Automating Scale Biosciences' Single Cell RNA Kit on SPT Labtech's firefly®

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Abstract

The ability to obtain and map transcripts from single cells has provided significant insight into the fundamentals of how life persists and how disease evolves. ScaleBio enables single cell analysis with an instrument-free method leveraging highly parallelized barcoding, making single cell RNA sequencing more approachable for researchers across both academic and industry spectrums.

While their plate-based workflows have increased accessibility significantly without requiring specialized instruments, automation of these methods can significantly reduce consumable consumption and streamline processing time while reducing user pipetting errors and strain. This application note discusses the approach, benefits, and results of automating the Scale Biosciences Single Cell RNA Sequencing Kit on firefly.

Introduction

ScaleBio's Single Cell RNA Sequencing Kit v1.1 provides an instrument-free method of single cell analysis that leverages the cell itself as the reaction compartment. Highly parallelized barcode combinations (figure 1) scale exponentially, allowing users to scale up sample and cell throughput.

Library preparation using the ScaleBio Single Cell RNA Kit v1.1 allows for modular output of up to 500,000 cells with Extended Throughput. Fixation enables long-term stability for samples and a streamlined workflow with multiple safe stopping points allows for maximized flexibility for the user. Without the constraints of specialized instrumentation, there are no limitations to the types, species, and size of input cells and nuclei.

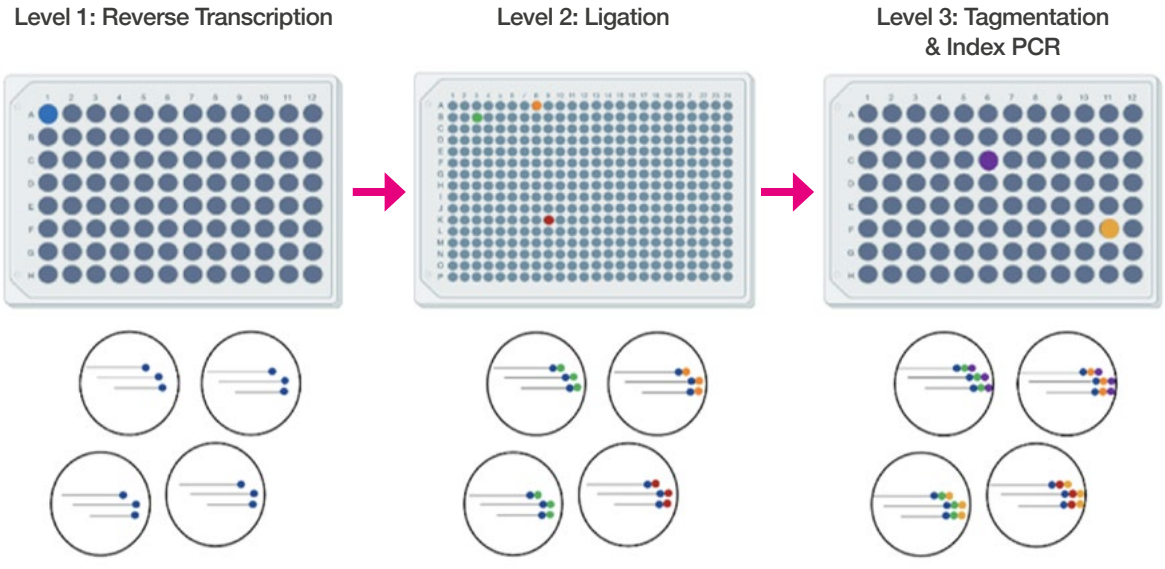


Figure 1: **ScaleBio's indexing workflow generates >3.5 million unique sequences, enabling streamlined identification of single cell transcripts. Barcodes are appended to each transcript during 1. Reverse Transcription, 2. Ligation, 3. Tagmentation and 4. Index PCR.**

As a plate-based technology, ScaleBio's method benefits from incorporating automation for its pipetting steps for ease of use and reduced hands-on time. SPT Labtech's firefly reduces manual processing time without compromising results.

Automating Scale Biosciences Single Cell RNA Sequencing Kit on firefly

SPT Labtech's firefly was used for all chilled incubation, dispense and vortex steps with minimal user interactions outside of master mix preparation and cell washing steps. For instance, ligation master mix transfer to the 384-well ligation plate was reduced to less than 2 minutes compared with an estimated 20 minutes required for manual pipetting. In addition, the dispense head within firefly utilizes true positive displacement (TPD) technology, which has been demonstrated as an ideal solution for working with both fixed and live cell cultures. It is a gentler approach than traditional air-displacement pipetting, mitigating concerns around cells settling in suspension and shearing.

ScaleBio's Single Cell RNA kit was designed with automation in mind, incorporating built-in reagent overages, automation-compatible pipetting steps, and safe stopping points to streamline method development. Automating ScaleBio's RNA sequencing kit with firefly reduced workflow time by 20% and hands-on time by over 60% (figure 2).

Processing Time Comparison

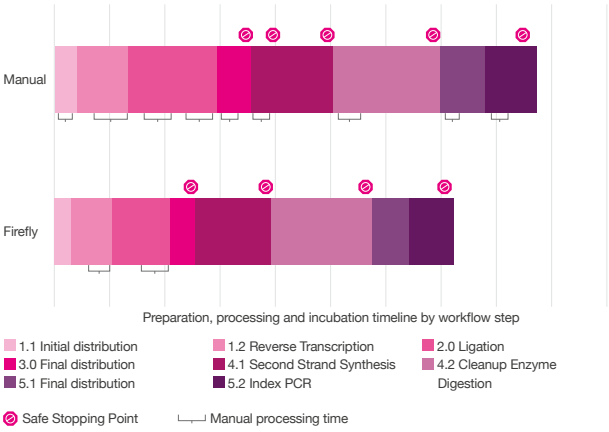


Figure 2. Processing time by workflow step. The method developed on firefly reduced overall processing time by 20% and manual processing by over 60%.

Reduction in Consumable Use

firefly significantly reduces plastics consumption compared to traditional pipetting methods through its non-contact reagent dispense head. Furthermore, this technology enables precise and dynamic dispensing without cross contamination. All reagent additions and cell redistribution steps were performed using firefly's dispense head, removing the requirement for over 1,000 pipette tips across the workflow (figure 4). firefly's reagent reservoirs also significantly reduce dead volumes compared to standard air-displacement liquid handlers. A single ScaleBio Single Cell RNA Sequencing kit had sufficient volume for automating this method on firefly, where all steps required the use of a single Low Dead Volume or Standard reservoir with dead volumes of 75 and 240 µL respectively (figure 3). Typical SBS footprint reservoirs require milliliters of dead volume.

A.

Liquid Handling Type	Reservoir Type	Source Plastic Type & Weight (g)	Reservoir Dead Volume (mL)	Tip/Syringe Plastic Type & Weight (g)
firefly®	Low Dead Volume Reservoir* Standard Reservoir*	PP, 1	0.75 0.240	PP/HDPE, 4
Standard Air-Displacement Automation	Low Profile SBS 96-well diamond bottom†	PP, 23.3	2.4	PP, 153.6§

B.

Figure 3. (a) Reservoir and tip type comparison. *SPT Labtech firefly/dragonfly reservoir (1 g), SPT Labtech firefly/dragonfly syringe (4 g). †LP SBS 96-well diamond bottom reservoir weight (23.3 g) based on Axygen (RES-SW96-LP), other SBS reservoir types may differ. §Single tip weight (1.6 g) based non-filter 125 µL apricot tips, other tip weights may differ. ‡Tip box not included in weight calculation. (b) SPT Labtech's firefly and dragonfly syringe and reservoirs.

Automated Single Cell RNA Sequencing Workflow Plastic (PP/HDPE) Consumption Comparison

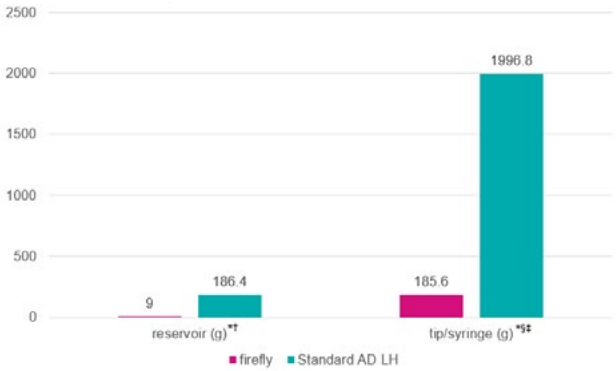


Figure 4. firefly's true positive displacement dispense head significantly reduces plastic consumption compared to traditional automated pipetting systems. †LP SBS 96-well diamond bottom reservoir weight based on Axygen® RES-SW96-LP, other SBS reservoir types may differ. §Single tip weight (1.6 g) based non-filter 125 µL apricot tips, total weight based on an of 96, other tip weights may differ. Tip box not included in weight calculation.

Method Development

Libraries were prepared using peripheral blood mononuclear cells (PMBCs). Cells were fixed using the ScaleBio RNA Fixation Kit and stored at -80°C until processed using firefly and the Single Cell RNA Sequencing Kit v1.1.

On firefly, plate and reservoir thermal modules were set to 4°C to keep both cells and reagents chilled during processing. All reagents were prepared according to ScaleBio's Single Cell RNA Sequencing Kit v1.1. Frozen fixed cells were thawed on ice and counted using a Denovix CellDro FL. Cell washing was performed with ScaleBio's spin funnel using Eppendorf 5810R centrifuge set to 4°C. Initial Distribution through to Ligation was performed on Day 1, and Final distribution through to Index PCR Pooling was performed on Day 2. Pooled Index PCR purification was performed manually. An additional library was prepared manually by Scale Biosciences.

Results

Preliminary QC

Initial library QC results were assessed prior to sequencing and manual processing. The final step of ScaleBio's protocol is a pooled purification of index libraries using SPRIselect beads. Four individual wells were purified in parallel to assess library consistency across wells. Pooled library and individual well samples fragment size and concentration were determined using the Agilent High Sensitivity DNA Kit and the 2100 Bioanalyzer instrument.

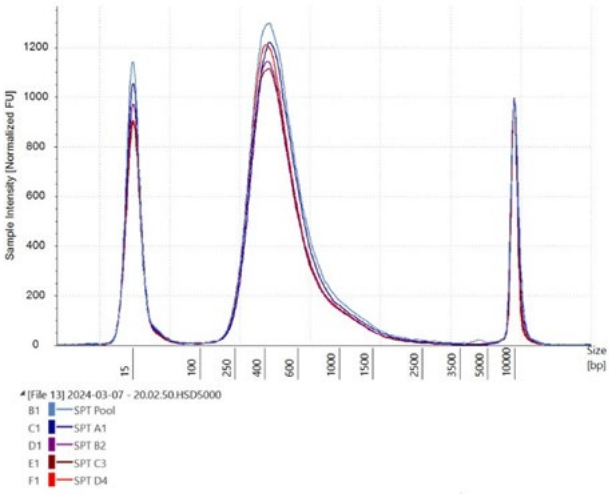


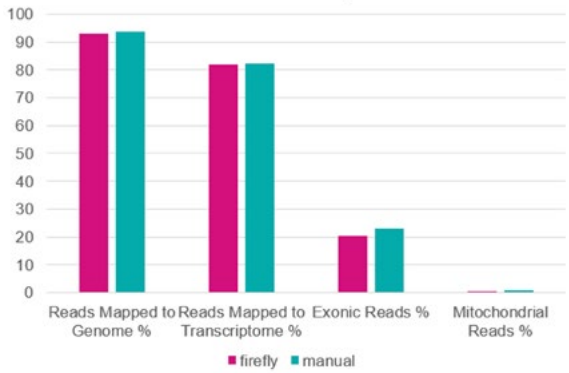
Figure 5: Overlayed sample trace of post Index PCR purified libraries prepared on firefly. 5 µL of each library was pooled by column using firefly prior to manual purification. Four individual wells were purified in parallel to assess fragment size and concentration consistency. Fragment size, average ~500 bp, was consistent between pooled and individually purified libraries.

Sequencing comparison of libraries prepared manually and on firefly

To conserve sequencing space and resources, 12-well sub-libraries from the manual and automated libraries were sequenced on NextSeq2000 instrument (Illumina). Sequencing data was processed with ScaleBio Seq Suite: RNA v1.5. Results from all 24 wells were analyzed, yielding 22,000 and 19,000 cells from each library respectively, with at least 15,000 reads per cell. Both data sets were mapped using the Azimuth PBMC reference data set.

Sequencing metrics between manual and firefly-prepared libraries were similar and within expected parameters. Over 90% of reads for both libraries were mapped to the reference data set, while over 80% mapped to the reference transcriptome. In addition, over 20% of reads were exonic, and less than 1% were mitochondrial in both libraries. These metrics demonstrate that libraries prepared with firefly were not only comparable to manual methods, but also did not increase technical artifacts or wasted sequencing reads.

A. Read Metric Comparison



B. Cell Metric Comparison

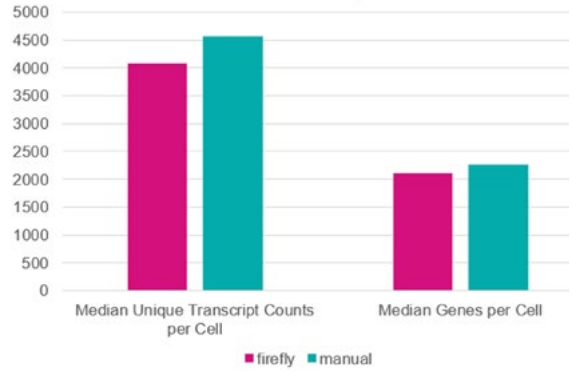


Figure 6: (a) Mapping metrics for firefly and manual libraries were similar, with 93.1% and 93.6% reads mapping to the x genome, 82.1 and 82.3 mapping to x transcriptome, 20.4% and 23.2% exonic and 0.5% and 0.7% mitochondrial reads. (b) Cell metrics for firefly and manual libraries varied slightly, but within error with median unique transcript counts of 4072 and 4563 and median gene per cell detection of 2106 and 2258.

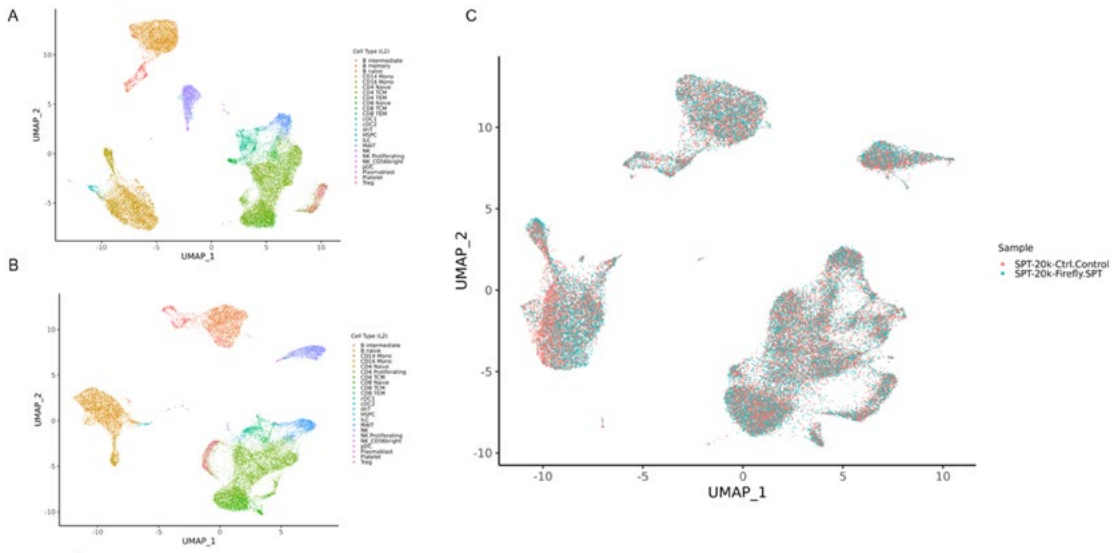


Figure 7: Library Clustering (A) firefly single cell RNA library data UMAP visualization. (B) manual single cell RNA library data UMAP visualization. (C) UMAP visualization using firefly and manual data sets.

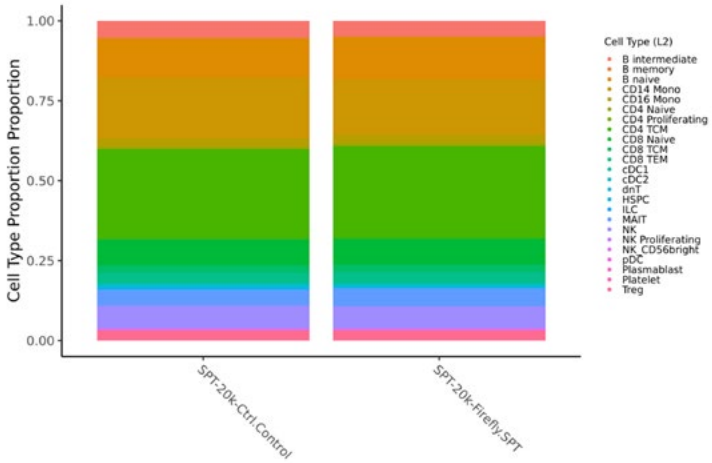


Figure 8: Cell type proportions for firefly and manual libraries. Similar cell type recovery observed across manual and automated methods showing no adverse effect on recovery or profiling of any specific cell type with automation.

Uniformity across expected metrics for automated and manually prepped libraries indicate the ScaleBio Single Cell RNA Sequencing Kit on firefly does not impact library quality or cell recovery.

Conclusion

Both manually prepared libraries and those generated with firefly showed comparable metrics, demonstrating that library preparation was not impacted by the use of automation with firefly. The easy-to-use, plate-based technology of the ScaleBio Single Cell RNA Sequencing Kit coupled with firefly's efficient and sustainable platform provide an automated workflow that can support scientists in scaling up single-cell studies.

The ScaleBio Single Cell RNA workflow is now available for all firefly users in the firefly community cloud.