

# Automating 10x Genomics Chromium 3' and 5' Gene Expression OCM on the Opentrons Flex®



Reproducible single nucleus RNA-seq libraries and  
8 hours of automated processing per run

## Written by

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DOI: <https://doi.org/10.5281/zenodo.23196863>

## ABSTRACT

Single-nucleus RNA sequencing (snRNA-seq) is foundational to neuroscience and translational research; however, its scale remains limited by the complexity, operator variability and hands-on demands of manual library preparation. To address these challenges, we developed a single Opentrons Flex protocol for the 10x Genomics Chromium 3' and 5' OCM workflows that automated post-GEM-RT cleanup, cDNA amplification and gene expression library construction. We evaluated the workflow in two pilot studies, with both sequenced on the Ultima Genomics platform. In Pilot 1, single nuclei isolated from mouse frontal cortex and processed with the 3' workflow showed strong manual-to-automated global gene-expression concordance ( $R^2 = 0.9945$ ), but automated library preparation produced a shift toward smaller fragment sizes. OpentronsAI enabled rapid, user-directed troubleshooting and implementation of a timing correction without external technical support. In Pilot 2, the updated automated 5' workflow produced highly consistent cDNA, NGS-library, and gene-expression profiles across human organoid replicates ( $R^2 = 0.9950$ ). Across both pilots, the Flex provided approximately eight hours of cumulative automated runtime, with operator involvement concentrated in reagent preparation, QC, and defined workflow handoffs.

## Key findings:

- Manual-to-automated concordance (Pilot 1): Global gene-expression  $R^2 = 0.9945$  between manual and automated 3' On-Chip Multiplexing (OCM) libraries from mouse frontal cortex.
- Automated reproducibility (Pilot 2): Global gene-expression  $R^2 = 0.9950$  between two automated replicates of the 5' OCM runs on human organoids.
- Time back to science: Approximately 8 hours of cumulative automated runtime across post-GEM-RT cleanup and cDNA amplification (~1.5h), cDNA cleanup (~2h), and NGS library construction (~4.5h).
- Protocol refinement with OpentronsAI: Rapid, user-directed troubleshooting and implementation of updated fragmentation conditions without external scripting support
- Broad assay support: A common Flex deck layout and automation framework supported both Chromium 3' and 5' Gene Expression OCM workflows using assay-specific parameters.

## INTRODUCTION

**Background.** 10x Genomics Chromium single-cell profiling has reshaped immunology, oncology, biomarker discovery, and translational research, placing growing pressure on labs to scale library preparation without sacrificing data quality.

**The reproducibility tax.** The 3' and 5' OCM protocol is lengthy, multistep, and technique intensive. Manual execution introduces operator-to-operator variability and run-to-run drift that inflate technical noise and can mask biological signal, often forcing additional replicates or re-runs to achieve publication-grade data.

**The time tax.** Manual single-cell library prep consumes the better part of a working day in active pipetting and watchful incubations, capping throughput to a handful of samples per technician per day and pulling scientists away from experimental design, data analysis, and downstream biology.

**Automation rationale.** The Opentrons Flex NGS Workstation automates post-GEM-RT processing for the 3' and 5' Gene Expression OCM workflows using standardized liquid handling, temperature control, and on-deck modules, reducing manual variability and hands on time.

**Summary.** This study evaluates automation of the post-GEM-RT Chromium Single Cell library-preparation workflow on the Opentrons Flex. A common deck layout and automation framework were used to support both Chromium 3' and 5' Gene Expression OCM workflows. Performance was evaluated by comparing automated preparation with a manual reference in Pilot 1 and by assessing reproducibility between independent automated runs in Pilot 2. Automated runtime was also evaluated.

## METHODS

**Pilot 1, A1/A2 manual-to-automated comparison:** Four mouse frontal-cortex samples were loaded at a nominal 9,000 nuclei per lane based on Vi-CELL BLU counts. GEM generation and reverse transcription (RT) produced one shared physical GEM-RT product, which was subsequently divided into two processing streams: post-GEM-RT processing was performed manually for A1 and on the Opentrons Flex for A2.

**Pilot 2, N1/N2 automated-replicate comparison:** Four human organoid models were processed in two independent automated 5' OCM runs, N1 and N2, using the updated fragmentation settings.

### cDNA generation and library input

cDNA yield and fragment size were assessed using the Agilent 4150 TapeStation. In Pilot 1, A1 and A2 had nearly identical cDNA concentrations, so a fixed 10 µL input was used for both. In Pilot 2, input volumes were adjusted based on measured cDNA concentrations to achieve comparable cDNA mass, resulting in 20 µL for N1 and 5 µL for N2.

### NGS Library preparation

- Manual workflow: performed per the 10x Genomics Chromium Single Cell user guide for the matching chemistry. Total runtime was recorded (Pilot 1).
- Automated workflow on Opentrons Flex: Required master mixes were prepared off deck and loaded onto the instrument. The Flex then automated reagent dispensing, GEM breakup and post-GEM-RT cleanup, cDNA amplification and cleanup, and gene-expression library construction (Figure 1). A common deck layout and automation framework supports the Chromium 3' and 5' Gene Expression OCM workflows using assay-specific parameters, and up to eight OCM libraries, representing up to 32 samples per run. Automated runtime was recorded for the demonstrated two-OCM-library workflow. Pilot 1 used 3' chemistry with the initial fragmentation settings. Pilot 2 used 5' chemistry and the updated fragmentation parameters (4°C reagent setup and 5 minute fragmentation at 32°C), implemented within the same automation framework.

## Automated Post GEM-RT Cleanup &cDNA Amplification (Day 1)

| Labware name                                            | Quantity | Recovery Agent                                              |
|---------------------------------------------------------|----------|-------------------------------------------------------------|
| Opentrons 96 Deep Well Heater-Shaker Adapter            | 1        | Recovery Agent for sample processing                        |
| Opentrons Flex 96 Filter Tip Rack 50 µL                 | 1        | SPRI Select Reagent                                         |
| Opentrons Flex 96 Filter Tip Rack 200 µL                | 1        | SPRI Select Reagent made in PEG for bead-based purification |
| Opentrons Flex 96 Filter Tip Rack 1000 µL               | 1        | Elution Solution I                                          |
| Opentrons 24 Tube Rack with NEST 2 mL Snapcap           | 1        | Elution Solution I for sample elution                       |
| NEST 96 Deep Well Plate 2mL                             | 1        | Elution Buffer                                              |
| Opentrons 24 Well Aluminum Block with NEST 2 mL Snapcap | 1        | Elution Buffer for final elution                            |
| NEST 12 Well Reservoir 15 mL                            | 1        | Dynabeads                                                   |
| Opentrons Tough 96 Well Plate 200 µL PCR Full Skirt     | 2        | Magnetic beads for sample capture                           |

- Post GEM-RT Cleanup – Dynabeads
- cDNA Amplification
- cDNA Cleanup - SPRIselect
- Post cDNA Amplification
- QC & Quantification

## Automated Gene Expression Library Construction (Day 2)

| Labware name                                            | Quantity | SPRI Beads                                |
|---------------------------------------------------------|----------|-------------------------------------------|
| Opentrons Flex 96 Filter Tip Rack 50 µL                 | 1        | SPRI Select Reagent in PEG                |
| Opentrons Flex 96 Filter Tip Rack 200 µL                | 1        | 80% Ethanol                               |
| Opentrons 24 Tube Rack with NEST 2 mL Snapcap           | 1        | 80% ethanol for washing                   |
| Opentrons Tough 96 Well Plate 200 µL PCR Full Skirt     | 2        | Elution Buffer                            |
| Opentrons 24 Well Aluminum Block with NEST 2 mL Snapcap | 1        | Buffer EB for elution                     |
| NEST 12 Well Reservoir 15 mL                            | 1        | Fragmentation Mix                         |
| Eppendorf Twin.tec 96 Well Plate 150 µL                 | 1        | Fragmentation mix prepared on ice         |
|                                                         |          | Adapter Ligation Mix                      |
|                                                         |          | Adapter ligation mix (viscous, keep cold) |

- GEX Fragmentation, End Repair & A-tailing
- GEX Post Fragmentation, End Repair & A-tailing Double Sided- SPRIselect
- GEX Adaptor Ligation
- GEX Post Ligation Cleanup -SPRIselect
- GEX Sample Index PCR
- Post Sample Index PCR Double Sided Size Selection - SPRIselect

**Figure 1.** Deck layout of the Opentrons Flex NGS Workstation configured for the Chromium Single Cell library-preparation protocol. The same deck layout was used for the 3' and 5' Gene Expression OCM workflows, with assay-specific settings and updated fragmentation parameters. Full protocols are available in the Opentrons Protocol Library.

## Sequencing

Libraries were sequenced on the Ultima Genomics platform at the University of Minnesota Genomics Center (UMGC). Libraries were prepared using the Ultima Flex Library Conversion protocol (including quality control). Sequencing was performed on a half wafer, yielding approximately 5-6 billion reads. No strict sequencing-depth target was prespecified; the libraries were sequenced deeply to obtain high coverage. Observed mean sequencing depths are reported in the sequencing-performance table.

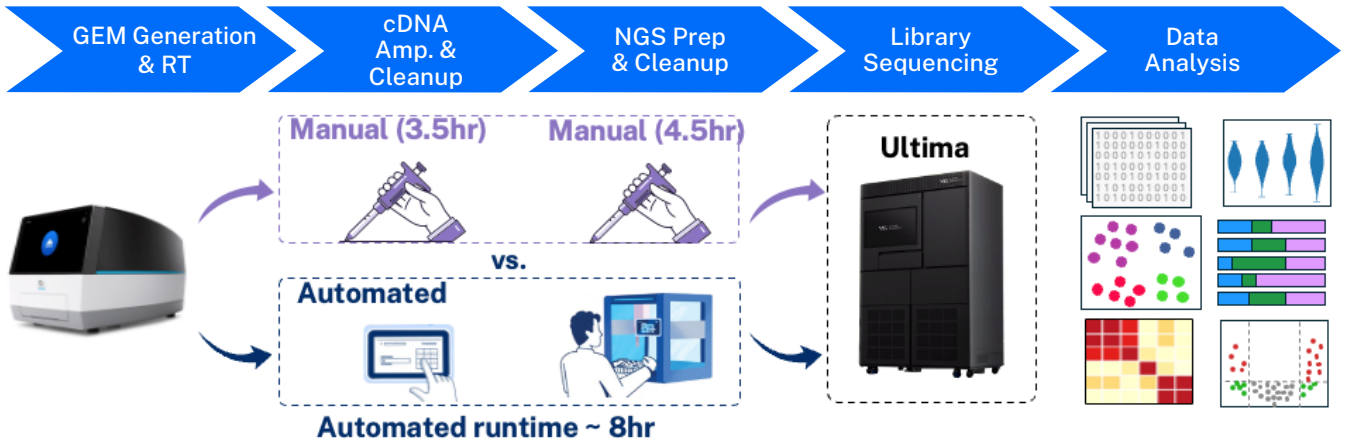
## Data analysis

Gene-expression matrices were analyzed separately within each pilot. Performance was evaluated using final retained nuclei, sequencing metrics, median genes and UMIs per nucleus at matched sequencing depth, pseudobulk gene-expression concordance, and annotated cell-population abundance. A1/A2 and N1/N2 were not combined because they represent separate studies, chemistries, and biological systems. The Pilot 1 composition analysis used the final cleaned A1 and A2 objects. Each pilot used an independent, study-specific QC and analysis pipeline tailored to its experimental design and data characteristics. All performance comparisons and sequencing-depth normalizations were therefore conducted within pilot rather than across pilots.

## RESULTS

### Time back to science: Automated runtime and workflow handoffs

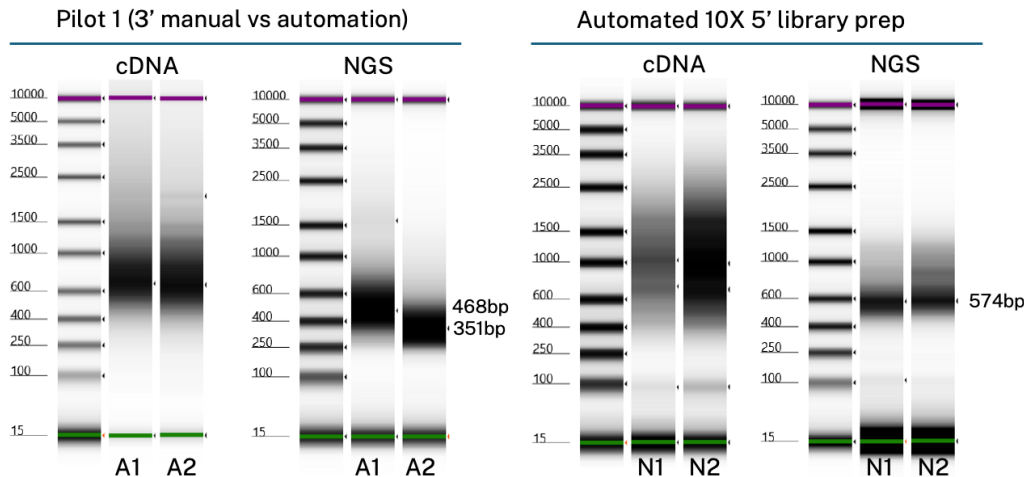
Automation shifted most post-GEM-RT processing from active manual handling to instrument-led execution. For the demonstrated two-OCM-library run, the workflow provided approximately eight hours of cumulative automated runtime covering post GEM RT cleanup, cDNA amplification and cleanup, and NGS library construction. Operator activity was limited to off-deck reagent preparation, deck setup, QC, and defined workflow handoffs.



**Figure 2.** Approximate manual-processing and automated-runtime requirements for post-GEM-RT processing. Automated execution limits operator activity to reagent preparation, deck setup, QC, and defined workflow handoffs.

### Automated and manual workflows generate comparable cDNA and NGS library quality metrics

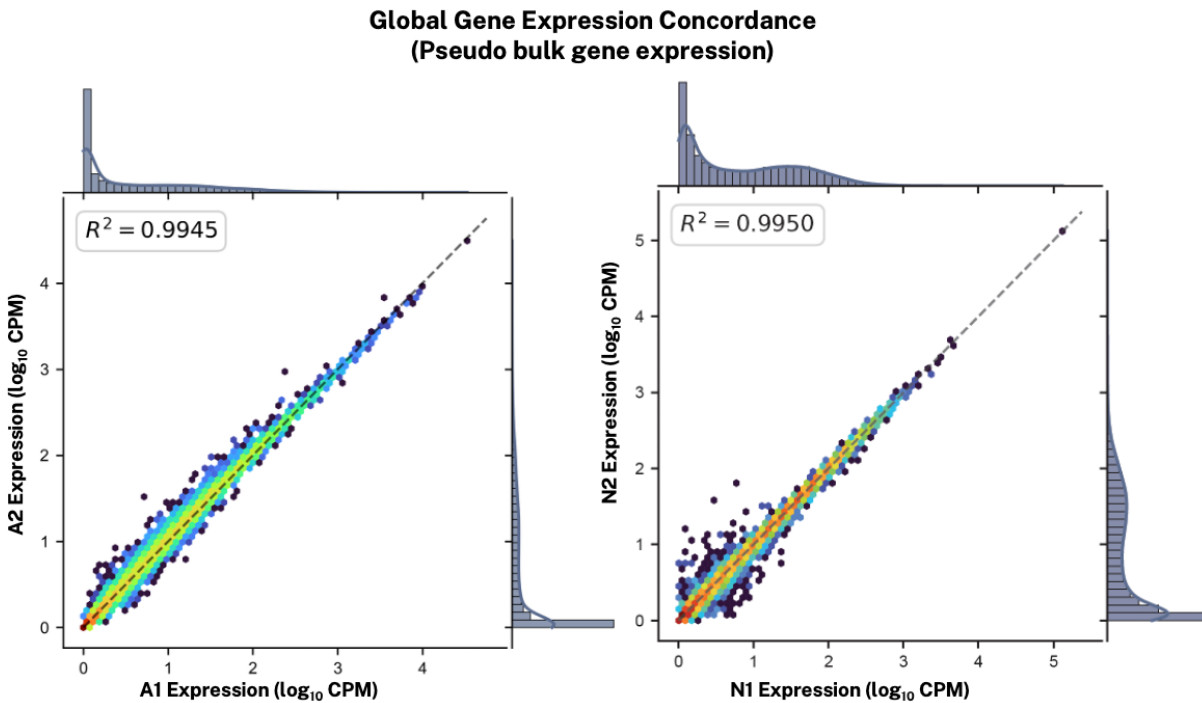
TapeStation analysis showed similar cDNA profiles between the manual and automated workflows in Pilot 1, but the A2 final library had a smaller average fragment size than A1, 351 bp versus 468 bp. Automated setup at 32°C exposed A2 to fragmentation conditions longer than the intended five-minute incubation, likely contributing to the shorter fragments. Using OpentronsAI, we modified the Flex protocol to perform reagent setup and pre-cooling at 4°C, followed by five minutes at 32°C, without external technical support. When deployed in Pilot 2, the two automated replicates produced nearly identical final-library fragment sizes of 574 and 576 bp, demonstrating consistent execution and the flexibility to rapidly optimize the workflow.



**Figure 3.** Fragment analysis of cDNA and final NGS libraries across both pilots. Left: Pilot 1, manual versus automated 3' workflows, with cDNA peaks near 650 bp and final library sizes of 468 bp and 351 bp, respectively. Right: Pilot 2, two automated 5' technical replicates, with cDNA profiles spanning 700-1,000 bp and final library sizes of 574 bp and 576 bp.

### Workflow Validation and Data Fidelity

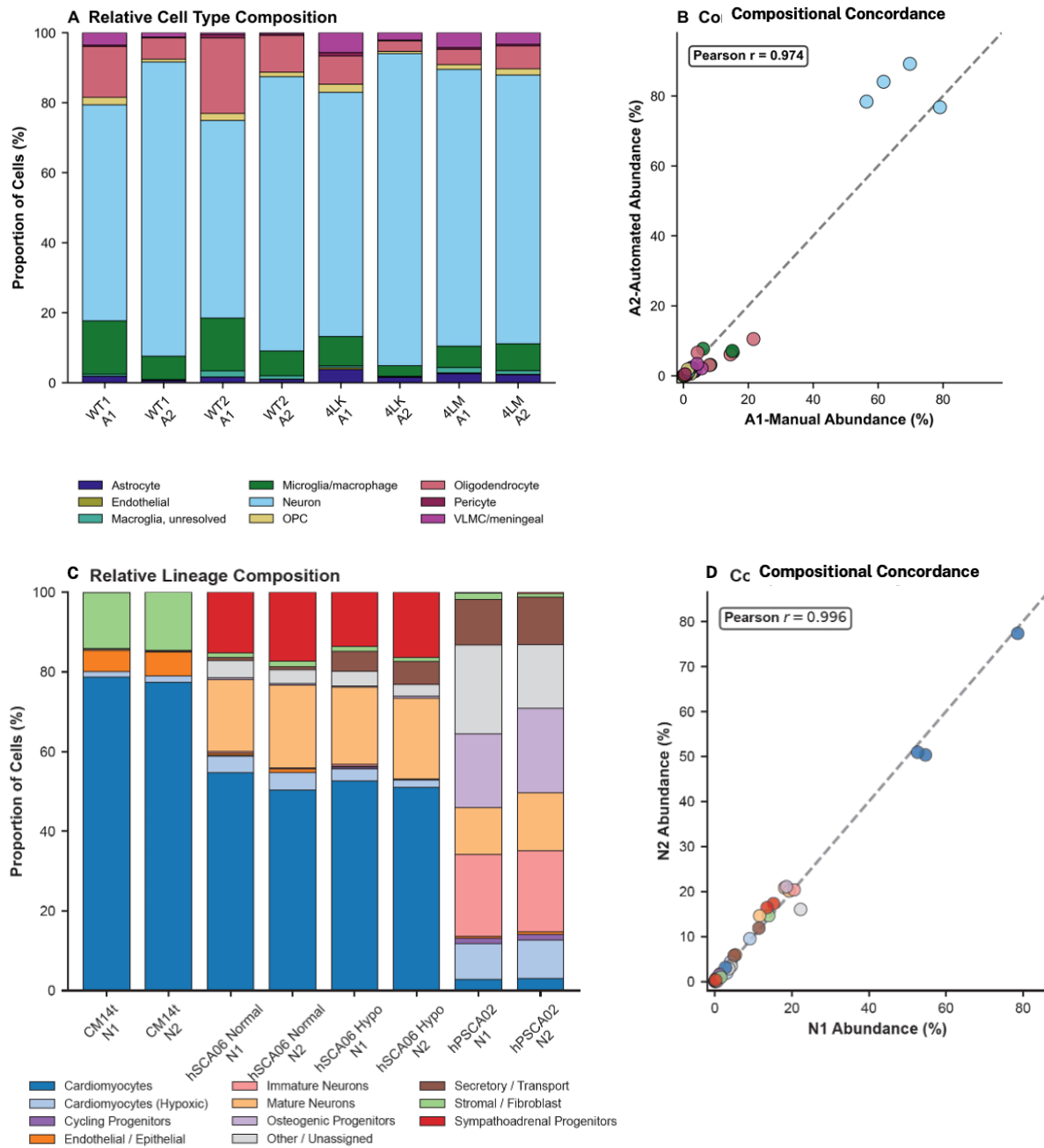
Global gene-expression profiles were highly concordant within both pilots. Pilot 1 showed strong agreement between the manual A1 and automated A2 workflows, with  $R^2 = 0.9945$ . Pilot 2 showed similarly high reproducibility between the two automated replicates, with  $R^2 = 0.9950$ . Neither comparison relied on batch correction or integration, supporting reproducible transcriptomic output from Flex automation.



**Figure 4. Global gene-expression concordance within each pilot.** Left: Pilot 1 comparison of manual A1 and automated A2 libraries,  $R^2 = 0.9945$ . Right: Pilot 2 comparison of automated N1 and N2 replicates,  $R^2 = 0.9950$ . Transcript abundance was quantified as log<sub>10</sub>-transformed counts per million.

### Cell-type and lineage composition concordance

A1 and A2 showed high overall compositional concordance (Pearson  $r = 0.974$ ), indicating that cell-type representation was largely preserved between the manual and automated workflows despite the shorter A2 fragment distribution. N1 and N2 showed similarly high lineage concordance across the four organoid models (Pearson  $r = 0.996$ ). Together, these results demonstrate robust preservation of cellular composition within both pilot studies, with only minor variation expected from matched-aliquot sampling, confirmatory QC, and sequencing-depth differences.



**Figure 5.** Cell-type and lineage composition concordance across matched workflows and technical replicates. **(A)** Relative abundance of annotated cell types in matched mouse brain samples processed using the manual A1 or automated A2 workflow. **(B)** Comparison of A1 and A2 cell-type abundances using the final cleaned objects. Each point represents one cell type; the dashed line indicates identity (Pearson  $r = 0.974$ ). **(C)** Relative abundance of annotated lineages in four human organoid samples processed as two automated 5' technical replicates (N1 and N2) from matched nuclei preparations. **(D)** Comparison of lineage abundances between the two automated replicates. Each point represents one lineage; the dashed line indicates identity (Pearson  $r = 0.996$ ).

## Sequencing performance

Sequencing and snRNA-seq performance metrics from the Ultima platform are summarized below. Pilot 1 gene and UMI metrics were calculated from the final QC-retained nuclei after read-level molecule-information subsampling of A1 and A2 to 64,087 reads per nucleus. Pilot 2 metrics were normalized to a simulated common depth of 100,000 reads per nucleus. Because the pilots differed in biological material, assay configuration, sequencing depth, and normalization procedure, absolute gene and UMI values are not directly comparable. The lower Pilot 2 medians are consistent with the lower RNA content and transcriptional complexity expected in immature human organoid nuclei compared with mature adult mouse brain nuclei. N1 and N2 nevertheless remained highly concordant, supporting strong technical reproducibility.

**Table 1.** Sequencing performance across Pilot 1 and Pilot 2. Pilot 1 compared manual A1 with automated A2, whereas Pilot 2 compared two automated replicates, N1 and N2. Gene-expression  $R^2$  and composition Pearson  $r$  represent within-pilot comparisons.

| Metric                                | Pilot 1 A1 manual | Pilot 1 A2 automated | Pilot 2 N1 automated | Pilot 2 N2 automated |
|---------------------------------------|-------------------|----------------------|----------------------|----------------------|
| Final nuclei retained                 | 29,970            | 32,608               | 21,611               | 16,406               |
| Original mean reads/nucleus           | 64,087            | 65,719               | 128,171              | 160,733              |
| Median genes/nucleus at matched depth | 3,884             | 3,389                | 468                  | 539                  |
| Median UMIs/nucleus at matched depth  | 14,899            | 9,701                | 1,097                | 1,000                |
| Gene expression $R^2$                 | 0.9945            |                      | 0.9950               |                      |
| Composition Pearson $r$               | 0.974             |                      | 0.996                |                      |

## DISCUSSION

**Reproducible transcriptomic output.** The Flex-automated libraries showed strong within-pilot gene-expression concordance. Pilot 1 produced  $R^2 = 0.9945$  between the manual and automated 3' workflows, while Pilot 2 produced  $R^2 = 0.9950$  between two automated 5' replicates. Cell-population analyses provided an additional biological-level assessment, with composition correlations of  $r = 0.974$  for A1/A2 and  $r = 0.996$  for N1/N2.

**Time back to science as a compounding benefit.** Automation shifted approximately eight hours of post-GEM-RT processing to instrument-led execution. Operator activity was concentrated in reagent preparation, deck setup, QC, and defined workflow handoffs, reducing the need for continuous manual pipetting.

**OpentronsAI enabled rapid protocol optimization and deployment.** Beyond reducing hands-on time, automation accelerated protocol development and optimization. Using OpentronsAI, we implemented a pre-cooling step and refined fragmentation timing. Subsequent automated replicates produced nearly identical final-library fragment sizes, demonstrating rapid protocol revision and reproducible execution under the updated settings. This ability to quickly revise, test, and deploy protocol updates lowers the technical barrier to workflow development and optimization and

enables laboratories to spend less troubleshooting automation code and more time on experimental design, data analysis, and biological discovery. As these gains accumulate across projects and teams, automation can increase both productivity and reproducibility.

**A common automation framework for 3' and 5' OCM workflows.** The same Flex deck layout and automation framework supported both Chromium 3' and 5' Gene Expression OCM workflows. Assay-specific settings, including the updated fragmentation parameters, can be incorporated without requiring separate automation platforms.

## CONCLUSION

The Opentrons Flex NGS Workstation automates post-GEM-RT cleanup through final gene-expression library construction for Chromium 3' and 5' Gene Expression OCM workflows. Across two pilot studies, the automated workflow provided approximately eight hours of cumulative automated runtime while producing reproducible library profiles, global gene-expression measurements, and cellular-composition results. These findings demonstrate that the Flex can reduce active manual handling while maintaining consistent snRNA-seq output.

## ACKNOWLEDGEMENT

This work was supported by a generous contribution by the Georgia Research Alliance, which funded the acquisition of the Opentrons Flex NGS Workstation, 10x Genomics Chromium platform, and Agilent 4150 TapeStation. Funding for reagents and consumables was provided by NIGMS award R35GM119850 (N.E.L.).

We thank Parrish Payne, Field Application Scientist at Opentrons, for contributions to workflow automation. We also thank Saroja Kolluru for platform training, Mohammad Rahimi for reviewing the application note, and Sara Tafoya for overall leadership, all from 10x Genomics.

## MATERIALS USED IN THIS STUDY

| Equipment / Reagent                                     | Vendor       | Catalog #  |
|---------------------------------------------------------|--------------|------------|
| Opentrons Flex NGS Workstation                          | Opentrons    | 991-00507  |
| GEM-X Universal 3' Gene Expression v4 4-plex 16 samples | 10x Genomics | PN-1000779 |
| GEM-X OCM 3' Chip Kit v4 4-plex                         | 10x Genomics | PN-1000747 |
| GEM-X Universal 5' Gene Expression v3 4-plex 16 samples | 10x Genomics | PN-1000780 |
| GEM-X OCM 5' Chip Kit v3 4-plex                         | 10x Genomics | PN-1000748 |
| Dual Index Kit TT Set A 96 rxns                         | 10x Genomics | PN-1000215 |
| D5000 ScreenTape assay                                  | Agilent      | 5067-5593  |
| D1000 ScreenTape assay                                  | Agilent      | 5067-5585  |

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