Evaluation of SMART-Seq® Human BCR (with UMIs) for unbiased BCR repertoire profiling

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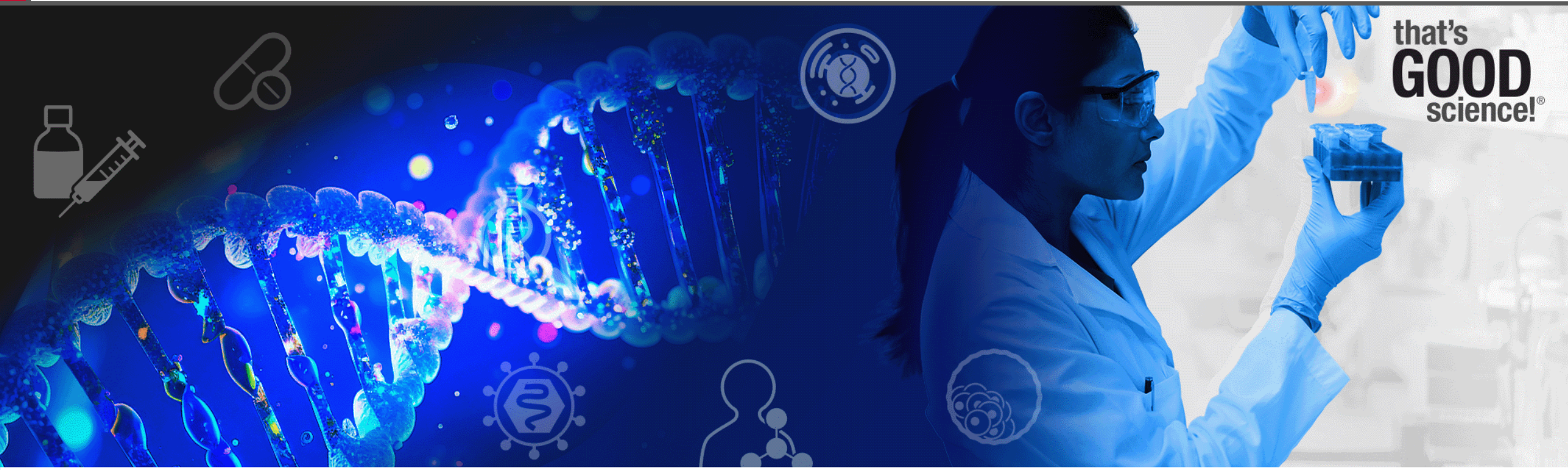
Takara Bio: core capabilities



NGS

PCR, qPCR, RT-PCR

Cloning

Nucleic acid purification

Gene delivery

Functional genomics

Protein expression & purification

OEM

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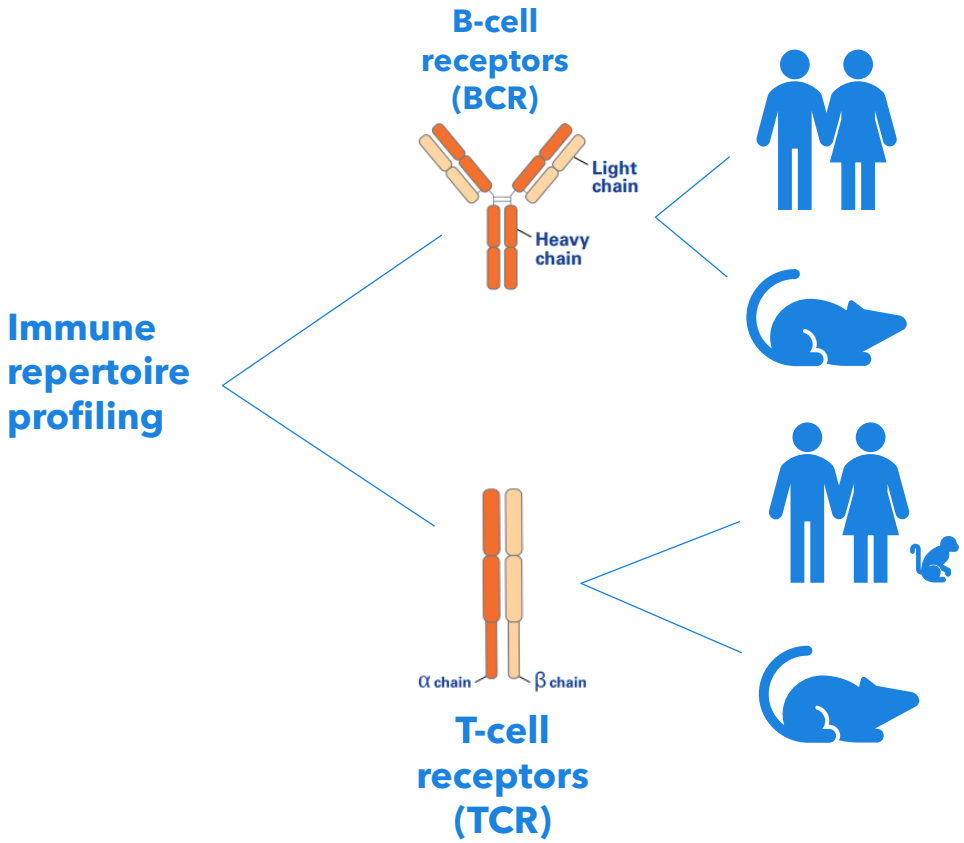
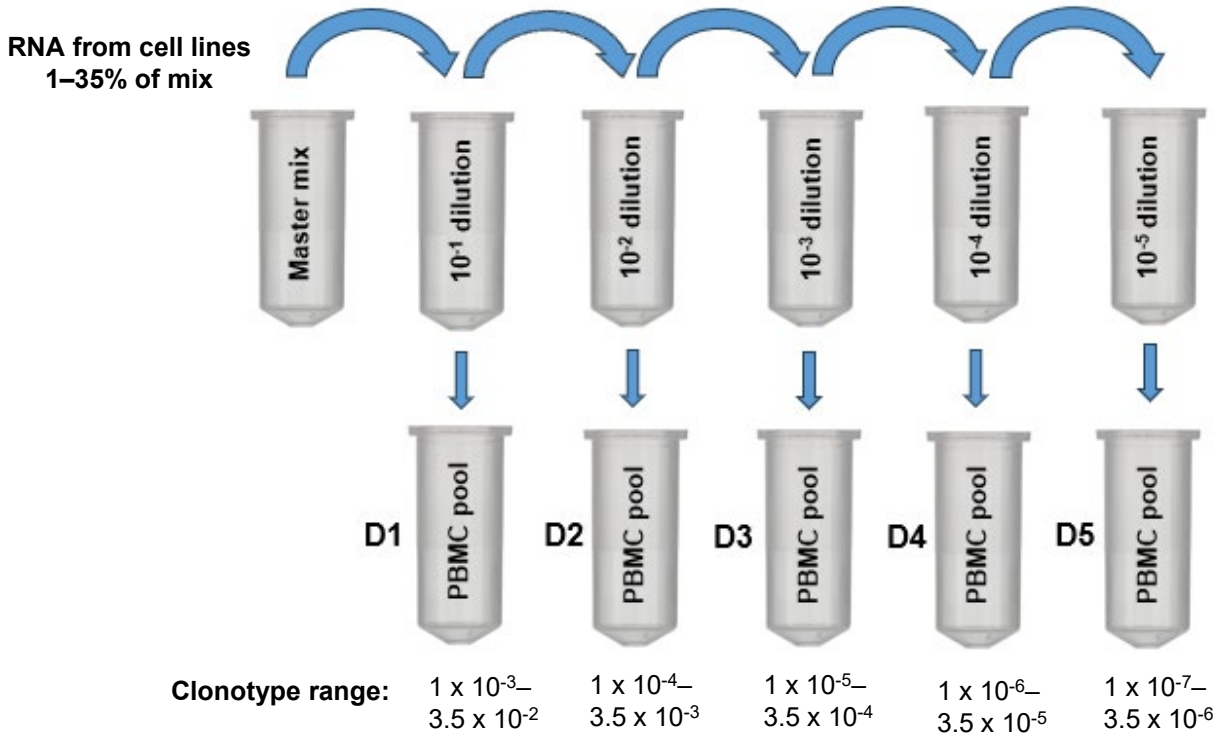
Takara Bio: immune repertoire profiling solutions



- Flexible **input range**
  - 1 ng–1 µg
- RNA from various **sample types**
  - B cells/T cells, PBMCs, whole blood, bone marrow, lymph node
- **UMIs** for error correction, confident clonotype calling
- **Sensitivity** to capture low-abundant clonotypes
- Capture all/most **isotypes**
  - BCR: IgA/D/E/G/M for heavy chain; IgK/L for light chain
  - TCR: TCRα, TCRβ
- Sequencing **flexibility**
  - Full length (300 x 2 bp)
  - CDR3 only (150 x 2 bp)
- UDIs for **multiplexing** capabilities
  - 384 samples in a single run

Evaluating bias in BCR repertoire profiling: FDA consortium study design

- The diversity of the BCR repertoire requires accurate analysis for correct biological information
- Bias from the assay (multiplex primers, PCR errors) or sequencing instruments could affect the outcome of immunological studies
- To understand the use of controls to check for such bias, we conducted a study in collaboration with the FDA*
  - Provided RNA samples from individual cell lines with known clonotype composition
  - Produced spike-in cell line mix using provided RNA samples

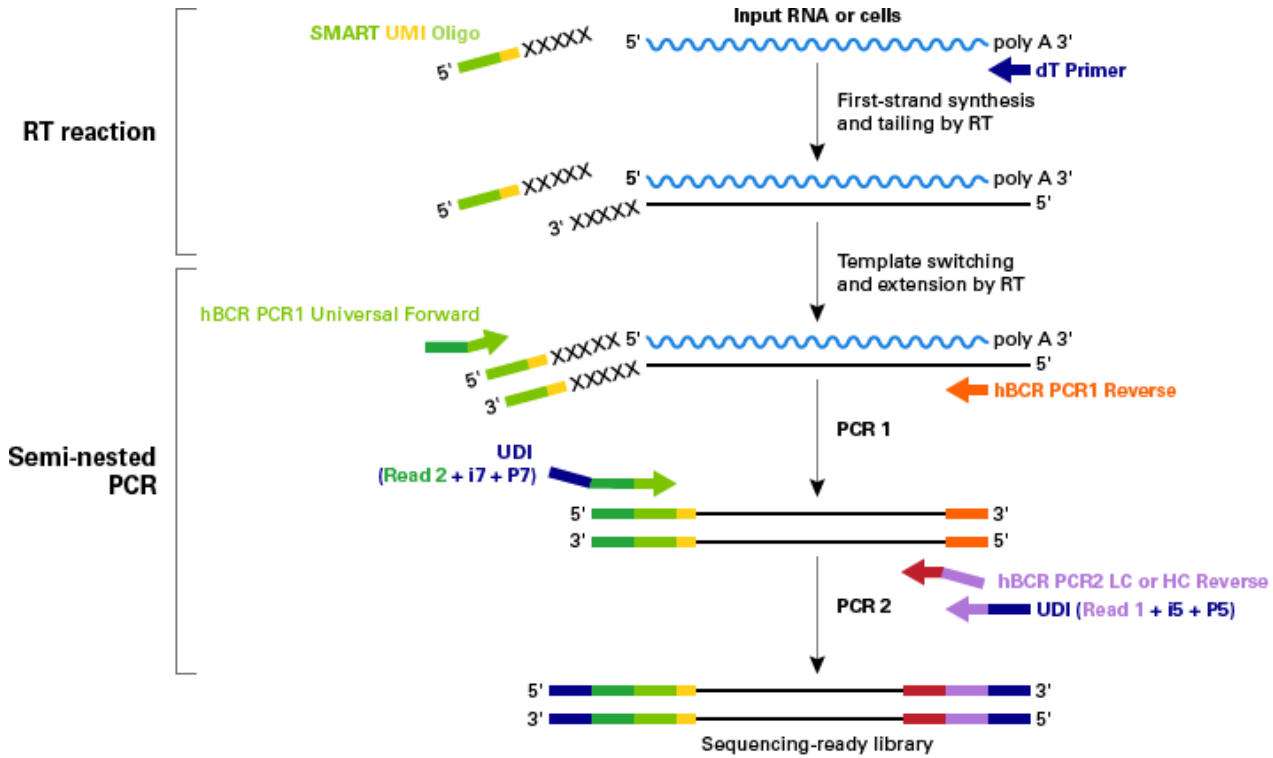


We would like to thank Wenming Xiao (FDA) for study design input and RNA samples.

FDA consortium study design

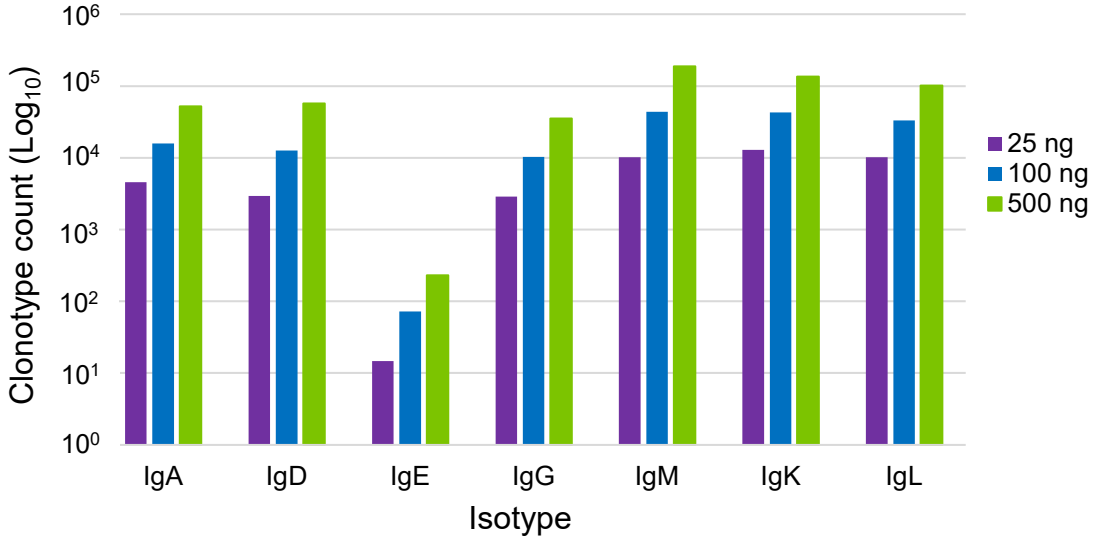
- Libraries from spike-in cell line mix dilutions were prepared using SMART-Seq Human BCR (with UMIs)
- To assess for bias in sequencing platform, libraries were sequenced on the Illumina® NextSeq® 2000, MiSeq®, NextSeq 2000-XLEAP, and the Element AVITI System*
- Sequencing data was analyzed using Cogent™ NGS Immune Profiler

SMART-Seq Human BCR (with UMIs) workflow



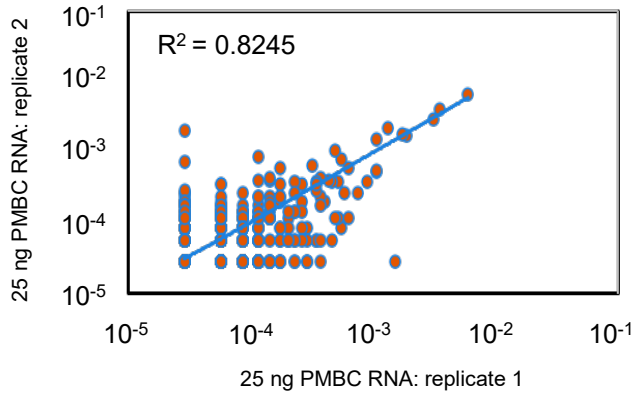
**We would like to thank Robin Bombardi (Illumina) for sequencing libraries on Illumina platforms and Element Biosciences for sequencing libraries on the AVITI System.*

Reproducible performance across a wide input range

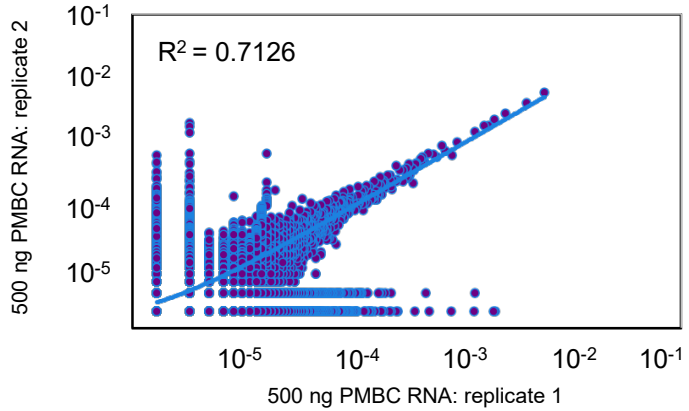


SMART-seq Human BCR (with UMIs) performs consistently at low and high inputs.

A



B



Clonotype frequencies correlate between technical replicates at low and high inputs.

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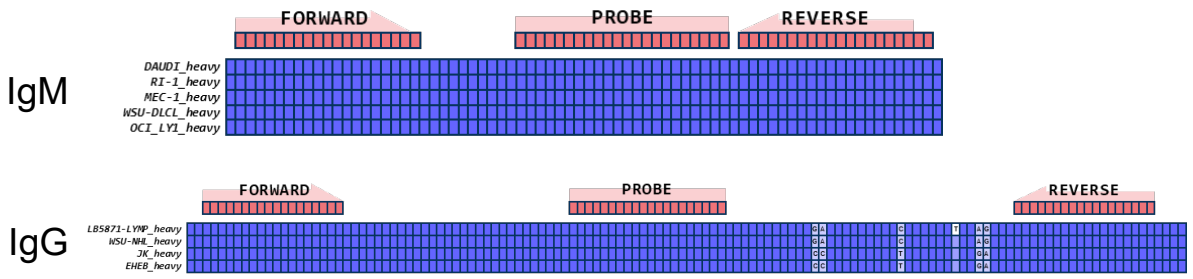
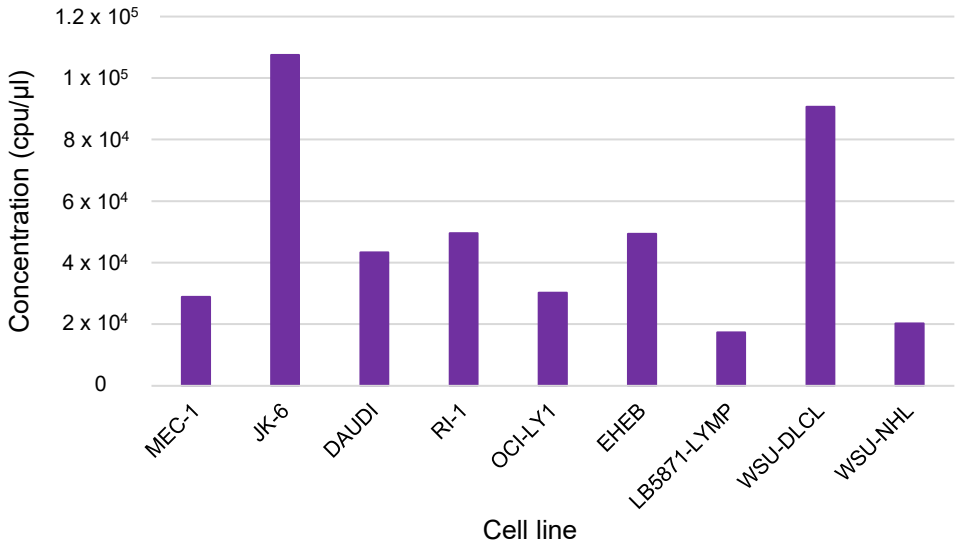
Expression abundance: ddPCR

Assay design

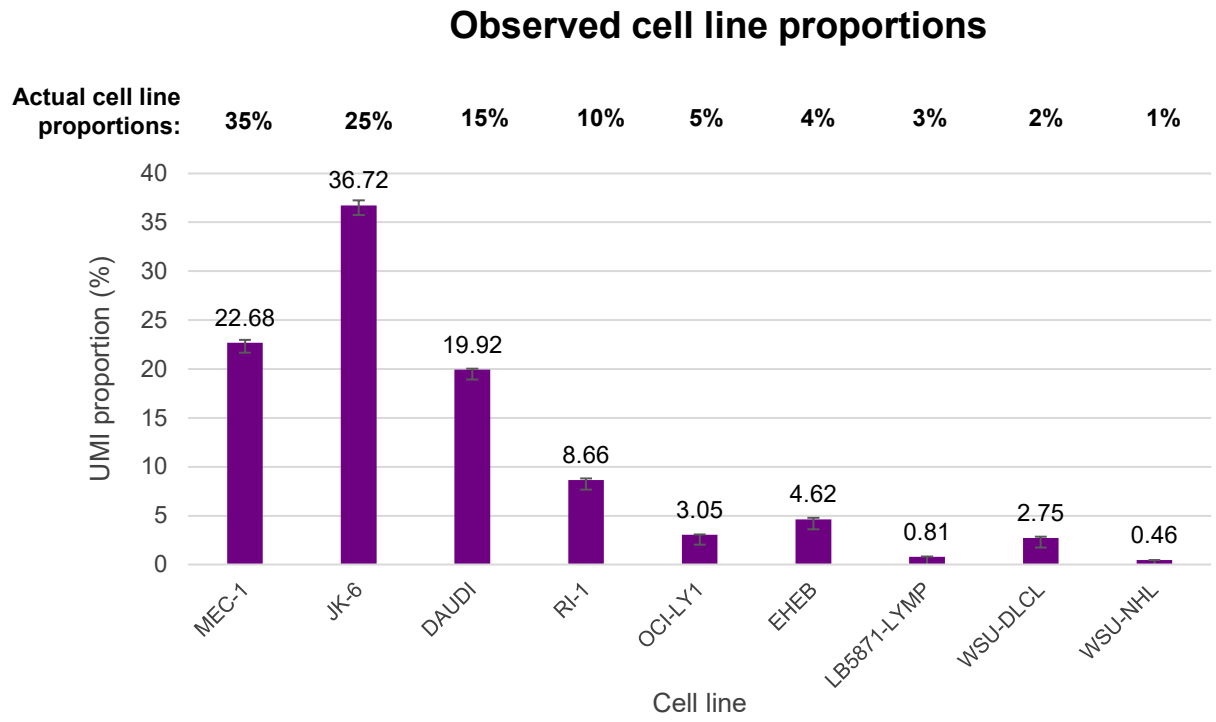


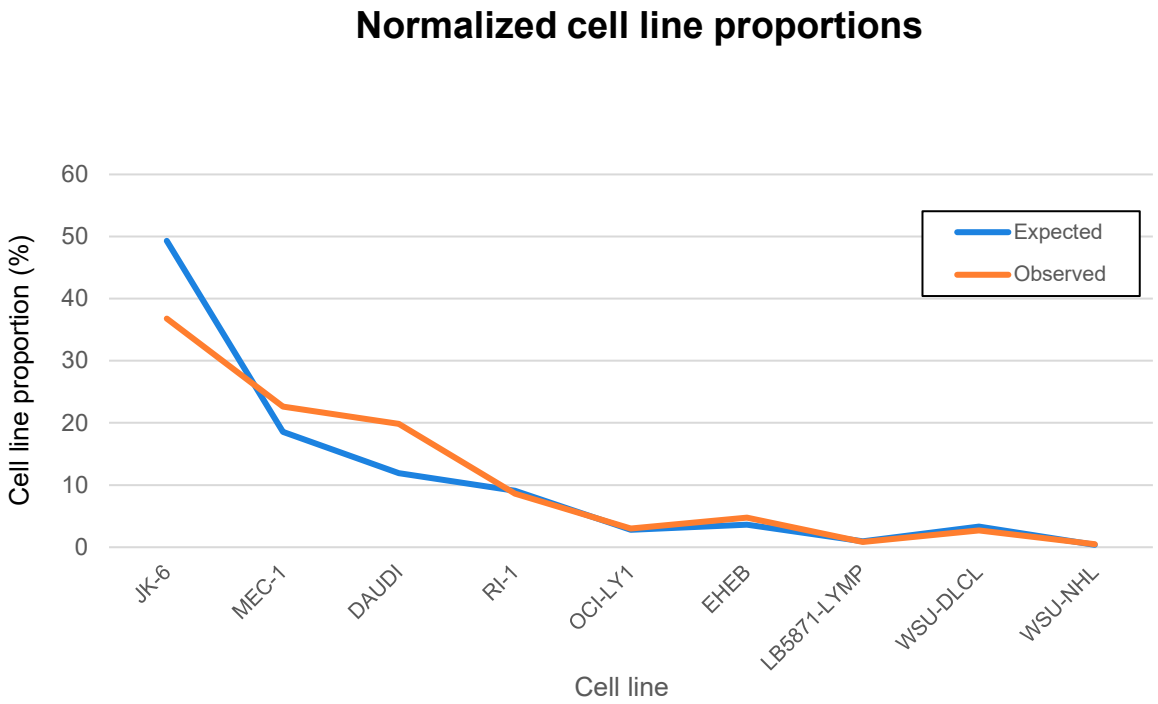
Expression abundance



Prior to producing the spike-in cell line mix, RNA from each individual cell line was analyzed using ddPCR for heavy chain expression (IgG or IgM). JK-6 exhibited the highest expression, followed by WSU-DLCL.

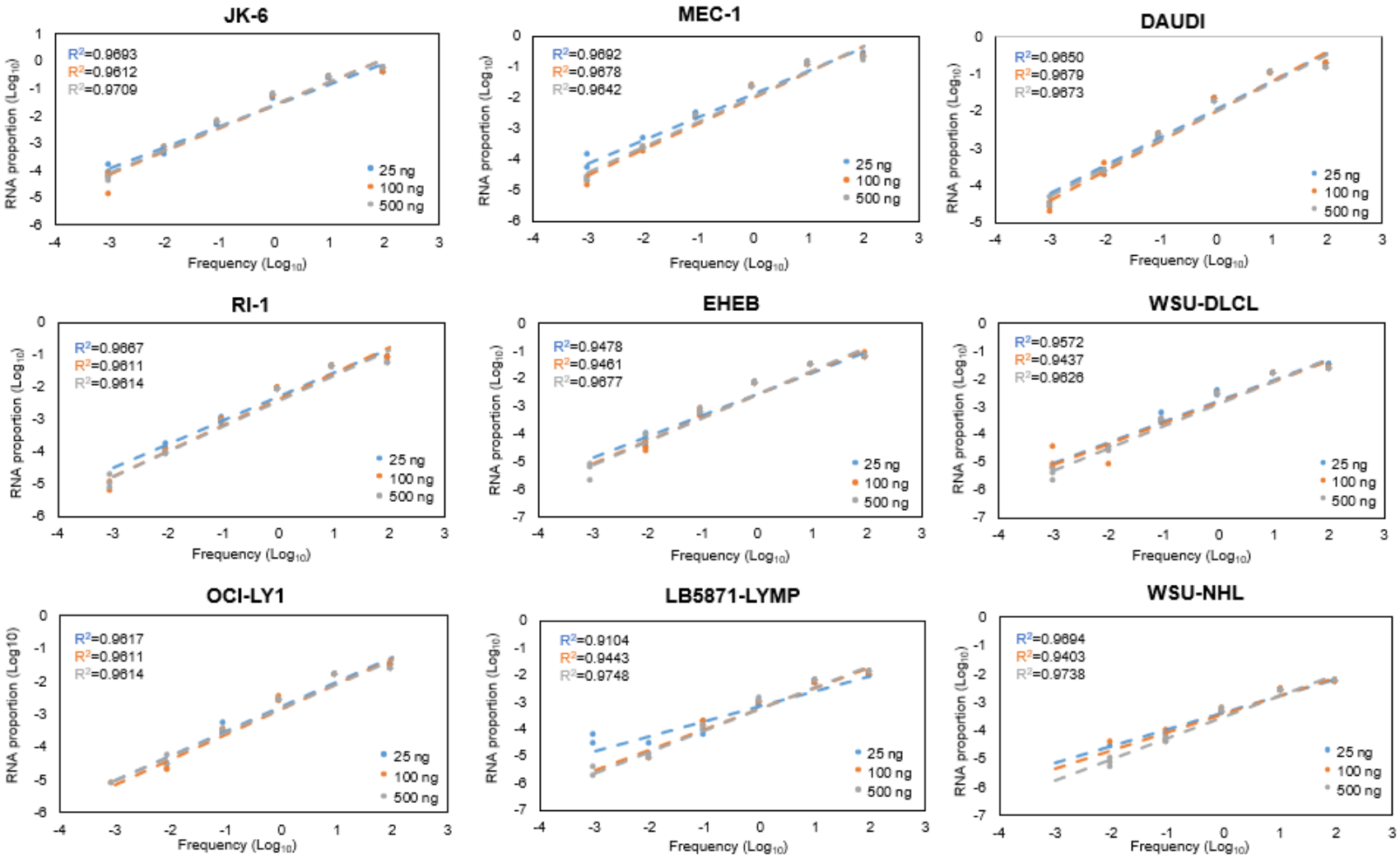
Unbiased amplification: spike-in cell line proportions





The normalized, observed spike-in cell line proportions calculated from libraries prepared with SMART-Seq Human BCR (with UMIs) are the same as the expected cell line proportions, demonstrating a lack of amplification bias.

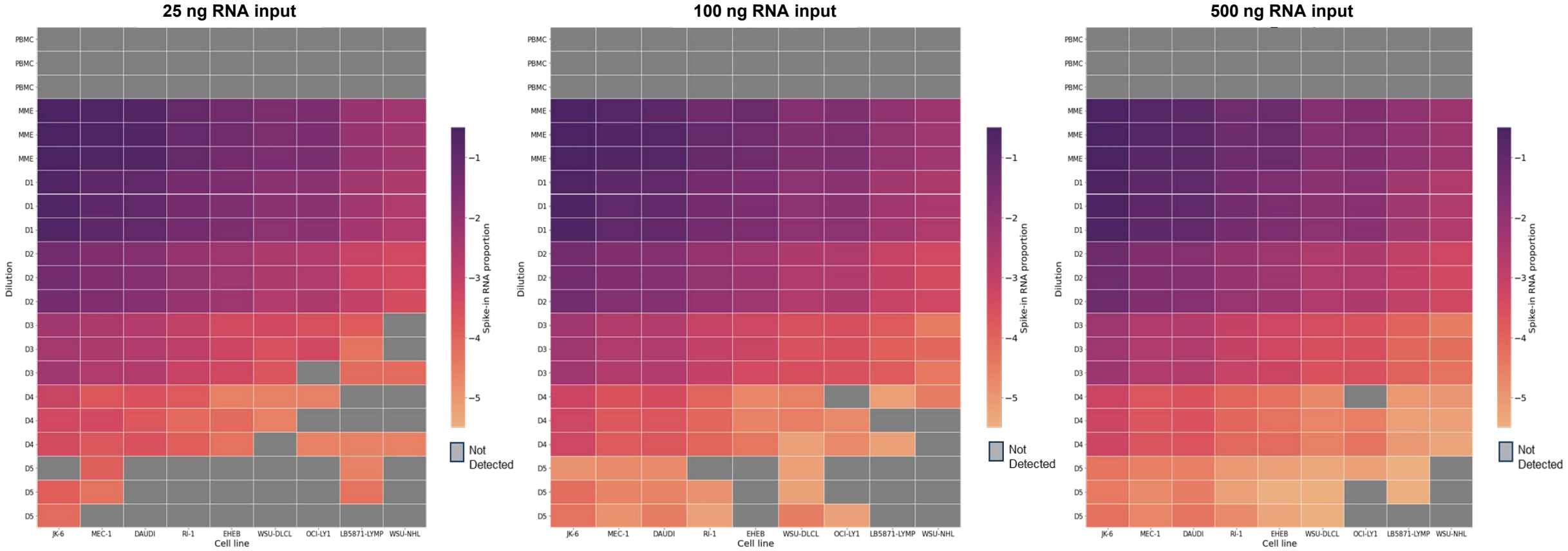
Precise quantification of low-abundant clonotypes



The number of UMI groups identified at tested concentrations is dependent on copies of target molecules.

- 25 ng RNA input: R^2 = 0.910–0.969
- 100 ng RNA input: R^2 = 0.940–0.967
- 500 ng RNA input: R^2 = 0.961–9.974

Reproducible detection of low-abundance clonotypes



Libraries prepared with SMART-Seq Human BCR (with UMIs) are sensitive enough to detect low-abundance clonotypes. The limit of detection (LOD) was 1 x 10^{-6} for RNA inputs of 25–100 ng and 4 x 10^{-7} for an RNA input of 500 ng.

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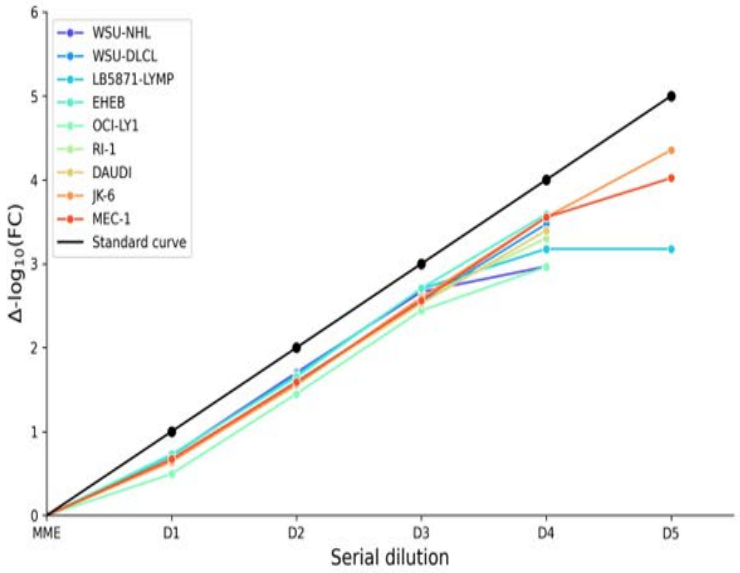
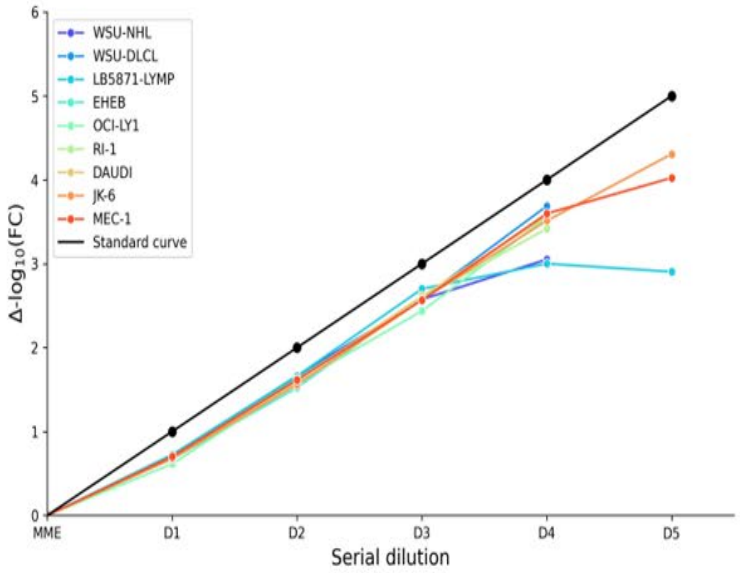
Consistency across Illumina sequencers

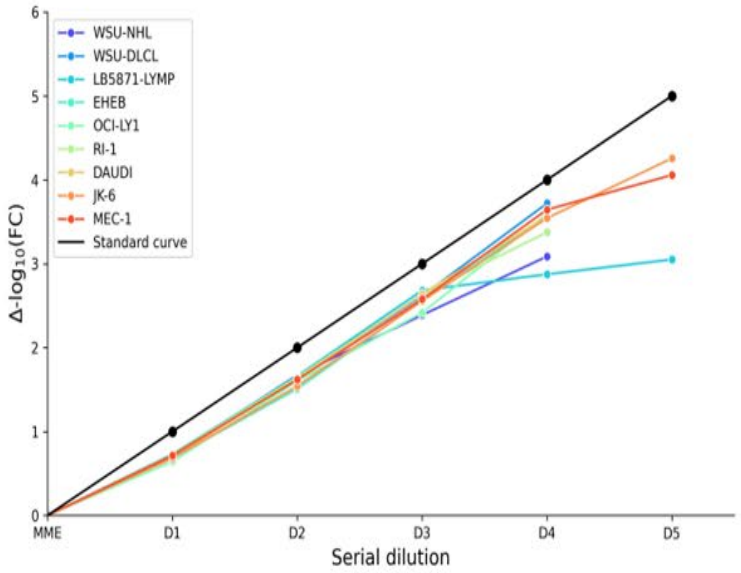
MiSeq



NextSeq 2000



NextSeq 2000-XLEAP



Libraries produced from 25 ng input with SMART-Seq Human BCR (with UMIs) performed comparably when sequenced on the MiSeq, NextSeq 2000, and NextSeq 2000-XLEAP.

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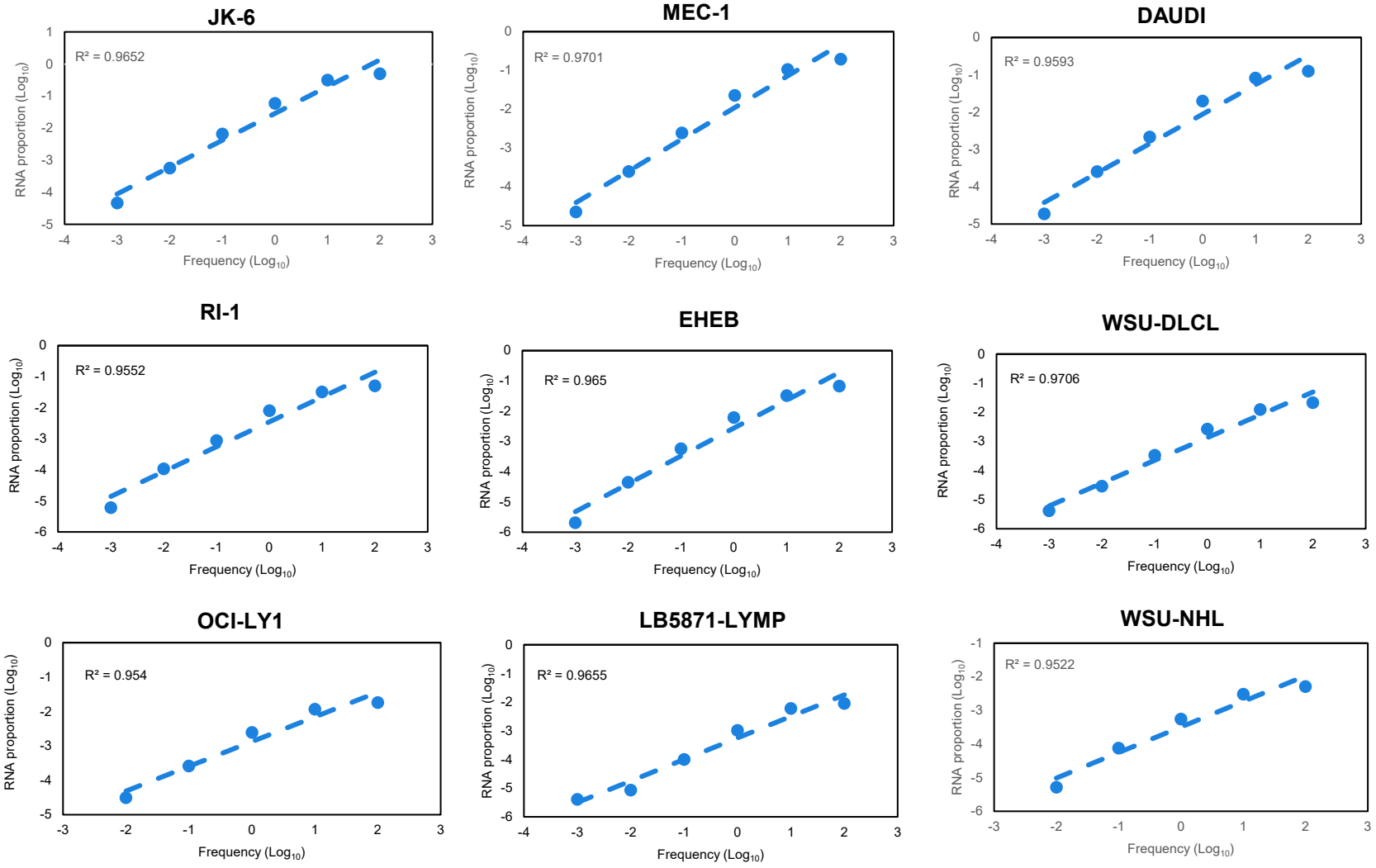
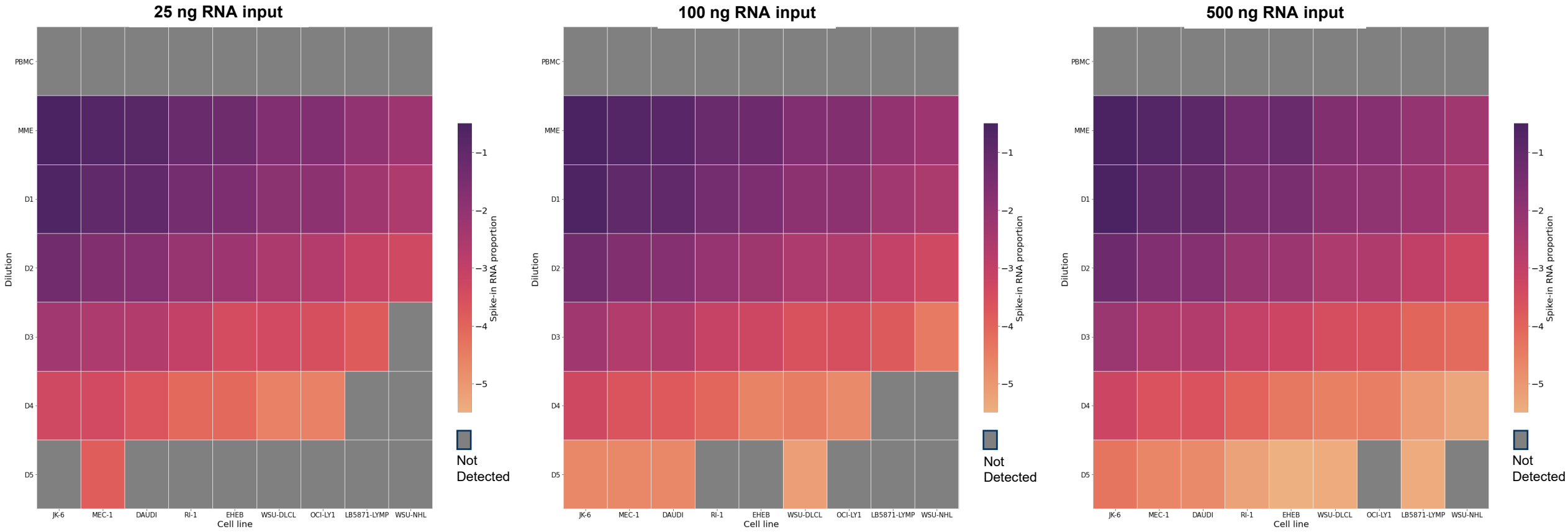
Precise quantification of low-abundant clonotypes on the Element AVITI System



Libraries sequenced on the Element AVITI system showed excellent linearity, as UMI groups corresponded to the target molecule copies.

- 500 ng RNA input R^2 = 0.952–0.970

Detection of low-abundant clonotypes on AVITI



For libraries sequenced on the AVITI System, the limit of detection (LOD) increased with an increase in RNA input, and a reproducible LOD of 4 x 10^{-7} was achieved at 500 ng RNA input. These data are comparable to data obtained for libraries sequenced on Illumina platforms.

Conclusions

Our data demonstrates that SMART-Seq Human BCR (with UMIs) is:

- **Free of bias**—the correlation between ddPCR expression data and NGS data shows that SMART-Seq Human BCR (with UMIs) is free from potential biases introduced by PCR, primers, or sequencing instruments
- **Reproducible**—libraries produced with SMART-Seq Human BCR (with UMIs) show high reproducibility between technical replicates
- **Accurate**—libraries produced with SMART-Seq Human BCR (with UMIs) show linear detection for spike-in clonotypes at various dilutions
- **Sensitive**—libraries produced with SMART-Seq Human BCR (with UMIs) have a limit of detection for tested spike-in concentrations of $\leq 10^{-6}$
- **Sequencing platform agnostic**—SMART-Seq Human BCR (with UMIs) performs similarly on all tested sequencing platforms

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