In single cell transcriptome studies, it is critical to obtain high-quality cDNA libraries from individual cells that represent the original in vivo mRNAs as closely as possible. To achieve this, we improved the commercially available mRNA-Seq chemistry for single cell transcriptome studies (the SMARTer Ultra® Low Input RNA Kit for the Fluidigm® C1™ System), which is incorporated in the “mRNA Seq” script in the Fluidigm C1 system.

The new chemistry employs locked nucleic acid (LNA) technology integrated in our proven SMART® technology, which greatly enhances the first strand cDNA synthesis reaction. The new chemistry also utilizes efficient SequAmp™ polymerase, which excels even in GC- and AT-rich regions. All procedures (including lysis, RT, and all PCR steps) are designed to be performed in the Fluidigm C1 System using Open App IFC.

In this poster, we present data derived from K562 cells (human leukemia cell line). The results show a higher yield of cDNA, a larger number of genes detected, and an improvement in high-GC gene detection compared to the existing chemistry. The new chemistry also produces higher consistency among replicates. This is important because the reduced technical variations will increase the likelihood of discovering true biological variations. Altogether, these results indicate that the new chemistry can robustly produce high-quality and highly reproducible cDNA from single cells for meaningful transcriptome analysis.

Introduction and Methods

Gaining the ability to identify and quantify the mRNA from a single cell has been a substantial benefit to many scientific fields, especially those where homogeneous populations are elusive, such as cancer research, developmental biology, neurobiology, and immunology. Here we introduce the “SMART-Seq v4” script for single-cell RNA-seq employing the Open App IFC and new chemistry (SMART®-Seq v4 Ultra Low Input RNA Kit for the Fluidigm C1 System), which has remarkable sensitivity and precision. In this poster, we will refer to the new chemistry used for the SMART-Seq v4 script as “UL-v4” and the previous chemistry used for the mRNA Seq script as “UL-v1.”

Conclusion

The SMART-Seq v4 Ultra Low Input RNA Kit for the Fluidigm C1 System (UL-v4) provides an improved single-cell, full-length mRNA-seq method. The new SMART-Seq v4 script with UL-v4 chemistry demonstrates great sensitivity and delivers high quality, robust and reproducible transcriptomic data.

- **High sensitivity**—the new chemistry (UL-v4) produced more cDNA, detected significantly more genes, and achieved a significantly higher exon to intron mapped ratio than the UL-v1 chemistry (Figure 3).
- **Improved detection of GC-rich genes**—more genes with high GC content were identified in the cDNA libraries constructed with UL-v4 relative to the cDNA libraries constructed with UL-v1 (Figure 4).
- **Reduced technical variability**—transcriptomic data generated by UL-v4 were more consistent between replicates with the data generated by UL-v1 (Figure 5). The protocol reduced technical variability, which may increase the opportunity to detect biological variations.

References