

Quality Control Report for RNA(cDNA) Counting Step

Metric Name	Experimental Value	Reference Range	Risk Level
barcode_detected_1_ratio	0.40	≥ 0.08	checked OK
barcode_detected_5_ratio	0.31	≥ 0.05	checked OK
barcode_detected_10_ratio	0.19	≥ 0.03	checked OK
mean_barcodes_detected_counts	14.63	≥ 5.00	checked OK
qualified_oligo_ratio	0.95	≥ 0.80	checked OK
qualified_oligo_ratio_with_bc_count3	0.94	≥ 0.78	checked OK
qualified_oligo_ratio_with_bc_count5	0.94	≥ 0.70	checked OK
qualified_oligo_ratio_with_bc_count10	0.90	≥ 0.50	checked OK
mean_barcodes_count_for_oligo	63.16	10.00 - 300.00	checked OK

Reference Explanation for Quality Control Results:

barcode_detected_1/5/10_ratio: The proportion of barcodes detected at least 1/5/10 times.

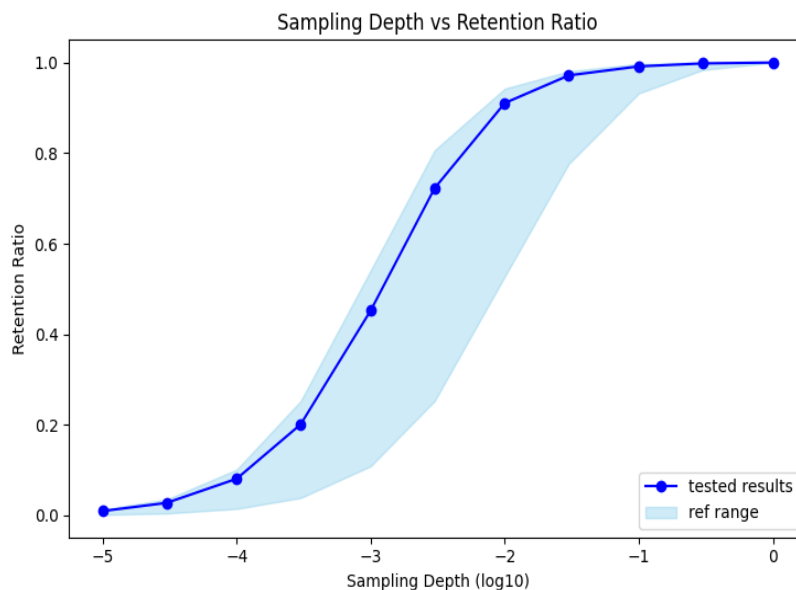
mean_barcodes_detected_counts: The average number of times barcodes are detected.

qualified_oligo_ratio/_with_bc_count3/5/10: The proportion of oligos linked with sufficient barcodes (default value is 3), using barcodes detected at least 1/3/5/10 times.

mean_barcodes_count_for_oligo: The average number of barcode types linked to each oligo.

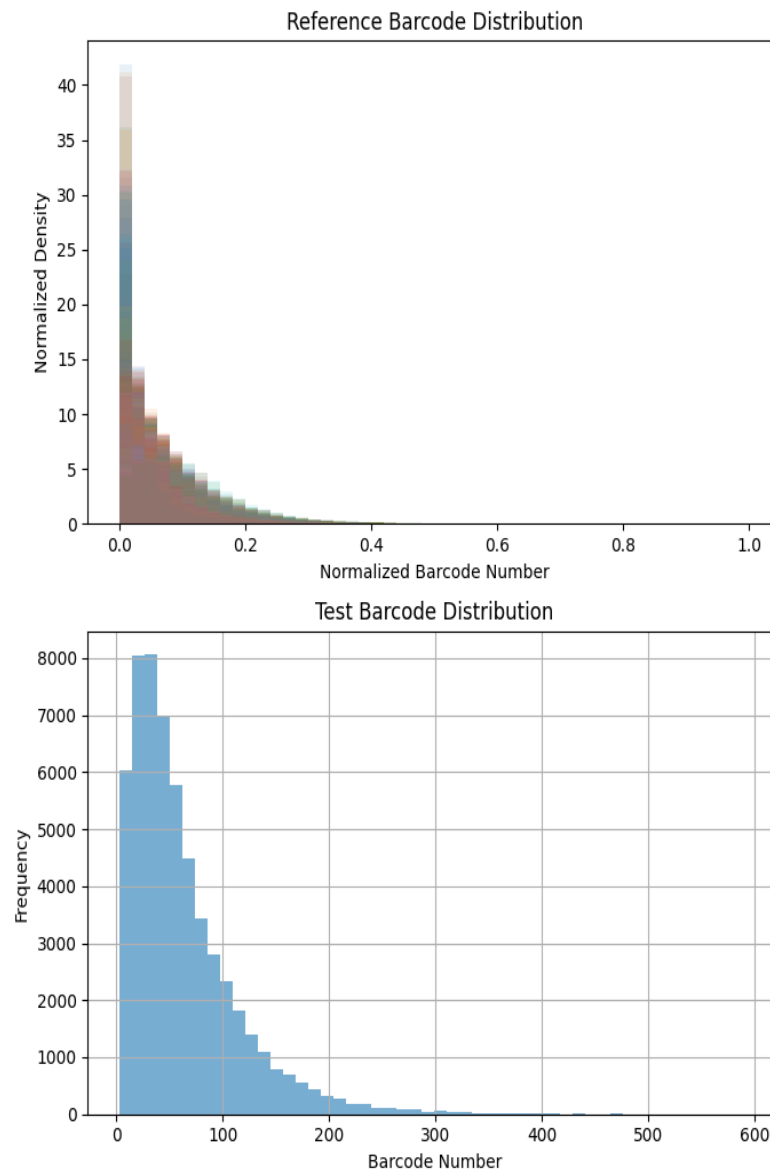
Abnormal values here are usually lower than expected, causing inaccurate quantification

Low values in above metrics may indicate insufficient sequencing depth or low reverse transcription efficiency. If so, check the reverse transcription experiment firstly. Insufficient total RNA used for reverse transcription is a common issue in this step.



Coverage of oligos at different downsampling ratios for sequencing results: The closer the curve is to the upper left, the more sufficient the sequencing depth. If the test result is in the lower right of the reference range, consider increasing the sequencing depth.

Distribution of barcodes number linked to oligos for reference:



The distribution of the tested barcodes should resemble one of the reference distributions. If a long-tail effect is significant here despite qualified QC in the previous step, it likely indicates uneven cDNA distribution due to issues in the reverse transcription process. If so, it is recommended to check the reverse transcription process and refer to the previous page of report to make sure the sequencing depth is enough. It is strongly recommend ensuring that the total amount of RNA used for reverse transcription is sufficient when encountering issues in this step.

Additional Quality Control Metrics

Metric Name	Experimental Value	Reference Range
mapped_seq_num	59142502.00	-
barcode_less_1	5251480.00	-
barcode_less_1_ratio	0.60	≤ 0.96
barcode_less_5	6096120.00	-
barcode_less_5_ratio	0.69	≤ 0.97
barcode_less_10	7095652.00	-
barcode_less_10_ratio	0.81	≤ 0.98
barcode_detected_1	3549410.00	-
barcode_detected_5	2704770.00	-
barcode_detected_10	1705238.00	-
barcode_detected_200	17036.00	-
total_barcodes_type	8800890.00	-
not_qualified_oligo_num	3209.00	-
not_qualified_oligo_ratio	0.05	≤ 0.20
qualified_oligo_num	56448.00	-
qualified_barcodes_num	3565017.00	-
qualified_barcodes_ratio	0.41	≥ 0.05
not_qualified_oligo_num_with_bc_count3	3430.00	-
not_qualified_oligo_ratio_with_bc_count3	0.06	≤ 0.30
qualified_oligo_num_with_bc_count3	56227.00	-
qualified_barcodes_num_with_bc_count3	3197218.00	-
not_qualified_oligo_num_with_bc_count5	3817.00	-
not_qualified_oligo_ratio_with_bc_count5	0.06	≤ 0.35
qualified_oligo_num_with_bc_count5	55840.00	-
qualified_barcodes_num_with_bc_count5	2719899.00	-
not_qualified_oligo_num_with_bc_count10	5903.00	-
not_qualified_oligo_ratio_with_bc_count10	0.10	≤ 0.60
qualified_oligo_num_with_bc_count10	53754.00	-
qualified_barcodes_num_with_bc_count10	1718317.00	-
cv_barcodes_count_for_oligo	0.84	0.60 - 1.50
mode_barcodes_count_for_oligo	21.00	3.00 - 300.00
mean_barcodes_counts	14.63	4.00 - 65.00
cv_barcodes_for_counts	1.24	0.60 - 2.10
mode_barcodes_for_counts	4.00	1.00 - 40.00

Reference for additional QC metrics:(see next page)

Reference for additional QC metrics:

`all_seq_num`: Total number of sequencing reads.

`mapped_seq_num`: Number of reads that meet the sequencing quality requirements and exactly match with the oligo library sequences.

`barcode_less_1/5/10`: The number of barcodes detected fewer than 1/5/10 times.

`barcode_less_1/5/10_ratio`: The proportion of barcodes detected fewer than 1/5/10 times. A high value may indicate insufficient sequencing depth or low reverse transcription efficiency.

`barcode_detected_1/5/10`: The number of barcodes detected at least 1/5/10 times

`barcode_detected_200`: The number of barcodes detected more than 200 times. This value usually represents outliers and may be associated with highly uneven plasmid distribution or not qualified reverse transcription.

`total_barcodes_type`: The total number of barcode types.

`not_qualified_oligo_num/_with_bc_count3/5/10`: The number of oligos that do not link to a sufficient amount (default is 3) of barcodes when using barcodes detected at least 1/3/5/10 times.

`not_qualified_oligo_ratio/_with_bc_count3/5/10`: The ratio of oligos that do not link to a sufficient amount (default is 3) of barcodes when using barcodes detected at least 1/3/5/10 times. A high value may indicate insufficient sequencing depth or low reverse transcription efficiency.

`qualified_oligo_num/_with_bc_count3/5/10`: The number of oligos that link to a sufficient amount (default is 3) of barcodes when using barcodes detected at least 1/3/5/10 times.

`qualified_barcodes_num/_with_bc_count3/5/10`: The number of barcodes linked to oligos that meet the required number of barcodes (default is 3) when using barcodes detected at least 1/3/5/10 times.

`cv_barcodes_count_for_oligo`: The coefficient of variation for the number of barcode types linked to each oligo.

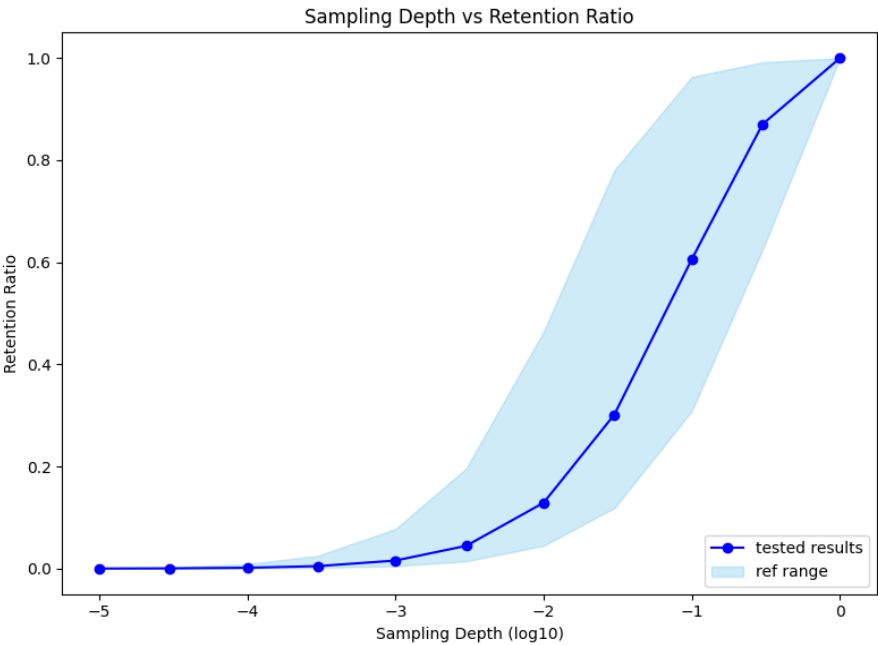
`mode_barcodes_count_for_oligo`: The mode for the number of barcode types linked to each oligo.

`mean_barcodes_counts`: The mean number of times each barcode is detected.

`cv_barcodes_for_counts`: The coefficient of variation for the number of times each barcode is detected.

`mode_barcodes_for_counts`: The mode for the number of times each barcode is detected.

Coverage of barcodes at different downsampling ratios for sequencing results (for reference):



Distribution for the number of times each barcode is detected (for reference):

