**CapTCR-seq experimental protocol**

CapTCR-seq is a hybrid capture TCR sequencing methodology developed by the Pugh lab to enrich for rearranged variable diversity joining (VDJ) regions. Typically, we extract DNA using Qiagen Allprep kit and depending on the sample type may shear to reach median 250 bp insert size. Illumina DNA libraries are then prepared using the KAPA HyperPrep Kit library preparation kit with custom adaptors including 2-base pair molecular identifiers. We pool two indexed libraries and perform hybrid capture using a custom TCR probeset following the Integrated DNA Technologies (IDT) hybridization capture protocol. We sequence the captured DNA pools minimum of ~2 million reads on the Illumina NovaSeq platform.

**Bioinformatic processing and analysis**

After barcode removal, we process FASTQ files using MiXCR immune repertoire pipeline. MiXCR aligns TCR and BCR reads to IMGT reference libraries (https://www.imgt.org/), assembles clonotypes defined by CDR3 sequences and outputs a tab-delimited file consisting of TCR/BCR information (VDJ usage, CDR3 nucleotide and amino acid sequences, clone counts and fractions). We use iNEXT R package and the sequence of clone counts as an input to measure immune diversity in each sample.

**Tracking recurring clones across samples**

We developed a custom R code to track specific clonotypes in a number of samples. A typical use case is to monitor T-cell clones in serial samples obtained from patients undergoing immunotherapy. In the typical clone tracking plot x-axis is specific samples and y-axis shows fraction of clones in each sample. The plot itself has two components. A stacked barplot that shows T-cell clonal distribution in each sample in x-axis, where the width of each stack represents clonal fraction. The tracking function is achieved by alluvial plot that colors and connects recurring T-cell clones together. By default, a recurring clone is defined as the specific CDR3 nucleotide sequence, but it can be relatively easily adjusted to track other entities such as amino acid sequence or specific VDJ gene segments.