10x GENOMICS®

TECHNICAL NOTE

Importance of Cell Stock Concentration for Accurate Target Cell Recovery

INTRODUCTION

10x Genomics® Single Cell Protocols require suspensions of viable, single cells (*Single Cell Protocols – Cell Preparation Guide* - CG00053). Accurate counting and assessment of cell viability are required as part of the workflow outlined in the Single Cell Protocols (*Chromium™ Single Cell 3' Reagent Kits v2 User Guide* – CG00052, *Chromium™ Single Cell V(D)J Reagent Kits User Guide* – CG000086) and are essential to determine the number of cells loaded onto the microfluidic chip for single cell partitioning. Manual (e.g. hemocytometer) and automated (e.g. Countess® II Automated Cell Counter) counting methods are common techniques to determine cell concentrations. A number of factors can affect the final cell count (Technical Note *Guidelines for Accurate Target Cell Counts Using 10x Genomics Single Cell Solutions* – CG000091). This Technical Note highlights cell stock concentration as one factor that can impact the accuracy of the final cell count and ultimately, the agreement between targeted and calculated cell recovery.

METHODS

We prepared four different single cell suspensions from HEK293T cells, each at the following cell concentrations:

- Cell Suspension #1: 340 cells/µl
- Cell Suspension #2: 500 cells/µl
- Cell Suspension #3: 780 cells/µl
- Cell Suspension #4: 1180 cells/µl

Samples were counted in four replicates with the Countess II Automated Cell Counter. For each single cell suspension, we prepared three Chromium Single Cell 3' v2 libraries with different target cell counts (1000, 3000 and 5000 cells) following the protocol outlined in the *Chromium Single Cell 3' Reagent Kits v2 User Guide* – CG00052 (see Table 1).

RESULTS

To assess the impact of different cell stock concentrations on cell recovery rates, we compared the *Estimated Number of Cells* reported by Cell Ranger with the targeted cell counts for each sample (see Figure 1). The difference between the targeted and calculated number of recovered cells is listed for each sample. Barcode Rank plots are also presented which illustrate the distribution of barcodes, ranked according to the number of UMIs that are associated with each barcode. Note that each cell partition is represented by a unique barcode. The y-axis represents the number of UMI counts that is associated with each barcode that is presented at the x-axis. Each barcodes are ranked from left to right based on the decreasing number of UMI counts associated for a given barcode. Cell-containing partitions are shown in green. Background partitions are shown in grey.

Cells Targeted	Volume Added to RT Master Mix	Cell Suspension #1 (340 cells/µl)	Cell Suspension #2 (500 cells/µl)	Cell Suspension #3 (780 cells/µl)	Cell Suspension #4 (1180 cells/µl)
1000	Cells	5.1 µl	3.5 µl	2.2 µl	1.5 µl
	Water	28.7 µl	30.3 µl	31.6 µl	32.3 µl
3000	Cells	15.3 µl	10.4 µl	6.7 µl	4.4 µl
	Water	18.5 µl	23.4 µl	27.1 µl	29.4 µl
5000	Cells	25.5 µl	17.3 µl	11.2 µl	7.4 µl
	Water	8.3 µl	16.5 µl	22.6 µl	26.4 µl

Table 1. Cell suspension and water volumes used for different cell suspensions to achieve three different cell target recovery counts.

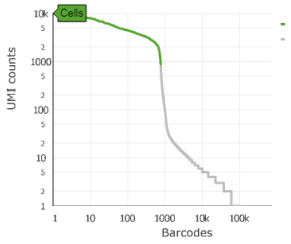
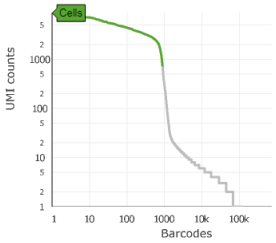
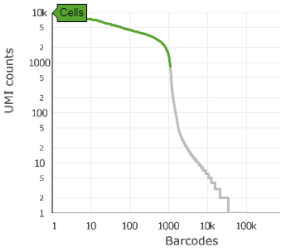
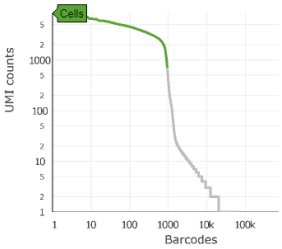
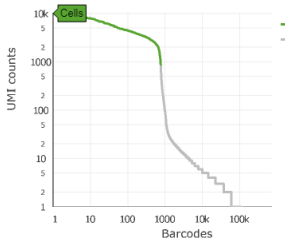
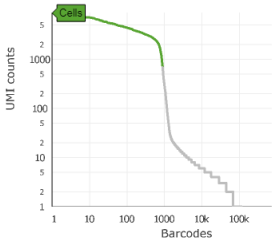
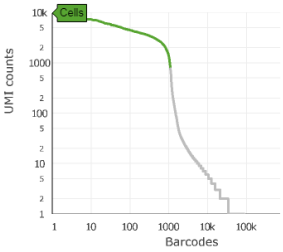
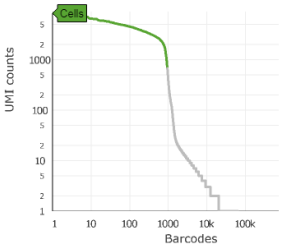
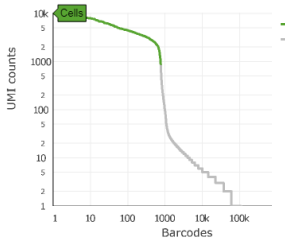
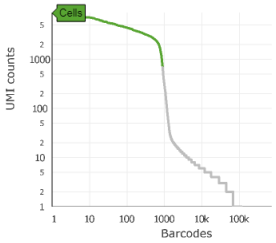
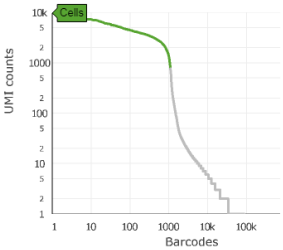
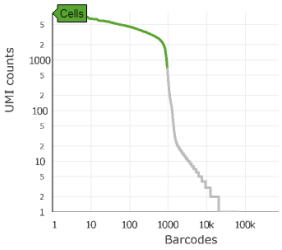
Cells Targeted	Cell Suspension #1 (340 cells/µl)	Cell Suspension #2 (500 cells/µl)	Cell Suspension #3 (780 cells/µl)	Cell Suspension #4 (1180 cells/µl)
1000				
	773 cells recovered Δ -227 cells	897 cells recovered Δ -103 cells	1104 cells recovered Δ +104 cells	952 cells recovered Δ -48 cells
3000				
	2503 cells recovered Δ -497 cells	2709 cells recovered Δ -291 cells	3445 cells recovered Δ +445 cells	3146 cells recovered Δ +146 cells
5000				
	4114 cells recovered Δ -886 cells	4601 cells recovered Δ -399 cells	5321 cells recovered Δ +321 cells	5136 cells recovered Δ +136 cells

Fig. 1. Ranked Barcode plots for all 12 libraries were prepared from four cell suspensions with different target cell counts.

Libraries were sequenced using paired-end sequencing (26bp Read 1 and 98bp Read 2) with a single sample index (8bp i7) on an Illumina® HiSeq® 4000 platform with approximately 5000 reads per cell. The resulting sequencing data was processed with Cell Ranger™ 2.0.

We determined the relative difference in the number of recovered cells calculated by Cell Ranger™ vs. the targeted cell counts for each library (Figure 2). Cell Suspensions #1 (340 cells/µl) and #2 (500 cells/µl) showed the greatest deviation from the targeted cell counts, 16 – 23% and 8 – 10% respectively. Target accuracy was improved with cell suspensions that were prepared at higher stock concentrations, Cell Suspensions #3 (780 cells/µl) and #4 (1180 cells/µl).

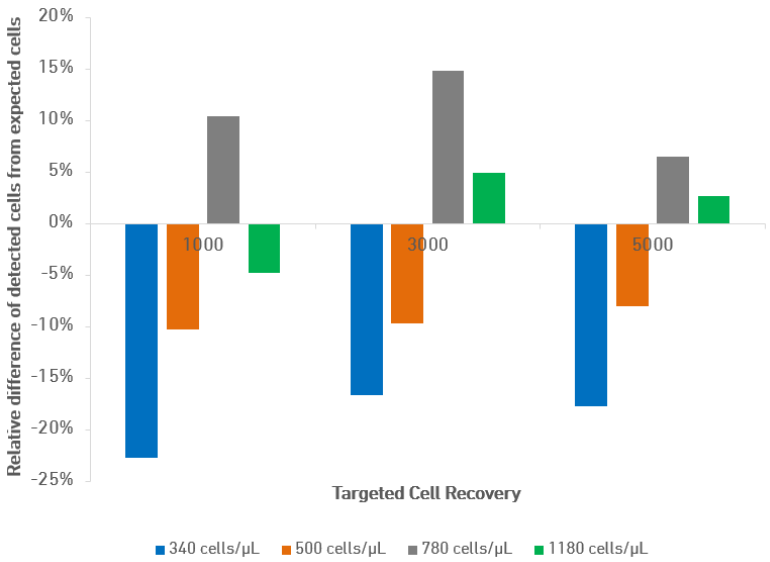


Fig 2. Relative difference of recovered cells from targeted cell counts.

The total number of cells required as input for 10x Genomics® Single Cell Solutions is determined by the User and their target for number of recovered cells. It is recommended that, for each assay, the input cell suspension be prepared to a concentration that allows pipetting of the desired number of cells in 2.5 µl to 15 µl. Transferring cell suspension volumes less than 2.5 µl increases variance due to pipetting inaccuracy, while using volumes larger than 15 µl increases the risk of introducing unwanted debris or inhibitors into the reactions.

We examined the relationship between the volume of single cell suspension added to the Master Mix for each Cell Suspension (#1-4) with the relative difference in the calculated recovered cells from the targeted number of cells (Figure 3). Expected cell recovery appeared positively correlated with both the input cell concentration and the volume of cells transferred into each reaction. Note that the lowest, targeted cell load (1000 cells) for Cell Suspension #4 required pipetting 1.5 µl into the Master Mix, which resulted in a 5% difference between the number of expected and recovered cells.

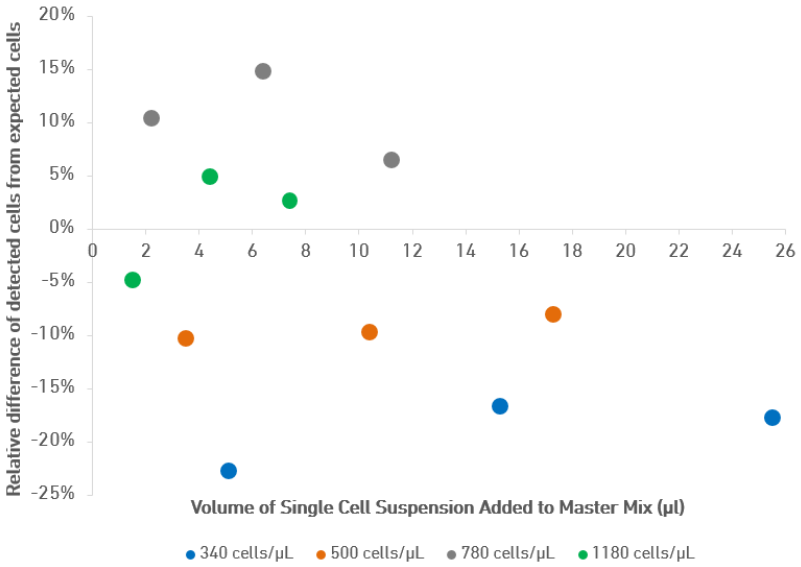


Fig. 3. Relative difference of recovered cells from expected cells as a function of the volume of single cell suspension added to the Master Mix.

DISCUSSION

Libraries were made from HEK293T cells at different cell stock concentrations. The impact of initial cell concentration on cell recovery targets was assessed in the analysis of sequencing data. To achieve the targeted number of recovered cells, the optimal input cell stock concentration was found to be between 700 and 1200 cells/µl. Cell suspensions that are outside this optimal concentration range may result in unreliable cell counts. If samples are outside this range, we recommend adjusting the cell stock concentrations accordingly. If the concentration is below this range, the number of cells counted may not accurately represent the total cell count. For example, if only 100 cells are counted, the variation can be as high as 10%. A 10% or higher variation in recovered cells will contribute to an unexpected deviation seen in the estimated number of cells reported by Cell Ranger™. If the concentration is above the range specified here the cells may be difficult to count.

CONCLUSION

Targeting the optimal cell number for analysis in 10x Genomics® Single Cell applications is dependent on multiple factors including the cell stock concentration. To minimize variability in cell counts loaded onto the microfluidic chip and to obtain an accurate recovered cell count, cell stock concentrations should be within the optimal range specified in this Technical Note. Adhering to the best practices described here will improve overall agreement of targeted cell counts and cell counts calculated by Cell Ranger.

REFERENCES

- *Chromium™ Single Cell 3' Reagent Kits v2 User Guide* (CG00052)
- *Single Cell Protocols – Cell Preparation Guide* - CG00053
- *Chromium™ Single Cell V(D)J Reagent Kits User Guide* (CG000086)
- *Guidelines for Accurate Target Cell Counts Using 10x Genomics® Single Cell Solutions* (CG000091)

Notices

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