06:01:01
19-02601	03:AHJG, 04:AHPJH	03:01:01/351:01 04:02:01/463:01:01	03:01:01 04:02:01
19-02635	04:EKAUU, 04:EDJXP	04:01:01/105:01:01 04:02:01/126:01:01	04:01:01 04:02:01

NMDP Codes:
ADCGE 04:01/350:01
BMSUG 06:01/914:01
AHJG 04/20/28/32/35/40/48
AHPJH 04:02/463:01
EKAUU 04:01/126:01/1321:01/1322:01/1436:01/1444:01Q
EDJXP 04:02/105:01/1346:01

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Objective

- Sequence the 11 classical HLA genes of 96 bone marrow registry donors using long-and short-read sequencing on the MinION, ONT and the MiSeq™ Illumina platform, respectively.
- Compare the methodologies and accuracy of the results between the two techniques.
- Resolve HLA-DPB1 allele ambiguities with long-read sequencing

Methodology

- HLA genotyping was subsequently carried out using the alpha testing protocol for the One Lambda LR-PCR LRS HLA typing kit assay on the MinION and the AllType™ FASTplex NGS 11 Loci Kit on the MiSeq™, according to the manufacturer's instructions.
- The ABO phenotype of each donor was determined in parallel to the HLA genotype using the One Lambda LR-PCR LRS HLA typing kit assay.
- The data was analysed at third field; the genotypes were compared for resolved allele ambiguities and phasing of samples.

Results

The ONT approach required minimal infrastructure expenditure, facilitated adaptive sampling ranging from 1 to 96 samples, and delivered same-day HLA typing results in approximately 6 hours for 16 samples. The 57.1% reduction in turnaround time from DNA extraction to HLA reporting for 48 samples further highlights how long-read sequencing can facilitate quick HLA genotyping.
The ONT achieved an accuracy of 99.2%. The ABO blood group phenotype was determined using the One Lambda LRS kit, offering additional data for transplantation patients.

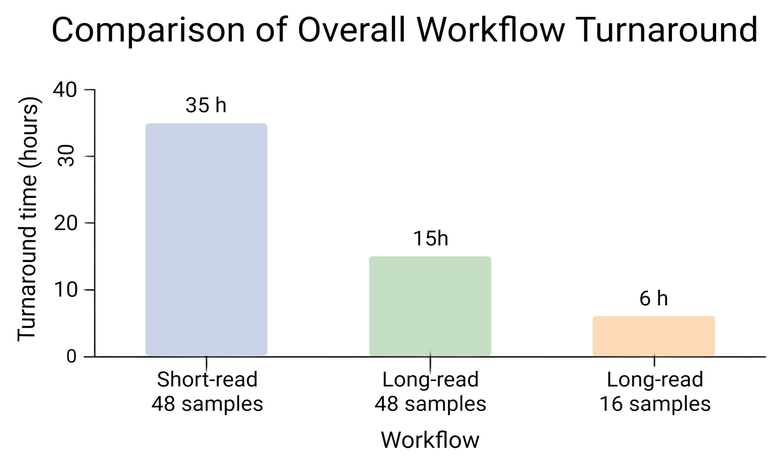


Figure 2. A comparison of workflow turnaround times for short-read and long-read sequencing. Created in BioRender. Reynolds, A. (2026)

Analysis

The long-read sequencing kit successfully resolved the DPB1 ambiguities, with an example of the results shown in Table 1. NMDP codes were used for the ambiguous AllType™ HLA genotype results. Short-read sequencing of DPB1 yielded a median of 24 alternative allele strings per genotype, whereas long-read sequencing reported a single allele pair. Among the 96 samples analysed, 40 (42%) exhibited ambiguous HLA-DPB1 allele assignments with the short-read FASTplex™ assay. These ambiguities were resolved through the application of long-read sequencing.

Discussion

This study serves as a proof of concept, demonstrating that the ONT methodology is cost-effective, scalable and highly accurate. Implementing the ONT methodology is expected to improve patient care by reducing turnaround times and enabling real-time analysis of urgent samples. Achieving high accuracy in the third field and resolving DPB1 and phasing ambiguities will enable the laboratory to provide more precise results, supporting the IUBMR at the IBTS with donor selection for stem-cell transplantations. The technology's accessibility will also support laboratory-based research and population studies. Moreover, adopting this new technology will promote future advancements in HLA genotyping at the NHIRL within the IBTS.

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