Efficient and sensitive, high-throughput mouse immune receptor repertoire profiling using SMART® technology

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Abstract

Objective: Immune receptor (BCR/TCR) repertoire profiling for biomarker discovery of host B cell and T cell responses are revealing more about the roles B cells and T cells play in viral and bacterial infections, autoimmune disorders, and cancer. Mice are one of the most common animal models used for both BCR - and TCR profiling. However, current technologies are limited in their ability to generate data accurately and reproducibly for all mouse BCR isotypes and for mouse TCR alpha and beta chains. To address this need, we developed two new mouse immune profiling kits: one to profile heavy and light-chains of all mouse BCR isotypes; the other to profile mouse TCR alpha and beta chains.

Methods: Both kits were developed as end-to-end solutions, streamlining library preparation to data analysis. Libraries were prepared using RNA purified from mouse spleens using our new mouse TCR repertoire profiling kit (1 ng – 1 ug purified mouse spleen RNA) or mouse BCR profiling kit (10 ng – 1 ug purified mouse spleen RNA). BCR and TCR libraries were sequenced on the Illumina® Miseq® benchtop sequencer with 300-bp paired-end reads. TCR libraries were sequenced on the Illumina® MiniSeq® and NextSeq® benchtop sequencer with 150-bp paired-end reads. Sequencing data were analyzed using our Cogent™ NGS Immune Profiler Software and MiXCR v3.

Results: Both the updated mouse TCR and BCR sequencing kits achieved a high on-target map rate across all RNA inputs tested. The updated mouse TCR sequencing kit demonstrated sequencing flexibility as clonotype counts were similar whether we generated full length (300bp x 2) or CDR3 only (150bp x 2) reads. The updated BCR-sequencing kit generated ~5x higher total clonotype count across various RNA inputs than the previous version of the kit. The most common clonotype was found to be consistent among technical duplicates regardless of the RNA input amount.

Conclusion: Our new mouse TCR profiling kit and mouse BCR profiling kit were observed to accurately and reproducibly profile T-cell and B-cell receptor sequences, respectively, and provide information on the diversity of immune repertoires in mouse samples.

1 TCR profiling kit performance: a range of RNA input amounts

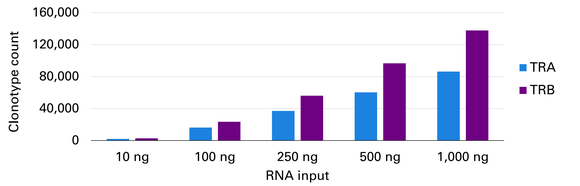

Figure 1. Our new mouse TCR profiling kit (mTCRv2) is compatible with a wide range of sample inputs. The mTCRv2 workflow was performed on five different amounts of mouse spleen RNA. The resulting libraries were sequenced on the Illumina NextSeq 550 platform, generating 2 x 150 bp reads. Sequencing outputs were downsampled to ~15 million reads. Data were processed using Cogent NGS Immune Profiler Software. TRA = TCR alpha chain, TRB = TCR beta chain.

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2 TCR profiling kit flexibility: full-length V(D)J or CDR3 only

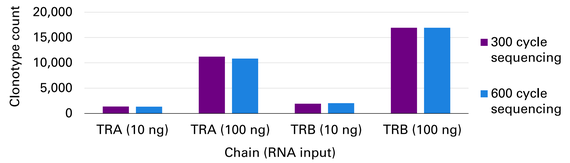

Figure 2. mTCRv2 kits can support full-length V(D)J or CDR3 only sequencing. Libraries generated with 10 ng or 100 ng RNA input were sequenced on the Illumina MiSeq platform with either 2 x 150 bp or 2 x 300 bp reads. Sequencing outputs were downsampled to 1 million reads. Data were processed using Cogent NGS Immune Profiler Software.

3 TCR profiling kit reproducibility: different inputs and cartridges

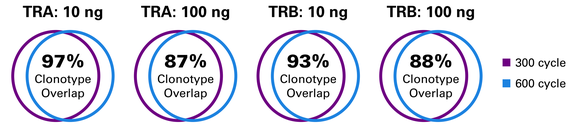

Figure 3. mTCRv2 kits have superior reproducibility with different sequencing configurations. Libraries (generated with 10-ng or 100-ng RNA input) were sequenced on the Illumina MiSeq platform with either 2 x 150 bp or 2 x 300 bp reads. Sequencing outputs were downsampled to 1 million reads. Data were processed using Cogent NGS Immune Profiler Software. Venn diagrams show clonotype overlap between TRA and TRB libraries sequenced with different cartridges. TRA = TCR alpha chain, TRB = TCR beta chain.

4 TCR profiling kit accuracy: UMI correction

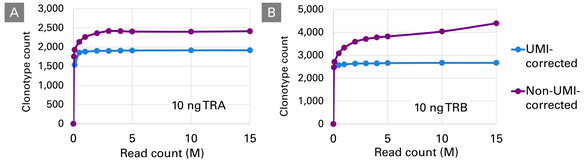

Figure 4. UMIs included with mTCRv2 enable removal of PCR duplicates and sequencing errors. TCR sequencing libraries were prepared from 10 ng of mouse spleen RNA. **Panel A.** TRA clonotype counts at different sequencing depths are shown with (blue line) and without (purple line) UMI-based error correction. **Panel B.** TRB clonotype counts at different sequencing depths are shown with (blue line) and without (purple line) UMI-based error correction.

5 TCR profiling kit performance: diverse sample types

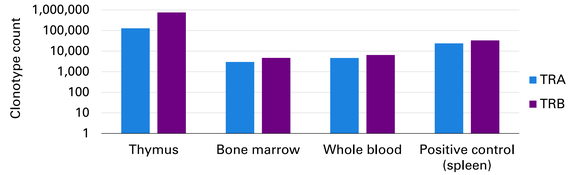

Figure 5. The mTCRv2 kit is compatible with a wide variety of sample types. The mTCRv2 workflow was performed using 200 ng of RNA isolated from four different sample types: thymus, bone marrow, whole blood, and spleen. The resulting TCR libraries were sequenced on the NextSeq 550, generating 2 x 150 bp reads. Sequencing outputs were downsampled to 20 million reads for each sample type. Data were processed using Cogent NGS Immune Profiler Software. The bar plot compares clonotype counts for each sample type. TRA = TCR alpha chain, TRB = TCR beta chain.

6 TCR profiling kit scale-up: adaptability to automation platforms

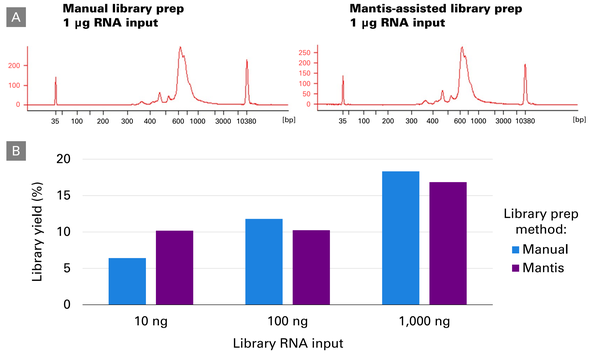

Figure 6. The mTCRv2 kit is compatible with automation platforms such as the Mantis system. The mTCRv2 workflow was performed using three different mouse spleen RNA inputs (10 ng, 100 ng, and 1,000 ng). **Panel A.** Electropherogram profiles of mTCRv2 sequencing libraries are shown. TCR libraries were generated using 1,000 ng of RNA obtained from mouse spleen either manually (left) or automatically on the Mantis liquid handler (right). Electropherogram profiles of the final libraries were obtained on an Agilent 2100 Bioanalyzer. **Panel B.** The bar plot shows library yield for each individual input amount prepared manually (blue) or automatically on the Mantis system (purple). Data were processed using Cogent NGS Immune Profiler Software.

7 BCR profiling kit performance: detection of all BCR isotypes

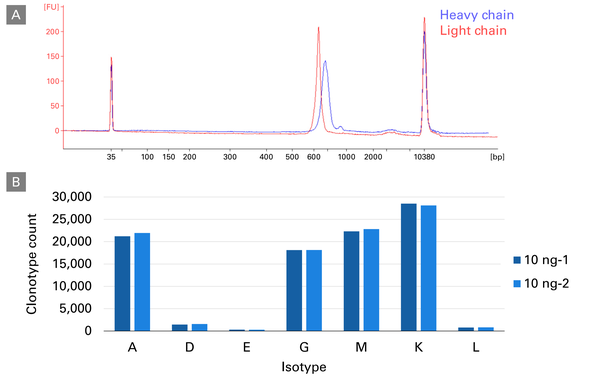

Figure 7. The full range of BCR isotypes is represented in sequencing libraries prepared with our new BCR profiling kit (mBCRv2). Panel A. Libraries containing both heavy-chain and light-chain isotypes were generated using 10 ng of RNA from mouse spleen. Electropherogram profiles of the final libraries were obtained on an Agilent 2100 Bioanalyzer. Peaks situated at the far left and right ends of each electropherogram correspond to DNA reference markers included in each analysis. A typical Bioanalyzer profile of heavy-chain isotypes and light-chain isotypes found in the mouse spleen libraries is shown. The peaks displayed in the Bioanalyzer profiles were ~700 bp for heavy-chain isotypes and a peak ~650 bp for light-chain isotypes, in line with the predicted peak sizes for those sequence fragments. **Panel B.** Clonotype distribution across different BCR isotypes is shown. To evaluate the performance of the kit, the mBCRv2 workflow was performed on 10 ng of spleen RNA. The resulting cDNA libraries were sequenced on Illumina NextSeq 550 (2 x 150). Sequencing outputs were downsampled to 2 million reads. The bar plot shows clonotype distribution for various isotypes within libraries made from 10 ng spleen RNA inputs.

8 BCR profiling kit performance: a range of RNA input amounts

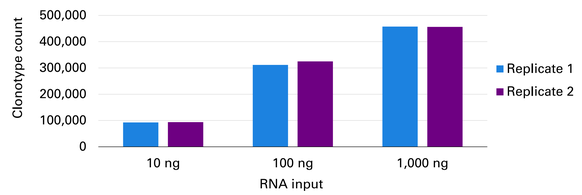

Figure 8. Clonotype counts for varying sample input amounts are shown. The mBCRv2 workflow was performed on three different amounts of spleen RNA (10 ng, 100 ng, and 1,000 ng). Resulting cDNA libraries were sequenced on an Illumina NextSeq 550 (2 x 150). Sequencing outputs were downsampled to ~2 million for each library (2 million for heavy chain and 2 million for light chain). Data were processed using MiLaboratories MiXCR3.0. The bar plot shows clonotype counts for spleen RNA at various RNA inputs.

9 BCR profiling kit performance: improved chemistry and sensitivity

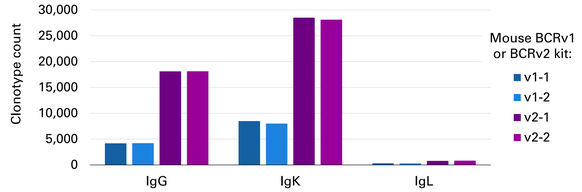

Figure 9. mBCRv2 outperforms an earlier kit. Libraries were generated using 10 ng of total RNA from mouse spleen. The resulting cDNA libraries were sequenced on an Illumina NextSeq 550 (2 x 150). Data were processed using MiLaboratories MiXCRv3.0. Through these data, it was evident that the updated BCR profiling kit (BCRv2) performed better than the previous version, SMARTer® Mouse BCR IgH/K/L Profiling Kit (BCRv1). Sequencing outputs were downsampled to ~1 million for all the IgG/K/L libraries for BCRv1; and ~2 million for heavy- and light-chain libraries for BCRv2. BCRv2 generated approximately 400% more clonotypes than BCRv1.

Conclusions

- The SMART-Seq® mouse TCR kit can capture the entire V(D)J region of the TCR alpha and beta chains.
- The SMART-Seq mouse BCR kit captures the entire variable region, allowing analysis of all heavy-chain isotypes and/or light-chain isotypes, all within the same experiment.
- Our new SMART-Seq mouse TCR kit and SMART-Seq mouse BCR kit enable highly sensitive, reproducible, and efficient detection of low-abundance clones over a range of total RNA input amounts, with the added confidence of UMIs.
- Both mouse immune profiling kits include unique dual indexes (UDIs) to prepare libraries for multiplexing. They offer the flexibility of selecting either 300 or 600 cycles for sequencing on any Illumina instrument. Both feature automation-friendly workflows.

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