T Cell Receptor (TCR)-HLA Interaction

The T cell receptor is the natural receptor of the HLA-peptide complex and interacts directly with MHC molecules, notably its CDR regions. The goal of this project will be to combine analysis of TCR and HLA in the context of disease, vaccination/immune alterations and populations. We would expect samples to be CD4 or CD8 T cells and to have full HLA class I and class II typing by NGS.

TCR-HLA NGS Survey Link:
http://goo.gl/forms/oMGxG6iJnKfGfOch1

A. Project leadership:

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B. Requirements Documentation:

1. **Objective/Goals:**
The T cell receptor is the natural receptor of the HLA-peptide complex and interacts directly with MHC molecules, notably its CDR regions. The goal of this project will be combine analysis of TCR and HLA in the context of disease, vaccination/immune alterations and populations. We would expect samples to be CD4 or CD8 T cells and to have full HLA class I and class II typing by NGS.

Subprojects:
- Disease Study: Meta-analysis of all autoimmune disease disorders for the report of the workshop and individual disease analysis that will be led by specific investigators. These may include case/control and longitudinal studies.
- TCR usage/HLA Study: Changes in usage and CDR regions as a function of HLA types. The analysis will be performed in control samples for anthropological/environmental population studies, and could possibly be applied to specific disease.
- Transplantation Study: Analysis of the TCR repertoire in relation to HLA in transplantation.
- Technology and Nomenclature normalization Study: Various commercially available techniques using the same samples will be tested and analyzed.
- GWAS-TCR Study: GWAS data, when available will be submitted to study genome wide and TCR cis QTL effects on the TCR repertoire

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

If samples are being submitted without NGS-HLA data, contact the Project Leaders to identify a collaborating laboratory who will perform the NGS testing. Based on information from the survey, a lab may be assigned by the Component Chair to perform NGS-HLA typing.

Sample requirements (if submitting samples only)

For NGS-HLA:

- 3µg DNA is required. For example, DNA at a concentration adjusted to 100 nanograms per microliter, in a volume of 20 microliters per tube.
- DNA must have > 10 kb fragments visible as a strong band when checked for quality.

For TCR NGS clone typing:

- **RNA concentration and quality specs**:
  - According to iRepertoire protocol, between 50 ng (minimum) and 1800 ng (maximum) of template RNA is recommended. **See protocol in annex**
  - RNA is extremely sensitive to degradation. Ensure that RNA is stored at -80°C prior to use and maintained at 4°C during template addition. Once thawed, gently flick RNA sample and agitate by pipetting to mix. DO NOT VORTEX. Avoid excessive freeze- thawing of RNA samples to prevent degradation.
- **Source**:
  - CD4, CD8. If samples are not available sourced from CD4 or CD8, PBMCs may be use
- **Shipment of material SOP**:
  - RNA samples should be shipped on dry ice, live cells store live cells in Qiagen's RNAprotect Cell Reagent (cat no. 76526). For the appropriate volume, please follow the manufacturer's recommendation.
  - See link below for additional information

http://www.irepertoire.com/#!shippingfaq/cp3e

3. **Test Requirements:**
   - **NGS HLA:**
     There are no specific requirements for NGS platform/reagent combinations.
     Required NGS HLA loci: HLA-A, B, C, DRB1, DRB3/4/5, DQB, HLA-DQA, DPA, DPB (GL string, min 4 digit)

     Genotype_GL (Required Field): a locus-level HLA genotype recorded using GL String format, as defined by Milius et al. 2013 (doi: 10.1111/tan.12150).
     Validated using web service https://gl.nmdp.org/ See an example below for GL string format and the appropriate delimiters.

Order	Delimiter	Description	GLString Example
1	^	Unphased multilocus genotype	`HLA-A*02:69+HLA-A*23:30|HLA-A*02:302+HLA-A*23:26/HLA-A*23:39^HLA-B*44:02:13+HLA-B*49:08`
2	|	Genotype list	`HLA-A*02:69+HLA-A*23:30|HLA-A*02:302+HLA-A*23:26/HLA-A*23:39`
3	+	Genotype	`HLA-A*02:302+HLA-A*23:26/HLA-A*23:39`

4	~	Haplotype	HLA-A*23:26~HLA-B*44:02:13
5	/	Allele list	HLA-A*23:26/HLA-A*23:39

- **NGS TCR**
  TCR molecules: TCR Beta (CDR3 as a minimum test), TCR Alpha, TCR Gamma, TCR Delta.

4. **Analysis and publications:**
   Raw sequences for TCR and HLA NGS will be hosted by the workshop so that multiple analytical techniques can be performed. Data analysis will be conducted separately for each study. It will be possible to publish separately these sub-studies providing the component heads agree and verify there is no conflict.

5. **Request for participation:**

   We are asking the scientific community for potential new projects, whether or not they have interest in being part of any of the existing projects, and if yes, would they contribute samples (how many and types of cells), data (what type), and will they be a site for NGS sequencing. The Workshop will do its best to get free or reduced prices for NGS HLA typing and TCR sequencing from current vendors**.**

6. **Proficiency Testing:**
   a. ***TCR NGS Proficiency Testing:***
      *Labs performing TCR typing are required to perform testing on **2 samples** to be supplied by the project leadership.*
      Specific instructions are TBD

   b. ***NGS HLA 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 NGS HLA 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		$	-

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)*

7. **Project Timelines**:

Nov 2016:	Due date for registration for project online at http://ihiws.org & response to Survey http://goo.gl/forms/oMGxG6iJnKfGfOch1
Dec 2016:	Scope NG HLA and TCR NGS testing: Core Lab testing labs & number of samples
Feb 2017:	Due date for data submission proficiency and reference DNA panel(s) to IHIWS database.
June 2017	Due date for submission of raw data files (uploaded to IHIWS SFTP, upload of NGS HLA data to the IHIW database and submission of TCR typing to database TBD
Sep 1 2017	Due date for Analysis results & draft abstracts

8. **Cost**:
   a. **HLA NGS**: 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.

   b. TCR NGS: 40$ agreement per gene, this do not include Kapa RT Quantification kit and flow cell (10 sample/flow cell)

9. **Data analysis and data entry**:

   a. HLA NGS: 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.
   b. Raw sequences for TCR and HLA NGS will be hosted by the workshop so that multiple analytical techniques can be performed. Data analysis will be conducted separately for each study. It will be possible to publish separately these subproject provided that the study heads agree and verify there is no conflict.

c. TCR NGS: fasq files need to be uploaded to the IHIWS SFTP site

<u>To transfer raw data files to the IHIWS secure FTP site</u>:
Note: You may need to install software to allow secure file transfer between your local computer and the IHIWS secure FTP site, such as WinSCP for windows or FileZilla for Mac:
File protocol = SFTP
Host Name = hidpl.stanford.edu
User Name = labcode (six digit ID provide to each lab when register to participate in the IHIWS
Password = *one per labcode. See Lab participation section of the Home Page for your Lab's SFTP password*

Each lab has a folder named "upload" under which all the raw data files are to be stored. You may create subfolders under this folder to store your raw data based on how you want to organize the files, by project or by software used.

Required for all projects for NGS HLA typing

- 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)

- HLAtyping: Allele in the GL String

- 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

- Reagent_Protocol

- 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.*

10. 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.

C. Other Information that may be requested:

Raw data