Name of Project: Study of haplotypes in families by NGS HLA

Anthropological study of families by NGS of full-length HLA genes to determine haplotype segregation in multiple populations. Samples from family quartets consisting of two parents and at least two non-HLA identical children or family trios consisting of one parent and at least two non-HLA identical children or 2 parents and one biological child are required. The families should be previously HLA typed at any level (serology, DNA).

NGS HLA Component Survey Link:
http://goo.gl/forms/OsoSM5SpTePSxEw33

A. Requirements Documentation:

Objective/Goals:
To pursue family based studies by NGS of full-length HLA genes to determine haplotype segregation and compare their distribution among multiple populations.

Samples type:
Samples from family quartets consisting of two parents and at least two non-HLA identical children or family trios consisting of one parent and at least two non-HLA identical children or 2 parents and one biological child are required. The families should be previously HLA typed at any level (serology, DNA)

NOTE: Investigators may submit any of the following:
- Samples
- NGS data
- Both samples and data

If samples are being submitted without NGS data, contact the Component Chair to identify a collaborating laboratory who will perform the NGS testing. NGS could also be performed by a core laboratory if your group does not have a collaborating laboratory identified.

DNA requirements (if submitting samples only)
- **3µg DNA** is required. Minimum concentration is 5ng/µl (e.g., 20 µL of 150 ng/µL DNA)
- DNA must have > 10 kb fragments visible as a strong band when checked for quality.

Test Requirements:
There are no specific requirements for NGS platform/reagent combinations.

Proficiency testing:
Labs performing NGS HLA testing are required to perform proficiency testing (NOTE: labs who successfully participated in the Pilot project are not required to perform proficiency testing)

Instructions to request panels:
1. Choose the number of DNA reference panels desired (each is composed of 24 DNAs). Each participant may select as many as four reference DNA panels in addition to one Proficiency DNA panel.
2. Complete the **IHWG Nonprofit Order Form** attached here. Be sure to complete all the information requested.

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Nonprofit Order
Form-IHIW-Referenc

3. Indicate the total number of panels being ordered (additional panels may be ordered at a later time by completing another form). Enter the total number of panels in the yellow box as shown below

| SPD40304 | HLA IHIWS Reference Panel | $ 120.00 | | | $ | - |

4. Once the form is completed, email to rcb@fhcrc.org or by fax to 206-667-5255 (please include tamara.vayntrub@stanford.edu)
5. Please contact the Fred Hutchinson Cancer Research Center IHWG Cell and DNA Bank for PO or other ordering information (please note: you must use the form attached here)
6. Make sure to indicate in the body of the email to rcb@fhcrc.org and tamara.vayntrub@stanford.edu if you are requesting a Proficiency DNA panel and how many additional reference DNA panels you would like (total max = 1 PT + 4 reference)

Project Timelines:

January 2016:   Start registration for project online at http://ihiws.org
                               Start ordering reference DNA panels
February 2016:   Start submitting study samples to NGS lab if not performing NGS testing
March 2016:   Start reference DNA panel distribution
                               Start testing study subjects and reference DNA panel
September-October 2016: Deadline for data submission proficiency and reference DNA panel(s) to IHIWS database.
April 30, 2017:   Deadline for all data submission of study samples.

IRB Requirements
Samples and data submitted must de-identified, it should not contain personally identifiable patient information and should not include "Protected Health Information" as defined under the United States Health Insurance Portability and Accountability Act (HIPAA), http://www.hhs.gov/hipaa/index.html.
An IRB submitted by Stanford University will cover de-identified samples and data from participating centers that contain no PHI or clinical data and samples were not collected specifically for this project.

Required NGS HLA loci: HLA-A, B, C, DRB1, DRB3/4/5, DQB
Optional NGS HLA Loci: HLA-DQA, DPA, DPB, MICA, MICB

Cost: participants of this project will be expected to perform NGS typing of a proficiency panel to be provided by the Workshop organizers. Panels consist of 24 DNAs. These will be shipped directly from the Fred Hutchinson Cancer Research Center IHWG Cell and Gene Bank. The cost of the panel is $150.31 plus shipping (shipping cost are about $60 within USA and $100 - $200 international) Participants may also provide an overnight courier shipping account number at the time the form with the number of panels requested is submitted.

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Data analysis and data entry:
Database requires entry of HLA types in GL string format. Depending on the analysis software, a macro may be used to convert the output to the desired format. Example of Output file if not in GL string format should be provided.

Required for all projects:
- LabCode: six character alphabetic code provided by the 17WS organizers
- SampleID: As labeled
- GL Genotyping: a locus-level HLA genotype recorded using GL String format, as defined by Milius et al. 2013 (doi: 10.1111/tan.12150)

GL String.pdf

- HLAtyping: Allele in the GL String

Project Specific data:
- Complete HLA typing of the subject included in the family quartet (or trios)
- Race/ethnicity/country of origin of the subjects included in the family quartet (or trios)
- Closest allele in the IMGT database to novel alleles and differences from the closest allele, when applicable
- Inclusion: Reason for including the sample in this study (E.g., any characteristics of interest of the haplotypes identified or the population of the subject carrying these haplotypes)
- Information pertinent to admixture when appropriate?

Other data entry:
- Does the procedure involve mixing Amplicons (if yes, at what step(s) of the testing protocol)
  – *This information will be captured in the accessory methodological information either provided as a text/pdf file or referenced via a DOI or a published citation when providing the method documentation as part of the typing submission*

- Software_related
  Software_Manufacturer: the manufacturer of the software
  Software_Name: the name of the software applied

- Hardware related
  Instrument_Firmware: the version number, or other identifier, defining the software used on the instrument for data-analysis
  Instrument_Model_Number: the model number, or other identifier, defining the type of instrument used for the typing
  Instrument_name

- Alignment_Reference_DB: the IMGT/HLA Database release version (e.g., IMGT/HLA Database 3.18.0) or Genome Reference Consortium release version (GRCh37) used for aligning reads for consensus generation

- BaseCalling_Reference_DB: the IMGT/HLA Database release version (e.g., IMGT/HLA Database 3.18.0) used to identify the genotype from the consensus sequence.

- Consensus_Sequence: A nucleotide sequence representing a contiguous phased region of DNA. This can correspond to a single feature, or to multiple contiguous features. If a locus is absent, this is not reported.

- Locus_name: (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, HLA-DPB1, MICA, MICB) the locus for which the sequence data and metadata in a given Locus element are reported

- NovelPolymorphism: describe any novel sequence polymorphisms resulting in a sequence that does not correspond to an allele in the reference database using a notation that identifies the reference database version, reference allele acccession number, feature in which the novel polymorphism is found, and difference from the referenced feature. For example, IMGT/HLA|3.18.0|HLA00001|1.4|G56C identifies a G>C transversion at position 56 of exon 2 in a sequence that is otherwise identical to the exon 2 sequence of HLA-A*01:01:01:01.

B. Other Information that may be requested:
Raw data