Single-Cell Services: Quality Control (QC) Standards

Every single-cell or single-nuclei sample submitted to Signios Bio undergoes a standardized primary Quality Control (QC) check. Single-cell analysis resolves cellular heterogeneity, but its success depends entirely on the quality of the starting suspension. This guide outlines our absolute cell/nuclei suspension metrics, explains passing versus failing baseline conditions, and establishes visual and molecular benchmarks for fresh, frozen, and fixed sample types.

Fresh Cells QC – Viability and Counting

For standard single-cell workflows (e.g., 10x Genomics Chromium 3' or 5' GEX), achieving a high percentage of viable cells with minimal debris or cell aggregation is the most critical factor for sample parsing and preventing microfluidic clogging.

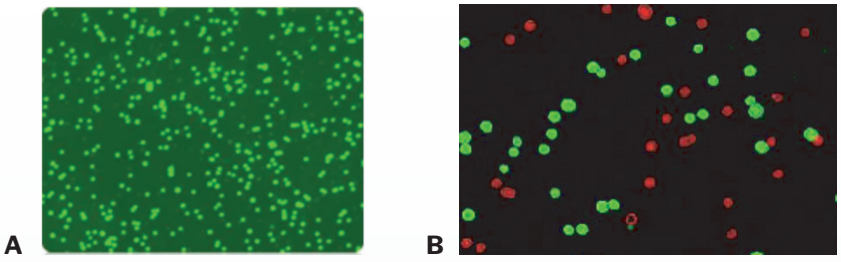
Assessment Method: Dual-Fluorescence AO/PI Staining

Signios Bio utilizes the Cellometer K2 automated cell imager running a dual-color fluorescence Acridine Orange/Propidium Iodide (AO/PI) protocol. This method stains viable nucleated cells green and dead nucleated cells red, guaranteeing accurate viability metrics even in the presence of heavy debris or red blood cell contamination.

- Minimum Cell Input: ≥ 100,000 total cells recommended to account for loss during microfluidic processing and washes.
- Buffer Conditions: Culture media with 10% FBS and/or PBS + 1% BSA.

Criteria for Fresh Cell Suspensions

Metric	Passing Condition	Failing Condition
Viability Rate	> 85% viable cells	< 80% viability (High dead cell ratio causes cell-free ambient RNA background)
Debris & Aggregates	Clean background; completely singulated cells	High debris, platelet clouding, or massive cell clumps (causes microfluidic wetting failures)



QC Image Reference (Figure 1):
(A) Passing Profile: Micrograph exhibits bright, round, green fluorescent signals denoting distinct individual live cells.
(B) Failing Profile: Micrograph shows a high density of red fluorescent signals (~50% dead cells) combined with amorphous particulate matter (debris) or multiple cells bound together.

Note: If a fresh sample lands in a marginal window (60–80% viability), an in-house dead-cell removal or debris cleanup step (e.g., Miltenyi MicroBeads or FACS sorting) must be applied prior to chip loading.

Nuclei QC – Visual & Microscope Assessment

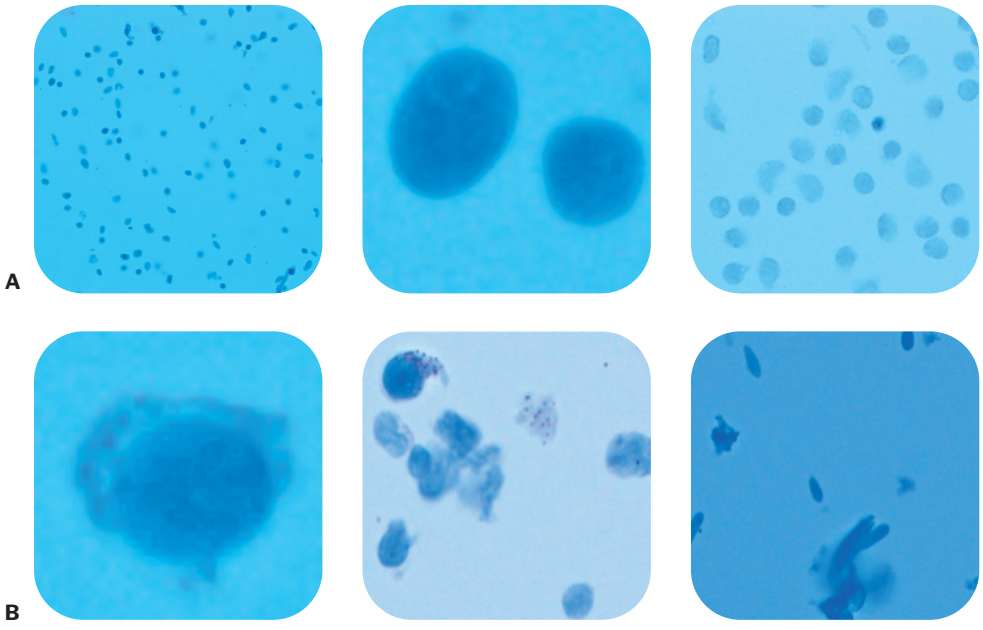
When working with frozen tissues or single-nuclei workflows (such as Multiome ATAC + GEX), cell viability checks are no longer applicable. Quality control shifts strictly to visual assessment via high-magnification microscope imaging.

Assessment Method: Trypan Blue / Brightfield Imaging

Isolated nuclei are stained with Trypan Blue or evaluated under high-contrast brightfield channels to assess structural integrity, membrane smoothness, and clumping.

Criteria for Single-Nuclei Suspensions

Metric	Passing Condition	Failing Condition
Nuclear Membrane	Desired Intact Nuclei: Perfectly round, smooth, well-defined borders indicating a completely intact nuclear envelope.	Poor Quality Nuclei: Nuclei show rough edges, significant blebbing, or partially lysed envelopes leaking genomic DNA.
Suspension State	Uniformly separated, individualized nuclei distributed across the field of view.	Nuclei Clumps & Debris: Massive aggregations of stuck nuclei or heavy non-nuclear tissue fragments.



QC Image Reference (Figure 2):
(A)Passing Profile: Clear, singulated dark-ring structures (intact nuclei) free of surrounding cellular debris, typical of successfully isolated PBMC or healthy frozen tissue suspensions. **(B) Failing Profile:** Visual clusters of un-lysed cell chunks, thick strands of free DNA webbed with nuclei clumps, or highly contaminated suspensions (common in challenging tissues like fresh skin or dense tumors).

Fixed Cell/Nuclei QC – Molecular Assessment

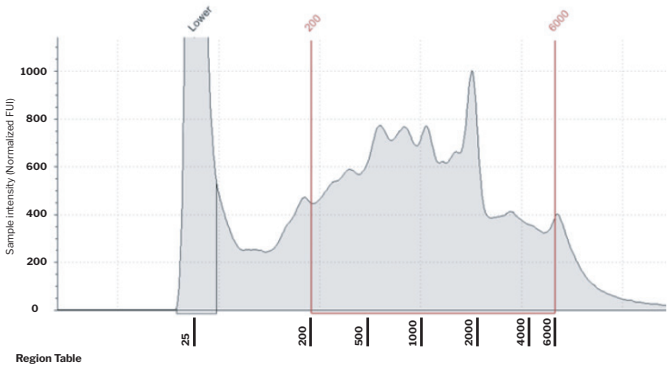
For Formaldehyde-fixed cells/nuclei, FFPE tissues, or 10x fixed tissues/cells/nuclei utilizing 10x Genomics Fixed RNA Profiling (Flex), standard cellular viability metrics do not apply. Quality is determined by verifying the preservation of fragment lengths from the starting material.

Assessment Method: RNA Extraction and TapeStation DV200

Prior to hybridization with probe sets, a subset or representative sample undergoes total RNA extraction. Quality is evaluated using the Agilent TapeStation to measure the DV200 score—the percentage of total extracted RNA fragments that are greater than 200 nucleotides in length.

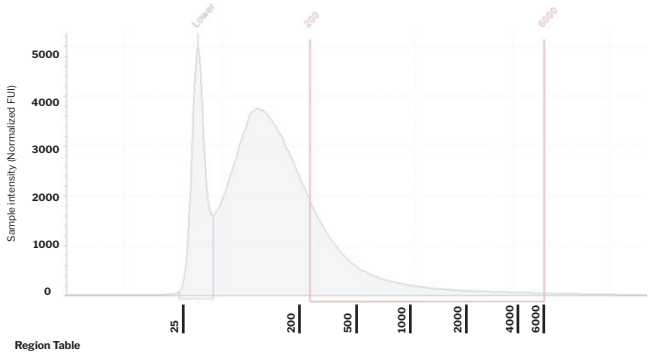
- Input Thresholds: For optimal fixation, sample sizes should range from 300,000 to 1M cells/nuclei.
- Low Input Risk: Samples falling below 100,000 cells/nuclei risk a 10–20% drop in usable genomic data complexity.

Criteria for Fixed Tissue/Cells/Nuclei QC



Region Table

From [nt]	To [nt]	Average Size [nt]	Conc. [ng/µl]	Region Molarity [n mol/l]	% of Total
200	6000	2666	30.8	33.9	76.13



Region Table

From [nt]	To [nt]	Average Size [nt]	Conc. [ng/µl]	Region Molarity [n mol/l]	% of Total
200	6000	1609	21.6	39.4	22.85

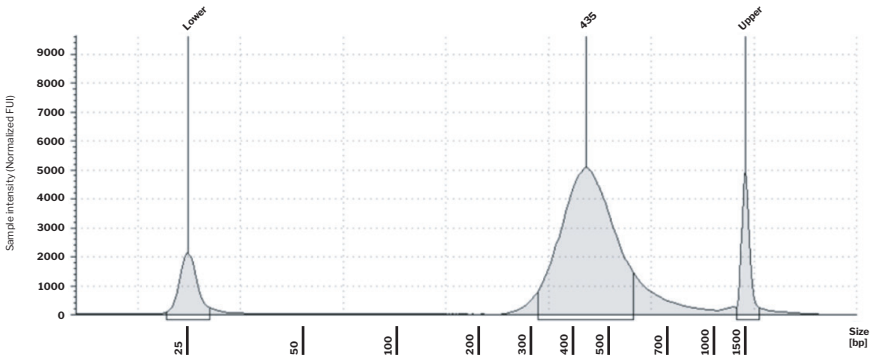
QC Image Reference (Figure 3):
(A)Passing Condition (High-Quality Flex Input): A TapeStation electropherogram showing a broad curve shifted toward larger fragments, achieving a DV200 > 76%. This confirms that the fixing or archiving process has preserved enough structural continuity for probe hybridization.

(B)Failing Condition (Highly Degraded Input): An electropherogram showing an extreme leftward shift, flatlining before the 200 nt marker (DV200 < 30%). Highly fragmented or over-fixed configurations cannot properly hybridize to the probe pairs, yielding high unmapped background reads.

Library and Data QC Benchmarks

Once a cell or nuclei suspension passes primary visual and molecular checks, it moves into library generation and sequencing. Downstream processing is benchmarked against the following quality specifications:

Library QC Check



Sample Table

Well	Conc. [ng/µl]	Sample Description	Alert	Observations
B1	4403	2011017_M1F_control_YFP_		

Peak Table

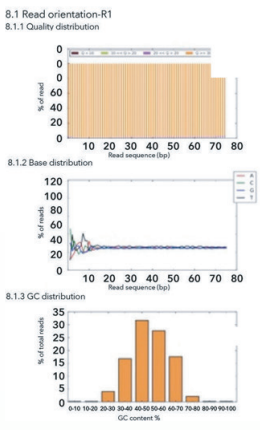
Size [bp]	Calibrated Conc. [ng/µl]	Assigned Conc. [ng/µl]	Peak Morality [nmol/l]	% Integrated Area	Peak Comment	Observations
25	6.24	-	384	-		Lower Marker
435	4403	-	157	100.00		
1500	6.50	6.50	6.67	-		Upper Marker

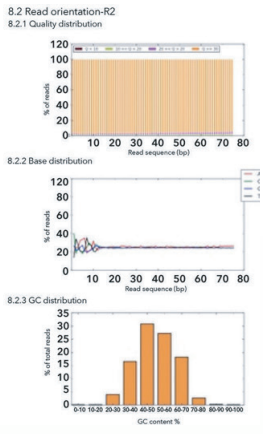
QC Image Reference (Figure 4): **TapeStation Analysis:** Confirms that final single-cell cDNA and target-enriched NGS libraries match precise size distributions (typically peaking between 300–500 bp) with zero presence of adapter dimers.

Data QC Reporting Requirements

Table 1: Raw reads summary

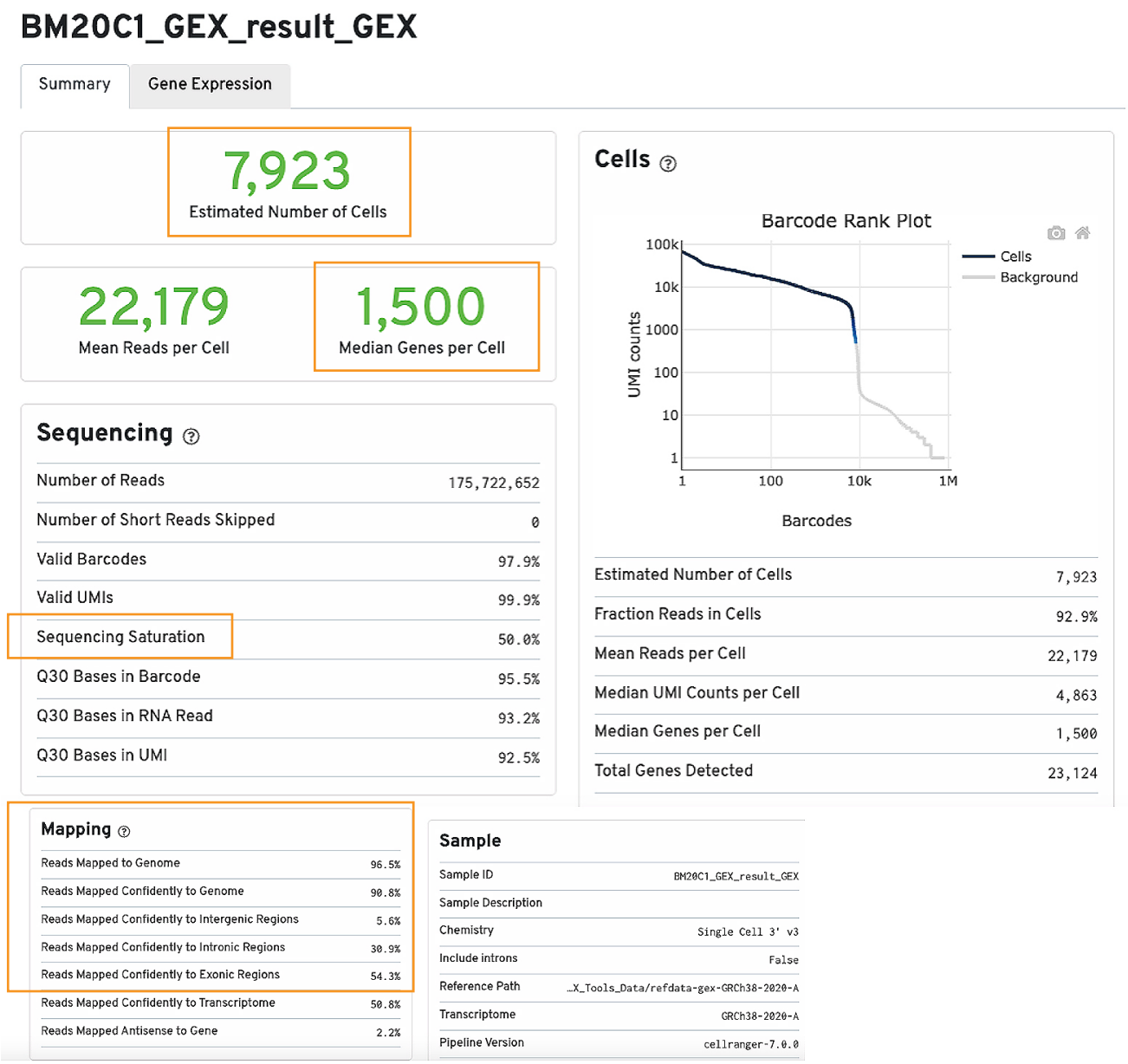
Run	Read orientation	Mean read quality (Phred score)	Number of reads	% GC	% Q < 10	% Q 10–20	% Q 20–30	% Q > 30	Number of bases (MB)	Mean read length (bp)
	R1	33.64	314650130	36.86	13.9	0.0	17.09	69.0	47197.52	150.0
	R2	40.24	314650130	43.21	1.11	0.0	2.39	96.5	47197.52	150.0
	R1	33.76	349299283	36.75	13.71	0.0	16.77	69.52	52394.89	150.0
	R2	40.22	349299283	42.77	1.16	0.0	2.39	96.45	52394.89	150.0





QC Image Reference (Figure 5): After sequencing is completed, the team will check the data QC report to verify read depth is achieved, Q30 scores are met along with run reports to confirm quality distribution, base distribution and GC distribution meet Illumina standards.

Data QC Reporting Requirements

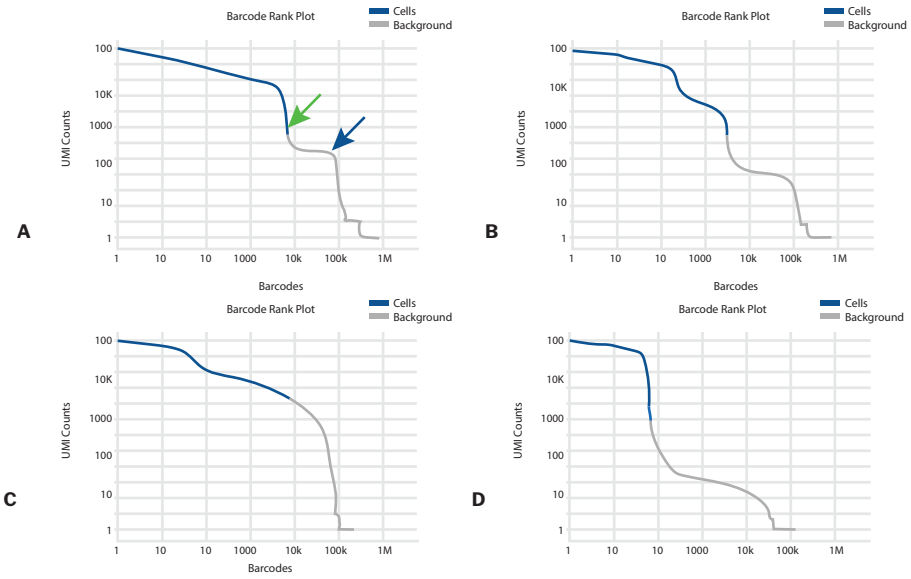


QC Image Reference (Figure 6):

- **Number of Cell/Nuclei:** Confirmation of capture goal is +/- 15% of target goal given passing sample QC
- **Mean Reads per cell:** Confirm sequencing depth meets targeted Reads/Cell expectations (I.e. 20,000 paired reads/cell).
- **Median Genes per Cell:** Serves as a primary indicator of sample complexity and transcriptional data quality.
- **Sequencing Saturation:** High sequencing saturation curves plot "Mean Reads per Cell" against "Sequencing Saturation." This determines whether sequencing deeper will yield significantly more unique transcripts (UMIs) or simply duplicate existing reads.
- **Mapping and Alignment Rates:** Check that the percentage of reads mapping confidently to the reference genome, exonic regions, and the targeted transcriptome meets baseline standards (≥ 50–70% for standard applications). High intergenic or intronic mapping in a standard fresh cell assay can indicate genomic DNA contamination or high ambient RNA.

Barcode Rank Plot Diagnostics

The Barcode Rank Plot (or "knee plot") is the most powerful diagnostic visual for separating real, high-quality cell-containing droplets from background ambient RNA. It can immediately pinpoint whether an issue stems from sample preparation, microfluidic instrument errors, or sequencing data anomalies.



Examples of QC Issue Evaluations (Figure 7): When presenting or reviewing your data, utilize the following diagnostic shapes from the analysis dashboard to troubleshoot atypical results: **(A)Typical Profile (Normal Run):** Shows a distinct, sharp horizontal "shoulder" followed by a steep vertical drop (the "cliff"). This clear separation indicates a highly successful run where cell-containing droplets have thousands of UMIs, while empty background droplets drop sharply to single-digit UMI counts. **(B)Atypical Profile:** Multi-Step Drop (Possible Heterogeneous/Different Cell Populations): The blue cell line contains a secondary step or notch before dropping off completely. This typically indicates a sample containing distinctly mixed cell populations of radically different sizes or RNA contents (e.g., small, quiescent T-cells mixed with large, highly active structural cells or tumor cells). **(C)Atypical Profile:** Gradual, Lazy Slope (Wetting Failure, Lysis, or Low Viability): The plot loses its sharp vertical cliff entirely and gradually slants downward across the background threshold.

- Root Cause: Often caused by microfluidic wetting failures (where oil and aqueous phases do not mix correctly in the chip channels), premature cell lysis during handling, or loading a sample with poor initial cell viability. This creates a high concentration of free-floating ambient RNA that bleeds into empty droplets.

(D)Atypical Profile: Premature Vertical Drop (Sample Clog or Inaccurate Cell Count): The curve drops sharply into the background background line very early on the x-axis, capturing a fraction of the intended target cells (e.g., less than a few hundred cells).

- Root Cause: Typically caused by a physical clog in the chip microchannels due to cellular aggregates/debris, or an underloaded channel resulting from an inaccurate initial cell counting step.

Contact Signios Bio: Have questions about your upcoming single-cell project, tissue dissociation options, or specific fixation kits? Contact our technical team today to plan your customized multiomic solutions.