Synthetic Spike-in Controls for Immune Repertoire Profiling

Ensure Accurate and Reproducible Immune Repertoire Results

Cellecta Spike-In RNA Immune Repertoire Profiling Controls enable researchers to produce consistent and reproducible clonotype profiling results. The inclusion of RNA Profiling controls in an RT-PCR Profiling Assay provides a calibration standard for the sequencing data to eliminate biases caused by PCR variations and different sequencing platforms.

Quantify Clonotype Variations with Multiplex RT-PCR

T cell receptors (TCRs), B cell receptors (BCRs) and antibodies are the key players of adaptive immune responses. Variable (V), diversity (D), and joining (J) gene segments combine to form the highly polymorphic complementarity region 3 (CDR3) involved to produce the functionally diverse repertoire of millions of different T and B cell clonotypes which are present in blood and tissues

Cellecta's DriverMap Adaptive Immune Repertoire (AIR) Profiling Assay uses multiplex RT-PCR and Next-Generation Sequencing (NGS) to measure both TCR and BCR functional CDR3 RNA. Both TCR and BCR variable (V) regions consist of two polypeptide chains involved in antigen recognition–either single-pair alpha (α), beta (β), gamma (γ) and delta(Δ) chains for TCRs or two pairs of heavy (H) and light (K or L) chains for BCRs. The DriverMap AIR assay simultaneously amplifies all seven TCR and BCR chains using a set of 300 experimentally validated PCR primers to yield Illumina-compatible NGS libraries.

Why are spike-in controls needed?

To obtain reliable and comparable AIR-seq data, we have designed and synthesized 48 BCR (Fig.1) and 39 TCR (Fig. 2) mRNA constructs that mimic all the different IGH and TRB variations. When these synthetic constructs are added to each sample, they are reverse-transcribed,

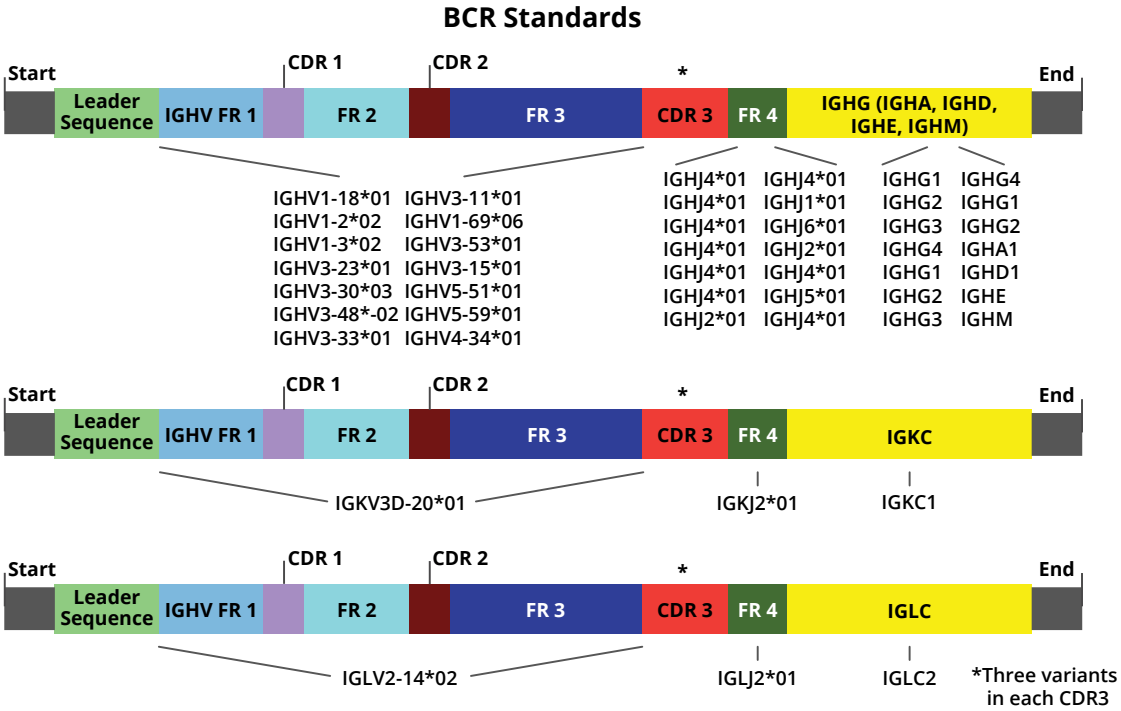

Figure 1

48 BCR mRNA spike-in constructs represent 10 different IGHVs (evenly distributed from IGHV1 through IGHV4); 1 for each of IGHA, IGHD, IGHE, IGHM, and 1 for each of IGKV and IGLV region.

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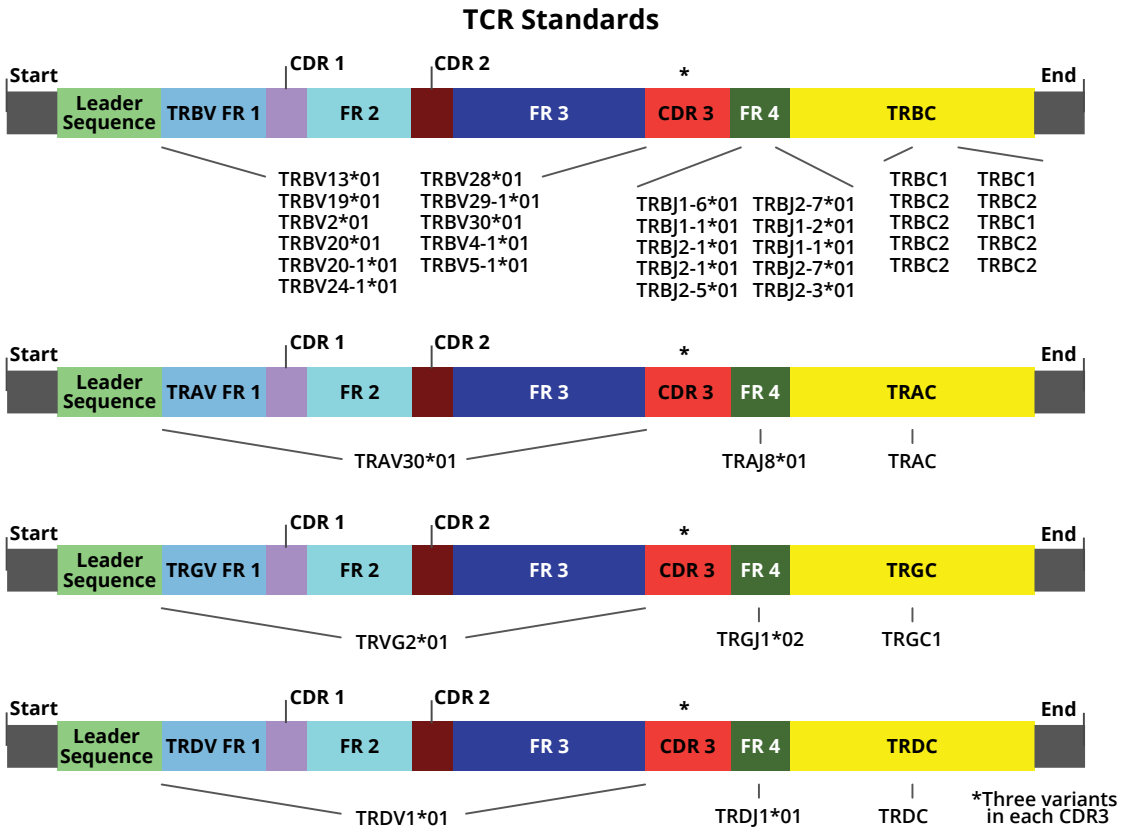

Figure 2

39 TCR spike-in constructs represent 10 different TRBVs, 1 for each of TRAV, TRGV and TRGV region.

amplified and sequenced with the endogenous TCRs and BCRs. As a result, they provide a known quantity of defined RNA transcripts in each sample, against which the NGS results can be normalized. This ability to standardize the results across samples with these RNA spike-in controls ensures the accuracy of sequencing data and eliminates bias caused by PCR and the sequencing platform.

- Each construct has the same sequence as those in the international ImMunoGeneTics information system (IMGT) database (except the CDR3 region)
- Each type of construct has three variations in the CDR3 region that differ by three nucleotides in fixed position, resulting in 48 BCR and 39 TCR sequences
- These spike-ins serve as controls by adding three sets of variants at different concentrations to the RNA sample and reverse-transcribing the C gene heavy- and light-chains.

High Quality Assays Yield High Quality Results

Standards and controls are needed for optimal AIR-seq data harmonization, interpretation, and sharing. Cellecta provides a comprehensive AIR profiling service and kits along with RNA spike-in controls to reliably quantify the adaptive immune repertoire. **Learn more at cellecta.com/DriverMapAIR**

Cellecta is beta-testing our RNA spike-in controls. We are looking for collaborators.
Please contact us at **collaborate@cellecta.com** for more information.