

Utilizing Blood from PAXgene Tubes for Single Nuclei Transcriptomic Analysis

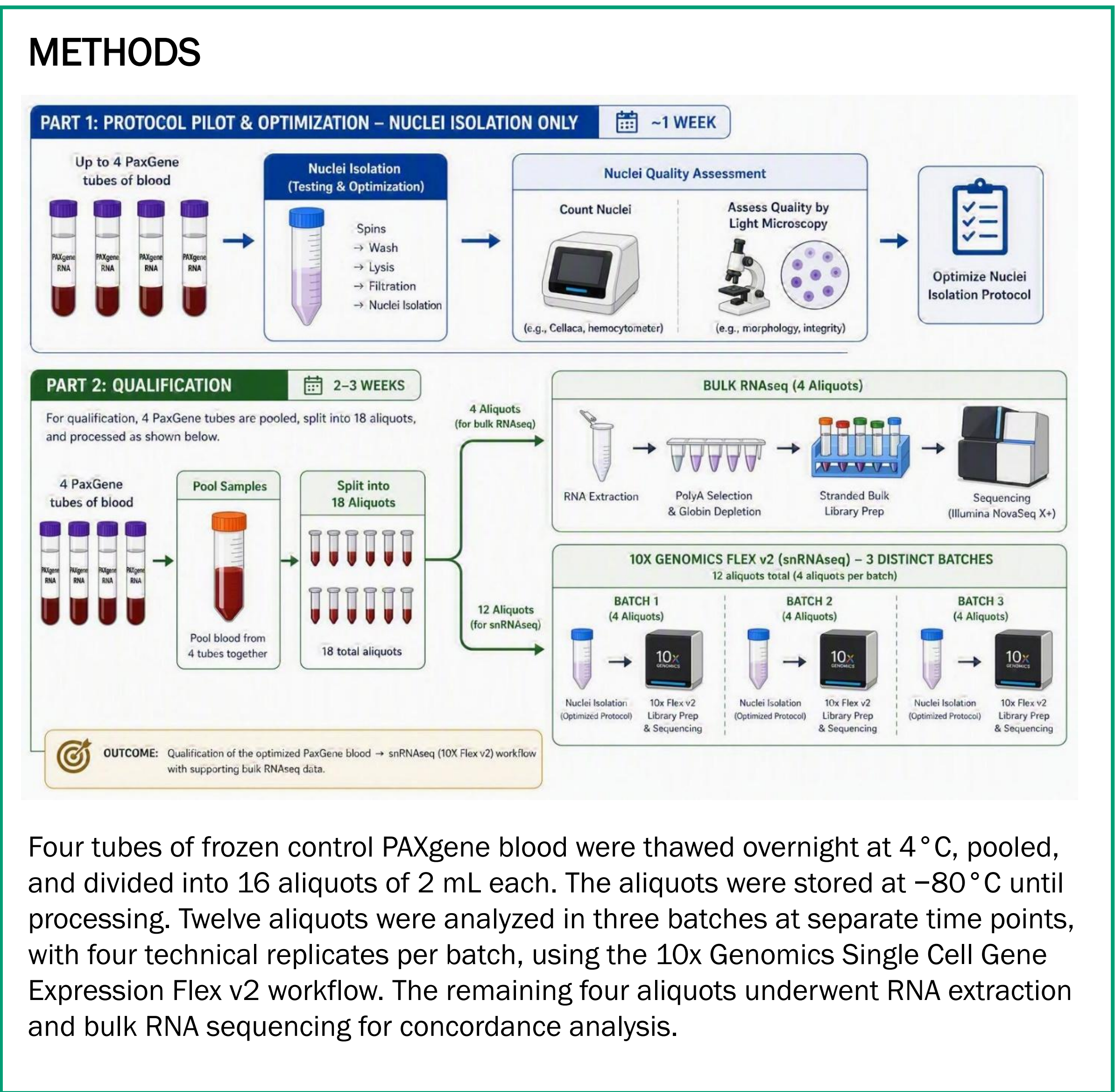
Bhaven Mehta, Katie Duval, Andrea O'Hara, Yongjun Fan, Yang Han, and Haythem Latif

¹GENEWIZ from Azenta Life Sciences, South Plainfield, NJ 07080

ABSTRACT

Single-cell RNA sequencing (scRNA-seq) and single-nuclei RNA sequencing (snRNA-seq) empower researchers to assess the unique transcriptomes of tens of thousands of individual cells and allow the detection of rare cell populations. scRNAseq on patient-derived blood samples is becoming more prevalent across a variety of disease areas but is restricted to cryopreserved PBMCs, which require specialized collection and storage. These complications can prevent some studies from being able to deploy scRNAseq, and those that do often see high levels of variability linked to PBMC processing, rather than true biology. Blood collection in PAXgene RNA tubes, by contrast, is highly standardized and requires no special equipment. Previously, whole blood samples collected in PAXgene RNA tubes have been used primarily for bulk RNA sequencing, which, while ideal for standard gene expression analysis, fails to interrogate the heterogeneity of complex cell populations. Here we describe a novel method for snRNA-seq on whole blood collected in PAXgene RNA tubes using the 10X Genomics® Flex fixed RNA profiling preparation.

In this proof-of-principle study, blood from four individuals was collected in PAXgene RNA tubes and stored at -20°C until processing. Each sample was processed in technical triplicates to test replicability and batch concordance. Nuclei isolation with a series of spin and wash steps was followed by visual inspection, counting, and then a variation of the 10X Flex protocol. This library preparation method utilizes a defined set of probes for whole transcriptome capture without requiring live cells or full-length intact RNA; it has previously shown to give comparable results to traditional live cell processing. Here we show that this method successfully achieves transcriptomic readout on a single-nucleus basis from whole blood in PAXgene RNA tubes with high levels of replicability. Whole blood in PAXgene RNA tubes is a commonly biobanked specimen type, and the presentation and rigorous evaluation of this novel protocol unlocks new potential insights for an untold number of studies and patients. Overall, this modified snRNA-seq approach allows for analysis of transcriptome-wide gene expression at high sensitivity, with simultaneous cellular readouts, from RNA PAXgene tubes creating new possibilities for prospective and retrospective clinical sample analysis.

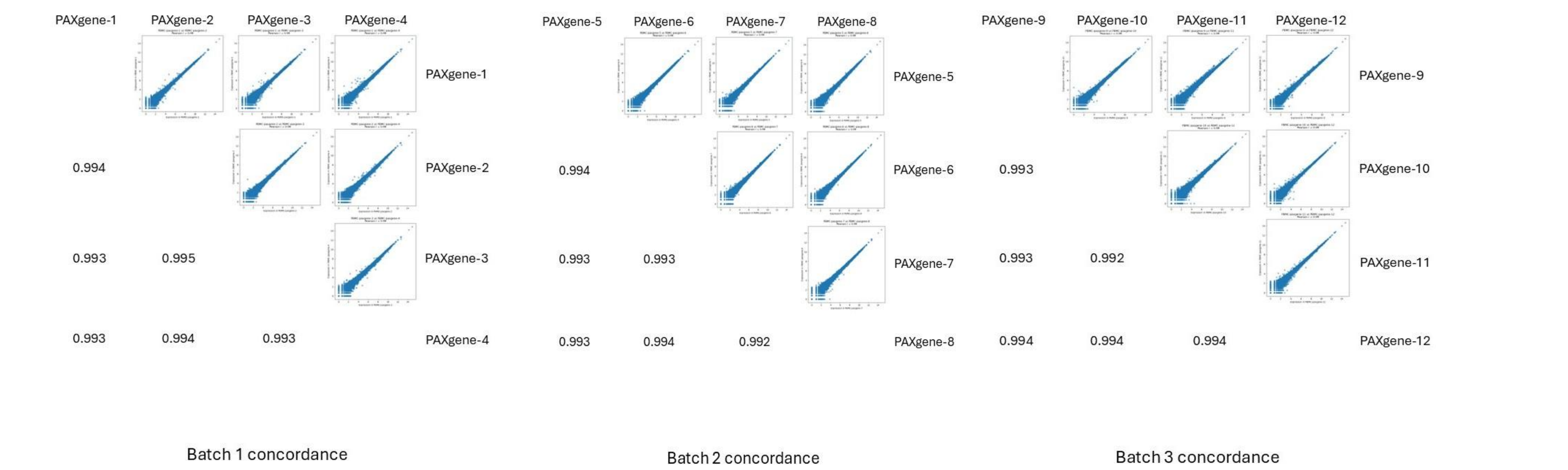


Replicate	Batch	Recovered Cell #	CV	Median Genes per Cell	CV	Median UMI Counts per Cell	CV
Paxgene-1	1	5606	12%	3210	4%	7884	8%
Paxgene-2		6162		3428		8288	
Paxgene-3		5221		3514		9164	
Paxgene-4		7105		3197		7402	
Paxgene-5	2	9916	16%	1859	15%	4511	9%
Paxgene-6		7969		2609		5334	
Paxgene-7		6262		1840		4279	
Paxgene-8		8139		1978		4450	
Paxgene-9	3	7250	15%	3107	5%	7632	4%
Paxgene-10		5759		3191		7331	
Paxgene-11		6527		2825		6807	
Paxgene-12		8670		3145		7138	

To assess intra-assay repeatability, four 10x Genomics GEM-X Flex v2 libraries targeting 10,000 recovered cells or nuclei were prepared in each of three batches. Within-batch coefficients of variation were calculated for key performance metrics (left). Major cell-type counts were normalized to the total number of cells detected in each sample and are presented as percentages (right).

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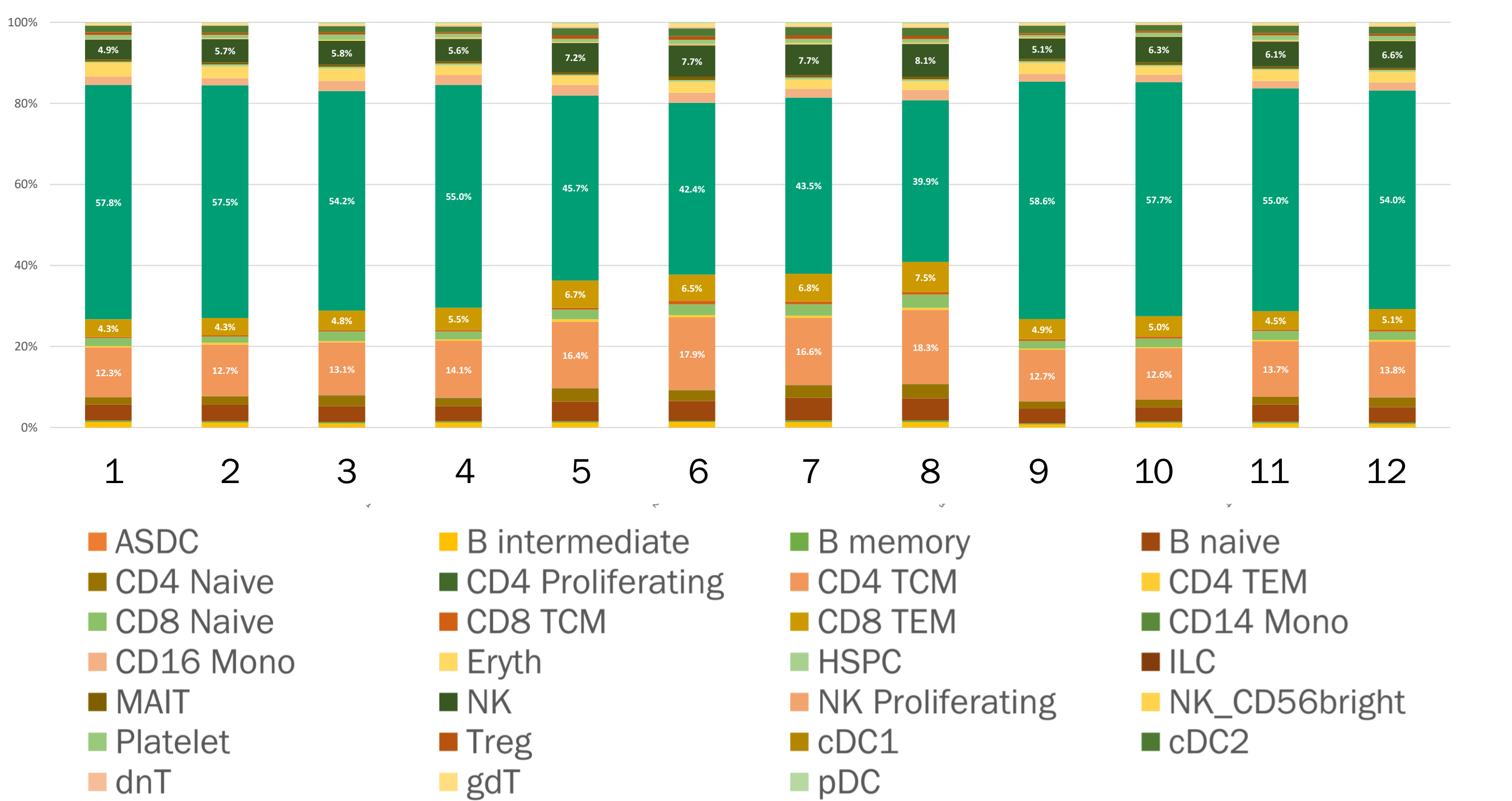
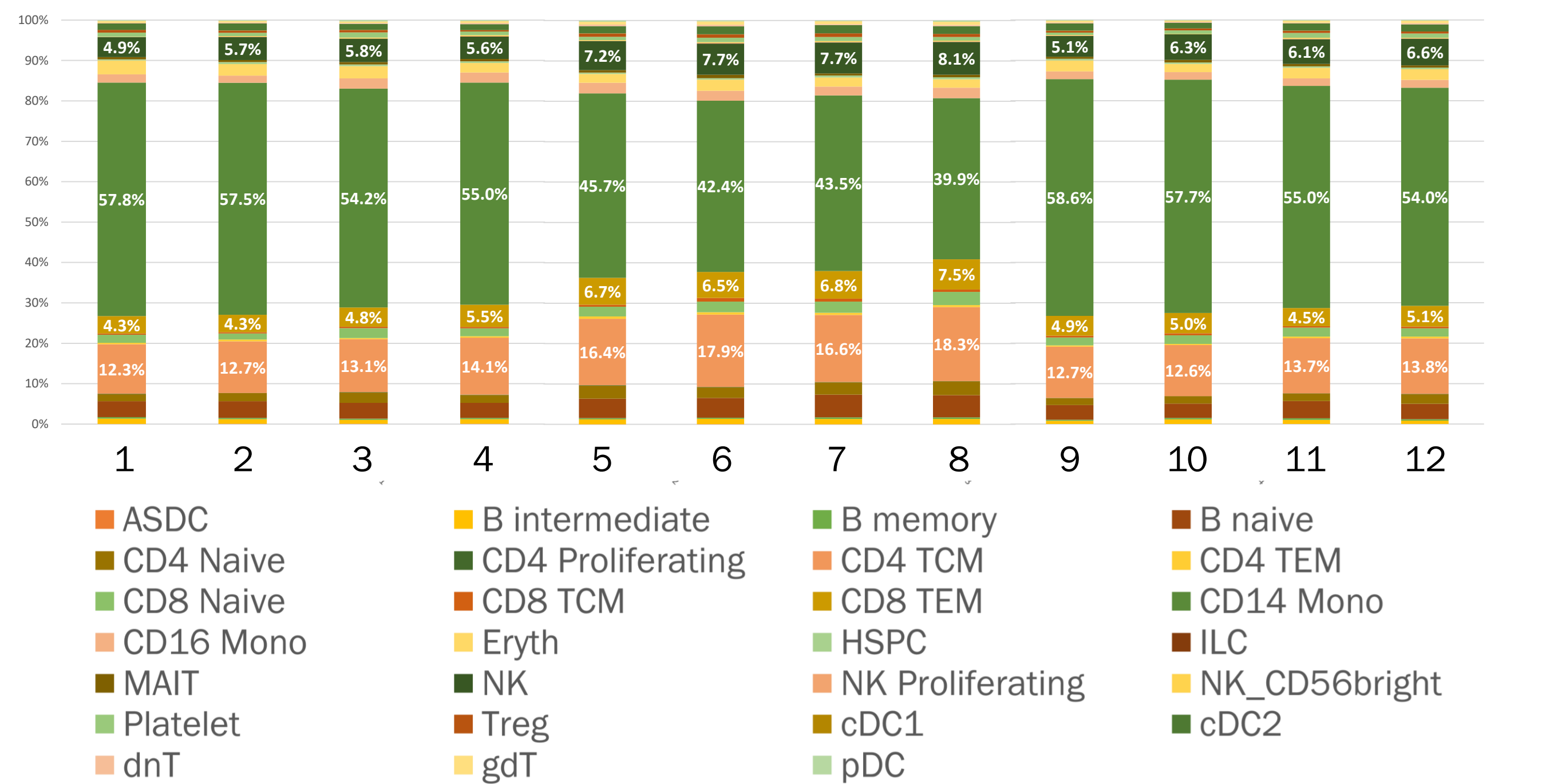
To assess inter-batch reproducibility, results were compared across three independently processed batches using the mean of four technical replicates per batch. Coefficients of variation for key performance metrics were generally below 20% (left). Cell-type composition was evaluated across all 12 replicates as described above (right). Despite observable batch effects, major cell types representing more than 1% of the total population showed low variability, with CVs below 20%.



To allow for a quantitative characterization of the overlap between technical replicates, pseudobulk gene expression analysis was used to quantify concordance among technical replicates. Gene expression counts were aggregated across all cells within each sample, followed by pairwise Pearson correlation analysis. Samples processed across Batches 1–3 showed high concordance (left), with $R^2 \geq 0.99$ for intra-batch replicates (right) and $R^2 > 0.99$ across all samples, demonstrating strong inter-run reproducibility.

CONCLUSIONS

- This proof-of-concept study evaluates whether cryopreserved whole blood collected in PAXgene RNA tubes can support reproducible single-nucleus RNA sequencing using the 10x Genomics GEM-X Flex v2 workflow. 10,000 cells were targeted for this test with replications at different batches. Libraries were sequenced and sequencing data were then analyzed.
- Across the single-nucleus libraries, within-batch coefficients of variation for cell recovery, median genes per cell, and median UMI counts per cell ranged from 4% to 16%. Inter-batch performance metrics generally showed CVs below 20% across the three independently processed batches.
- Cell-type profiles were generally consistent across the 12 replicates. Although some batch-related differences were observed, major cell populations representing more than 1% of detected cells maintained CVs below 20%. Pseudobulk gene expression profiles showed particularly strong agreement, with pairwise correlations of approximately $R^2 = 0.99$ or greater within and across batches.
- The optimized workflow produced consistent, reproducible single-nucleus transcriptomic data from frozen PAXgene whole blood. PAXgene blood may enable retrospective and prospective single-cell studies when immediate PBMC isolation or live-cell processing is not feasible.



	1	2	3	4	5	6	7	8	9	10	11	12
1	1.000	0.994	0.993	0.993	0.993	0.993	0.992	0.993	0.993	0.992	0.993	0.994
2	0.994	1.000	0.995	0.994	0.995	0.994	0.993	0.994	0.995	0.994	0.994	0.995
3	0.993	0.995	1.000	0.993	0.994	0.993	0.992	0.993	0.993	0.993	0.993	0.994
4	0.993	0.994	0.993	1.000	0.993	0.993	0.991	0.993	0.993	0.992	0.993	0.994
5	0.993	0.995	0.994	0.993	1.000	0.994	0.993	0.993	0.994	0.993	0.993	0.994
6	0.993	0.994	0.993	0.993	0.994	1.000	0.993	0.994	0.993	0.992	0.992	0.994
7	0.992	0.993	0.992	0.991	0.993	0.993	1.000	0.992	0.992	0.992	0.992	0.993
8	0.993	0.994	0.993	0.993	0.993	0.994	0.992	1.000	0.993	0.992	0.992	0.994
9	0.993	0.995	0.993	0.993	0.994	0.993	0.992	0.993	1.000	0.993	0.993	0.994
10	0.992	0.994	0.993	0.992	0.993	0.992	0.992	0.992	0.993	1.000	0.992	0.994
11	0.993	0.994	0.993	0.993	0.993	0.992	0.992	0.992	0.993	0.992	1.000	0.994
12	0.994	0.995	0.994	0.994	0.994	0.994	0.993	0.994	0.994	0.994	0.994	1.000

BONUS: Utilizing Blood from PAXgene Tubes for 5' Gene Expression with T-VDJ

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