Bulk sequencing of antibody heavy chain gene rearrangements (IGH)

Luning Prak Lab

Date of revision: June 15, 2021

I. IGH sequencing

1. Antibody heavy chain (IGH) gene rearrangements were bulk sequenced from genomic DNA.
2. Genomic DNA was isolated from sorted or bulk cell populations using the QIAGEN Gentra Puregene Kit (Qiagen, Cat. No. 158388). For further details on the sorted cell populations, please see PMID 33545036.
3. Antibody heavy-chain variable region rearrangements were amplified using framework 1 region (FR1) + JH primers that were adapted from EuroClonality primers for next-generation sequencing, as described in Meng *et al*., 2017.
4. Typically 100 ng of input DNA was amplified per replicate. Sequencing libraries were prepared using 2 × 300 bp paired-end kits (Illumina MiSeq Reagent Kit v3, 600-cycle, Illumina Inc., San Diego, CA). For a more detailed description of the wet bench methods, please see PMID 28829438.
5. For data analysis, we recommend using the **ImmuneDB pipeline** (PMID 30298069), with database installation instructions available on GitHub: https://immunedb.readthedocs.io/en/latest/.
   Note: A detailed step-by-step protocol for all of the wet and dry bench methods is expected to be available in Methods in Molecular Biology later in 2021.

II. TRB sequencing

1. T cell receptor beta chain gene rearrangements were bulk sequenced from gDNA using similar methods to IGH sequencing.
2. Further details, including PCR primers, can be found in PMID 30111633 and Meng *et al*., 2021.