DriverMap™ Adaptive Immune Receptor (AIR) Profiling RNA Spike-In Controls

Ensure Accurate and Reproducible Immune Repertoire Profiling Results

Cellecta DriverMap™ AIR RNA Spike-In Controls are a set of synthetic B-cell receptor (BCR) and T-cell receptor (TCR) mRNA constructs that represent all TCR and BCR chain variations. They enable researchers to produce consistent and reproducible clonotype profiling results.

Immune Repertoire Profiling by AIR Sequencing (AIR-Seq)

T cell receptors (TCRs), B cell receptors (BCRs), and antibodies are the key players of the adaptive immune response. Variable (V), diversity (D), and joining (J) gene segments combine to form the highly polymorphic complementarity region 3 (CDR3) involved in producing the functionally diverse repertoire of millions of different T and B cell clonotypes which are present in blood and tissues. NGS-based methods (AIR-Seq) are commonly used to profile clonotypes.

Why Are Spike-In Controls Needed?

To obtain reliable and comparable AIR-Seq data, Cellecta designed and synthesized 48 B-cell (Fig. 1) and 39 T-cell mRNA constructs (Fig. 2) that represent all seven TCR (TRA, TRB, TRG, TRD) and BCR (IGH, IGK and IGL) chain variations. These are designed to serve as universal RNA positive controls for validating, calibrating, and standardizing commercial and home-brew AIR-Seq assays using multiplex RT-PCR or 5′-RACE (SMART) assays.

- 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

The spike-in controls are available in two formats:

a. Premixed Controls have the three CD3 variants from each V(D)JC isoform pool mixed in a 16:4:1 ratio, present int three discrete concentrations: 4000, 1000, and 250 molecules (i.e. UMIs) per microliter.

b. Triplex Isoform Pools are sets of the 16 BCRs or 13 TCRs that have three synthetic RNA constructs with the same V(D)JC isoform receptor structure, although each construct has a unique CD3 sequence. The three variants are mixed in a 1:1:1 ratio, with 2500 molecules per microliter, each.

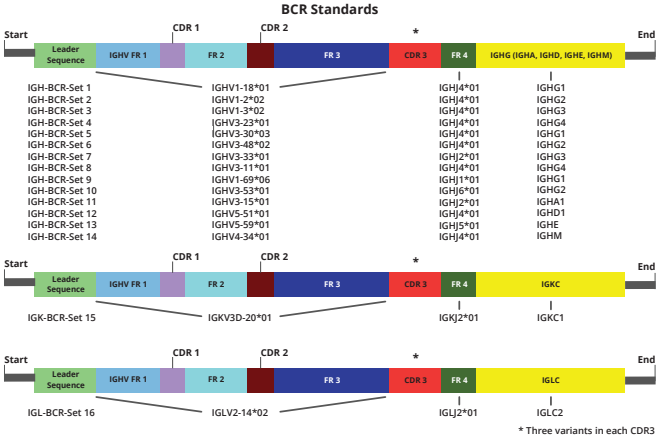

Figure 1

48 BCR mRNA spike-in constructs represent 10 different IGHs; 1 for each IGHA, IGHD, IGHE, IGHM, IGK and IGL genes. Each construct has 3 variations in the CDR3 region that differ by 3 nucleotides in fixed position.

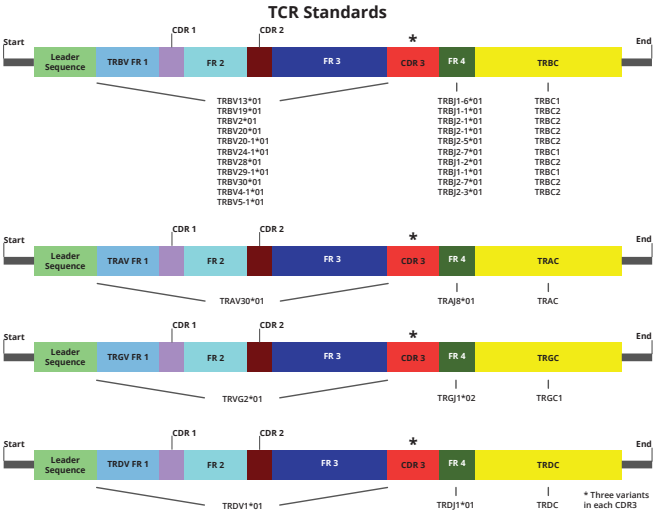

Figure 2

39 TCR spike-in constructs represent 10 different TRBs, 1 for each of TRA, TRG and TRD genes. Each construct has 3 variations in the CDR3 region that differ by 3 nucleotides in fixed position.

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Sample Applications

- **Assessing Immune Receptor Profiling Assay Performance** Pre-mixed RNA Spike-In Controls added to the RT-PCR Immune Profiling assay provide a calibration standard for the sequencing data to eliminate biases caused by PCR variation and the different sequencing platforms (Fig. 3).

- **Measuring Sequencing Errors** Due to the enormous complexity and variability in sequences of the TCR / BCR repertoire, any biases generated in the enzymatic, NGS, or data processing steps, can significantly affect the AIR profiling results. Using Spike-In Control Mixes with defined reference sequences is an excellent strategy to accurately measure sequence errors and evaluate mechanisms responsible for generating these errors.

- **Identifying Cross-Contamination** Cross-contamination between different samples is a serious problem for any PCR-based assay. Bench-level cross- contamination due to sample mixing, contamination with PCR products from previous experiments, or barcode jumping during sequencing are some common issues that could significantly affect AIR experimental results. The RNA Spike-In Control Pools enable the measurement of cross-contamination levels between different samples (Fig. 4).

High Quality Assays Yield High Quality Results

Together with **Cellecta DriverMap AIR Profiling RNA Assay**, a multiplex RT-PCR and NGS based technique, you can accurately profile TCR and BCR functional CDR3 or full-length receptor regions. The DriverMap AIR assay can simultaneously amplify all seven TCR and BCR chains using a set of 300 experimentally validated PCR primers to yield NGS-ready, Illumina-compatible libraries. Standards and controls allow 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 enable researchers to reliably quantify the adaptive immune repertoire. **Learn more at cellecta.com/DriverMapAIR**

Ordering Information

Catalog #	Product Description	Format
DMAIR-HTR-SC-M	DriverMap AIR TCR Spike-in Premixed Controls (Human RNA)	mix
DMAIR-HTR-SC-P	DriverMap AIR TCR Spike-in Isoform Pools (Human RNA)	pool
DMAIR-HBR-SC-M	DriverMap AIR BCR Spike-in Premixed Controls (Human RNA)	mix
DMAIR-HBR-SC-P	DriverMap AIR BCR Spike-in Iso-form Pools (Human RNA)	pool

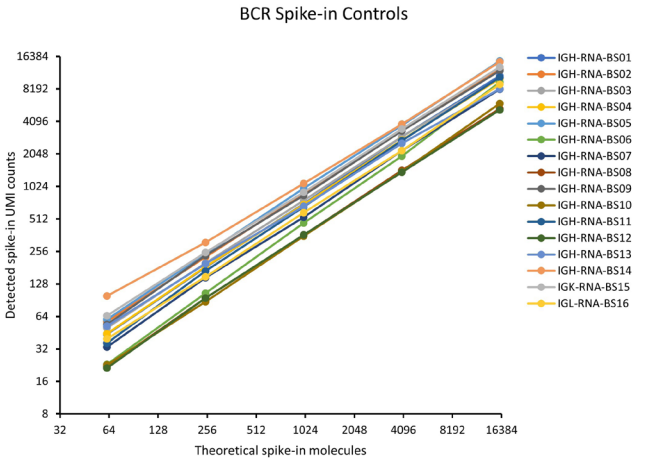

Figure 3

BCR Spike-in Control Mix was added to PMBC Control RNA (50 ng) at different input amounts (0.5µl, 2µl and 8 µl). DriverMap AIR Assay was run and the clonotypes were sequenced at 20M read per sample. MiXCR pipeline was used to calculate detected UMI as compared to spike-in amount (theoretical amount). Results show a linear trend for each construct as the input amount increases. construct as the input amount increases.

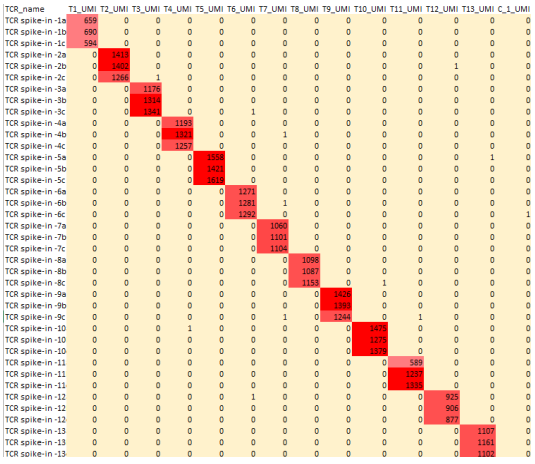

Figure 4

Individual TCR Triplex Isoform Control Pools (2 µl each) were spiked in at different experimental samples of whole blood RNA and control PBMC RNA (50 ng each). DriverMap AIR Assay for each experimental RNA sample was performed in separate test tubes and spike-in control UMI numbers for each spike-in control construct were calculated by MiXCR. The results show that most samples have zero or limited crossover and only detect the respective spiked-in control set allowing us to measure cross contamination or crossover between multiple samples.

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