Assay Methods: Human and Mouse T-cell Receptor Sequencing

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The cellular adaptive immune system generates a remarkable breadth of diversity in antigen-specific T-cell receptors (TCR) by combinatorial recombination of gene segments in lymphocytes. The TCR is composed of two peptide chains, encoded by the T-cell receptor alpha and beta (TCRA, TCRB) or T-cell receptor gamma and delta (TCRG, TCRB) genes. There are thus two types of T-cell receptors, αβ and γδ, that differ by the TCR heterodimer type and immune function. The antigenic specificity of T-lymphocytes is in large part determined by the amino acid sequence in the hypervariable complementarity-determining region 3 (CDR3) of T-cell receptors. The existence of multiple V and J gene segments at the T-cell loci permits a large combinatorial diversity in receptor composition; and the non-templated insertion or deletion of nucleotides at the V-J, V-D, and D-J junctions further adds to the potential diversity of receptors that can be encoded. Because of the potential diversity of receptors (a healthy adult has approximately 10 million different TCRB chains contained within their $10^{1,2}$ circulating T-cells[1]) it is highly improbable to randomly converge on the same TCRB nucleotide CDR3 sequence, effectively making each CDR3 sequence a unique tag for a T-cell clone.

Adaptive Biotechnologies' immunoSEQ Assay, a multiplex PCR-based method that amplifies rearranged TCR CDR3 sequences (Fig. 1) and exploits the capacity of highthroughput sequencing technology, characterizes tens of thousands of TCRB CDR3 chains simultaneously. The primary obstacle to addressing amplification bias in repertoire analysis has been the lack of any gold standard in which the exact concentration of each target is known prior to amplification. To address this problem, Adaptive Biotechnologies developed a rigorous PCR amplification bias-control process, ensuring a quantitative read-out of the adaptive immune repertoire[2]. Additionally, because the technology utilizes genomic DNA, the frequency of sequenced CDR3 chains is highly representative of the relative frequency of each CDR3 sequence in the sample population of T cells. Thus, the assay captures the full TCR repertoire including specific individual clones. The immunoSEQ Assay provides a novel method to identify and track the presence and frequency of common and rare clones in the context of the total adaptive immune system.
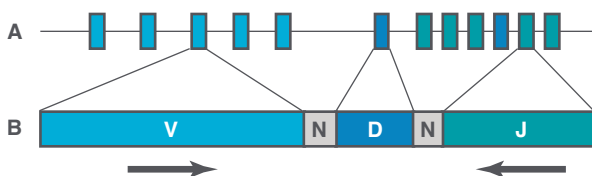

Fig 1: TCR sequencing assay. (A) Multiple V, D and J segments exist in the germline genome, non-templated diversity is introduced at the junctions by insertion of random nucleotides (shown as N). (B) The assay uses a multiplex PCR with forward primers in each V segment and reverse primers in each J segment.

1. **Comprehensive assessment of T-cell receptor β-chain diversity in αβ T cells.**
   Blood, Nov 2009. Robins HS, Campregher PV, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, Riddell SR, Warren EH, Carlson CS.

2. **Using synthetic templates to design an unbiased multiplex PCR assay.** *Nature Communications*, October 2013. Christopher S Carlson, Ryan O Emerson, Anna M Sherwood, Cindy Desmarais, Moon-Wook Chung, Joseph M Parsons, Michelle S Steen, Marissa A LaMadrid-Herrmannsfeldt, David W Williamson, Robert J Livingston, David Wu, Brent L Wood, Mark J Rieder & Harlan Robins.

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