

Report for dataset PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell1-Cell3

Dataset 0385b7fb-f292-4da5-8555-e4eab77fcc2a

Summary

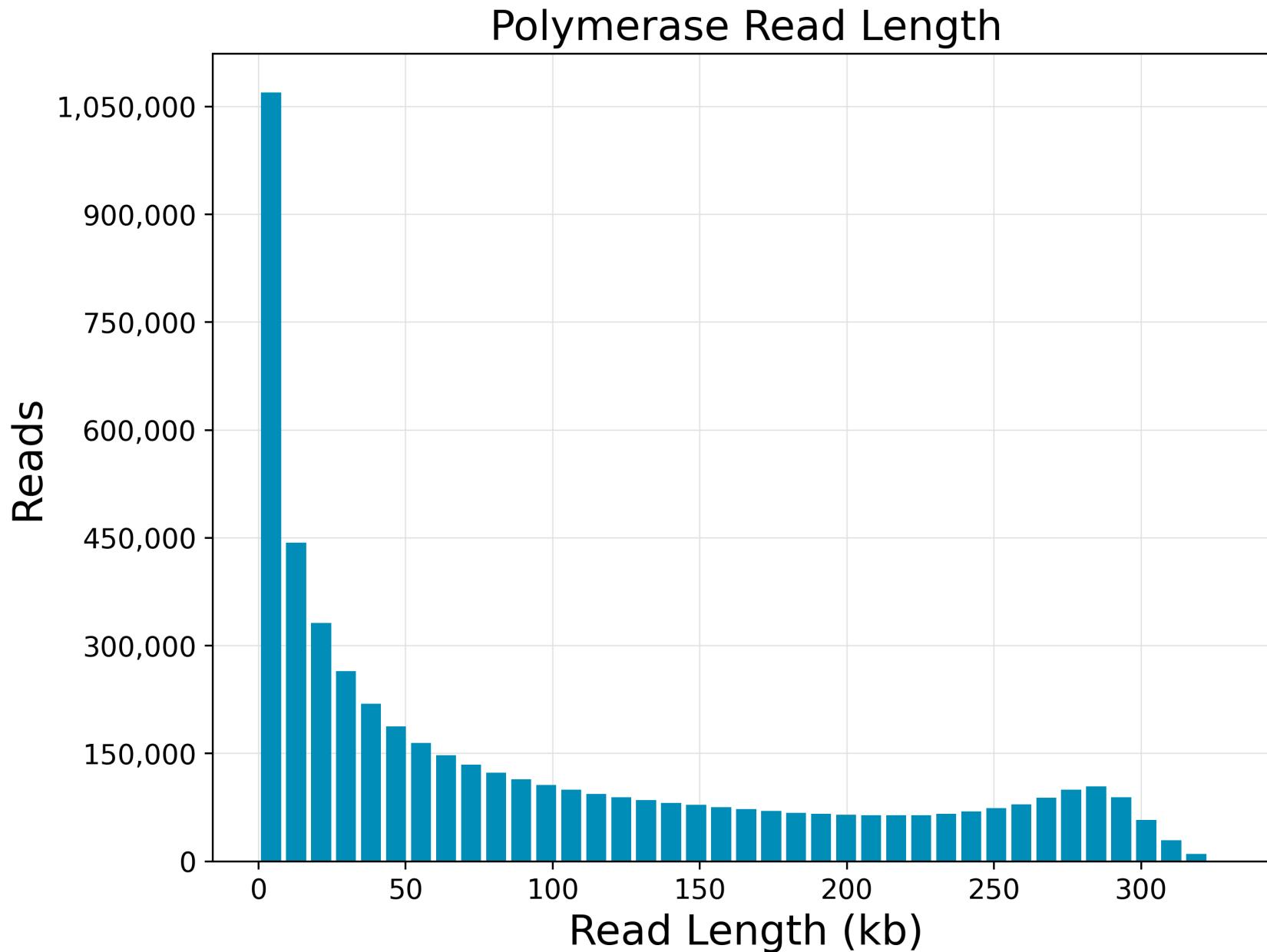
Name	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell1-Cell3
Created At	2022-07-26 17:57:34.243
Number of Records	41476319
Total Length	488863175815
Movie ID	m64069_220729_164623
ICS Version	11.0.0.144466
Well Sample	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell1
Biological Sample	a

Raw Data Report

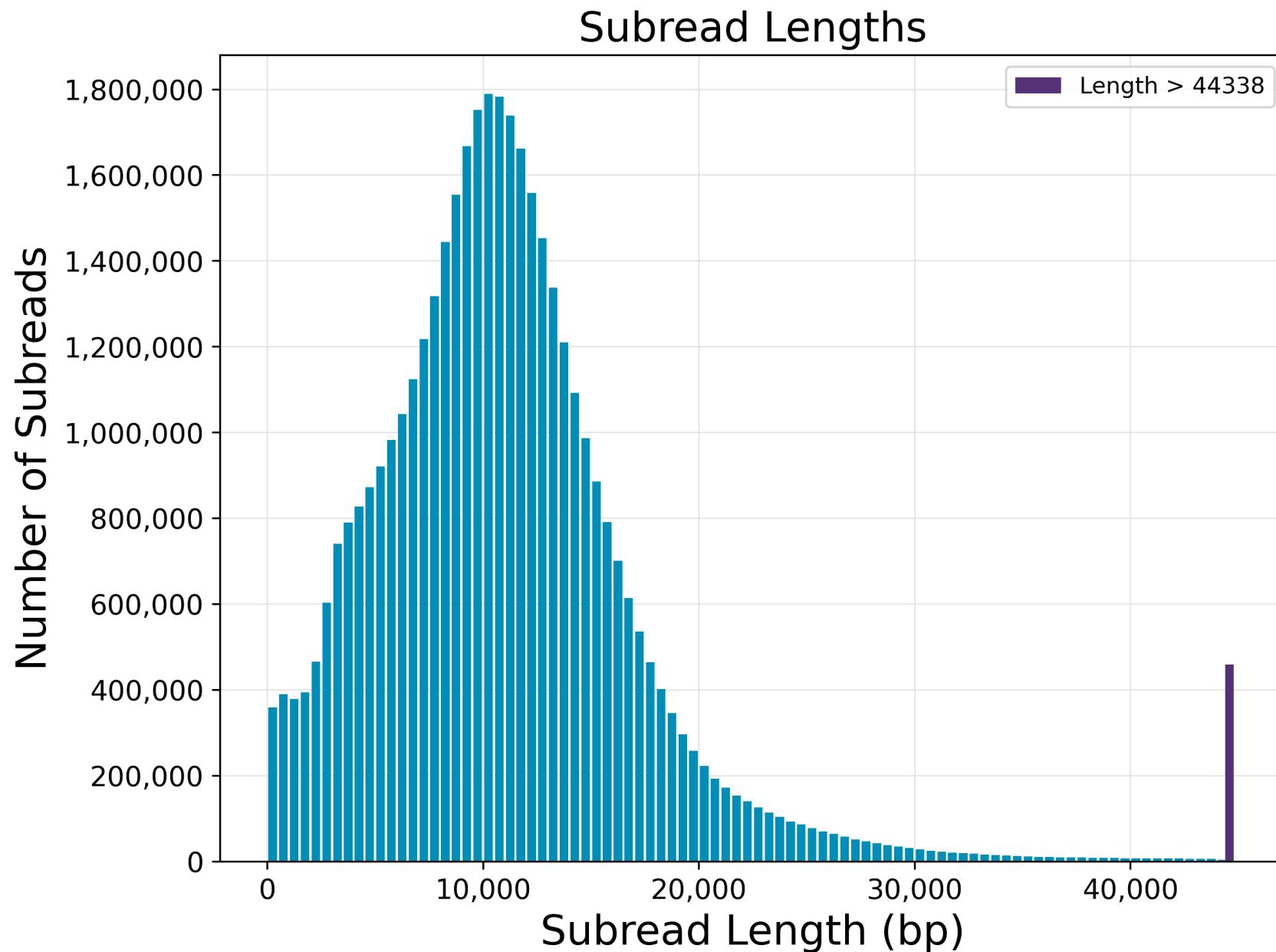
Summary

Polymerase Read Bases	490,413,044,938
Polymerase Reads	5,183,477
Polymerase Read Length (mean)	94,610
Polymerase Read N50	207,437
Subread Length (mean)	11,786
Subread N50	13,313
Longest Subread Length (mean)	20,217
Longest Subread N50	28,752
Unique Molecular Yield	92,424,527,872

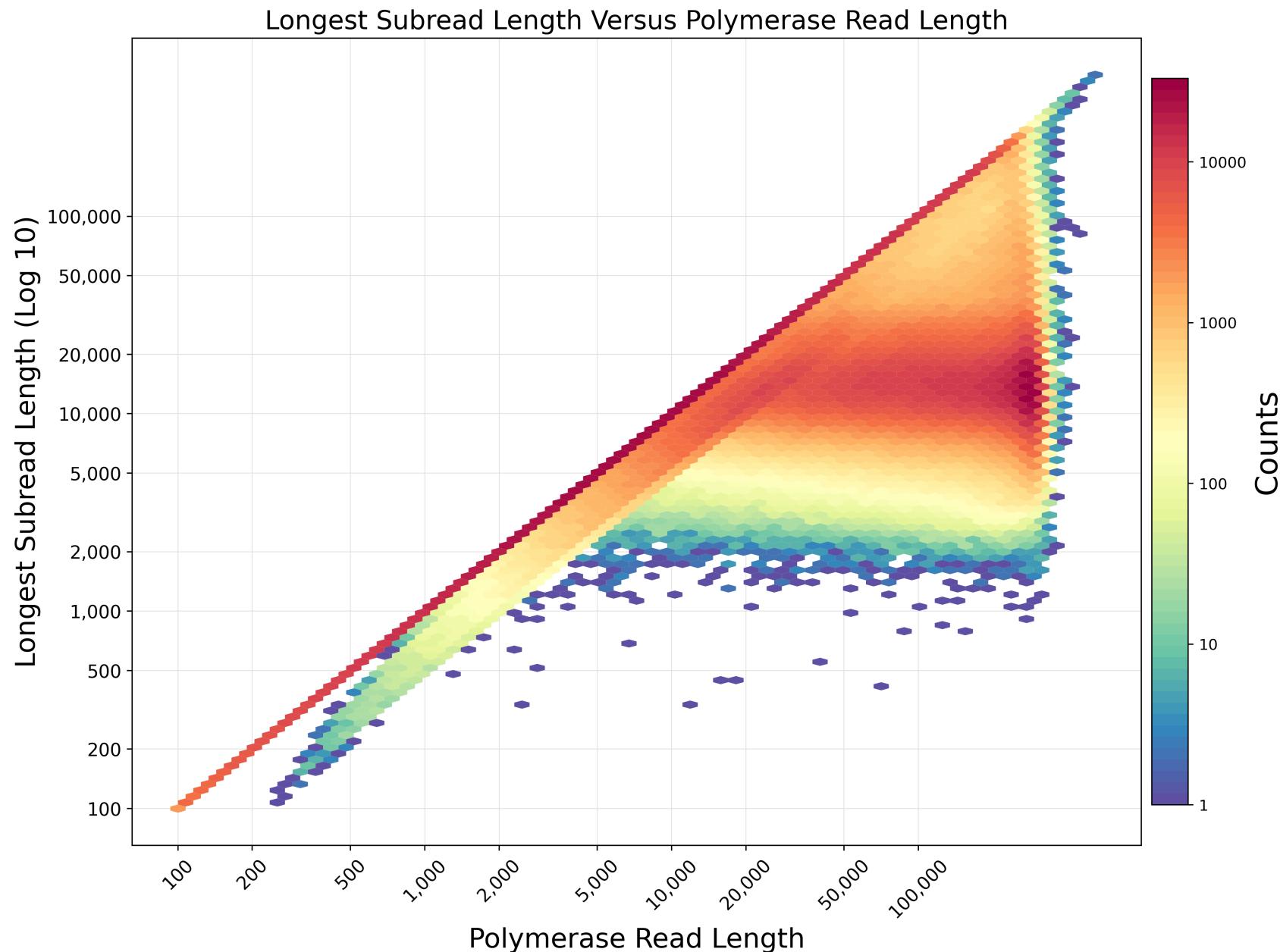
Polymerase Read Length



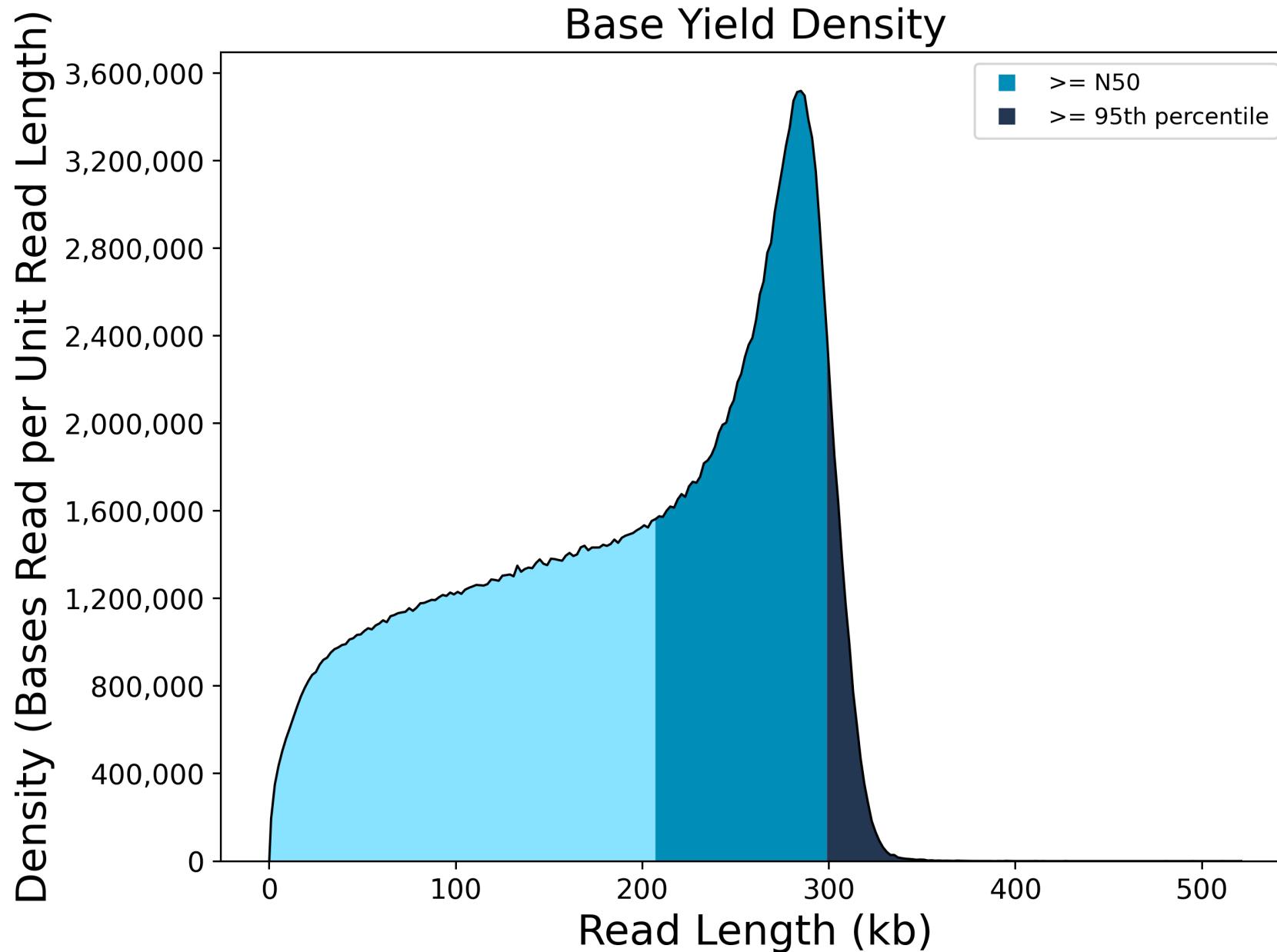
Subread Length



Longest Subread Length Versus Polymerase Read Length



Base Yield Density



Loading Report

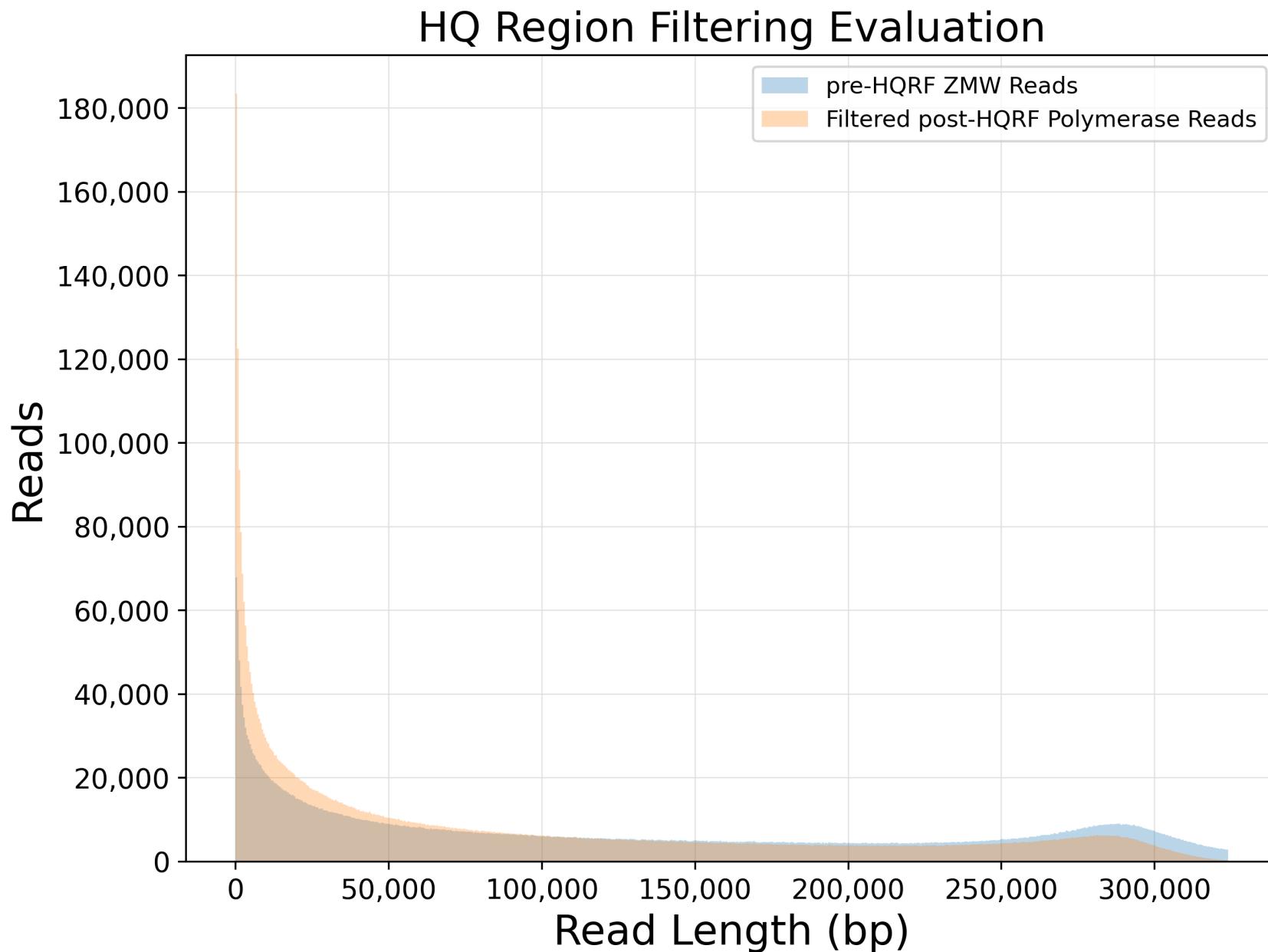
Summary

Productive ZMWs	8,013,164
Productivity 0	2,625,167
Productivity 1	5,212,347
Productivity 2	177,157

Loading Statistics

Collection Context	Productive ZMWs	Productivity 0	(%)	Productivity 1	(%)	Productivity 2	(%)	Loading type
m64069_220729_164623	8013164	2625167	32.76	5212347	65.04	177157	2.21	Diffusion

HQ Region Filtering Evaluation



Adapter Report

Summary

Adapter Dimers (0-10bp) %	0
Short Inserts (11-100bp) %	0
Local Base Rate	2.76

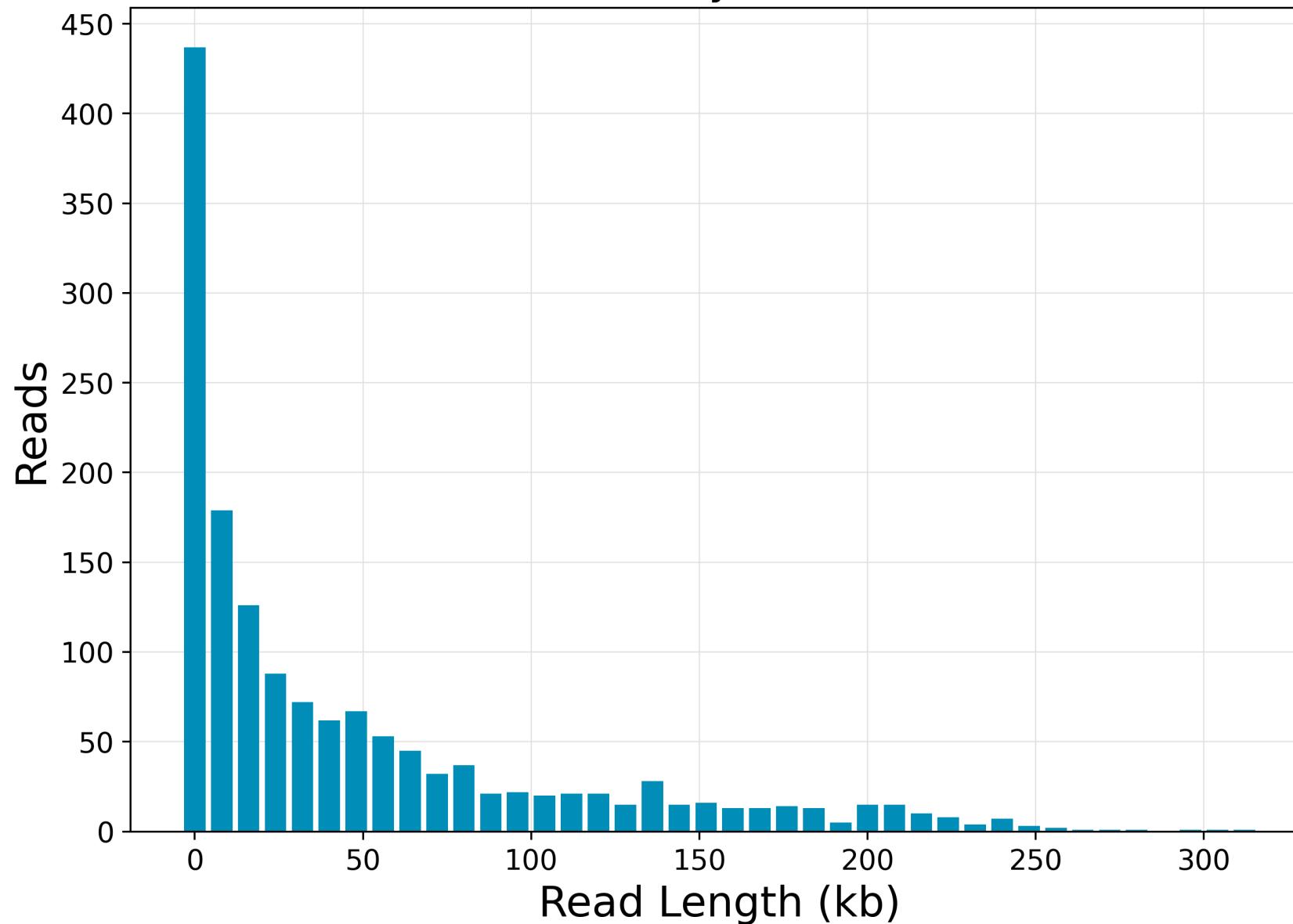
Control Report

Summary

Number of Control Reads	1,507
Control Read Length Mean	51,648
Control Read Concordance Mean	0.86
Control Read Concordance Mode	0.89

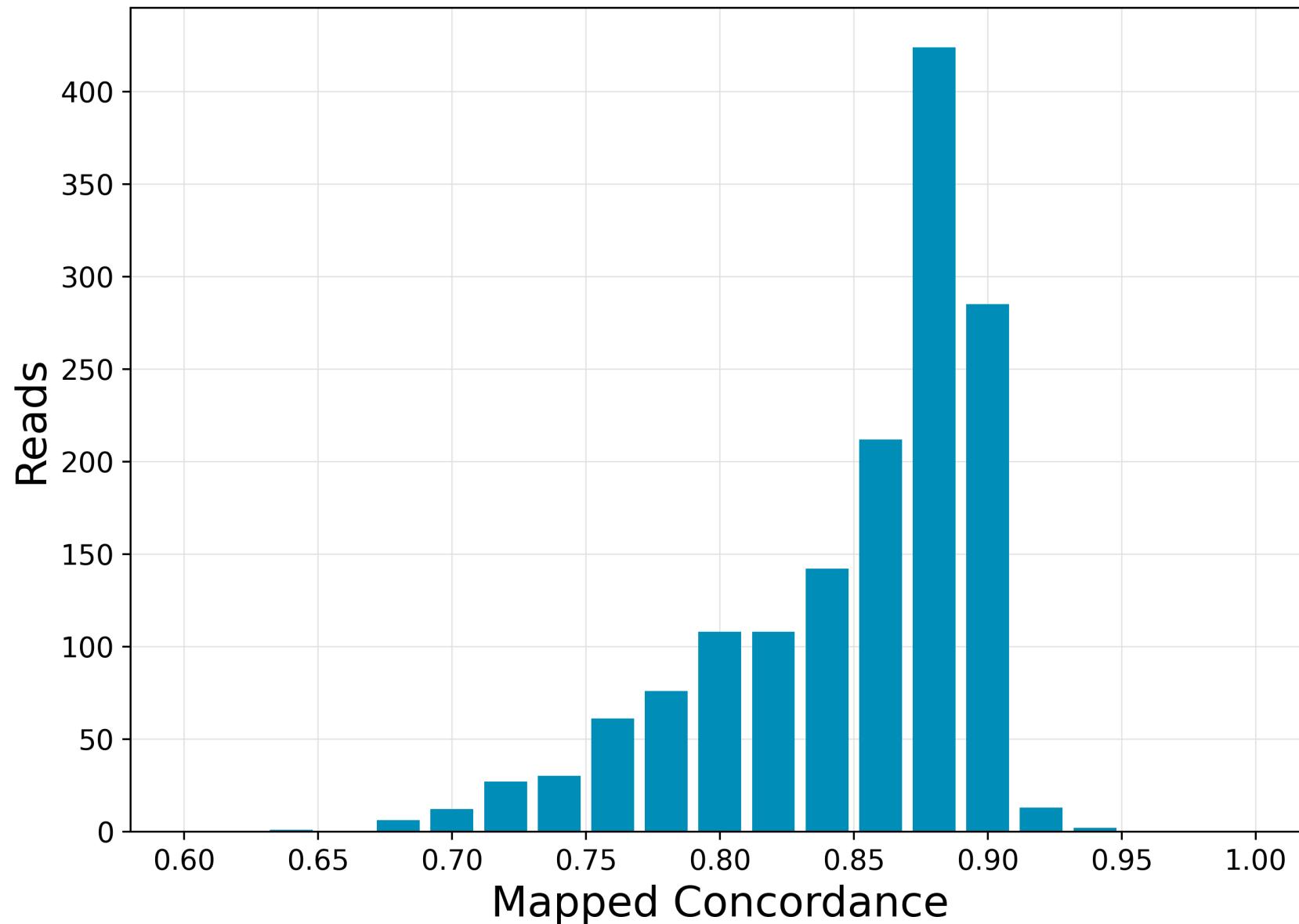
Control Polymerase RL

Control Polymerase RL



Control Concordance

Control Concordance



Sample Setup

Sample 7ae80a03-d560-9b01-044f-ab2b7c3be1f1

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_cell1
Sample Comment	
Sample Volume to Use	3.4 uL
# of SMRT Cells	2
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	58 pM
Instrument Type	Sequel II
Application	HiFi Reads
	19.9 nM 1.0 nM

Primer Concentration Template Concentration	
	5.1 nM 0.5 nM

Polymerase Concentration	
Template Concentration	
Minimum Pipetting Volume	1 μ L
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	<ul style="list-style-type: none"> • Due to high OPLC or a large number of requested SMRT Cells, the annealing reaction volume (and reagent consumption rate) is higher than normal. • The entered sample quantity exceeds 125% of the theoretical maximum. (126%)

Conditioning Primer

Reagent	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell1	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_cell1	Notes
Water	12.0 uL	
10x Primer Buffer v2	4.5 uL	
Sample	3.4 uL	
Conditioned Sequencing Primer v5	2.7 uL	
Total Volume	22.6 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_cell1	Notes
Sequel® II Polymerase 2.2	1.0 uL	
Sequel® Binding Buffer	9.0 uL	
Total Volume	10.0 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_cell1	Notes
Sequel® Binding Buffer	7.0 uL	
Water	8.3 uL	
Sequel® dNTP	4.1 uL	
Annealed Sample	20.5 uL	
Diluted Sequel® II Polymerase 2.2	1.4 uL	
Total Volume	41.3 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_cell1	Notes
Volume of Binding Reaction (uL)	41.3 uL	
Volume of Complex Dilution Buffer (uL)	58.7 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_cell1	Notes
Volume of Diluted Bound Complex (uL)		

100 uL
100 uL

Diluted Bound Complex Concentration (ng/uL)

	2.89 ng/uL 2.89 ng/uL	
Warnings	The entered sample quantity exceeds 125% of the theoretical maximum. (126%)	

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

	PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell1	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_cell1	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_cell1	Notes
Volume of Purified Complex (uL)		

50 μ L
50 μ L

Purified Complex Concentration (ng/ μ L)

	4.47 ng/uL 4.47 ng/uL	
Molar Concentration of Purified Complex (pM)	404.5 pM	
AMPure Cleanup Yield (%)	77.0 %	

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_cell1	Notes
Adaptive Loading Buffer	94.4 uL	
Prepared sample	16.8 uL	
Diluted Internal Control (Dilution 2)	3.8 uL	
Total Volume	115.0 uL	
# of SMRT Cells requested	2	

Show values for a different number of cells

	1 1	
On Plate Loading Concentration requested	58 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 6aba79ee-83ea-4977-8851-2eb451761e51

Summary

Name	07.26.2022_SQ2
Status	RUNNING
Created	2022-07-26 17:57:34.243
Started	2022-07-26 18:43:39.229
Context	r64069_20220726_183843
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 4405

Summary

Job Type	import-dataset
Pipeline	cromwell.workflows.sl_dataset_reports
Name	import-dataset
Comments	Description for job Import PacBio DataSet
Created At	2022-07-31 17:18:09.283
SMRT Link Version	11.0.0.146107

Child jobs (1)

Job 4375

Summary

Job Type	analysis
Pipeline	cromwell.workflows.pb_ccs
Name	CCS of PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell1
Comments	
Created At	2022-07-26 17:57:34.358
SMRT Link Version	11.0.0.146107

Output files

File name	Size	Type
PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell1-Cell3 (CCS)	20,636	ConsensusReadSet
m64069_220729_164623.hifi_reads.fasta.gz	8,554,639,158	Fasta
m64069_220729_164623.hifi_reads.bam	18,287,467,993	ConsensusReadBamFile
m64069_220729_164623.hifi_reads.fastq.gz	19,399,127,459	Fastq
PacBio.FileTypes.Datastore file	772	Datastore
All Reads (BAM)	38,920,216,442	bam
PacBio.FileTypes.Datastore file	783	Datastore
PacBio.FileTypes.Datastore file	773	Datastore
PacBio.FileTypes.json file	66,034	json
PacBio.FileTypes.json file	67,105,328	json
CCS Analysis Per-Read Details	34,830,643	zip
Analysis Log	1,592,900	log
SMRT Link Log	29,851	log

CCS Analysis Report

Summary

HiFi Reads	1,972,754
HiFi Yield (bp)	24,639,312,353
HiFi Read Length (mean, bp)	12,489
HiFi Read Quality (median)	Q36
HiFi Read Quality (median)	36
HiFi Number of Passes (mean)	14
<Q20 Reads	340,906
<Q20 Yield (bp)	4,796,273,987
<Q20 Read Length (mean, bp)	14,069
<Q20 Read Quality (median)	Q17
<Q20 Read Quality (median)	17

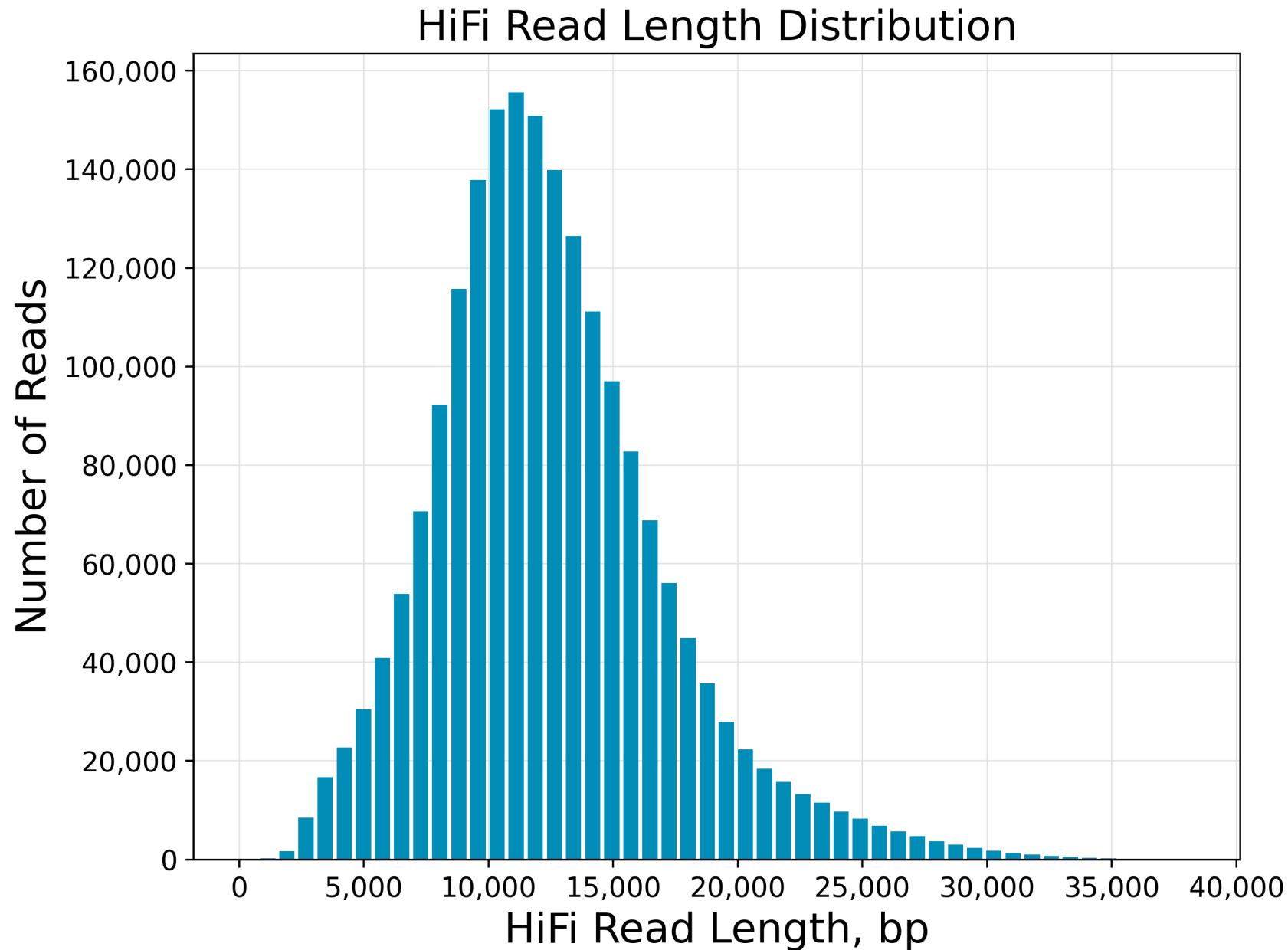
HiFi Read Length Summary

Read Length (bp)	Reads	Reads (%)	Yield (bp)	Yield (%)
0	1972754	100	24639312353	100
5,000	1908152	97	24387106645	99
10,000	1374121	70	20044915952	81
15,000	487898	25	9137079257	37
20,000	129554	7	3064595268	12
25,000	35575	2	990763400	4
30,000	6159	0	197928898	1
35,000	494	0	18315671	0
40,000	55	0	2378403	0

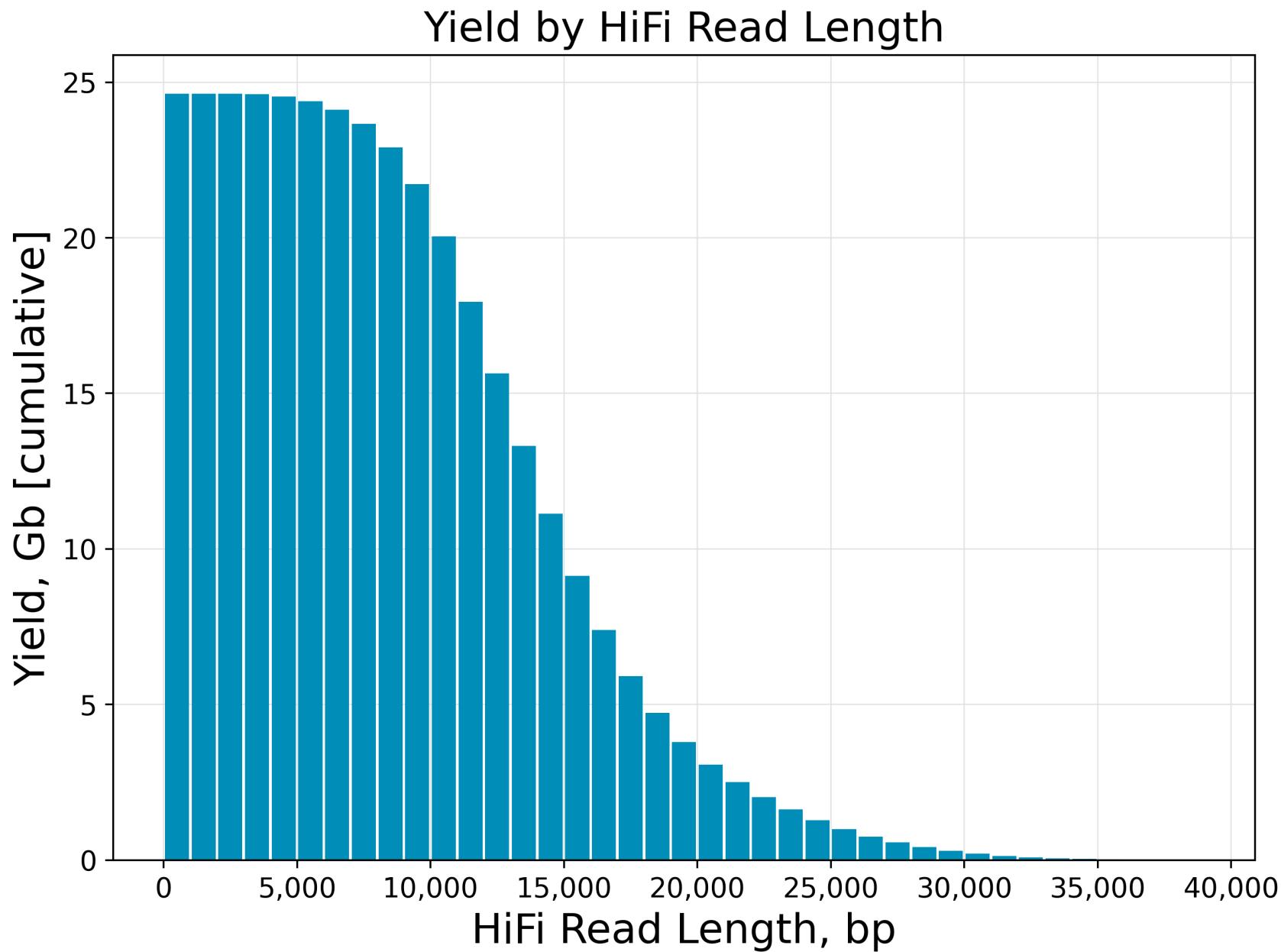
HiFi Read Quality Summary

Read Quality (Phred)	Reads	Reads (%)	Yield (bp)	Yield (%)
Q20	1972754	100	24639312353	100
Q30	1367194	69	16261881587	66
Q40	652373	33	6767500299	27
Q50	223312	11	1901346220	8

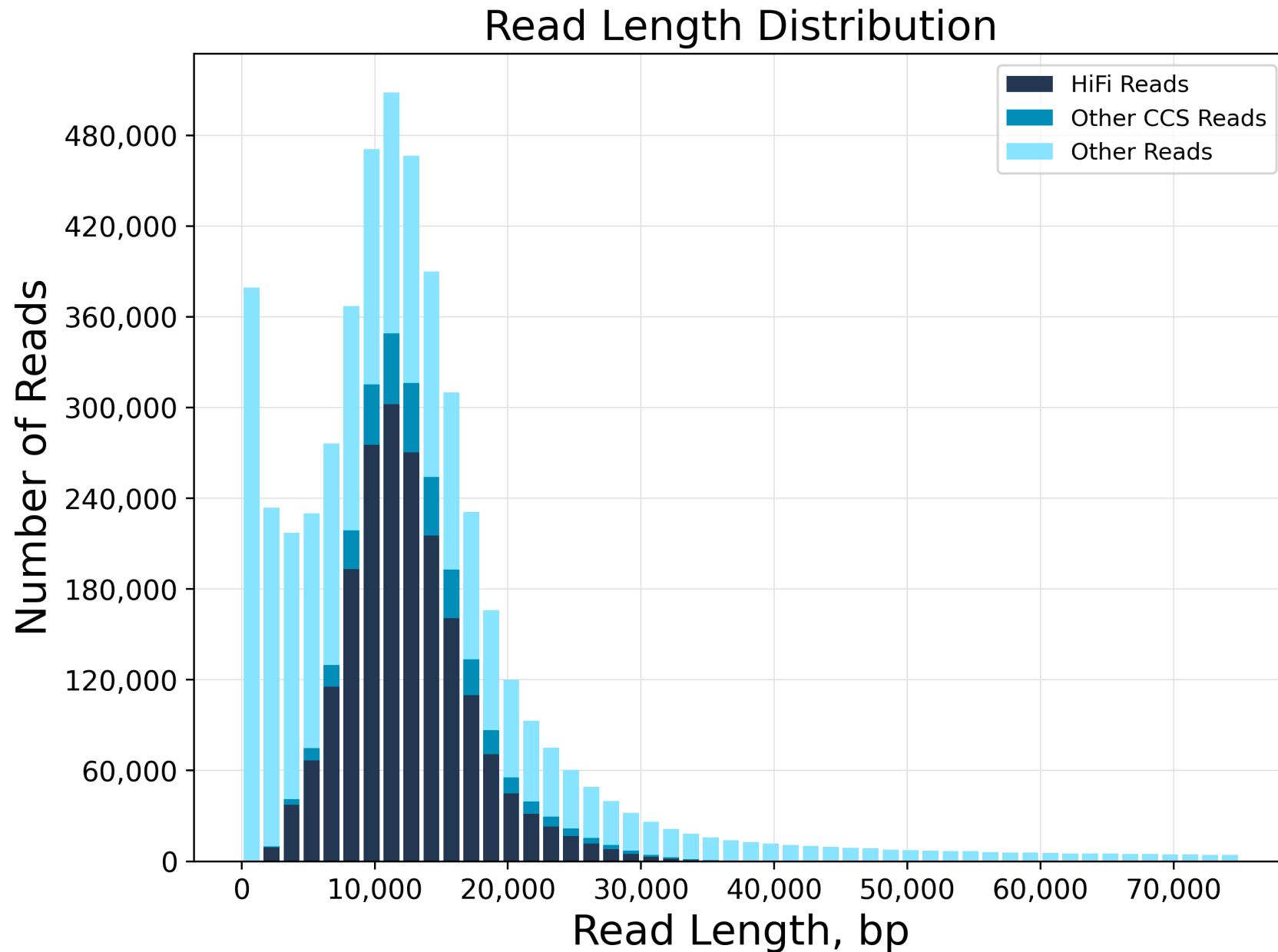
HiFi Read Length Distribution



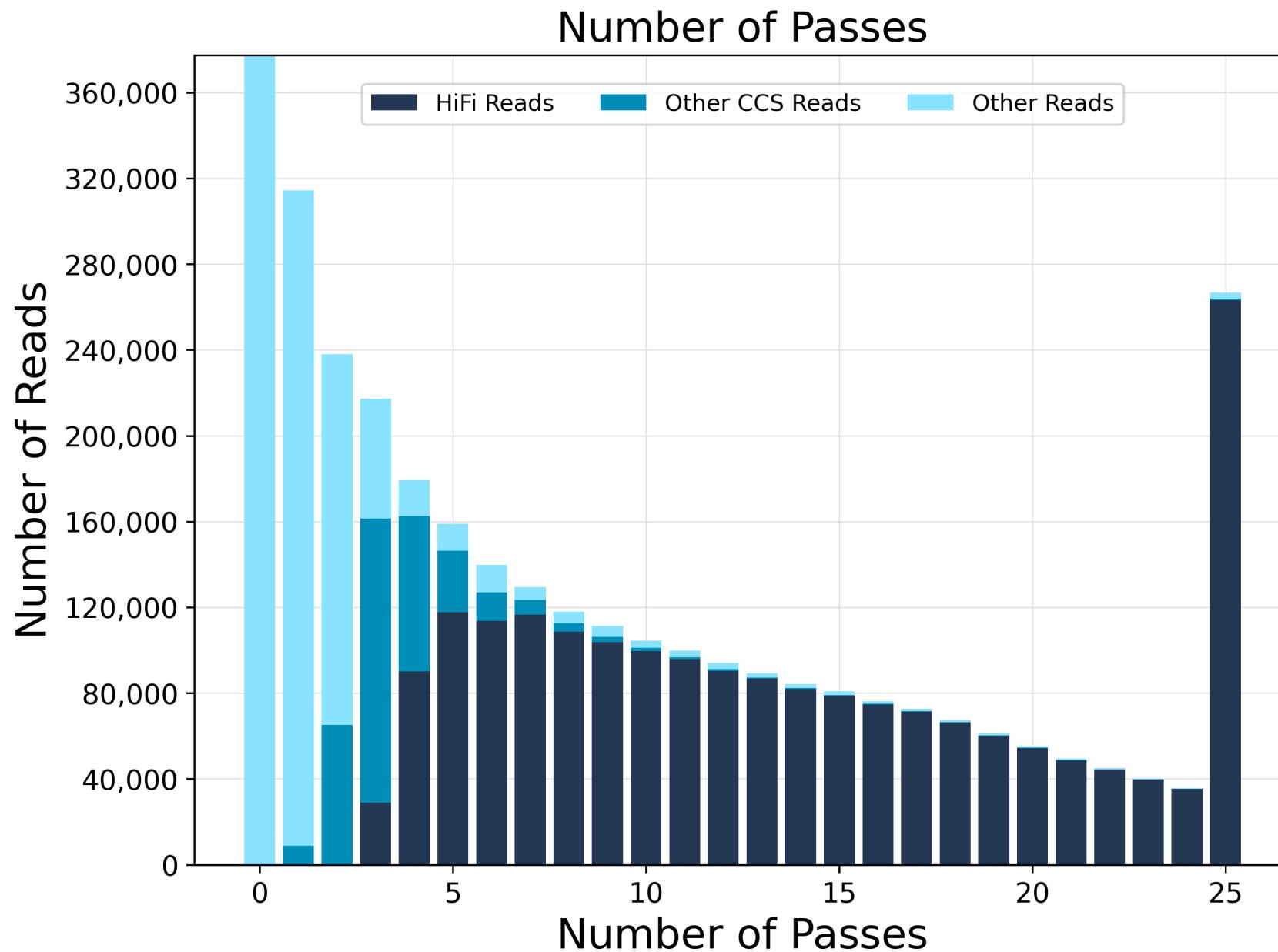
Yield by HiFi Read Length



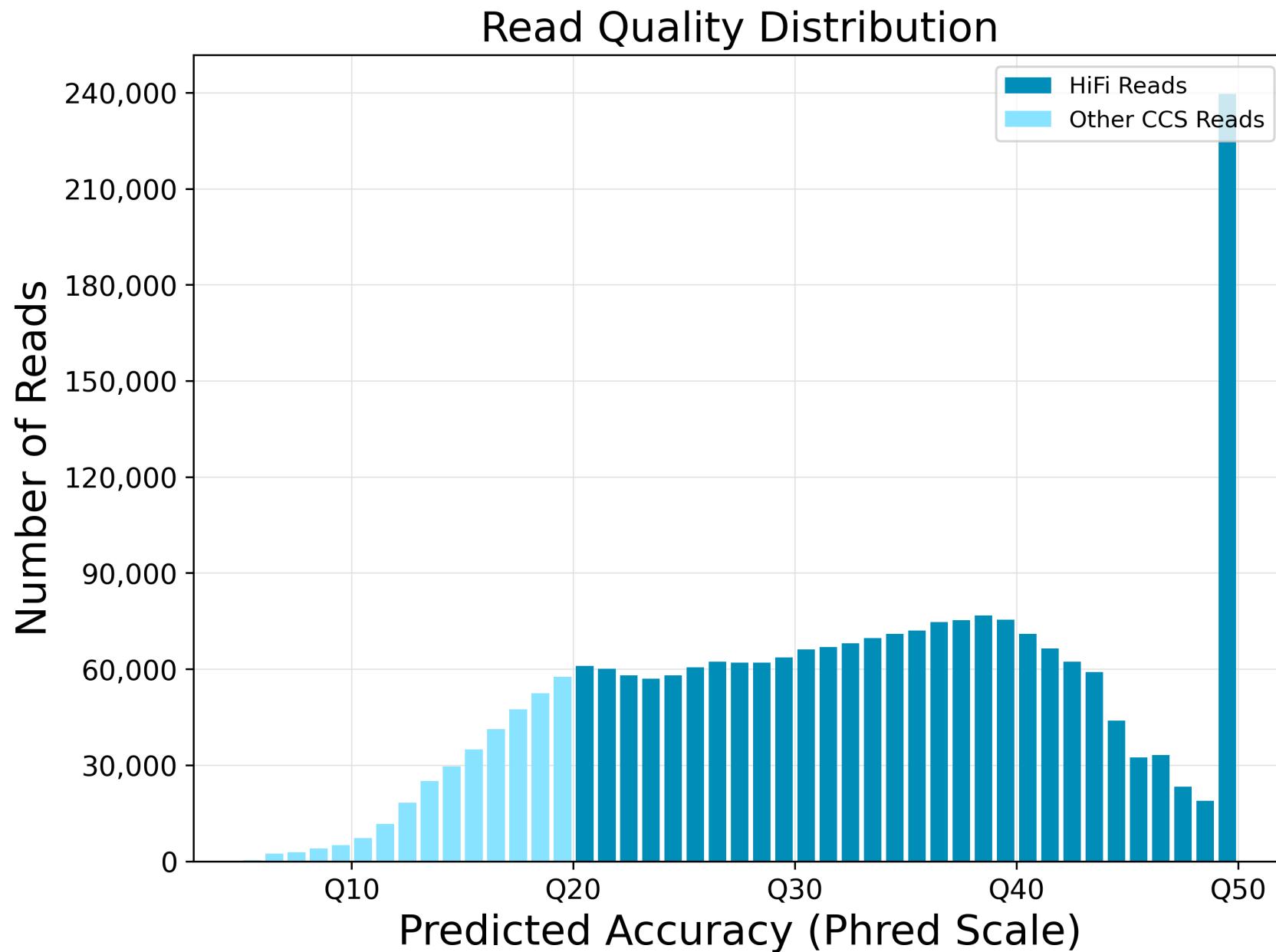
Read Length Distribution



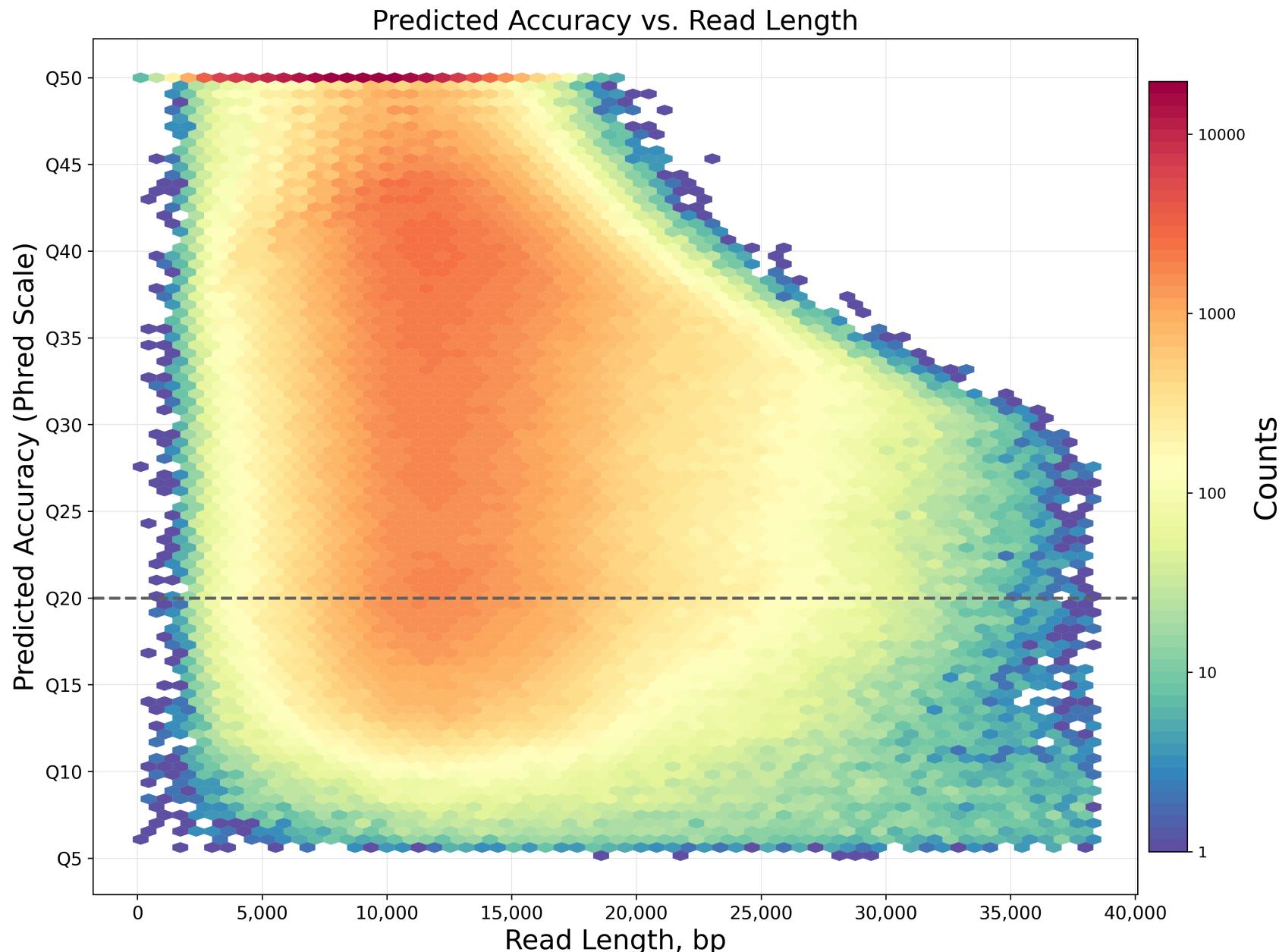
Number of Passes



Read Quality Distribution



Predicted Accuracy vs. Read Length



CCS Processing

Summary

ZMWs input	5,183,477
ZMWs pass filters	2,313,660
ZMWs fail filters	463,312
ZMWs shortcut filters	2,406,505
ZMWs with tandem repeats	23,110
Below SNR threshold	167,431
Median length filter	0
Lacking full passes	0
Heteroduplex insertions	36,473
Coverage drops	7,252
Insufficient draft cov	185,625
Draft too different	0
Draft generation error	64,134
Draft above --max-length	0
Draft below --min-length	10
Reads failed polishing	1
Empty coverage windows	793
CCS did not converge	1,593
CCS below minimum RQ	0
Unknown error	0
ZMWs missing adapters	155,935

