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TruSight® HLA v2 Sequencing Panel

Unambiguous, phase-resolved HLA typing in a single assay using proven Illumina NGS technology and Assign™ 2.1 analysis software.

Highlights

- **Comprehensive Assay**
  One assay provides high-resolution sequencing of 11 HLA loci
- **Unambiguous Results**
  Deep sequencing enables accurate, unambiguous HLA typing
- **DNA-to-Report Solution**
  Complete workflow includes library preparation, Illumina sequencing, and data analysis with TruSight HLA Assign 2.1 Software

Introduction

Human leukocyte antigen (HLA) plays a significant role in the ability of the immune system to recognize and remove invasive, foreign, infected, and malfunctioning cells to fight disease and maintain overall health. HLA mutations can produce aberrant immune response and have been associated with autoimmune disorders, cancer, transplant rejection, and drug sensitivity.[1-4]

Sequencing the HLA region can provide critical insight into various immune disorders. Unfortunately, HLA sequencing has been difficult due to the high levels of sequence homology and dense variability found within this region of the genome. Past attempts at deciphering this region required multiple, tedious assays and produced highly ambiguous results.[5,6] The TruSight HLA v2 Sequencing Panel overcomes these challenges in a single assay. Using proven Illumina next-generation sequencing (NGS) technology, labs can generate unambiguous, phase-resolved HLA sequencing results for 11 HLA loci. TruSight HLA Assign 2.1 Software supports intuitive, rapid analysis and reporting of HLA typing results.

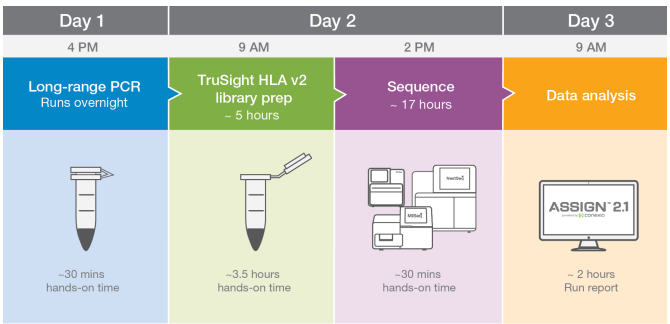

Figure 1: An Integrated DNA-to-Report Workflow for HLA Typing—The TruSight HLA v2 Sequencing Panel enables accurate and efficient HLA typing going from sample to report in less than 48 hours with 4 hours hands-on time.

Table 1: The TruSight HLA v2 Panel Types 11 HLA Loci

Loci	Target Sequence
HLA-A	4.1 kb (entire gene)
HLA-B	2.8 kb (exon 1 — intron 6)
HLA-C	4.2 kb (entire gene)
HLA-DRB1/3/4/5	4.6 — 5.1 kb (exon 2 — intron 4)
HLA-DQB1	6.9 kb (entire gene)
HLA-DPB1	9.7 kb (exon 2 — 3' UTR)
HLA-DQA1	7.3 kb (entire gene)
HLA-DPA1	10.3 kb (entire gene)

Capture Full HLA Gene Sequences

The TruSight HLA v2 Sequencing Panel covers all commonly typed HLA loci, plus those with emerging relevance (Table 1). This expands gene coverage beyond the classical loci (class I exons 2, 3, and 4; and class II exons 2 and 3), providing additional information for higher resolution typing. In addition, The TruSight HLA v2 Panel achieves full gene coverage, enabling discovery of new alleles without the need to design new primers.

DNA-to-Report Workflow

The TruSight HLA v2 Sequencing Panel offers a comprehensive DNA-to-report solution for HLA typing that includes reagents and software optimized for HLA analysis (Figure 1). A combination of long-range PCR and Nextera® library preparation produces long inserts with paired-end reads that enable accurate phasing of exons and introns in a single assay.[7] There's no need to order follow-up assays to identify specific HLA alleles. The simplified workflow enables turnaround time of less than 48 hours and increased efficiency with less than 4 hours of hands-on time. Intelligent assay design and the flexible portfolio of sequencing systems and reagents enables any level of multiplexing of 4–384 samples per run.

Advanced NGS Chemistry for Library Preparation

The TruSight HLA v2 Sequencing Panel harnesses long-range PCR and HLA-specific Nextera library preparation technology to produce high-accuracy, unambiguous HLA typing in a single assay (Figure 2). In addition, unique multiplexing capabilities and integrated sample barcodes enable sample pooling for analyzing up to 384 samples simultaneously.

The TruSight HLA v2 workflow starts with the amplification of HLA genes using locus-specific primers in long-range PCR (Figure 2A). Next, a rapid Nextera library preparation step converts amplified DNA into fragmented, adapter-tagged libraries (Figure 2B) without the need for additional end repair. Using a proprietary, bead-based normalization

technique, the TruSight HLA v2 workflow enables normalization of all amplicons *en masse*. This eliminates the need to quantify and normalize each amplicon individually, significantly reducing hands-on time. Other NGS-based HLA typing methods circumvent this by normalizing only a few samples and then applying that average to the entire pool. However, this presents a problem as not all samples, loci, and alleles will amplify equally, possibly introducing errors into the HLA typing. The proprietary, bead-based normalization technique employed by the TruSight HLA v2 Panel is fast, requires no specialized instrumentation, and is more amenable to automation. The entire library prep protocol (Figure 3) requires less than 4 hours of hands-on time.

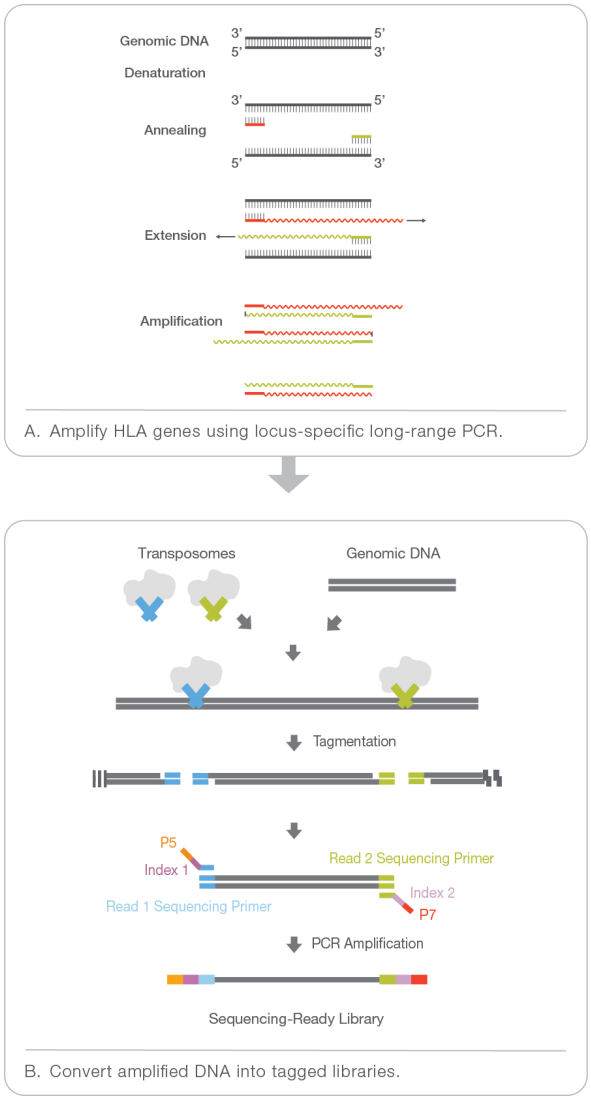

Figure 2: The TruSight HLA v2 Assay—The TruSight HLA v2 Sequencing Panel harnesses long-range PCR and HLA-specific Nextera library preparation technology to produce high-accuracy, unambiguous HLA typing in a single assay.

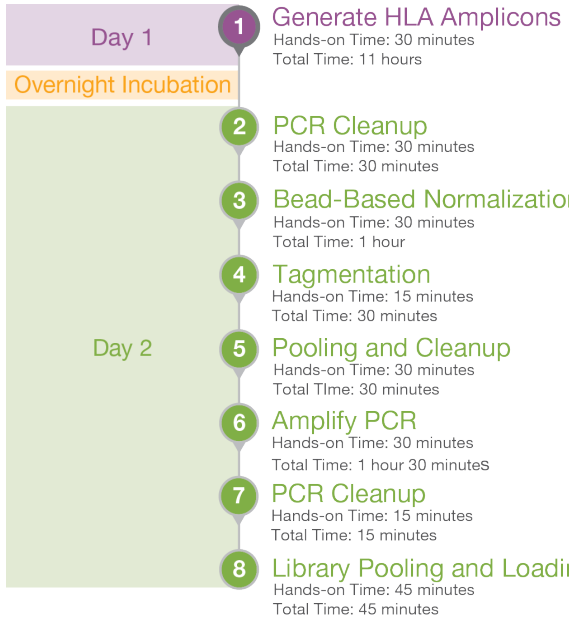

Figure 3: TruSight HLA Library Preparation Protocol—The TruSight HLA v2 library preparation protocol proceeds from DNA to sequencer in ~16 hours with <4 hours of hands-on time.

Versatile Sequencing Options with Illumina Desktop Sequencers

Prepared libraries are loaded directly onto a MiniSeq™, MiSeq®, or NextSeq® System for sequencing (Figure 4). The HLA locus is sequenced with high-quality, paired-end 2 × 150 bp reads, enabling use of dense polymorphisms to assign phase accurately. This allows unambiguous HLA typing results to be derived directly from the sequencing data. From sample to report, the process is completed in less than 48 hours.

Optimized Data Analysis with TruSight HLA Assign 2.1 Software

On-instrument software analyzes sequence data generated from TruSight HLA v2 libraries. The HLA amplicons for each sample are pooled into a single barcoded library resulting in one pair of FASTQ files per sample. After demultiplexing and FASTQ file generation, files are loaded directly into TruSight HLA Assign 2.1 Software for alignment.

Figure 4: Flexible Sequencing Options with TruSight HLA—The TruSight HLA v2 Sequencing Panel is compatible with the Illumina portfolio of desktop sequencers.

After the initial alignment, all heterozygote positions are phased. Phased alignments are then referenced against the International ImMunoGeneTics Information System (IMGT)/HLA database[8] to produce high-confidence HLA typing results. The Assign software is optimized specifically for use with the TruSight HLA v2 Sequencing Panel. It allows the import of sequences from multiple samples and loci into a user-friendly interface with:

- A summary panel providing a unified view for rapid identification of loci requiring more in-depth analysis
- A results panel displaying the most closely matched alleles, allowing for quick confirmation of rare alleles without having to search for related (common, well-documented) CWD alleles
- Reads, alignments, and reference views with a rich set of tools for in-depth analysis, alleviating the need for external resources and tools

TruSight HLA Assign 2.1 Software provides flexible options for postanalysis reporting of results independent of backend system compatibility of the user. The sample report highlights CWD alleles and includes P and G groups, audit trails, and user edits reporting selections. By harnessing TruSight HLA Assign 2.1 Software, the TruSight HLA v2 Sequencing Panel offers a complete solution for HLA typing.

High-Accuracy HLA Typing

To demonstrate the high-quality typing achieved with the TruSight HLA v2 Sequencing Panel, 72 samples with a total of 959 reference alleles were analyzed for the calculations of accuracy and ambiguity rate using the panel. Generated results were compared to typing results from previous experiments and alternate methods provided with the International Histocompatibility Working Group (IHWG)[9] reference panels (Table 2).

Samples

Samples from the following sources were used for the comparison study:

- 11 samples from the IHWG Consanguineous Reference Panel comprised of cell lines from 48 DNA samples from the 10th workshop indicated to be HLA homozygous by descent
- 37 samples from the IHWG Sequence Polymorphism (SP) Reference Panel, a combination of 51 DNA samples (also includes 4 samples that overlap with the Consanguineous Reference Panel) typed using the highest frequency sequence-specific methods possible at the time of the 13th workshop
- 13 samples from the IHWG Anthropology Reference Panel composed of 15 samples (also includes 10 samples from the SP Reference Panel) from different regions of the world
- 9 samples from Centre d'Etude du Polymorphisme Humain (CEPH) cell lines with SBT-derived high-resolution HLA typing available
- 1 additional sample from the 10th IHWG Workshop, 4 additional samples from the 12th IHWG Workshop, 11 additional samples from the 13th IHWG Workshop, and 2 samples from the IHWG Null Sample Repository

Results

TruSight HLA v2 typing results show high accuracy (Figure 5A) compared with the reference panel. Of the 72 samples, 959 alleles had published reference typings available for calculations of concordance and accuracy, 907 (94.58%) alleles were concordant with the reference typing, 42 (4.38%) references were modified based on increased coverage, literature references, or both and have been shown to be accurate results, 5 (0.52%) alleles had novel exonic variants, 5 (0.52%) alleles required editing of 1 to 3 base postions, and these last 5 alleles were not considered accurate. Ambiguity rate was calculated using all 1294 alleles typed in the study, resulting in a 3.17% ambiguity rate (Figure 5B).

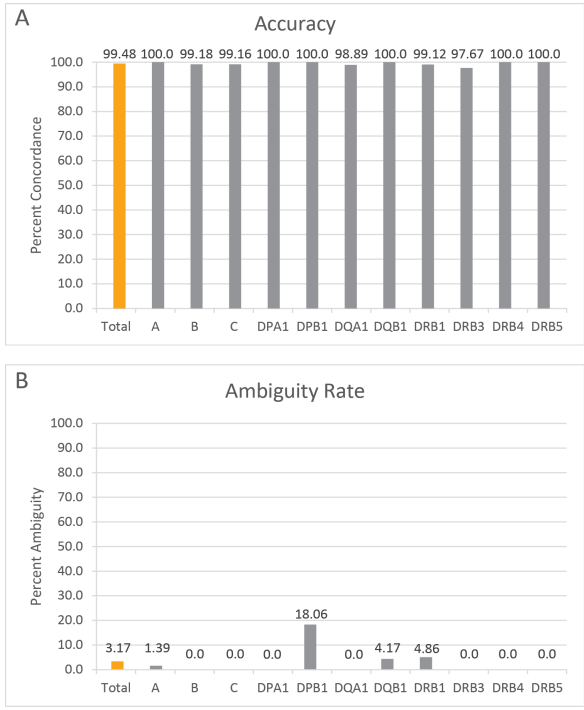

Figure 5: TruSight HLA v2 Panel Typing Performance—TruSight HLA v2 typing results show high accuracy (A) and low ambiguity (B) compared to available reference typing results.

Summary

The TruSight HLA v2 Sequencing Panel provides labs with a broad-coverage, ultrahigh resolution HLA typing solution for simple, rapid assessment of the HLA region in a single assay. The expanded coverage of the TruSight HLA v2 Sequencing Panel provides the highest level of resolution, eliminating the need for follow-up testing to obtain a confident typing result.

Learn More

To learn more about the TruSight HLA v2 Sequencing Panel, TruSight HLA Assign 2.1 Software, and the Illumina sequencing portfolio, visit www.illumina.com/hlaseq.

Ordering Information

Library Prep Kits	No. of Samples	Catalog No.
TruSight HLA v2 Sequencing Panel (24 samples)	24	20000215
TruSight HLA v2 Sequencing Panel (24 samples, Automated)	24	20005170
Indexing Kits	**No. of Samples**	**Catalog No.**
Nextera XT Index Kit (24 indexes, 96 samples)	96	FC-131-1001
Nextera XT Index Kit v2 Set A (96 indexes, 384 samples)	384	FC-131-2001
Nextera XT Index Kit v2 Set B (96 indexes, 384 samples)	384	FC-131-2002
Nextera XT Index Kit v2 Set C (96 indexes, 384 samples)	384	FC-131-2003
Nextera XT Index Kit v2 Set D (96 indexes, 384 samples)	384	FC-131-2004
Sequencing Kits (for all compatible systems)	**No. of Samples**	**Catalog No.**
MiSeq Reagent Kit v2 Nano (300 cycles)	6	MS-103-1001
MiSeq Reagent Kit v2 Micro (300 cycles)	24	MS-103-1002
MiSeq Reagent Kit v2 (300 cycles)	96	MS-102-2002
MinSeq High Output Kit (300 cycles)	144	FC-420-1003
MiniSeq Mid Output Kit (300 cycles)	48	FC-420-1004

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