Switch to NGS

Omixon Holotype HLA™

Holotype HLA is a combination Assay and Software product that leverages the power of Next Generation Sequencing (NGS), for use on the Illumina® MiSeq. The Assay is licensed from The Children's Hospital of Philadelphia and provides amplification and library preparation reagents for comprehensive gene coverage of multiple HLA loci. The Software, Omixon HLA Twin™, is included in Holotype HLA and features two independent algorithms for double validation by data analysis and the most accurate, high-resolution genotyping available, with no reflexive testing required.

Holotype HLA™ X2

Holotype HLA X2 amplifies 7 loci (HLA-A, B, C, DQB1, DRB1, DPB1 & DQA1) and is available in two sizes: 96 samples and 24 samples.

In the X2 configuration, all seven loci are pooled together in a single sequencing library indexed with a single molecular barcode.

The X2 configuration scales from 8 samples per run to 96 samples per run to effectively handle any throughput.

With the X2 configuration, you can process 24 samples per run with less than five hours hands on time.

The highest possible confidence is obtained by running two independent algorithms for a double (X2) check of every locus for every sample.

Holotype HLA™ X4

Holotype HLA X4 amplifies 5 loci (HLA-A, B, C, DQB1, DRB1) for 16 samples.

The X4 configuration employs a "5+1" approach for generating amplicon libraries with five individual libraries (one per locus) and 1 pooled library (all loci combined for a single sample).

With the X4 configuration, you can process 16 samples with approximately four hours of hands on time.

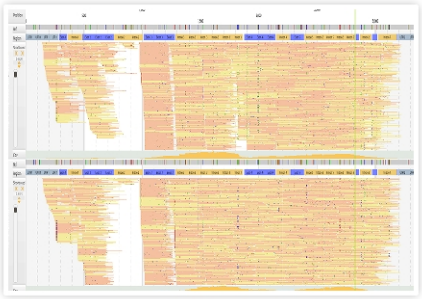
Four-fold genotyping is achieved by analyzing the two sequencing results of the "5+1" approach with two independent algorithms to provide a quadruple (X4) check of every locus for every sample.

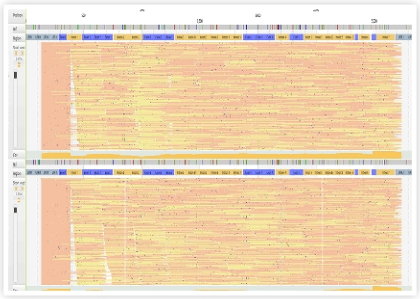
Details & Features

- Whole gene coverage of HLA-A, B, C, DQB1, DQA1 and partial coverage for HLA-DRB1 and DPB1
- 2x 250 bp Illumina paired end sequencing to resolve 99.8% of allele pairs unambiguously (0.2% ambiguity* at the 2 field level)
- Scalable from 8 to 96 samples per MiSeq run
- Balanced coverage between alleles at a locus, between loci in a sample and among samples
- Deep and even coverage across the whole length of the targeted region
- Two independent algorithms in HLA Twin for orthogonal validation by bioinformatic analysis
- Automatic genotyping after MiSeq run
- 100% concordance with known samples with HLA Twin re-analysis of double-blind Alpha Study data

* *Ambiguity not including HLA-DPB1 and HLA-DQA1 - data not yet available at time of print*

Competitive Advantages





Coverage plot of HLA-C*07:01,12:03 for a competing library preparation technique (left) sample and with Holotype (right)

Other techniques have low coverage of key exons. Loss of coverage of intron 2 may cause loss of phase between key exons 2 and 3. Shorter DNA fragments lead to further phase ambiguities, with 25 possible alleles reported for the example on the left.

Holotype HLA provides deep and even coverage of the whole region, with balance between alleles at the same locus, all loci within a sample and among samples - for up to 96 samples per run enabling better phasing, less ambiguity, and optimal utilization of sequence output.

Competing Products	Holotype HLA
Occasional allele dropout	No allele dropout (following extensive validation)
Uneven coverage, dips in coverage at key exons	Even coverage across each locus
Allele imbalance	Balanced coverage depth for both alleles at each locus
Fragmented amplicon libraries too small	Optimized size-selection of fragmented amplicon libraries to help resolve phase
One algorithm	Two orthogonal / independent algorithms
Up to 24 samples per run	Up to 96 samples per run
8+hrs hands-on time	Less than 5hrs hands-on time
Higher rate of ambiguity	Lowest rate of ambiguity

Omixon HLA Twin

Two Independent Algorithms for Orthogonal Bioinformatic Validation

Omixon HLA Twin was co-developed with the Assay and includes two independent algorithms (Statistical Genoyping and Consensus Genotyping) that use orthogonal methods (database alignment vs a de novo assembly technique) to determine the genotype of each sample.

Consensus Genotyping provides full consensus sequences of known alleles, even if these are not available in the HLA database, and exceptional capability for identifying null and novel alleles at the 3 or 4 field level.

Statistical Genotyping provides a concordance check for every locus of every sample

Omixon HLA Twin is designed for ease-of-use, computational performance, accurate genotyping, comprehensive quality metrics and detailed visualization.

For more information:
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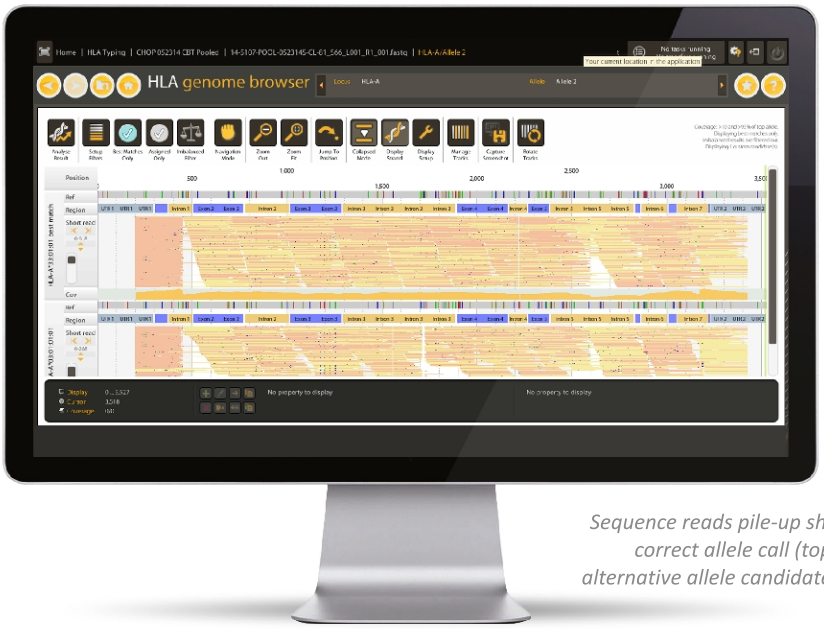
"This assay solves many challenges including preferential allele amplification, uneven amplification of key exons and fragment size problems that most people who develop HLA assays struggle with. It simply gives the most beautiful HLA data we have ever seen."

"This new approach addresses a 60-year-old problem," says Professor Dimitri Monos. "Since the discovery of HLAs in the early 1950s, it has been a challenge to accurately and thoroughly characterize HLA gene sequences. We have now used next - generation sequencing tools to significantly advance HLA typing."



Sequence reads pile-up showing the correct allele call (top) and one alternative allele candidate (bottom)