Technical Note

Variable Cell Input on RT Barcode Plate of the Single Cell RNA Kit

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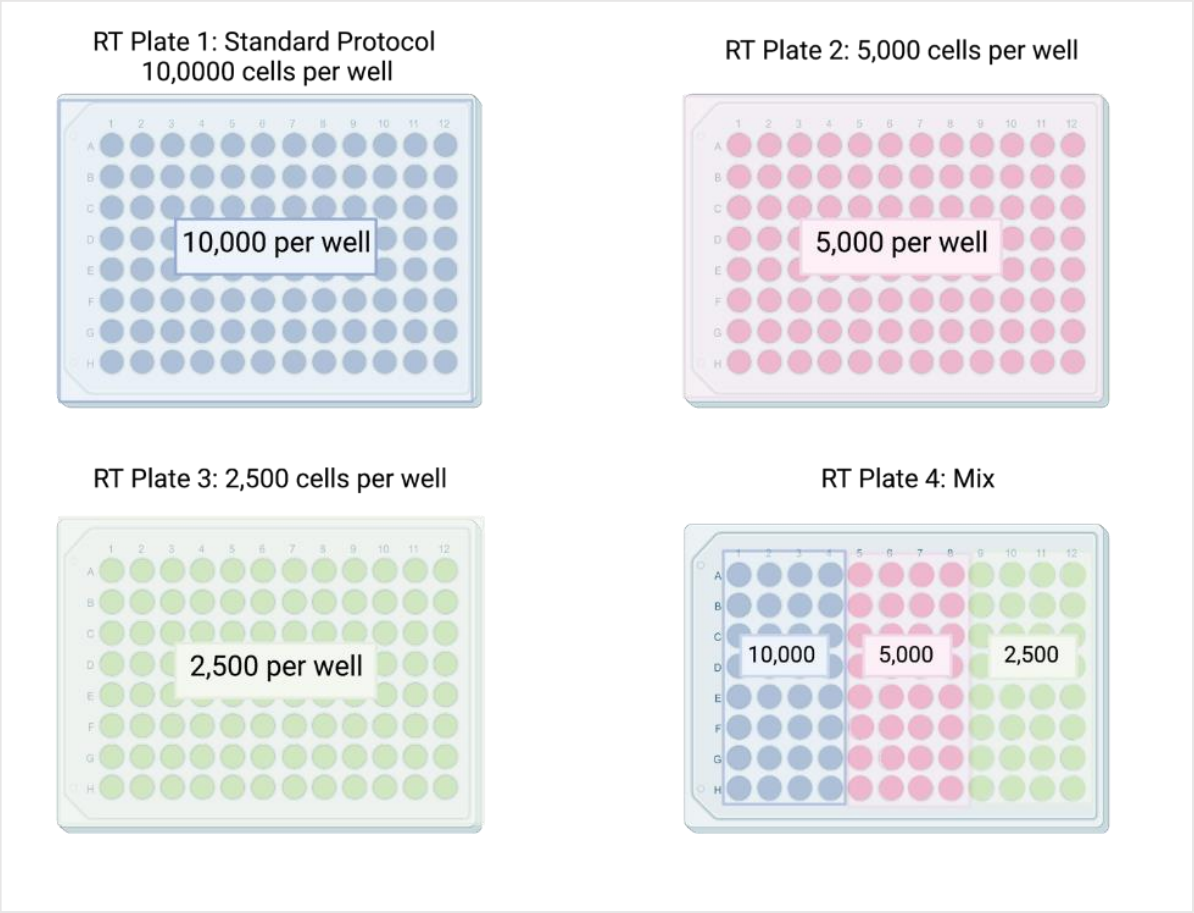
Introduction

Although the ScaleBio Single Cell RNA Sequencing Kit recommends an input of 10,000 cells per well of the initial (RT) Plate, sample and experiment limitations can make this difficult to achieve for all experiments. This Technical Note showcases the data quality observed when fewer than 10,000 cells and more than 10,000 cells were loaded into each well of the RT Plate compared to the standard recommended input.

Materials and Methods

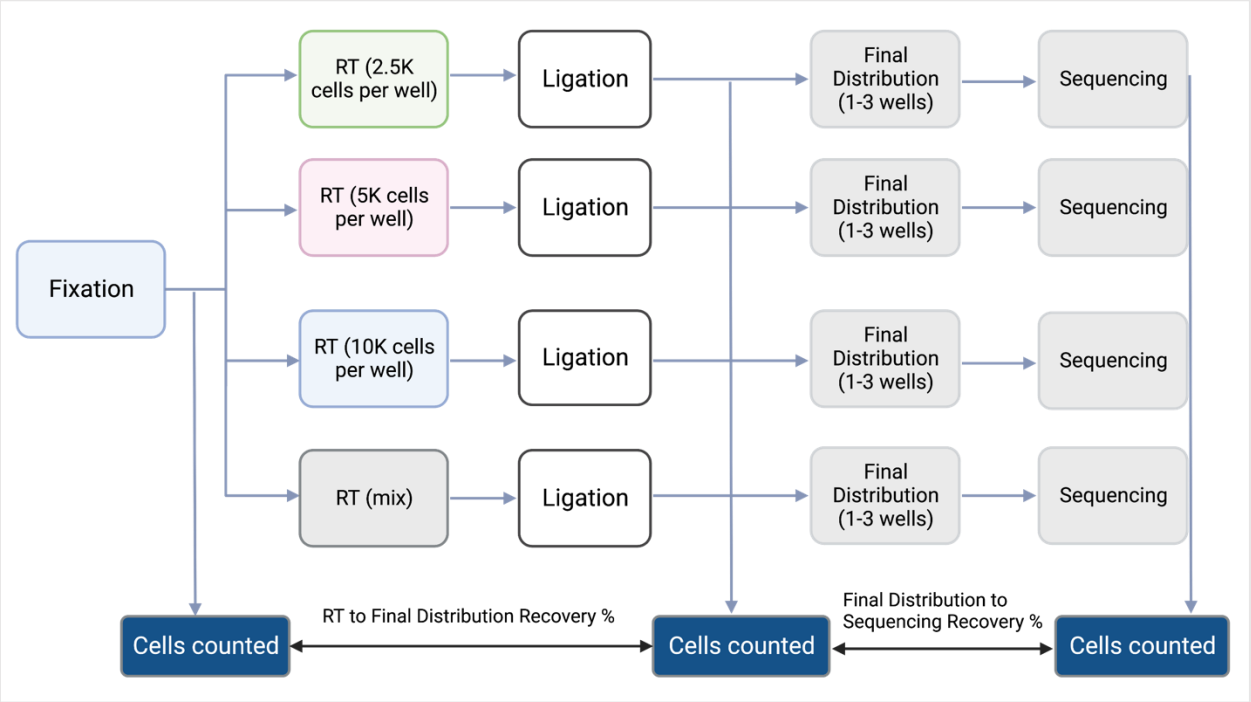
Peripheral Blood Mononuclear Cells (PBMCs) were fixed as a single sample using the ScaleBio Sample Fixation Kit protocol (RevB). To test low cell input, fixed cells were split across 4 plates and taken through the ScaleBio Single Cell RNA Sequencing Kit protocol (RevB). The cell input was varied according to the scheme shown in Figure 1, with the first plate using the recommended 10,000 cells/well, the 2nd and 3rd plates using 5,000 and 2,500 cells, respectively, and the final plate mixing inputs across the plate.

Figure 1: Layout of each of the four RT plates used for low cell input.



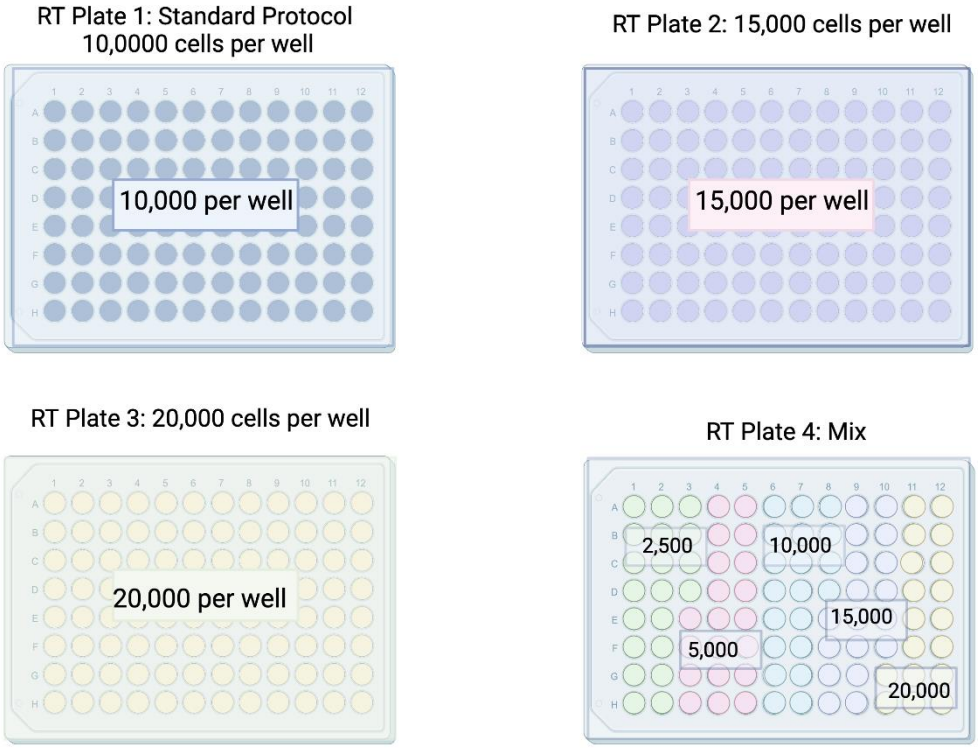
A subset of wells from the Final Distribution plate were then taken through sequencing, for a final expected yield of approximately 1,500-4,500 cells from each initial RT Plate (Figure 2). Library quality, sequencing metrics, and data quality were then compared across inputs. This was performed twice, and the results were averaged across both replicates.

Figure 2: Full plate workflow for low cell input.



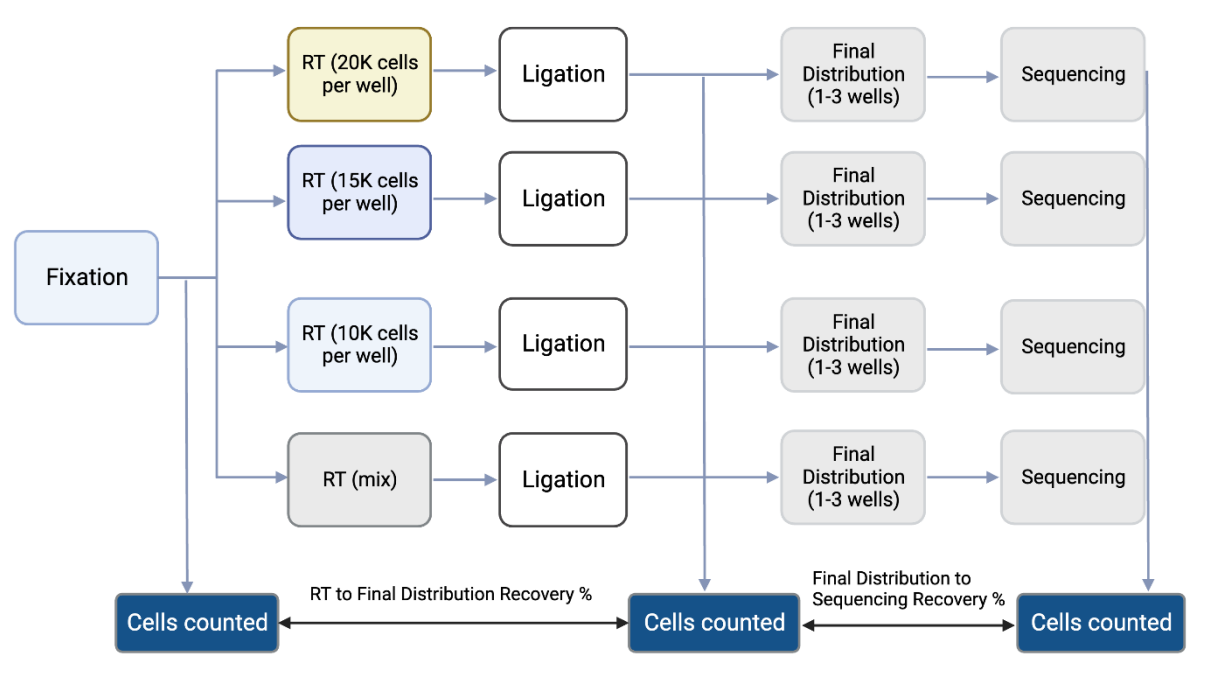
Similarly, to test high input greater than 10,000 cells/well, fixed cells were split across four plates and taken through the ScaleBio Single Cell RNA Sequencing Kit protocol (RevB). The cell input was varied according to the scheme shown in Figure 3, with the first plate loaded with the recommended input of 10,000 cells/well, the second and third plate with higher input amounts of 15,000 and 20,000 cells/well.

Figure 3: Layout of each of the four RT plates used for high cell input.



Cells were quantified post-ligation. A subset of cells from each RT plate were plated onto a Final Distribution plate at a concentration of 1600 cells/well and then taken through sequencing. Library quality, sequencing metrics, and data quality were then compared across inputs.

Figure 4: Full plate workflow for low cell input.

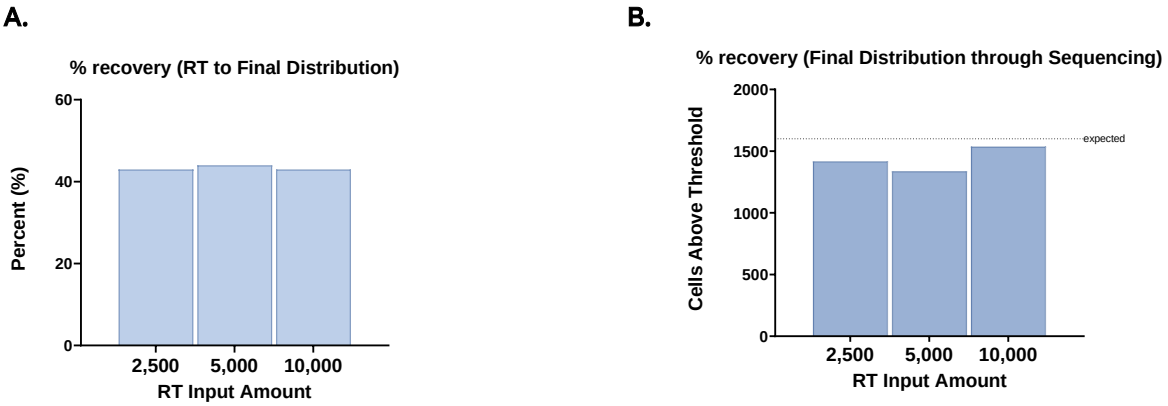


Results

Low Cell Input

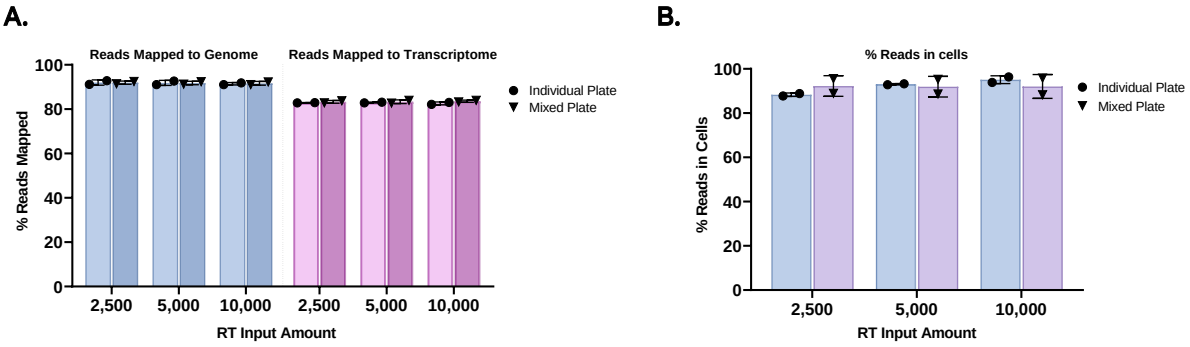
When testing low cell input into the RT plate, good cell recovery was observed throughout pooling/centrifugation for all cell inputs, with no substantial drop in cell recovery when lower cell input was used (Figure 5A). The percentage of cells loaded into the Final Distribution Plate that were observed in sequencing was also similar across cells from all input plates (Figure 5B). Together, these results suggest that with careful handling a similar proportion of cells put into the workflow should be recoverable in the final library regardless of lower cell input.

Figure 5: (A) Percentage of cells loaded into the RT Plate that were recovered before going into the Final Distribution Plate. (B) Percentage of cells loaded into the Final Distribution plate that were observed in the sequencing data. Data from replicate A shown for both figures.



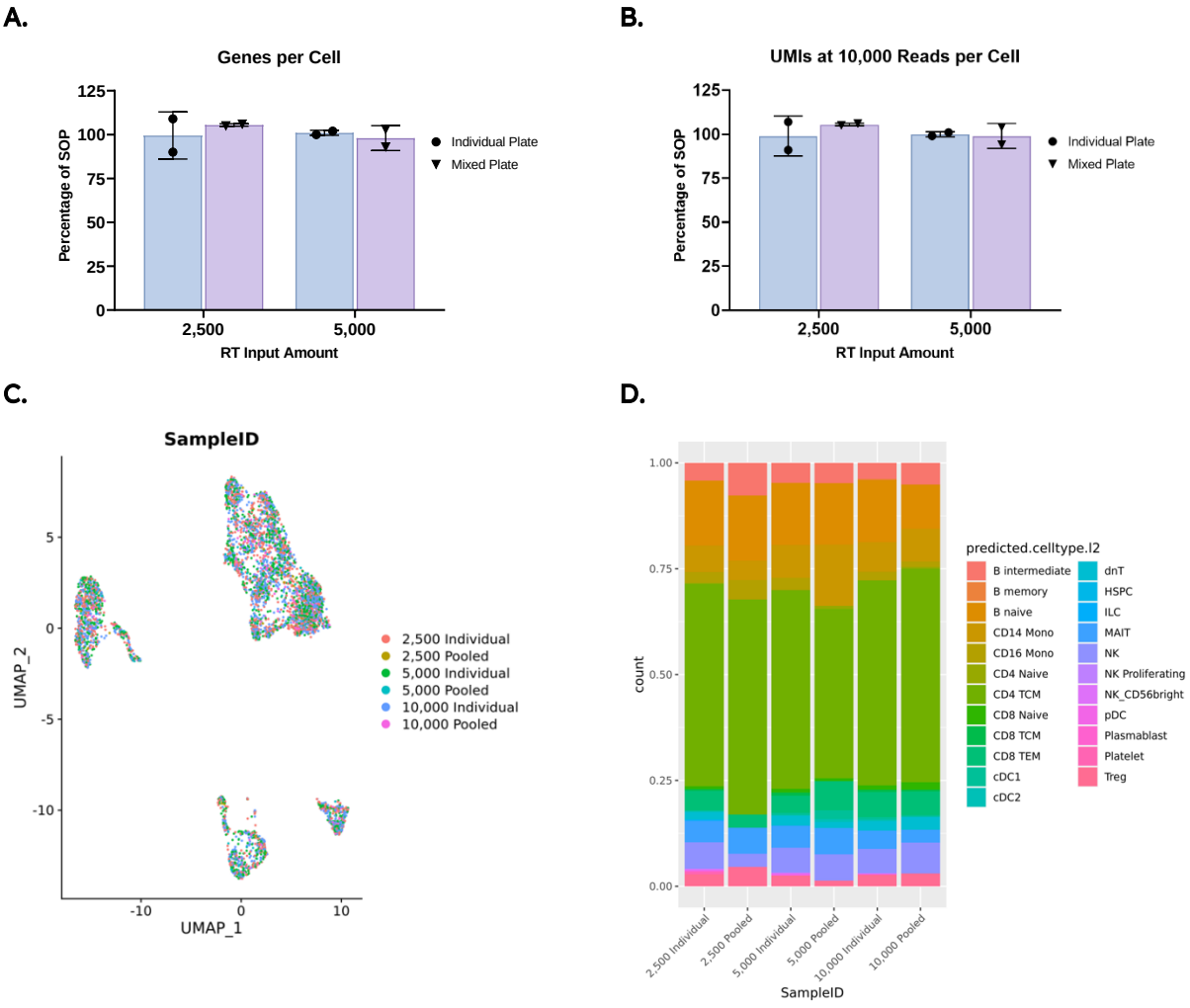
To determine if library quality or background was impacted by the RT cell input, we analyzed the percentage of usable reads. Analysis showed no change in the percentage of reads mapping to the genome or transcriptome as cell input decreased (Figure 6A). Although a slight decrease in the percentage of reads in cells is observed, this drop was marginal with a <10% drop observed from 10,000 to 2,500 cell inputs. (Figure 6B). Taken together, this uniformity suggests that lowering cell input does not increase technical artifacts or wasted sequencing reads.

Figure 6: (A) Percentage of reads mapping to the genome and transcriptome across cell input plates. Bars show the average of two experiments (whiskers +/- standard deviation) with individual datapoints shown as circles or triangles. (B) Percentage of reads associated with cell barcodes called as real cells. Bars show average of two experiments (whiskers +/- standard deviation) with individual datapoints shown as circles or triangles.



Finally, to determine if sensitivity was impacted by the RT cell input, we examined the genes and transcripts recovered across cell inputs. For each experiment, raw gene and UMI counts from the 2,500 and 5,000 cell input plates were normalized to recovery in the control (10,000 cell input) and averaged. Results show no pattern of decreasing sensitivity as cell input decreases, though variability may increase with lower cell inputs. (Figure 7A-B). Seurat analysis of these data show no batch effects (Figure 7C) and similar proportions of each cell type recovered from different RT cell inputs (Figure 7D).

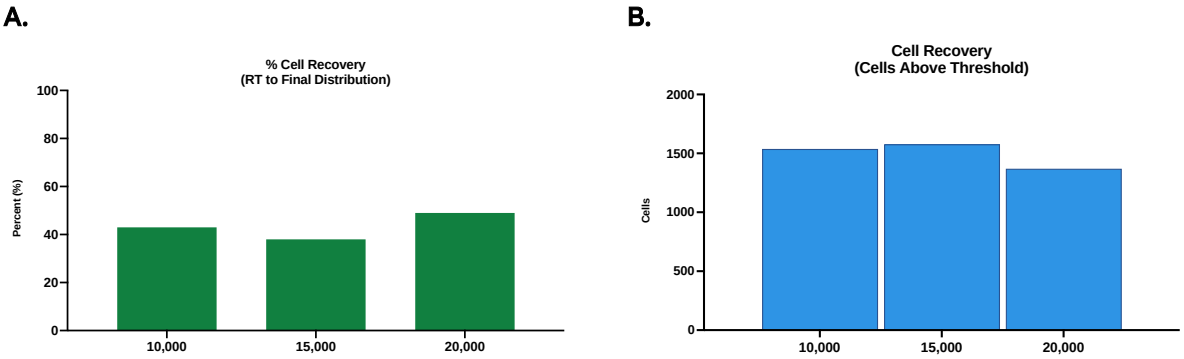
Figure 7: (A-B) Normalized gene and UMI recovery across cell inputs into RT Plates. Number of UMIs and genes for each sample was normalized to the UMI and gene recovery for the control (10,000 cell input) for each sample. Bars show average of two experiments (whiskers +/- standard deviation) with individual data points shown as circles or triangles. (C) UMAP projection was generated using Seurat with each cell colored based on the RT Plate from which it originated. (D) Cell types were identified using Azimuth "reference-based mapping". The proportion of cell types identified from each RT Plate is plotted.



High Cell Input

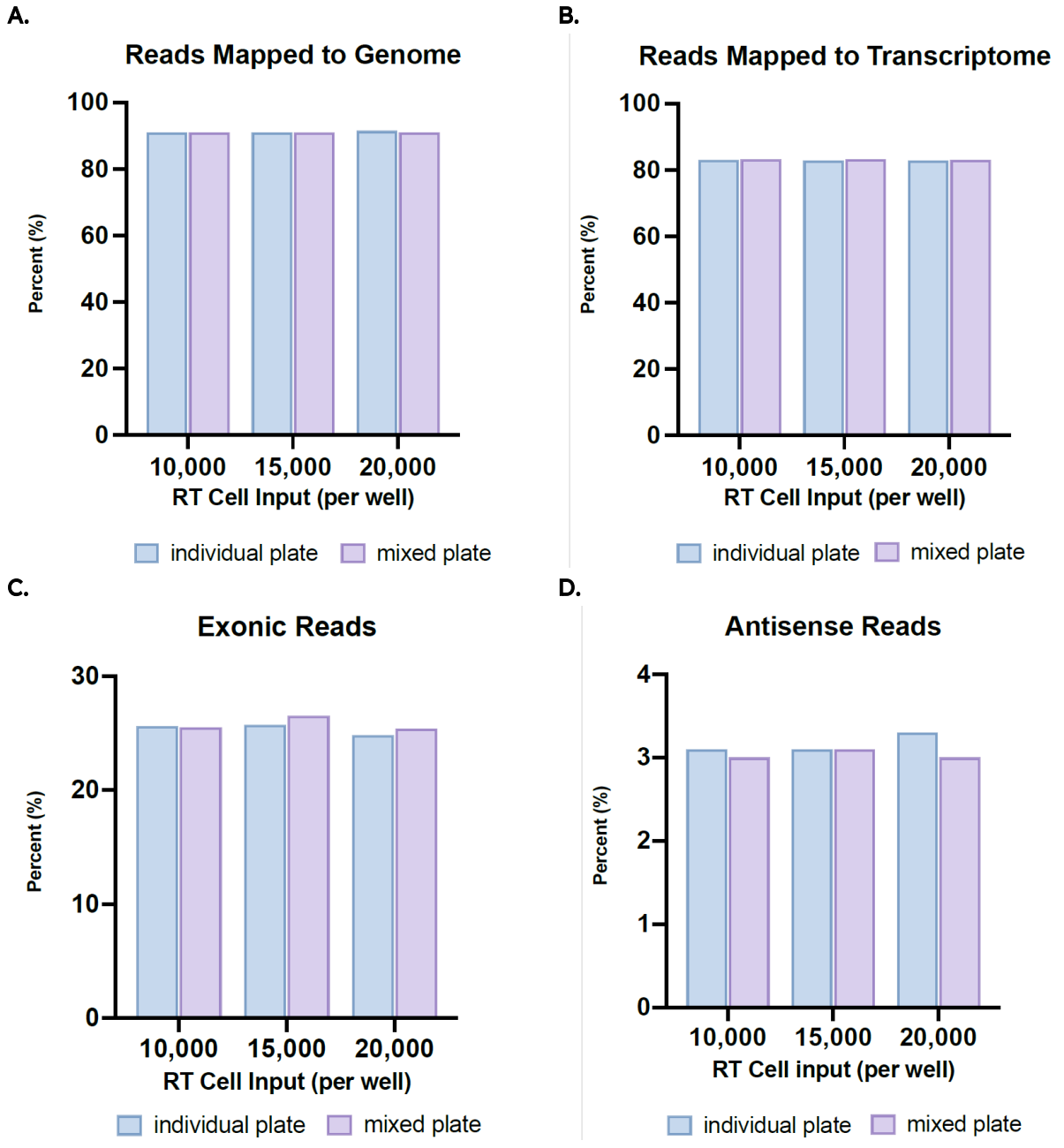
With higher cell inputs, good cell recovery was observed in all conditions throughout pooling/centrifugation, with no substantial drop in percent cell recovery when higher cell input was used. The number of cells recovered from each well of the Final Distribution Plate and observed in sequencing was also similar across cells from all input plates. Together, these results suggest that with careful handling a similar proportion of cells put into the workflow should be recoverable in the final library regardless of cell input.

Figure 8: (A) Percentage of cells loaded into the RT Plate that were recovered before going into the Final Distribution Plate. (B) Number of cells per well of the Final Distribution plate that were observed in the sequencing data.



Analysis showed no change in the percentage of reads mapping to the genome or transcriptome as cell input increased. This uniformity suggests that increasing cell input does not increase technical artifacts or wasted sequencing reads.

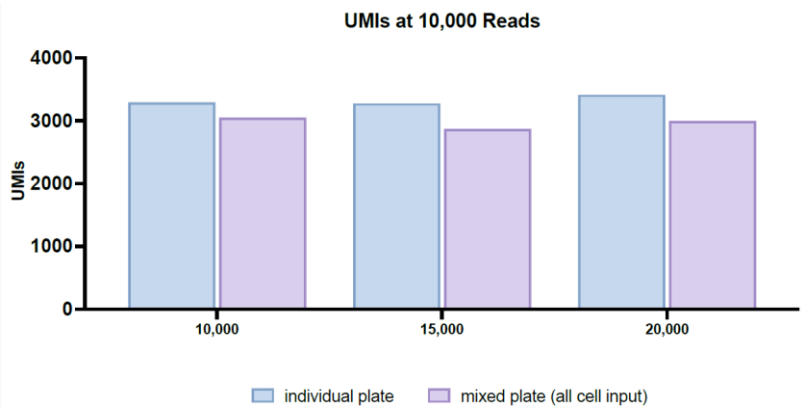
Figure 9: (A-B) Percentage of reads mapping to the genome and transcriptome across cell input plates. (C-D) Percentage of exonic reads and antisense reads across cell input plates.



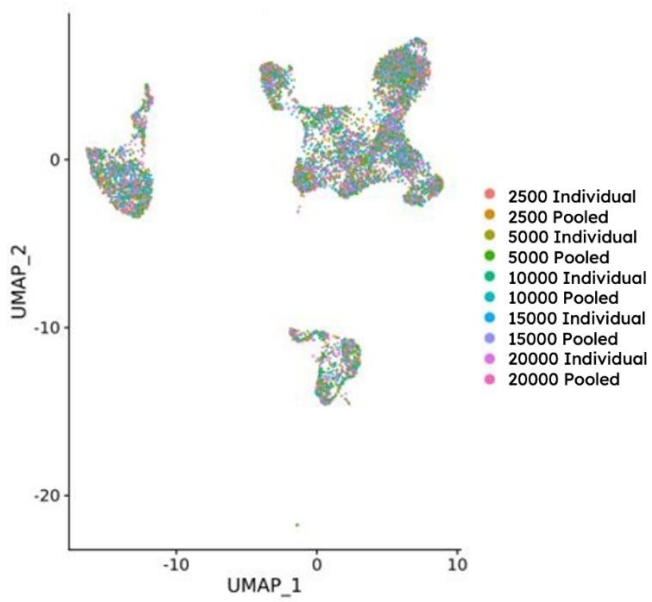
To determine if sensitivity was impacted by the RT cell input, the transcripts recovered across cell inputs were examined. The full plates of higher cell inputs had a UMI count within 10% of standard input. Results show no pattern of decreasing sensitivity as cell inputs decrease or increase.

Figure 10: (A) UMI recovery across cell inputs into RT Plates. Number of UMIs for each sample was compared to the UMI recovery for the control (10,000 cell input). (B-C) UMAP projection was generated using Seurat with each cell colored based on the RT plate from which it originated.

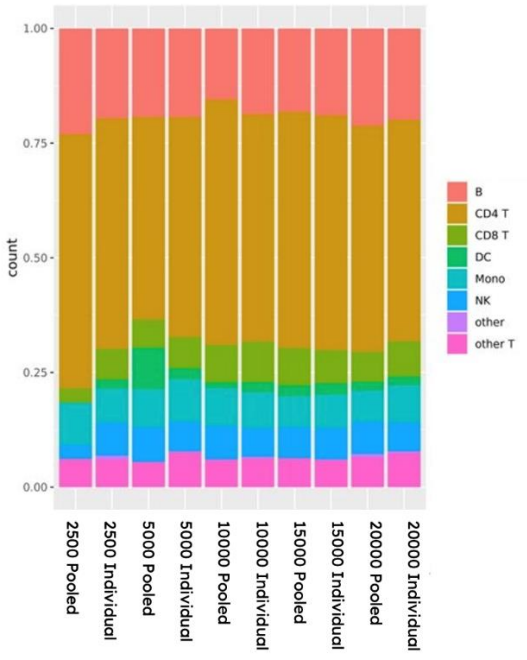
A.



B.



C.



Conclusions

- ScaleBio recommends starting the assay with 5,000-10,000 cells per well of the RT Plate for best assay performance.
- Lower cell inputs into the RT Plate may lead to a slightly lower percentage of reads mapping to cells.
- With careful handling, similar cell recovery across all pooling steps can be achieved even with lower cell inputs.
- Recovery is comparable for low and high cell input from RT to final distribution.
- Cell recovery from final distribution for the individual plates are within acceptable limits of the expected recovery.
- Recommended to use approximately the same number of cells per well in the RT plate as mixed cell quantities must be investigated and characterized further.

Note: Given expected recovery rates from RT through Final Distribution, ScaleBio does not recommend loading 2,500 cells per well across a full RT plate, as this will not yield enough cells to run the assay. Data shown here suggests that decreasing the cell input for a small subset of samples on the RT Plate should result in similar quality data as running the assay at recommended cell loads.

Document Revision History

Revision	Revision Date	Document ID	Changes
Rev A	Mar 2024	1020808	Initial release.