**A complete ultra-low input RNA-seq solution for full-length transcriptome analysis and RNA counting**

**Genomics**

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OBJECTIVE: RNA sequencing (RNA-seq) is a powerful way to investigate transcriptional highs and lows, allelic origins, and isoform preferences in the transcriptome that can underlie key biological states. One current limitation of single-cell RNA-seq methodologies is either the absence of unique molecular identifiers (UMIs), or the inability to maintain the yield, sensitivity, and reproducibility when UMIs are employed.

METHODS: To test the yield, sensitivity, and reproducibility, we benchmarked this new SMART-Seq® method against the existing SMART-Seq v4 Ultra® Low Input RNA Kit for Sequencing (SSv4), and two homebrew methods, Smart-seq2 (SS2) and Smart-seq3 (SS3). We included our novel library prep method in the testing to determine if a complete, end-to-end solution improved the data outcome.

RESULTS: Total cDNA yield was significantly higher for the new SMART-Seq method using UMIs compared to SSv4, SS2, and SS3, while Gene count and read distribution across major RNA-seq output components were comparable between the new SMART-Seq method using UMIs and SSv4. However, the new SMART-seq method showed significantly increased sensitivity compared to the SS2 and SS3 homebrew methods. In addition, we demonstrate that our new SMART-Seq method can enable RNA counting, and while optimized for low RNA input, is compatible with single-cell RNA-seq analysis.

CONCLUSION: Our data demonstrate that the new SMART-seq method leveraging SMART technology with UMIs for cDNA generation and our unique library preparation protocol, combined with our Cogent™ NGS analysis software, is a complete, robust, and sensitive solution for full-length transcriptome studies. The inclusion of UMIs allowed for RNA counting without compromising data quality, and lead to superior sensitivity compared to homebrew SS2 and SS3 chemistries.