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FDA

Evaluation of BCR control cell-lines and synthetic controls using DriverMap Adaptive Immune Receptor (AIR) profiling technology

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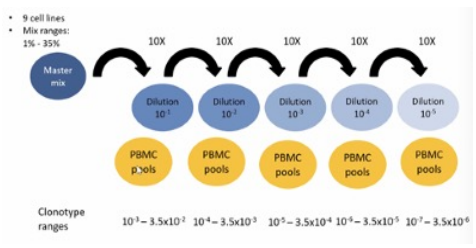
Abstract

The DriverMap™ Adaptive Immune Receptor (AIR) repertoire profiling assay employs multiplex RT-PCR for comprehensive profiling of CDR3 or full-length receptor profiling of all variable heavy and light chains of T-cell receptors (TCR) and B-cell receptors (BCR) from either RNA or DNA. In this study, initiated by a U.S. Food and Drug Administration (FDA) consortium to compare performance of different BCR repertoire profiling technologies, we tested spike-ins in peripheral blood mononuclear cell (PBMC) RNA and DNA control mixtures prepared from nine different B-cell lines. We measured the sensitivity, linearity and accuracy of AIR RNA and DNA BCR profiling for the IGH, IGK and IGL chains of the spike-in controls. Our comprehensive analysis, encompassing CDR3 profiling and full-length receptor profiling (CDR1, CDR2, and CDR3) sequencing, revealed a 100X increase in sensitivity of detection of spike-in controls in RNA compared to the DNA assay. The high sensitivity of the full-length DriverMap™ AIR RNA assay allowed the detection of controls from all nine cell lines, with 7 out of 9 cell lines detected in the DNA assay, which demonstrates the robustness of the DriverMap™ AIR profiling technology. In parallel, we also validated Cellecta's synthetic spike-in controls to measure assay performance and measure cross contamination between experimental samples. This study highlights the remarkable sensitivity of the AIR RNA assay in quantifying transcripts. This approach enables the detection of low-frequency BCR clonotypes, which is particularly advantageous when working with samples containing low numbers of B-cells. Furthermore, our AIR DNA assay in combination with controls offers a quantitative tool for minimal residual disease (MRD) applications, providing accurate insight into cell numbers, which then facilitates tracking clonal expansion of immune cells. Moreover, the synthetic spike-in controls are commercially available, easy-to-use controls to evaluate any PCR based immune profiling assay performance.

Introduction

- Cellecta participated in BCR-seq benchmarking study led by FDA consortium.
- 9 well-characterized monoclonal B-cell-lines were used as candidates for generating standard reference material.
- A series of material was generated that had a wide range of clonotype fractions, from 35% (10^-3) down to 1 %(10^-7).
- DNA/RNA was extracted and shared with sequencing providers.
- Cellecta ran DriverMap™ AIR RNA and DNA assay for BCR repertoire profiling of IGH, IGK and IGL chains.

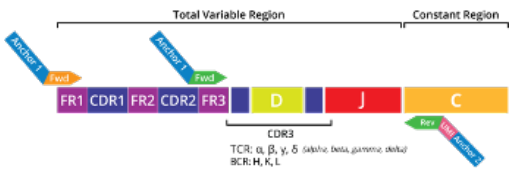
Cell line	Heavy V Gene
EHEB	IGHV1-18
RI-1	IGHV3-7
OCI-LY1	IGHV3-23
WSU-NHL	IGHV3-48
LB5871-LYMP	IGHV3-15
WSU-DLCL	IGHV4-61
GUMBUS	IGHV4-31
MEC-1	IGHV4-59
JK-6	IGHV1-2



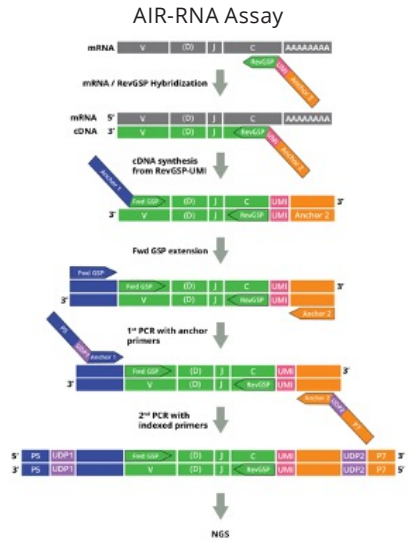
Method

Design of AIR RNA & DNA Assay

AIR-RNA



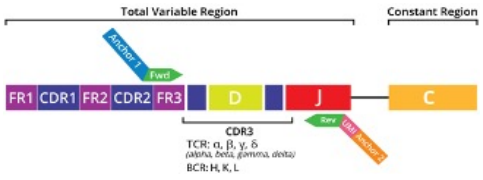
AIR-RNA Assay



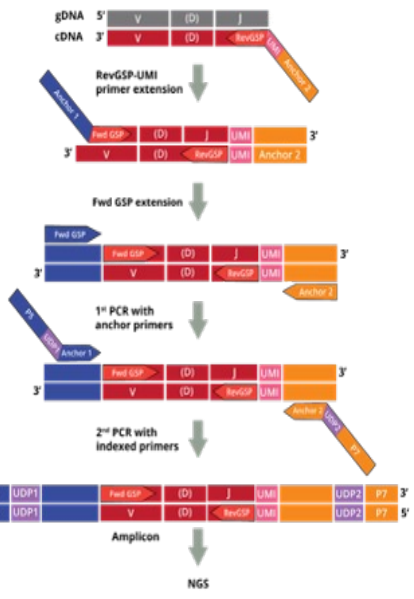
- **Improved coverage** of CDR3 or CDR1-CDR2-CDR3 regions (full-length) with highly validated, redundant V primer sets
- **Unbiased amplification** with universal anchor primers
- **No primer dimers and off-target products**
- **Quantitative clonotype analysis** with UMI and AIR RNA calibration standards
- **CellDirect AIR-RNA assay** > could be run directly in cell lysate (single cell, sorted cells)

Method (cont.)

AIR-DNA



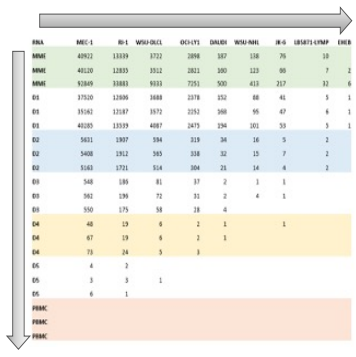
AIR-DNA Assay



- **Separate reactions for TCR (TRB, TRA, TRD,TRG) and BCR (IGH, IGK, IGL) genes** > due to differences in T/B-cell composition
- **Universal anchor primers for amplification step** J and FR3 gene-specific primers used only for primer extension
- **UMIs to measure copy number of template DNA/cells** to quantify frequency of clonotypes associated with cell number

Results

RNA Assay



DNA Assay

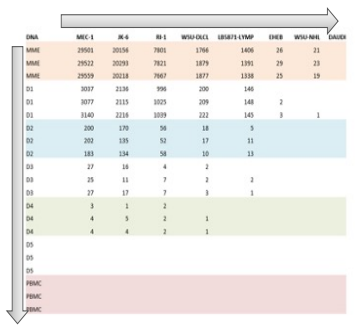
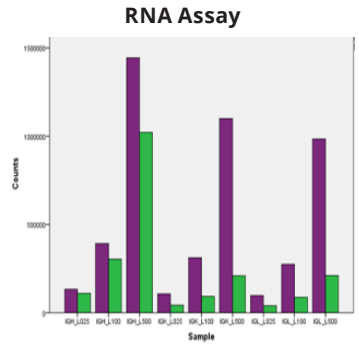


Fig 1: RNA-based assay is more sensitive than DNA based assay as it detects to lowest dilution point of 10^-5 than DNA-based assay which has a lowest dilution point of 10^-4. RNA-based assay detected 9/9 cell lines while DNA-based assay detected 7/9 cell lines (due to constraints in primer design or mutations in cell-lines).

RNA Assay



DNA Assay

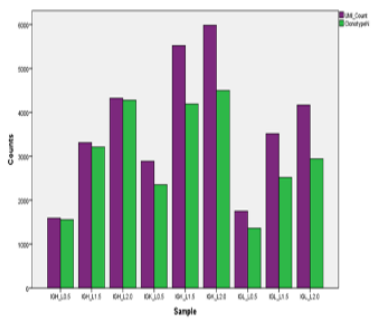


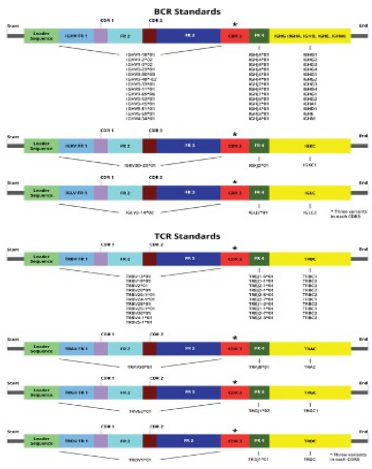
Fig 2: Analysis of CDR3 Regions using AIR-RNA and DNA assays with varied input amounts. 25 ng, 100 ng and 500 ng of RNA was used, and 0.5µg, 1.25µg and 2µg of input DNA was used. AIR-RNA assay (100 ng) is more sensitive than AIR-DNA assay (2 µg).

Cellecta offers Synthetic Spike-in Controls (RNA)

Collection of synthetic mRNA constructs that may be used as universal controls for both commercial and home-brewed AIR sequencing assays based on multiplex RT-PCR or 5'-RACE (SMART) PCR techniques.

Product Format:
- Premixed Controls (DMAIR-HBR-SC-M)
- Triplex Isoform Pools (DMAIR-HBR-SC-P)

Applications:
- **Ensure Sensitivity and Specificity:** Premixed Controls may be spiked into your experimental sample to measure the accuracy and linear range of your PCR assay.
- **Error Quantification:** Premixed Controls may be used to assess sequencing errors introduced at PCR and NGS stages.
- **Sample Quality Control:** Triplex Isoform Pools or Premixed Controls may be used to assess sample quality such as in degraded FFPE samples.
- **Cross-Contamination Detection:** Triplex Isoform Pools can measure cross-contamination between different samples.



48 BCR mRNA spike-in constructs represent 10 different IGHVs; 1 for each IGHA, IGHD, IGHE, IGHM and 1 for IGKV and IGLV region

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

*Each construct has 3 variations in the CDR3 region that differ by 3 nucleotides in fixed position

Applications of Synthetic Spike-In Controls

Evaluate Assay Performance

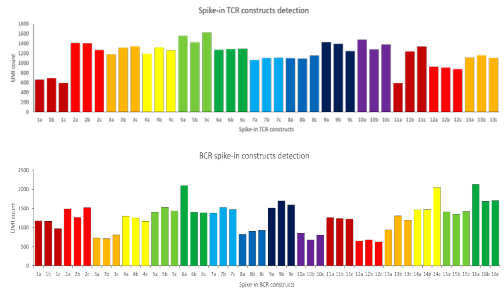


Fig 3: UMI count of 39 TCR and 48 BCR control constructs spike-in at 8000 molecules in 50 ng of PBMC RNA. Efficiency of detection of control molecules is in 30-80% range which aligns with the efficiency of multiplex RT-PCR technology (i.e. DriverMap™ AIR assay).

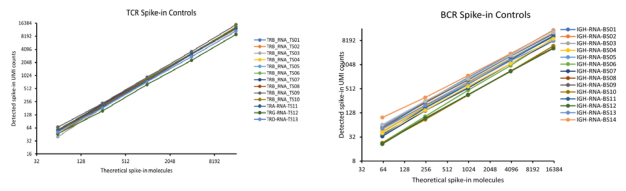


Fig 4: Detection of TCR and BCR spike-in control constructs at different number of molecules. The figure illustrates linearity and dynamic range as the number of molecules increases.

Measure cross-contamination

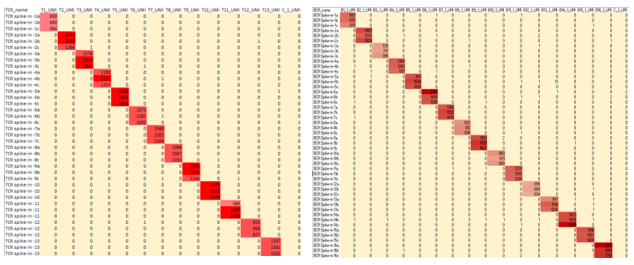


Fig 5: Detection of TCR and BCR spike-in control sets in multiple experimental samples vs. control. Most samples show zero or limited cross-over and only detect the respective spiked-in control set.

Summary

- **Adaptive Immune Receptor (AIR) profiling assay** → comprehensive TCR/BCR repertoire analysis (all seven chains) in single multiplex PCR reaction in bulk RNA and DNA samples
- **Quantitative clonotype analysis** → clonotypes with UMI >10, technical replicates
- **Optimal conditions:** PBMC RNA (50-100 ng), DNA (5-10 µg), Triplicates, 5M reads /replicate
- **Application:**

→ AIR-RNA is > 10-100-fold more sensitive than AIR-DNA for detection of activated clones.

→ AIR-DNA can be used for MRD detection to accurately quantify frequency of clonotypes associated with disease.

→ AIR-DNA assay is a better choice for degraded RNA samples such as FFPE samples.

- Cellecta developed **universal, commercially available** 36 TCR and 48 BCR **spike-in synthetic RNA controls** to evaluate performance and optimize conditions of different commercial immune receptor profiling assays.

AIR-RNA and AIR-DNA profiling assays are available as kits and custom services. RNA Spike-in controls available for testing.