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TruSight® HLA v1 Sequencing Panel

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

Highlights

- **Comprehensive Assay**
  One assay provides high-resolution sequencing of 11 HLA loci
- **Unambiguous Results**
  Deeper sequencing with long inserts and paired-end reads enables phasing of all exons and introns for more accurate, unambiguous, high-resolution HLA typing
- **Sample-to-Report Solution**
  Complete workflow includes library preparation and sequencing on Illumina technology and data analysis and reporting with TruSight HLA Assign Software

Introduction

Human leukocyte antigen (HLA) plays a significant role in the ability of the immune system to recognize invasive, foreign, infected, and malfunctioning cells. The immune system efficiently removes such 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 v1 Sequencing Panel overcomes these challenges in a single assay. Using proven Illumina next-generation sequencing (NGS) technology, researchers can generate unambiguous, phase-resolved HLA sequencing results for 11 HLA loci. The TruSight HLA v1 Sequencing Panel is supported by intuitive, rapid analysis and reporting with TruSight HLA Assign Software.

Capture Full HLA Gene Sequences

TruSight HLA covers all commonly typed HLA loci, plus those with emerging relevance (Table 1). This expands gene coverage beyond class I exons 2, 3, and 4 and class II exons 2 and 3, providing additional information that can inform how and when immune responses occur. In addition, full coverage means that new alleles can be identified as they are discovered. Using TruSight HLA, laboratories can take advantage of new data without designing new primers.

Table 1: TruSight HLA Types 11 HLA Loci

Loci	Target Sequence
HLA-A	4.1 kb (entire gene)
HLA-B	2.6 kb (exons 1-7 + introns)
HLA-C	4.2 kb (entire gene)
HLA-DRB1/3/4/5	4.1 kb (exon 2-intron 4)
HLA-DQB1	7.1 kb (exon 1–3"UTR)
HLA-DPB1	9.7 kb (exon 2–3"UTR)
HLA-DQA1	7.3 kb (entire gene)
HLA-DPA1	10.3 kb (entire gene)

Key Features of TruSight HLA v2

The TruSight HLA v2 Sequencing Panel has been optimized to improve accuracy, increase efficiency, and reduce cost (Table 2). Primers for sequencing HLA-B and -DRB loci have been redesigned to reduce ambiguities and testing burden. Library preparation incorporates amplicon pooling, enabling a ~40% reduction in pipette tips and 25–30% in overall savings. Sequencing read length has been reduced from 2 × 250 bp to 1 × 150 bp, providing faster turnaround time and lower sequencing cost while maintaining the same proven data quality. Collectively, TruSight HLA v2 provides a reduction in total sample-to-report time from 72 hours with 6 hours of hands-on time to 48 hours with 4 hours of hands-on time.

Table 2: TruSight HLA Version Comparison

Parameter	v1	v2	Benefit
HLA-B Primer	Primer encroaches exon 1	New primer in UTR	Reduced ambiguities and testing burden
HLA-DRB Primer	Primer encroaches exon 2	New primer outside exon boundary	Reduced ambiguities and testing burden
Amplicon Pooling?	No	Yes	Reduced pipette tip usage and cost
Read Length	2 × 250	2 × 150	Faster turnaround time and lower cost
Sample-to-Report Time	Total: 72 hrs Hands-on: 6 hrs	Total: 48 hrs Hands-on: 4 hrs	Faster turnaround time

Sample-to-Report Workflow

TruSight HLA offers a comprehensive sample-to-report solution for high-throughput 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. There's no need to order follow-up assays to identify the specific HLA allele. The simplified workflow enables multiplexing of up to 24 samples, reducing turnaround time and increasing productivity.

Advanced NGS Chemistry for HLA Typing

TruSight HLA harnesses long-range PCR and HLA-specific Nextera library preparation technology to produce high-accuracy, unambiguous HLA typing in a single assay (Figure 2). One assay yields a complete result. In addition, unique multiplexing capabilities enable sample pooling for analyzing up to 24 samples simultaneously.

The TruSight HLA workflow starts with amplification of HLA genes using locus-specific primers in long-range PCR (Figure 2A). Then a rapid Nextera library preparation step converts amplified DNA into fragmented, adapter-tagged libraries (Figure 2B). Using a proprietary, bead-based normalization technique, the TruSight HLA workflow enables quantification and 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 quantifying only a few samples and then applying that average to the entire pool. However, this presents a problem as not all alleles will amplify equally, possibly introducing errors into the HLA typing. The proprietary, bead-based normalization technique employed by the TruSight HLA v1 solution represents a significant advantage.

Prepared libraries are loaded directly onto the MiSeq® System for sequencing. The HLA locus is sequenced with high-quality, paired-end 2 × 250 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 3.5 days.

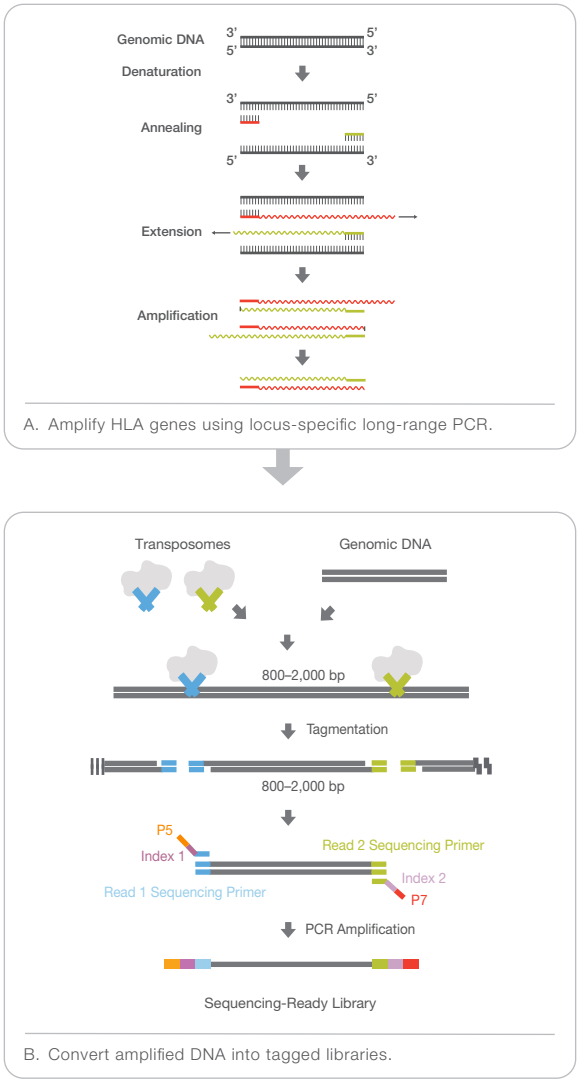

Figure 2: The TruSight HLA Assay —TruSight HLA v1 harnesses long-range PCR and HLA-specific Nextera library preparation technology to produce high-accuracy, unambiguous HLA typing in a single assay.

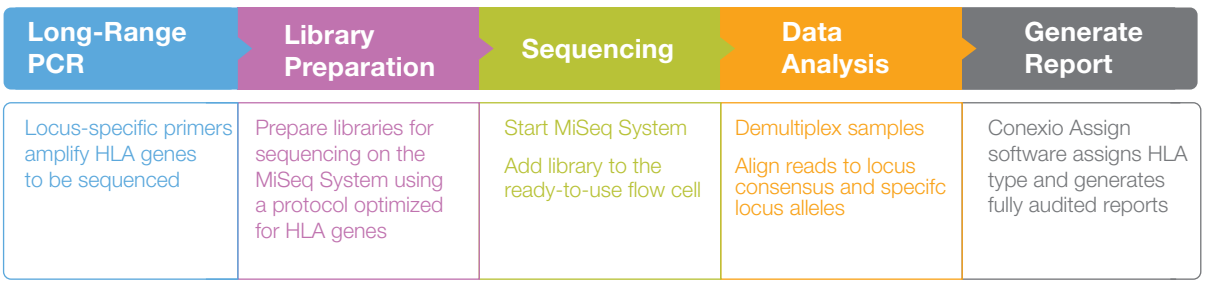

Figure 1: An Integrated Sample-to-Report Workflow for HLA Typing —The TruSight HLA v1 Sequencing Panel offers an efficient, integrated workflow from library preparation to data analysis and reporting for accurate HLA typing.

Optimized Data Analysis with Assign Software

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

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

- A summary panel provides a unified view for rapid identification of loci requiring more in-depth analysis
- A results panel displays the most closely matched alleles allowing for quick confirmation of rare alleles without having to search for related CWD alleles (Figure 3)
- Reads, alignments, and reference views are a rich set of tools for in-depth analysis, alleviating the need for external resources and tools

Assign 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 the Assign software, Illumina offers a complete solution for HLA typing with the TruSight HLA v1 Sequencing Panel.

High-Accuracy HLA Typing

To demonstrate the high-quality typing achieved with TruSight HLA v1 Sequencing Panel, 117 samples with a total of 1481 reference alleles were analyzed* for the calculations of identity and concordance using the panel. Generated results were compared to those of previously typed samples and data from International Histocompatibility Working Group (IHWG) reference panels (Table 3).

Samples

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

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

Results

TruSight HLA typing results show high concordance and high sample identity with each reference panel (Table 3).

* Of the 117 samples, 1510 alleles had published reference typings available for calculations of concordance and identity, 29 reference alleles were removed from the analysis, and 11 reference alleles modified. 26 (89%) of the removed alleles were DQ and DP in which the difference between TruSight HLA typing and reference were outside of exon 2. Of the remaining 3 alleles, 1 was in B (consanguineous samples of 27:03 reference versus a 27:05 result), 1 in C (06:06 reference versus 06:02 typing), and 1 in DRB1 (14:01 reference versus a 14:54 typing). Reference alleles were modified after confirmation by CE sequencing or literature evidence. Therefore, 1481 reference alleles were analyzed for the calculations of identity and concordance.

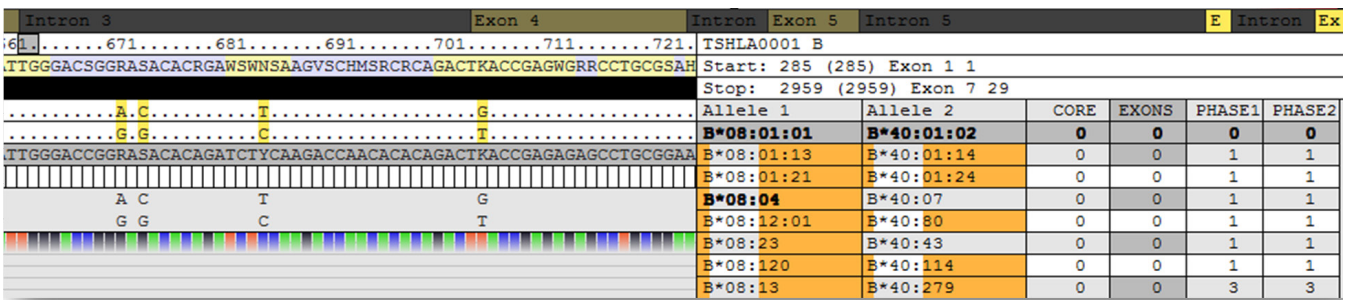

Figure 3: An Integrated Sample-to-Report Workflow for HLA Typing —Unambiguous HLA typing results derived directly from sequencing data using TruSight HLA Assign Software.

Table 3: TruSight HLA Typing Results for 117 Samples

		HLA Loci										
	No./ Percent Alleles	A	B	C	DPA1	DPB1	DQA1	DQB1	DRB1	DRB3	DRB4	DRB5[a]
Alleles with Reference Typing	1481[b]	234	233	233	119	156	104	155	232	6	9	0
Alleles Concordant[c]	1466[b]	233	229	233	119	156	104	155	222	6	9	0
Percent Concordant[c]	99.0%	99.6%	98.3%	100.0%	100.0%	100.0%	100.0%	100.0%	95.7%	100.0%	100.0%	NA
Alleles CWD Identical[d]	1459	233	229	233	119	156	104	155	222	6	2	0
Percent CWD Identical[d]	98.5%	99.6%	98.3%	100.0%	100.0%	100.0%	100.0%	100.0%	95.7%	100.0%	22.2%	NA
Alleles Identical[e]	1342	229	215	231	118	139	104	146	152	6	2	0
Percent Identical[e]	90.6%	97.9%	92.3%	99.1%	99.2%	89.1%	100.0%	94.2%	65.5%	100.0%	22.2%	NA

a. NA: Not applicable
b. There were 15 alleles in which the TruSight HLA-generated results did not match the reference typings. Each sample was run multiple times on TruSight HLA and the mismatches persisted from run to run. 11 of the 15 discordant alleles reproducibly demonstrated novel sequences. Three of the 4 remaining discordant alleles were homozygote references called as heterozygotes based on a single base mismatch, and the final discordant allele required manual editing of 3 base positions.
c. Alleles were considered concordant when the TruSight HLA result matched the reference typing with no variation in the core exons (Class I exons 2, 3, and 4 and Class II exons 2 and 3). It is possible for a concordant result to be ambiguous.
d. Common and well-documented (CWD) identical indicates that the TruSight HLA result is concordant with no ambiguities between CWD alleles.
e. Identical means that the result is concordant with no ambiguities (common or rare).

Ordering Information

Product	Catalog No.
TruSight HLA v1 Sequencing Panel (24 samples) Includes long-range PCR reagents, HLA-specific Nextera reagents, and Conexio Genomics Assign software for 192 libraries (24 samples, 11 loci each). Indexes for multiplexing, sequencing reagents, and MiSeq System sold separately	FC-142-1001
MiSeq System	SY-410-1003
MiSeq Reagent Kit v2 (500 cycles) Supports up to 24 TruSight HLA-prepared samples per MiSeq sequencing run	MS-102-2003
MiSeq Reagent Nano Kit v2 (500 cycles) Supports up to 6 TruSight HLA-prepared samples per MiSeq sequencing run	MS-103-1003
Nextera XT Index Kit v2 Set A (96 indexes, 384 samples) Supports up to 96 unique TruSight HLA-prepared libraries (12 samples, 11 loci each). Includes sufficient reagents to perform 4 library preparations (384 libraries)	FC-131-2001
Nextera XT Index Kit v2 Set B (96 indexes, 384 samples) Supports up to 96 unique TruSight HLA-prepared libraries (12 samples, 11 loci each). Includes sufficient reagents to perform 4 library preparations (384 libraries). Combine with Set A to multiplex up to 192 unique libraries (24 samples, 11 loci each)	FC-131-2002
Nextera XT Index Kit v2 Set C (96 indexes, 384 samples) Supports up to 96 unique TruSight HLA-prepared libraries (12 samples, 11 loci each). Includes sufficient reagents to perform 4 library preparations (384 libraries). Combine with Sets A and B to multiplex up to 288 unique libraries (36 samples, 11 loci each)	FC-131-2003
Nextera XT Index Kit v2 Set D (96 indexes, 384 samples) Supports up to 96 unique TruSight HLA-prepared libraries (12 samples, 11 loci each). Includes sufficient reagents to perform 4 library preparations (384 libraries). Combine with Sets A, B, and C to multiplex up to 384 unique libraries (48 samples, 11 loci each)	FC-131-2004

Summary

The TruSight HLA v1 Sequencing Panel provides clinical researchers with a broad-coverage, ultra-high–resolution HLA typing solution for simple, rapid assessment of the HLA region in a single assay. The sequencing panel's expanded coverage provides the highest level of resolution, eliminated the need for follow-up testing to obtain a confident typing result.

Learn More

To learn more about the TruSight HLA Sequencing Panel, Assign TruSight HLA software, and the MiSeq System, visit www.illumina.com/hlaseq.

References

1. Center for Comparative Genomics. HLA associated diseases and phenotypes (ccg.murdoch.edu.au/private/yurek_kulski/mhc/Table4.html).
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