

Report for dataset PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell2_0726-Cell1

Dataset 7a36f321-3940-4bd0-b1c3-7088499198a6

Summary

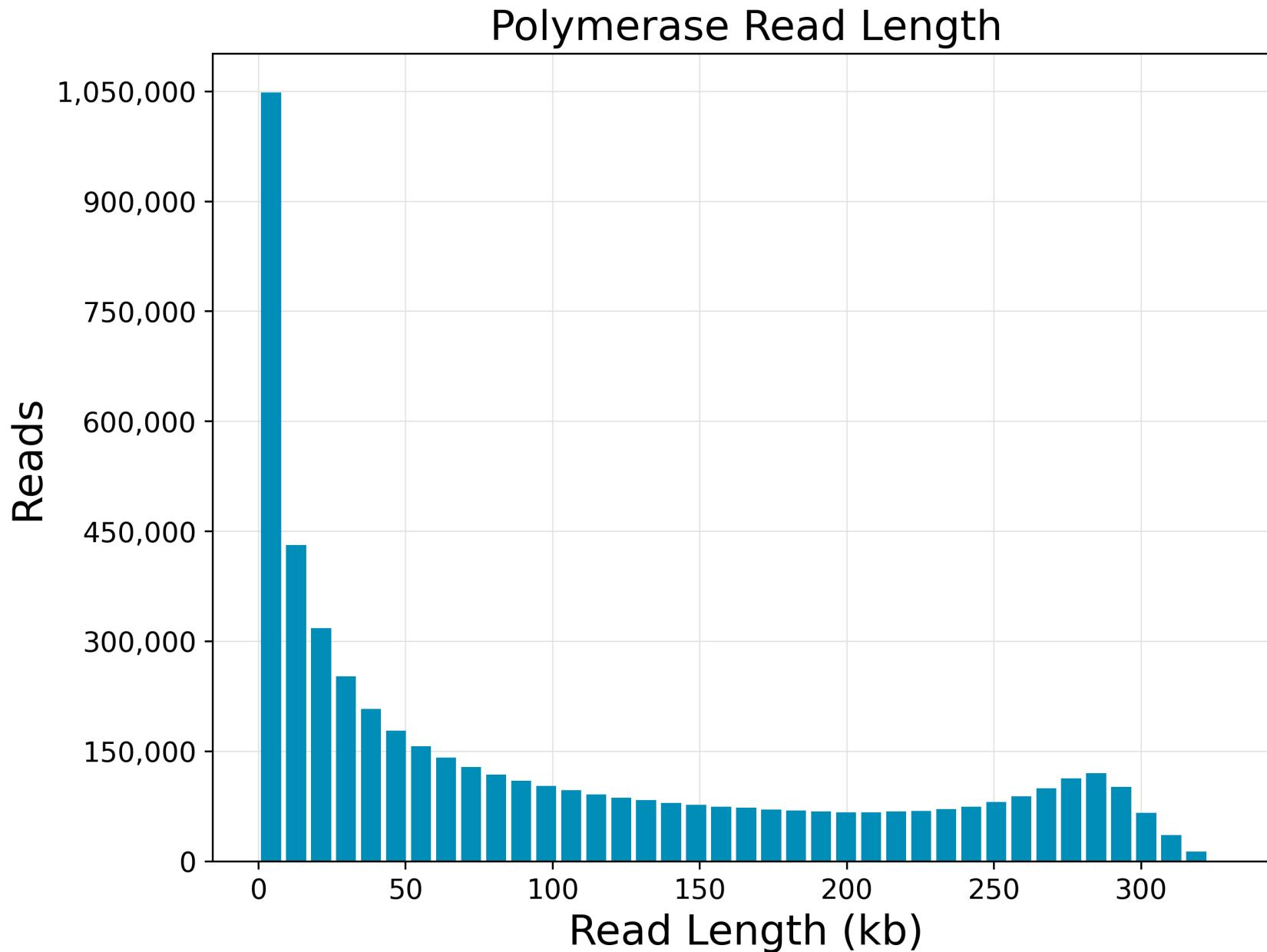
| | |
|-------------------|---|
| Name | PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell2_0726-Cell1 |
| Created At | 2022-08-09 18:34:50.975 |
| Number of Records | 46822117 |
| Total Length | 513980311075 |
| Movie ID | m64069_220809_200722 |
| ICS Version | 11.0.0.144466 |
| Well Sample | PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell2_0726 |
| Biological Sample | a |

Raw Data Report

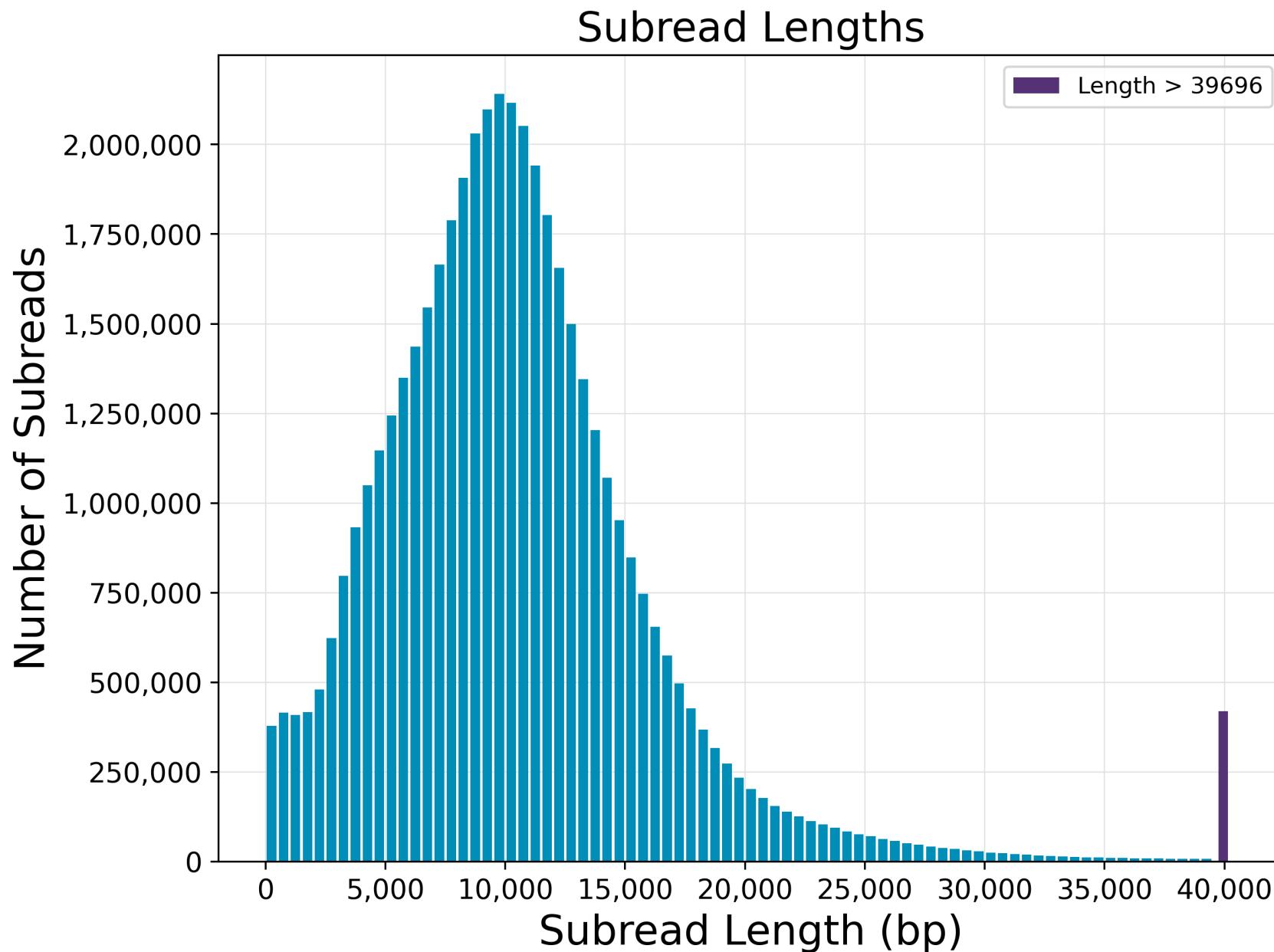
Summary

| | |
|-------------------------------|-----------------|
| Polymerase Read Bases | 515,762,623,143 |
| Polymerase Reads | 5,176,147 |
| Polymerase Read Length (mean) | 99,642 |
| Polymerase Read N50 | 217,508 |
| Subread Length (mean) | 10,977 |
| Subread N50 | 12,399 |
| Longest Subread Length (mean) | 18,303 |
| Longest Subread N50 | 24,095 |
| Unique Molecular Yield | 82,871,541,760 |

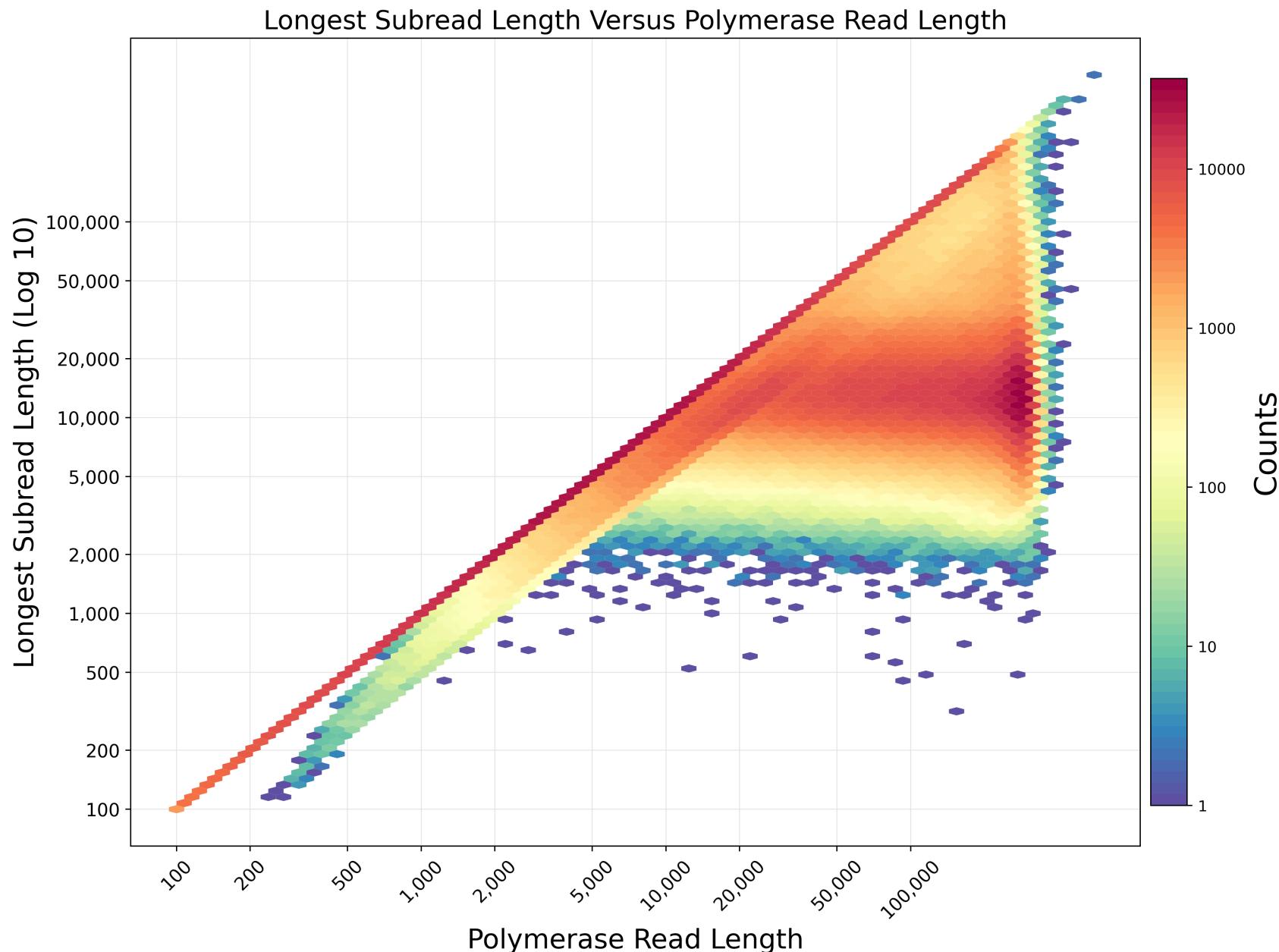
Polymerase Read Length



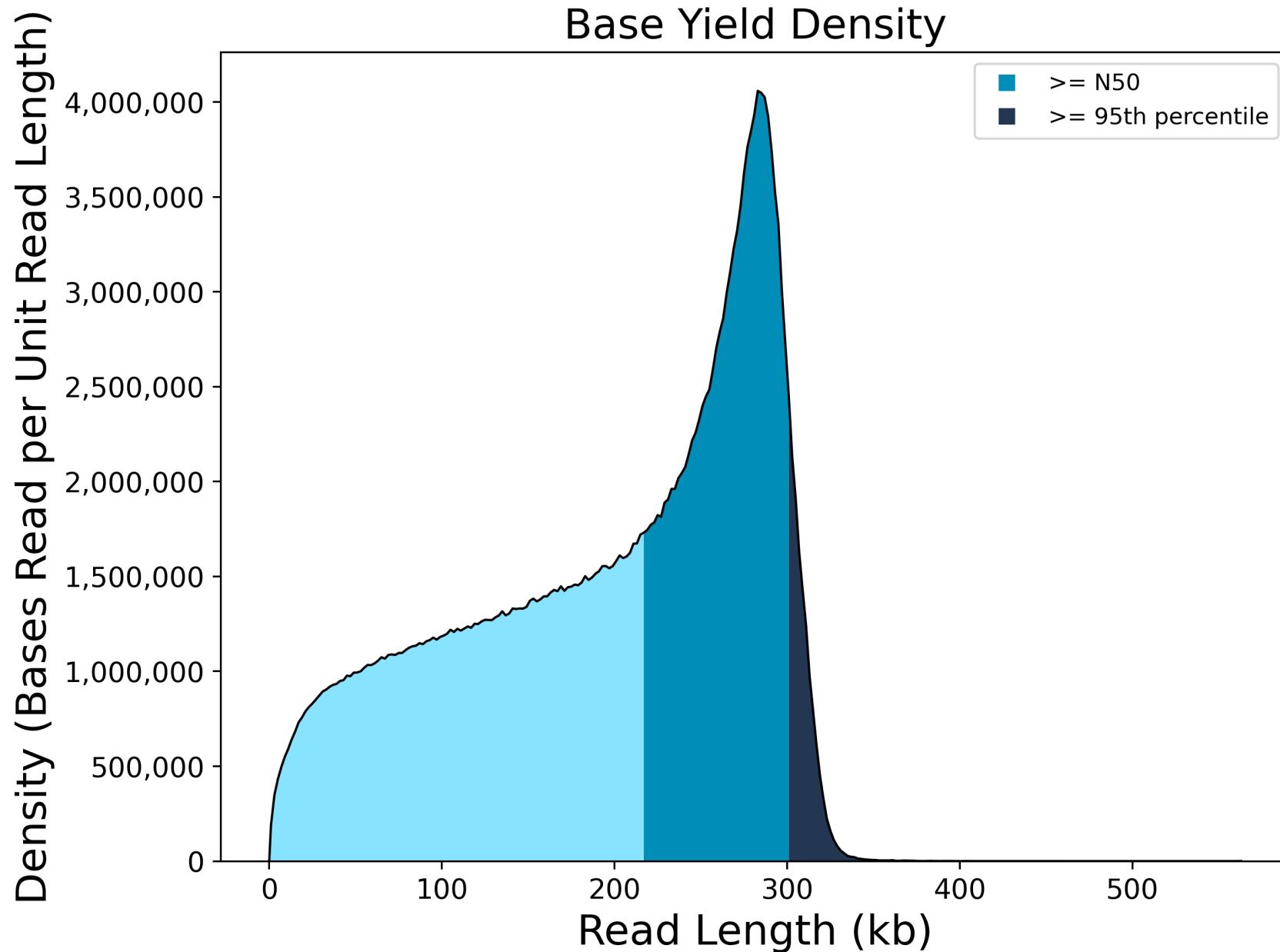
Subread Length



Longest Subread Length Versus Polymerase Read Length



Base Yield Density



Loading Report

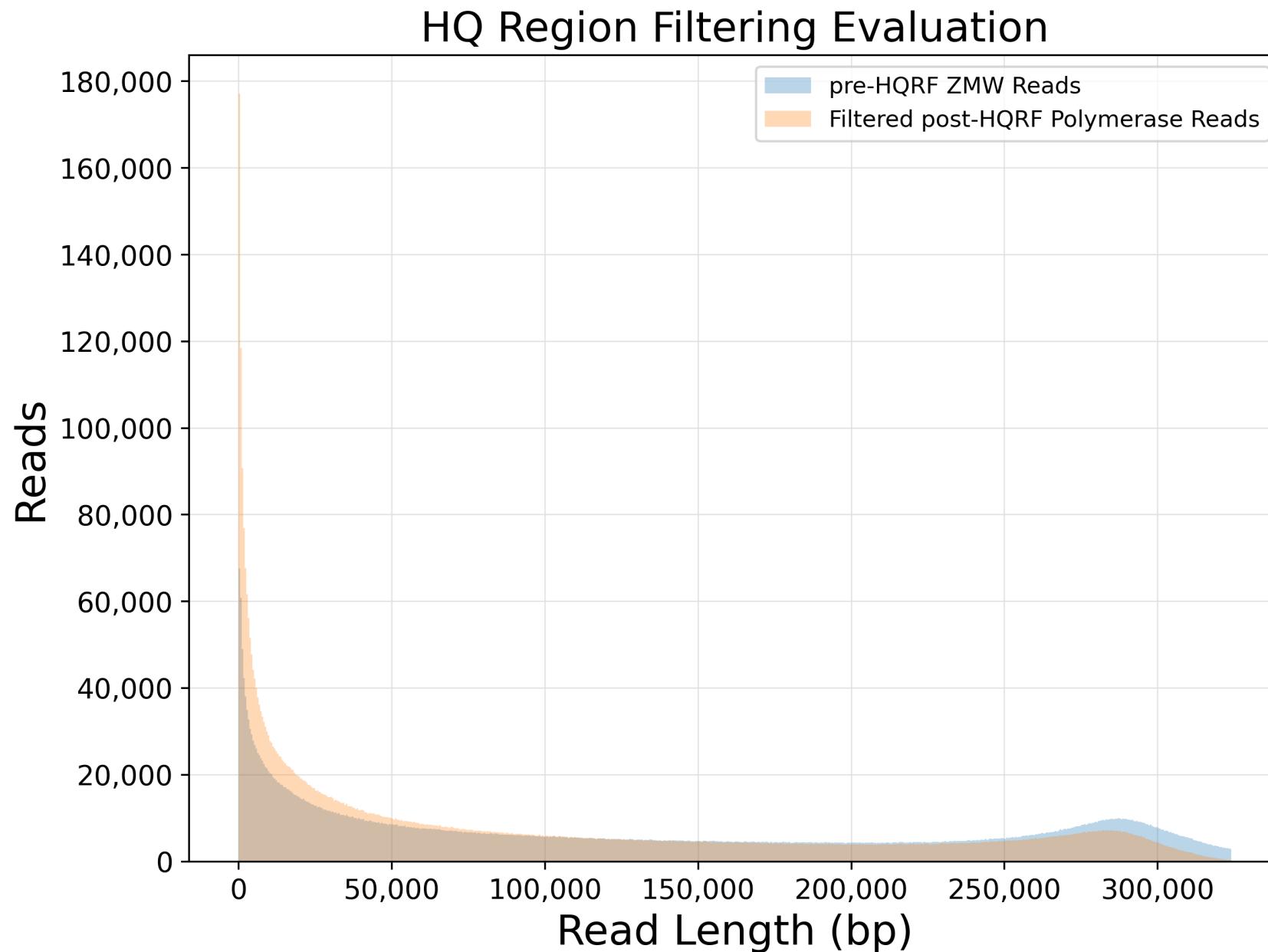
Summary

| | |
|-----------------|-----------|
| Productive ZMWs | 8,013,339 |
| Productivity 0 | 2,649,506 |
| Productivity 1 | 5,203,922 |
| Productivity 2 | 161,243 |

Loading Statistics

| Collection Context | Productive ZMWs | Productivity 0 | (%) | Productivity 1 | (%) | Productivity 2 | (%) | Loading type |
|----------------------|-----------------|----------------|-------|----------------|-------|----------------|------|--------------|
| m64069_220809_200722 | 8013339 | 2649506 | 33.06 | 5203922 | 64.93 | 161243 | 2.01 | Diffusion |

HQ Region Filtering Evaluation



Adapter Report

Summary

| | |
|----------------------------|------|
| Adapter Dimers (0-10bp) % | 0 |
| Short Inserts (11-100bp) % | 0 |
| Local Base Rate | 2.78 |

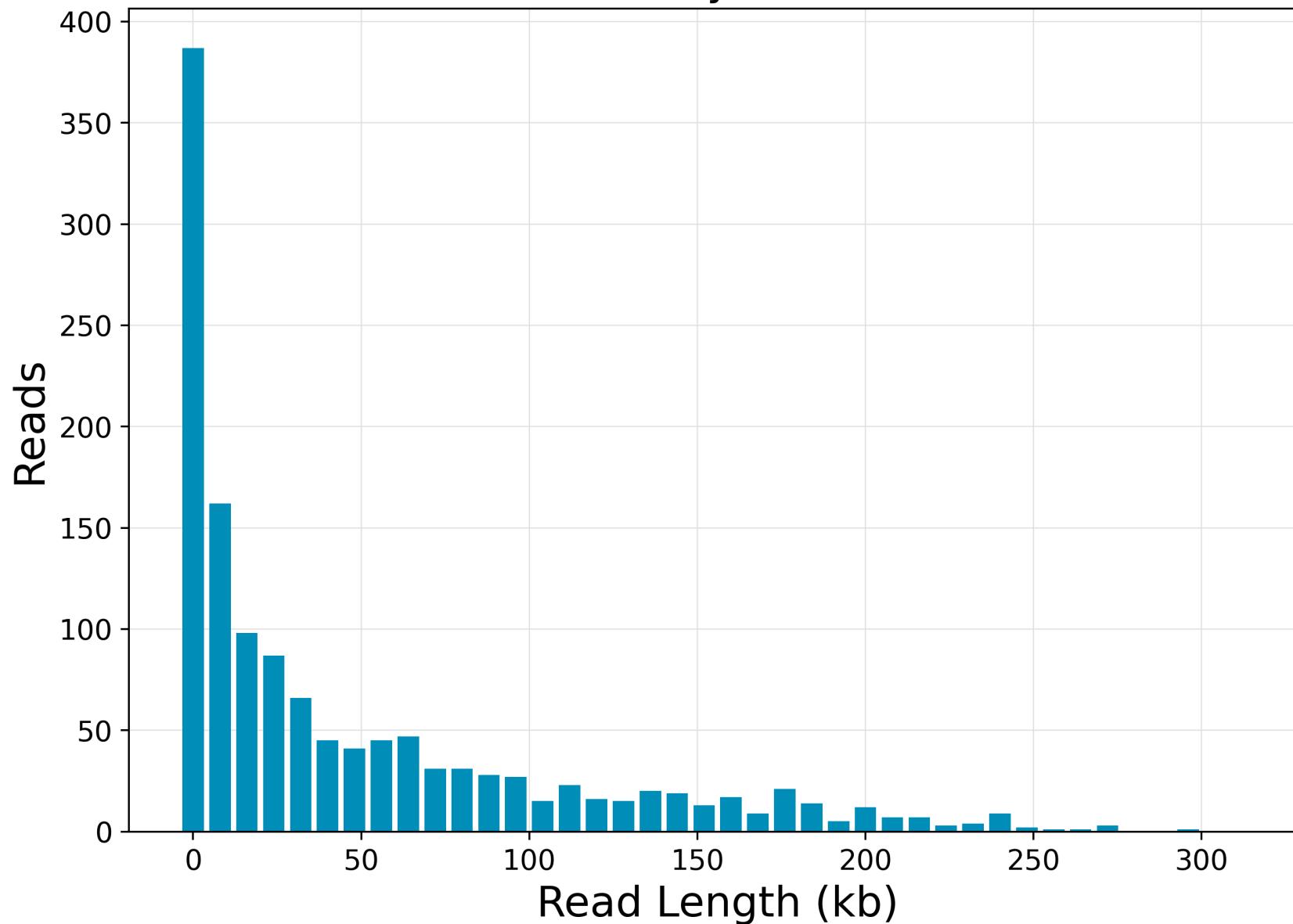
Control Report

Summary

| | |
|--------------------------------------|--------|
| Number of Control Reads | 1,332 |
| Control Read Length Mean | 52,069 |
| Control Read Concordance Mean | 0.86 |
| Control Read Concordance Mode | 0.89 |

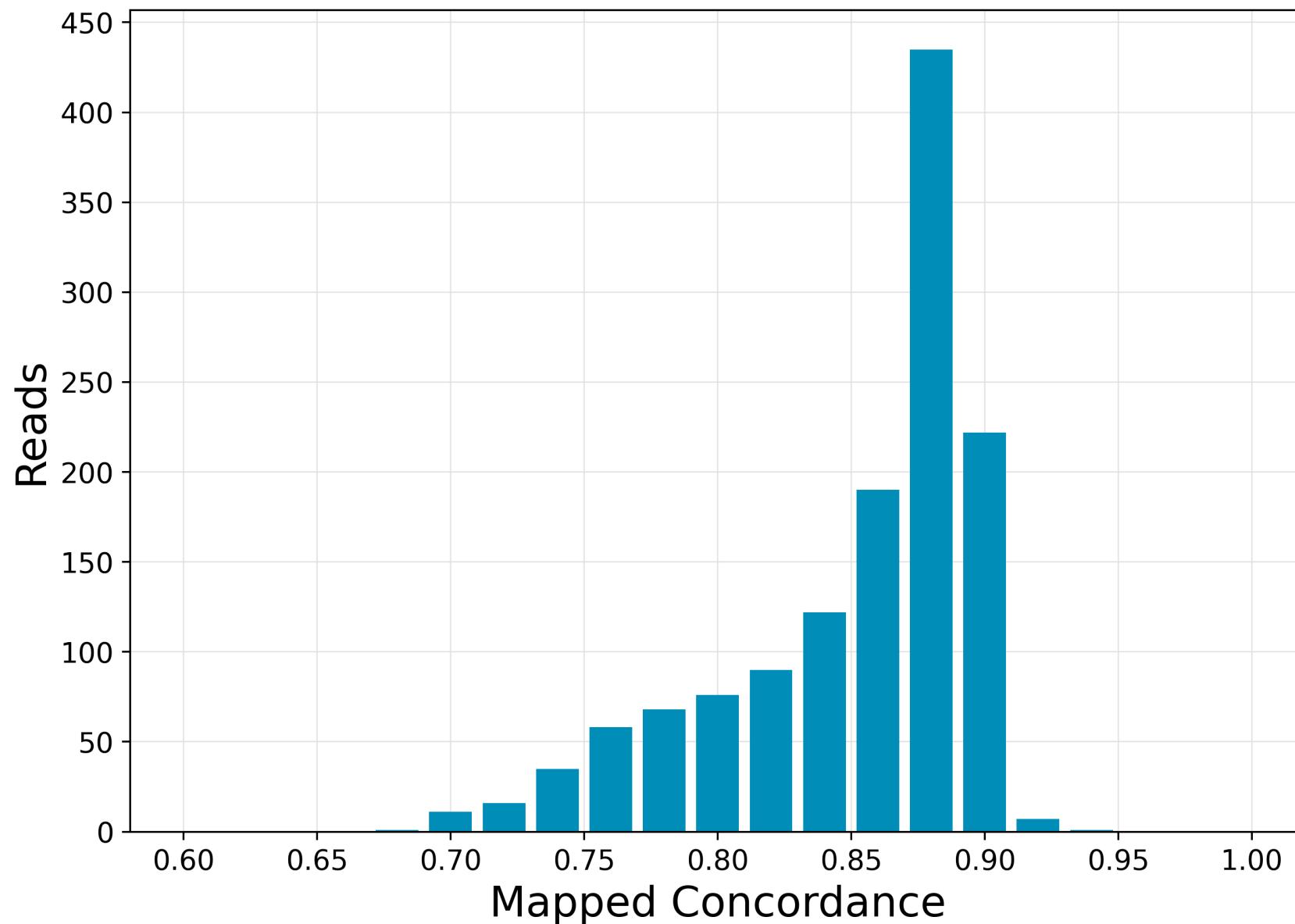
Control Polymerase RL

Control Polymerase RL



Control Concordance

Control Concordance



Sample Setup

Sample 4e810ea3-a523-576b-4dc5-93b39dee2e36

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_cell2_0726 |
| 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_cell2_0726 | 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_cell2_0726 | 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_cell2_0726 | 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_cell2_0726 | 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_cell2_0726 | 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_cell2_0726 | 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_cell2_0726 | 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_cell2_0726 | 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_cell2_0726 | Notes |
|---------------------------------|---|-------|
| Volume of Purified Complex (uL) | | |

50 uL
50 uL

Purified Complex Concentration (ng/uL)

| | | |
|--|--------------------------|--|
| | 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_cell2_0726 | Notes |
|---------------------------------------|---|-------|
| Adaptive Loading Buffer | 94.1 uL | |
| Prepared sample | 17.1 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 | | |

60 pM
60 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 4457

Summary

| | |
|-------------------|---|
| Job Type | import-dataset |
| Pipeline | cromwell.workflows.sl_dataset_reports |
| Name | import-dataset |
| Comments | Description for job Import PacBio DataSet |
| Created At | 2022-08-11 19:36:02.801 |
| SMRT Link Version | 11.0.0.146107 |

Child jobs (1)

Job 4443

Summary

| | |
|-------------------|--|
| Job Type | analysis |
| Pipeline | cromwell.workflows.pb_ccs |
| Name | CCS of PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell2_0726 |
| Comments | |
| Created At | 2022-08-09 18:34:50.404 |
| SMRT Link Version | 11.0.0.146107 |

Output files

| File name | Size | Type |
|---|----------------|----------------------|
| PB776_GVTP-1_Gaviota Tarplant_HiFiv3_cell2_0726-Cell1 (CCS) | 20,643 | ConsensusReadSet |
| m64069_220809_200722.hifi_reads.fasta.gz | 8,898,968,307 | Fasta |
| m64069_220809_200722.hifi_reads.bam | 18,379,861,796 | ConsensusReadBamFile |
| m64069_220809_200722.hifi_reads.fastq.gz | 19,488,125,139 | Fastq |
| PacBio.FileTypes.Datastore file | 772 | Datastore |
| All Reads (BAM) | 36,670,597,989 | bam |
| PacBio.FileTypes.Datastore file | 783 | Datastore |
| PacBio.FileTypes.Datastore file | 773 | Datastore |
| PacBio.FileTypes.json file | 66,031 | json |
| PacBio.FileTypes.json file | 68,024,004 | json |
| CCS Analysis Per-Read Details | 34,729,941 | zip |
| Analysis Log | 1,629,706 | log |
| SMRT Link Log | 30,041 | log |

CCS Analysis Report

Summary

| | |
|------------------------------|----------------|
| HiFi Reads | 2,162,014 |
| HiFi Yield (bp) | 25,700,506,383 |
| HiFi Read Length (mean, bp) | 11,887 |
| HiFi Read Quality (median) | Q37 |
| HiFi Read Quality (median) | 37 |
| HiFi Number of Passes (mean) | 14 |
| <Q20 Reads | 344,741 |
| <Q20 Yield (bp) | 4,630,061,302 |
| <Q20 Read Length (mean, bp) | 13,430 |
| <Q20 Read Quality (median) | Q17 |
| <Q20 Read Quality (median) | 17 |

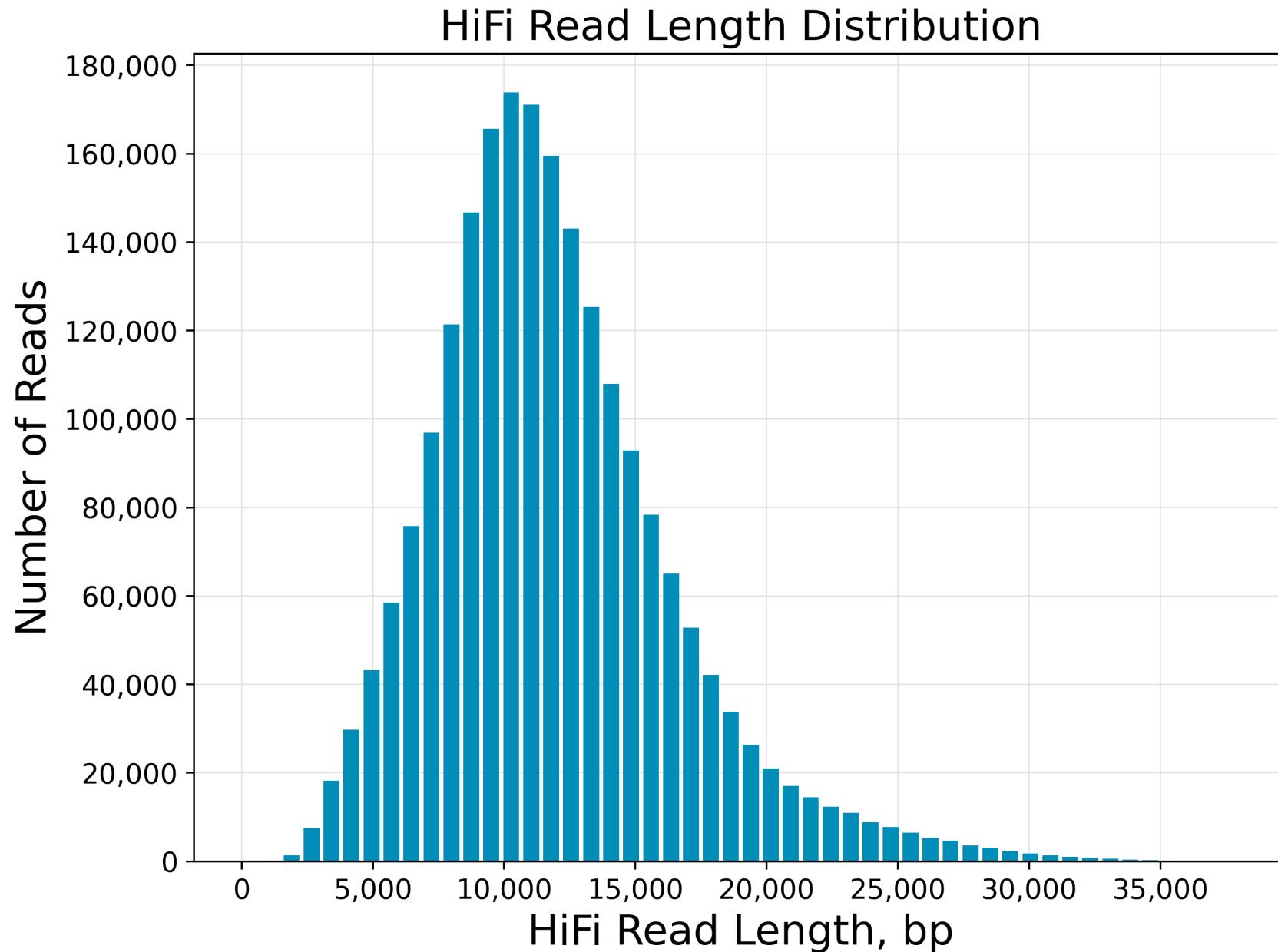
HiFi Read Length Summary

| Read Length (bp) | Reads | Reads (%) | Yield (bp) | Yield (%) |
|------------------|---------|-----------|-------------|-----------|
| 0 | 2162014 | 100 | 25700506383 | 100 |
| 5,000 | 2082050 | 96 | 25378842334 | 99 |
| 10,000 | 1372145 | 63 | 19668150479 | 77 |
| 15,000 | 447871 | 21 | 8377211274 | 33 |
| 20,000 | 117582 | 5 | 2784192932 | 11 |
| 25,000 | 32780 | 2 | 913277586 | 4 |
| 30,000 | 5684 | 0 | 182677533 | 1 |
| 35,000 | 464 | 0 | 17148391 | 0 |
| 40,000 | 38 | 0 | 1689387 | 0 |

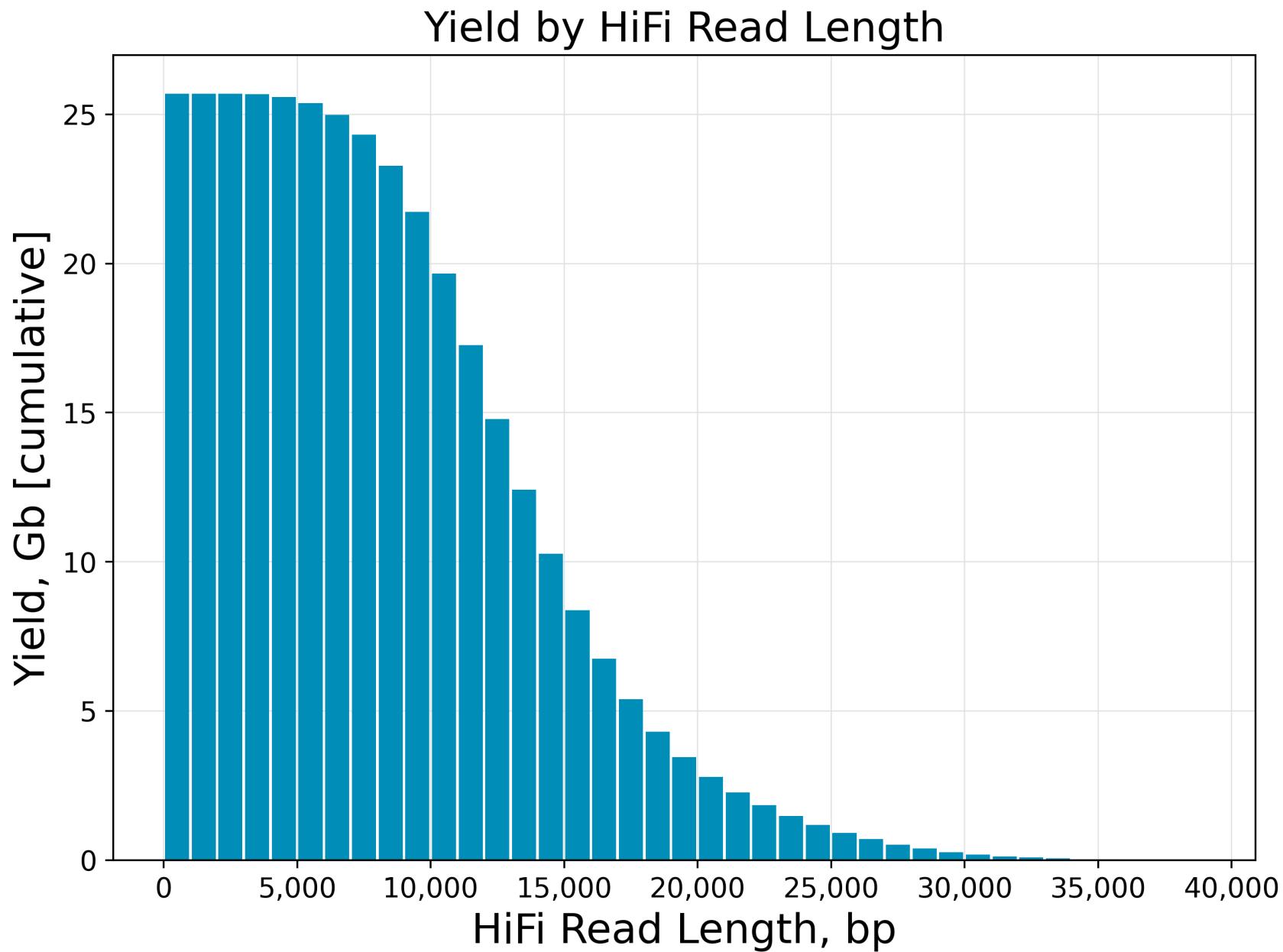
HiFi Read Quality Summary

| Read Quality (Phred) | Reads | Reads (%) | Yield (bp) | Yield (%) |
|----------------------|---------|-----------|-------------|-----------|
| Q20 | 2162014 | 100 | 25700506383 | 100 |
| Q30 | 1568835 | 73 | 17850363317 | 69 |
| Q40 | 846093 | 39 | 8546384578 | 33 |
| Q50 | 334456 | 15 | 2843256225 | 11 |

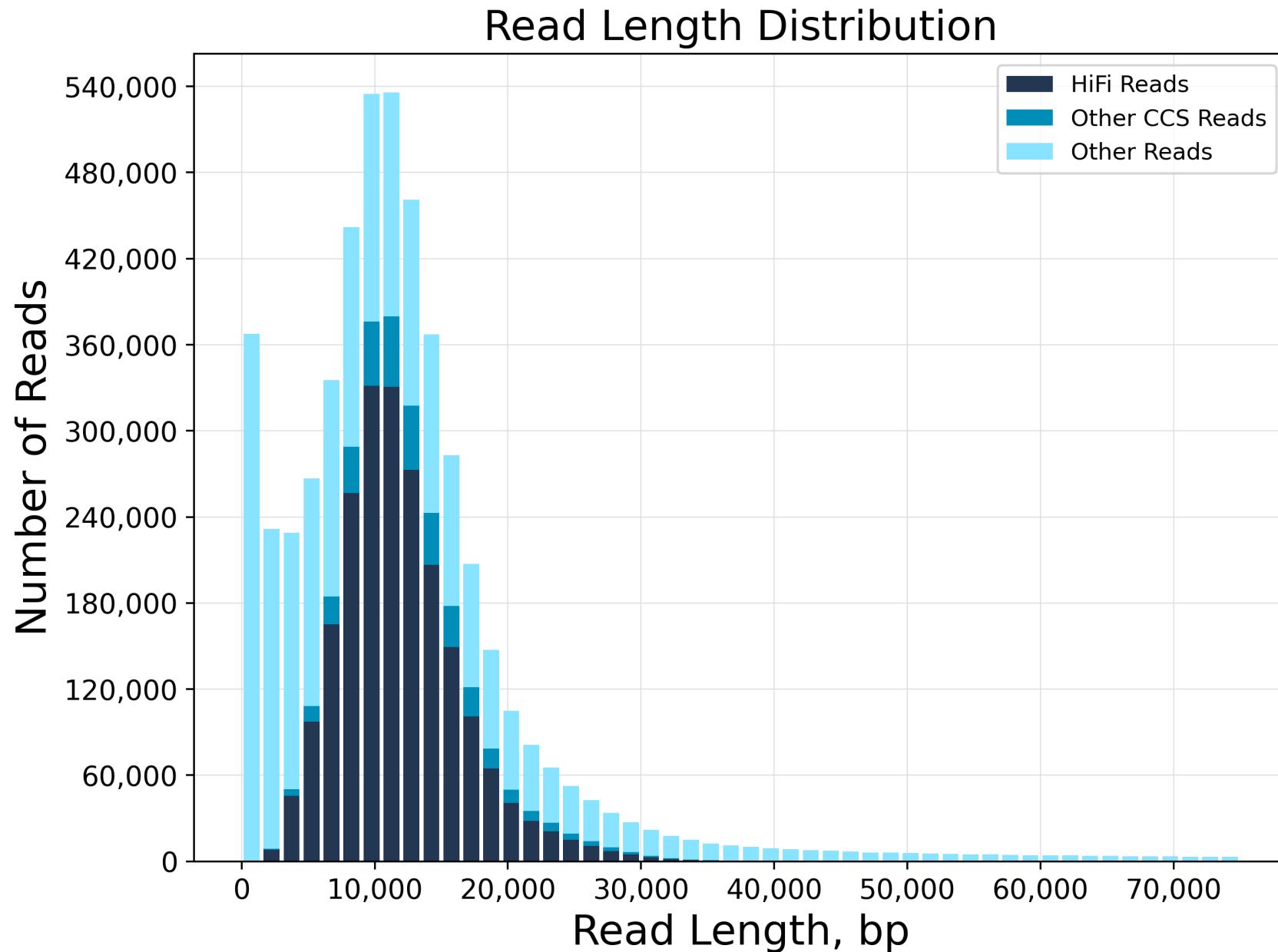
HiFi Read Length Distribution



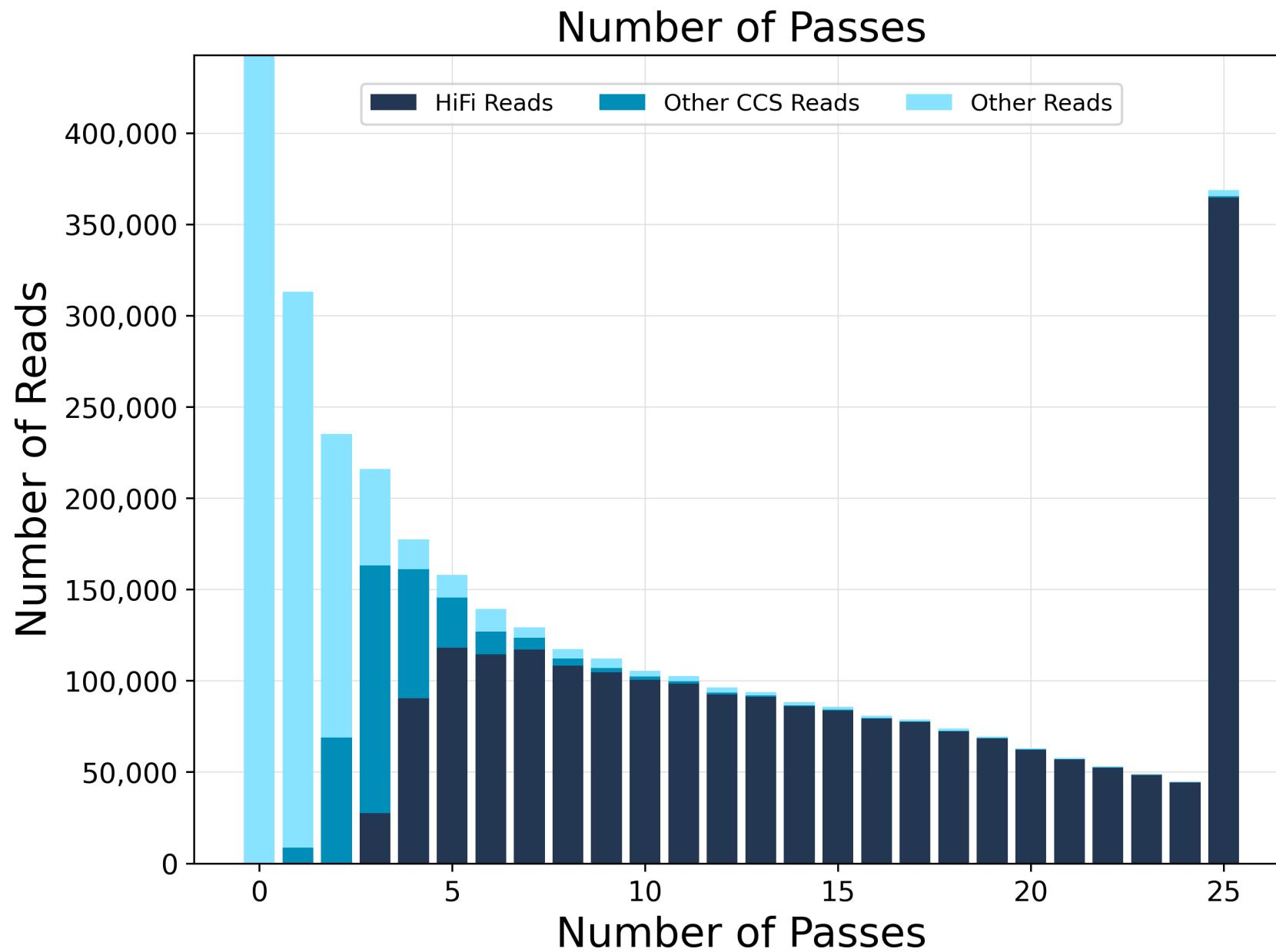
Yield by HiFi Read Length



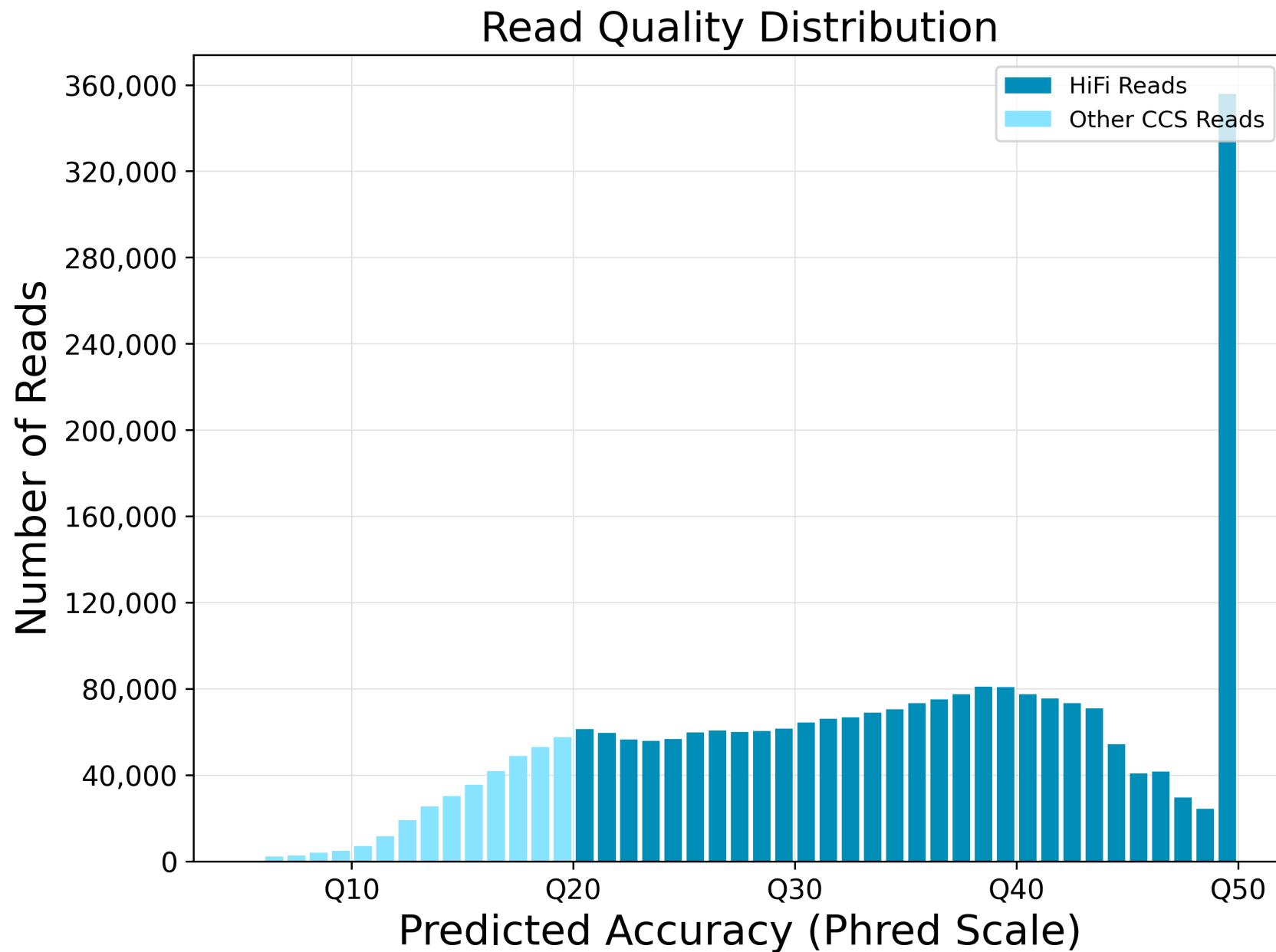
Read Length Distribution



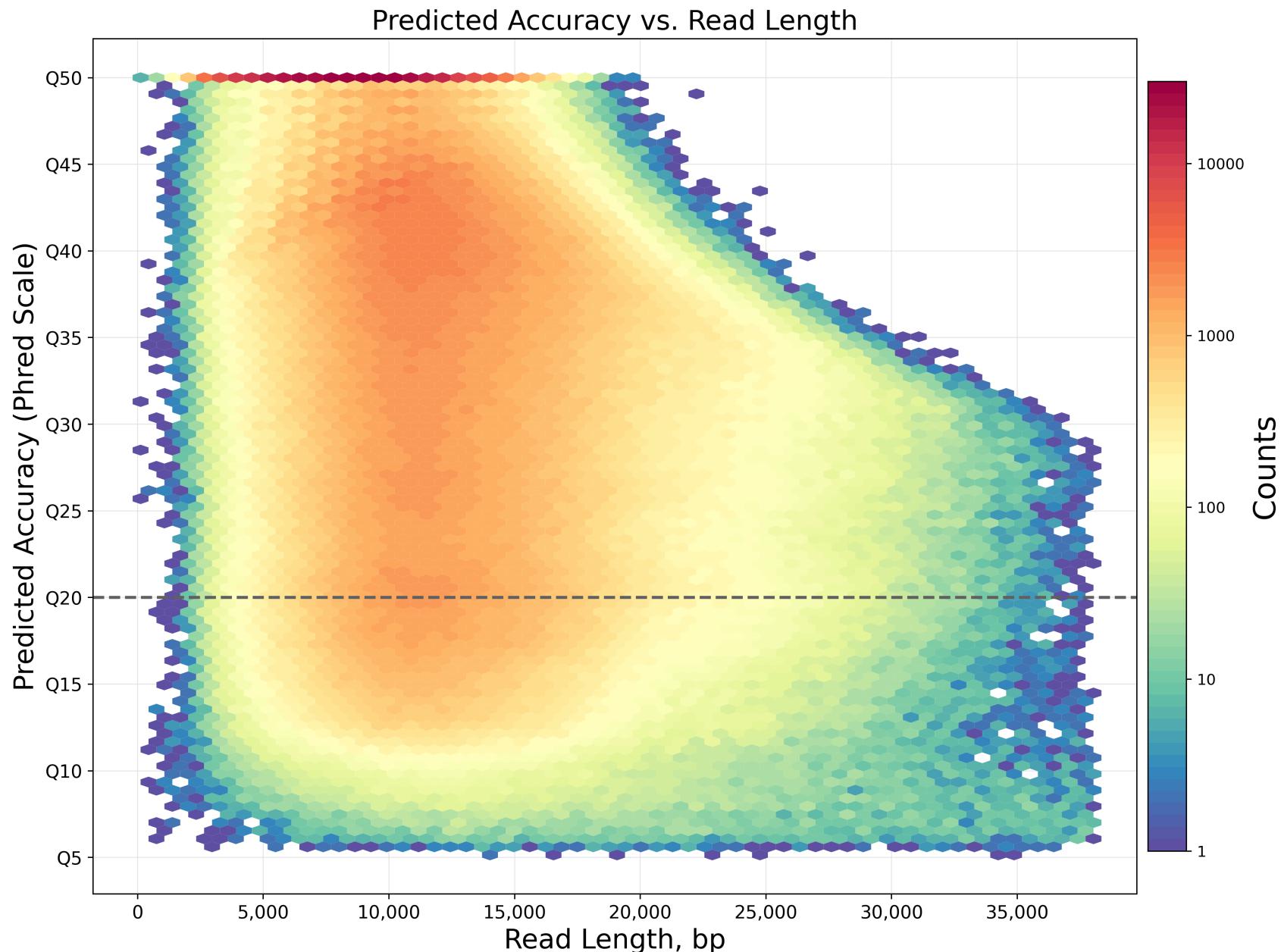
Number of Passes



Read Quality Distribution



Predicted Accuracy vs. Read Length



CCS Processing

Summary

| | |
|--------------------------|-----------|
| ZMWs input | 5,176,147 |
| ZMWs pass filters | 2,506,755 |
| ZMWs fail filters | 409,474 |
| ZMWs shortcut filters | 2,259,918 |
| ZMWs with tandem repeats | 23,526 |
| Below SNR threshold | 122,590 |
| Median length filter | 0 |
| Lacking full passes | 0 |
| Heteroduplex insertions | 34,695 |
| Coverage drops | 7,853 |
| Insufficient draft cov | 179,484 |
| Draft too different | 0 |
| Draft generation error | 62,459 |
| Draft above --max-length | 0 |
| Draft below --min-length | 13 |
| Reads failed polishing | 3 |
| Empty coverage windows | 878 |
| CCS did not converge | 1,499 |
| CCS below minimum RQ | 0 |
| Unknown error | 0 |
| ZMWs missing adapters | 166,648 |

