eppendorf

Changing to NGS-based HLA Typing?

Illumina® qualified automated NGS library preparation with the TruSight® HLA v2 Sequencing Panel on the ep*Motion*® 5075t workstation

HLA typing by next-generation sequencing (NGS) is about to revolutionize the field of histocompatibility research. NGS increases coverage and resolution of the HLA region compared to conventional sequencing methods. The TruSight HLA v2 Sequencing Panel uses Illumina NGS technology to generate phase-resolved sequencing results for eleven HLA loci, which cover all commonly typed HLA loci, plus those with emerging relevance. The ep*Motion* automates this pipetting-intensive protocol into a ready-to-run procedure with minimal interventions and setup time.

The ep*Motion* 5075t from Eppendorf offers many benefits that make it an ideal choice. This easy-to-use automated library preparation tool results in the immediate ability to produce HLA libraries. The sequencing results are comparable to those from manual preparation. Thus automated procedure reduces the risk of human errors and frees up time for other tasks.

Key highlights

> Two to three day workflow (8 or 24 samples)
> Minimized hands-on time
> On deck incubation (e.g. tagmentation)
> Maximized library yield and quality with high quality Eppendorf LoBind® consumables
> Standardized process minimizes risk of human errror
> Minimized plate usage
> All equipment and consumables are available through Eppendorf

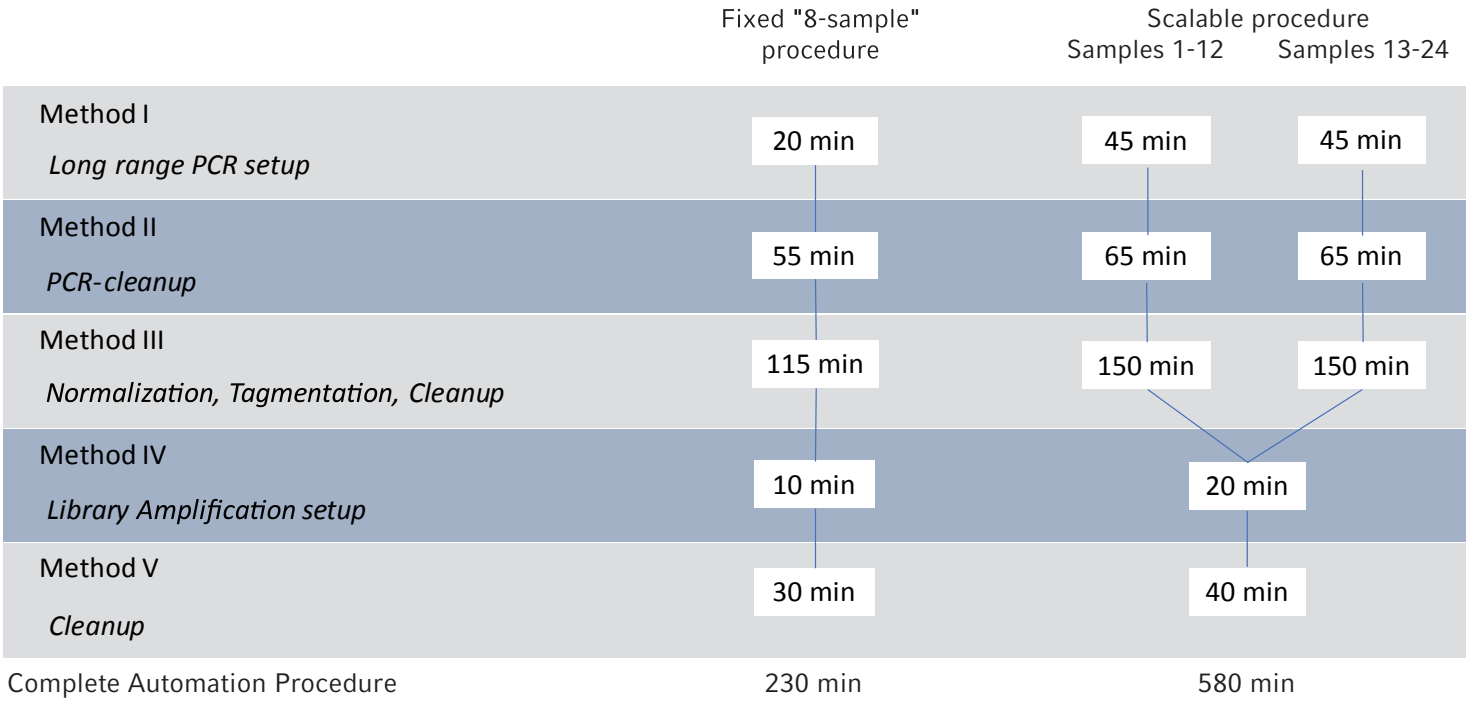
Illumina® TruSight® HLA Sequencing Panel V2

For Research Use Only. Not for use in diagnostic procedures.

www.eppendorf.com/automation

Standardize your NGS library preparation and significantly reduce hands-on time with the Eppendorf automated solution for TruSight® HLA v2 Sequencing Panel.

Generate up to 24 libraries from genomic DNA:



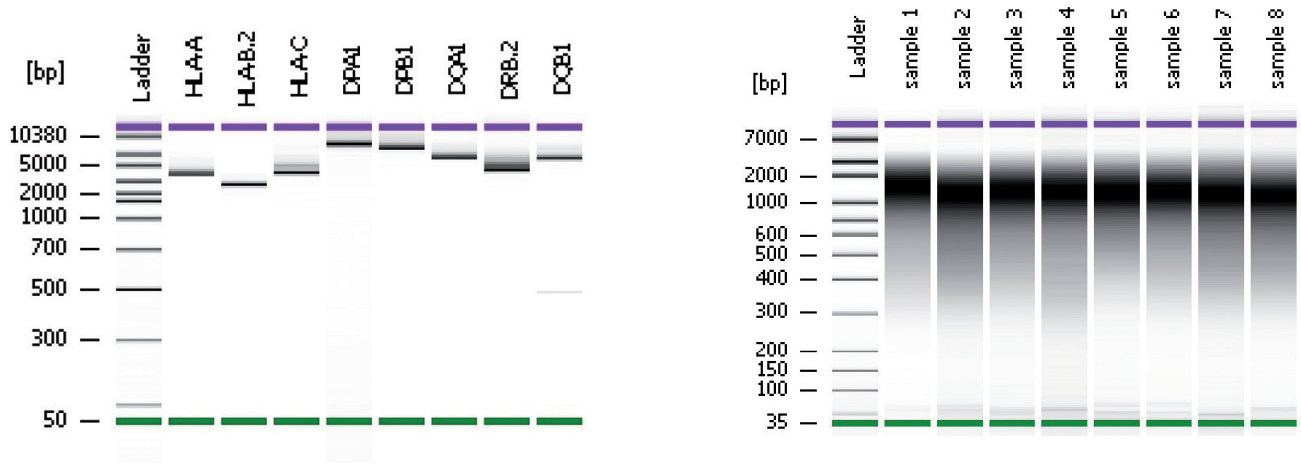
The Illumina TruSight HLA v2 Sequencing Panel targets 11 genetic loci for genotyping using 8 long-range PCR products. To increase the amplification efficiency, each DNA sample is equally divided into 8 fractions that undergo separate, parallel preparations.

The Illumina qualified workflow is divided into five (sub)methods on the ep*Motion* 5075t as shown in the figure. There are two adaptations of the method available: (i) A fixed "8-sample" procedure is optimized towards efficiently processing 8 samples and therefore has a fixed sample number and (ii) a scalable procedure, which can process up to 12 samples per run. Methods I to III can be run twice to process up to 24 samples in one experiment.

> Further information available at: www.eppendorf.com/automation

Results

The quality of enrichment steps and the final libraries can be demonstrated at different steps of the process on an Agilent® Technologies 2100 BioAnalyzer® or similar. An example of these QC steps is shown on the left hand-side in the figure below starting from the 8 long-range PCR products for 1 sample to the final libraries from a typical 8-sample run on the right-hand side. In this example, 1 µL of the final libraries run on a BioAnalyzer using a High Sensitivity DNA chip. A distribution of DNA fragments with a size range up to ~2 kbp is expected.



Example of a 2100 BioAnalyzer electropherogram of initial amplicons of one sample (left) and the post-enriched fragment distribution obtained with automated library preparation of 8 samples on the ep*Motion* (right).

These libraries can be readily sequenced on a MiniSeq™ or MiSeq® system and analyzed using Illumina's Assign™ 2.0 or 2.1 TruSight HLA Analysis Software. The resulting genotype calls for above example are shown below.

	HLA-A	*HLA-B*	*HLA-C*	*DPA1*	*DPB1*	*DQA1*	*DQB1*	*DRB1*	*DRB3*	*DRB4*
Sample 1	01:01:01	07:02:01	07:02:01	01:03:01	04:01:01	03:01:01	03:02:01	04:02:01		01:03:01
(IHW01136)	01:01:01	51:01:01	05:13	01:03:01	15:01:01	03:01:01	03:02:01	04:04:01		01:03:01
Sample 2	02:01:01	18:01:01	07:01:01	01:03:01	04:01:01	01:04:01	05:03:01	15.54:01	02:02:01	
(IHW09061)	02:01:01	18:01:01	07:01:01	01:03:01	04:01:01	01:04:01	05:03:01	15.54:01	02:02:01	
Sample 3	02:17:02	15:01:01	03:03:01	01:03:01	04:02:01	05:03	03:01:01	14:02:01	01:01:02	
(IHW09099)	02:17:02	15:01:01	03:03:01	01:03:01	04:02:01	05:03	03:01:01	14:02:01	01:01:02	
Sample 4	02:02:01	15:03:01	02:10:01	01:03:01	04:01:01	03:01:01	03:02:01	11:01:02	02:02:01	01:03:01
(IHW09114)	29:02:01	41:01:01	17:01:01	02:01:01	13:01:01G	05:05:01	03:19:01	04:03:01		
Sample 5	02:03:01	38:02:01	02:02:01	02:02:02	05:01:01	03:02	03:03:02	09:01:02		01:03:01
(IHW09367)	11:02:01	46:01:01	12:02:01	02:02:02	05:01:01	03:02	03:03:02	09:01:02		01:03:02
Sample 6	03:01:01	40:01:02	08:04:01	01:03:01	04:01:01G	03:01:01	03:01:01	04:01:01		01:03:01
(IHW09388)	11:01:01	40:01:02	08:04:01	01:03:01	04:02:01G	03:03:01	03:02:01	04:04:01		01:03:02
Sample 7	36:01	53:01:01	04:01:01	01:03:01	11:01:01	02:01:01	02:02:01	08:04:01	02:02:01	
(IHW09398)	74:01:01	57:03:01	07:01:01	02:01:01	104:01	04:01:02	03:19:01	13:03:01	02:02:01	
Sample 8	30:01:01	53:01:01	04:01:01	01:03:01	11:01:01	02:01:01	02:02:01	11:08:02	03:01:01	01:03:01
(IHW09431)	33:01:01	81:01	08:04:01	02:01:01	104:01	03:03:01	02:02:01	07:01:01		

Example of Genotype calls using Illumina's Assign 2.0 TruSight HLA Analysis Software on 8 libraries that were automatically prepared with the ep*Motion*.

To demonstrate the concordance of automated procedure with manual library preparation we prepared, sequenced and analyzed a total of 24 samples with both methods. Among the 426 alleles analyzed in those 24 samples 100% concordance was obtained.

»Ease of use for small- and medium-scale sequencing throughputs.«

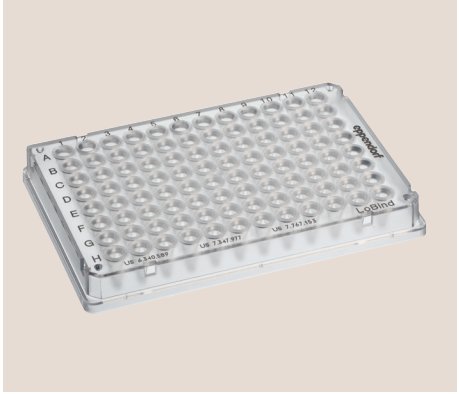
Eppendorf ep*Motion*® 5075t

Additional Illumina qualified methods for ep*Motion* 5075t:
TruSeq® Stranded Total RNA (RiboZero), TruSeq Stranded mRNA, TruSeq Nano DNA, TruSeq DNA PCR-Free, TruSeq Rapid Exome, TruSeq RNA Access, TruSight Tumor 15, TruSight Enrichment (Cancer), and Nextera® XT DNA

Other Eppendorf products that are beneficial for the NGS based HLA workflow

Eppendorf Mastercycler® X50
> Reduced sample loss with high pressure lid against evaporation during long-range PCR
> Consistent results due to excellent block temperature regulation

LoBind Tubes and Plates
> Increase quality and quantity of NGS libraries
> No sample loss due to DNA recovery rates up to 100% after long-term incubation steps



Eppendorf twin.tec® PCR Plates
> Excellent compatibility to PCR cyclers, magnets and the ep*Motion*
> Highly uniform well geometry and rigid design
> LoBind capability

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