MEDGENOME

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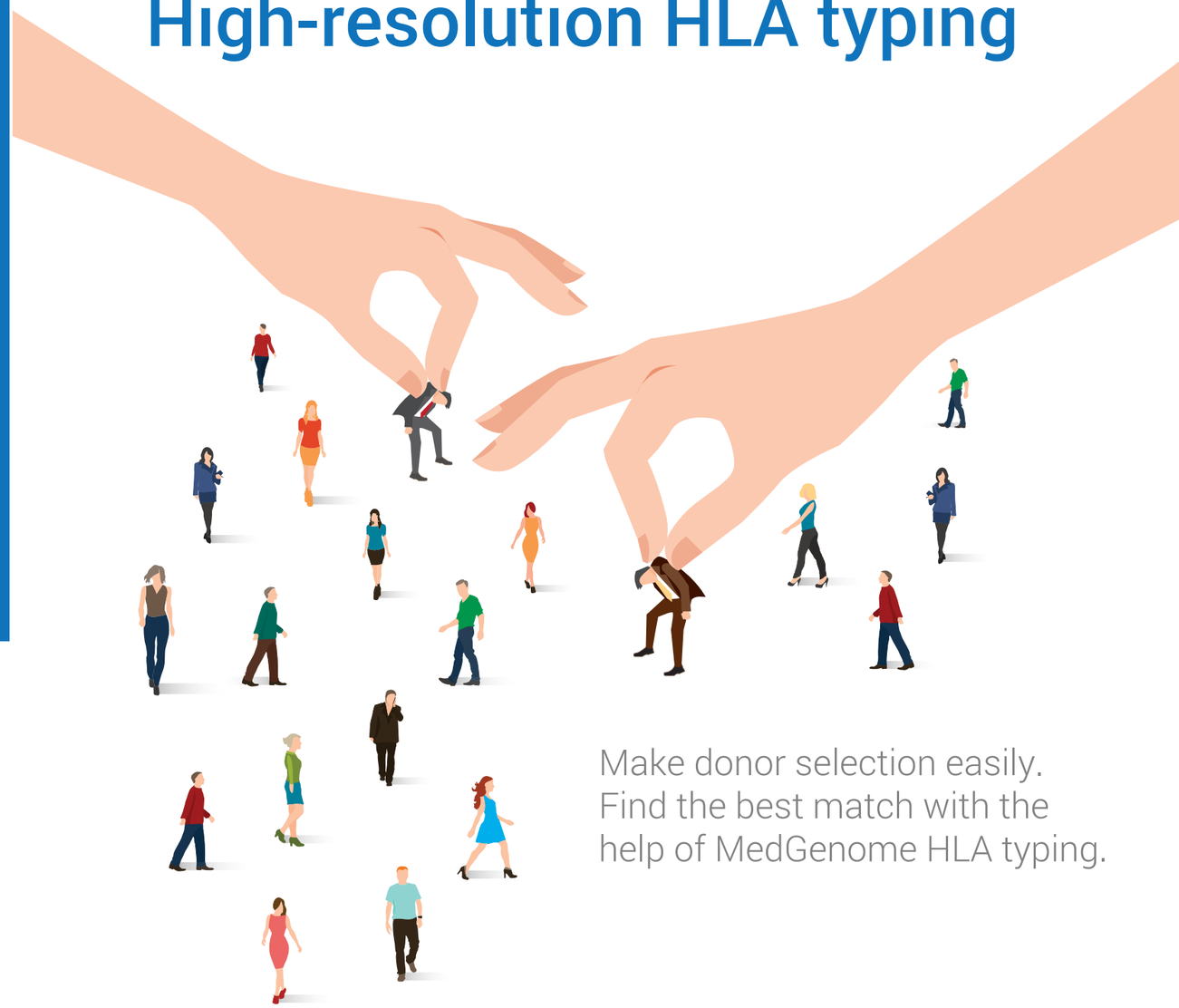
Prima
Cancer Genetics

High-resolution HLA typing



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Find the best match with the
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What is HLA typing !

The HLA genes which code for the HLA antigens are located within the short arm of human chromosome 6 and contains more than 220 genes of diverse function – This portion of the human genome is known as the **Major Histocompatibility Complex (MHC) region**. Many of the genes encode the proteins of the immune system. In cases of allogenic bone marrow transplantation, identification of specific HLA alleles is of vital significance in matching the immune signature of a potential donor with that of a recipient. 50% or higher match in the HLA gene allelic sequence indicates that such a transplant will have least possibility of ***graft versus host disease*** or in other words lowest risk of a transplant rejection.

HLA genes are the most polymorphic regions of the human genome. Ever since, the discovery of HLA genes, over 12,000 different types of alleles have been found in the HLA genes in the human population.

Since the initiation of HLA typing in 1950s it has been a challenge to accurately characterise the HLA genes. But now through high resolution HLA typing via next generation sequencing, it is possible to select the best possible donor for stem cell transplantation.

Advantages over conventional typing methods

HLA typing is challenging due to its high polymorphic nature and high levels of sequence homology between the loci. Using conventional typing methods, Serology, SSP, SSO and SBT, are laborious and time consuming and may not resolve ambiguities between homologous regions at allelic resolutions. Besides, different HLA loci (genes and pseudogenes) share nucleotide sequences which is difficult to map with conventional techniques. NGS allows a single nucleotide based sequencing to derive an accurate map of each HLA gene loci.

Limitations of Sanger Sequencing for HLA typing

- HLA genotyping by sanger sequencing is derived from few exons of HLA class I and Class II genes. NGS covers entire gene & has no allelic ambiguities
- Sanger sequencing fails to solve cis/trans chromosomal polymorphic positions of HLA alleles

NGS methods facilitate complete, unambiguous & high throughput HLA gene sequences.

Advantages of NGS based HLA typing

- Replaces preliminary and reflexive HLA typing methods with a single test providing the highest possible resolution making donor selection faster, more precise & less expensive
- Improves transplant outcomes

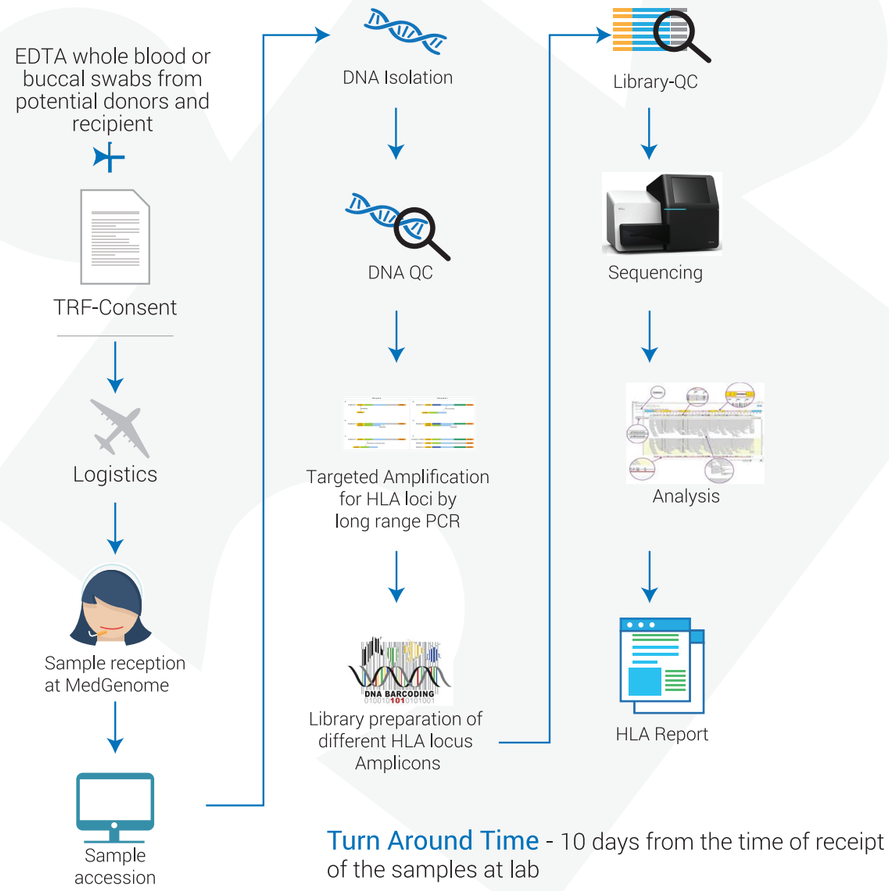
NGS based typing

1. Eliminates the problem of sequencing heterozygous DNA through clonal template amplification *in vitro*
2. Sufficiently long read length (300+ bp) to cover entire exon (or more) in phase
3. Increased sequence coverage of HLA genes
4. Capability to multiplex patient specimens
5. Short turn around time for analysis
6. Identification of novel alleles

What does NGS based HLA typing data yield

1. Identification of novel allele that may result in mismatch
2. Detection of null alleles (resulting from mutations in untested/uncovered areas; Identification of null alleles allows the identification of non-self-epitopes that may be recognized as foreign
3. Identification of mismatches
4. Detection of sequence variations that regulate expression levels (permissible and deleterious if alleles are mismatched)

NGS-HLA workflow at MedGenome



Turn Around Time - 10 days from the time of receipt of the samples at lab

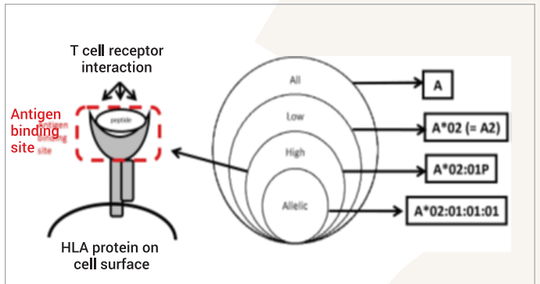
Blood sample should not be collected for HLA typing if whole blood transfusion has been given in the past 120 days.

Blood: 5—10 ml Whole blood in EDTA (purple top). Store and ship at 2—8 degree celsius. Do not collect soon after dialysis or Chemotherapy. Collect after 72 hours.

Buccal swabs: 3 buccal swabs must be collected from each subject incase blood sample is not being submitted

Ultra high-allelic resolution HLA typing

HLA typing resolution. The Venn diagram illustrates increasing levels of HLA typing resolution. The figure below shows the antigen binding site of a HLA class I molecule. Through NGS, the specific DNA sequence of the region of genome that codes for the 'antigen binding site' is deciphered.

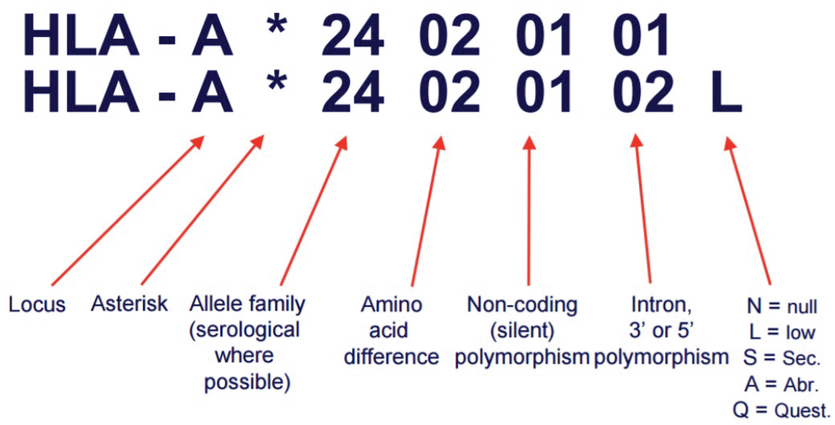


Ref: Definitions of histocompatibility typing terms - Eduardo Nunes; Blood -2011-05-353490.

The highest degree of resolution to which a HLA coding gene can be deciphered is known as 'Allelic resolution'. Allelic resolution coding enables to indentify whenever there are 'synonymous DNA substitutions within the coding region as well as major introns' leading to change in expression of HLA antigen.

Such resolution of gene sequence is depicted as four digits separated by colons eg A*01:01:01:01.

HLA Allele Nomenclature



Comparison of methods of HLA

Level of resolution	
Low level of resolution	A*02
Medium level	A*0201/0205/0209/0240
High level	A*02010101

Method	About method	Pros	Cons
Serotyping	Non-sequencing based typing method where antibodies specific to HLA proteins are used to identify the proteins on the cell surface.	- Low Cost - Rapid - Tradition	- Crude Method - Protein based detection - Inaccurate typing - Protein binding to more than one serotype
Sequence Specific Oligonucleotide Hybridization (SSO)	Typing .method where specific oligos are first designed for genes of interest and then hybridized to patient or donor DNA to check for hybridization.	- Checking of specific target - Efficient	- Cannot account for unrecorded alleles - Hybridization errors - Need to know target sequence - Cannot phase
Sanger Sequencing	Sanger sequencing or Sequencing by Termination (SBT) is a classical method used for sequencing specific regions of the MHC.	- Used to sequence regions of interest - Fast - Base pair resolution - Coverage only 2X	- Different HLA alleles share similar sequences, difficulty aligning - Cannot phase
Next-gen Sequencing	Performing long range PCR to amplify HLA genes in MHC region, fragmenting the amplified genes.	- Deep coverage (1000x) - Total MHC coverage - Rapid high throughput - Accurate and efficient - Phasing	- Data Analysis

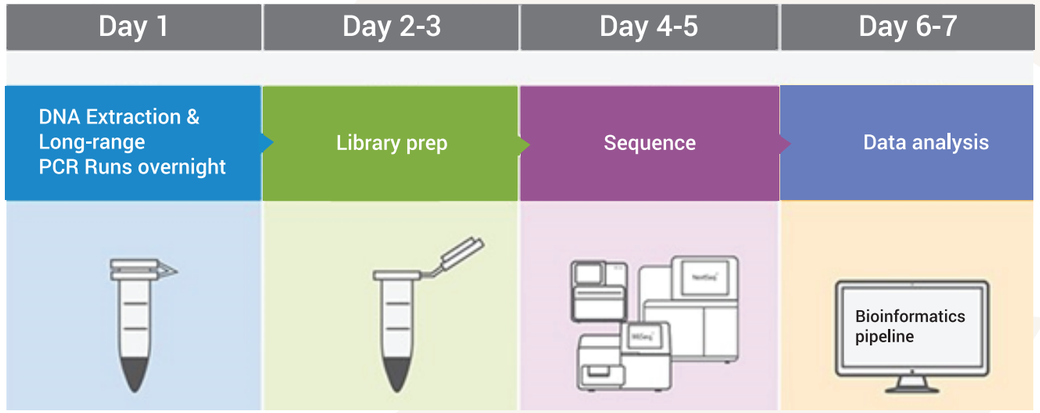
MedGenome's NGS based ultra high-resolution allelic HLA typing and matching provides transplant specialists with a broad-coverage, ultra-high—resolution human leukocyte antigen (HLA) typing solution for simple, rapid assessment of the HLA region in a single assay. It features:

1. Comprehensive Assay: One assay provides high-resolution sequencing of 5 HLA loci
2. Highest quality standards in sample-to-report Workflow: Includes library preparation, sequencing, and data analysis/reporting
3. Unambiguous Results: Deeper sequencing enables accurate and high-resolution HLA typing

Capture Full HLA Gene Sequences

MedGenome's NGS based ultra high-resolution allelic HLA covers all commonly typed HLA loci, plus those with emerging relevance. This expands gene coverage beyond the classical loci, providing additional information that can inform how and when immune responses occur. In addition, it achieves full gene coverage, enabling reporting of new alleles.

Comparison of methods of HLA



MedGenome uses customised curettage and annotation tools for filtering the sequencing data followed by IPD-IMGT/HLA database parsing for postanalysis reporting of results.

High-Accuracy HLA Typing

The customised high coverage of MedGenome's high resolution allelic HLA Sequencing Panel provides the highest level of resolution, eliminating the need for follow-up testing to obtain a confident typing result.

The HLA locus is sequenced with high-quality, paired-end 2 × 100 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.

Advantages of HLA Analysis with NGS

The human leukocyte antigen (HLA) is the most densely polymorphic region of the genome. HLA genes have been strongly associated with transplant rejection, autoimmune disease, vaccine pharmacogenomics (vaccinomics), cancer and mate selection.

Other methods fail to fully characterize HLA genes due to dense variability and sequence homology between the genes and pseudogenes. MedGenome's Next-generation sequencing (NGS) with Illumina sequencing by synthesis (SBS) chemistry overcomes these challenges enabling simple, high-quality analysis of the key HLA genes.

In NGS millions of nucleotide base pairing reactions occur in a massively parallel fashion, rather than being limited to a single or a few DNA fragments. This advance enables rapid sequencing of large stretches of DNA, with the latest instruments capable of producing hundreds of gigabases of data in a single sequencing run.

MedGenome's multiplex sequencing enables large numbers of loci to be sequenced simultaneously during a single experiment. To accomplish this, individual "barcode" sequences are added to each locus so they can be differentiated during the HLA data analysis.

Sample Reporting

TYPING RESULT			
		(Patient)	(Donor)
CLASS I	HLA-A	A*24:02:01:01 / A*11:01:01:01	A*24:02:01:01 / A*24:02:01:01
	HLA-B	B*40:06:04 / B*51:01:01:01	B*40:06:04 / B*44:03:02
CLASS II	HLA-C	C*01:02:01 / C*07:02:01:01	C*01:02:01 / C*07:06
	DRB1	DRB1*04:03:01 / DRB1*08:03:02	DRB1*04:03:01 / DRB1*07:01:01G
	DQB1	DQB1*03:02:01 / DQB1*03:01:01G	DQB1*03:02:01 / DQB1*02:02:01:01

G code: G code is a group of alleles that have identical nucleotide sequences in the antigen recognition site.

Please refer Appendix in this report for the alleles bearing suffix 'G' on them.

INTERPRETATION
The HLA typing of patient matches 50% at each locus with potential donor.

APPENDIX		
Locus	**(Reported Allele)**	**Included Alleles**
DRB1	DRB1*07:01:01G	DRB1*07:01:01:01, DRB1*07:01:01:02
	DRB1*08:03:02	DRB1*08:14
DQB1	DQB1*03:01:01G	DQB1*03:01:01:02, DQB1*03:01:01:03