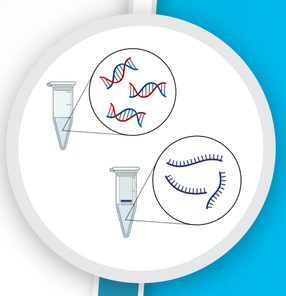
Getting Started with NGS-Based Immune Profiling

Next-generation sequencing has revolutionized immune profiling by allowing researchers to gain a more complete picture of B-cell and T-cell receptor repertoires. Whether you're conducting an immune profiling experiment to study immune cell development, explore autoimmune disease or cancer mechanisms, or identify new biomarkers for immune-mediated diseases, the workflow requires attention to the same key success factors.



1 DO YOU PLAN TO USE RNA OR DNA?

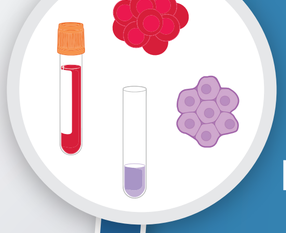
Whether you choose genomic DNA (gDNA) or RNA as input for your NGS-based immune profiling experiment depends on your research needs.

USE RNA FOR

- Increased sensitivity with very low/single-cell inputs
- Expression-based studies beyond clonotype counts
- Isoform/splicing analysis
- Capturing functional receptor sequences
- Minimal residual disease analysis

USE DNA FOR

- Clonality analysis
- Minimal residual disease analysis



2 ISOLATE HIGH-QUALITY RNA OR DNA FROM YOUR SAMPLES

Although you can go straight from individual cells to immune profiling data, isolating RNA or DNA from your samples allows for multiple types of analysis. Use a robust, reliable protocol to isolate RNA/DNA from your samples and verify quality before moving on to library preparation.

Takara Bio tip: Get high-quality gDNA or total RNA with NucleoSpin kits for small-to-medium scale extractions or NucleoMag kits for high-throughput or automated extractions. Both types of kits are available for various sample sources, with fast and easy workflows and guaranteed quality.

Takara Bio tip: If using RNA for immune profiling experiments, RNA samples should be DNAse-treated to avoid genomic DNA (gDNA) contamination. Many RNA purification kits include gDNA removal as part of the protocol; be sure to check if your RNA purification method includes gDNA removal or if it needs to be performed separately.

Takara Bio tip: Verify RNA quality by measuring the RIN number. RNA samples with a RIN greater than 7 are considered high-quality samples. Verify DNA quality by measuring the $A_{260/280}$ and $A_{260/230}$ values.

3 PREPARE YOUR SEQUENCING LIBRARIES

Sequencing libraries can be prepared using a multiplex approach or a 5'-RACE-based approach.

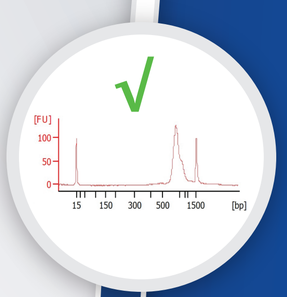
Multiplex PCR:

- Needs many degenerate primers to amplify different TCR/BCR sequences
- Works with both DNA and RNA inputs
- Introduces significant PCR bias and can miss important mutations
- Does not capture full BCR/TCR sequence information

5′ RACE:

- Ensures high on-target rate with semi-nested PCR to reduce sequencing costs
- Works with RNA only
- Reduces PCR bias compared to multiplex PCR approaches
- Captures full BCR/TCR sequence information

Takara Bio tip: Avoid primer bias with SMART-Seq® immune profiling kits, which employ a 5′-RACE-based approach along with unique molecular identifiers (UMIs) for bias-free, sensitive immune profiling.



4 QC YOUR LIBRARIES

Use a combination of library quantification and electrophoresis technology, such as a Bioanalyzer or Tapestation, to assess your libraries' concentration and size, respectively. Accurate concentration and size measurements are essential for calculating library molarity and properly diluting libraries to maximize sequencing read counts.

Takara Bio tip: qPCR is regarded as the most accurate library quantitation method. Ensure accurate sequencing library loading with the Library Quantification Kit from Takara Bio.

5 SEQUENCE YOUR LIBRARIES

The number of cycles and length of reads you use during sequencing will depend on the information you want to obtain from your immune profiling experiment. To obtain full-length V(D)J information, sequence using more cycles and longer reads than when interested in only obtaining CDR3 region information.

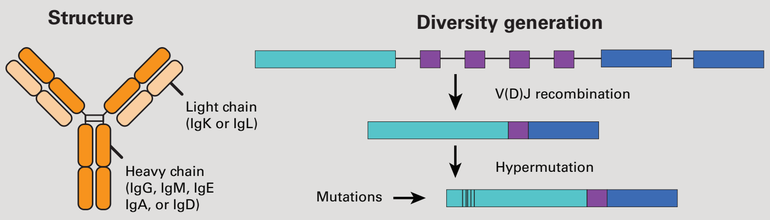
Takara Bio tip: SMART-Seq immune profiling kits give you the flexibility to sequence either the full-length V(D)J region or the CDR3 region only.

6 ANALYZE YOUR SEQUENCING DATA

Using bioinformatics tools or a pipeline that allows for data visualization enables you to identify TCR/BCR sequence information and frequency of different TCR/BCR clones with confidence.

Takara Bio tip: Avoid the hassle of downloading different pipelines and spending time troubleshooting by using Cogent™ NGS Immune Profiler and Immune Viewer, which allow for analysis and visualization of immune profiling data. No bioinformatics expertise required!

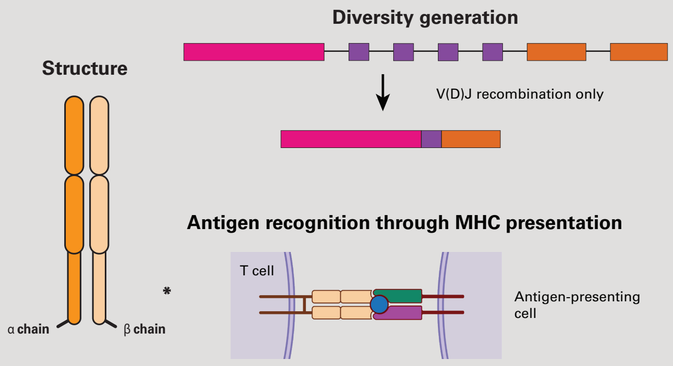
B-CELL RECEPTORS (BCRs)



BCR PROFILING

- Understanding antibody response to pathogens
- Identifying B-cell clones associated with autoimmune disease symptoms or severity
- Developing new antibody therapies

T-CELL RECEPTORS (TCRs)



TCR PROFILING

- Studying tumor-infiltrating T cells in cancer immunotherapy
- Monitoring immune response to viral infection
- Investigating safety and clonal diversity of T cells used in T-cell/CAR-T/adoptive transfer therapies

*A minor subset of T cells, gamma-delta T cells, have TCRs that are composed of a gamma chain and a delta chain.

View data generated with our immune profiling products

TaKaRa