

Quality Control Report for Plasmid Counting Step

Metric Name	Experimental Value	Reference Range	Risk Level
barcode_detected_1_ratio	0.47	≥ 0.28	checked OK
barcode_detected_5_ratio	0.15	≥ 0.10	checked OK
barcode_detected_10_ratio	0.04	≥ 0.02	checked OK
mean_barcodes_detected_counts	4.11	≥ 3.00	checked OK
qualified_oligo_ratio	0.95	≥ 0.92	checked OK
qualified_oligo_ratio_with_bc_count3	0.93	≥ 0.90	checked OK
qualified_oligo_ratio_with_bc_count5	0.88	≥ 0.85	checked OK
qualified_oligo_ratio_with_bc_count10	0.58	≥ 0.60	Medium Risk
mean_barcodes_count_for_oligo	72.91	40.00 - 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.

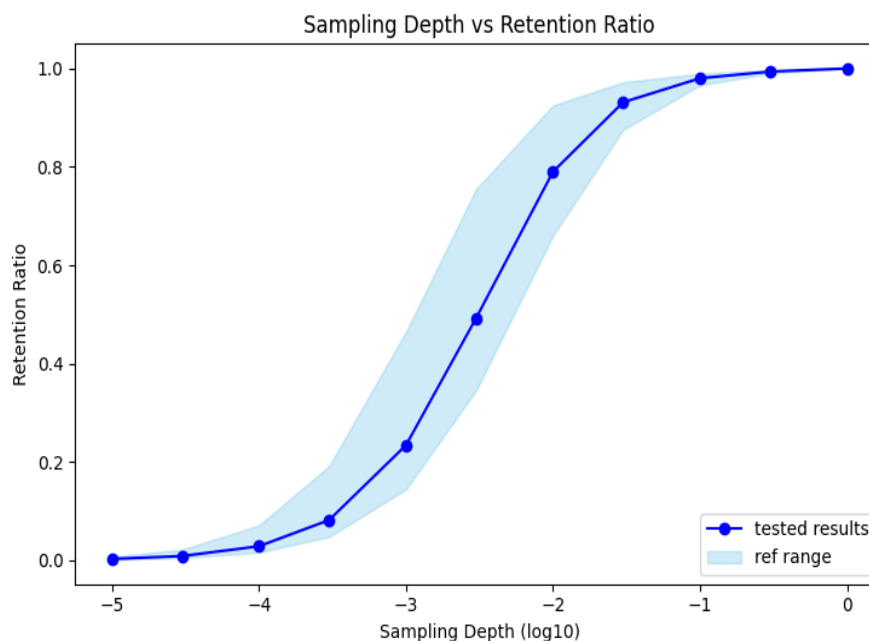
mean_barcodes_detected_counts: The average number of times barcodes are detected.

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.

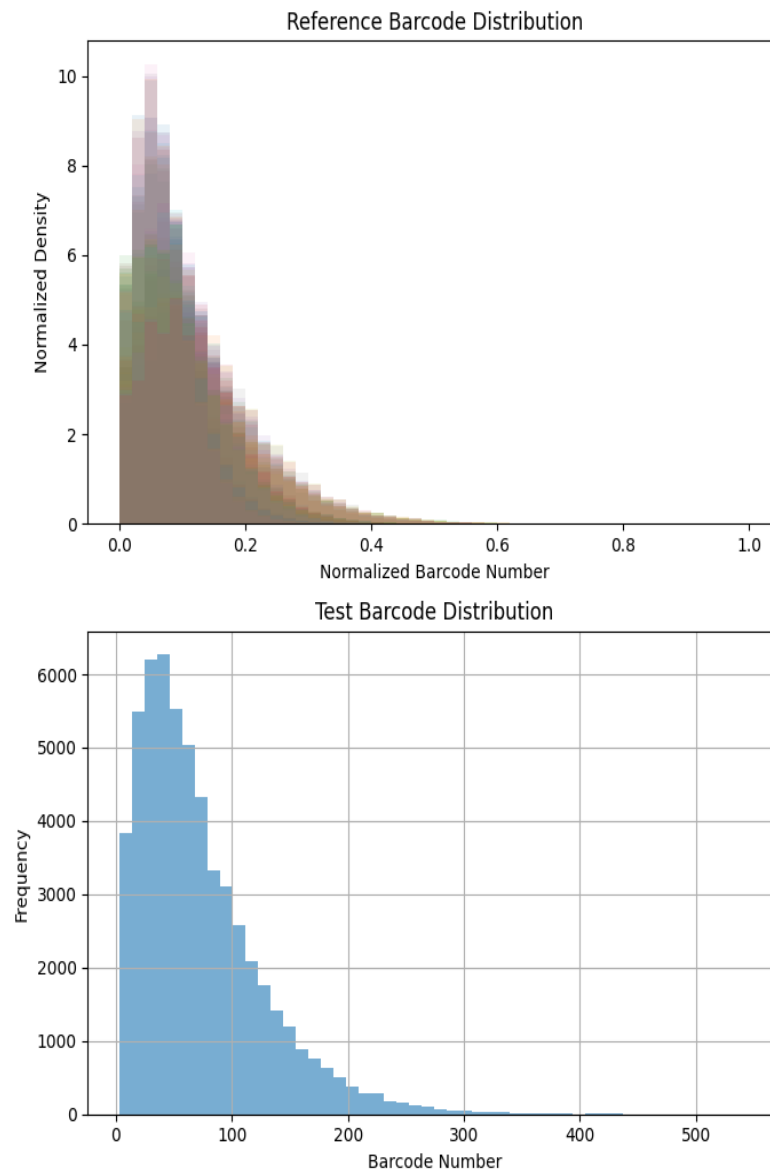
Abnormal values here are usually lower than expected, causing inaccurate quantification

Low values in above metrics may indicate insufficient sequencing depth or low plasmid construction efficiency. If so, check the plasmid construction experiment firstly.



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 plasmid distribution due to issues in the target gene insertion process. If so, it is recommended to check the plasmid construction process and refer to the previous page of report to make sure the sequencing depth is enough.

Additional Quality Control Metrics

Metric Name	Experimental Value	Reference Range
mapped_seq_num	17032362.00	-
barcode_less_1	4676479.00	-
barcode_less_1_ratio	0.53	≤ 0.72
barcode_less_5	7462271.00	-
barcode_less_5_ratio	0.85	≤ 0.90
barcode_less_10	8487330.00	-
barcode_less_10_ratio	0.96	≤ 0.98
barcode_detected_1	4141447.00	-
barcode_detected_5	1355655.00	-
barcode_detected_10	330596.00	-
barcode_detected_200	0.00	-
total_barcodes_type	8817926.00	-
not_qualified_oligo_num	2872.00	-
not_qualified_oligo_ratio	0.05	≤ 0.08
qualified_oligo_num	56785.00	-
qualified_barcodes_num	4140243.00	-
qualified_barcodes_ratio	0.47	≥ 0.25
not_qualified_oligo_num_with_bc_count3	4161.00	-
not_qualified_oligo_ratio_with_bc_count3	0.07	≤ 0.10
qualified_oligo_num_with_bc_count3	55496.00	-
qualified_barcodes_num_with_bc_count3	2390919.00	-
not_qualified_oligo_num_with_bc_count5	7182.00	-
not_qualified_oligo_ratio_with_bc_count5	0.12	≤ 0.15
qualified_oligo_num_with_bc_count5	52475.00	-
qualified_barcodes_num_with_bc_count5	1350655.00	-
not_qualified_oligo_num_with_bc_count10	25153.00	-
not_qualified_oligo_ratio_with_bc_count10	0.42	≤ 0.50
qualified_oligo_num_with_bc_count10	34504.00	-
qualified_barcodes_num_with_bc_count10	311355.00	-
cv_barcodes_count_for_oligo	0.75	0.60 - 0.80
mode_barcodes_count_for_oligo	39.00	40.00 - 300.00
mean_barcodes_counts	4.11	2.00 - 10.00
cv_barcodes_for_counts	0.86	0.80 - 1.00
mode_barcodes_for_counts	1.00	1.00 - 5.00

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 plasmid construction 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.

total_barcode_types: 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 plasmid construction 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_barcode_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_barcode_count_for_oligo: The coefficient of variation for the number of barcode types linked to each oligo.

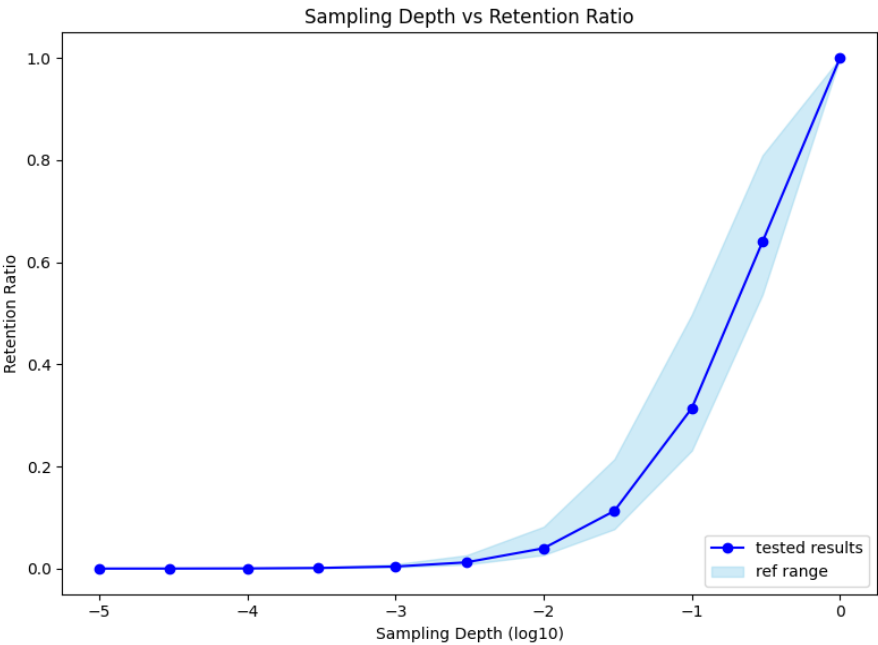
mode_barcode_count_for_oligo: The mode for the number of barcode types linked to each oligo.

mean_barcode_counts: The mean number of times each barcode is detected.

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

mode_barcode_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):

