Penn Human Immunology Core

Sample Preparation and Submission Guidelines for BCR/TCR Sequencing

SAMPLE PREPARATION

Sorting instructions

We use this kit (https://web.emmes.com/study/hbb/public/EN-Gentra-Puregene-Handbook-2011.pdf) to do DNA extraction for BCR/TCR sequencing.

- For fewer than 50,000 cells, please sort cells directly into a 1.5 ml RNase/DNase free Eppendorf tube with 300 ul of cell lysis buffer (QIAGEN cat. no. 158113 for a 125 ml bottle, or 158116 for 1000 ml). Vortex well (4-5 seconds) immediately following sort. You can pick up cell lysis buffer from us.

- For very rare populations of fewer than 5,000 cells, please use Eppendorf™ DNA LoBind Microcentrifuge Tubes (EMSCO/FISHER 13-698-791 or Eppendorf™ 022431021) for sorting. You can also pick up DNA LoBind tubes from us if you do not have any. *For samples with fewer than 1,000 cells, due to the increased likelihood of PCR jackpots, we cannot guarantee adequate library quality.*

- For more than 50,000 cells, if only 1-2 fold more cells, please switch to a new tube with 300 ul lysis buffer and continue the sort. For example, if you estimate 90,000 cells, please sort 50,000 into one tube of 300 uL lysis buffer, and the remaining 40,000 into a second tube of 300 uL lysis buffer. Vortex each tube well immediately following sort.

- If you estimate your cell population is a lot more than 50,000 cells, please sort them into regular FACS buffer (1X DPBS without Ca++ & Mg++ with either FBS (1%) or BSA (0.5%) without any sodium azide (NaN_3) or EDTA) in flow tubes instead of sorting into lysis buffer. Then spin down flow tubes at 1300 rpm for 5 minutes to pellet the cells. Aspirate the supernatant, leave around 50 ul FACS buffer, vortex the flow tube vigorously, then transfer the cells (remove cell clump if any) into a RNase/DNase free 1.5 mL Eppendorf tube and add lysis buffer (less than 2 million, 300 ul lysis buffer; 2-7 million 600 ul lysis buffer, 7-10 million, 1ml lysis buffer).

- You can leave sorted samples in lysis buffer at room temperature for up to a month. Otherwise, please store samples in lysis buffer at -20C to prevent evaporation.

We will do DNA extraction on our end, if desired for your project, and this is included in the sequencing cost. External customers please ship samples to us in **dry ice** to prevent leakage. Our shipping address is below.

Any leftover DNA samples will be stored for six months then discarded if you do not retrieve them. Please contact Dr. Wenzhao Meng (wmeng@pennmedicine.upenn.edu) with sample preparation or submission questions, to arrange pickup of tubes or lysis buffer, etc. at SCL (Stellar Chance Labs) room 408. Regular HIC hours are Monday-Friday 9 a.m. to 5 p.m., except for University-defined holidays.

FFPE tissue block cutting instructions

Since PCR is very sensitive, picking up possible contamination between cutting of different blocks, please follow these instructions for all downstream RNA or DNA sequencing analyses.

- Blades - Please change blades every time for a different tissue block. If you want to save blades (since a blade is likely 2.5 inches long), use one side for one block and the other side for a different

block. Then change blades when both sides are used. (Blades can be used to cut other tissue blocks for other projects which will not involve downstream sequencing work).

- Tweezers - Always use new tweezers for a new block when putting FFPE curls into tubes. Even if you think tweezers only touch the wax portion, they may still get contaminated with tissue that cannot be seen by eye.
- Eppendorf tubes - Always use RNase/DNase free Eppendorf tubes. It is better to UV-treat them first for half an hour if they are not stored properly.
- Please discard the first two slices of FFPE tissue curls, since they may have become contaminated during storage.
- Please provide at least 5 curls (5-10 micron per tissue curl) per block.

SCHEDULE AND SUBMIT SAMPLES

Once you have set up a project, scheduling and submitting samples is a three-step process.

1. Email **Wenzhao Meng** (wmeng@pennmedicine.upenn.edu) to schedule sequencing work and submit your **sequencing sample worksheet** (available on our website or from Wenzhao).

2. Submit an online request. If your project will be billed through PBR, visit https://pathbio.med.upenn.edu/pbr/secure/submission_immu_coord_blood.php to select your project and submit specimens/samples - PennKey login required. If your project will be billed through iLab, visit https://med-upenn.corefacilities.org/landing/264 to login, select your project, and submit specimens/samples.

Fill out a **sequencing sample worksheet** for your project and email to Wenzhao before you bring (or ship) your samples to SCL 408. The sample shipping address and contact info is below.

The **sequencing sample worksheet** is an Excel spreadsheet where you will provide the PI and contact names, sample type and species, time point, and other essential information (see below for more). Download a copy from the HIC website or request one from Wenzhao (wmeng@pennmedicine.upenn.edu). Sample fields in the worksheet follow:

A	B
Project Name	
Project Number	
PI Name	
PI E-mail	
PI Phone	
Contact Name	
Contact E-mail	
Date Generated	

identifier	name	status	species	sequencing_type	molecule_type	umi	IgH	IgL	IgK	TCRa	TCRb
do not fill	letters, numbers, hyphens only	do not fill									

time_point	sample_origin	concentration_ng_per_microliter	volume_microliters
	e.g. SPL, PBL, BM	if DNA or RNA	if DNA or RNA

cell_subset_description	cell_subset_flow_markers	cell_counts	comments
e.g. naïve, memory	e.g. CD38+CD27-		e.g. # slices of tissue curls (10 micron per slice)

3. Drop off samples at Stellar-Chance Labs (SCL) room 408 after reviewing our **sample submission guidelines** (available on our website or from Wenzhao).

Shipping address for BCR/TCR Sequencing

Attn: Dr. Wenzhao Meng
408 Stellar Chance Labs
422 Curie Blvd.
Philadelphia, PA 19104 Phone # 215-746-5769 Email wmeng@pennmedicine.upenn.edu