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TruSight™ HLA Sequencing Panel

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

Highlights

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

Introduction

Human leukocyte antigen (HLA) plays a significant role in the body's ability to recognize invasive, foreign, infected, or malfunctioning cells and mount an immune response, helping to fight disease and maintain overall health. When working optimally, this system is efficient at removing these cells, but there are instances where this system produces an unwanted, or unwarranted, response. In these cases, HLA mutations have been associated with autoimmune disorders, cancer, transplant rejection, and drug sensitivity[1–4].

Table 1: TruSight HLA Types 11 HLA Loci.

Loci	No. Alleles[a]	Sequenced Region
HLA-A	2,579	4.1 kb (entire gene)
HLA-B	3,285	2.6 kb (exons 2–7 + introns)
HLA-C	2,133	4.2 kb (entire gene)
HLA-DRB1/3/4/5	1,512	4.1 kb (exon 2–intron 4)
HLA-DQB1	509	7.1 kb (exon 1–3'UTR)
HLA-DPB1	248	9.7 kb (exon 2–3'UTR)
HLA-DQA1	51	7.3 kb (entire gene)
HLA-DPA1	37	10.3 kb (entire gene)

[a] As defined by hla.alleles.org/nomenclature/stats.html (27 May 2014)

Learning more about the HLA region can provide critical insight into various diseases and situations. Unfortunately, sequencing HLA has been notoriously difficult due to the high levels of sequence homology and dense variability found within the region of the genome that codes for HLA. Current attempts at deciphering this region require multiple, tedious assays and produce highly ambiguous results. The TruSight HLA Sequencing Panel overcomes these challenges with a single assay that uses proven Illumina next-generation sequencing (NGS) technology to generate unambiguous, phase-resolved HLA sequencing results for 11 HLA loci.

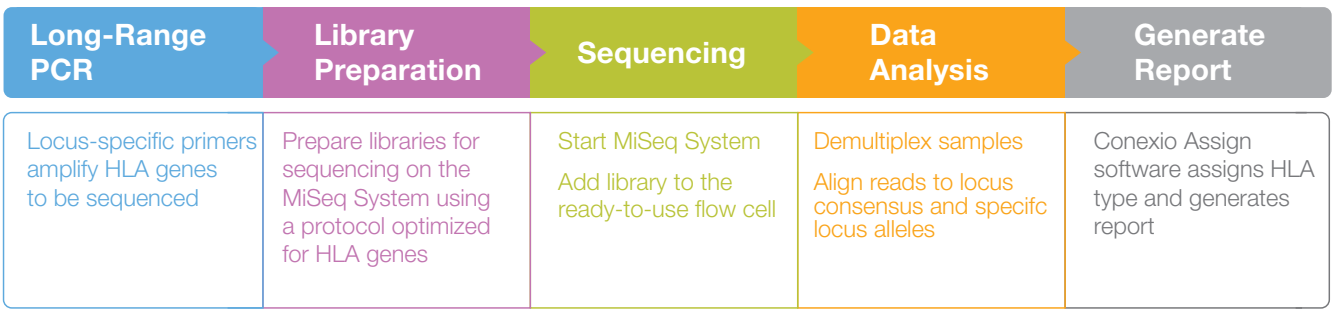

Figure 1: An Integrated Sample-to-Report Workflow for HLA Typing.

Capture Full HLA Gene Sequences

TruSight HLA covers all commonly typed HLA loci, plus those loci 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, there is no need to design new primers and implement new assays to take advantage of new data.

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 type. The simplified workflow enables multiplexing of up to 24 samples, reducing turnaround time and increasing productivity.

Flexible to Meet Specific Lab Needs

Flexible kit configurations enable labs to readily meet their sample throughput needs. Kits can accommodate from 2 to 24 samples.

Advanced NGS Chemistry for HLA Typing

TruSight HLA leverages long-range PCR and HLA-specific Nextera library preparation technology to produce high-accuracy, unambiguous HLA typing in a single assay (Figure 2). There's no need to perform multiple assays to obtain a correct HLA type. One assay yields a complete result. In addition, unique multiplexing capabilities enable sample pooling for analyzing up to 24 samples simultaneously.

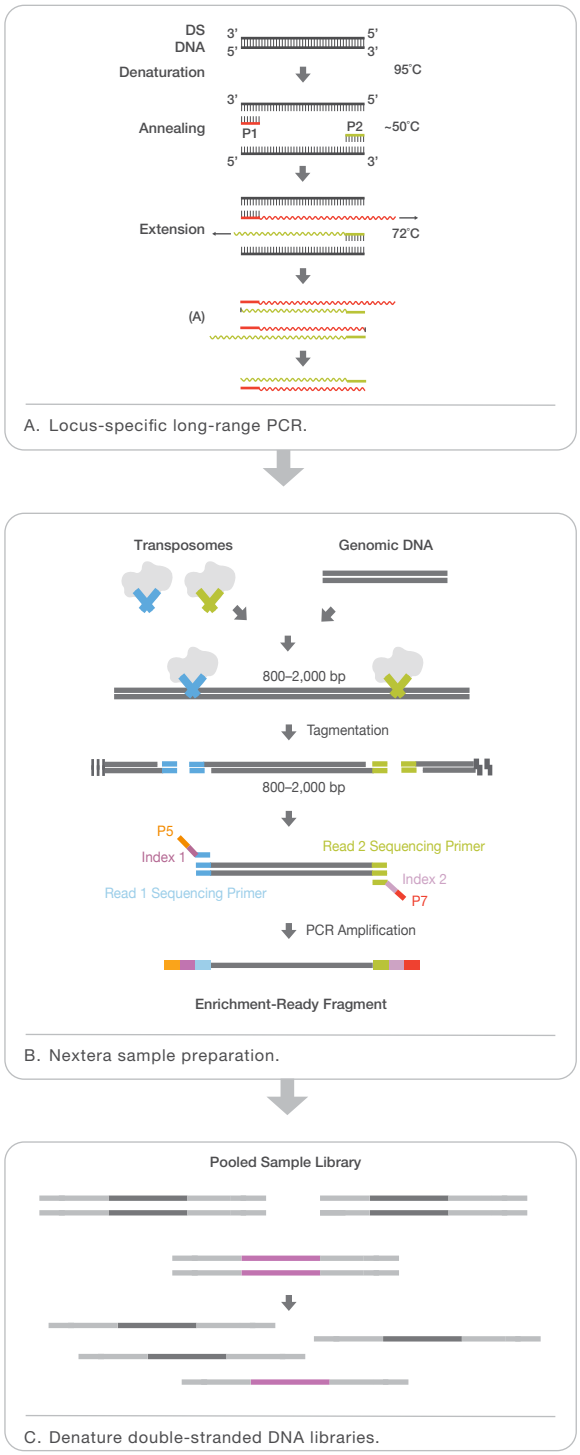

Figure 2: Illumina NGS Chemistry Powers the TruSight HLA Assay.

Following the TruSight workflow, the process starts with amplification of the HLA genes using locus-specific primers in long-range PCR (Figure 2A). Next, a rapid library preparation step optimized for HLA typing converts amplified DNA into adapter-tagged libraries (Figure 2B). Nextera tagmentation of DNA simultaneously fragments and tags DNA without the need for mechanical shearing. Integrated sample barcodes allow pooling of up to 24 samples for a single pulldown. Next, libraries are denatured into single-stranded DNA (Figure 2C) and ready to load 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. Unambiguous HLA typing results are derived directly from the sequencing data. From sample to report, the process is completed in less than 3.5 days.

Proven MiSeq® System

The MiSeq System (Figure 3) offers unparalleled reliability and accuracy, making it the system of choice for HLA typing. Using the most widely published NGS technology, this desktop sequencer enables any laboratory to perform various sequencing applications.

Figure 3: The MiSeq System. TruSight HLA leverages Illumina NGS technology on the MiSeq System for reliable HLA typing.

Optimized Data Analysis

The on-instrument MiSeq Reporter software analyzes sequence data generated from TruSight HLA–enriched libraries. Individual barcoding of each locus during library preparation generates two FASTQ files per locus (one for each paired read), resulting in the highest alignment accuracy. After demultiplexing and FASTQ file generation, files are loaded directly into Conexio Assign for alignment of the sequence reads to the HLA loci. After this initial alignment, sequences are realigned to closely matched alleles characterized in the IMGT/HLA database. Conexio Assign software is optimized specifically for use with TruSight HLA. It provides a unified view of the sequence data for rapid analysis and assignment and flexible reporting of HLA typing results.

Summary

TruSight HLA provides clinical researchers with a broad-coverage, ultra-high–resolution HLA typing solution for simple, rapid assessment of the HLA region. Using TruSight HLA, accurate, unambiguous, phase-resolved HLA typing can be achieved in a single assay. The sequencing panel's expanded coverage provides the highest level of resolution. There's no need to spend time and resources on follow-up testing to obtain a confident typing result.

Learn More

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

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References

1. Center for Comparative Genomics. HLA associated diseases and phenotypes (ccg.murdoch.edu.au/private/yurek_kulski/mhc/Table4.html).
2. Ayala García MA, González Yebra B, López Flores AL, Guaní Guerra E (2012) The major histocompatibility complex in transplantation. J Transplant. 2012: 842141.
3. Anasetti C, Amos D, Beatty PG, Appelbaum FR, Bensinger W, et al. (1989) Effect of HLA compatibility on engraftment of bone marrow transplants patients with leukemia or lymphoma. N Engl J Med. 320: 197–204.
4. The International HIV Controllers Study (2010) The major genetic determinants of HIV-1 control affect HLA Class I peptide presentation. Science 330: 1551–1557.

Ordering Information.

Product	Catalog No.
TruSight HLA Sequencing Panel (24 samples) Includes long-range PCR reagents, HLA-specific Nextera reagents, and Conexio Genomics Assign software for 192 loci (24 samples, 8 loci each)[a]. Indexes for multiplexing, sequencing reagents, and MiSeq System sold separately	Coming soon
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 loci (12 samples, 8 loci each)[a]. Includes sufficient reagents to perform 2 library preparations (192 loci)	FC-131-2001
Nextera XT Index Kit v2 Set B (96 indexes, 384 samples) Supports up to 96 unique TruSight HLA–prepared loci (12 samples, 8 loci each)[a]. Includes sufficient reagents to perform 2 library preparations (192 loci). Combine with Set A to multiplex up to 192 unique loci (24 samples, 8 loci each)[a]	FC-131-2002
Nextera XT Index Kit v2 Set C (96 indexes, 384 samples) Supports up to 96 unique TruSight HLA–prepared loci (12 samples, 8 loci each)[a]. Includes sufficient reagents to perform 2 library preparations (192 loci). Combine with Sets A and B to multiplex up to 288 unique loci (36 samples, 8 loci each)[a]	FC-131-2003
Nextera XT Index Kit v2 Set D (96 indexes, 384 samples) Supports up to 96 unique TruSight HLA–prepared loci (12 samples, 8 loci each)[a]. Includes sufficient reagents to perform 2 library preparations for a total of 192 loci. Combine with Sets A, B, and C to multiplex up to 384 unique loci (48 samples, 8 loci each)[a]	FC-131-2004

[a] For capacity planning purposes, DRB1/3/4/5 are amplified by a single primer set and considered a single locus.

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