Report for dataset PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3-Cell2

Dataset 1fc87921-a4ad-4eac-a52f-9141cc94ea34

Summary

|	
Name	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3-Cell2
Created At	2022-08-09 18:34:50.975
Number of Records	45450400
Total Length	527324769474
Movie ID	m64069_220811_070224
ICS Version	11.0.0.144466
Well Sample	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3
Biological Sample	a

Raw Data Report

Summary

|	
Polymerase Read Bases	529,026,127,567
Polymerase Reads	5,460,790
Polymerase Read Length (mean)	96,877
Polymerase Read N50	210,037
Subread Length (mean)	11,602
Subread N50	12,849
Longest Subread Length (mean)	19,365
Longest Subread N50	26,300
Unique Molecular Yield	91,944,984,576

Polymerase Read Length
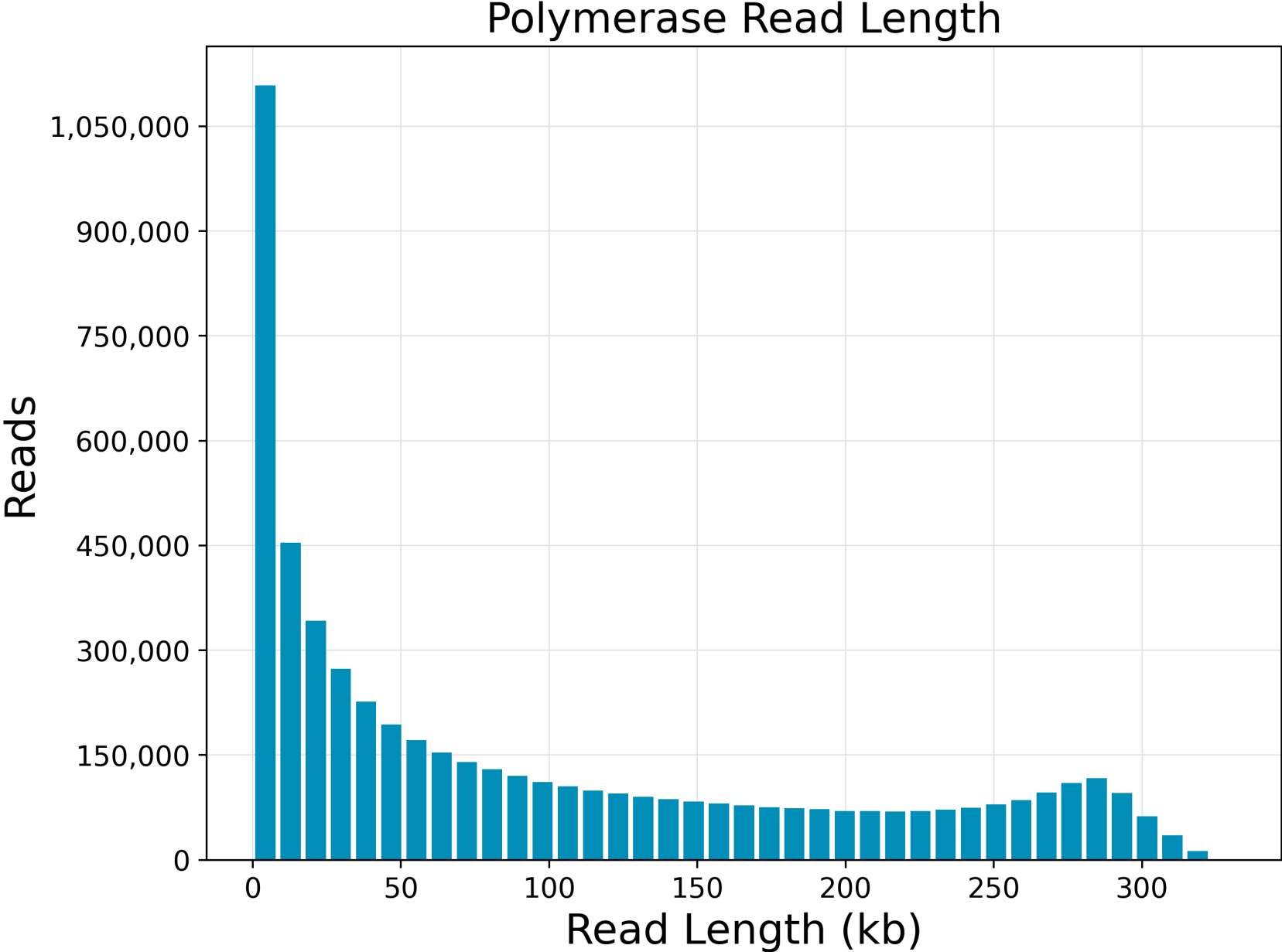
Subread Lengths
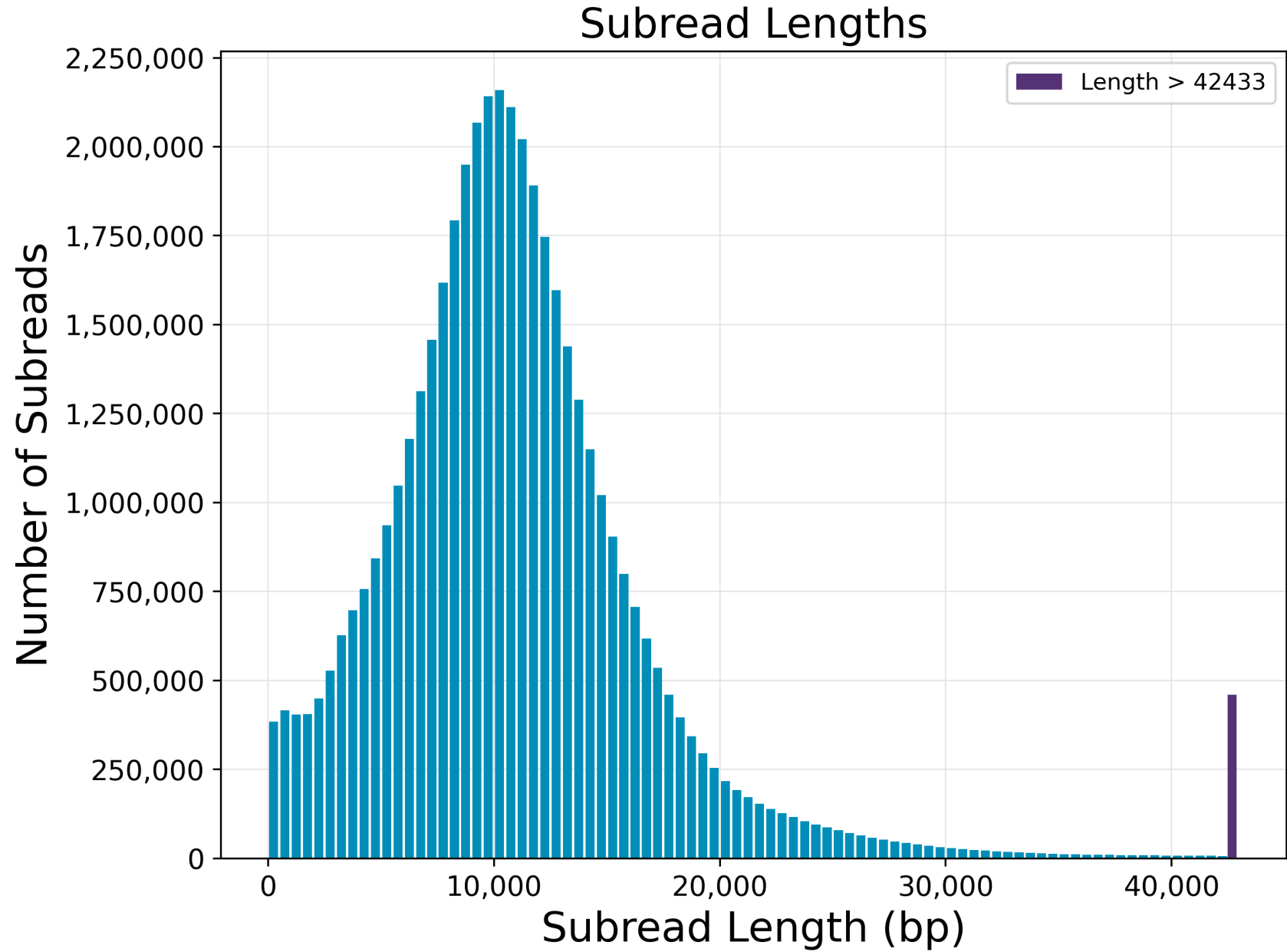
Longest Subread Length Versus Polymerase Read Length
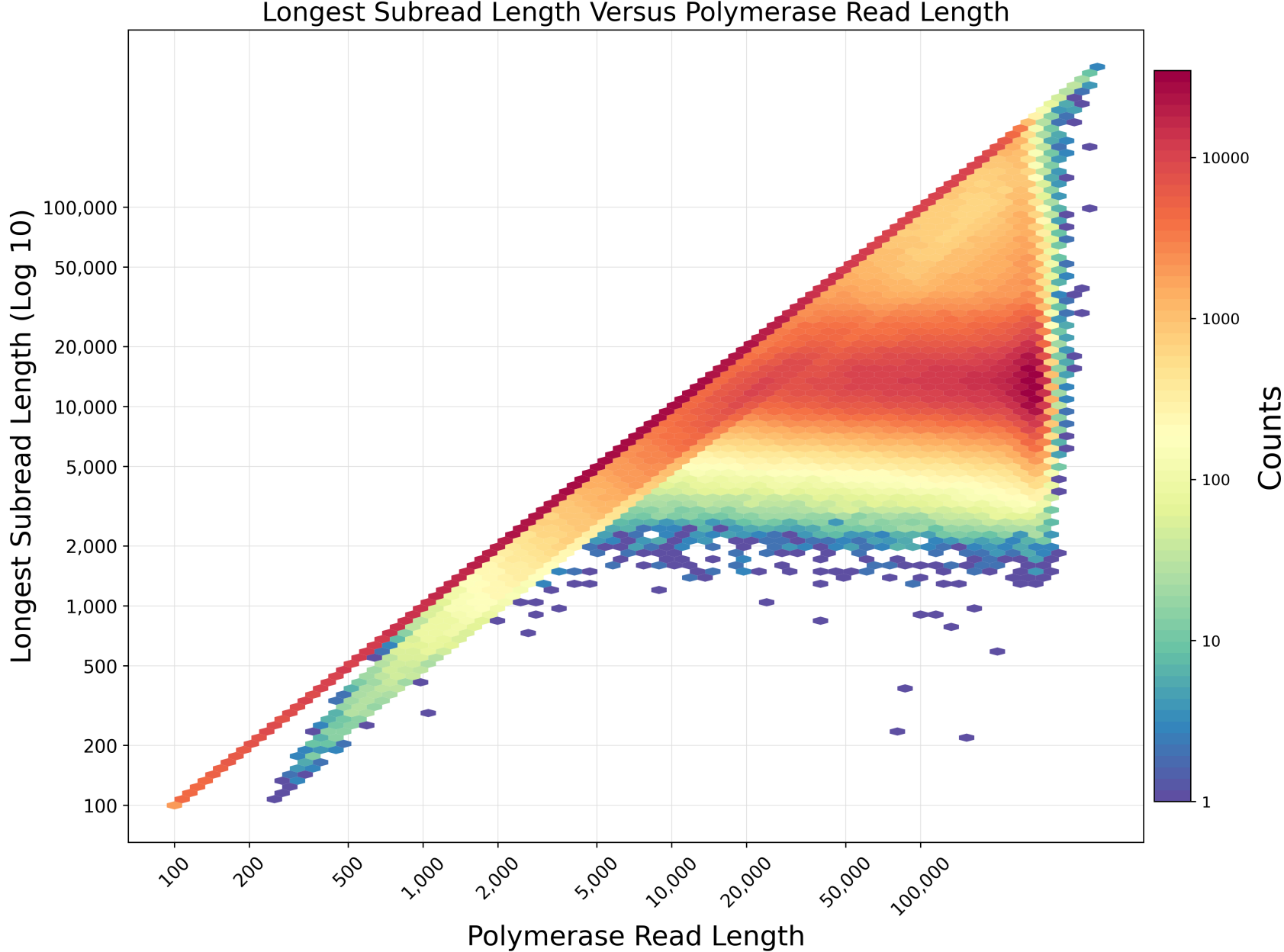
Base Yield Density
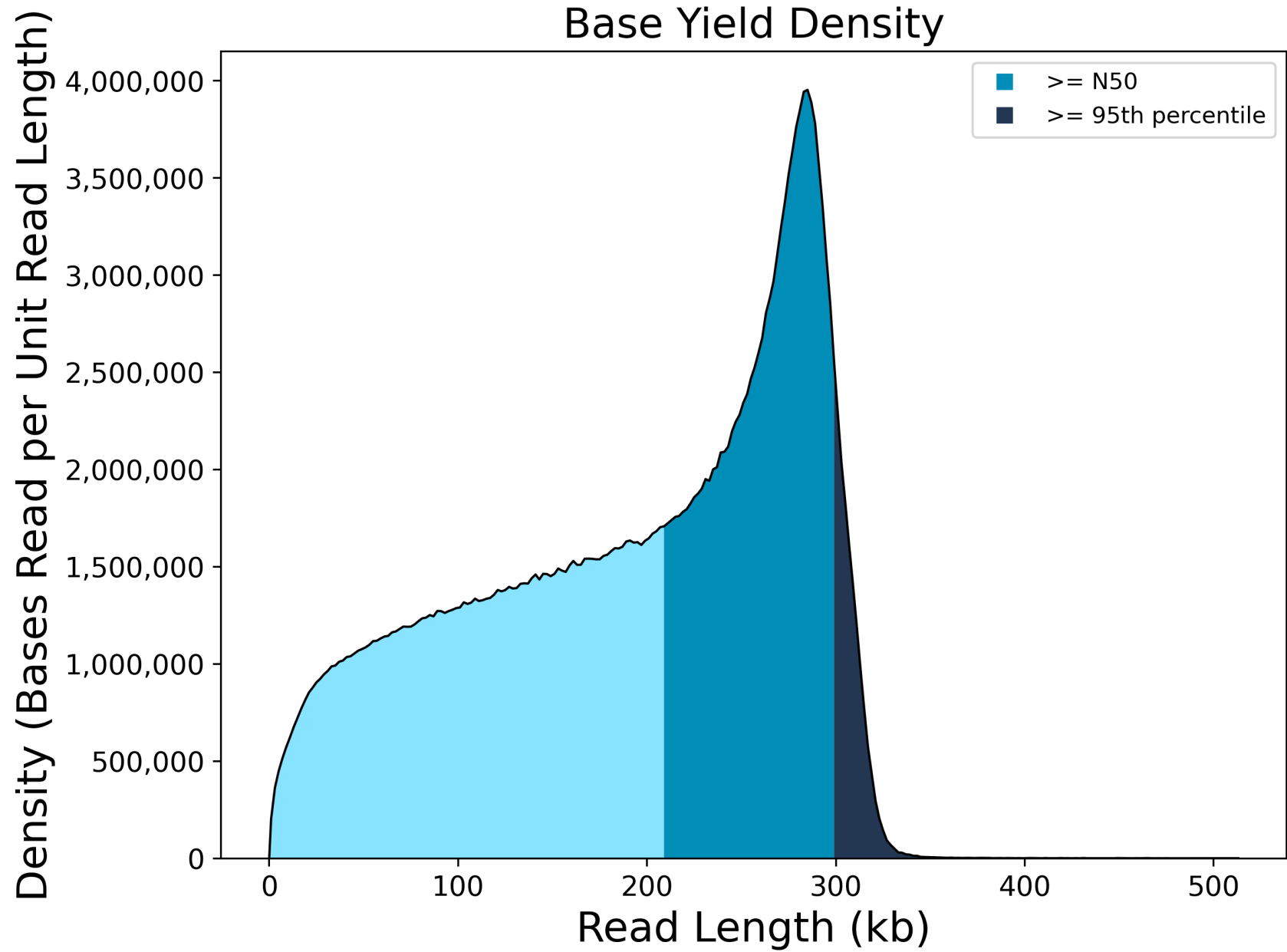
Loading Report

Summary

Productive ZMWs	8,013,797
Productivity 0	2,345,258
Productivity 1	5,490,122
Productivity 2	179,291

Loading Statistics

Collection Context	Productive ZMWs	Productivity 0	(%)	Productivity 1	(%)	Productivity 2	(%)	Loading type
m64069_220811_070224	8013797	2345258	29.26	5490122	68.50	179291	2.24	Diffusion

HQ Region Filtering Evaluation
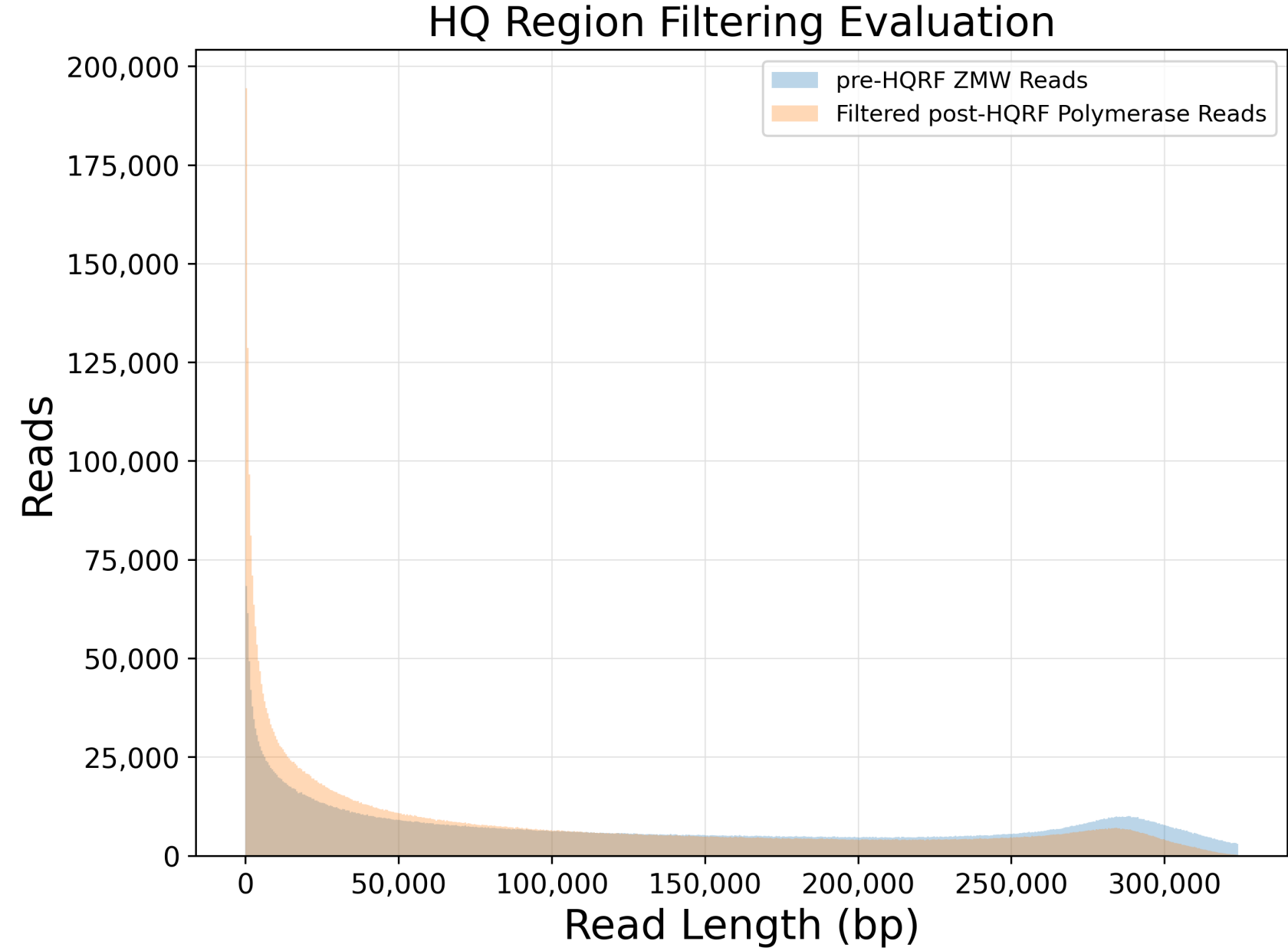
Adapter Report

Summary

Adapter Dimers (0-10bp) %	0
Short Inserts (11-100bp) %	0
Local Base Rate	2.77

Control Report

Summary

Number of Control Reads	874
Control Read Length Mean	52,677
Control Read Concordance Mean	0.86
Control Read Concordance Mode	0.89

Control Polymerase RL
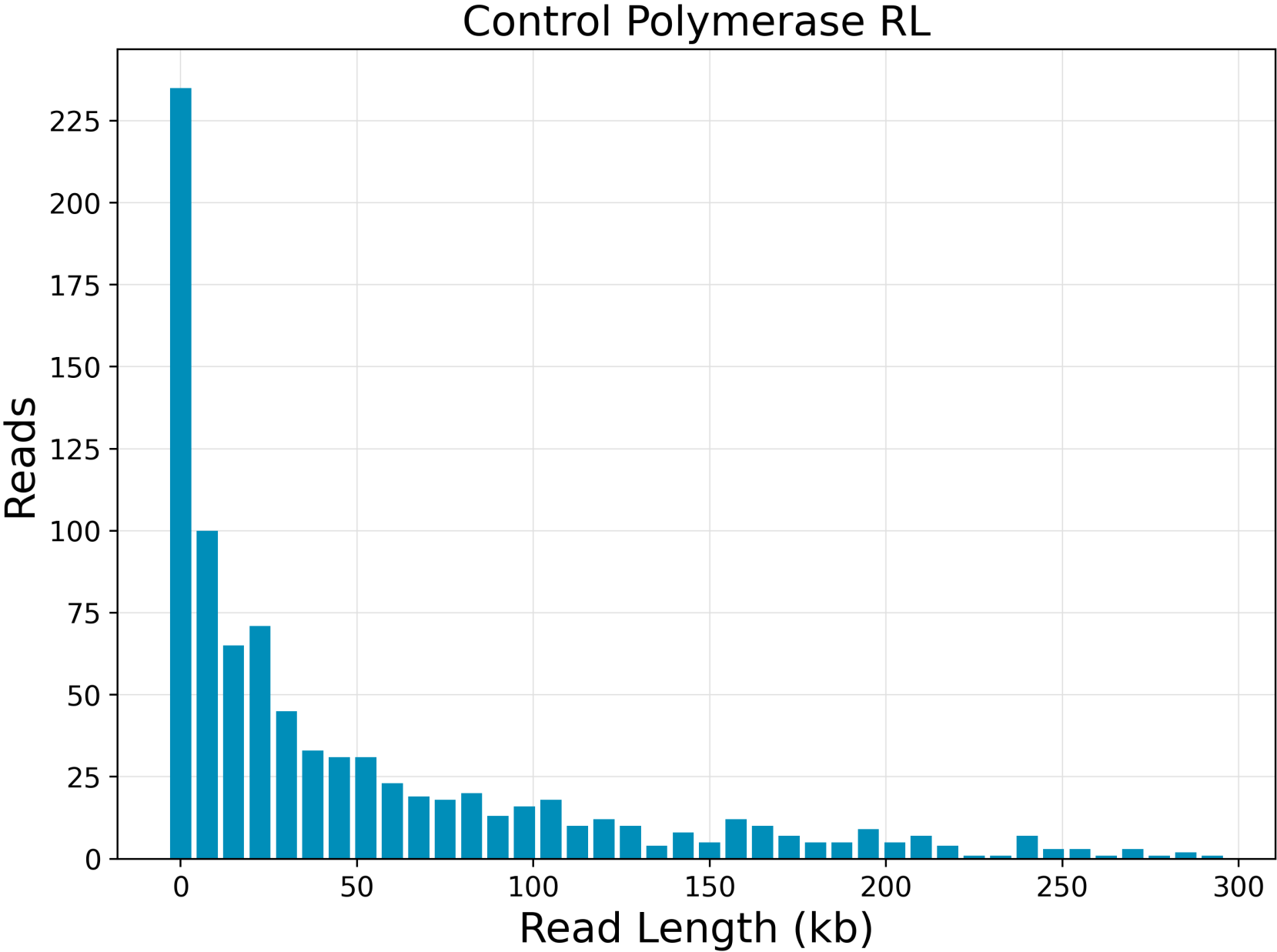
Control Concordance
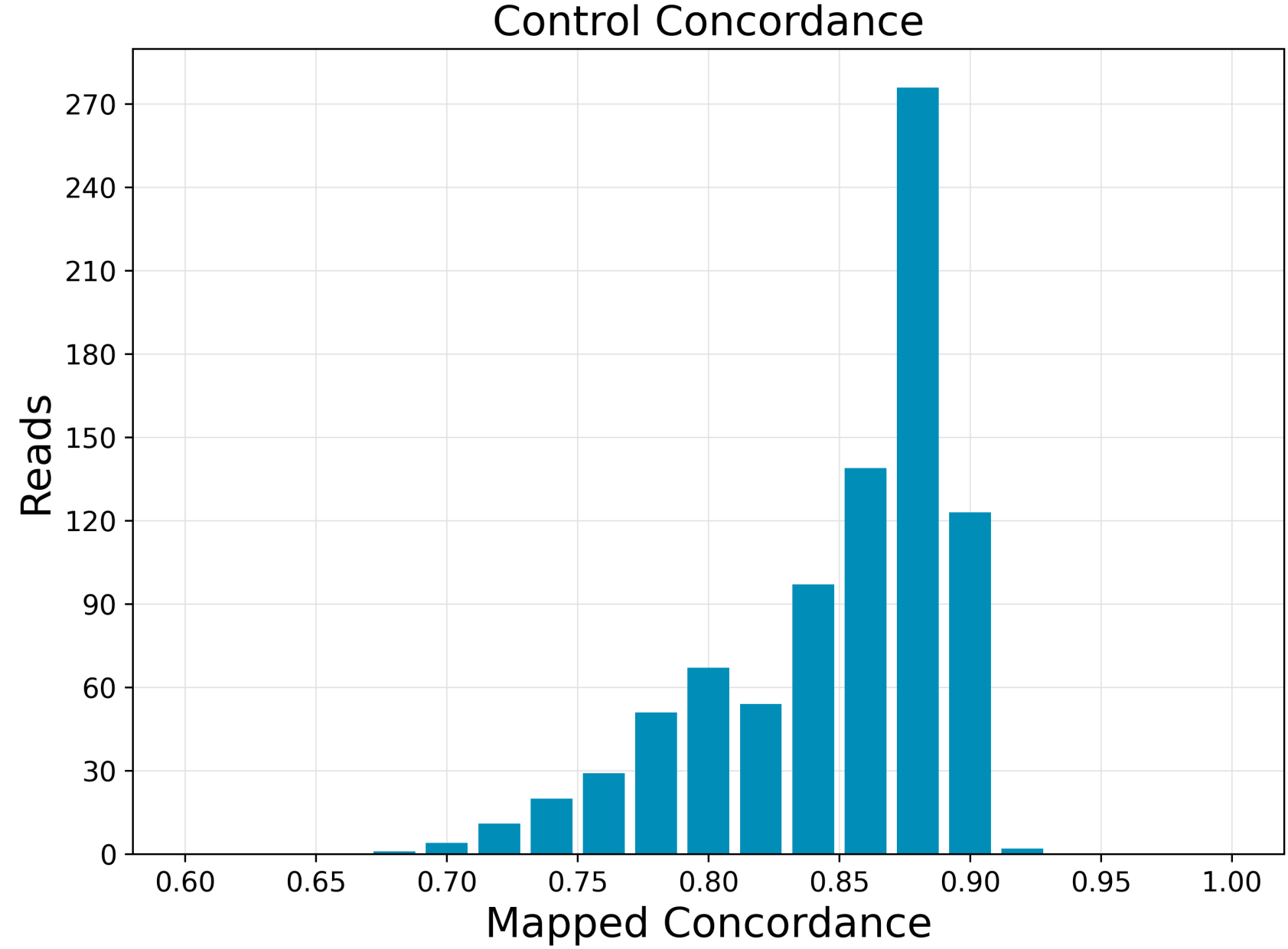
Sample Setup

Sample 87f73ffd-18c2-4255-ffdb-960c8c20a7eb

Sample Setup Instructions for Loading

SMRT Link: 11.0.0.146107; Chemistry Bundle: 11.0.0.143406; Params: 11.0.0

Sample Information

	Sample 1
Sample Name	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3
Sample Comment	
Sample Volume to Use	1.7 uL
# of SMRT Cells	1
Concentration	74 ng/uL 6.70 nM
Insert Size	17000 bp
Sequencing Primer	Sequencing Primer v5
Loading	Diffusion
Iso-Seq Experiment	No
Binding Kit	Sequel® II Binding Kit 2.2
Cleanup	AMPure
AMPure Cleanup Anticipated Yield	65 %
Sample Dilution Buffer	Adaptive Loading Buffer
Concentration On Plate	60 pM
Instrument Type	Sequel II
Application	HiFi Reads
	19.9 nM 1.0 nM

Primer Concentration Template Concentration	
	5.2 nM 0.5 nM

Polymerase Concentration Template Concentration	
Minimum Pipetting Volume	1 uL
Target Annealing Sample Concentration	1 nM
Target Annealing Primer Concentration	20 nM
Target Binding Concentration	0.5 nM
Target Polymerase Concentration (Relative)	10 X
% of Annealing Reaction to Use in Binding	90 %
Warnings	

Conditioning Primer

Reagent	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Sequencing Primer v5	1.0 uL	
1x Elution Buffer	29.0 uL	
Total Volume	30.0 uL	

Incubate @80C for 2 minutes, then hold at 4C. Conditioned primer may be stored at -20C and used for up to 30 days.

Annealing primer

Reagent	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Water	6.3 uL	
10x Primer Buffer v2	2.3 uL	
Sample	1.7 uL	
Conditioned Sequencing Primer v5	1.4 uL	
Total Volume	11.7 uL	

Incubate at room temperature for 15 minutes then transfer to a 4C location for immediate use, or store at -20C for long-term use.(*)

Bind polymerase to annealed SMRTbell

Polymerase Dilution

Reagent	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Sequel® II Polymerase 2.2	1.0 uL	
Sequel® Binding Buffer	9.0 uL	
Total Volume	10.0 uL	

A secondary polymerase dilution of the previous dilution is used (rather than initial dilution directly to the target concentration) so that the primary dilution may used for additional samples if desired.

Reagent	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Primary dilution of Sequel® II Polymerase 2.2	2.5 uL	
Sequel® Binding Buffer	1.0 uL	
Total Volume	3.5 uL	

Diluted Polymerase must be used immediately, any remaining should be discarded.

Binding

Add annealed sample to dNTPs, water and Binding Buffer.
Finger tap or pipet to mix before adding diluted polymerase

Reagent	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Sequel® Binding Buffer	3.2 uL	
Water	3.7 uL	
Sequel® dNTP	2.1 uL	
Annealed Sample	10.6 uL	
Diluted Sequel® II Polymerase 2.2	1.0 uL	
Total Volume	20.6 uL	

Incubate at room temperature for 15 minutes. Finish all preparations with a hold at 4C. Bound complex can be used right away or stored at 4C for 7 days.(*)

AMPure® PB Purification of Polymerase Bound SMRTbell® Complexes

1. Equilibrate the AMPure PB beads, Complex Dilution Buffer, and Adaptive Loading Buffer to room temperature.

2. Add Complex Dilution Buffer to binding reaction.

	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Volume of Binding Reaction (uL)	20.6 uL	
Volume of Complex Dilution Buffer (uL)	79.4 uL	
Total Volume (uL)	100.0 uL	

3. Measure the concentration with a Qubit® High Sensitivity kit. Enter concentration and volume of diluted bound complex into the yellow boxes below:

	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Volume of Diluted Bound Complex (uL)		

	100 uL	
	100 uL	
Diluted Bound Complex Concentration (ng/uL)		

	1.34 ng/uL
	1.34 ng/uL

4. Add AMPure PB beads and gently pipette-mix.

	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Volume of AMPure PB beads (uL)	120.0 uL	

5. To bind the polymerase-bound complexes to AMPure PB beads, incubate the mixture on the benchtop for 5 minutes. Note: Longer incubation times have not been tested and may have a negative impact on polymerase-template complex stability due to high salt concentration.

6. Place the tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear. Discard the supernatant. DO NOT wash the collected bead pellet with ethanol.

7. Immediately resuspend the beads in room temperature Adaptive Loading Buffer and pipette-mix.

	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Volume of Adaptive Loading Buffer (uL)	50.0 uL	

8. To elute the polymerase-bound complexes, incubate the sample on the benchtop for at least 15 minutes at room temperature.

9. Place the tube in a magnetic bead rack until the beads collect to the side of the tube and the solution appears clear.

10. Transfer eluate to a new LoBind microcentrifuge tube and protect it from light. Place the tube (containing the now-purified polymerase-bound complexes) on ice.

11. Measure the recovered DNA concentration with a Qubit® High Sensitivity kit. Enter concentration and volume of the purified complex into the yellow boxes below:

	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Volume of Purified Complex (uL)		

	50 uL	
	50 uL	
Purified Complex Concentration (ng/uL)		

	2.11 ng/uL	
	2.11 ng/uL	
Molar Concentration of Purified Complex (pM)	191.0 pM	
AMPure Cleanup Yield (%)	79.0 %	

Internal Control Dilution

Perform two sequential 1:100 dilution steps using Sequel® Complex Dilution Buffer. The resulting solution contains 30X internal control complex.

1. First Dilution

Reagent	Internal Control		Notes
Sequel® Complex Dilution Buffer	99.0 uL		
Internal Control Stock	1.0 uL		
Total Volume	100.0 uL		

Mix well by flicking tube by hand and pulse spin to collect contents and keep on ice.

2. Second Dilution

Reagent	Internal Control		Notes
Sequel® Complex Dilution Buffer	99.0 uL		
Diluted Internal Control (Dilution 1)	1.0 uL		
Total Volume	100.0 uL		

Mix well by flicking tube by hand and pulse spin to collect contents and keep on ice.

Final Loading Dilution

Reagent	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3	Notes
Adaptive Loading Buffer	75.7 uL	
Prepared sample	35.5 uL	
Diluted Internal Control (Dilution 2)	3.8 uL	
Total Volume	115.0 uL	
# of SMRT Cells requested	1	

Show values for a different number of cells

	1 1	
On Plate Loading Concentration requested	60 pM	

Show values for a different OPLC

| | | 59 pM | |
| | | 59 pM | |

Load 115 uL of sample per well and store at 4C for up to 24 hours before use.

(*) Sequencing performance after storage is dependent on DNA sample quality/type and cannot be guaranteed.

Instrument run(s)

Run e5451f5d-65ad-460b-8409-df561548c8b1

Summary

Name	08.09.2022_SQ2
Status	UNKNOWN
Created	2022-08-09 18:34:50.975
Started	2022-08-09 19:57:07.434
Completed	2022-08-22 01:00:00.209
Context	r64069_20220809_195002
Instrument Name	64069
Instrument Serial Number	64069
ICS Version	11.0.0.144466
Primary Analysis Version	11.0.0.144466
Chemistry Version	11.0.0.143406

Parent jobs (1)

Job 4459

Summary

Job Type	import-dataset
Pipeline	cromwell.workflows.sl_dataset_reports
Name	import-dataset
Comments	Description for job Import PacBio DataSet
Created At	2022-08-13 07:28:09.043
SMRT Link Version	11.0.0.146107

Child jobs (1)

Job 4445

Summary

Job Type	analysis
Pipeline	cromwell.workflows.pb_ccs
Name	CCS of PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3
Comments	
Created At	2022-08-09 18:34:50.660
SMRT Link Version	11.0.0.146107

Output files

File name	Size	Type
PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell3-Cell2 (CCS)	20,636	ConsensusReadSet
m64069_220811_070224.hifi_reads.fasta.gz	9,191,073,346	Fasta
m64069_220811_070224.hifi_reads.bam	19,593,517,525	ConsensusReadBamFile
m64069_220811_070224.hifi_reads.fastq.gz	20,773,325,110	Fastq
PacBio.FileTypes.Datastore file	772	Datastore
All Reads (BAM)	40,707,049,089	bam
PacBio.FileTypes.Datastore file	783	Datastore
PacBio.FileTypes.Datastore file	773	Datastore
PacBio.FileTypes.json file	66,020	json
PacBio.FileTypes.json file	71,587,072	json
CCS Analysis Per-Read Details	36,709,031	zip
Analysis Log	1,643,411	log
SMRT Link Log	30,428	log

CCS Analysis Report

Summary

HiFi Reads	2,157,413
HiFi Yield (bp)	26,475,173,531
HiFi Read Length (mean, bp)	12,271
HiFi Read Quality (median)	Q36
HiFi Read Quality (median)	36
HiFi Number of Passes (mean)	14
<Q20 Reads	390,840
<Q20 Yield (bp)	5,427,228,135
<Q20 Read Length (mean, bp)	13,886
<Q20 Read Quality (median)	Q17
<Q20 Read Quality (median)	17

HiFi Read Length Summary

Read Length (bp)	Reads	Reads (%)	Yield (bp)	Yield (%)
0	2157413	100	26475173531	100
5,000	2107619	98	26275329189	99
10,000	1460213	68	20973902395	79
15,000	478774	22	8965519234	34
20,000	127243	6	3013844330	11
25,000	35400	2	986399672	4
30,000	6219	0	199666494	1
35,000	484	0	17902301	0
40,000	46	0	1983589	0

HiFi Read Quality Summary

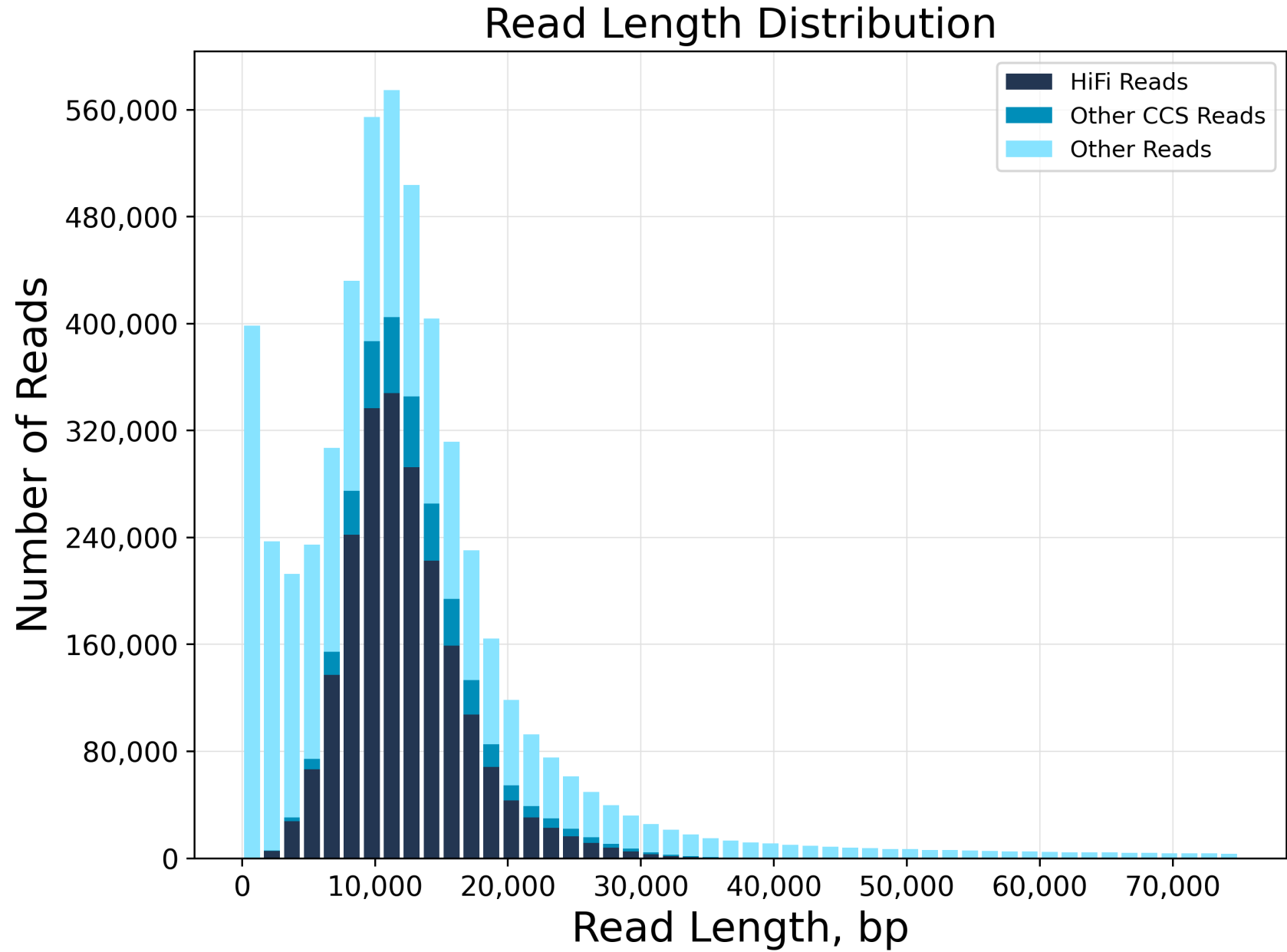| Read Quality (Phred)	Reads	Reads (%)	Yield (bp)	Yield (%)
Q20	2157413	100	26475173531	100
Q30	1509460	70	17697124638	67
Q40	750848	35	7803675939	29
Q50	265188	12	2334944793	9

HiFi Read Length Distribution
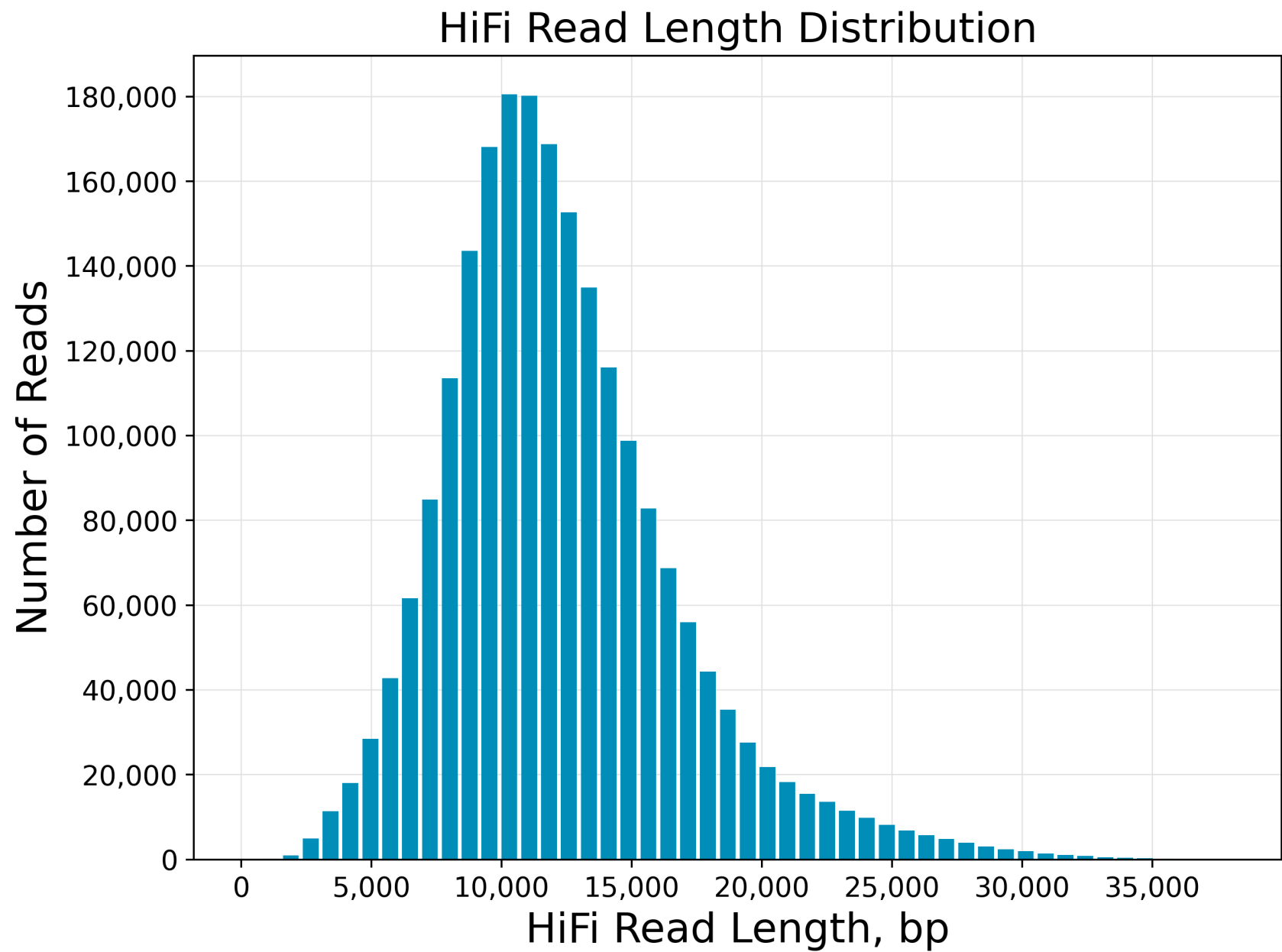
Yield by HiFi Read Length
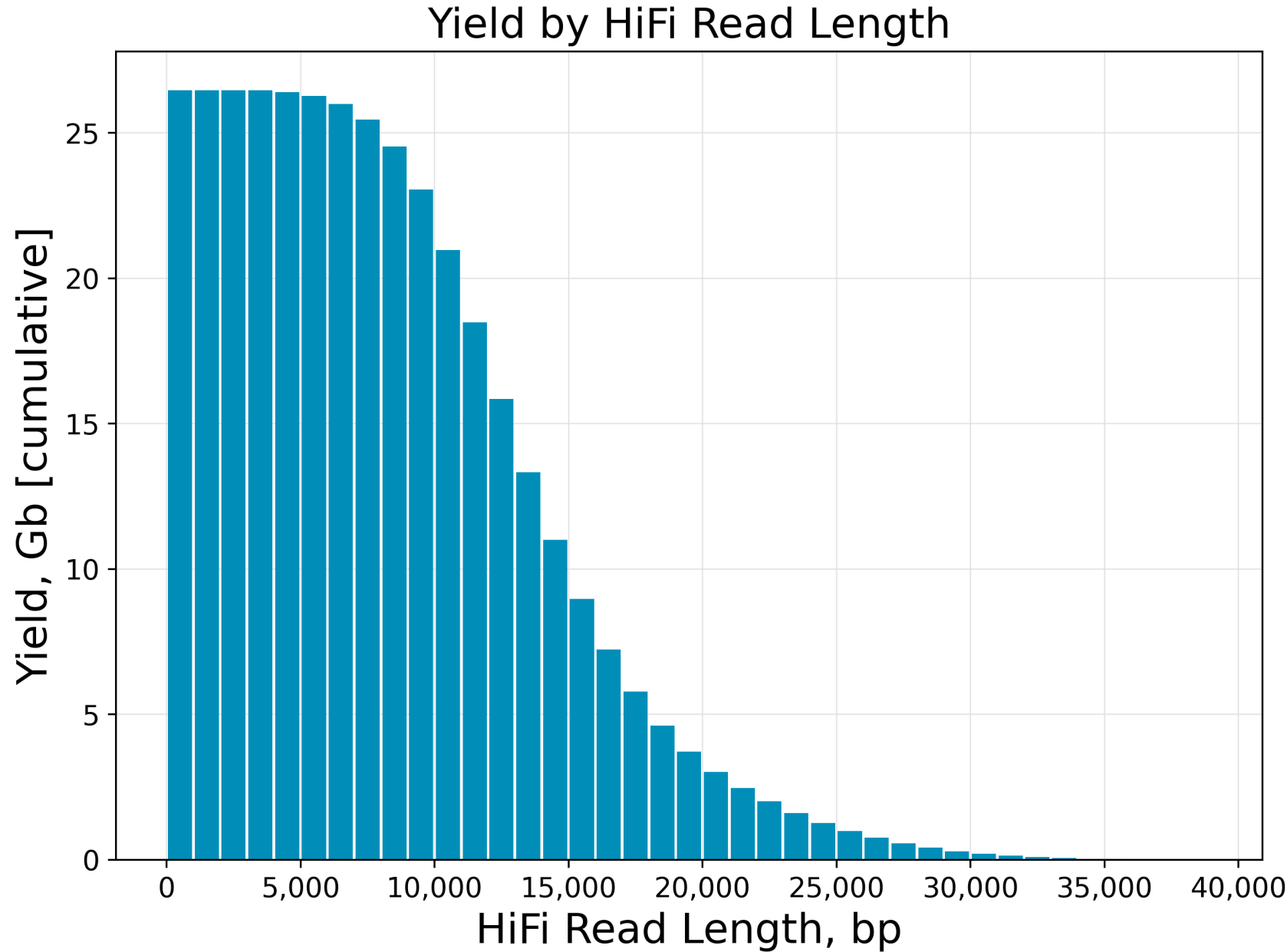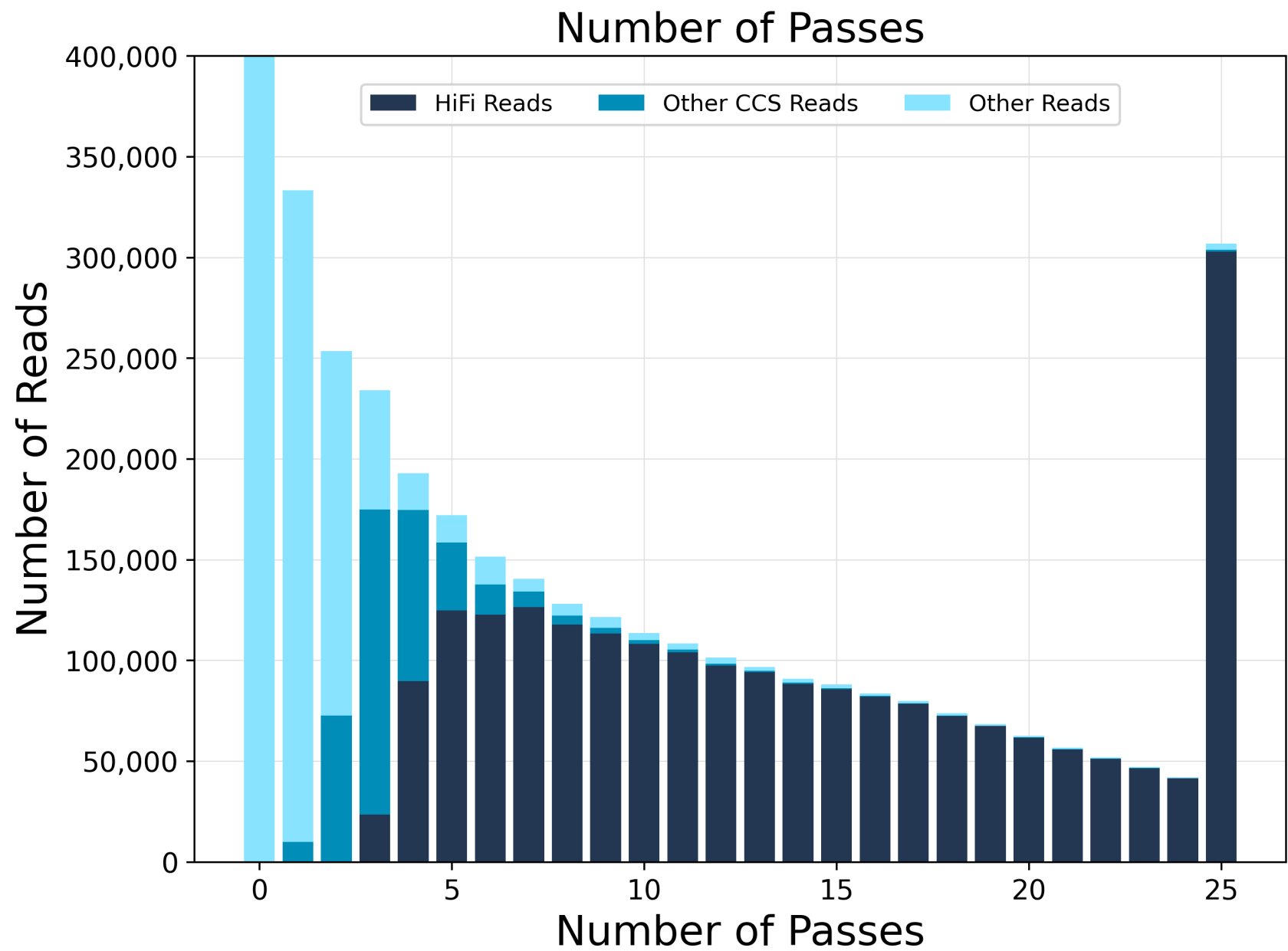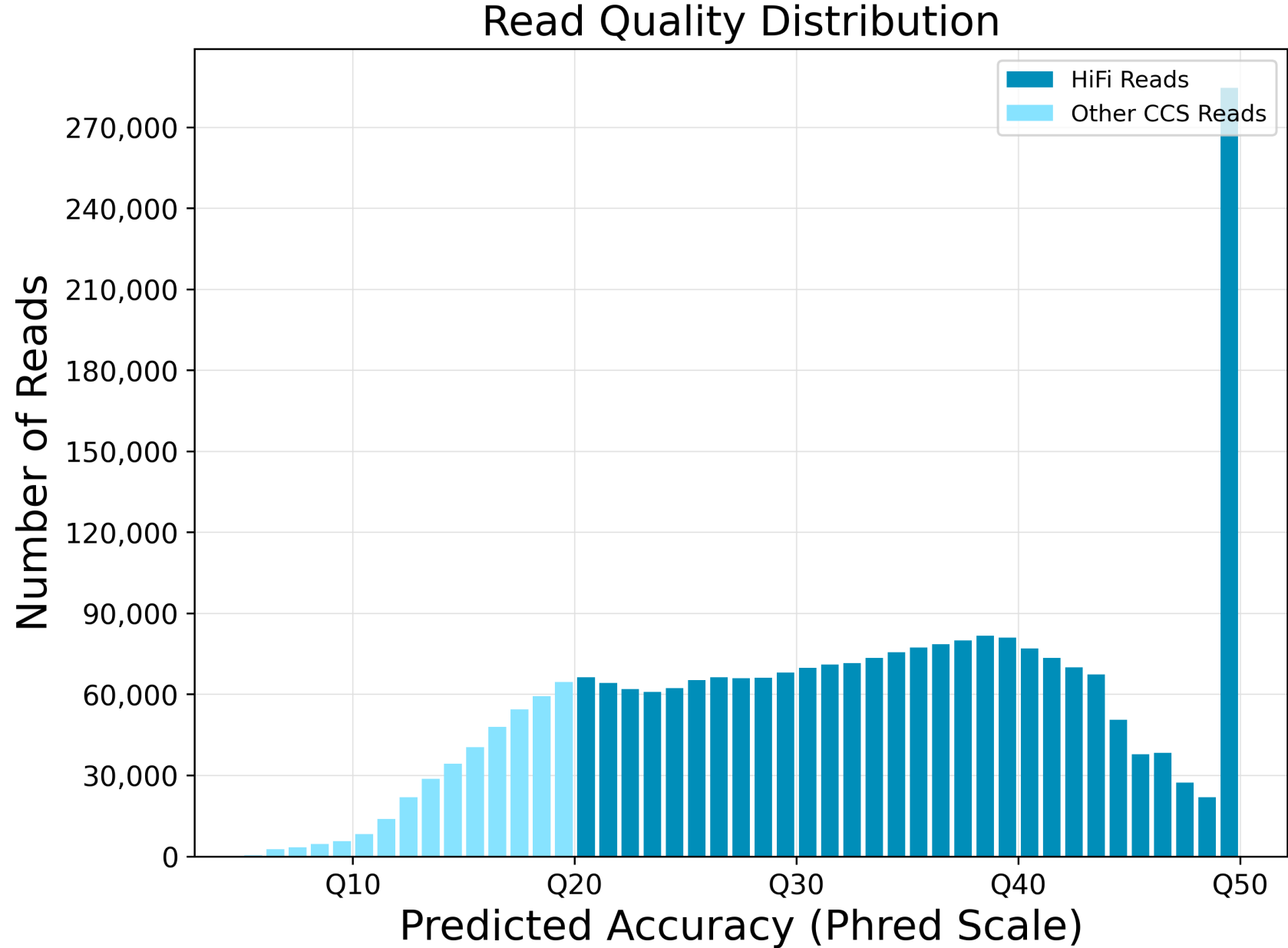
Read Length Distribution

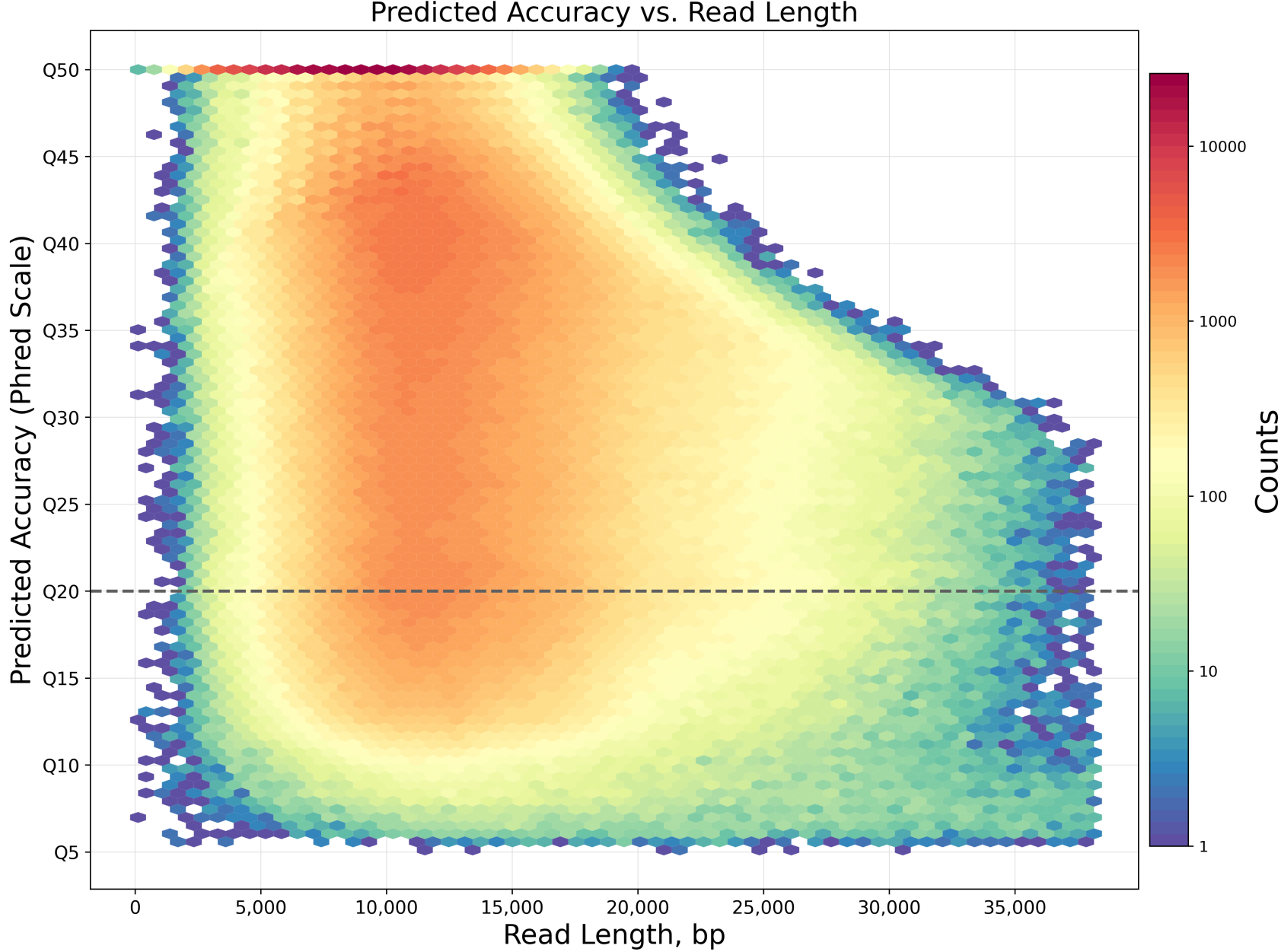Number of Passes

Read Quality Distribution

Predicted Accuracy vs. Read Length

CCS Processing

Summary

ZMWs input	5,460,790
ZMWs pass filters	2,548,253
ZMWs fail filters	458,937
ZMWs shortcut filters	2,453,600
ZMWs with tandem repeats	25,416
Below SNR threshold	145,985
Median length filter	0
Lacking full passes	0
Heteroduplex insertions	38,044
Coverage drops	7,755
Insufficient draft cov	195,486
Draft too different	0
Draft generation error	69,232
Draft above --max-length	0
Draft below --min-length	10
Reads failed polishing	1
Empty coverage windows	882
CCS did not converge	1,542
CCS below minimum RQ	0
Unknown error	0
ZMWs missing adapters	185,958