

NGS of Full-length HLA genes of Reference Cell Lines

Short description:

NGS of full-length HLA genes typing of a reference panel of Cell Lines collected historically by the previous workshops. The panels will consist of various number of Cell lines depending on the specific objective, such as performance validation for the 17th IHIWS or for accreditation by the various certification boards

A. Requirements Documentation:

Objective/Goals:

Sequence a reference panel of Cell Lines (collected historically by the previous workshops) with complete NGS sequence of HLA genes performed by multiple laboratories, the results will serve as an unambiguous reference of the evaluation of each reagent/platform used. The panels consisting of various number of DNAs from Cell lines will be used for performance validation, building NGS reference panels that may be used for accreditation by the various certification boards.

Samples provided:

DNA supplied by the Fred Hutchinson Cancer Research Center IHWG Cell and DNA Bank. Each participating organization will be required to perform typing on at least 24 DNA panel. Four panels of 24 DNAs each specially designed by the 17th IHIWS may be requested in addition to the required PT panel.

The cell panels will be shipped to the labs requesting participation directly from the IHWG Cell and DNA Bank, along with recommended Handling Instructions for Genomic DNA. Each cell line DNA will be provided at a concentration adjusted to 100 nanograms per microliter, in a volume of 20 microliters per tube. Each sample will therefore contain a total of 2 micrograms of DNA.

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.



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		\$ -
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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)

Panels:

1. Workshop Proficiency Testing: DNA panel of 24 DNAs with known HLA by NGS will be used for Proficiency Testing to qualify labs requesting to participate in NGS HLA projects.
2. Additional reference panels are available. Participants may request as many as 4 additional DNA reference panels of 24 DNAs each.
3. Laboratories who participated in the Pilot Project and perform NGS full-length HLA gene testing, with $\geq 95\%$ of the samples being discrepancy free at all 5 loci (A, B, C, DRB1 and DQB1) are not required to perform the testing on the Proficiency DNA panel. These laboratories may wish to request additional reference DNA panels of 24 DNAs each.

Accreditation:

Labs who wish to do so, may submit the results of the Proficiency Testing panel to the accrediting agencies for NGS accreditation (see the appropriate agency for minimum validation requirements and blind parallel testing requirements)

Test Requirements:

To participate in the NGS HLA component, Labs are expected to perform NGS full-length HLA gene testing of the Proficiency Panel, with $\geq 95\%$ of the samples being discrepancy free at each of the 5 required loci (A, B, C, DRB1 and DQB1).

For laboratories participating in the accreditation, it is required that laboratories agree to submit results to both accrediting agency and the IHIWS and provide attestation that information is not being shared between labs. The IHIWS will send the consensus result for the PT panels to the corresponding commissioner.

Project Timelines:

January 2016:	Start registration for project online at http://ihiws.org Start ordering reference DNA panels
March 2016:	Start Proficiency and reference DNA panel distribution Start Proficiency and reference DNA panel testing
September-October 2016:	Deadline for data submission proficiency and reference DNA panel(s) to IHIWS database.
November 30, 2016:	Project closure

Required NGS HLA loci: HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1

Optional NGS HLA Loci: HLA-DQA1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DPA1, HLA-DPB1, MICA, MICB

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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.

Data requested

NGS of full-length HLA genes typing: Allele in GL String. (Alternative reporting method may be provided by the Workshop organizers)

Data analysis and data entry:

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)



- HLA typing: Allele in the GL String

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.

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- **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 accession 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

C. Other Documents included with the DNA panels :

- Terms and Conditions of Use Statement For Material Requested From The Fred Hutchinson Cancer Research Center IHWG Cell and DNA Bank.
- IHWG Cell and DNA Bank Recommended Handling Instructions for Genomic DNA