SCALE biosciences
CELL TO INSIGHT

ScaleBio™ Technical Note

Loading fewer than 10,000 cells per well

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 were loaded into each well of the RT Plate.

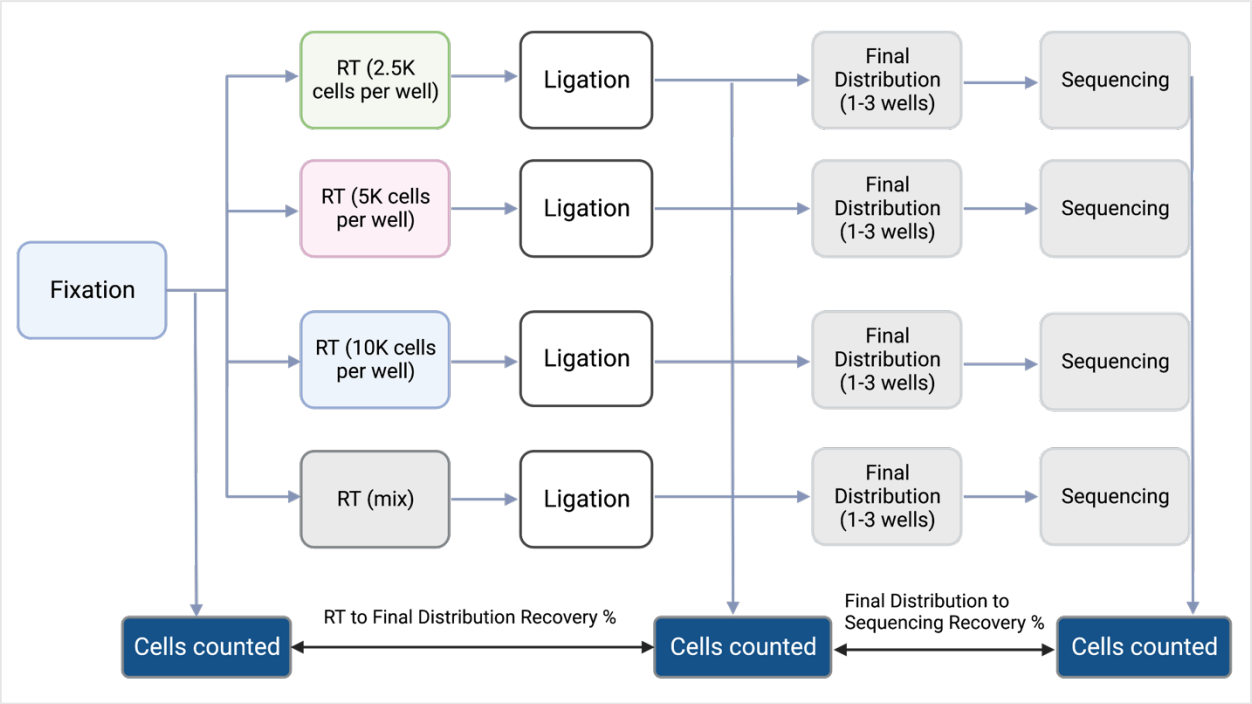
Methods

Peripheral Blood Mononuclear Cells (PBMCs) were fixed as a single sample using the ScaleBio Sample Fixation Kit protocol (RevB). Fixed cells were then 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.

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



Results

In both replicates 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 3A). The percentage of cells loaded into the Final Distribution Plate that were 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 lower cell input.

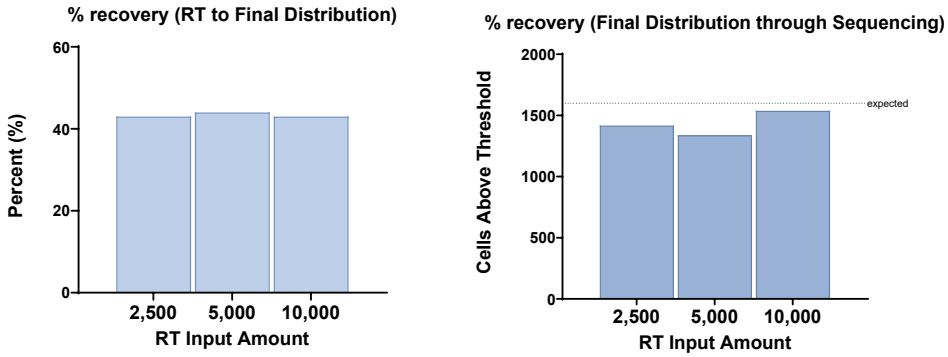


Figure 3: (A) Percentage of cells loaded into the RT Plate that were recovered before going into the Final Distribution Plate. (B) Percentage of cells loaded into the Final Distribution plate that were observed in the sequencing data. Data from replicate A shown for both figures.

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 4A). 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 4B). Taken together, this uniformity suggests that lowering cell input does not increase technical artifacts or wasted sequencing reads.

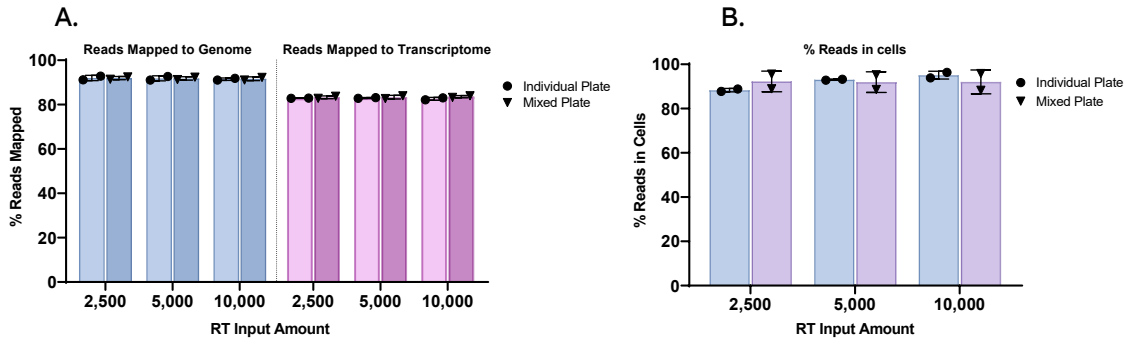


Figure 4: (A) Percentage of reads mapping to the genome and transcriptome across cell input plates. Bars show the average of two experiments (whiskers +/- standard deviation) with individual datapoints shown as circles or triangles. (B) Percentage of reads associated with cell barcodes called as real 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 5A-B). Seurat analysis of these data show no batch effects (Figure 5C) and similar proportions of each cell type recovered from different RT cell inputs (Figure 5D).

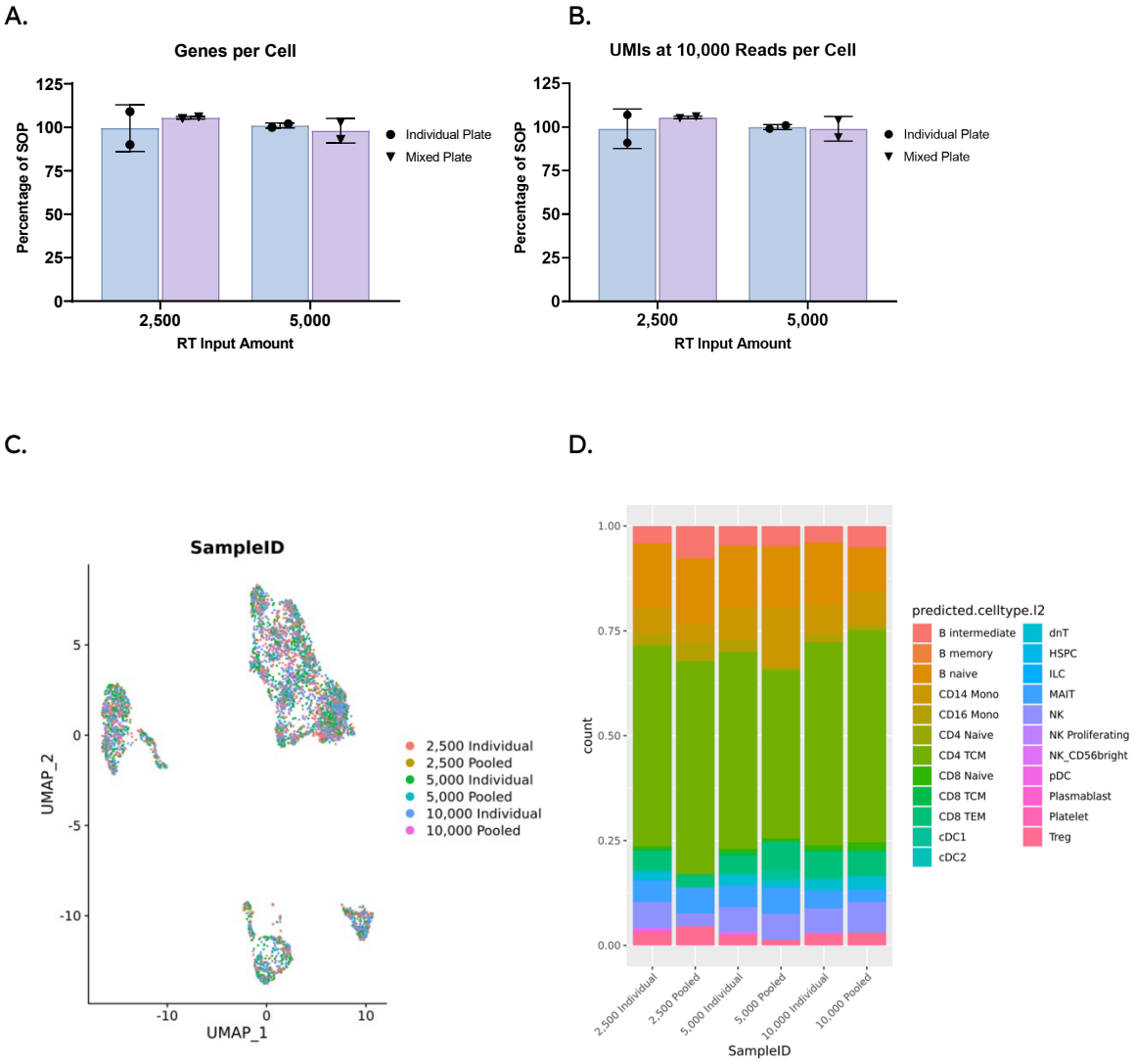


Figure 5. (A-B) Normalized gene and UMI recovery across cell inputs into RT Plates. Number of UMIs and genes for each sample was normalized to the UMI and gene recovery for the control (10,000 cell input) for each sample. Bars show average of two experiments (whiskers +/- standard deviation) with individual 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.

Conclusions

- Decreasing cell inputs in the RT Plate to 2,500 cells per well does not result in decreased sensitivity and does not create any technical artifacts that could lead to batch effects or the appearance or loss of specific cell populations.
- Lower cell inputs into the RT Plate may lead to a slightly lower percentage of reads mapping to cells.
- With **careful handling** similar cell recover across all pooling steps can be achieved even with lower cell inputs.

Note: Given expected recovery rates from RT through Final Distribution, ScaleBio recommends an input of >350,000 total cells into the RT Plate to utilize the full throughput of the kit (125,000 cells going into Final Distribution). Delicate samples or those that do not show good recovery during spins may require higher inputs.