High-throughput, high-resolution HLA typing using NGS-Pronto nanopore sequencing – a multicentre performance study

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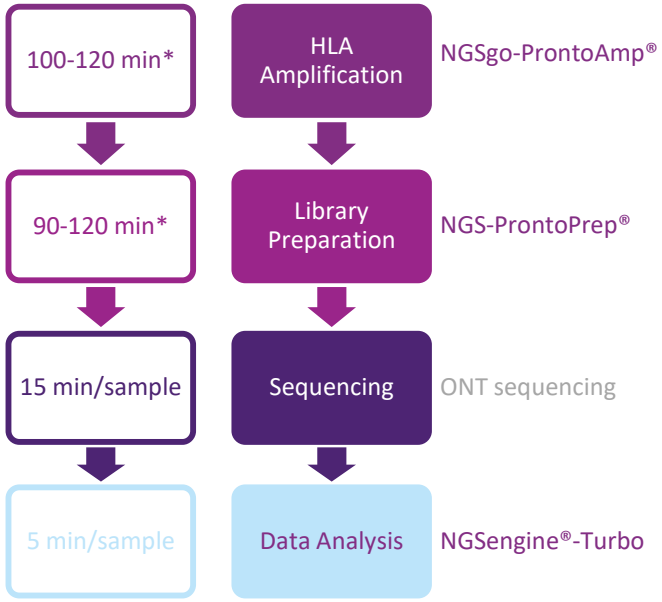
Introduction:
NGS-Pronto® is a scalable, high-throughput, high-resolution HLA typing solution using nanopore sequencing . Using three amplification mixes, whole gene amplicons for 11 HLA loci can be generated using the generic amplification method NGSgo-ProntoAmp®. Subsequently, library preparation is performed with NGS-ProntoPrep®, allowing for 4 - 96 samples per library.

In this study, eight different research centers located across Europe, North-America and Australia without prior experience with NGS-Pronto were guided by a GenDx representative while performing the workflow. Final libraries were sequenced on either a MinION or GridION sequencing device using the R10.4.1 sequencing chemistry and Super-accurate basecalling followed by analysis in NGSengine-Turbo.



Results:
In total 139 samples encompassing 1315 loci were sequenced, from which 13 putative new HLA alleles were identified. The robustness of the assay allowed for successful HLA typing using gDNA samples from both blood (n=121) and buccal swaps (n=18). A concordance of 99,8% was reached at 3-field resolution for all 1315 tested loci. In general, the workflow was experienced as easy, with especially the room temperature incubations at the bench found to be very convenient.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Total
Samples	6	24	24	8	24	21	8	24	139
Loci	58	229	224	75	230	199	74	226	1315
Buccal Swab Samples	1	12	1	0	3	1	0	0	18
Concordance % (3rd field)	100	100	100	97,3	100	100	100	100	99,8

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* Depending on the number of samples