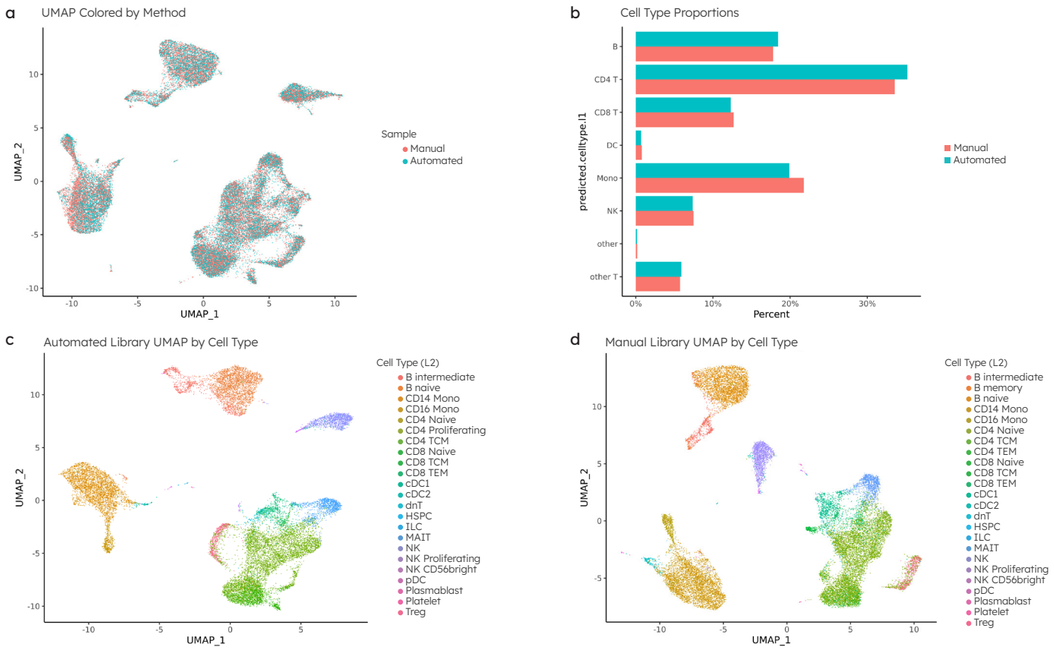
To examine whether these slight differences in sensitivity had any biological impact, we compared the cells recovered from both methods in Seurat. 18,577 cells from the automated workflow and 22,494 cells from the manual workflow were analyzed together to create a single UMAP projection with both samples. This analysis revealed no observable batch effect between the cells recovered from the automated vs. manual methods **(Figure 5a)**. Furthermore cell-type calling using Azimuth revealed similar proportions of each cell type recovered from manual and automated workflows, showing no bias in cell type recovery when using automation **(Figure 5b-d)**.

Figure 5: a) UMAP projection with cells colored based on method. b) Proportion of major cell types recovered from both methods. c) and d) UMAPs of cells recovered from automated (left) and manual (right) annotated by cell type.



Conclusion

Both manual and automated libraries shared comparable results, demonstrating that there was no impact to library preparation or biological results when introducing automation into the ScaleBio scRNA workflow using the firefly. The easy-to-use, plate-based technology of the ScaleBio Single Cell RNA Sequencing Kit coupled with the firefly's efficient and sustainable platform provide an automated workflow that can support scientists in scaling up their single cell studies.

Check out the ScaleBio Single Cell RNA workflow on the firefly Community Cloud! Interested in automating your single cell analysis? Reach out to us!

ScaleBio Single Cell RNA Sequencing
Support@scale.bio

NGS Workflow Automation
community@sptlabtech.com

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

® 2024 Scale Biosciences
SPT Lab Tech + Scale Bio App Note _ RevB_19Apr24

SCALE biosciences | sptlabtech

Automating ScaleBio Single Cell RNAseq on the SPT Labtech firefly

Abstract

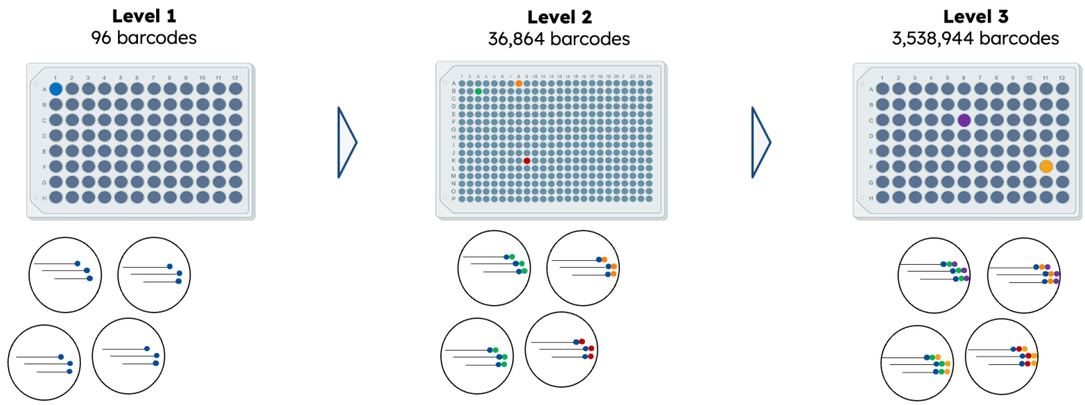
Obtaining transcriptomes from single cells can provide valuable insights into complex biological systems. ScaleBio is making this more accessible to researchers by offering an instrument-free method for single cell analysis using highly parallelized barcoding. While this plate-based workflow already provides an easy-to-use and low-cost solution, automation of this library preparation can further streamline the process and reduce hands-on time. Here we show that automation of the ScaleBio scRNA workflow with SPT Labtech's firefly further simplifies and shortens the workflow while delivering comparable results to manual methods.

Introduction

Scale Bioscience's™ Single Cell RNA Sequencing Kit provides an instrument-free method for single cell analysis. Leveraging the cell itself as the reaction compartment, three rounds of highly parallelized barcoding are performed, providing a plate-based method to uniquely barcode hundreds of thousands of single cells **(Figure 1)**. While this provides an accessible, high throughput and fast workflow, automation can further increase the scale and throughput of these single cell experiments.

To explore how an automated workflow can increase usability of the ScaleBio scRNA Kit we integrated the ScaleBio workflow with the SPT Labtech firefly. Notably the ScaleBio scRNA Kit was developed incorporating built-in reagent overages, automation-compatible pipetting steps, and safe stopping points; this, in combination with the firefly's unique minimization of dead volume, enabled seamless integration of these workflows with no changes to the ScaleBio protocol or master mixes.

Figure 1: Three levels of indexing generate >3.5 million unique combinations.



Methods

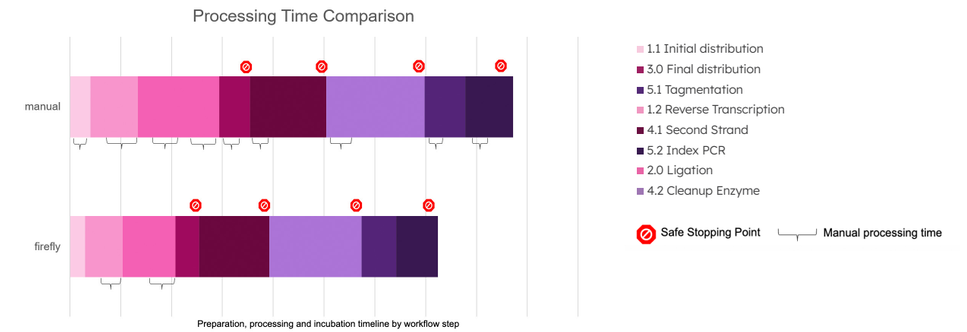
Frozen PBMCs that had been fixed using the ScaleBio RNA Fixation Kit and stored at –80°C were thawed on ice and counted using a Denovix CellDro FL. All reagents and master mixes throughout were prepared according to the ScaleBio Single Cell RNA Sequencing Kit (v1.1). The firefly was used for all chilled incubation, dispense, and vortex steps; plate and reservoir thermal modules on the firefly were set to 4°C to keep both cells and reagents chilled during processing. Cell washing throughout the protocol was done manually using the ScaleBio spin funnel, as well as purification of the final pooled indexed PCR library from a subset of wells. Fragment size and concentration were determined using the Agilent High Sensitivity DNA Kit and the 2100 Bioanalyzer instrument before sequencing on a NextSeq2000. An additional library was prepared manually by Scale Biosciences in parallel for comparison using the same PBMCs. Sequencing data from automated and manual libraries was processed with ScaleBio Seq Suite: RNA v1.5.

Results

Reduced hands-on time and reagent usage

To evaluate whether automation shortens the ScaleBio scRNA workflow, we compared total workflow as well as hands-on time with and without the automation. Results showed that the firefly reduced hands-on time during all levels of barcoding, with the time to pipette 384 wells notably decreasing from 20 minutes to less than 2 minutes. In total, addition of the firefly reduced workflow time by 20% and hands-on time by over 60% **(Figure 2)**.

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

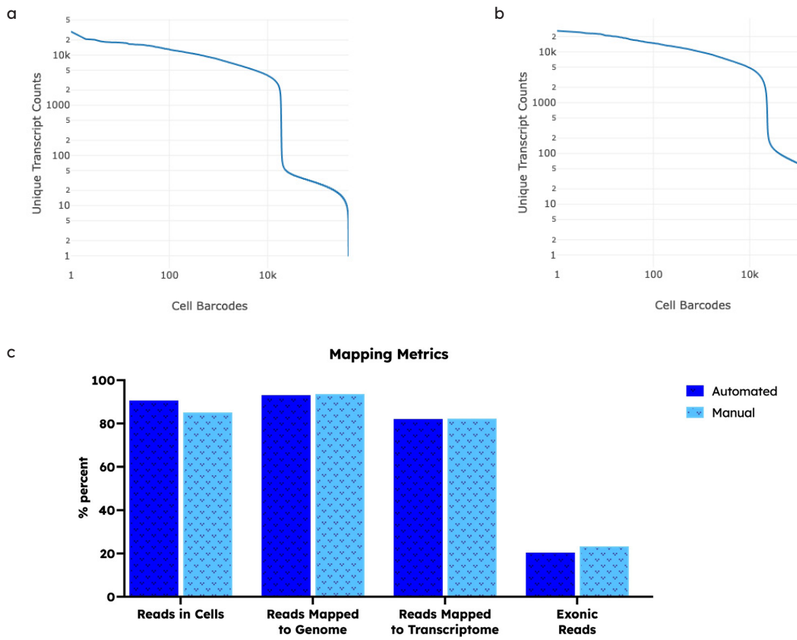


Next we evaluated reagent usage with and without automation. All reagent additions and cell redistribution steps were done using the firefly's dispense head, which enables precise and dynamic dispensing without cross contamination, maintaining barcode integrity. The firefly's reagent reservoirs also saved significantly on dead volumes (75 and 240 uL respectively), ensuring that a single ScaleBio Single Cell RNA Sequencing Kit had sufficient volume for automation on the firefly.

Cell recovery and library purity

To examine library quality and yield, we looked at mapping metrics and cell calling from the automated and manual samples. Knee plots showed similarly clean cell calling for both libraries with clean delineation between signal and background. Metrics also showed good library quality across both libraries with a high percentage of usable reads, showing that addition of automated pipetting does not increase debris or RNA crosstalk in the ScaleBio scRNA data. Notably the firefly dispense head's true positive displacement (TPD) has been shown to be an ideal solution for working with both fixed and live cell cultures, alleviating issues with cells settling in suspension and reducing shearing.

Figure 3. a) and b) Libraries from automated (left) and manual (right) library preparation methods yielded similar and clean cell calling. c) Percent reads assigned to each category shown for the respective library types.



Sensitivity and cell-type recovery

To further examine the data recovered from the automated workflow, we then looked at transcript and gene detection across the two methods. First a subset of cells was deeply sequenced to generate saturation curves, which revealed slightly higher sensitivity in the manual libraries compared to the automated libraries **(Figure 4a)**. This slight difference was also observed in the number of genes detected **(Figure 4b)**.

Figure 4: a) Saturation curves show similar sensitivity for both methods. b) Comparable gene detection between manual and automated methods. Note: Mean passing reads per cell for the manual library and automated library were 15,287 and 19,430.