Whole Transcriptome Services: Quality Control (QC) Standards

Every bulk RNA sample submitted to Signios Bio undergoes a standardized, two-tiered primary Quality Control (QC) check to verify absolute concentration and structural integrity before library preparation begins. This guide outlines our assessment metrics, explains how we handle low-quality or degraded samples, and provides a clear benchmark for the data quality you can expect based on your starting material.

Sample QC Total RNA Assessment –Qubit™ and TapeStation™

Signios Bio measures two critical metrics for sample QC to ensure quality control throughout processing. Concentration is assessed via Qubit and Quality via Tapestation. Quality metrics are assessed by two methods:

- RNA Integrity Number (RIN): Evaluation of 18S to 28S ribosomal RNA (rRNA) peaks to determine structural continuity for high quality sample assessment.
- DV200: Percentage of RNA fragments that are greater than 200 nucleotides in length. A more reliable metric for degraded samples.

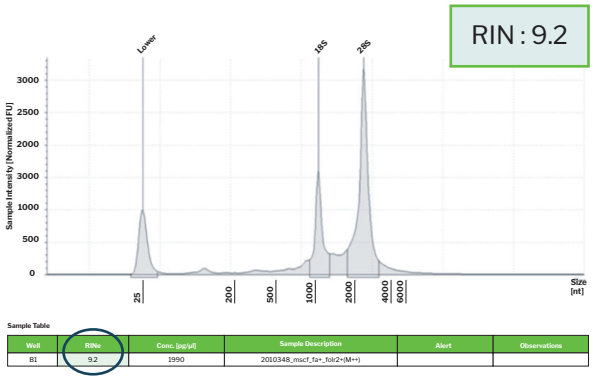


Figure 1 : Representative Electrophoregram generated from TapeStation shows profile of high quality RNA input from micro-dissected tissue with intact 18S & 28S peaks.

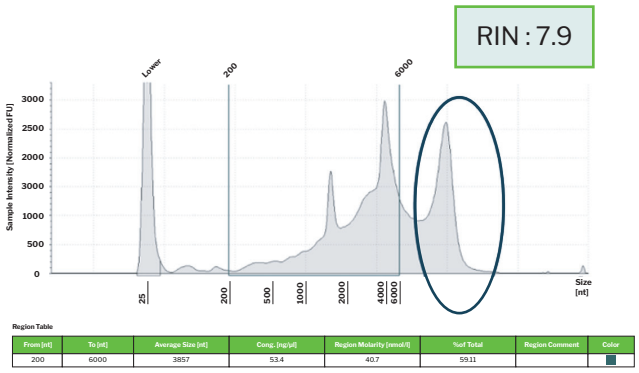
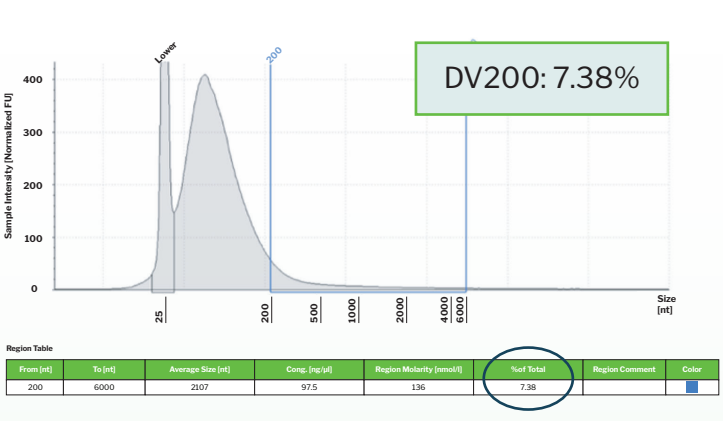


Figure 2: Representative Electrophoregram generated from TapeStation showing gDNA contamination artificially distorting RIN. If gDNA is present, an in-house DNase treatment ($30/sample) is recommended.



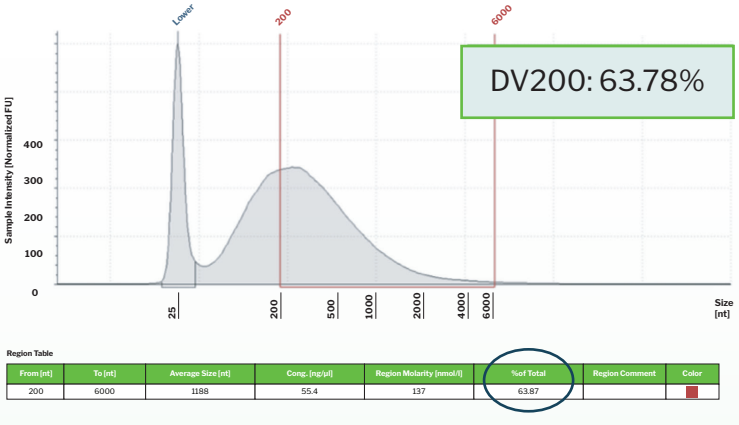
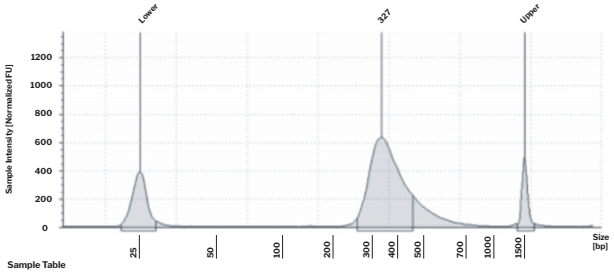


Figure 3 & 4: Representative Electrophoregram generated from TapeStation showing profiles of low quality Total RNA. Both have RIN 1.6 and no presence 18S & 28S peaks. Evaluation moves to DV200 to assess intact fragments >200bp for proper quality assessment.

Library QC Assessment –Qubit™ and TapeStation™

Sample QC will determine the appropriate library prep workflow for your samples based on Qubit quantity and Tapestation RIN/DV200. After library preparation, all libraries will undergo a quality assessment. Review of the tapestation file will confirm library peak is the appropriate size and there are no adapter dimers.



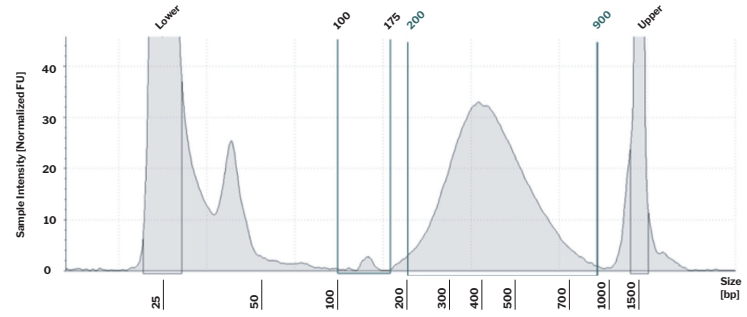
Sample Table

Well	Conc. [pg/µl]	Sample Description	Alert
D1	1730		

Peak Table

Size [bp]	Calibrated Conc. [pg/µl]	Assigned Conc. [pg/µl]	Peak Molarity [pmol/l]	% Integrated Area	Height	Peak Comment	Observations
25	512	-	31500	-	332.020		Lower Marker
327	1730	-	8140	100.000	538.945		
1500	250	250	256	-	423.554		Upper Marker

A



Peak Table

From [bp]	To [bp]	Assigned Size [bp]	Conc. [pg/µl]	Region Molarity [pmol/l]	%of Total	Region Comment	Color
100	175	141	2.75	35.6	0.72		
200	900	417	245	985	64.43		

B

Figure 5: **(A)** Representative Electrophoregram generated from TapeStation shows profile of NGS library generated using the RNA isolated from fresh-frozen tissue. **(B)** Representative Electrophoregram generated from TapeStation showing a library QC profile that contains adapter dimers.

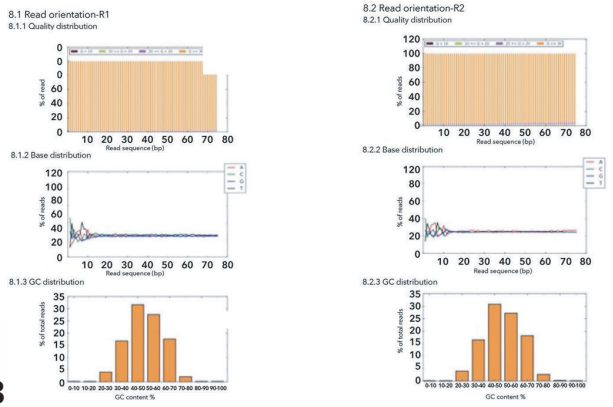
Data QC Assessment –Illumina Reporting

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.

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	39.56	38127191	49.53	0.01	1.13	2.18	96.69	3812.72	100.0
	R2	37.97	38127191	49.84	0.27	3.49	5.02	91.21	3812.72	100.0
	R1	39.61	43217226	48.98	0.01	1.11	2.07	96.81	4321.72	100.0
	R2	38.11	43217226	49.26	0.26	3.34	4.73	91.67	4321.72	100.0
	R1	39.53	39173659	49.32	0.01	1.22	2.19	96.58	3917.37	100.0
	R2	37.86	39173659	49.71	0.3	3.7	5.18	90.82	3917.37	100.0

A



B

Figure 6: **(A)** Representative FastQC report showing quantity and quality of reads obtained from the sequencing run. **(B)** Representative Illumina Run Metrics showing instrument performance.

Contact us today for questions about our QC processes, services or planning your customized solution.

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