Performance of Blood Collection Tubes and PBMC Isolation Methods for Single Cell Gene Expression

Introduction

Blood products such as Peripheral Blood Mononuclear Cells (PBMCs), Bone Marrow Aspirates (BMAs) and leukocytes provide insightful information for assessing disease and immune states across patients. They are easily accessible, minimally invasive, and provide readouts reflective of other organ states. Although existing methods for isolating these cell types are broadly used across traditional bulk assays, the impact of anticoagulants and isolation methods on single cell assay performance is not fully understood. This Technical Note compares different blood collection tubes and PBMC isolation methods, demonstrating how they can be implemented into single cell workflows.

To assess the performance of different anticoagulants and separation methods, whole blood was collected in either BD Vacutainer tubes (ACD-A, Sodium Citrate, Heparin, or EDTA anticoagulants) or CPT tubes (Heparin or Citrate anticoagulants). PBMCs were isolated from the whole blood samples using CPT or SepMate density centrifugation methods as described in the Isolation of Leukocytes, Bone Marrow, Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing Demonstrated Protocol (CG000392). Two replicates were performed for each experimental condition.

Figure 1 delineates the sample preparation for these experiments. Samples were processed using the Chromium Single Cell 3' v3.1 (Dual Index) Reagent Kits User Guide (CG000315) and Single Cell 5' v2 Reagent Kits User Guide (CG000331).

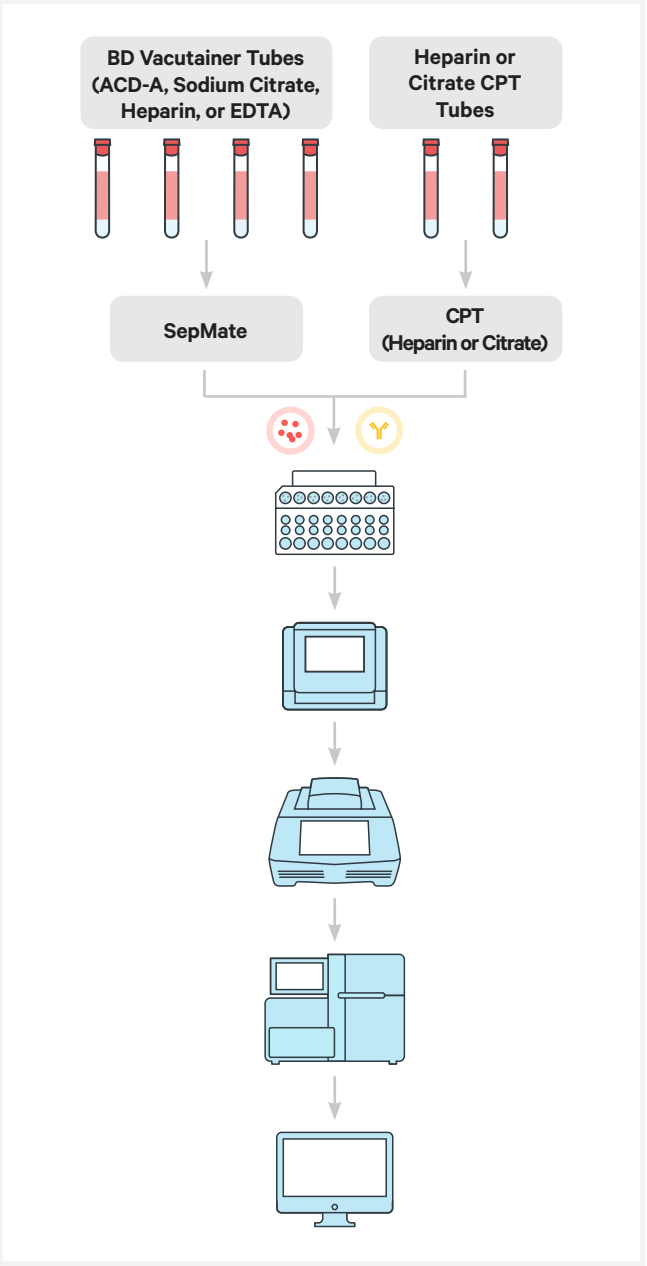

Figure 1. Sample preparation involving different collection tubes and isolation methods. Analysis may be performed with Cell Ranger and Loupe Browser Software

Blood Anticoagulant Comparison

Cell Viability and Single Cell Behavior

PBMCs isolated from whole blood vacutainer tubes showed high cell viability across anticoagulants and isolation methods. Single cell behavior can be assessed by the barcode rank plot and fraction of reads in cells. In the barcode rank plots, a distinct knee and cliff are expected, showing good separation between cells and background. This also correlates to the high fraction of reads in cells, demonstrating that most reads are coming from called cells rather than background. Barcode rank plots overlap well across all anticoagulants and show consistent high fraction of reads in cells.

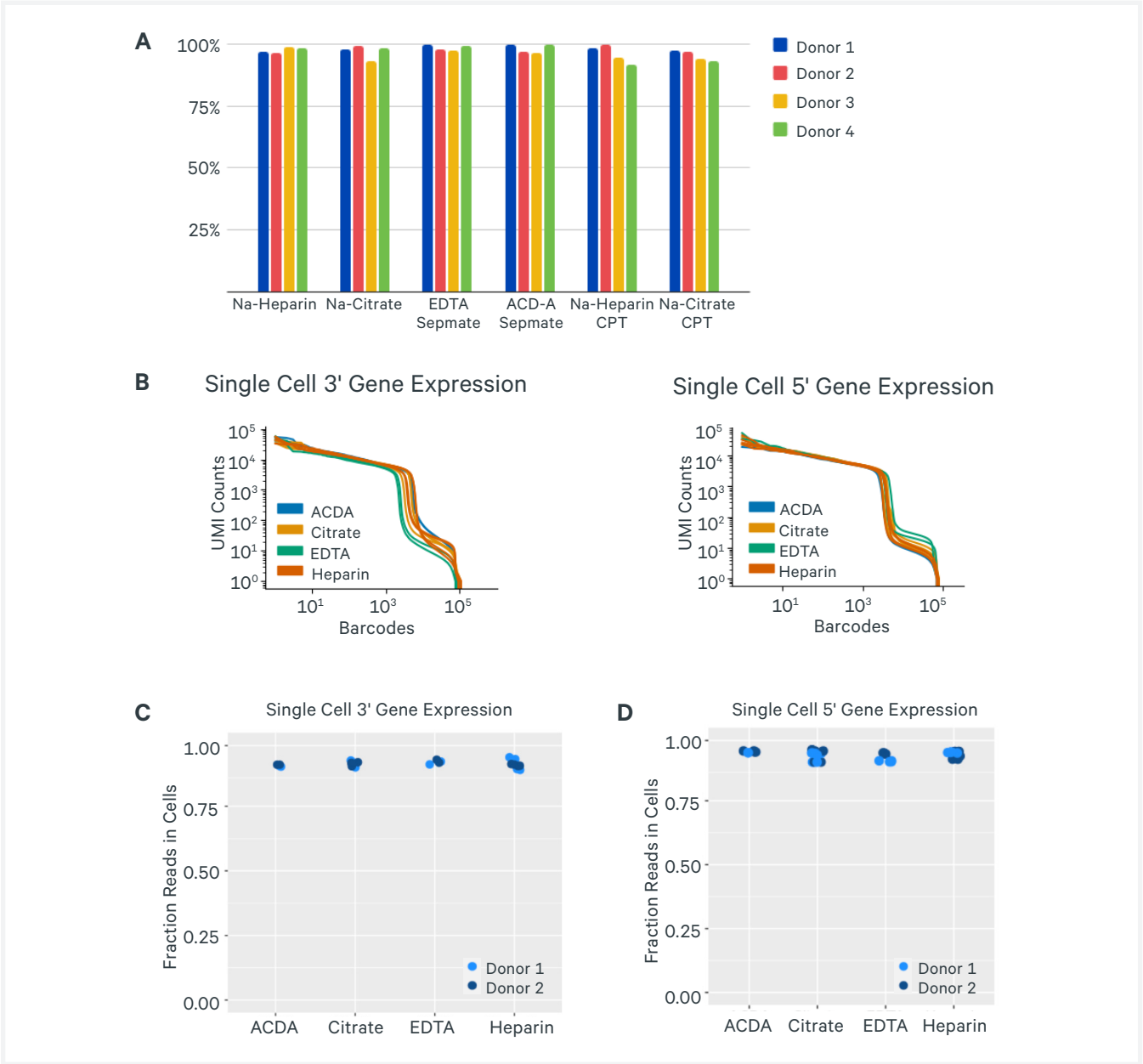

Figure 2. Cell Viability and single cell behavior data for Single Cell 3' and 5' libraries. Rank plots contain data from both Single Cell 3' and 5' libraries (B). Fraction Reads in Cells plots are for Single Cell 3' libraries (C) and Single Cell 5' libraries (D).

Sensitivity and Sequencing Saturation

To compare sensitivity, median genes and UMIs are plotted against sequenced raw read pairs. Median genes and UMIs captured across different anticoagulants and across replicates are comparable for both Single Cell 3' v3.1 and Single Cell 5' v2 libraries. At 20,000 read pairs per cell, both assays reach ~50% sequence saturation.

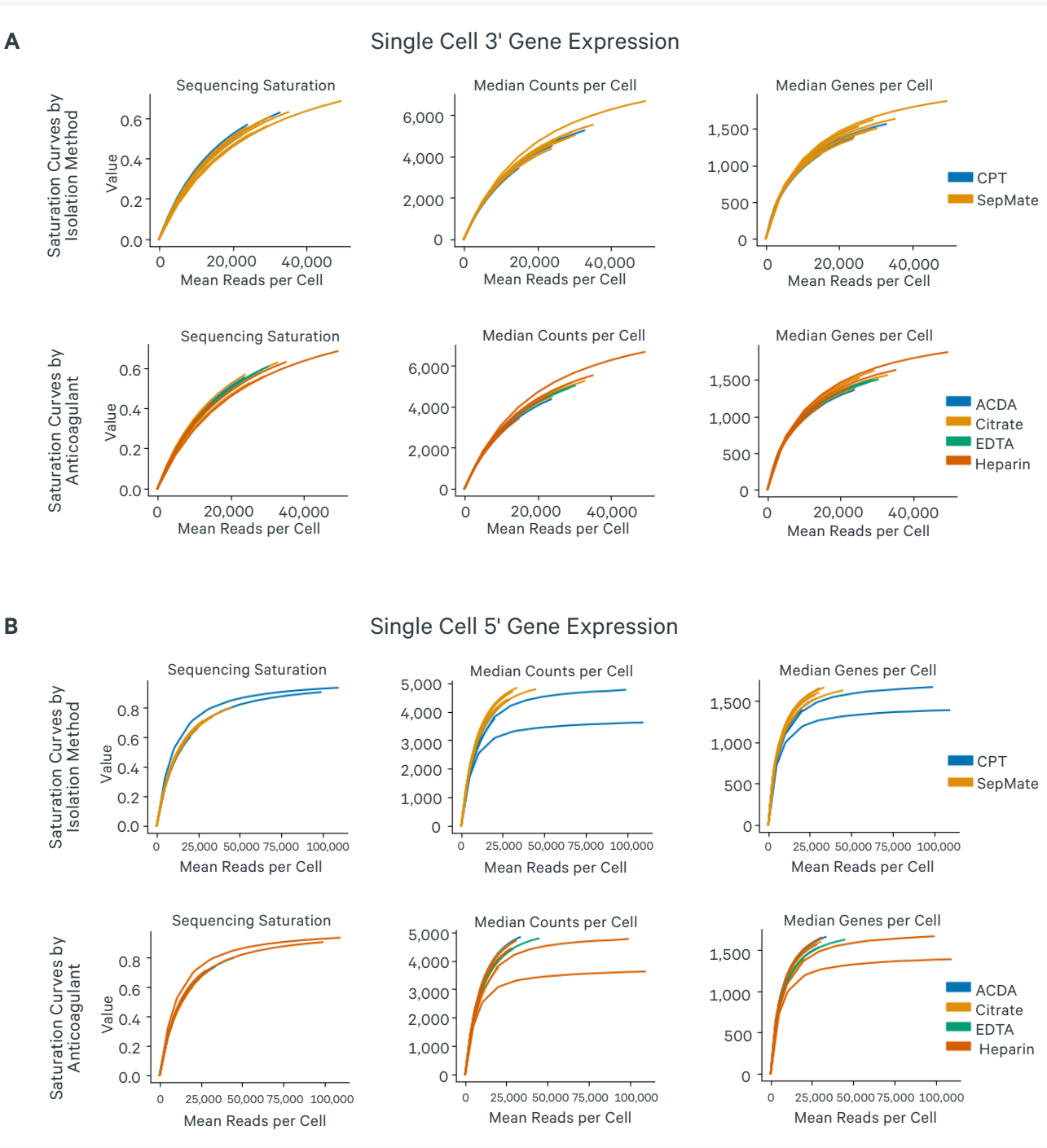

Figure 3. Sensitivity and sequencing saturation data for Single Cell 3' v3.1 (A) and 5' v2 (B) libraries. Sensitivity and sequencing comparisons for Multiome and Single Cell 3' v3.1 are shown in (C). Sequencing saturation curves for Single Cell 3' v3.1 (D) and Single Cell 5' v2 (E) assays.

Gene Expression Profiles Are Consistent Across Anticoagulants

The single cell barcode UMAP plots below show gene expression data analyzed and visualized using Cell Ranger aggr and Loupe Cell Browser, respectively. These UMAP plots for PBMCs collected with different anticoagulants show significant overlap as evidenced by the consistent cluster structure across both Single Cell 3' v3.1 and Single Cell 5' v2 assays.

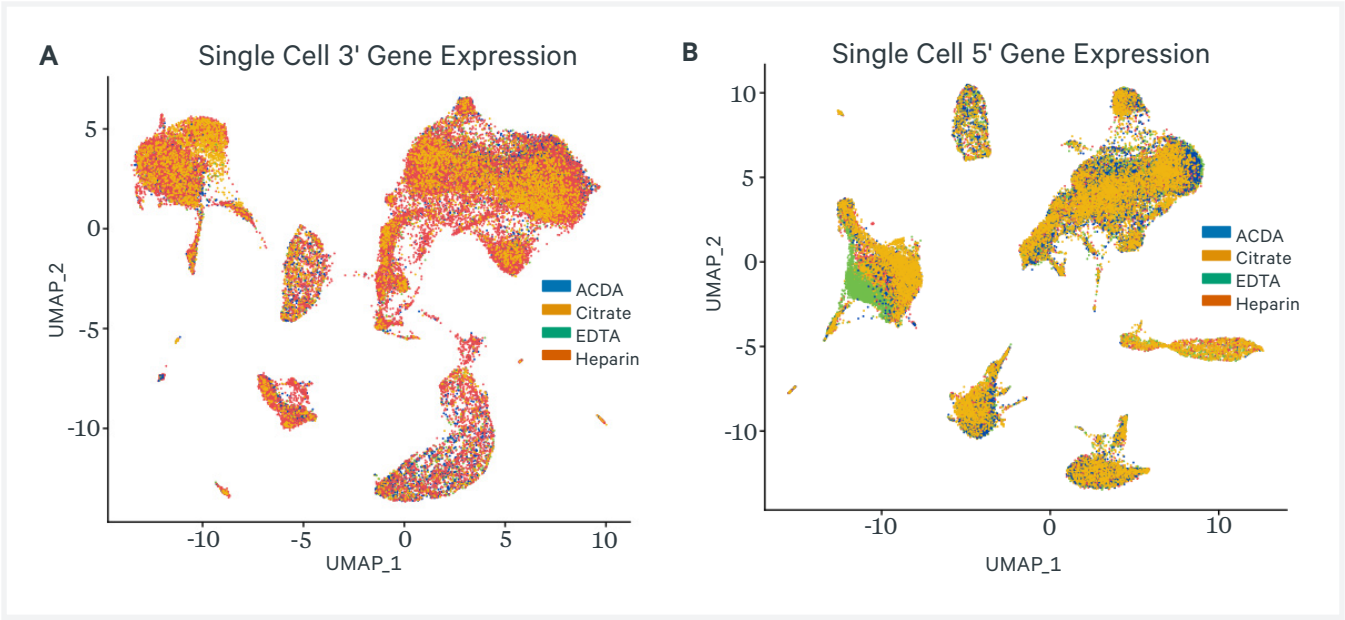

Figure 4. UMAP plots for Single Cell 3' v3.1 (A) and 5' v2 (B) libraries.

Cell Populations Are Consistent Across Anticoagulants

Major cell populations in each cluster were identified with known PBMC markers. Comparable cell type proportions amongst the different anticoagulants are shown in the figure below, demonstrating that most anticoagulants conserve main PBMC cell populations. Slight variation in red blood cell (RBC) retention is observed across anticoagulant conditions.

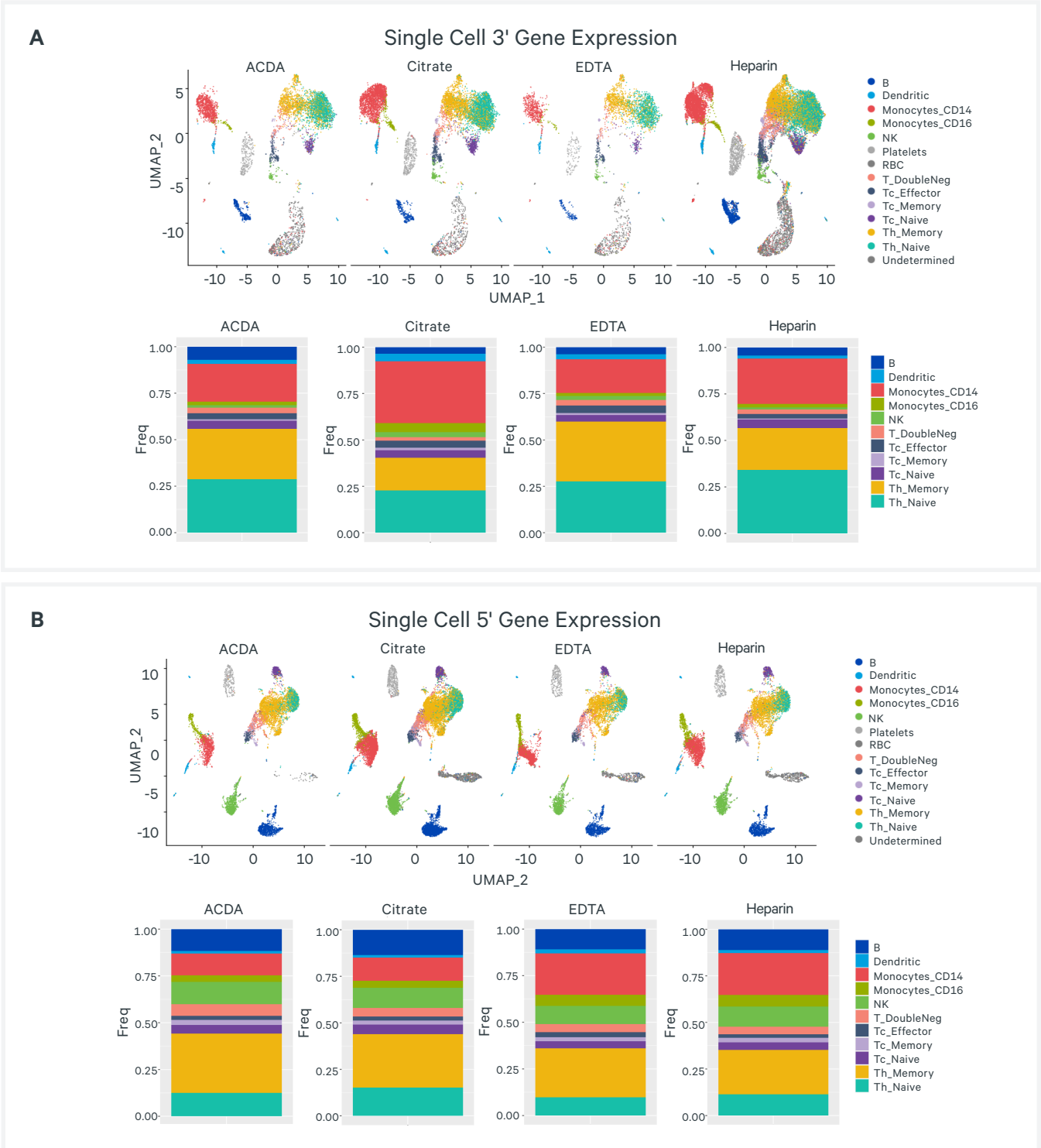

Figure 5. Cell type proportions for Single Cell 3' v3.1 (A) and 5' v2 (B) libraries.

Global Gene Expression Profiles Are Consistent Across Anticoagulants

Global gene expression across different anticoagulants is highly correlated. Differentially expressed genes due to differential RBC contamination are observed.

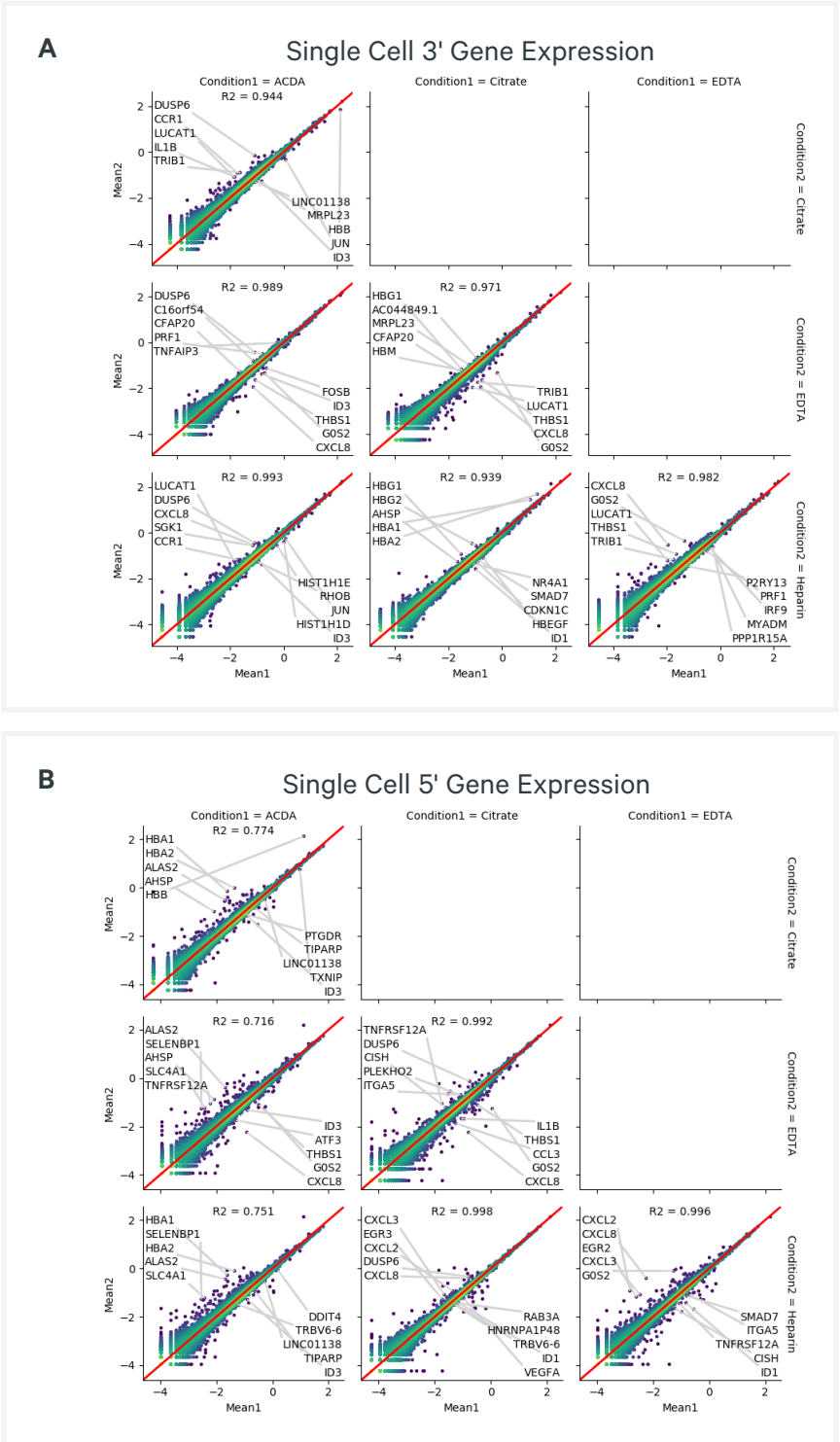

Figure 6. Global gene expression profiles for Single Cell 3' v3.1 (A) and 5' v2 (B) libraries.

Isolation Method Comparison

Gene Expression Profiles Are Consistent Across Isolation Methods

Whole blood was isolated using CPT and SepMate tubes. Major cell populations in each cluster were identified with known PBMC markers. Comparable cell type proportions amongst the different isolation methods are observed.

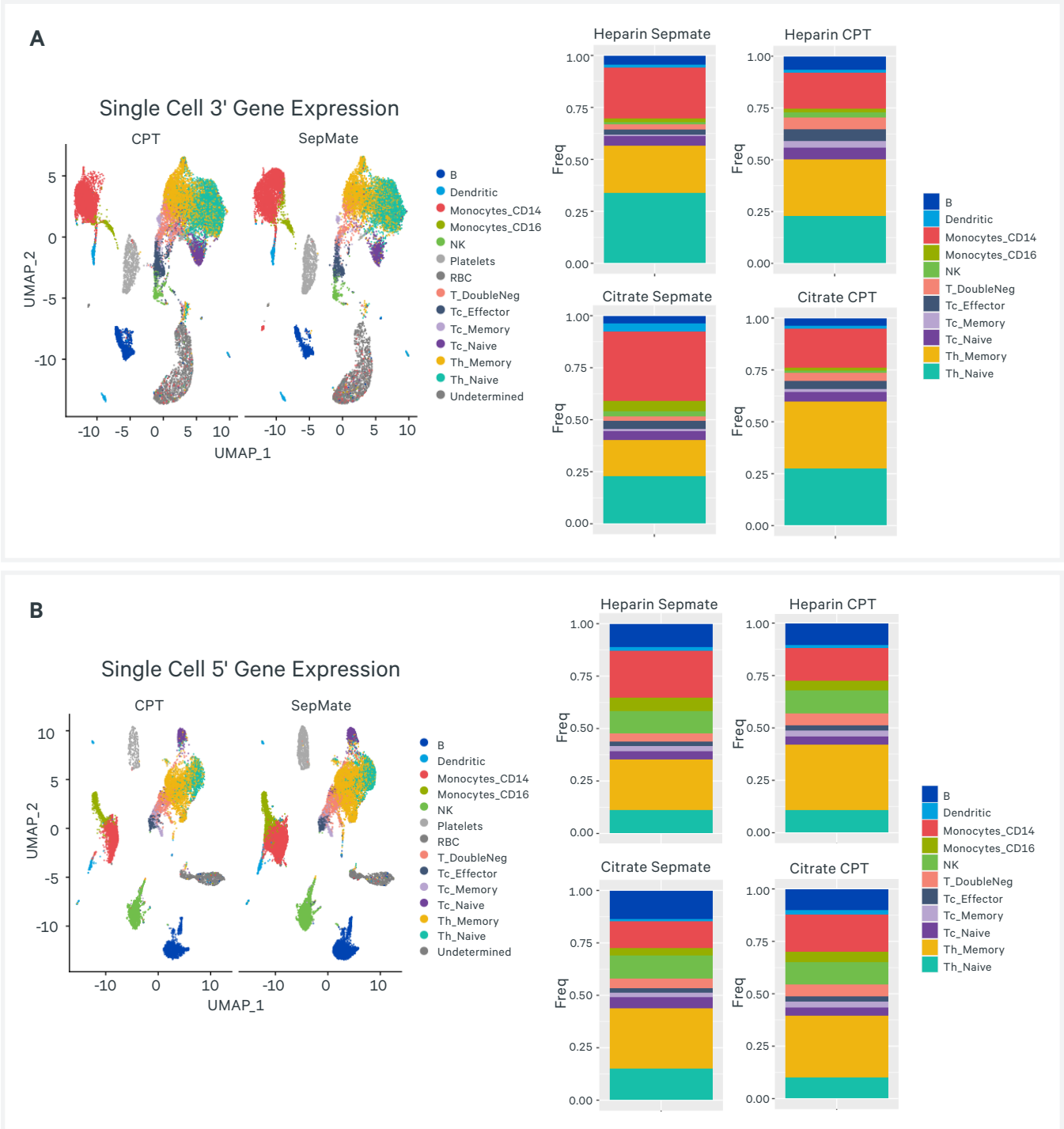

Figure 7. Global gene expression clustering and cell population frequencies for Single Cell 3' v3.1 (A) and 5' v2 (B) libraries .

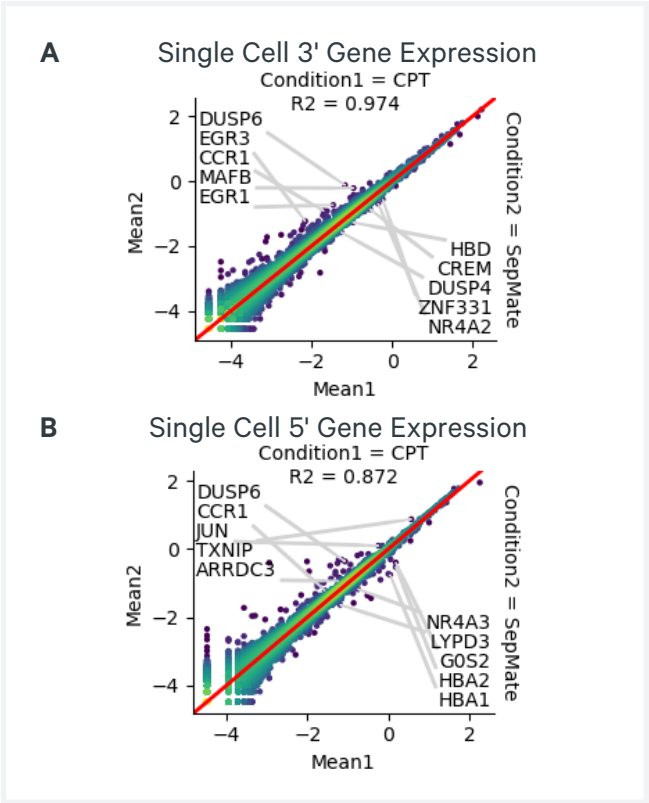

Figure 7. Global gene expression for Single Cell 3' v3.1 (A) and 5' v2 (B) libraries .

Global Gene Expression Profiles Are Consistent Across Isolation Methods

Conclusion

In summary, blood collection tubes of different, widely available types as well as different isolation methods are compatible with 10x Genomics Single Cell Gene Expression solutions. As described in this Technical Note, utilizing different collection tubes and isolation methods yielded comparable gene expression profiles and cell population clustering between samples. Users who are already using these blood collection tubes and isolation methods can continue to do so knowing they are fully compatible with 10x Genomics Single Cell Gene Expression assays.

References

1. Garvin, Tyler, Robert Aboukhalil, Jude Kendall, Timour Baslan, *et. al.*, Interactive analysis and assessment of single-cell copy-number variations. *Nature methods* 12, no. 11 (2015): 1058
2. Chromium Single Cell 3' v3.1 (Dual Index) Reagent Kits User Guide (CG000315)
3. Chromium Single Cell 5'v2 (Dual Index) Reagent Kits User Guide (CG000331)

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA