immunoSEQ®

Reference Guide

Experimental Design

Adaptive Biotechnologies' immunoSEQ Assays can identify millions of T- and B-cell receptors in a single sample. This quantitative sequencing data advances our collective understanding of the adaptive immune system and drives innovation toward breakthrough discoveries across translational and clinical applications.

Rigorous experimental design sets the foundation for successful immunosequencing projects. This Reference Guide contains insights and tips from Adaptive's data analysis and immunology experts to assist you in your research preparation and ensure that the quantitative results generated from your samples are accurate and meaningful.

Adaptive biotechnologies®

CHOOSING A STARTING MATERIAL

Determining the best sample type to work with depends on the overall goals for your experiment. It is important to tailor your selection to the specific tissues or sites of interest that are relevant to the disease state you are studying.

"To detect a biomarker of disease, a sample from the site of pathogenesis is recommended."

To detect a biomarker of disease:

- A sample from the site of pathogenesis is recommended.

To detect a treatment effect:

- Multiple timepoints are essential to monitor the dynamics of the immune system over time.
- Timing of sample collection should be considered.
  - Ensure that pre-treatment sampling can enable the establishment of a baseline.
  - Plan post-treatment sampling with sufficient frequency to capture the immune response to specific therapies.
  - For retrospective analyses, consider data that indicates when peak immunologic responses occur within treatment cycles and associate these time points with sample collection or analysis.

Consultation with your local statistician during the experimental design process is highly recommended.

STARTING MATERIALS (gDNA VS cDNA)

The immunoSEQ Assay is compatible with both genomic DNA (gDNA) and complementary DNA (cDNA).

"cDNA measures TCR or BCR expression rather than clonality."

- If you require quantitative assessment of individual clonal T or B cells, gDNA is an ideal starting material since each cell has the same "productive" template copy number. When sequencing from gDNA, T-cell receptor (TCR) reads can be quantitated to provide an actual cell count, making it possible to accurately assess clonal expansion or T- or B-cell density in tissues.
- mRNA copies can vary widely from cell to cell, and copy numbers are highly dependent on cellular activation. Therefore, cDNA measures TCR or B-cell receptor (BCR) expression, and it is not an accurate measure of clonality. Furthermore, the RT-PCR process during cDNA generation may introduce bias.
- cDNA is a more relevant starting material when studying relative antibody production across a B cell repertoire, given the expression of potentially thousands of copies of mRNA per B cell vs. the single productive rearrangement present as gDNA, and considering the amount of diversity of the naive B cell population.
- Sequencing cDNA from B cells may increase the chances of detecting a signal in a diverse starting material (e.g., peripheral blood, PBMCs) or in samples with low cell counts, like cerebral spinal fluid (CSF) and synovial fluid.

Samples from different mice should not be pooled; each individual mouse has a unique immune repertoire (even inbred mice).

Every project should include relevant control samples.

In order to sequence only rearranged TCRG receptors originating from gamma/delta T cells in PBMCs and tissue samples, a cell sort must be performed. Since alpha/beta T cells and gamma/delta T cells carry a rearranged gamma locus, sequencing straight from tissue or from unsorted cells will result in data from both cell populations. (Sherwood, et al. Science Translational Medicine, 2011)

"Cell sorting techniques can be paired with immunosequencing to identify specific sequences from T- or B-cell sub-populations."

SAMPLE SIZE

If your goal is to show a statistically significant difference between groups, include enough cases and controls for the experiment to have high statistical power. The likelihood of detecting a statistically-significant signal is dependent on both the size of the signal and the number of samples.

For pre-clinical studies:

- A minimum of five mice per group is required to attempt detection of a difference between groups, but ten mice per group is recommended.
- Samples from different mice should not be pooled as even individual inbred mice have unique immune repertoires.

For human studies:

- An absolute minimum of 20 subjects is recommended, more samples may be required depending on study parameters.

CONTROLS

Every project should include control samples which produce an expected outcome that serves as a baseline for the experiment; other test samples and groups can then be compared to the control group. Always minimize the number of variables between the controls and the test samples, and ensure that all samples are processed in an identical manner.

- If your scientific goal is to identify a biomarker of disease, a healthy control group should be included in the study.
- If your goal is to monitor the effects of a treatment or identify a biomarker of response to treatment, a pre-treatment sample is the best control.
- For murine studies, a naïve or isotype control group is recommended.

SAMPLE TYPES

Peripheral blood mononuclear cells (PBMCs) and whole blood vs. sorted cells: If a signal is expected in a specific subset of cells (e.g., Tregs), we recommend enriching the sample for that particular cell type. However, if you want to know the abundance of a single clone or set of clones within a T-cell population, you may want to consider running both PBMCs and the sorted subset in parallel to allow for clone tracking among the larger population.

Tissue vs sorted cells: T- and B-cell density must be determined by sequencing bulk tissue. Alternatively, cell sorting techniques can be paired with immunosequencing to identify specific sequences from T- or B-cell sub-populations.

SEPARATING SIGNAL FROM NOISE

If you are starting with very diverse material, like peripheral blood or bone marrow, you should consider enriching for the population of interest (i.e. bead sort a specific T-cell subpopulation). If a signal is predicted in a specific subpopulation of T or B cells, enriching the sample for the population of interest will increase the chances of detecting a signal.

POWER OF PAIRING

If the experiment involves tissue, pairing tissue with peripheral blood is often recommended.

- T- or B-cell sequences may be detected in multiple tissues, making it possible to track clones "trafficking" from tissue into blood and vice-versa.

For example, a pre-treatment tissue sample matched with a pre- and post-treatment blood sample permits tracking of "the expansion" tumor-associated clones in the blood and enables you to evaluate changes in the immune repertoire (e.g., peripheral T-cell clonality).

Greater sequencing depth increases the likelihood of detecting rare clones in a population, however, the optimal sequencing depth will depend on the particulars of your experimental design (i.e. Deep resolution is not always necessary).

CHOOSING A SEQUENCING DEPTH

immunoSEQ Service:

- Survey resolution: Target of 1 in 60,000 cells.
  - This is appropriate for cell populations with fewer than 200,000 cells, clonal specimens, and tissues with low T- or B-cell infiltration.
- Deep resolution: Target of 1 in 200,000 to 1 in 1 million cells.
  - This is appropriate for cell populations with more than 200,000 cells, extremely diverse specimen such as naïve B cells, PBMCs or lymphoid tissue.

immunoSEQ Kit:

- Sequencing depth and number of PCR reactions can be customized based on your research question, cell counts, and diversity of the population of interest.

Evenness metrics are indexing measures which summarize how many types of unique clones are present and their relative magnitudes.

METRICS AND ENDPOINTS

Analysis metrics and endpoints should be chosen to best support the research question or hypothesis being investigated. Consulting with a statistician is highly recommended to help define the data requirements for an appropriate analysis.

Diversity metrics can be used to monitor changes in the immune repertoire. The resulting information can be further analyzed to assess immune response to treatment, identify and follow biomarkers of disease, and survey immunomodulation.

- Clonality is a measure of how evenly receptor sequences (clones) are distributed amongst a set of T or B cells. Clonality ranges from 0.0 to 1.0, where 0.0 represents a completely even sample and 1.0 represents a sample with a single dominant clone.
- Richness estimators can be used to normalize samples of different absolute sizes and profile unique clones by providing confidence intervals and total count estimates.
- T- or B-cell density (as a fraction of nucleated cells) is a measure that can be used to assess immune response.

Richness estimators can be used to normalize samples of different absolute sizes and profile unique clones.

VIEW ONLINE

Publication. Origin and evolution of the T cell repertoire after posttransplantation cyclophosphamide.

VIEW ONLINE

Published Project. For 35 alloBMT transplant patient and 16 donors, Kanakry et al. with the Warren lab at the Fred Hutchinson Cancer Research Center sequenced antigen receptors to assess diversity of TRB and IGH repertoires and tracked T cell clones in blood and GVHD target tissues for up to 5 years post transplant.

VIEW ONLINE

Published Project. TCR repertoire sequencing identifies synovial Treg cell clonotypes in the bloodstream during active inflammation in human arthritis.

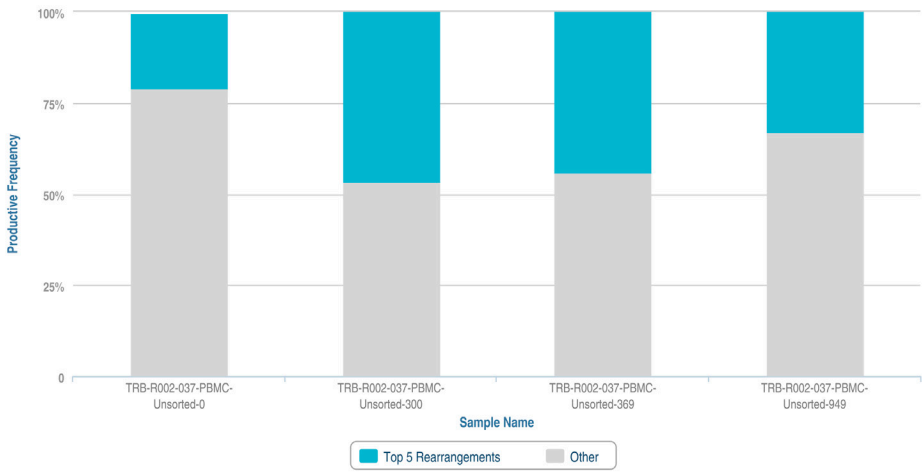

Figure 1: Using a pre-treatement sample as a control, the top 5 most frequent rearrangements made up significant portions of the repertoire in a pre-transplant sample and three post-transplant timepoints. (http://adaptivebiotech.com/pub/Kanakry-2016-JCIInsight)

Sharing metrics are valuable to measure the degree of repertoire turnover/change due to treatment. They can also be used to characterize the tissue specificity of a repertoire. To best utilize sharing metrics, compare analyses from multiple time points or different sample types from the same patient.

- Compare shared clones in one patient group or sample type vs. another
  - NOTE: It is unlikely to find many shared rearrangements unless subjects are restricted by HLA type.
- Examine repertoire similarity using TCR overlap, the Morisita index and/or the Jaccard index.

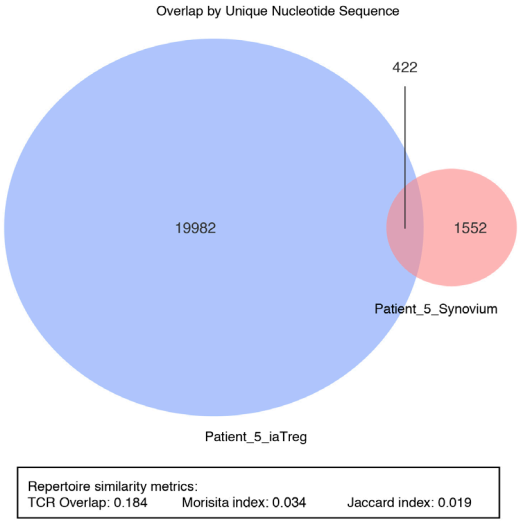

Figure 2: The power of pairing—overlapping unique nucleotide sequences between the site of pathogenesis and the peripheral Treg repertoires. The three repertoire similarity metrics provided by the Analyzer differential abundance tool are inset. (http://adaptivebiotech.com/pub/Rossetti-2015-annrheumdis)

Track specific and even rare T- and B-cell clones in a very complex background. We can track specific and even rare clones in a very complex background. Plotting the data using the immunoSEQ Analyzer enables you to visualize clonal expansion and contraction over time and allows you to overlay treatment cycle and therapy information to monitor response.

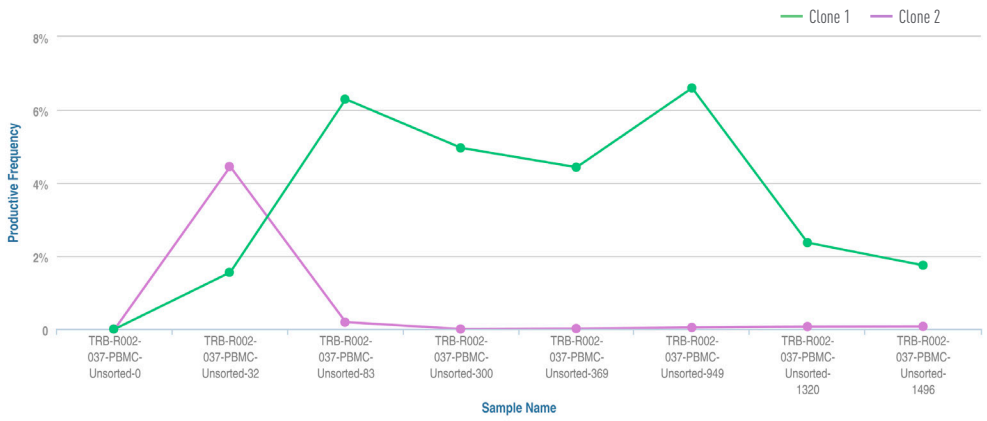

Figure 3: Monitoring a specific clone in PBMC samples over multiple timepoints (http://adaptivebiotech.com/pub/Kanakry-2016-JCIInsight)

VIEW ONLINE

Published Project.
For 35 alloBMT transplant patient and 16 donors, Kanakry et al. with the Warren lab at the Fred Hutchinson Cancer Research Center sequenced antigen receptors to assess diversity of TRB and IGH repertoires and tracked T cell clones in blood and GVHD target tissues for up to 5 years post transplant.

CONCLUSION

Adaptive's immunoSEQ Platform is a powerful immunosequencing solution that is specifically designed to identify and enumerate the repertoire of T and B cells within a sample. immunoSEQ Technology enables researchers to track specific T- or B-cell clones, monitor immune repertoire diversity, determine therapeutic mechanism of action and identify biomarkers of response to therapy.

Robust experimental design, including proper sample selection, is critical to ensure the generation of high-quality results.

If you would like assistance with experimental design or to discuss the application of immunosequencing in your research please contact researchsales@adaptivebiotech.com.

For any questions, please contact: researchsales@adaptivebiotech.com

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