APPLICATION NOTE

Discover more and maximize lab efficiency without sacrificing data quality

- Sequence Takara Bio's full-length SMART-Seq® solutions with single-day turnaround on the Singular Genomics G4™ Sequencing Platform
- Achieve equivalent SMART-Seq data with the G4 Sequencing Platform and industry-standard sequencing platforms

Introduction

Researchers require sensitive and reproducible methods to understand the biological mechanisms underlying disease pathogenesis and to develop novel therapeutics. One of the most popular approaches to biomarker discovery is through single-cell and low-input RNA-seq technologies, many of which rely on 3′ end-counting methods. While these methods allow for detection of gene expression signatures in single cells, they are unable to detect important biomarkers such as single nucleotide polymorphisms (SNPs), isoforms, and gene fusions, which can only be identified using full-length RNA-seq methods.

To improve the quality of biomarker discovery workflows, it is critical to efficiently maximize laboratory operations, from library prep through sequencing and analysis. Takara Bio is well-positioned, with its portfolio of industry-leading single-cell and low-input library preparation solutions, to support customers in uncovering novel biomarkers in oncology and infectious disease. With streamlined workflows from samples through sequencing data analysis, SMART-Seq technology delivers more data from every sample. This facilitates the discovery of novel RNA isoforms, gene fusions, and SNPs, leading to new biological discoveries.

Biomarker discovery also benefits from faster sequencing turnaround times. Singular Genomics has developed an innovative benchtop sequencer, the G4 Sequencing Platform, that leverages a 4-color rapid sequencing by synthesis (SBS) chemistry with advanced optics and fluidics engineering to provide single-day turnaround times across all applications. By combining fast run times and the ability to run up to 4 flow cells, with 16 independently addressable lanes, the G4 enables highly efficient laboratory operations.

Three sets of experiments performed on control RNA and single cells show that the combined Takara Bio and Singular Genomics workflow yields similar sequencing metrics and data quality as industry-standard sequencing platforms. These results show that researchers now have access to a fast, accurate, and cost-effective solution to power their biomarker discovery research.

Methods

Two kits—SMART-Seq mRNA LP (Cat. # 634769) and SMART-Seq Single Cell PLUS Kit (Cat. # R400751)—were used to generate cDNA from six mouse brain total RNA (MBR) samples each (obtained from the SMART-Seq mRNA LP kit). 10 pg of MBR was used as an input for the SMART-Seq mRNA kit, running at 17 cycles. 2 pg of MBR was used for the SMART-Seq Single Cell kit, running at 18 cycles. In addition, six K562 cell samples were single-cell sorted into 96-well plates using the Sony SH800 Cell Sorter before running with the SMART-Seq Single Cell kit at 18 cycles. The resulting cDNAs were quantified using a Qubit 2.0 Fluorometer and Agilent BioAnalyzer.

Afterward, 1 ng of cDNA inputs were used to generate all libraries using the library preparation kits included in the SMART-Seq mRNA LP and SMART-Seq Single Cell PLUS kits. All libraries were prepared according to established protocols at 16 cycles using Singular Genomics or Illumina® unique dual index (UDI) primers, where applicable. All completed libraries were quantified using the Qubit 2.0 Fluorometer and Agilent BioAnalyzer. The libraries were pooled at equimolar ratios before the libraries with SG UDIs were sequenced on the G4, and the libraries with the Illumina UDIs were sequenced on the NextSeq® 500 sequencer only (below). Once the sequences were generated, both sets of sequencing data were downsampled to 6.5 million reads. The sequence matrices were then generated using Takara Bio Cogent™ NGS Analysis Pipeline v.2.0.

Learn more about the G4 Sequencing Platform at
www.singulargenomics.com/G4

Results

Similar cDNA library quality for RNA-seq

The ability to prepare high-quality RNA-seq libraries is essential for generating robust RNA-seq runs for low-input and single-cell RNA-seq assays. Comparing RNA-seq libraries generated using both SMART-Seq mRNA LP and SMART-Seq Single Cell PLUS kits revealed nearly identical cDNA fragment length distributions (Figure 1). The Bioanalyzer traces yielded similarly sized libraries generated using both kits for all three experiments (Figure 1, Panels A–C).

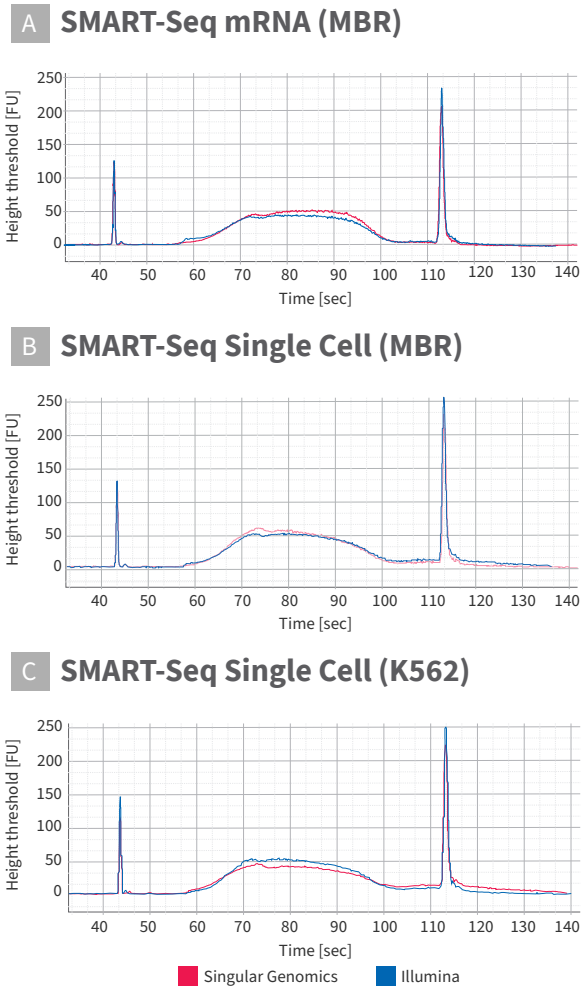

Figure 1: Library preparation generates similarly robust RNA-seq libraries.

All cDNA libraries were prepared using the cDNA library prep protocols in the Takara Bio kits. The Illumina RNA-seq libraries for all three sets of cDNA (Panels A, B, and C) were generated either with the SMART-Seq mRNA LP ("library prep" kit) or the SMART-Seq Single Cell PLUS library prep kit. The Singular Genomics RNA-seq libraries were prepared in an identical manner except that the standard Illumina PCR primers were replaced with Singular Genomics unique dual index (UDI) primers. Each panel comprises a single representative library for each platform. The red and blue lines denote Singular Genomics and Illumina libraries, respectively. **Panel A.** Bioanalyzer graph of RNA-seq libraries generated using SMART-Seq mRNA cDNA. **Panel B.** Bioanalyzer graph of RNA-seq libraries generated using SMART-Seq Single Cell cDNA. **Panel C.** Bioanalyzer graph of RNA-seq libraries generated using SMART-Seq Single Cell (K562) cDNA.

Switch sequencers without impacting gene identification, sensitivity, or read distribution

SMART-Seq libraries sequenced using the Singular Genomics G4 produced RNA-seq data similar to the industry-standard sequencing platform. First, a similar mean number of genes was detected between the two platforms used for each of the three experiments (Figure 2, Panel A). The distribution of reads mapped to genes, introns, intergenic regions, mitochondrial regions, and ribosomal regions were also nearly identical across these platforms (Figure 2, Panels B–D). Finally, the Pearson's and Spearman's correlations calculated from raw counts for the genes were robust between the two sequencing workflows. These correlations were observed across the SMART-Seq mRNA-processed MBR (Figure 3, Panel A), the SMART-Seq Single Cell-processed MBR (Figure 3, Panel B), and the SMART-Seq Single Cell-processed K562 cell (Figure 3, Panel C) samples.

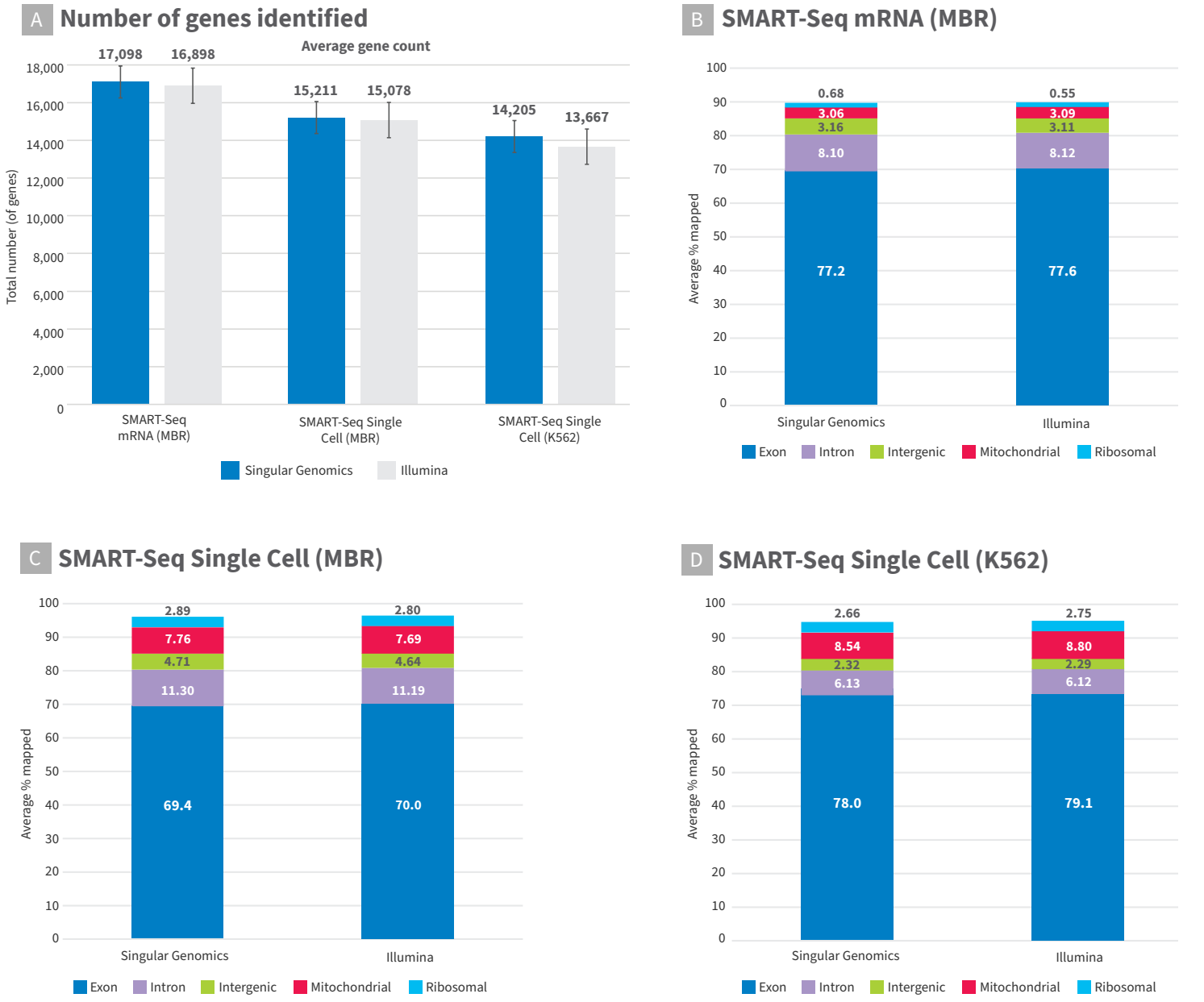

Figure 2. Comparable number and proportion of detected transcript features between RNA-seq libraries sequenced with the G4 and NextSeq 500 sequencers.

Panel A. The total number of detected genes was similar between the libraries sequenced with the G4 and the NextSeq 500 sequencer for all three sets of cDNA libraries. The error bars represent standard deviations from the six samples for each experiment. **Panels B–D.** The distribution of reads mapped to different regions of the genome was similar between the two library preparation methods for the cDNA prepared from SMART-Seq mRNA and single-cell mRNA extracts.

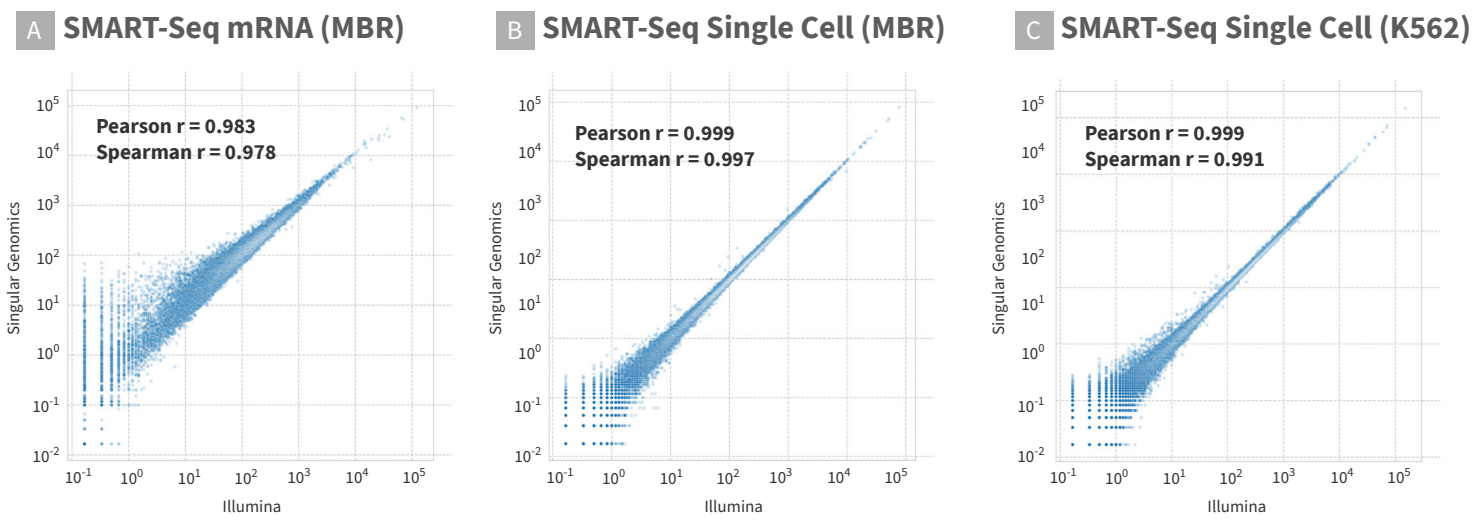

Figure 3. Strong average correlations between the transcript abundances generated from RNA-seq libraries prepared with the G4 and NextSeq 500 sequencers. **Panel A.** Correlation plot between SMART-Seq mRNA kit-generated cDNA libraries sequenced with the G4 and NextSeq 500 sequencers. **Panel B.** Correlation plot between SMART-Seq Single Cell (MBR) cDNA libraries sequenced with the G4 and NextSeq 500 sequencers. **Panel C.** Average correlation plots between SMART-Seq Single Cell cDNA libraries generated from sorted K562 cells and sequenced with the G4 and NextSeq 500 sequencers. All scatter plots depict the mean raw counts for all genes with a log10+1 scale. Each point for every correlation plot represents a single gene.

Conclusion

Generating high-quality RNA-seq data from low-input and single-cell samples is essential for discovering novel biomarkers. Full-length RNA-seq methods address many of the challenges of 3′ end-counting methods, allowing the detection of SNPs, isoforms, and gene fusions. Combining the power of the G4 Sequencing Platform with SMART-Seq technologies allows researchers to achieve high-quality, reproducible detection of important genetic features at a high throughput and low cost.

Get in touch

Takara Bio USA, Inc. is a wholly owned subsidiary of Takara Bio Inc. that manufactures and distributes kits, reagents, and instruments for the life sciences, including NGS, PCR, gene delivery, genome editing, stem cell research, nucleic acid and protein purification, and automated sample preparation.

Singular Genomics is a life science technology company that develops next-generation sequencing and multiomics technologies. Our mission is to empower researchers and clinicians to advance science and medicine.

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