BECKMAN COULTER Life Sciences

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

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Introduction

Chromium™ GEM-X Single Cell Gene Expression v4 chemistry and Chromium™ Single Cell Immune Profiling v3 chemistry 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 Single Cell Gene Expression 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. Highlights of the Chromium™ GEM-X Single Cell Gene Expression include:

- Exploring the influence of cell heterogeneity on development, disease, and more with scalable transcriptional profiling across a few hundred to tens of thousands of cells
- Further characterizing cell subtypes and states with multiomic readouts of gene and cell surface protein expression for the same cell
- Uncovering hidden complexity and revealing more accurate, comprehensive views of biology using technology that captures fragile cells and low-expressed transcripts
- Adding confidence to your results by increasing sample number to improve statistical significance and accelerate time to results

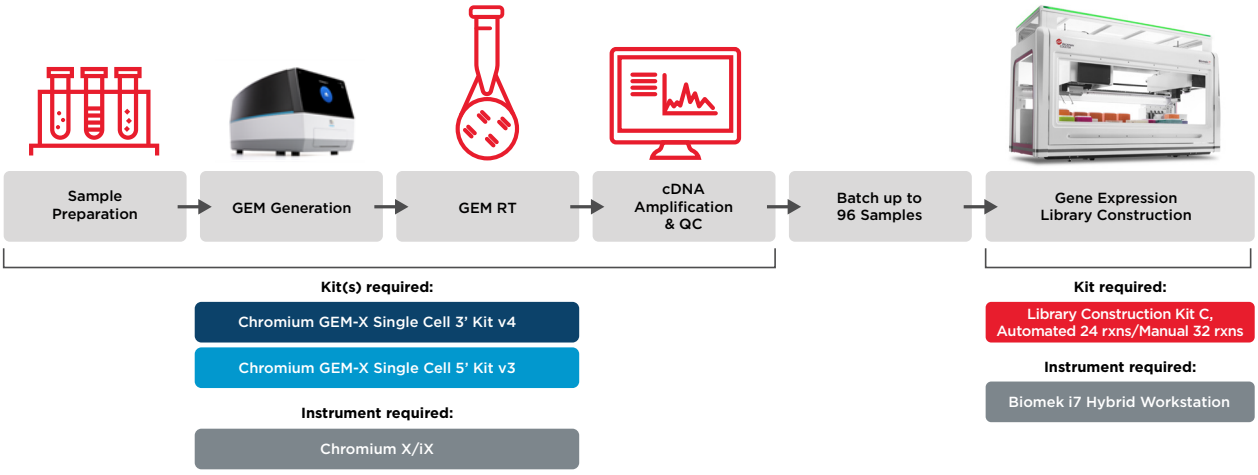

Figure 1. Chromium™ GEM-X Single Cell Workflow with automation and kit alignment.

The GEM-X Single Cell Gene Expression and Single Cell Immune Profiling workflows consist of multiple steps (Figure 1). This automated workflow is for either 3' or 5' Gene Expression (GEX) Library Construction. 10x Genomics has created a reagent kit formatted specifically for this method – Library Construction Kit C, Automated 24 rxns / Manual 32 rxns, (10x, 1000774).

Spotlight

The automated method for GEM-X GEX Library Construction features the Biomek i7 Hybrid Workstation and 10x Genomics Library Construction Kit for Automation, Library Construction Kit C.

Chemistry Spotlight

Features of the 10x Genomics GEM-X Library Construction Kit C, Automated 24 rxns/ Manual 32 rxns (10x Genomics, 1000774) include:

- Automation-friendly dead volumes to guarantee 24 reactions on the Biomek i7 hybrid workstation
- Sufficient fill volumes to allow three 8-sample runs from a single kit
- Standard reagent containers for direct deck loading to enable fully automated on-deck master mix creation
- Modular format (24 rxn per kit) to fit common throughput needs and avoid excessive freeze and thaw

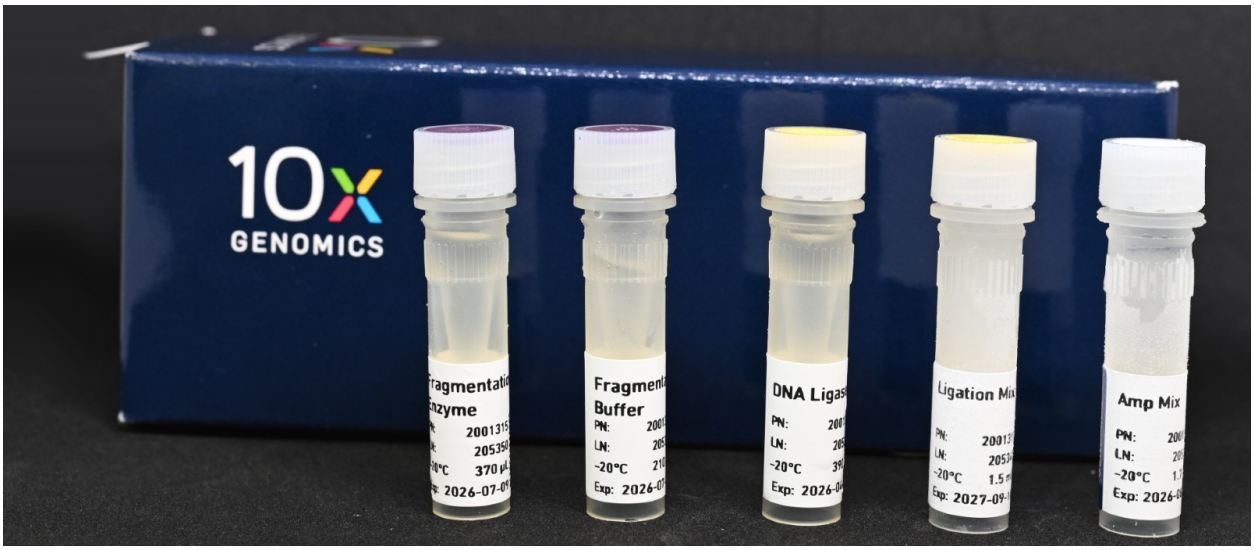

Figure 2. 10x Genomics GEM-X Library Construction Kit C, Automated 24 rxns/ Manual 32 rxns (10x Genomics, 1000774).

Automation Spotlight

The Biomek i7 Hybrid Workstation deck for GEM-X GEX Library Construction features 2 ColdPlates (QInstruments, CP96PCR1 and CP1), a BioShake (QInstruments, BS1), and an optional on-deck thermal cycler (Applied Biosystems, ATC1).

Benefits of automating GEM-X GEX Library Construction on the Biomek i7 Hybrid Workstation include:

- Knowledgeable technical support from experts at 10x Genomics and Beckman Coulter Life Sciences
- High-throughput scalable solutions for library preparation
- User-friendly Method Option Selector (MOS) interface for real-time workflow selections
- Guided Labware Setup (GLS) to provide runtime setup instructions, reagent volumes, and user-friendly labware and deck visuals
- Reduced hands-on time and pipetting errors
- Extensively tested and 10x-validated method

Automated Method

Chromium™ GEM-X Gene Expression (GEX) Library Construction of the single cell workflow has been automated using the Biomek i7 Hybrid Workstation. This automated method includes a single user interaction to set up the instrument deck and contains 6 method sections that can be processed as 2 workflows (Figure 3) aligned with the 10x Genomics user guides (CG000731 for 3' Gene Expression and CG000733 for 5' Gene Expression). The complete library construction workflow, Workflow 1, for 96 samples can be run start to finish in under 7 hours with full walk-away capability when the on-deck thermocycling is enabled. The automated GEM-X GEX Library Construction Biomek method includes automated master mix creation, foil piercing of the index plate foil seal, and reduces overall processing time and operator handling.

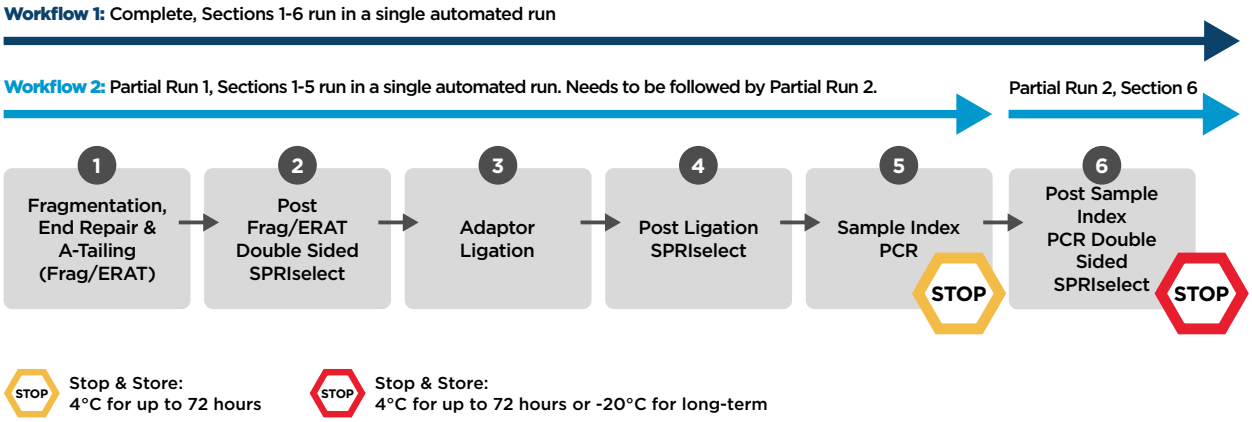

Figure 3. Supported workflows for the GEM-X GEX Library Construction automated method for the Biomek i7 Hybrid Workstation. The entire workflow can be processed from start to finish in under 7 hours for 96 samples.

Method Features

Automated Master Mix Formulation

To minimize preparation time and opportunities for operator error, the formulation of all library construction master mixes has been automated by the Biomek i7 Hybrid Workstation. The kit reagent tubes can be loaded directly onto the Biomek instrument deck for processing.

Foil Piercing

Piercing of the foil seal on the 10x Dual Index plate has been automated for the GEM-X GEX Library Construction Biomek i7 Hybrid Workstation automated method. The automated foil piercing will be performed using the Biomek i7 Hybrid MC head during preparation for Sample Index PCR.

Method Options Selector (MOS)

The GEM-X GEX Library Construction automated method for Biomek i7 Hybrid Workstation provides an intuitive user interface, the MOS, showcasing all workflow options available at the start of method run. The 10x Genomics approved workflow options are:

- Number of samples 8-96; increments of 8
- On-deck vs off-deck thermocycling*
- Number of Sample Index PCR cycles*
- Starting well position for the Dual Index Plate

*On-deck Thermocycler option required

Enhanced Logging Capability

Additional logging capability has been built into the GEM-X GEX Library Construction automated Biomek i7 Hybrid Workstation method for MOS selections and on-deck thermocycler use tracking. These logs increase visibility and support by 10x Genomics and Beckman Coulter Life Sciences technical support teams.

Guided Labware Setup (GLS)

The Guided Labware Setup (GLS) provides clear step-by-step instructions during the deck setup for the GEM-X GEX Library Construction automated method. Dynamic reagent volumes and tube locations based on sample number will be displayed via visual representations to provide additional help to the operator.

DeckOptixFinal Check (DFC)

The DeckOptixFinal Check will use instrument cameras to analyze the final instrument deck for missing or misplaced labware to reduce opportunity for operator setup errors.

Experimental Design

To test the performance of the automated GEM-X GEX Library Construction an extensive 10x-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) and 500 human peripheral blood mononuclear (hPBMCs) cell lines. A total of 216 libraries, across 7 independent end-to-end runs, were generated from the automated method on the Biomek i7 Hybrid Workstation to compare with 80 libraries generated from 3 library construction runs manually. 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. Two users were recruited to evaluate robustness of the setup process. These users 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. Both users executed the automated method, and their results were then compared with results from relevant experts (i.e., an expert on the manual process and an expert on Biomek automation).

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

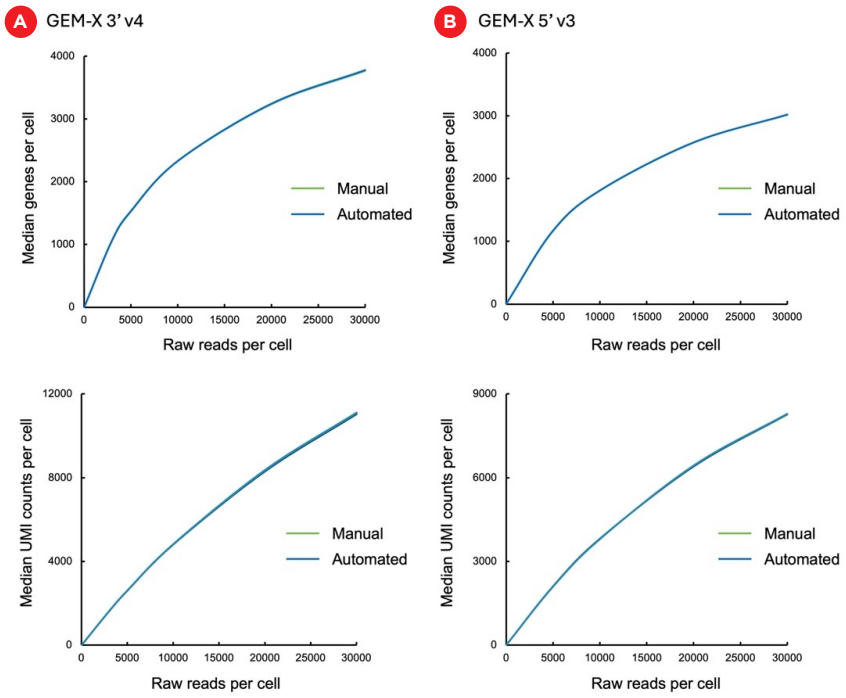

Figure 4. Libraries generated by the automated workflow have complexity and sensitivity at parity with manual workflow. Comparable median genes per cell and median UMI counts per cell between manual and automated workflows across multiple sequencing depths for (A) GEM-X 3' v4 libraries and (B) GEM-X 5' v3 libraries.

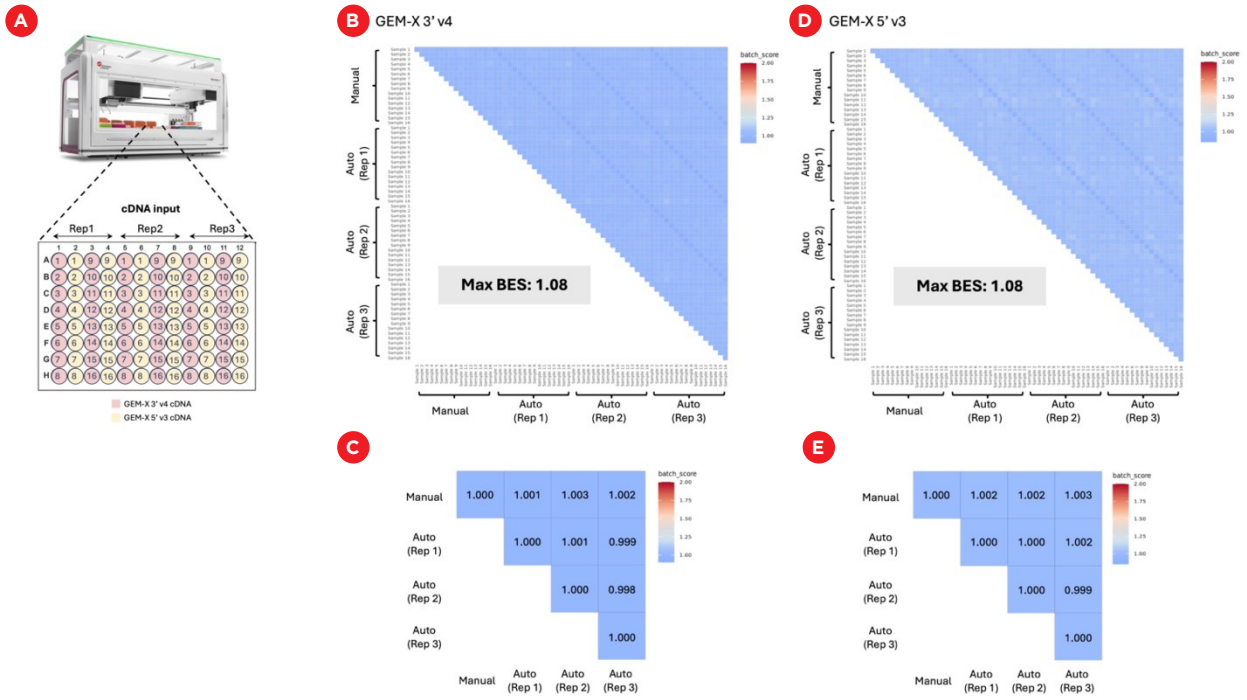

Figure 5. Library performance consistency across a 96-sample run with minimal batch effect. (A) Fully loaded cDNA input plate for the automated GEM-X GEX automated method. 16 unique GEM-X 3' v4 cDNAs and 16 unique GEM-X 5' v4 cDNAs were present in 4 consecutive columns and 3 automated replicates from the same cDNA aliquot were set up to fill the entire plate. The same cDNA aliquots were also processed manually for library performance comparison. Each cDNA was generated from 500 human PBMC samples. (B) Batch effect score comparison between 48 GEM-X 3' v4 libraries generated from the automated workflow and 16 libraries generated

manually. Maximum batch effect is 1.08. (C) 16 libraries from each workflow and/or replicate are clustered and analyzed for grouped batch effect score. (D) Batch effect score comparison between 48 GEM-X 5' v3 libraries generated from the automated workflow and 16 libraries generated manually. Maximum batch effect is 1.08. (E) 16 libraries from each workflow and/or replicate are clustered and analyzed for grouped batch effect score.

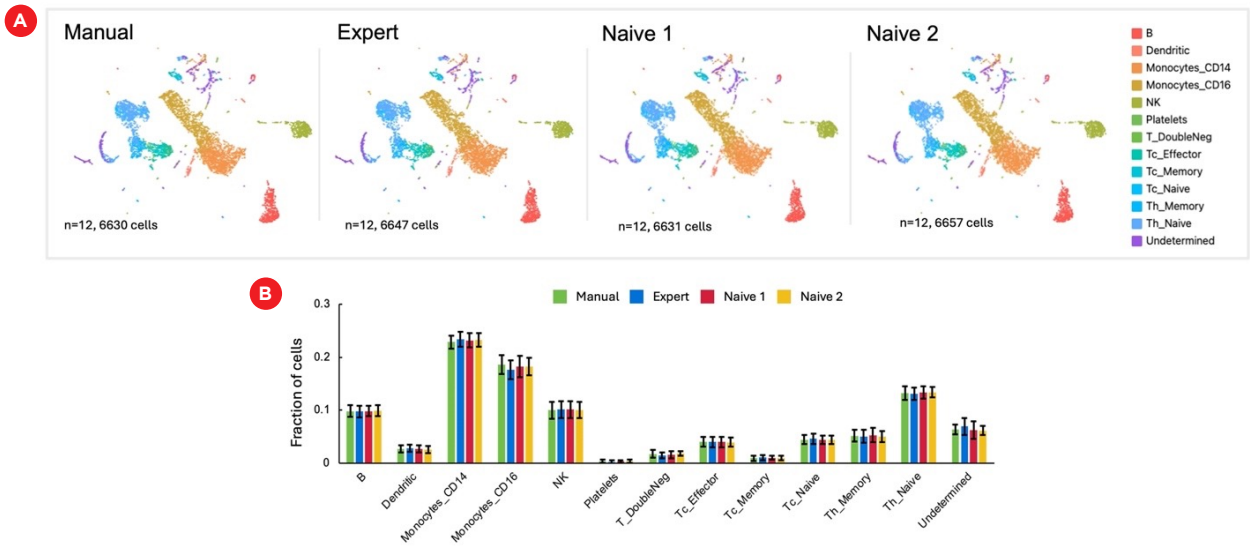

Figure 6. Consistent cell type profiling of human PBMC cells derived from GEM-X 3' v4 gene expression library construction between the manual workflow and multiple independent automated runs executed by users of different experience levels. (A) UMAP shows the gene expression data analyzed and visualized 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. (B) Bar graph demonstrates comparable average distribution of each cell population from the 12 libraries for each category, with error bars showing the standard deviations.

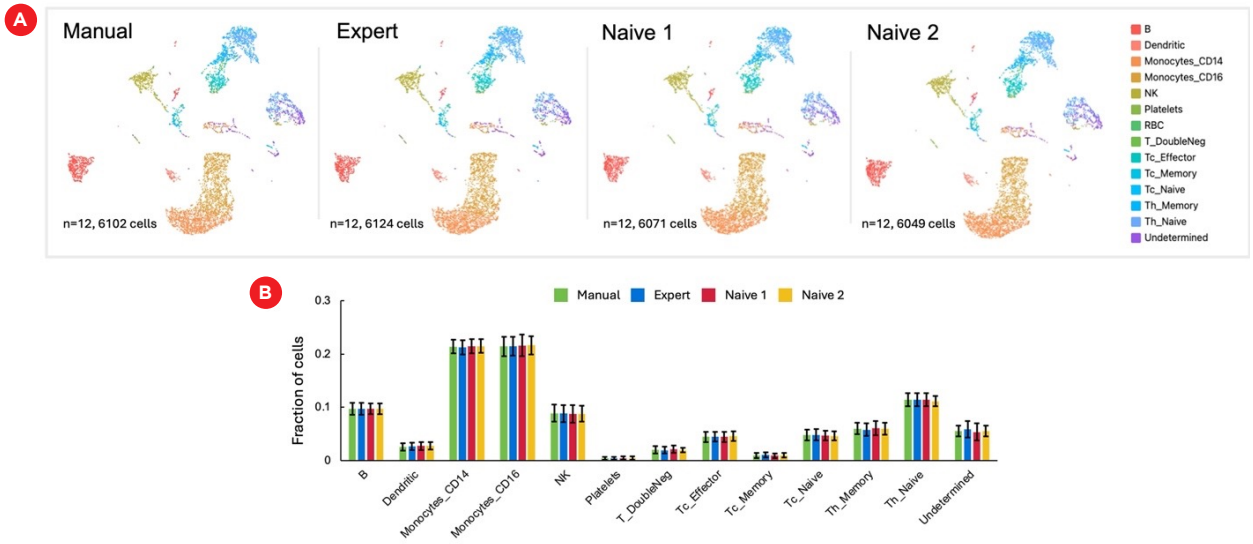

Figure 7. Consistent cell type profiling of human PBMC cells derived from GEM-X 5' v3 gene expression library construction between the manual workflow and multiple independent automated runs executed by users of different experience levels. (A) UMAP shows the gene expression data analyzed and visualized 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 runs) were analyzed. (B) Bar graph demonstrates comparable average distribution of each cell population from the 12 libraries for each category, with error bars showing the standard deviations.

Summary

Overall, this data shows automating the GEM-X GEX Library Construction on the Biomek i7 Hybrid Workstation delivers a robust, reliable solution for preparing high-quality, sequencing-ready libraries for single cell analysis.

GEM-X GEX Library Construction kit when automated on the Biomek i7 Hybrid Workstation delivers:

- Gene Expression libraries with performance at parity to libraries manually prepared.
- Libraries with consistent performance across all 96 samples with minimal batch effect.
- Highly consistent library generation across multiple independent automated runs.
- Robust setup process to enable success by users of different experience levels.

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