Automating single-cell profiling of fixed tissue enables academic core and CRO-scale single-cell genomics

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Introduction

Archives of fixed samples are a vast, underutilized resource for single-cell genomics. The 10x Genomics GEM-X Flex v2 (Apex) assay leverages these samples, offering logistical advantages, cost savings, reproducibility, and the ability to multiplex dozens to hundreds of samples. Conventionally, the pre-partitioning steps for the Flex Apex assay rely on repeated centrifugal wash cycles. These spin-washes are not automation-friendly and exert mechanical and osmotic stress, which reduces nuclei yield with every spin. Furthermore, manual centrifugation is labor-intensive, user skill-dependent, and prone to sample loss. As sample volumes scale up, these limitations create a significant bottleneck, making plate-scale profiling cost-prohibitive or impractical for core facilities and contract service providers without dedicated, highly skilled staff.

To overcome these challenges, we developed an automated pre-partitioning strategy for the Flex Apex assay that replaces traditional spin-washes with Concanavalin A paramagnetic beads (EpiCypher, 21-1411) for the hybridization, washing, and pooling steps. Implemented on the SPT Labtech firefly liquid handling system, this bead-based clean-up transforms a staff-intensive bottleneck into a highly streamlined workflow. SPT Labtech and 10x Genomics collaborated with the University of Michigan's Advanced Genomics Core to optimize the protocol and benchmark it against manual spin-based and mag-bead-based methods.

This work culminated in the processing of 96 samples in a single automated run. To rigorously assay cross-species and cross-tissue hybridization performance, the run included replicates of chop-fixed mouse tissues (liver, spleen, kidney, lymph node, thymus, and pancreas), diverse rat and rabbit tissues (liver, kidney, pancreas, spleen, bladder, heart, muscle, and adipose), and fixed rabbit PBMCs. The automated method successfully matched the expected median genes and UMIs per cell, as well as clustering resolution, across all tissue and species types tested. Importantly, this approach reduced hands-on processing time by 70%, delivering high consistency with no recovery penalty regardless of sample origin. By removing the throughput ceiling imposed by manual processing, this workflow provides academic genomics cores and CROs a validated, greatly reduced hands-on time path to population-scale, multi-tissue, and multi-species single-cell studies.

Automated GEM-X Flex Apex v2

Spin-based

- Centrifuge steps required to pellet cells/nuclei to exchange buffers
- This will require centrifugation of samples during fixation, hybridization, washing and pooling.

Bead-based

- Bead-based approach uses Concanavalin A-Conjugated Paramagnetic Beads
- Automation-friendly that does not require extra on-deck equipment or user intervention to transfer plates.

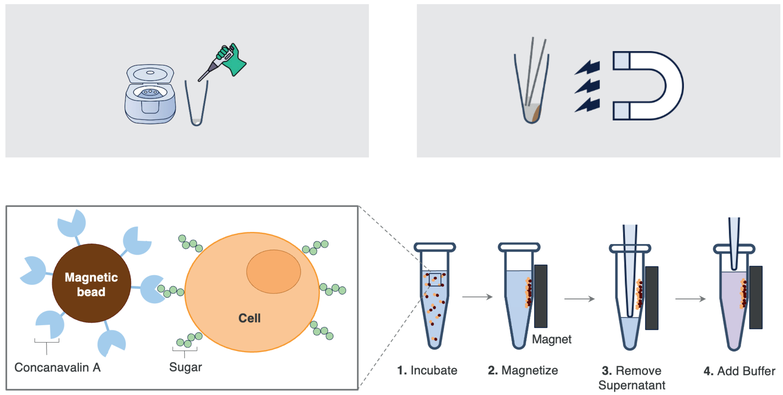


Figure 1. ConA paramagnetic beads replace centrifugation in the GEM-X Flex Apex v2 workflow. (a) Spin-based workflows pellet cells or nuclei by centrifugation for buffer exchange, which is needed at fixation, hybridization, washing and pooling. The bead-based workflow uses Concanavalin A (ConA)-conjugated paramagnetic beads. It needs no extra on-deck equipment and no user intervention to transfer plates, so it can run on an automated liquid handler. **(b)** ConA is a lectin covalently conjugated to magnetic nanospheres. It binds α-D-mannose and α-D-glucose on the cell or nuclear surface and captures cells and nuclei. Buffer exchange: (1) incubate sample with beads, (2) magnetize, (3) remove supernatant, (4) add buffer.

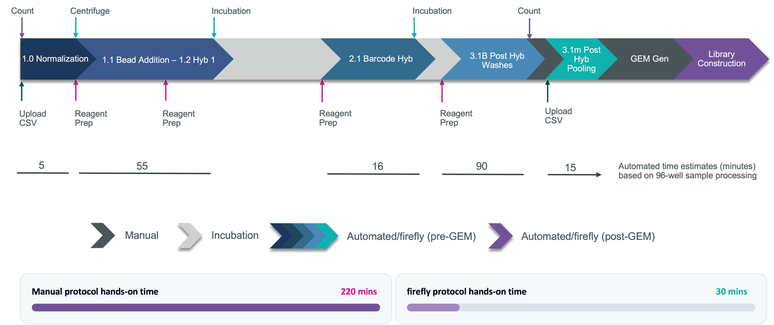


Figure 2. Automated GEM-X Flex v2 pre-GEM workflow on the firefly platform. (a) Workflow from normalization through library construction. Arrows mark manual steps (cell count, centrifugation, reagent prep, CSV upload, incubation). Chevron colors distinguish manual steps, incubations, and automated firefly steps before and after GEM generation. Automated times (minutes) are estimates for 96-well processing: normalization 5, bead addition/hybridization 1 55, barcode hybridization 2.1 16, post-hyb washes 90, post-hyb pooling 15. **(b)** Hands-on time for a 96-sample run was 220 min for the manual protocol and 30 min on firefly, with a >85% reduction in hands-on time.

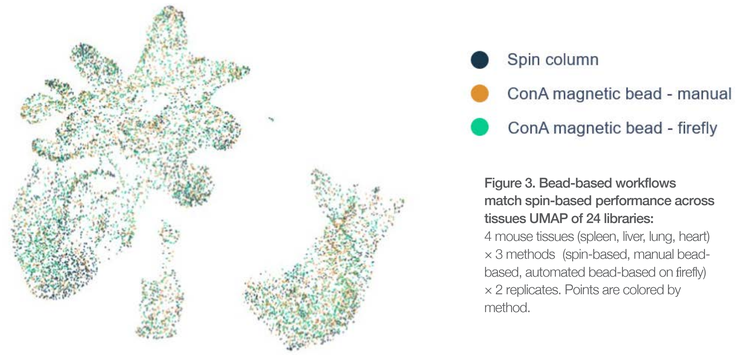
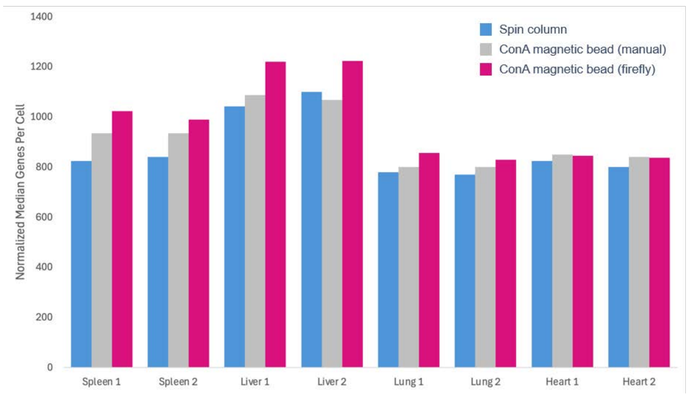


Figure 3. Bead-based workflows match spin-based performance across tissues UMAP of 24 libraries: 4 mouse tissues (spleen, liver, lung, heart) × 3 methods (spin-based, manual bead-based, automated bead-based on firefly) × 2 replicates. Points are colored by method.



4: Figure 4. Automated ConA magnetic bead method matches or traditional spin-based washes. Four different mouse tissues (spleen, liver, lung and heart) were processed in duplicate using three different methods, traditional spin-based washing, a manual ConA bead-based method and automated ConA bead-based washing on the SPT firefly. Shallow sequencing was performed on the libraries and normalized median genes per cell were calculated across all samples. (Normalized depths (read pairs per cell): spleen, 2500; liver, 5000; lung and heart, 3500.

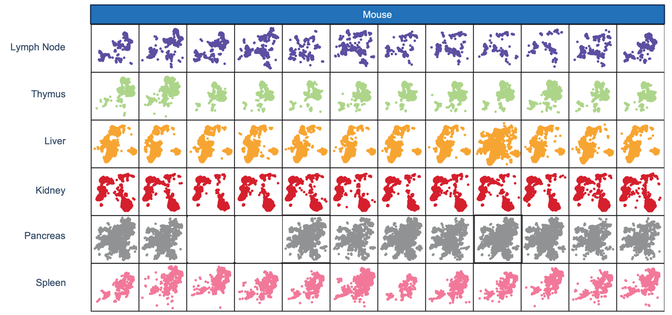


Figure 5. Reproducibility across multiple tissue types. Six different tissues were harvested from 12 individual mice and run as part of a 96-sample set processed on firefly. All 72 samples were processed in aggregate and then split to show UMAPs across the tissues and mice. Two pancreas samples failed data processing and have been excluded here (white boxes). Note that because tissue types of varying transcriptional activity were run together, cell numbers vary across tissues due to an imbalance of reads across sample types. Normal best practice would be to run tissue types of differing transcriptional activity in separate pools. Rat and rabbit tissues were also run as an internal experiment to test cross-hybridization with mouse probes (data not shown).

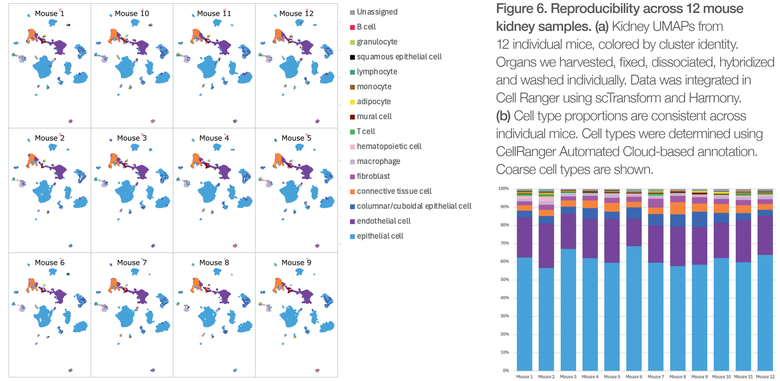


Figure 6. Reproducibility across 12 mouse kidney samples. (a) Kidney UMAPs from 12 individual mice, colored by cluster identity. Organs we harvested, fixed, dissociated, hybridized and washed individually. Data was integrated in Cell Ranger using scTransform and Harmony. **(b)** Cell type proportions are consistent across individual mice. Cell types were determined using CellRanger Automated Cloud-based annotation. Coarse cell types are shown.

Summary

- ConA paramagnetic beads replace centrifugation for buffer exchange in the GEM-X Flex v2 workflow, which makes it compatible with automated liquid handling.
- Libraries from automated bead-based, manual bead-based, and spin-based processing were comparable on key Cell Ranger metrics and overlapped in UMAP space across four mouse tissues.
- A 96-sample automated run covered multiple mouse tissues and rat and rabbit tissues. Replicate mouse livers clustered consistently across 12 animals with no batch-effect correction.
- Automation cut hands-on time from 220 to 30 minutes per 96-sample run (>70% less) and manual pipetting by >90%.
- Together, these results give academic cores and CROs a hands-off route to plate-scale single-cell profiling of fixed tissue without specialized labor or infrastructure.

Next steps

- 10x Flex v2 (Apex) available on firefly community for download
- Deploy validated protocol to additional core facility and CRO sites
- Validate Flex Apex v2 library construction on firefly
- Develop and validate upstream fixation workflow on firefly

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