Automating 10x Genomics GEM-X 5’ Gene Expression V3 Library Prep with SPT Labtech’s firefly® to enable screening of over 12M activated primary human T cells

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Abstract

Efficient and reproducible RNA sequencing of primary human T cells is essential for immunological research and translational studies. In this study, we evaluated the performance of SPT Labtech’s firefly liquid handling system for automated 10x Genomics GEM-X 5’ Gene Expression v3 library preparation across three T cell polarization methods. Three distinct polarization and activation conditions were evaluated, with each yielding ~4.5M activated T cells. For each polarization condition, cells were sorted in batches of 2 followed by Gel Beads-in-emulsion (GEM) generation and library preparation. Key performance metrics, including library yield, cell recovery, sequencing saturation, and gene detection, demonstrate that firefly generates consistent, high-quality RNA-seq libraries across a range of conditions and workflows.

Methods

Over 12M primary T cells were isolated from human PBMCs and activated in-vitro across 3 distinct polarization and activation methods (E1, E2, E3). One day (24 h) after activation, 45,000 cells were partitioned per lane of a GEM-X chip, followed by automated library preparation using SPT Labtech’s firefly to minimize manual variability. Using firefly, 288 library preparations were performed in three batches, then pooled into 13 distinct pools and sequenced on Illumina sequencers. Libraries 1 & 3 were run using a 150/10/10/150 configuration, while Library 2 was run using 28/10/10/90 configuration. Data sets were then combined and analyzed for transcriptome-wide changes.

This data was obtained from an existing firefly and 10x Genomics customer, while they have been using both products for several years, this data was generated in just two months. The data has been anonymized to protect intellectual property.

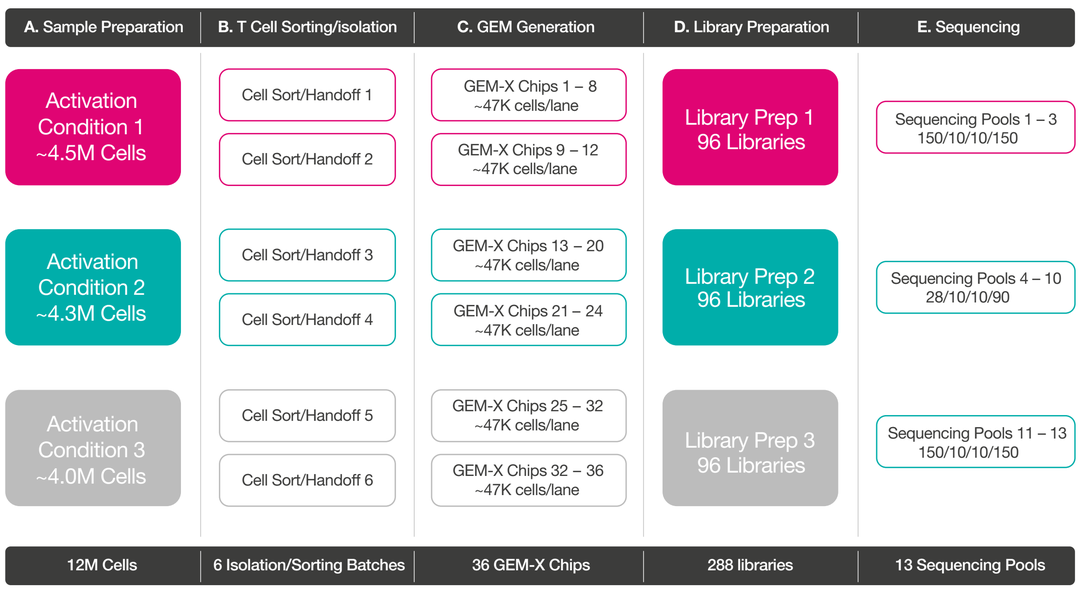


Figure 1. Experimental workflow for T cell activation and single-cell gene expression library generation. This schematic outlines the processing of ~12M T cells under three distinct activation conditions (Condition 1: ~4.5M cells; Condition 2: ~4.3M cells; Condition 3: ~4.0M cells). Following sample preparation (A), cells were sorted into six isolation/processing batches (B, Cell Sort/Handoff 1–6). Each batch was loaded across a total of 36 GEM-X Chips (C, ~47,000 cells per lane) for GEM generation and cDNA synthesis. Libraries were then prepared in three sets of 96 libraries each (D, Library Prep 1–3), yielding a total of 288 libraries. Finally, libraries were combined into 13 sequencing pools (E) with balanced sequencing depths across pools (e.g., Pools 1–3 at 150/10/10/150; Pools 4–10 at 28/10/10/90; Pools 11–13 at 150/10/10/150). This high-throughput design enabled parallel processing of multiple activation conditions, ensuring balanced representation across cell culture batches, library preps, and sequencing runs.

Workflow on firefly

firefly’s novel community feature enables users to download pre-validated protocols without the need for additional liquid class optimization or teaching. Labware and sample numbers can be seamlessly updated once downloaded by the customer to enable full customization for individual user needs. firefly’s use of true positive displacement dispensing reduces liquid class optimization and dead volume requirements. Unlike other systems, additional reagents are not required to be purchased to enable automation as dead volumes for kit reagents can be as low as 55 µL.

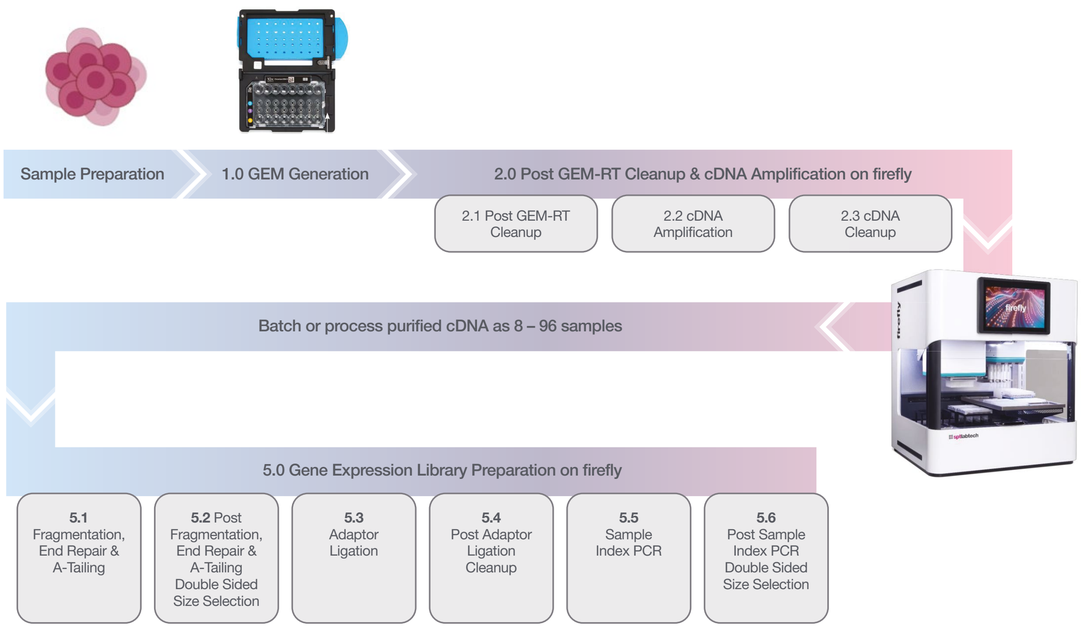


Figure 2. Automating 10x Genomics’ 5’ Gene Expression Library Construction on firefly. This workflow illustrates the end-to-end process of single cell RNA library preparation combining 10x Genomics GEM technology with automated liquid handling on SPT Labtech’s firefly. Following sample preparation, cells are encapsulated into GEMs (Gel Bead-in-Emulsion) for reverse transcription (1.0 GEM Generation). Firefly automates cDNA cleanup (2.1 – 2.3) and library preparation, from fragmentation and adapter ligation through sample indexing and double-sided size selection (5.0 – 5.6), producing sequence ready libraries with minimal hands-on time.

Library QC Metrics by Automated Library Preparation & Cell Culture

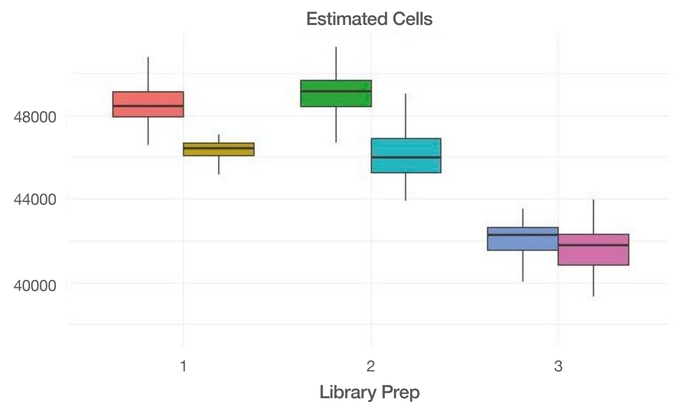


Figure 3. Estimated Cells Per Library. 45K Cells were targeted per library. Estimated cell distributions are within range of cell counting errors prior to loading with an average of 47K cells per lane.

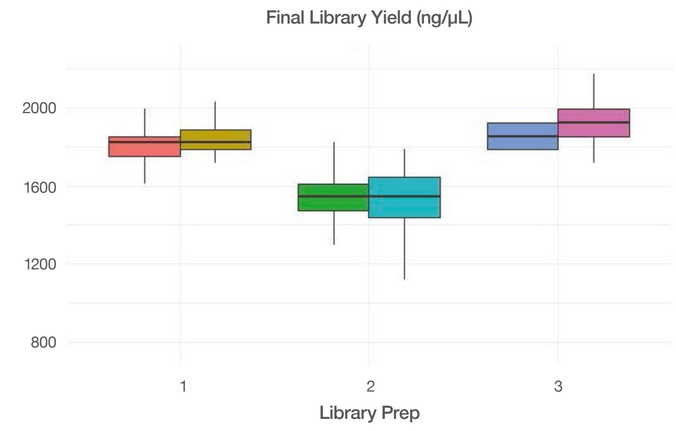
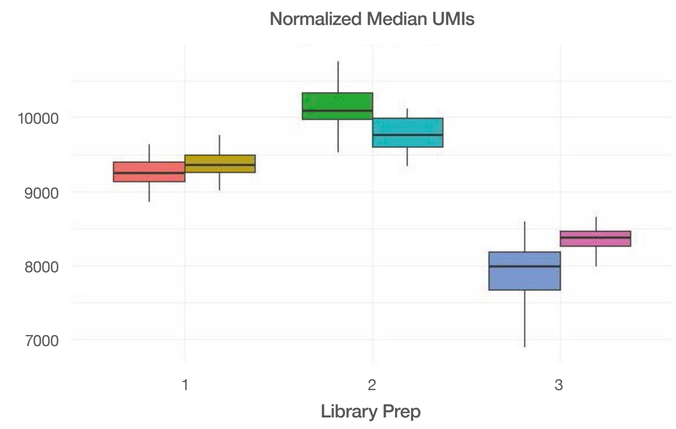


Figure 4. Library yield across library preparations and cell culture batches. Boxplots show the distribution of library yields (ng) for 3 96-sample library preparations, colored by cell culture batch (1-6). Library Preps 1 and 3 yields were higher (~1800 - 2000 ng) while Library Prep 2’s yields were slightly lower (~1300 - 1600 ng). Given T Cell Polarization condition differences across the 3 library preps, results represent consistent yields within and across automated library preparations.

Normalized Gene Expression Metrics by Automated Library Preparation & Cell Culture



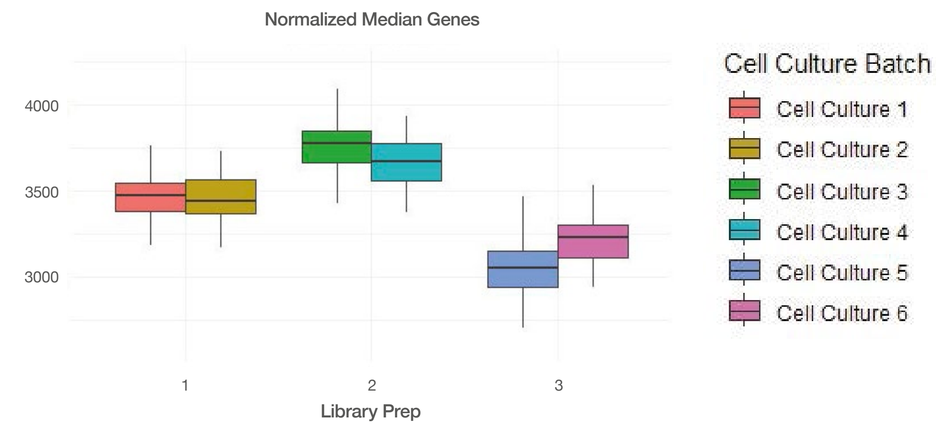


Figure 5. Gene expression metrics across library preparations and cell culture batches. Box-plots show the distribution of normalized single-cell sequencing metrics— Normalized (by total reads) Median Genes* per Cell, and Normalized Median UMI Counts per Cell —stratified by library preparation method (1–3) and cell culture batch (1–6). Each box represents the interquartile range (IQR), with medians shown as horizontal lines and whiskers extending to 1.5× the IQR. Results highlight differences in cell recovery, gene detection sensitivity, and UMI complexity across both library preparations and T Cell polarization conditions.

*Genes were annotated using HG38, which contains over 54K genes and pseudogenes, including 20,203 protein-coding genes and >17,800 non-coding genes.

Conclusions

SPT Labtech’s firefly liquid handling platform enabled reproducible, high-throughput RNA library preparation from primary human T cells, with performance metrics demonstrating robust yields, strong transcript detection, and excellent consistency across diverse activation protocols. These results support firefly as a reliable solution for automated 10x Genomics library prep, and its flexibility makes it well-suited for researchers looking to automate library preparation for their single cell workflows.

- High-quality libraries with consistent yield and complexity.
- firefly produced robust final library yields ranging from ~1400 - 2400 ng across library preparations (L1, L2, L3 - 96 samples per preparation). Variability across library preparations may correlate to cell culture batches & polarization conditions.
- Across polarization methods (P1, P2, P3) provided mean reads per cell of 15247, 14915, and 19316 respectively; median genes per cell of 3199, 3329, 3607 respectively; total genes detected of 28451, 30271 and 31297 respectively.
- Automation reduced hands-on time by >50% while maintaining library preparation consistency within treatment conditions.