The cellular adaptive immune system generates a remarkable breadth of diversity in antigen-specific B-cell receptors (BCR) by combinatorial recombination of gene segments in lymphocytes. The BCR is composed of two peptide chains, encoded by the immunoglobulin heavy chain (IGH) and immunoglobulin kappa/lambda (IGK/L) genes. The antigenic specificity of B-lymphocytes is in large part determined by the amino acid sequence in the hypervariable complementarity-determining region 3 (CDR3) of B-cell receptors. The existence of multiple V and J gene segments at the B-cell loci permits a large combinatorial diversity in receptor composition; the non-templated insertion or deletion of nucleotides at the V-J, V-D, and D-J junctions as well as somatic hypermutation further adds to the potential diversity of receptors that can be encoded. Because of the potential diversity of receptors it is highly improbable to randomly converge on the same IGH nucleotide CDR3 sequence, effectively making each CDR3 sequence a unique tag for a B-cell clone.

Adaptive Biotechnologies’ immunoSEQ Assay, a multiplex PCR-based method that amplifies rearranged BCR CDR3 sequences (Fig. 1) and exploits the capacity of high-throughput sequencing technology, characterizes tens of thousands of IGH or IGK/L 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. 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 B cells. Thus, the assay captures the full BCR 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.

**Assay Methods: Human and Mouse B-cell Receptor Sequencing**

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![Fig 1: IGH 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.](image-url)
