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From Slow to Lightning: HLA Typing Using NanoTYPE™ for High Throughput Laboratories and Registries

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Introduction

Nanopore sequencing has rapidly gained traction as a transformative next-generation sequencing technology, offering unique advantages over traditional platforms like Illumina. Its ability to sequence single molecules in real time—by detecting changes in ionic current as DNA passes through nanopores—provides unmatched speed, flexibility, and scalability. While previous work has emphasized nanopore sequencing's adaptability for low- and medium-throughput laboratories, this poster shifts the spotlight to high-throughput applications. We present the use of **NanoTYPE™**, Werfen's robust HLA genotyping solution, tailored for the needs of large registries and high-volume laboratories. This study showcases a 59 samples run using the **PromethION® 2 Solo** and the **transposase-based Rapid Barcoding Kit (Chemistry 14)**, eliminating complex ligation steps washing, and third-party reagents. PCR and library preparation were performed manually on Day 1, sequencing launched that afternoon, and results were delivered by early afternoon on Day 2—highlighting a highly streamlined, cost-efficient workflow. Critically, this is enabled by **NanoTYPE™ 11 Plus (RUO) kit**, the **first and only solution providing full coverage and complete phasing across all HLA exons**. This ensures the highest possible resolution and confidence in genotyping results, without compromising speed or throughput.

Material & Methods

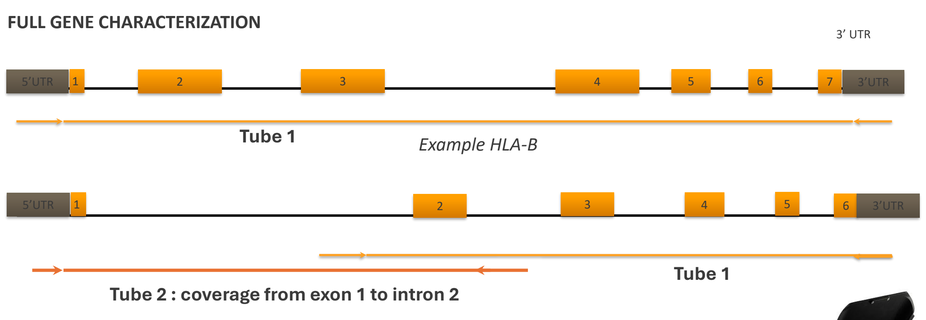
The **NanoTYPE™ 11 Plus (96)** kit (product code: NT9611v3) is a next-generation solution for high-resolution HLA genotyping across 11 genes: *HLA-A,B,C,DRB1,DRB3,DRB4,DRB5,DQA1,DQB1,DPA1,*DPB1) and is planned for release as RUO in **2025.**

It uses a **two-tube amplification strategy** designed to provide laboratories with both **maximum flexibility and complete coverage**:

- **Tube 1**: Amplifies all 11 genes with broad exon coverage and is fully compatible with high-throughput workflows.
- **Tube 2** : Specifically targets exon 1 and intron 1 of *DRB1,3,4,5* and *DPB1*, ensuring uninterrupted 5'–3' coverage and full phasing.

Labs operating in **high-throughput mode** can choose to use **Tube 1 alone**, as it already includes complete amplification for all 11 loci. In this configuration, the workflow remains streamlined and efficient while still delivering high-resolution genotyping.

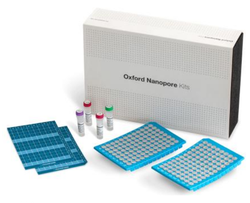
In comparison to previous NanoTYPE™ versions, **reverse primers for DRB1,DRB3 and DRB4 have been extended** to include **exons 5 and 6**, enhancing allele discrimination and enabling improved resolution at the 3' end of these genes.



The **PromethION® 2 Solo (P2 Solo**) is one of Oxford Nanopore Technologies' most compact high-throughput platforms, designed to bring powerful sequencing capabilities to the benchtop. Like the **MinION®**, it operates via USB-C and interfaces directly with a laptop or external workstation. However, due to its significantly higher data output—thanks to the increased number of nanopores in PromethION flow cells—the P2 Solo requires a more robust GPU for basecalling and downstream analysis.

Supporting up to **two flow cells concurrently**, the P2 Solo delivers a substantial increase in throughput while adding flexibility to the HLA laboratories. Each flow cell can be run independently, enabling labs to scale runs according to sample volume. This modular design is ideal for high-throughput labs aiming to integrate HLA typing with other applications such as deceased donor typing, disease association samples, all without the footprint or investment required for an Illumina sequencer.

We used **PromethION R10.4.1** flow cells for the sequencing runs. Flow cell quality control was performed during the flow cell check revealing **6,437** available pores for flow cell PBC20521. After sequencing the flow cell had **4,284** pores remaining. The flow cell lost **2,153** pores. This would allow to perform another 2 to 3 runs making the consumable cost a fraction of Illumina's cartridges.



The **Rapid Barcoding Kit 96 V14 (SQK-RBK114.96)** from Oxford Nanopore Technologies represents a major improvement in library preparation for high-throughput HLA genotyping. Utilizing a **transposase-based, single-tube protocol**, this kit enables the barcoding and sequencing of up to 96 samples simultaneously, with minimal hands-on time and **no intermediate purification steps**. This streamlined workflow contrasts sharply with traditional ligation-based methods, which involve ligation and bead-cleanup steps—adding complexity, preparation time, and increasing the risk of sample loss.

The **V14 chemistry** incorporates the enhanced **E8.2 motor protein**, providing improved basecalling accuracy and robust performance. Combined with the 96-barcode transposase approach, this kit streamlines workflows and is ideal for labs looking to scale rapidly without compromising data quality. Sequencing results using the Rapid Barcoding Kit show **N50 read lengths of 2–3 kb for Class I** genes and **3–4 kb for Class II**, with many reads extending to nearly full amplicon length. This enables excellent phasing across exons and delivers the full-gene resolution needed for confident allele assignment—**without the added complexity or time burden of traditional ligation workflows**.

In this run, A total of **59 samples** were extracted from whole blood of various origins, including **ASHI and NEQAS External Proficiency Testing (EPT) samples**, anonymized clinical specimens, and in-house extractions. DNA extraction for in-house samples was performed using the **QIAamp DNA Mini Kit** (Qiagen, 51104), while the extraction methods for the remaining samples were not documented. The workflow followed **IFU Rev6**, with **PCR amplification** carried out on a **Veriti™ Thermocycler** (Applied Biosystems, ThermoFisher), **gDNA quantification** performed using a **NanoDrop spectrophotometer** (ThermoFisher), and **amplicon concentration** measured using the **Qubit dsDNA BR Assay Kit** (ThermoFisher). Results of the quantification and amplification steps are not shown.

MinKNOW run parameters were as follows:
Sequencing was performed using **MinKNOW version 24.02.10** with the **High-Accuracy (HAC) basecalling model** set at **400 bases per second (bps)**. The **mux scan** was configured with the default duration of **90 minutes**. A **minimum read length of 1000 bp** and a **minimum Q score of 10** were applied for filtering.

Results

MinKNOW Run Summary

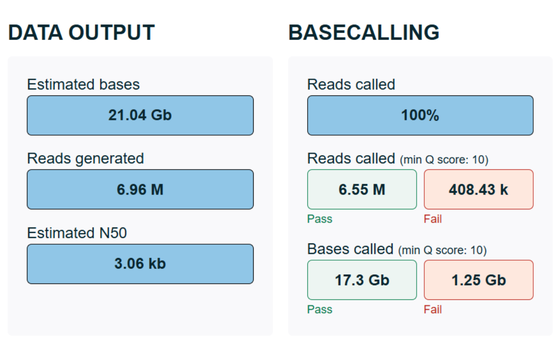


Figure 1. *MinKNOW run summary report.*
MinKNOW generates an HTML-format summary report that includes key run metrics, such as the total number of reads called and the estimated N50 value.

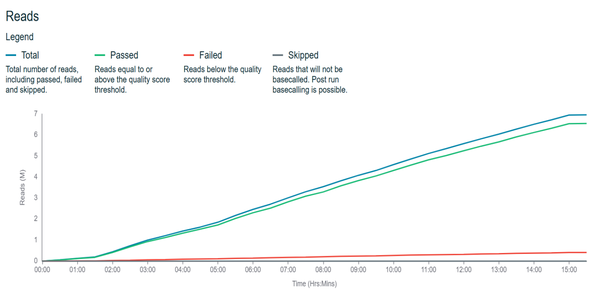
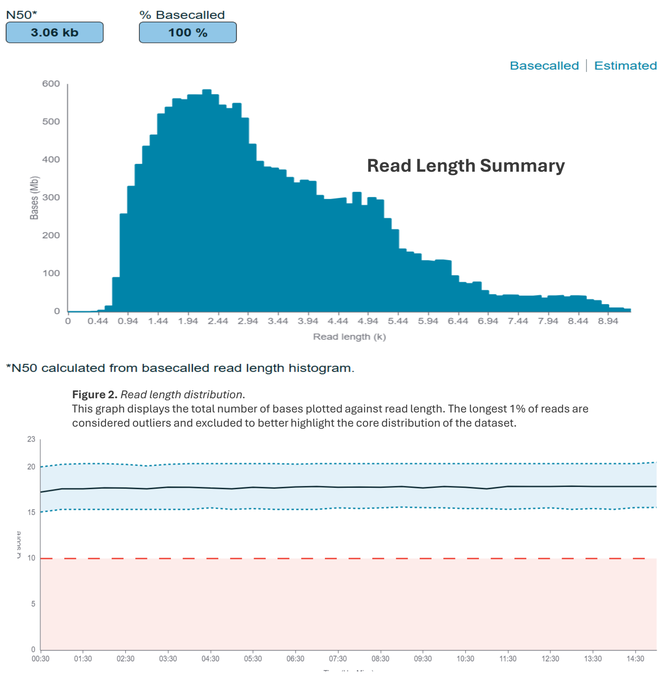


Figure 3. *Total, passed, and failed reads (in millions) sequenced over time.*



*N50 calculated from basecalled read length histogram.

Figure 2. *Read length distribution.*
This graph displays the total number of bases plotted against read length. The longest 1% of reads are considered outliers and excluded to better highlight the core distribution of the dataset.

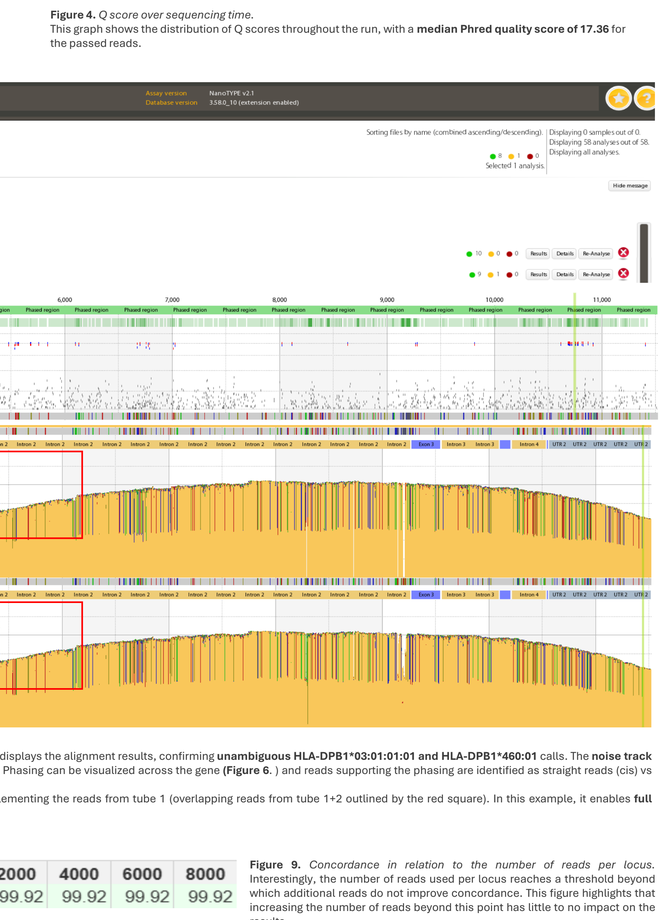


Figure 4. *Q score over sequencing time.*
This graph shows the distribution of Q scores throughout the run, with a **median Phred quality score of 17.36** for the passed reads.

NanoTYPER V2.2 Analysis

Fig 5

Fig 6

Fig 7

Figure 5-6-7. *Sample analysis using NanoTYPER™ 2.2.*
The **traffic light system (Figure 5.**) provides a quick visual overview of locus quality, while the **Gene Browser (Figure 6-7**)displays the alignment results, confirming **unambiguous HLA-DPB1*03:01:01:01 and HLA-DPB1*460:01** calls. The **noise track (Figure 6.**) illustrates signal-to-noise levels and highlights **allelic balance at heterozygous positions** (blue and red dots). Phasing can be visualized across the gene **(Figure 6.**) and reads supporting the phasing are identified as straight reads (cis) vs cross reads (trans).
Figure 7. The **NanoTYPE™ 11 Plus** assay includes a second tube covering **exon 1, intron 1, exon 2, and intron 2**, complementing the reads from tube 1 (overlapping reads from tube 1+2 outlined by the red square). In this example, it enables **full phasing of DPB1** from the **5' UTR to the 3' UTR**.

Results Analysis

Fig 8

	3h	6h	9h	12h	15h
HLA-A	100.00	100.00	100.00	100.00	100.00
HLA-B	100.00	100.00	100.00	100.00	100.00
HLA-C	100.00	100.00	100.00	100.00	100.00
HLA-DPA1	98.28	100.00	100.00	100.00	100.00
HLA-DPB1	99.14	100.00	100.00	100.00	100.00
HLA-DQA1	98.21	98.21	98.21	99.09	100.00
HLA-DQB1	97.41	97.41	99.14	97.37	99.12
HLA-DRB1	99.14	100.00	100.00	100.00	100.00
HLA-DRB3	100.00	100.00	100.00	100.00	100.00
HLA-DRB4	100.00	100.00	100.00	100.00	100.00
HLA-DRB5	100.00	100.00	100.00	100.00	100.00

Figure 8. *Concordance over time for the 59 samples per locus.*
Class I loci reached full concordance across all 59 samples after just 3 hours of sequencing, whereas Class II loci required a longer runtime. This delay can often be attributed to PCR amplification with unbalanced alleles and/or variability in barcoding efficiency affecting a few samples. In majority of the cases, sequencing longer will not improve the results. In such cases, and for cost-efficiency, it may be preferable to stop sequencing and re-amplify the unresolved loci using **NanoTYPE MONO™**, then combine it with your next library preparation.

Fig 9

Reads	2000	4000	6000	8000
Concordance	99.92	99.92	99.92	99.92

Figure 9. *Concordance in relation to the number of reads per locus.* Interestingly, the number of reads used per locus reaches a threshold beyond which additional reads do not improve concordance. This figure highlights that increasing the number of reads beyond this point has little to no impact on the results.

Fig 10

SAMPLE	LOCUS	EXPECTED_RESULT_IN_REFERENCE_FILE	EXTRACTED_RESULT	Concordance_text	failed_qc_metrics
All	/	All	All	All	All
HA-2803 HLA 4	HLA-DQB1	HLA-DQB1*03:02:01 + HLA-DQB1*05:01:01	HLA-DQB1*03:02:01:01 + HLA-DQB1*05:01:01:03#1 |	partially concordant	

Figure 10. *Summary of results for the 59 samples.*
We achieved a concordance rate of 99.92%, with a single discrepancy arising from one sample where the 4th field of the HLA-DQB1 alleles was not reported. The reference sample had **HLA-DQB1*03:02:01 + *HLA-DQB1*05:01:01**, while the result included the 4th field: **HLA-DQB1*03:02:01:01 + *HLA-DQB1*05:01:01:03**.

Discussion & Conclusion

This study demonstrates that the combination of the **NanoTYPE 11 Plus kit**, **PromethION® 2 Solo**, and the **Rapid Barcoding Kit v14** offers a scalable, high-throughput solution for HLA genotyping without compromising data quality or phasing accuracy. The use of a **transposase-based protocol** significantly reduces hands-on time, eliminates complex ligation steps, and enhances workflow simplicity—key factors for large registries and clinical laboratories aiming for efficiency and reproducibility. Our analysis of 59 samples revealed rapid and robust concordance across Class I loci within just 3 hours, while Class II loci required a longer runtime for some samples due to PCR performance or barcode imbalance. Notably, Figure 9 shows that **adding more sequencing time or reads does not significantly improve concordance**, reinforcing the idea of an optimal read threshold. This observation supports a **cost-efficient decision-making process**: instead of prolonging sequencing unnecessarily, it is more strategic to stop the run and re-amplify challenging loci using **NanoTYPE MONO™**, which can then be pooled into a future library preparation. Importantly, the inclusion of **Tube 2 in the NanoTYPE 11 Plus assay** enables extended coverage for DR and DP loci and delivers unambiguous phasing from the 5' to 3' ends. Combined with **NanoTYPER 2.2's analytical capabilities**, the system offers powerful visualization tools like the **Noise and Phasing Track** to help troubleshooting and interpretation.

The NanoTYPE workflow presented here enables **full-gene, high-resolution HLA typing** in under 24 hours with **99.92% concordance** across 59 diverse samples. This performance validates its suitability for high-throughput environments while maintaining the quality expected for clinical-grade genotyping. Together, the streamlined PCR protocol, single-tube library preparation, and PromethION's scalable sequencing capacity offer an **optimized solution for registries and donor centers**, bridging the gap between speed, accuracy, and throughput. The flexibility to reprocess difficult samples using **NanoTYPE MONO™** ensures no compromise in data quality, making this platform ideal for the future of large-scale HLA typing.

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