Tracing of residual DNA when reusing MinION flow cells with a barcoded version of NGS-Turbo

E. ter Steege, B. Rood, S. Creutzburg, P. van der Weele
GenDx, Utrecht, The Netherlands

Introduction

High-resolution HLA typing of deceased donors for solid organ transplantation is pivotal for optimal matching of donor and recipient to minimize the rate of graft rejection. NGS-Turbo in combination with Oxford Nanopore Technologies (ONT) sequencing has a turnaround time of <3 hours from DNA to results for a single sample, allowing NGS-Turbo to be used when time is paramount (Fig. 1). An additional advantage of ONT sequencing is the reusability of flow cells making it highly cost-effective.

To accommodate new guidelines with respect to the reuse of ONT flow cells, the design of NGS-Turbo was improved to include barcoding of samples. To get a perspective on the carry over between NGS-Turbo sequencing runs, this study determines the percentage of residual DNA runs when pooling multiple samples.

Methods

Twelve gDNA samples originating from cell-lines were amplified for the eleven HLA loci with NGS-TurboAmp. Subsequently, libraries suitable for ONT sequencing were generated with NGS-TurboPrep.
The twelve cell-line samples were pooled per two in one library, each sample containing its own barcode. The six libraries were sequenced sequentially on a single R10.4.1 MinION flow cell, using a GridION sequencing device and super high accuracy base-calling. For each sample, at least 10.000 reads were generated. After each run, the flow cell was washed according to ONT instructions and pore degradation was determined. Additionally, the number of reads generated for each barcode included in the current and all previous runs was identified to calculate the percentage of carry over. Generated data was subsequently analysed in NGSengine® to determine typing concordance.

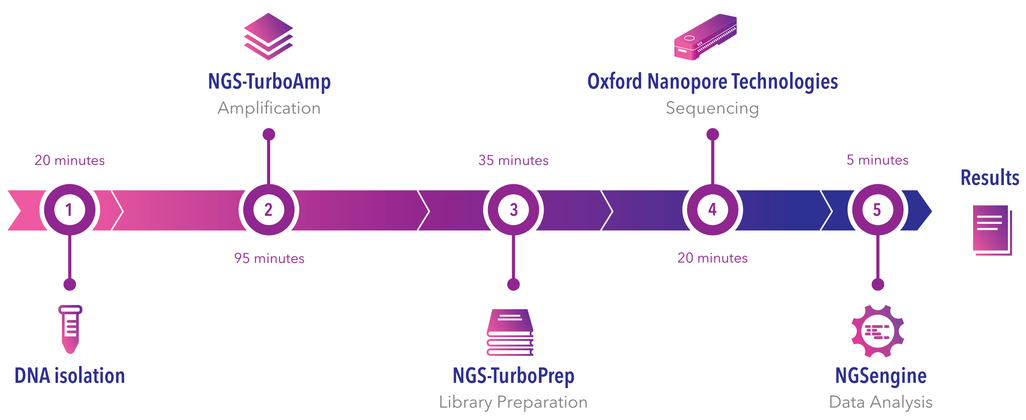

Figure 1. NGS-Turbo has a turn-around time of <3 hours from DNA to results for one sample.

Run #	# reads generated	Sequencing time (min)
1	32609	28
2	30653	28
3	42388	39
4	29155	30
5	28317	31
6	49015	52

Table 1. Sequencing time and number of generated reads for each sequencing run.

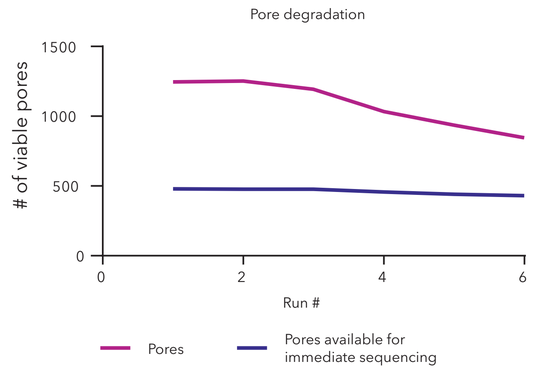

Figure 2. Number of pores available for immediate sequencing remains constant across the six sequencing runs.

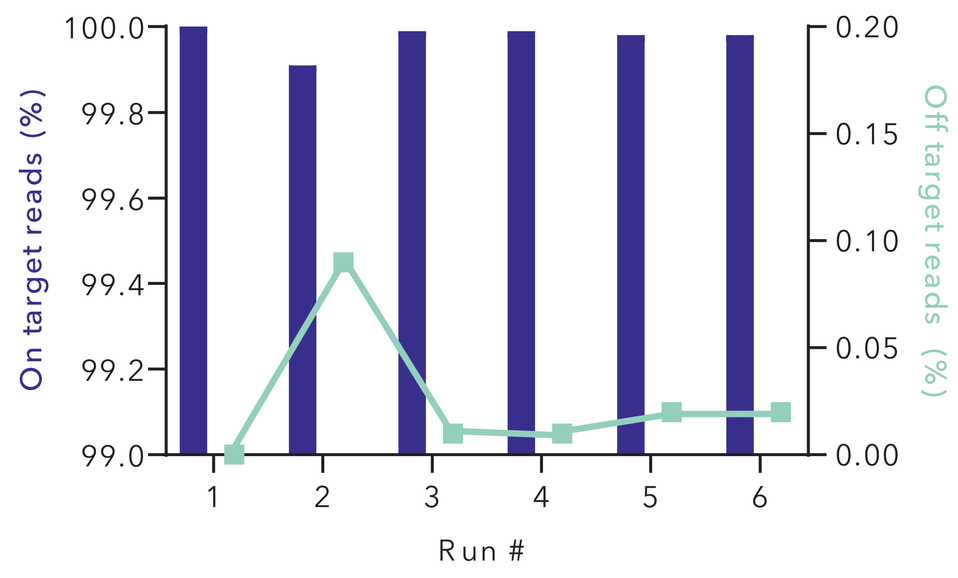

Figure 3. Run-to-run carry-over is <0.1% between sequencing runs.

Table 2. 100% HLA typing concordance at 3rd, and 4th field resolution.

	n	3 field	%3 field	4 field	%4 field
HLA-A	12	12	100	12	100
HLA-B	12	12	100	12	100
HLA-C	12	12	100	12	100
HLA-DRB1	12	12	100	12	100
HLA-DRB3	3	3	100	3	100
HLA-DRB4	3	3	100	3	100
HLA-DRB5	2	2	100	2	100
HLA-DQA1	12	12	100	12	100
HLA-DQB1	12	12	100	12	100
HLA-DPBA1	12	12	100	12	100
HLA-DPB1	12	12	100	12	100

Results

For each sequencing run, at least 10.000 reads per sample were generated (Table 1). The number of viable pores declined with each sequencing run but the number of pores available for immediate sequencing remained stable throughout the six sequential runs (Figure 2). Residual DNA levels from previous sequenced samples were fully traceable and <0,1% between runs (Figure 3). An HLA typing concordance of 100% at third and fourth field resolution was obtained (Table 2). Most importantly, residual DNA did not interfere with the sequencing results of any subsequent runs (Table 2).

Conclusion

The new design of NGS-Turbo allows for combining of multiple samples in one NGS-Turbo run. Residual DNA from previous runs is fully traceable and <0.1% between runs. These low levels of residual DNA do not interfere with data quality and can therefore be neglected. Overall, GenDx NGS-Turbo allows for robust high-resolution HLA typing results within three hours, making it an optimal solution for HLA typing of deceased donors.

eurobio gendx