10x Chromium Cell Suspension Sample QC

Date and Time Received: _____

Submitter and PI/Lab: _____

Project ID: _____

RTSF Genomics Core Summary:

Tube Label	Cell Conc. (Cells/µl)	Percent Viability (%)	Percent Aggregates (%)	Target Cell #	Volume Water for Library Input (µl)	Volume Cell Suspension for Library Input (µl)

MSU Genomics Core data based on manual hemocytometer counts using Trypan Blue viability staining.
*A corner square is outlined in red in the hemocytometer counting chamber schematic.
**Trypan Blue staining adds a factor of 2X.

☐ Slowly and gently mix cell suspension up and down with a wide-bore pipet tip 5 times. Aliquot 12µl of cells into a new tube using a narrow-bore pipet tip, taking the aliquot from the center of the suspension.

☐ Add an equal volume (12µl) 0.4% Trypan Blue solution to the aliquoted cells. Gently mix the dyed cells 5X with a wide-bore pipet tip (P200). Incubate at RT for 5 minutes and again mix gently pipetting up and down with a wide-bore pipet tip 5X. Trypan Blue is an exclusion dye; therefore, stained cells are not viable.

☐ Clean hemocytometer and cover slip with 70% ethanol and wipe dry with lens paper. Position the cover slip over the counting chamber.

☐ Pipette 10µl of Trypan Blue stained cell suspension into each chamber of the hemocytometer via the V-shaped well using a narrow-bore pipet tip. The area under the cover slip will fill by capillary action.

☐ Place hemocytometer on microscope stage and focus with low magnification (10X objective) first before increasing objective power, if required.

☐ Count all four corner squares of the hemocytometer grid, including the total number of cells (viable + non-viable), the non-viable cells alone (stained), and any cells forming aggregations (3 or more cells that appear closely associated, stuck together, or overlapping).

☐ Complete calculations to determine cell suspension concentrations, the percent viability, and the percent aggregation. If cell viability is <90% filter cell suspension with a Flowmi filter to help remove dead cells and cell debris and repeat the count. If the cell suspension concentration, viability, and aggregation is acceptable, proceed to determine input volume.

☐ Flowmi filtration may be completed by setting a P1000 to the cell suspension volume, aspirating the sample, and while the sample is in the tip, push the tip into a Flowmi tip strainer (this should take a force equivalent to the attachment of a regular tip). Slowly dispense the sample from the pipette into a new tube.

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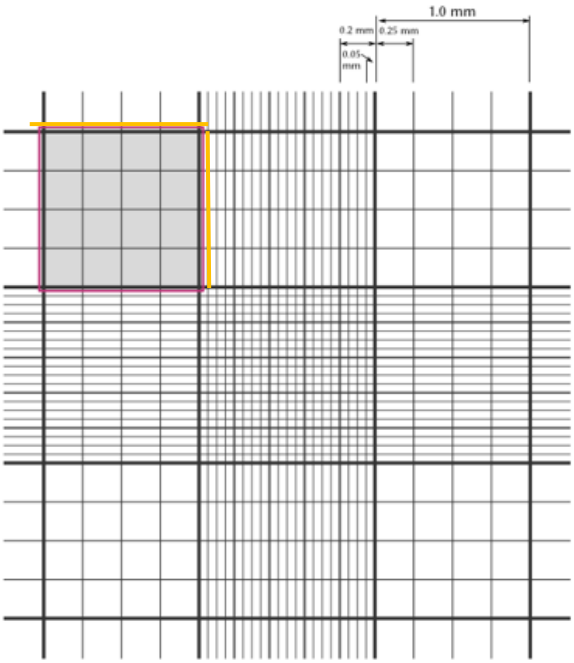
Count #: ____ Type: ☐ Disposable ☐ Standard Start Time: _____ End Time: _____



Total #: _____
Non-Viable #: _____
Aggregate #: _____

Total #: _____
Non-Viable #: _____
Aggregate #: _____

Total #: _____
Non-Viable #: _____
Aggregate #: _____

Total #: _____
Non-Viable #: _____
Aggregate #: _____

Only count top and right lines of any box. ***Non-viable cells will be stained blue.
Each corner square (red) is 1mm x 1mm x 0.1mm (depth) = $0.1mm^3 = 10^{-4}cc = 10^{-4}ml$

Total cell count (all 4 squares): ☐ Total # Non-viable cells (all 4 squares): ☐

$$\text{Total \# cells/µl} = \left(\frac{\text{Total cell count}}{\text{\# squares counted}}\right) \times \left(\text{Dilution factor}\right) \times 2 \times 10{,}000 \times \left(\frac{1}{1{,}000}\right)$$

_____ X _____ X 2 X 10,000 X $\frac{1}{1{,}000}$ = ☐

Total # viable cells = Total cell count - Total # Non-viable cells

_____ - _____ = ☐

$$\text{\% Viability} = \left(\frac{\text{Total \# viable cells}}{\text{Total cell count}}\right) \times 100$$

_____ X 100 = ☐

$$\text{\% Aggregates} = \left(\frac{\text{Total \# Aggregate cells}}{\text{Total cell count}}\right) \times 100$$

_____ X 100 = ☐

Volume (µl) cell suspension input: ☐ Volume (µl) water input: ☐