Accelerating Single Cell Research by Automating Gene Expression Library Construction for 10x Genomics GEM-X Chemistry on the Biomek i7 Hybrid Workstation

Kelly Parliament[1], Aimee Zhao[2], Suhas Gandhi[2], Mike Rose[2], Jill Herschleb[2], Crystal Girod[1], Partha Banerjee[1], and Mohammad Rahimi[2]

[1]Beckman Coulter Life Sciences, [2]10x Genomics

Introduction

The Chromium GEM-X Universal 3' and 5' Gene Expression assays were designed using advanced GEM-X technology to help uncover biological complexities with unmatched sensitivity and cell recovery efficiency for transcriptomic profiling. The Chromium GEM-X chemistry can be used to characterize rare cell types, identify biomarkers, capture fragile or low-RNA-content cells, and reveal hidden heterogeneity in more samples.

Here, we present an automated solution to streamline and accelerate the throughput of Gene Expression library construction on the Biomek i7 Hybrid Workstation utilizing the 10x Genomics automation-friendly GEM-X Gene Expression Library Construction Kit C, Automated 24 rxns / Manual 32 rxns. The GEM-X Universal Gene Expression Library Construction automated solution for the Biomek i7 Hybrid Workstation generates high-quality sequence-ready libraries in under 7 hours for a full 96-library run. The QC and sequencing results demonstrate libraries generated from the automated workflow perform at parity with those generated by expert manual users, with low batch effect scores.

Accelerating GEM-X with Automation

To enhance the user experience 10x Genomics has developed a new library construction reagent kit, Library Construction Kit C, Automated 24 rxns / Manual 32 rxns, with automation-friendly volumes for easy instrument loading and on-deck master mix preparation. Library Construction Kit C ensures volumes for 24 reactions when automated on the Biomek i7 Hybrid Workstation or 32 reactions for manual preparation.

Here, we present an automated solution to streamline and accelerate the throughput of Gene Expression library construction for the 10x Genomics GEM-X Universal Gene Expression assays on the Biomek i7 Hybrid Workstation while maintaining high-quality sequence-ready libraries. This automated workflow offers a flexible and scalable solution for library preparation following cDNA generation (**Figure 1**). This automated method can generate up to 96 high-quality sequence-ready libraries in less than 7 hours.

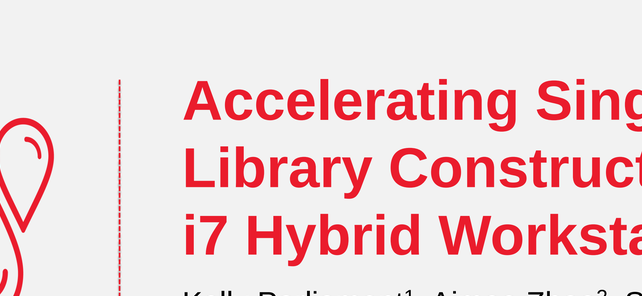
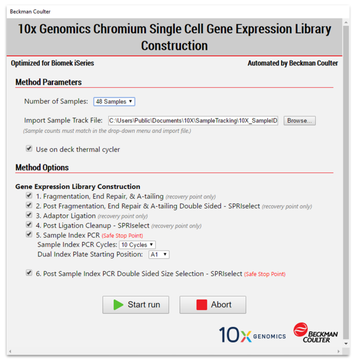


Figure 1. *GEM-X Universal Gene Expression Workflow. The automated workflow (top, shown in blue) shows Step 2, Gene Expression Library Construction, with walk-away time for 96 samples compared to the manual workflow executed by manual users (bottom, shown in grey).*

The Biomek i7 Hybrid Workstation automated method for GEM-X GEX Library Construction features automated mastermix formulation, multichannel head foil piercing of the index plate, enhanced device and method logging, and a user-friendly interface for runtime options (**Figure 2, left**).



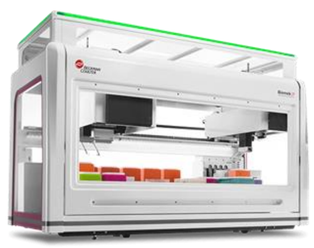
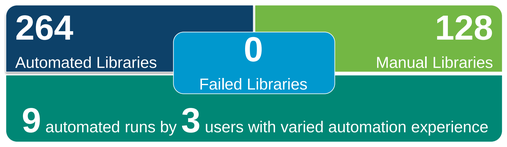


Figure 2. *User Interface for the Biomek i7 Hybrid Workstation GEM-X GEX Library Construction automated method (left) and the Biomek i7 Hybrid Workstation (right).*

Experimental Design

To test the performance of the automated GEM-X Universal Gene Expression Library Construction an extensive verification and validation test plan of manual and automated library preparations for 3' and 5' GEX library construction was performed. Sample inputs included cDNA manually prepared from 20,000 HEK293T (293T) cells and 500 human peripheral blood mononuclear cells (hPBMCs). A total of 264 libraries, across 9 independent end-to-end runs, were generated from the automated method on the Biomek i7 Hybrid Workstation to compare with 128 libraries generated from manual library preparations. Same aliquot of cDNA, same lot of library construction reagents, and the same Sample Index PCR cycle were used for both automated and manual workflows to ensure a well-controlled side-by-side comparison. To evaluate the robustness of the setup process, 2 users who were familiar with the GEM-X Gene Expression library construction manual workflow, but completely naïve to the Biomek i7 Hybrid Workstation, the automated method, and the Library Construction Kit C, Automated 24 rxns/ Manual 32 rxns, were recruited to execute the automated method for comparison with results generated by an expert manual user along with an expert automation user.



Following library preparation, the libraries were analyzed using High Sensitivity DNA Kit (Agilent, 5067-4626) on the Agilent Bioanalyzer instrument for library size and preliminary assessment of library quality. To determine concentrations of the GEX libraries for library pooling and flow cell loading prior to sequencing, qPCR was performed with the KAPA Library Quantification Kit for Illumina Platforms (Roche, KK4824). Sequencing was performed following the 10x Genomics GEM-X GEX user guides. hPBMC libraries were sequenced to 20K read pairs per cell while the 293T libraries were sequenced to 50K read pairs per cell. All data analysis was performed by 10x Genomics using Cell Ranger and visualized by Loupe Browser.

Results

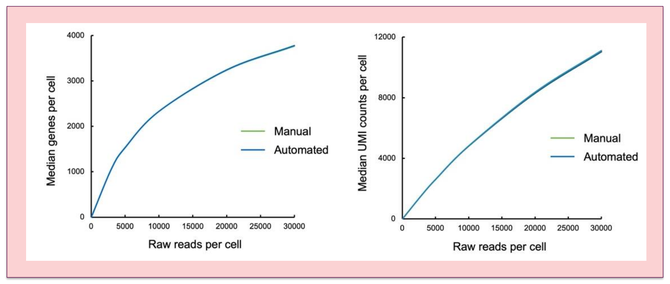


Figure 3*. Libraries generated by the automated workflow have complexity and sensitivity at parity with the manual workflow. Comparable median genes per cell (left) and median UMI counts per cell (right) between manual and automated workflows across multiple sequencing depths for GEM-X Universal 3' Gene Expression libraries.*

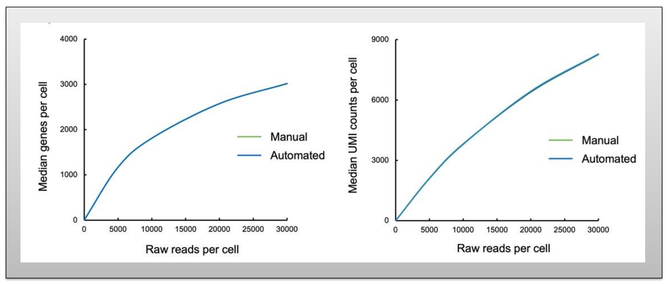


Figure 4*. Libraries generated by the automated workflow have complexity and sensitivity at parity with the manual workflow. Comparable median genes per cell (left) and median UMI counts per cell (right) between manual and automated workflows across multiple sequencing depths for GEM-X Universal 5' Gene Expression libraries.*

Results Continued

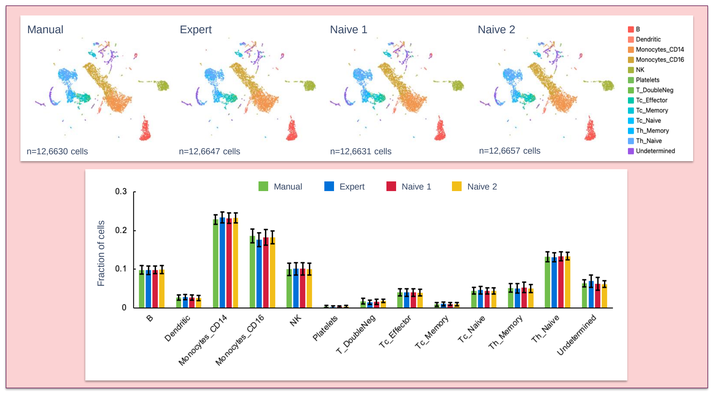


Figure 5. Consistent cell type profiling of human PBMC cells derived from GEM-X Universal 3' Gene Expression library construction between the manual workflow and multiple independent automated runs executed by users of different experience levels. *UMAP shows the gene expression data analyzed and visualized using Cell Ranger and Loupe Browser, respectively. Major immune cell populations in each cluster were identified via marker gene expression. 500 human PBMC cells in each library and 12 libraries (from manual workflow as well as in each independent automated run) were analyzed. Bar graph (bottom) demonstrates comparable average distribution of each cell population from the 12 libraries for each category, with error bars showing the standard deviations.*

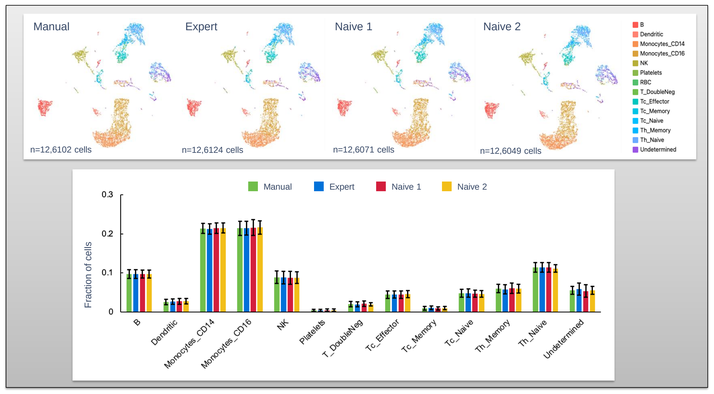


Figure 6. Consistent cell type profiling of human PBMC cells derived from GEM-X Universal 5' Gene Expression library construction between the manual workflow and multiple independent automated runs executed by users of different experience levels. *UMAP (top) shows the gene expression data analyzed and visualized using Cell Ranger and Loupe Browser, respectively. Major immune cell populations in each cluster were identified via marker gene expression. 500 human PBMC cells in each library and 12 libraries (from manual workflow as well as in each independent automated run) were analyzed. Bar graph (bottom) demonstrates comparable average distribution of each cell population from the 12 libraries for each category, with error bars showing the standard deviations.*

Conclusion

We demonstrated the performance parity, consistency and system robustness of the automated solution for GEM-X Universal 3' and 5' Gene Expression assays across different cDNA inputs (different RNA content cells like PBMC and 293T), different cell loads (500-20,000 per sample), different sample throughputs (8 to 96), and users with different levels of experience. Overall, this data shows the automated solution for the GEM-X Universal 3' and 5' Gene Expression library construction on the Biomek i7 Hybrid Workstation is robust and reproducible and delivers high-quality libraries for single cell analysis.

In conclusion, automating GEM-X Gene Expression Library Construction on the Biomek i7 Hybrid Workstation delivers:

- Library quality and sensitivity at parity with a manual workflow.
- Library performance is consistent across all 96 samples in the automated GEM-X Universal Gene Expression run.
- Library performance is consistent across multiple automated runs.
- Users naïve to automation can generate high-quality data.

For additional information on 10x Genomics, please visit Contact Us at https://10xgen.com/automation
2025-AMER-EN-107172-v1