Universal synthetic spike-in controls for accurate adaptive immune receptor profiling

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Abstract

The results of adaptive immune receptor (AIR) repertoire diversity assays can be affected by various biases from differences in conditions in the RT-PCR and NGS sequencing steps. Spike-in synthetic controls can be used as calibration standards to address these biases. In this study, we synthesized near full-length BCR and TCR constructs that mimic seven different IGH, IJK, IGL, TRB, TRA, TRG, and TRD genes. To test our spike-in controls, three sets of variants at different concentrations were added to the RNA samples before the reverse transcription reaction with Cellecta’s DriverMap™ Adaptive Immune Receptor (AIR) Profiling Assay. The DriverMap™ protocol uses reverse gene-specific primers with unique molecular identifiers (UMI), allowing UMI-based correction of amplification biases during data analysis. Calculating UMI-based correction and comparing that with spike-in controls enabled us to differentiate between real and background sequences and estimate the average sequencing error rate at 0.4%-0.8% per base, which is within the reported range of Illumina sequencing. This suggests that our spike-in controls are reliable and may be used as a universal tool to correct AIR protocol biases and calculate error and mutation rates in different AIR profiling assays.

Method

Synthetic TCR/BCR mRNA Control Constructs
- Linear TCR and BCR plasmids were transcribed to make total RNA for each construct.
- A mixture of 48 BCR constructs and 39 TCR constructs was spiked at different concentrations to 50 ng PBMC RNA at a 1:1 ratio.
- DriverMap™ AIR-RNA TCR/BCR assay was run for immune receptor profiling of all TCR (TRA, TRB, TRD, and TRG) and BCR (IGH, IGL, and IJK) chains.
- Pooled library was sequenced on Illumina NextSeq 500, 300-n paired-end kit for 10M reads per sample.
- Data was analyzed using the MiXCR 3.0 software.

Calibration Standard Workflow for RNA Spike-in

TCR/BCR Calibration Standards
- Synthetic TCR/BCR mRNA Control Constructs
- BCR Calibration Standards
- 48 BCR constructs with unique CDR3 region

Results

TCR Calibration Standards
- 39 TCR constructs with unique CDR3 region
- Heatmap of UMI counts in TCR spike-in control RNAs in 13 TCR isoforms (39 sequences) in various concentrations spiked in 50 ng of PBMC RNA.

BCR Calibration Standards
- 48 BCR constructs with unique CDR3 region
- Box plot analysis of BCR spike-in control constructs: results show a linear trend as the number of spike-in molecules increases.

Discussion

Summary
- Cellecta developed a panel of synthetic, near full-length TCR/BCR RNA and DNA constructs for universal calibration standard application in immune receptor profiling assays.
- TCR/BCR calibration standards can be used for measuring the sensitivity and accuracy (by mutation rate analysis) of multiple RT-PCR AIR-RNA profiling data using UMI-powered MiXCR analysis in universal PCR-based immune receptor profiling assays.
- The design of the calibration standards enables them to be used with any standard AIR-PCR assay.
- Calibration provides a basic test to measure primer performance in AIR-PCR assays.

Download Demo

Access on GitHub
Access on NCBI: BioProject ID PRJNA975292

Fig 1: 48 BCR mRNA spike-in constructs represent 10 different IGHs, 1 for each IGHA, IGHD, IGHE, IGHM, IGK and IGL genes.

Fig 2: 39 TCR spike-in constructs represent 10 different TRBs, 1 for each TRA, TRB, TRG and TRD genes.

Fig 3: Heatmap of UMI counts in TCR spike-in control RNAs in 13 TCR isoforms (39 sequences) in various concentrations spiked in 50 ng of PBMC RNA.

Fig 4: Box plot analysis of TCR spike-in control constructs: results show a linear trend as the number of spike-in molecules increases.

Fig 5: UMI count of 39 TCR control constructs spike-in at 8000 molecules in 50 ng of PBMC RNA.

Fig 6: Heatmap of UMI counts in BCR spike-in control RNAs in 16 BCR isoforms (48 sequences) at 8000 and 4000 molecules in 50 ng of PBMC RNA.

Fig 7: Box plot analysis of BCR spike-in control constructs: results show a linear trend as the number of spike-in molecules increase.

Fig 8: UMI count of 48 BCR control constructs spike-in at 8000 molecules in 50 ng of PBMC RNA.

Fig 9: Correlation between BCR control constructs in replicates for 8000 molecules in 50 ng of PBMC RNA.