Penn Human Immunology Core

BCR/TCR Sequencing and Data Analysis Methods

Bulk DNA TCR/BCR Sequencing

We provide human TCR/BCR and mouse (C57BL/6 and BALB/c strains) BCR sequencing (we DO NOT offer mouse TCR sequencing). We also provide customized TCR/BCR sequencing to look at specific immune response (such as the NP response in mice).

For human BCR sequencing methods, please see our publications listed on our website (one example below). We provide primers that can amplify near full length (FW1), full length (leader region), or short length (FW3) regions of the heavy chain. We also have an in-house sequencing method for kappa and lambda light chains.

Meng et al., Nat Biotechnol. 2017 Sep;35(9):879-884. PMID: 28829438
https://pubmed.ncbi.nlm.nih.gov/28829438/

Rosenfeld et al., Methods Mol Biol. 2022;2453:317-343. PMID: 35622334
https://pubmed.ncbi.nlm.nih.gov/35622334/

For human TCR sequencing methods, please see our publication:

Ritz et al., Blood Adv. 2020 Apr 14;4(7):1378-1382. PMID: 32267929
https://pubmed.ncbi.nlm.nih.gov/32267929/

For mouse BCR sequencing methods, please see our publication:

Johnson et al., Immunity. 2020 May 19;52(5):842-855.e6. PMID: 32353250
https://pubmed.ncbi.nlm.nih.gov/32353250/

Bulk RNA TCR/BCR Sequencing

- RNA will be extracted using the Qiagen RNeasy mini or micro kit
- Human TCR sequencing: SMARTer Human TCR α/βprofiling kit from Takara Bio
- Human BCR sequencing: SMARTer Human BCR IgG IgM H/κ/λ profiling kit from Takara Bio
- Mouse TCR sequencing: SMARTer Mouse TCR α/βprofiling kit from Takara Bio
- Mouse BCR sequencing: SMARTer Mouse BCR IgG H/κ/λ profiling kit from Takara Bio

If your experiment requires other kit types or experimental methods (e.g. UMIs), please contact Dr. Wenzhao Meng at wmeng@pennmedicine.upenn.edu

Single-cell BCR/TCR Sequencing

For single cell sequencing, we accept isolated and/or sorted viable cells on the same day of the experiment (cut-off times for sample submission/delivery apply). Please contact Dr. Wenzhao Meng (wmeng@pennmedicine.upenn.edu) to schedule the experiment with us in advance.

We offer
◊ Human/Mouse single cell immune profiling (VDJ (BCR or TCR) and 5' GEX) by 10X Genomics

◊ CITE-seq for single cell immune profiling with TotalSeq™–C antibodies from Biolegend, compatible with the 10X Genomics 5' library construction kit
◊ Hashtags for single cell immune profiling with TotalSeq™–C hashtags from Biolegend, compatible with the 10X Genomics 5' library construction kit

Data Analysis

We offer basic data analysis, including an IMGT-style clonal output table, D20 (D50) indices, clonality/diversity, CDR3 spectratypes, clonal overlap analysis, V/J gene usage, CDR3 amino acid usage, somatic hypermutation (SHM) analysis (BCR only), etc. - ask us for guidance on the analysis that will make your point(s). We also offer

- Retrieval of high quality full-length sequences of heavy and light chain pairs of dominant clones for downstream cloning projects.
- Project-specific analyses, including lineage tree analysis (BCR only), public clone analysis, clone distance mapping analysis, etc.
- Assistance with data visualization, manuscript preparation and data deposition to public data repositories, and AIRR-compliant data submissions for an additional fee.

We use ImmuneDB for our in-house data analysis pipeline. Ongoing contributions to ImmuneDB include 358 databases, >34,000 sequencing libraries and over 4.2 billion antigen receptor reads.

For single cell data analysis, we offer pre-processing of the raw data right off the sequencer with Cell Ranger from 10X genomics. We also offer further quality control and data analysis with Seurat.

What we recommend if you would like to DIY (Do It Yourself)

- For a single sequence alignment, we recommend IgBLAST
- For batch sequence alignment, we recommend IMGT High V quest
- For the nomenclature for IG and TR genes from human, mouse and other species, we recommend IMGT/GENE-DB and OGRDB
- For NGS data QC and pair-end reads processing, we recommend pRESTO (Python)
- For clone collapsing and data analysis of bulk TCR/BCR sequencing, we recommend
  - ImmuneDB (Python)- (software can be installed via GitHub)
  - MiXCR (Java) and VDJtools (Java and R)
  - Immunarch (R)
- For public clone and epitope analysis we recommend VDJmatch, VDJdb, IEDB, and IMGT
- For single cell data analysis, we recommend Cell Ranger (10X Genomics), Seurat (R), scRepertoire (R), Scanpy (Python)