type HLA

a hassle-free professional HLA typing service for any study

How HLA tissue typing your study-cohort can make all the difference

Over 99% of our genes are the same
Our HLA type accounts for much of the difference

It is not surprising that many diseases, their progression, prognosis, and - as we are beginning to discover - the success of their treatments, are linked to certain HLA types. Such linkages were first discovered long before the molecular immunology of HLA was fully understood. HLA typing analysis continues to be important today even for studies with no obvious immunology link.

How it can make all the difference

Many clinical or other statistical studies of human cohorts fail their primary endpoints because they do not reach statistical significance on their core hypothesis. Studies are usually designed to cover a diverse group of individuals with a broad distribution of HLA tissue types. However, there is the possibility that when the study is reanalyzed on the basis of detailed HLA typing data, that statistically-significant trends become visible. Such analysis could make all the difference in recovering the success of a study for at least a sub-population in the cohort or for formulating important new hypotheses to be investigated.

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Why typeHLA?

The driving force in the development of HLA typing technologies continues to be the field of organ and stem cell transplantation. As a consequence, it is predominantly transplant centers and tissue donor registers that carry out HLA typing routinely. Other scientific research into the HLA linkage of diseases and their treatments has often been carried out in collaboration with such transplantation-focused laboratories. While this can be a practical *ad hoc* solution it is often also problematic for projects that are not obviously linked to transplantation.

Recognizing the need for a reliable contract service for HLA tissue typing, typeHLA is designed to deliver a complete solution for any study based on state-of-the-art typing technology. Our service covers study planning, sample shipping, data reporting and interpretation as well as an appropriate contractual relationship.

typeHLA in summary

typeHLA is ProImmune's straightforward, dependable core facility tissue typing service with the following features:

- Carried out using the most up-to-date genotyping technologies
- Tier 1 Typing using PCR-SSOP, resolves major allele groups to 4 digits, with some degeneracy
- Tier 2 Typing using PCR-SSP or PCR-sequencing for higher resolution
- Order any or all of the following loci: Class I (A, B, C) and Class II (DRB1, DRB3/4/5, DPB1, DQA1&DQB1)
- Detailed results typically sent in 15 working days

The typeHLA service in detail

Tier 1, Class I and Class II

- Typing by PCR-sequence specific oligonucleotides (PCR-SSOP) to resolve major allele groups to 4 digits, with some degeneracy e.g. HLA-A*23:01 /03/ 05/ 06). PCR-SSOP: The genomic DNA is amplified using PCR, then incubated with a panel of different oligonucleotide probes, which have distinctive reactivities with different HLA-types. The Luminex xMAP® technology is used, where oligonucleotide probes are individually attached to up to 100 distinctly fluorescent microspheres. This allows the measurement of 100 different reactions in a single tube.

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Tier 2, Class I and Class II

- Can be carried out if needed following Tier 1 typing to achieve higher resolution. Typing by PCR-sequence specific primers (PCR-SSP) or PCR-sequencing, will usually resolve to a specific 4-digit allele, with only occasional degeneracy. PCR-SSP: the PCR reaction is used to define whether the targeted HLA allele is present or absent by using reagents in the PCR reaction specific for individual HLA alleles. PCR-sequencing: the DNA sequence of the HLA allele can be directly analyzed by performing nucleotide sequence analysis of the amplified DNA.

Sample types and order handling

- We accept genomic DNA, Saliva, fresh whole blood or frozen cells, and will provide you with details of how to ship each of these to us. There is an order handling charge for non-gDNA samples due to the additional processing needed for these sample types.
- We offer a worldwide service, available for one sample or hundreds - our high throughput service enables us to process tens to hundreds of samples at a time. You can confidently send your samples from any location worldwide using our experience in shipping globally. We can accept genomic DNA, whole blood or cryopreserved PBMCs (depending on origin and infectivity).
- Results are delivered in MS Word and Excel formats. The report contains 'strings' of results; see the examples below for how to interpret the data. The files are made available to you for download from your secure online customer account.
- Discuss your requirements with our customer service team of highly trained immunologists. They will work out the best service for you, and manage the whole process from sample handling to delivery of the final report. Typically, the results are available 15 working days following receipt of the samples, and you can download them from our secure server.

typeHLA customer reviews

Dr. Jennifer Kirchherr at the Duke Human Vaccine Institute in North Carolina, USA affirms the importance of correlating the immune response observed with HLA tissue type for their researchers carrying out clinical studies. In addition she comments, *"We looked at a range of companies offering tissue typing, and also at using an on-campus typing facility, and we found that ProImmune were able to offer us the best pricing and turnaround time."* Dr. Kirchherr and her colleagues send regular batches of samples to ProImmune for typing. *"They have been wonderfully easy to work with, if we ask anything they get right back to us, and overall it has been a really good experience"* she added.

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Highlighting the relevance of tissue typing, and the global availability of typeHLA to researchers all over the world, **Dr. Navapon Techakriengkrai** at Chulalongkorn University, Thailand has also had success with the service. *"I outsource my tissue typing to ProImmune because they provide me with the fastest service at the best price. Since the main theme of my research is on T cell immunology, HLA-type is inevitably needed. The customer service from ProImmune is of the best quality. Their tissue typing service allowed me to unambiguously resolve the HLA type of many of my samples."*

How do I interpret HLA tissue typing data?

Data will be reported as 'strings' of results, and are rarely unambiguous, so it is important to know what you are looking for. The typing process uses oligonucleotide primer sets to narrow down the possible tissue types to a limited number. However, in recent years, the number of HLA alleles being discovered has increased exponentially, as technology advances. Most of these newly-identified alleles are present in the global population at vanishingly small frequencies and are very unlikely to occur in a clinical trial cohort of a few hundred patients.

Medium resolution typing (such as ProImmune's Tier 1 service) will frequently give clear data, indicating just one or two possible 'four digit' HLA-types. This service should be sufficient for most users unless they are studying a very particular aspect of HLA linkage in their study. Usually the first answer is the correct tissue type, for the simple reason that alleles have been named as they have been discovered, and more common alleles were discovered first. In the following set of results for Tier 1 typing of the MHC Class II HLA-DRB1 locus, the sample typed could be DRB1*13:03 or DRB1*13:95, but the individual is very unlikely to be DRB1 *13:95 as this variant is so uncommon.

Example 1: **MHC Class II HLA-DRB1 *13:03/95**

The same logic also holds true for longer redundant strings of results. For example, the following string was produced from Tier I typing of the MHC Class I HLA-C locus.

Example 2:

MHC Class I HLA-C
***07:01/06/09/18/20/21/24/35/36/44/52/55N/57/59**

However, at least in Northern European populations, *07:01 is by far the most common variant and most likely to be the tissue type of the individual.

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HLA-linked diseases and treatments

An individual's immune response to a foreign agent, such as a vaccine, drug or allergen, will be strongly influenced by their HLA tissue type. In addition there are numerous examples of diseases with known HLA association – for instance, Type 1 Diabetes is associated with HLA-DRB1*04:01, Multiple Sclerosis with HLA-DRB1*15:01 and protection from HIV infection with B*57:01 and B*35:01. It seems absurd, but HLA type is ignored in most clinical trials where the presence of a novel therapeutic is known to influence the immune response.

One of the most relevant examples is recombinant factor VIII, used to treat suffers of hemophilia. There is clear bias towards an anti-drug response in a subset of recipients with common HLA alleles. Efforts are underway to understand this bias fully, and to develop variants of Factor VIII that will be less immunogenic for these individuals.

Autoimmune diseases including Rheumatoid Arthritis (RA) show a strong association with HLA DRB1*01:01 and HLA-DRB1*04:01. A therapeutic target in RA is CD20, and the anti-CD20 antibody Rituxan® (Rituximab), (a chimeric mouse/human monoclonal antibody developed by IDEC Pharmaceuticals) is used in RA treatment. However, Rituxan® contains DR1 and DR4 restricted T cell epitopes, which in the RA population contribute to a high rate of reported immunogenicity.

These examples illustrate the case for understanding your patient population in terms of HLA type, and thus knowing at an early stage how your biologic may perform in different populations. Even if your clinical trial doesn't result in your therapy going forward, you can maximize the amount of information you gain from the trial by having HLA typing data available.

ProImmune offers HLA Tissue Typing as a service, using PCR-SSOP, PCR-SSP and PCR sequencing. Whether you are engaged in a clinical trial or experiments for discovery research, in advance of starting functional cellular assays (such as ELISpot or flow cytometry testing), we highly recommend you find out the tissue type of your donor population, to add value and greater depth to your results.

How frequent are different HLA Alleles?

Information about allele frequencies can be found at the following links

http://www.allelefrequencies.net/default.asp

http://bioinformatics.nmdp.org/

http://www.ebi.ac.uk/imgt/hla/allele.html

For further detailed information on allele frequencies please contact us.

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