Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures

Introduction

Chromium Next GEM technology enables profiling the whole transcriptome of hundreds to tens of thousands of cells per sample. In combination with multiomic approaches such as Feature Barcode technology, CellPlex technology, and Immune profiling, a broad landscape of biological questions can be interrogated. This Technical Note highlights sample preparation best practices to minimize microfluidic chip clogs and wetting failures to enable optimal 10x Genomics Single Cell assay performance.

Single cell analysis using Chromium Next GEM technology is based on partitioning of barcoded gel beads and single cells in suspension into droplets called Gel Beads-in-emulsion (GEMs) using a microfluidic chip (Figure 1).

When running samples in the Chromium Controller or Chromium X/iX, a clog may occur. This is evidenced by recovering less than the expected volume of GEMs from the recovery well or an excess of Partitioning Oil in the recovered GEMs. If other samples are run in parallel, the clogged sample/bead wells may have uneven volumes of remaining reagents compared to other sample wells. Figure 2 shows representative images of a successful and failed GEM generation run.

Clogs are primarily caused by:

- Gel Beads clumping due to improper storage/handling
- Suboptimal sample preparations including mincing of tissue in plastic petri dishes
- Non-sterile work environments
- Use of unrecommended plasticware (i.e. pipette tips) that can introduce fibers into microfluidic channels

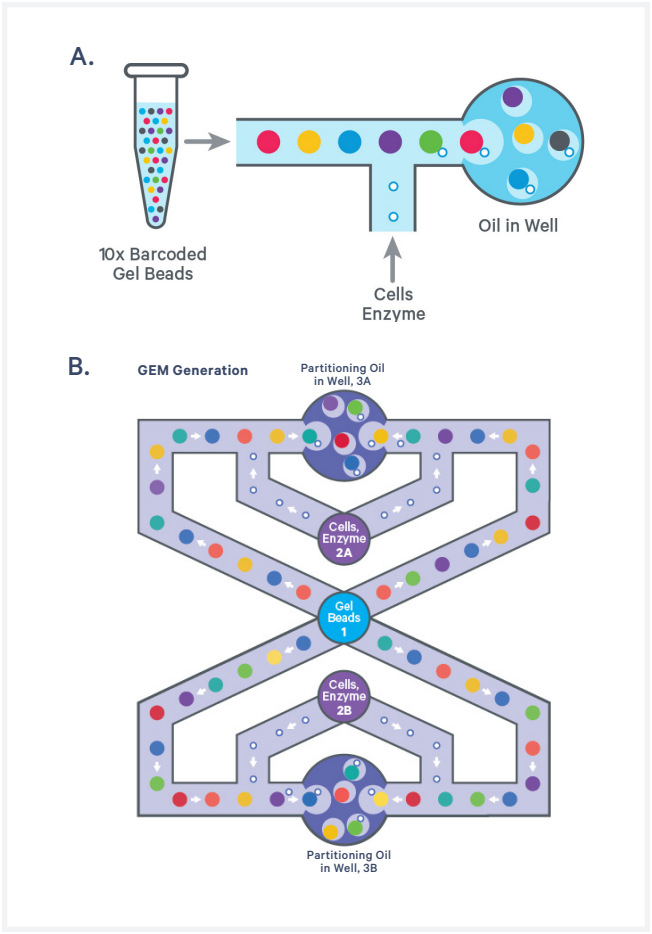


Figure 1. GEM generation in Chromium Next GEM chips (A) and Chromium Next GEM HT chips (B).

Gel Bead clogs are caused by improper storage or handling of Gel Beads. A clog in the Gel Bead line causes Gel Beads to flow slower, leaving an excess of Gel Beads behind.

Best practices to minimize Gel Bead clogs:

- Gel beads must be stored at -80°C (DO NOT store at -20°C)
- Ensure Gel Beads are completely thawed (30 min at room temperature), vortexed, and then centrifuged prior to loading
- Handle Gel Beads in a sterile environment
- Wear a lab coat to minimize exposure of reagents and chips to clothing that sheds fibers
- Store chips in an area free of dust

Sample clogs are caused by suboptimal sample preparation which can result in faster than usual depletion of Gel Beads, decreased cDNA yield, and decreased cell recovery. High quality single cell suspensions that lack large clumps, debris, and dead cells are recommended to minimize microfluidic clogs and wetting failures.

Best practices to minimize sample clogs:

- Avoid mincing tissue on plastic consumables (i.e. plastic petri dishes) to minimize generation of non-biological debris
- Remove large biological debris by using cell strainers/filters; visually inspect cells before loading the chip
- Remove debris while enriching for viable populations of interest using Fluorescence-Activated Cell Sorting (FACS)
- DO NOT overload chip with more cells than recommended for any supported application
- Minimize the volume of single cell suspension to reduce likelihood of debris carryover (i.e. increase the cell stock concentration)

Wetting failures are caused by incorrect priming of reagents in microfluidic channels, presence of surfactant or other contaminant in loaded reagents, or instrument/reagent temperatures outside of recommended ranges. Wetting failures can result in improperly formed GEMs leading to loss of partitioning of single cells and are recognized by the absence of a uniform GEM emulsion.

Best practices to minimize wetting failures:

- Add reagents in correct reagent well and in the correct order according to respective User Guide; wait 30 seconds between each reagent
- Avoid introducing air bubbles into the microfluidic chip through careful pipetting; eliminate pipette blowout when dispensing reagents in the chip
- Use only recommended plasticware
- Run Chromium Controller or X/iX between 18°C–28°C ambient lab temperatures
- Minimize chip handling after opening the package to avoid static buildup and minimize risk of contamination

Run chips promptly after loading. If a Gel Bead clog, sample clog, or wetting failure occurs during GEM generation, the sample should be rerun. For rare sample types, however, cDNA may be generated, but the yield and cell recovery will be lower.

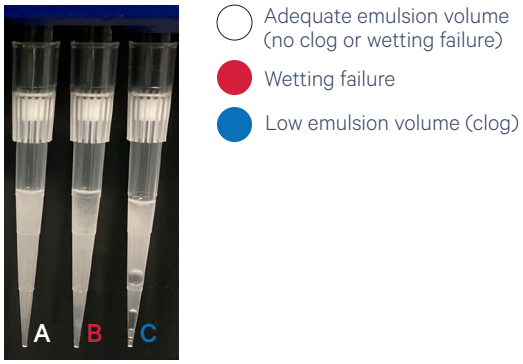


Figure 2. Representative images of clogs and wetting failures. Pipette tip A shows normal GEM generation, pipette tip B indicates a wetting failure, and pipette tip C shows a clog and wetting failure.

Tables 1–3 and Figures 3–11 highlight the key strategies along with single cell suspension images for minimizing clogs and wetting failures. Sample quality impacts both GEM generation and data quality. For additional considerations to improve sequencing data quality (e.g. minimizing ambient RNA), refer to the 10x Genomics Support website.

	Strain Cell Suspension	Slow Centrifugation	Cell Sorting	Dead Cell Removal	Density Gradient	Reduce Cell Volume into Master Mix	Use Glass Petri Dishes when Mincing Tissue
Large debris (>30 µm)	✓	⊘	✓	⊘	✓	✓	✓
Small debris (<30 µm)	⊘	✓	✓	⊘	⊘	✓	✓
Dead cells	⊘	⊘	✓	✓	⊘	✓	⊘

Table 1. Strategies to reduce the rate of Next GEM chip clogs and wetting failures.

Transfer optimal cell suspension volume

If possible, bring the input cell suspension to a concentration that is optimal for the dynamic range of the counting technique used (manual or automated), allows for at least 2 reproducible counts (3–4 preferred) where the standard deviation of these counts is <25%, and keeps the transfer volume of the cell suspension into the Single Cell Master Mix to less than 40% (and ideally less than 20%) of the overall reaction volume. Please note, pipetting cell suspension volumes <2.5 µl increases variance due to pipetting inaccuracy, while volumes >15 µl (for standard throughput assays and >30 µl for high throughput assays) increases the risk of introducing unwanted debris or inhibitors (e.g. Mg2+ and EDTA) into the GEM reaction. If loading low volumes, ensure to use appropriate pipettes (2 µl) that are calibrated.

Transfer Volume Example:

To optimally target 12,000 cells recovered in Chromium Next GEM Single Cell 3' with Feature Barcode technology for Cell Multiplexing, target a cell suspension concentration of 1,300–1,600 cells/µl (based on the Cell Suspension Volume Calculator Table). To further reduce the risk of introducing unwanted debris or inhibitors, a concentration of 1,500–1,600 cells/µl is recommended to keep the volume of cell suspension less than 20% of total reaction volume (15 µl for standard assays).

Removal of large debris from single cell suspensions

Filtering dissociated tissue and cell suspensions with cell strainers of appropriate pore size helps to remove cellular debris and aggregates. Depending on the degree of aggregation and the strainer type, the number of cells and the amount of wash solution retained in the strainer can vary (Table 2). The MACS SmartStrainer is recommended as it generally causes minimal changes to the cell concentration. However, a volume loss of 100 µl or more can occur. For low cell suspension volumes, the Flowmi™ Tip Strainer is recommended to minimize volume losses. Cell concentrations may decrease by up to 40%, depending on suspension volume and strainer type, as shown in Figure 3. It is therefore important to measure the concentration of the cell suspension before and after straining.

Strainer	Size	Bore	Average Volume Loss	Average Decrease in Concentration
Flowmi™ Cell Strainer	40 µm	Narrow	20.0 ± 9.4 µl	21 ± 6%
		Wide	45.0 ± 7.1 µl	31 ± 14%
MACS SmartStrainers	30 µm	Wide	130.0 µl ± 36.0 µl	3 ± 11%

Table 2. Comparison of recommended strainers.

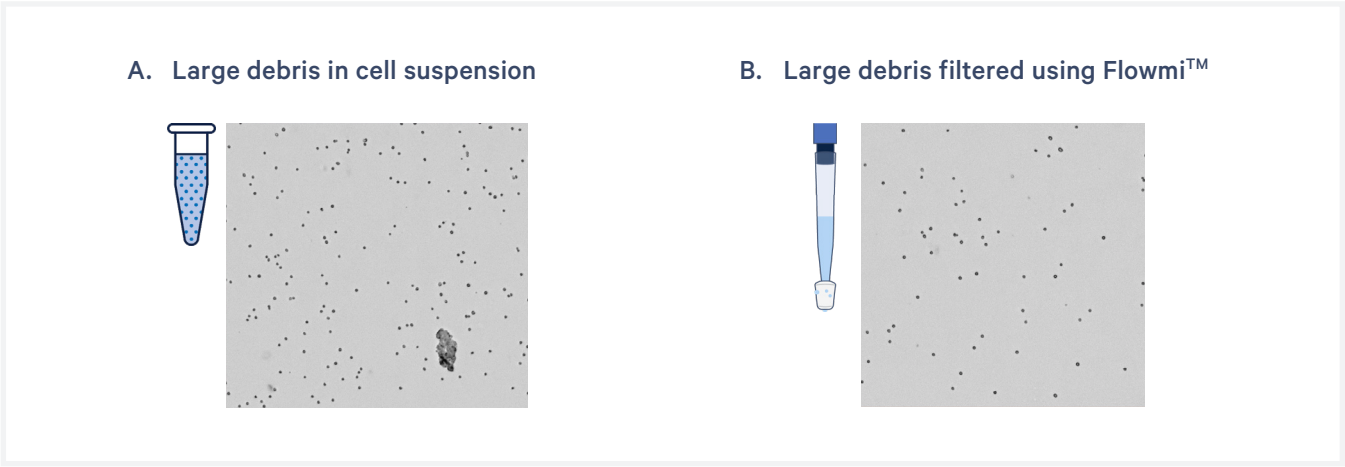


Figure 3. Single cell suspensions from embryonic mouse brains were obtained following the Dissociation of Mouse Embryonic Neural Tissue For Single Cell RNA Sequencing Demonstrated Protocol (CG00055) and labeled with cell multiplexing oligos following the Cell Multiplexing Oligo Labeling for Single Cell RNA Sequencing Protocols Demonstrated Protocol (CG000391). Cells were pooled, stained with AO/PI, and imaged in a Cellaca MX High-Throughput Automated Cell Counter (Nexcelom Bioscience). Viable cells fluorescing in the green channel are shown. False positive AO/PI staining may result from the large debris/cell cluster. Several instances of large clumps/debris were observed (A). To remove large clumps, samples were filtered with a 40 µm Flowmi™ Tip strainer; imaging showed a reduction in clumps and debris (B).

Removal of small biological debris from single cell suspensions

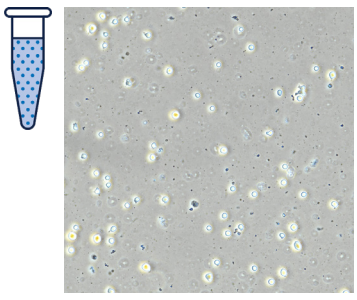
In addition to large clumps/debris, debris smaller than 30 µm may also impact efficient cell recovery/partitioning when transferring a large volume of cell suspension into Single Cell Master Mix (Table 3). Transferring >15 µL (for standard throughput assays) or >30 µL (for high throughput assays) of cell suspension into Single Cell Master Mix can increase the risk of introducing unwanted debris into the GEM reaction, leading to potential wetting failures. Removal of small biological debris is recommended in these cases.

Removing small debris is usually not feasible through filtration, but depending on the density of the cells and the nature of the debris, centrifugation may be an option (Figure 4). As a starting point, a single centrifugation in a swinging bucket rotor at 150 rcf for 10 min is recommended to pellet the cells and to keep the small debris remaining in the supernatant. Centrifugation times and speeds may need to be optimized depending on sample type. Smaller cells may require faster speeds and/or longer times to ensure cells are pelleted sufficiently while debris remains in the supernatant.

		Relative Volume of Cell Suspension Transferred to Single Cell Master Mix	
		Low Volume	High Volume
Relative Level of Debris Present in Cell Suspensions	Minimal debris	✓	✓
	Elevated debris	✓	⊘

Table 3. Transferring a high volume of cell suspension with elevated levels of debris into Single Cell Master Mix is NOT recommended.

A. Small debris in cell suspension



B. Small debris removed using MACS filter + slow centrifugation

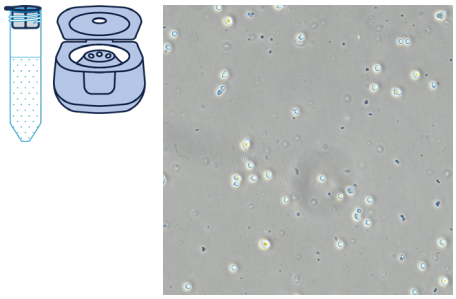


Figure 4. Single cell suspensions were imaged on a Nikon Eclipse Ts2R microscope with a 20x objective (A). Both large and small debris were observed. Cells were filtered through a 30 µm MACS SmartStrainer (to remove large debris) and centrifuged in a swinging bucket rotor at 150 rcf for 10 minutes (to remove small debris). After resuspension of the cells in fresh PBS + 0.04% BSA, both large and small debris in the cell suspension were reduced (B).

Flow-based removal of dead and dying cells

The presence of large amounts of dead cells can impact microfluidic performance. If sample concentration and volume allows, removal of non-viable cells/debris with a fluorescence-activated cell sorter (FACS) is recommended (Figures 5-6). 7-aminoactinomycin D (7-AAD) associates with DNA in cells with compromised cell membranes and can be used to label and remove non-viable and partially dying cells (7-AAD+). Other live/dead stains may also be used to enrich for viable cell populations of interest in single cell suspensions. After sorting cell suspensions, it is strongly recommended to recount cells prior to loading chips.

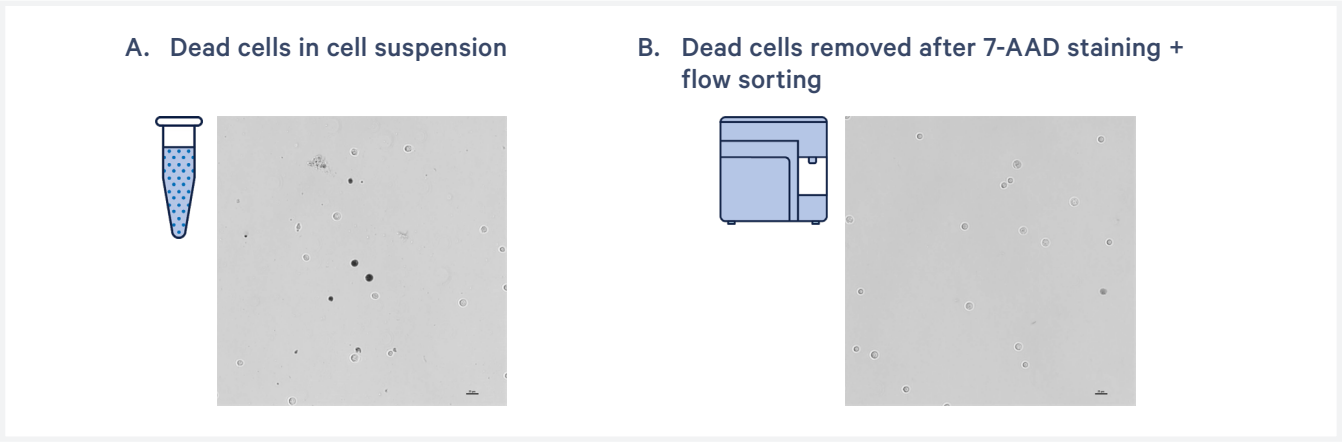


Figure 5. A single cell suspension was stained with Trypan Blue and viability was determined to be ~54% using the Countess II Automated Cell Counter (Thermo Fisher Scientific). The cell suspension was imaged on a Nikon Eclipse Ts2R microscope with a 20x objective and abundant dead cells and debris were observed (A). To remove dead cells and debris, cells were stained with 7-AAD and 7-AAD negative cells were selected using an MA900 Multi-Application Cell Sorter (Sony Biotechnology). Removal of dead/dying cells and debris resulted in a final sample viability of 93% post sort (B).

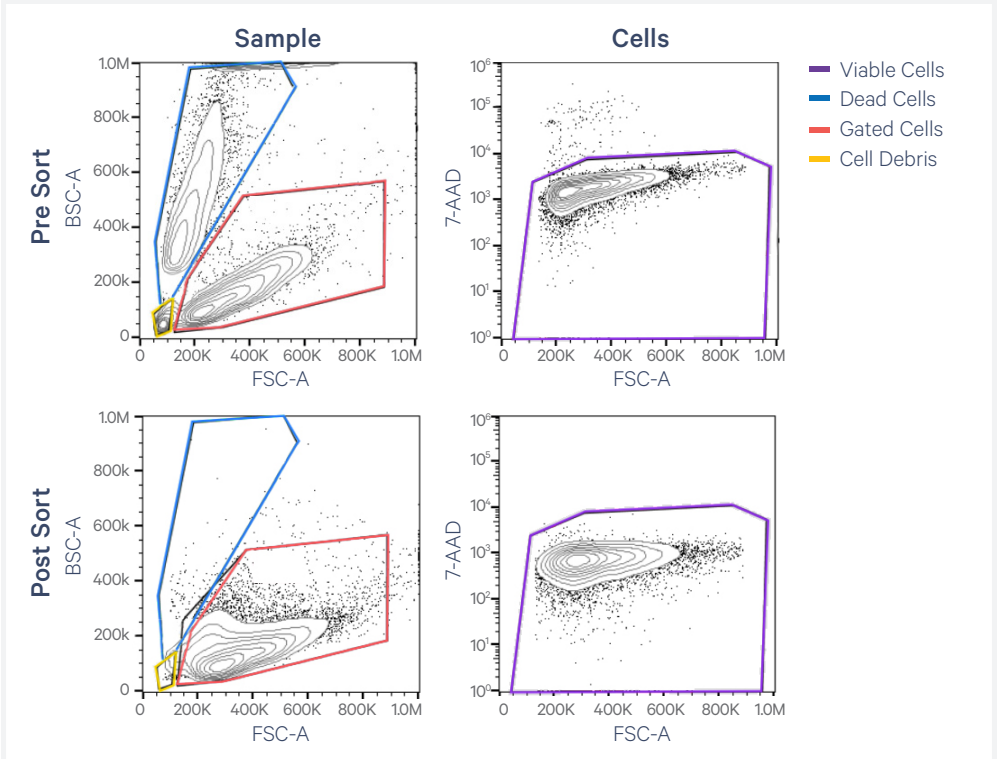


Figure 6. Flow gating scheme. Forward and backward scatters (FSC and BSC) were used to define cell size and granularity (viable/dead) and debris (left plots). The characterized cell population (red) was further narrowed down into viable and dead/dying cells by using the dead cell marker 7-AAD. Viable cells (purple) were sorted/collected and post sort reanalyzed (bottom plots). Sorted samples were composed of 93% viable cells, 2% dead cells, and 2% debris (accounting for a sort efficiency of >90%).

Bead-based removal of dead or dying cells

Dead and dying cells may leak their contents, making them sticky, which in turn can cause cell clumping and aggregation. If flow cytometry is unavailable, a Dead Cell Removal Kit can be utilized to remove dead cells. Figure 7 delineates the dead cell removal process using the Miltenyi Dead Cell Removal Kit (PN-130-090-101). Dead Cell Removal Annexin V MicroBeads recognize a phosphatidylserine moiety in the plasma membrane of apoptotic and dead cells. During dead cell depletion, dead cells are labeled with these magnetic MicroBeads and are passed through a separation column. The magnetically labeled dead cells are retained within the column. The unlabeled living cells run through; this cell fraction is thus depleted of dead cells and can be used in Single Cell protocols. Representative images of cell suspensions before and after dead cell removal are highlighted in Figure 8.

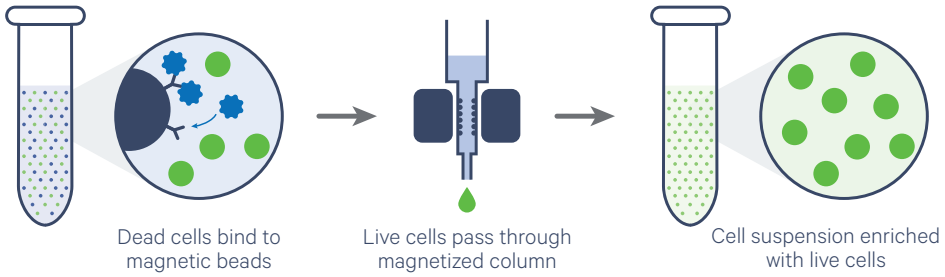


Figure 7. Dead cell removal process.

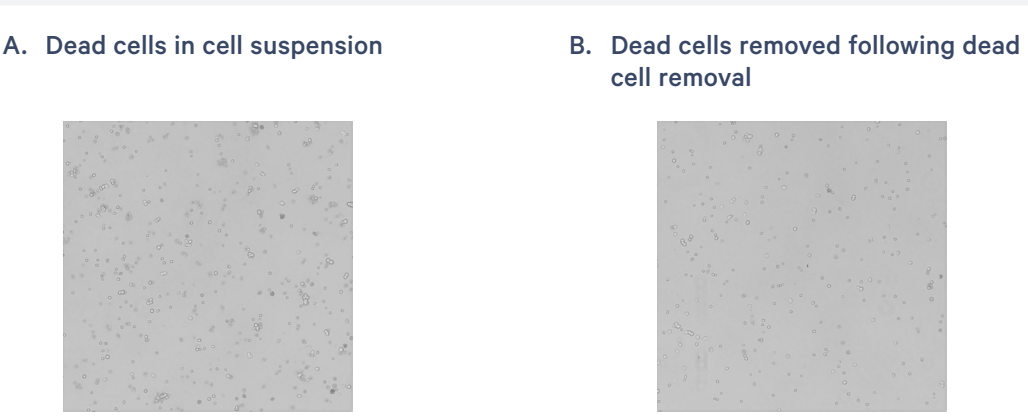


Figure 8. Dissociated tumor cells (DTCs) from human kidney were thawed, stained with Trypan Blue, and counted with a Countess II Automated Cell Counter (Thermo Fisher Scientific). Viability was determined to be 63%. Trypan Blue-stained cells were also imaged on a Nikon Eclipse Ts2R microscope (A). The Miltenyi Dead Cell Removal Kit was used and cell viability was increased to 82% (B).

Impact of solid debris on GEM generation

Solid debris in the cell suspension can impede the flow of GEM generation. Small debris can pass through 35 µm filters and accumulate in microfluidic channels, impacting GEM generation. A vast majority of solid debris does not originate from reagents, buffers, or chips. However, sample preparation that employs mechanical dissociation (e.g. tissue mincing, crushing, or scraping) can generate solid plastic debris when the scalpel, blade, or scissors come in contact with and scrape plastic consumables (i.e. cell culture dishes) (Figure 9).

Solid debris generated during mincing steps may lead to an elevated clogging rate, and the amount of plastic debris in the cell suspension correlates with both the rate and severity of the clog. Depending on the severity, clogs may result in a reduced emulsion volume and fewer GEMs recovered. Generation of solid debris during mincing on plastic consumables is highly variable and depends on parameters chosen by each user (e.g. the pressure on the plastic, the number of strokes used, and length of time of the dissociation).

To minimize the presence of solid debris in cell suspensions, the use of uncoated clean glass dishes instead of plastic is recommended for tissue mincing. Petri dishes with glass bottoms and plastic sides can still generate solid debris if blades come in contact with the plastic sides and therefore are not recommended. Glass cell culture dishes from VWR, Cat. No. 75845-542 (or equivalent) did not generate debris, even with excessive scratching. Mincing on glass petri dishes significantly reduced clog rates by minimizing solid debris in the cell suspension (Figure 10).

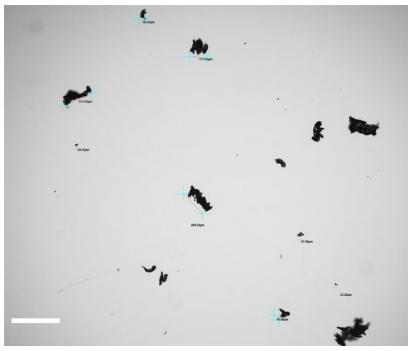


Figure 9. Solid debris generated while mincing on plastic consumables (Scale bar, 500 µm).

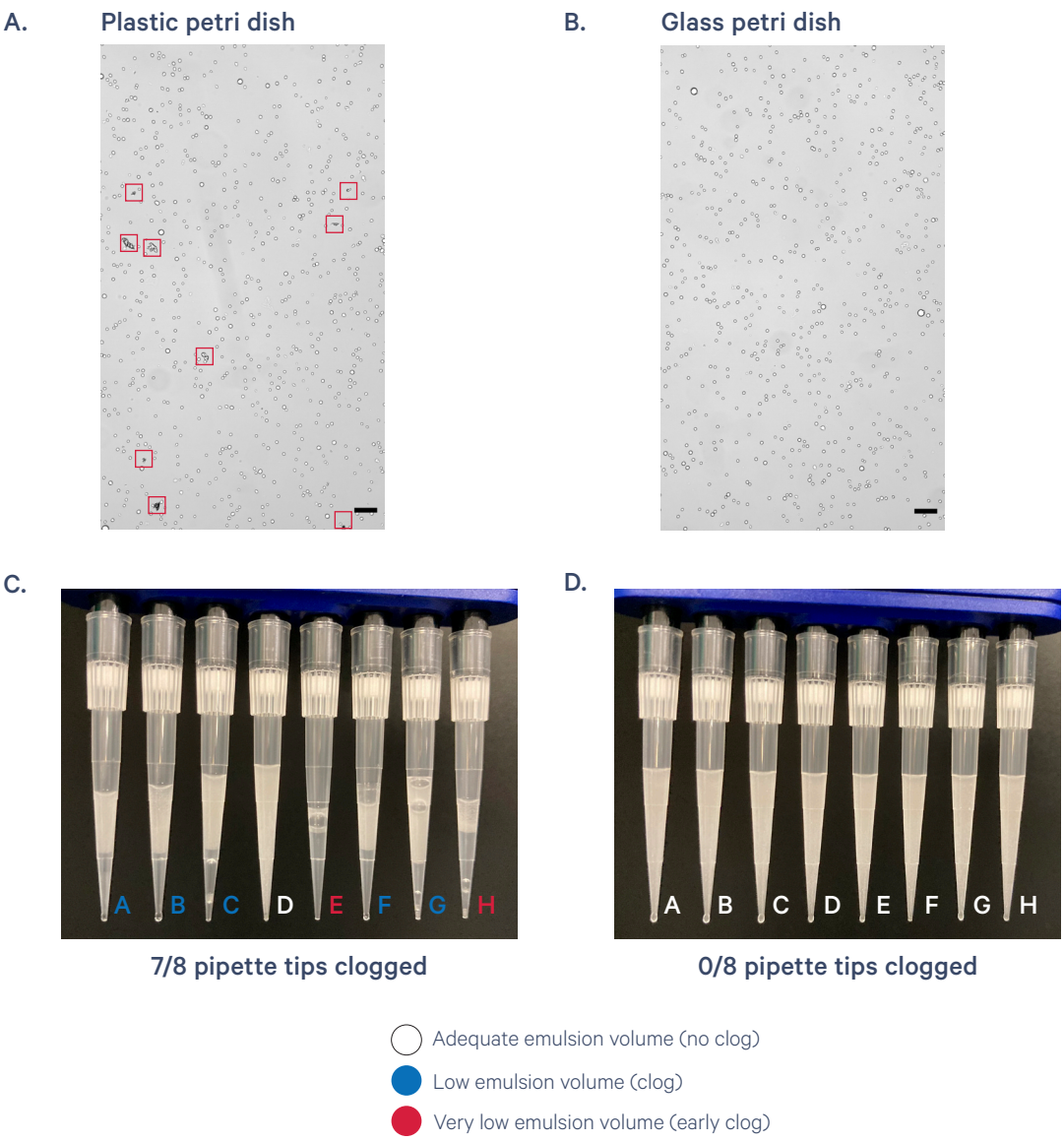


Figure 10. Impact of solid debris on GEM generation. Mock mincing was performed in a mincing buffer solution on either a plastic (A) or glass (B) petri dish. Solutions were filtered through a 35 µm FACS cell strainer and a 40 µM Nylon cell strainer, spiked into Jurkat cell suspensions, and imaged on a Nikon Eclipse Ti2 with 10x objective (Scale bar = 100 µm). Red boxes are indicative of solid plastic debris generated during the mincing process. GEMs generated with suspensions containing solid debris resulted in several microfluidic clogs of varying severity (C). Clogs were observed in all pipette tips except tip D, with tips E and H showing very low emulsion volume, suggesting a clog occurred early in the GEM generation process. GEMs generated with cell suspensions without solid debris showed high quality and uniform emulsions (D).

Comparing the impact of solid and biological debris

Large biological debris generated during dissociation may lead to microfluidic clogs or wetting failures and are largely mitigated through filtration of the cell suspension. However, filtering cell suspensions with solid debris may not be sufficient in eliminating clogs because smaller debris may accumulate in the microchannels of the chips, impeding the generation of GEMs over time.

Rigid plastic debris (even after filtration) may elevate the risk of microfluidic clogs (Figures 11A, 11C). Conversely, unfiltered cell suspensions with high abundance of biological debris result in fewer and less severe microfluidic clogs and wetting failures (Figures 11B, 11D). Larger fibers are more likely to lead to wetting failures than clogs.

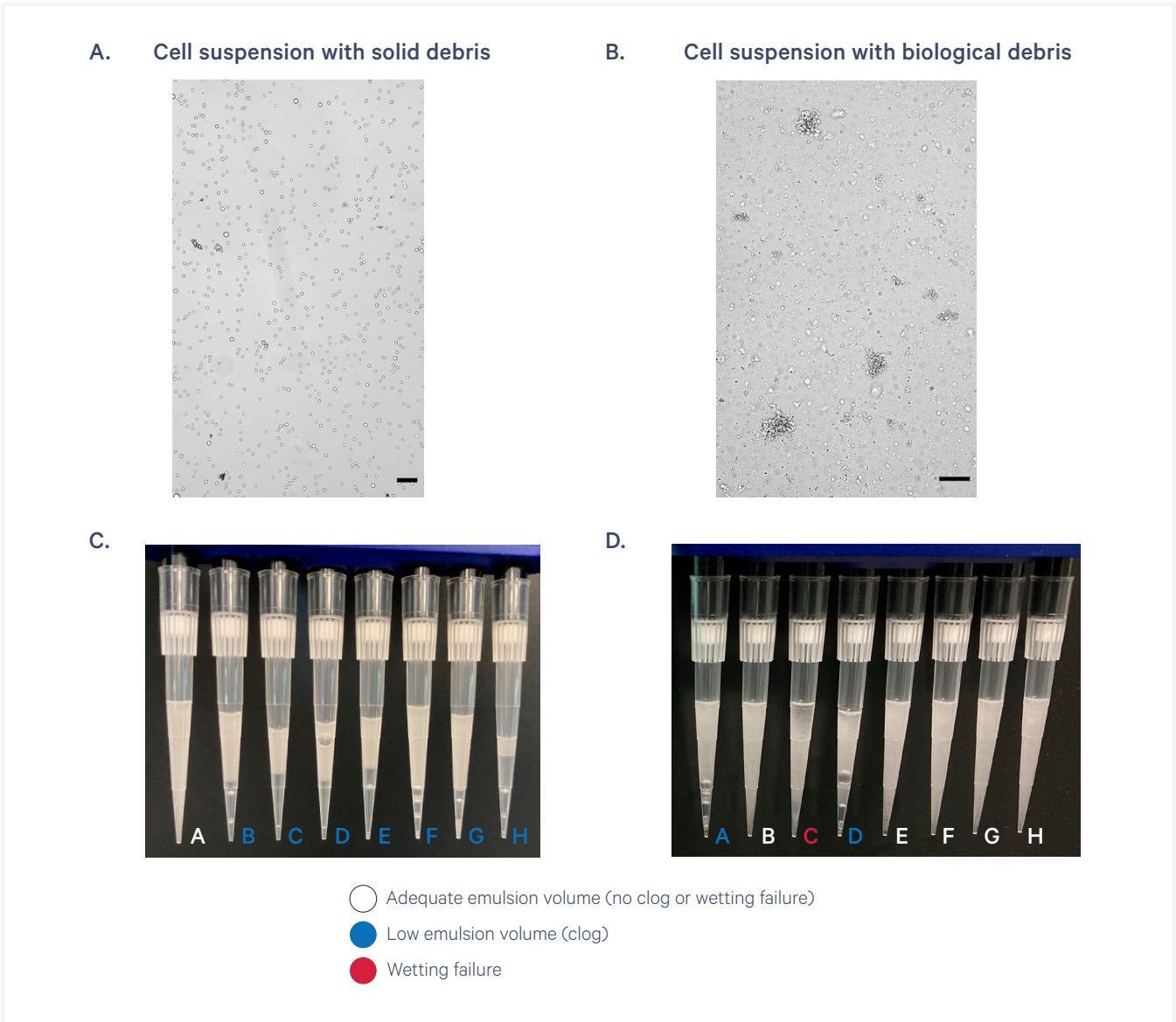


Figure 11. Impact of solid debris vs. large biological debris. Jurkat cell suspension spiked with filtered (2x filtration with 40 µm Flowmi™ filter) mincing buffer solution containing solid debris (A, Scale bar 100 µm) and unfiltered mouse liver nuclei suspension with biological cell/tissue clumps (B, Scale bar 100 µm) was imaged on a Nikon T2 Eclipse with a 10x objective. GEMs generated from cell suspensions with solid debris resulted in clogs of varying severity (C), whereas GEMs generated from cell suspensions with large biological debris displayed fewer and less severe microfluidic clogs and wetting failures (D).

This Technical Note is compatible with the following products:

Reagent Kit	Document Number
Chromium Next GEM Single Cell 3' HT	
Chromium Next GEM Single Cell 3' HT Reagent Kits User Guide (v3.1 - Dual Index)	CG000416
Chromium Next GEM Single Cell 3' HT Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcode Technology for Cell Surface Protein	CG000417
Chromium Next GEM Single Cell 3' HT Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcode technology for CRISPR Screening	CG000418
Chromium Next GEM Single Cell 3' HT Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcode technology for Cell Multiplexing	CG000419
Chromium Next GEM Single Cell 3' HT Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcode technology for Cell Surface Protein and Cell Multiplexing	CG000420
Chromium Next GEM Single Cell 3' HT Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcode technology for CRISPR Screening and Cell Multiplexing	CG000421
Chromium Next GEM Single Cell 3' v3.1 (Dual Index)	
Chromium Next GEM Single Cell 3' Reagent Kits User Guide (v3.1 - Dual Index)	CG000315
Chromium Next GEM Single Cell 3' Reagent Kits User Guide (v3.1- Dual Index) with Feature Barcoding technology for Cell Surface Protein	CG000317
Chromium Next GEM Single Cell 3' Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcoding technology for CRISPR Screening	CG000316
Chromium Next GEM Single Cell 3' Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcoding technology for Cell Multiplexing	CG000388
Chromium Next GEM Single Cell 3' Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcoding technology for Cell Surface Protein and Cell Multiplexing	CG000390
Chromium Next GEM Single Cell 3' Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcoding technology for CRISPR Screening and Cell Multiplexing	CG000389
Chromium Next GEM Single Cell 3' v3.1 (Single Index)	
Chromium Next GEM Single Cell 3' Reagent Kits v3.1	CG000204
Chromium Next GEM Single Cell 3' Reagent Kits v3.1 with Feature Barcoding technology for Cell Surface Protein	CG000206
Chromium Next GEM Single Cell 3' Reagent Kits v3.1 with Feature Barcoding technology for CRISPR Screening	CG000205
Chromium Next GEM Single Cell 3' LT v3.1	
Chromium Next GEM Single Cell 3' LT Reagent Kits User Guide (v3.1 - Dual Index)	CG000399
Chromium Next GEM Single Cell 3' LT Reagent Kits User Guide (v3.1 - Dual Index) with Feature Barcode technology for Cell Surface Protein	CG000400
Chromium Next GEM Single Cell 5' HT v2	
Chromium Next GEM Single Cell 5' HT Reagent Kits User Guide (v2 - Dual Index)	CG000423
Chromium Next GEM Single Cell 5' HT Reagent Kits User Guide (v2 - Dual Index) with Feature Barcode technology for Cell Surface Protein and Immune Receptor Mapping	CG000424
Chromium Next GEM Single Cell 5' HT Reagent Kits v2 (Dual Index) with Feature Barcode technology for CRISPR Screening	CG000512
Chromium Next GEM Single Cell 5' HT Reagent Kits v2 (Dual Index) with Feature Barcode technology for CRISPR Screening and Cell Surface Protein	CG000513

Compatible products continued:

Chromium Next GEM Single Cell 5' v2	
Reagent Kit	**Document Number**
Chromium Single Cell 5' Reagent Kits User Guide (v2 - Dual Index)	CG000331
Chromium Single Cell 5' Reagent Kits User Guide (v2 - Dual Index) with Feature Barcoding technology for Cell Surface Protein and Immune Receptor Mapping	CG000330
Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) with Feature Barcode technology for CRISPR Screening	CG000510
Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) with Feature Barcode technology for CRISPR Screening and Cell Surface Protein	CG000511
Chromium Next GEM Single Cell 5' v1.1	
Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1	CG000207
Chromium Next GEM Single Cell V(D)J Reagent Kits v1.1 with Feature Barcode technology for Cell Surface Protein	CG000208

References

1. Single Cell Protocols - Cell Preparation Guide (CG00053)
2. Thawing Dissociated Tumor Cells for Single Cell RNA Sequencing Demonstrated Protocol (CG000233)
3. Removal of Dead Cells for Single Cell RNA Sequencing Demonstrated Protocol (CG000093)

Document Revision Summary

Document Number	CG000479
Title	Best Practices to Minimize Chromium Next GEM Chip Clogs and Wetting Failures
Revision	Rev B
Revision Date	June 2022
Specific Changes	Added solid debris impact on GEM generation sections Updated Compatible Products list
General Changes	Updated for general minor consistency of language and terms throughout

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