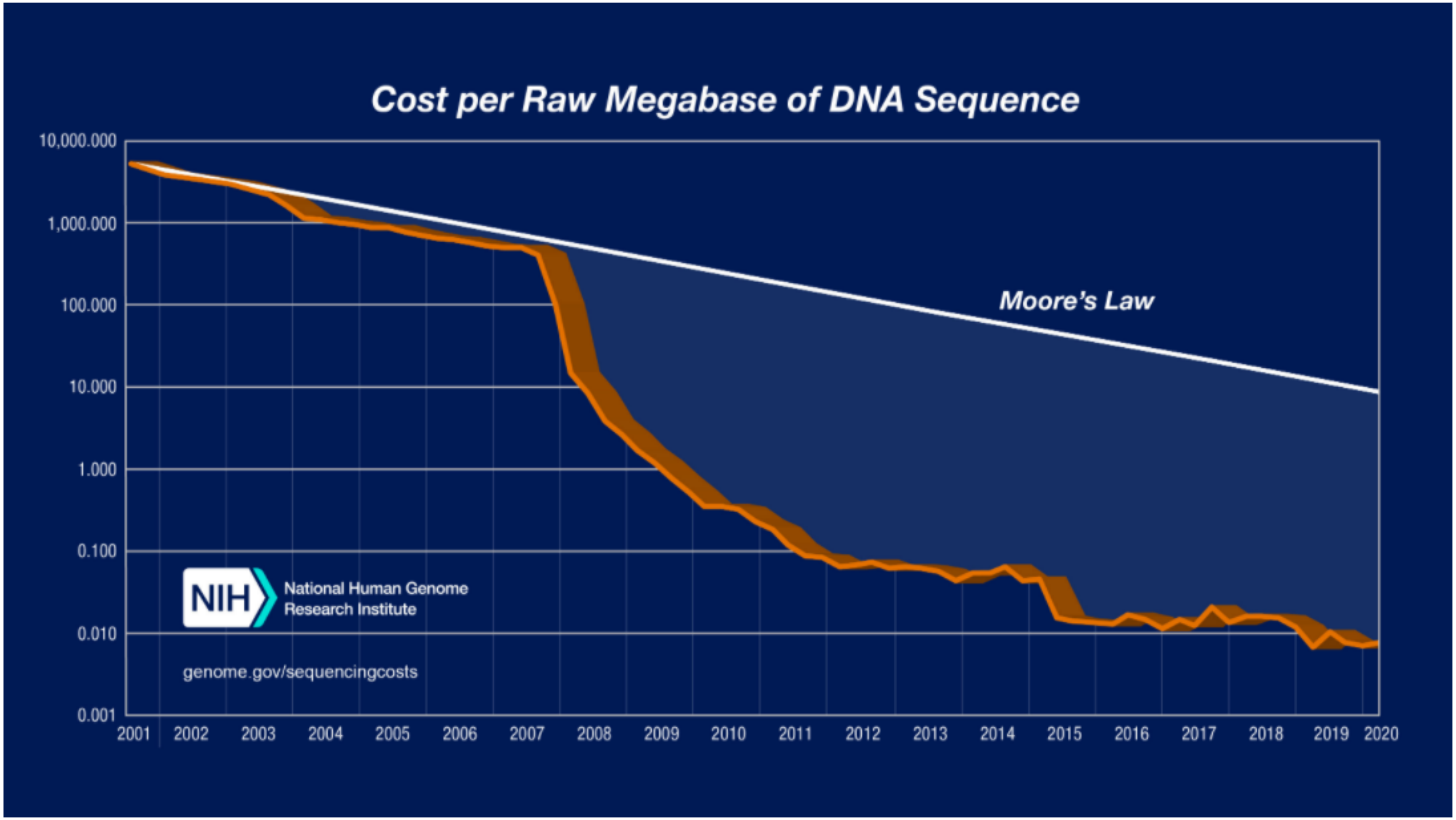
Long Read Sequencing

Dick McCombie
Davis Family Professor of Human Genetics
Cold Spring Harbor Laboratory

Advanced Sequencing Technologies and Applications course
Cold Spring Harbor Laboratory
2022

Significant advances in genome sequencing over last 16 years

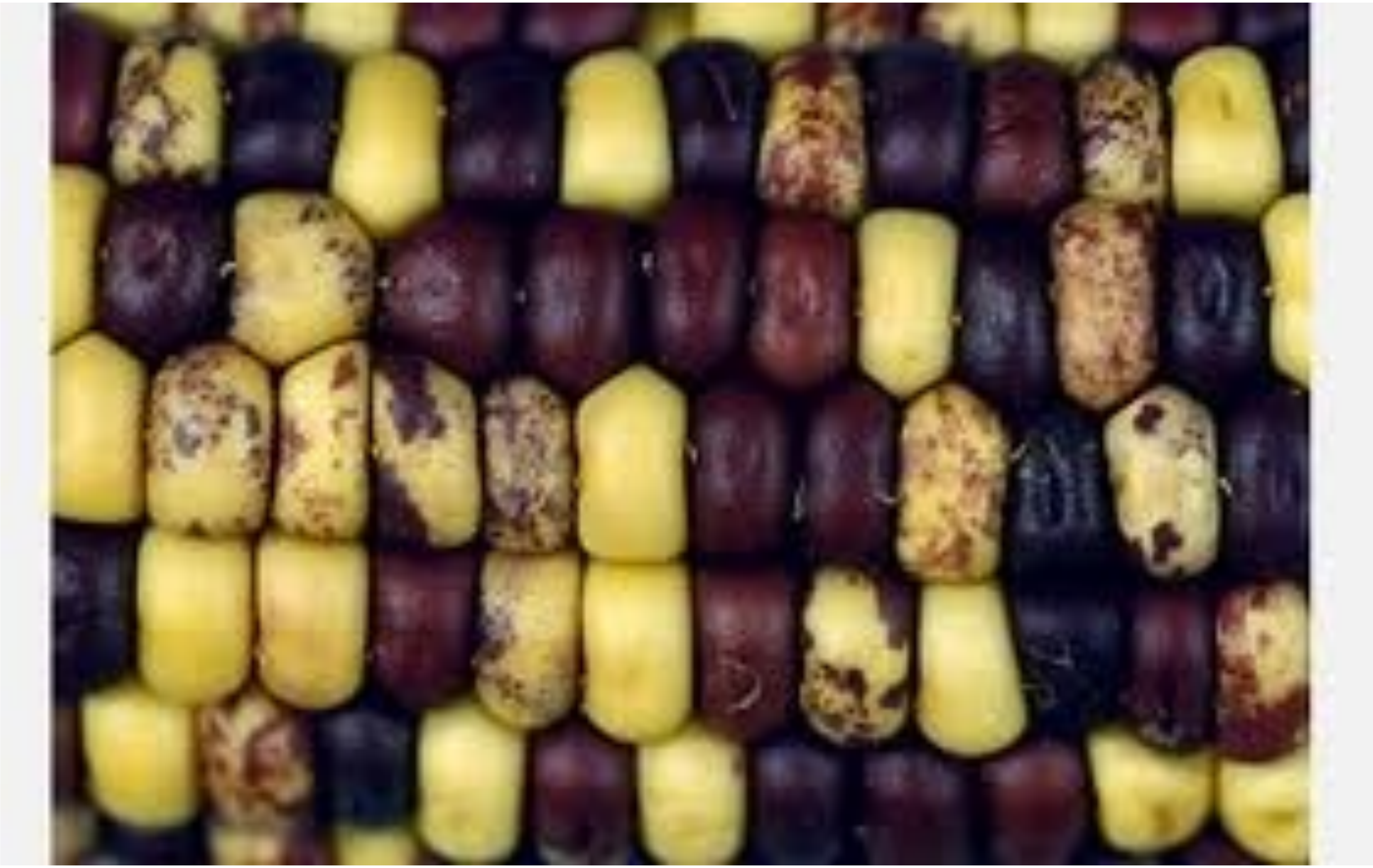


Evolution of genome assemblies

- Initial references – very high quality – extremely expensive
- Period of lower quality Sanger assemblies (~2001-2007)
- Next gen assemblies (short read) – 2007- now
- Third generation – long read assemblies -2013/2014 –now – what can we do currently?
- T2T extremely complete genomes

Goodwin, McPherson and McCombie. Nat. Rev. Genetics. 2016

??



Short vs long reads

- Short read NGS has revolutionized resequencing
- *De novo* assembly is possible but not optimal with short reads
- Long reads improve the ability to do *de novo* assembly dramatically
- Even in organisms with a good reference, such as humans, resequencing misses many structural differences relative to the reference

- Plant genomes are very large in general
- There are significant structural differences between different strains of the same plant such as rice
- These structural differences contribute to salient biological differences

Advantages of Long Read length

Full scale of genetic variation
Repetitive regions
Structural variants
Enables higher quality alignments and assembly
Gapless genomes - T2T

The Telomere-to-Telomere Consortium

Long read sequencing of the hydatidaform mole CHM13 with multiple technologies

-PacBio HiFi

- ONT ultralong reads

- Illumina Arima Genomics Hi-C (Hi-C)

- BioNano optical maps

- single-cell DNA template strand sequencing (Strand-seq)

Earth's heart of iron begins to yield its secrets p. 18

Microglia in chronic pain recovery and relapse pp. 33 & 86

Particle acceleration in a nova explosion p. 77

Science

$15
1 APRIL 2022
SPECIAL ISSUE
science.org

AAAS

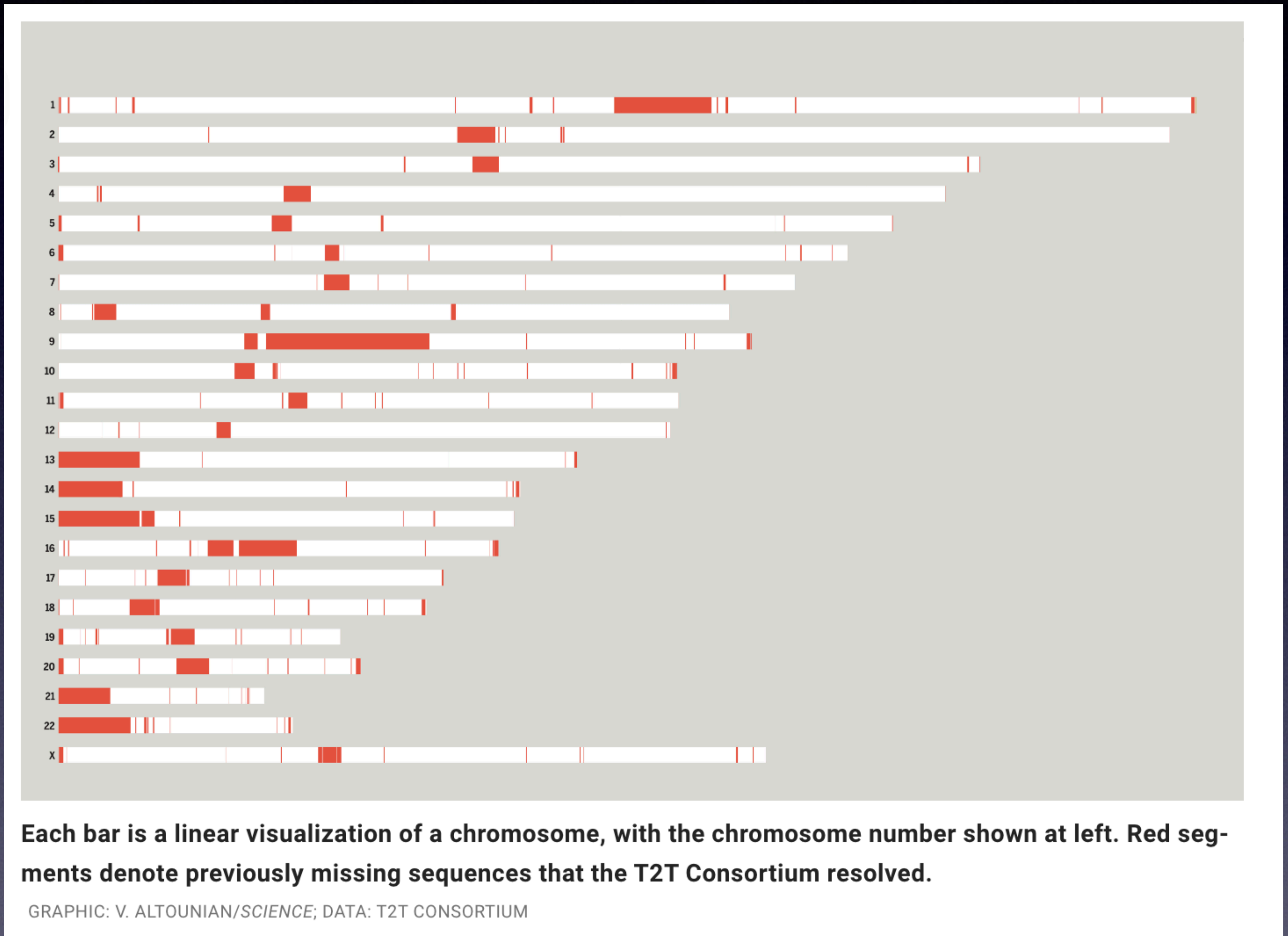
FILLING THE GAPS

Closing in on a complete human genome p. 42

CHM13 reference

Vastly improves upon the previous "gold standard" reference genome GRCh38

- Introduces nearly 200 million base pairs of sequence
- 1956 new gene predictions, 99 of which are predicted to be protein coding
- Gapless assemblies for all chromosomes except Y
- Corrects errors in the prior reference
- Resolves highly repetitive/ complex regions



Each bar is a linear visualization of a chromosome, with the chromosome number shown at left. Red segments denote previously missing sequences that the T2T Consortium resolved.

GRAPHIC: V. ALTOUNIAN/*SCIENCE*; DATA: T2T CONSORTIUM

Filling the gaps

Laura M. Zahn

Science, 376 (6588), • DOI: 10.1126/science.abp8653

Limitations of long reads

- Cost
- Throughput*
- Accuracy*
- DNA amount required
- DNA quality required

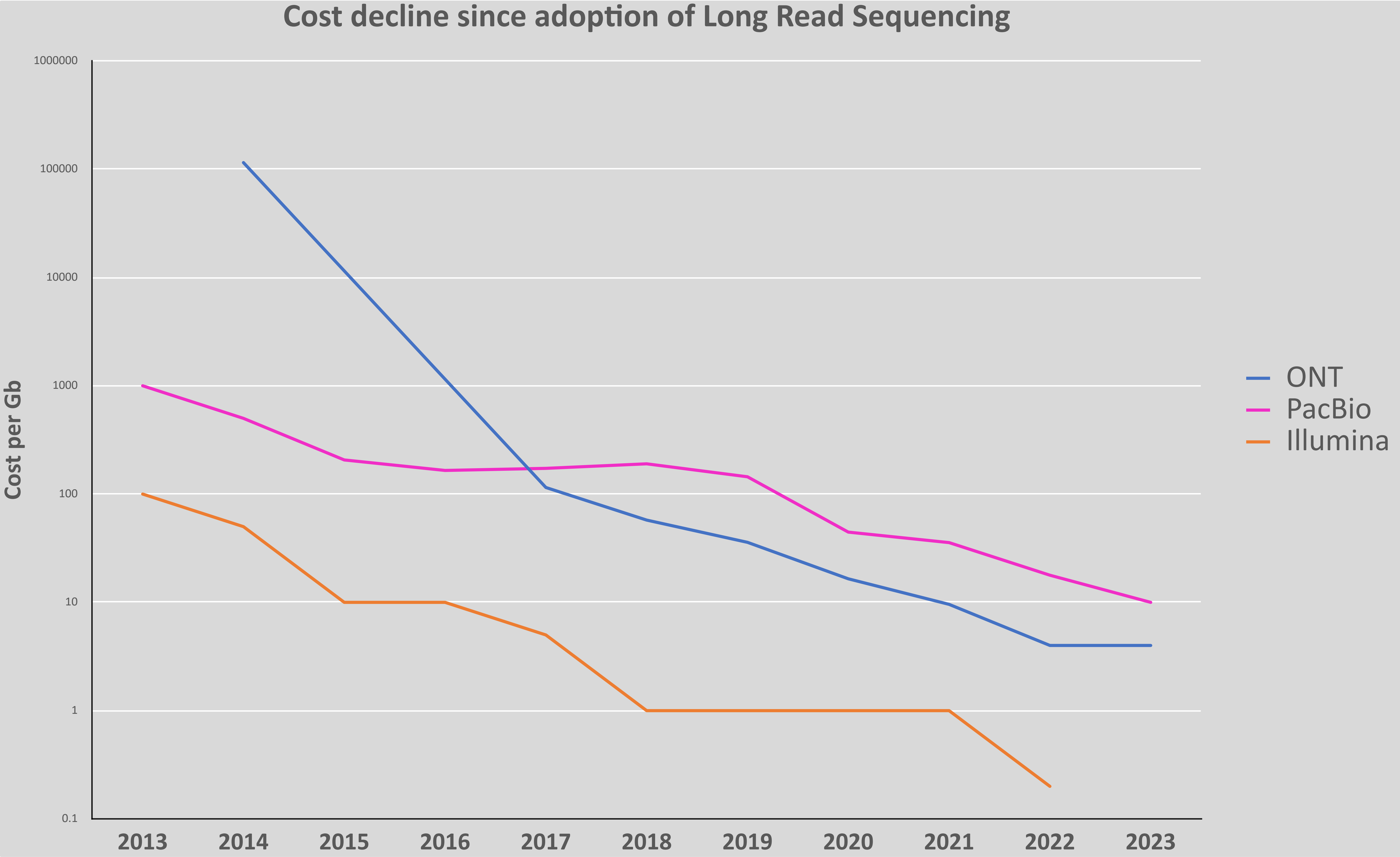
*This is rapidly changing

Two "flavors" of long read sequencing

PACIFIC
BIOSCIENCES®

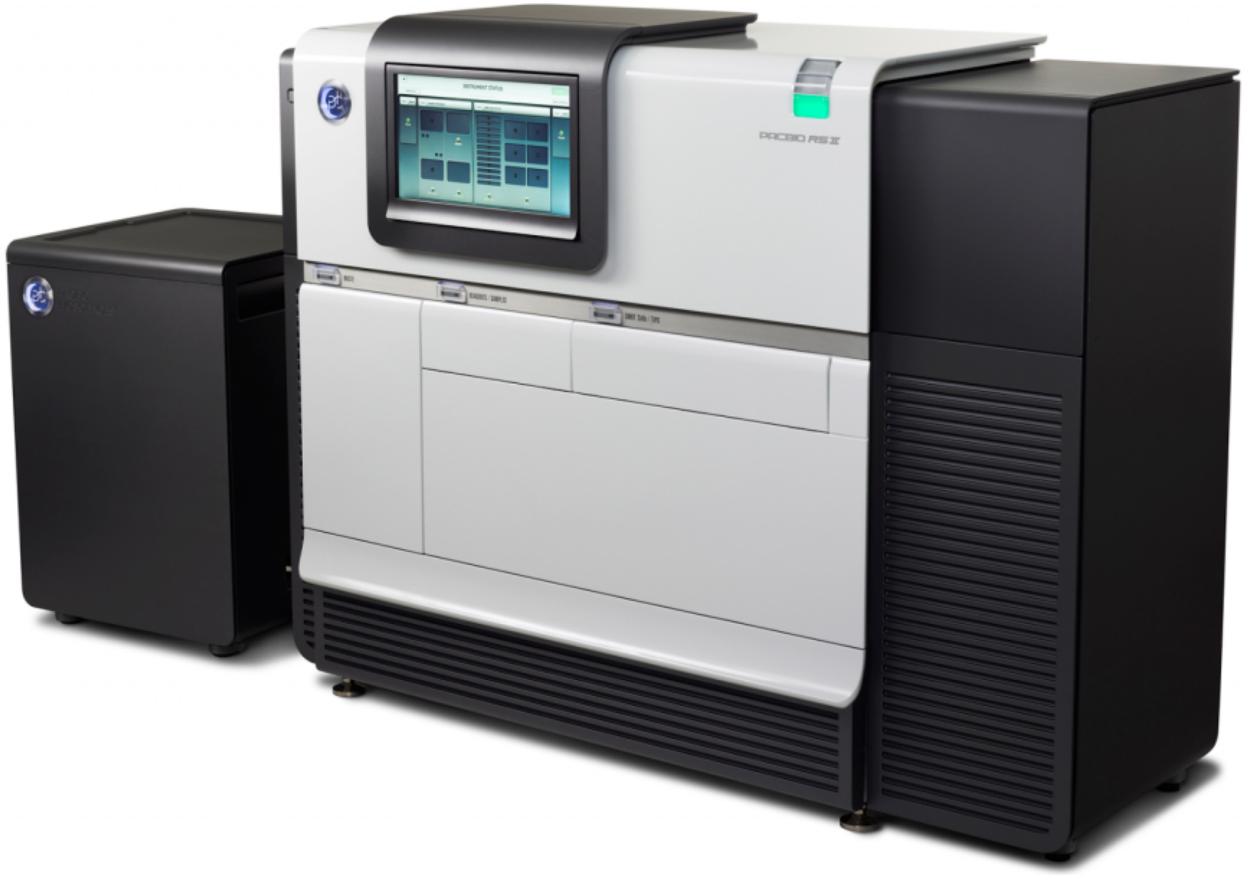
Oxford
NANOPORE
Technologies

Significant advances in long read sequencing over last 9 years



PACIFIC
BIOSCIENCES®

PacBio



RSII

- ~85% single pass accuracy
- “short read” CCS accuracy >99.999%
- Up to 2Gb per SMRTcell
- Read lengths up to 60kb

Pacific Biosciences Sequel II

Released in 2018

Smaller, lower cost instrument

8 Million ZMW (155k RSII,1M Sequel I)

Early runs were rocky

Substantial recent improvement in performance up to 200Gb of CLR data or 30Gb of HiFi data

Upto 800Gb CLR or 120Gb HiFi in one week

Pacific Biosciences Revio (Available 2023)

Similar in size to Sequel

25M ZMW (1M Sequel I, 8M Sequel II)

Main focus is HiFi data

Runs 4 chips in parallel

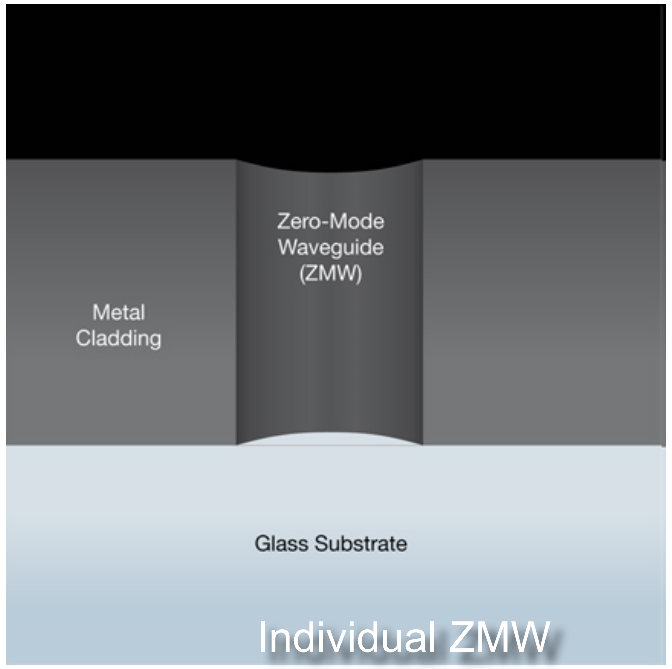
Estimated up to 3Tb of HiFi data per week

Zero-Mode Waveguides Are the Observation Windows

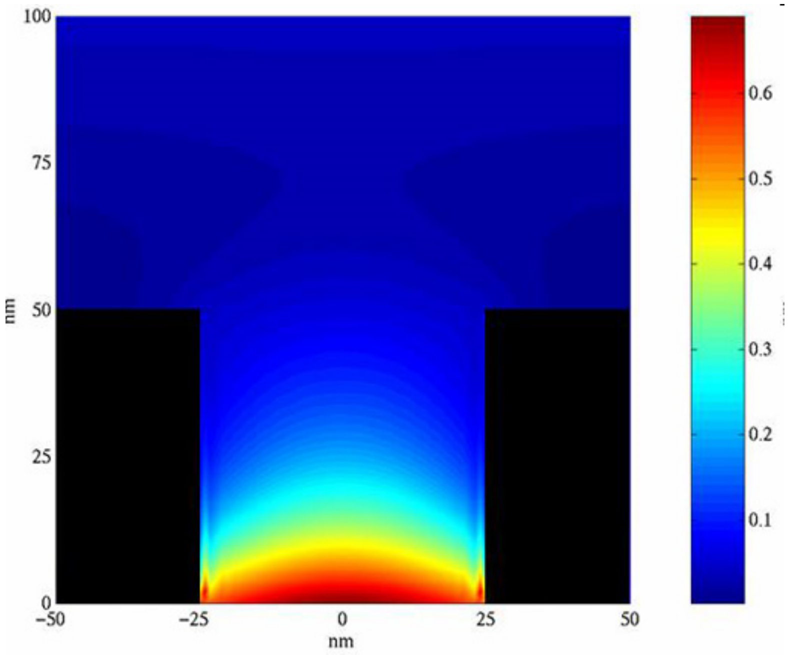
DNA sequencing is performed on SMRT™ Cells, each containing tens of thousands of zero-mode waveguides (ZMWs)

A ZMW is a cylindrical hole, hundreds of nanometers in diameter, perforating a thin metal film supported by a transparent substrate

The ZMW provides a window for observing DNA polymerase as it performs sequencing by synthesis



Individual ZMW

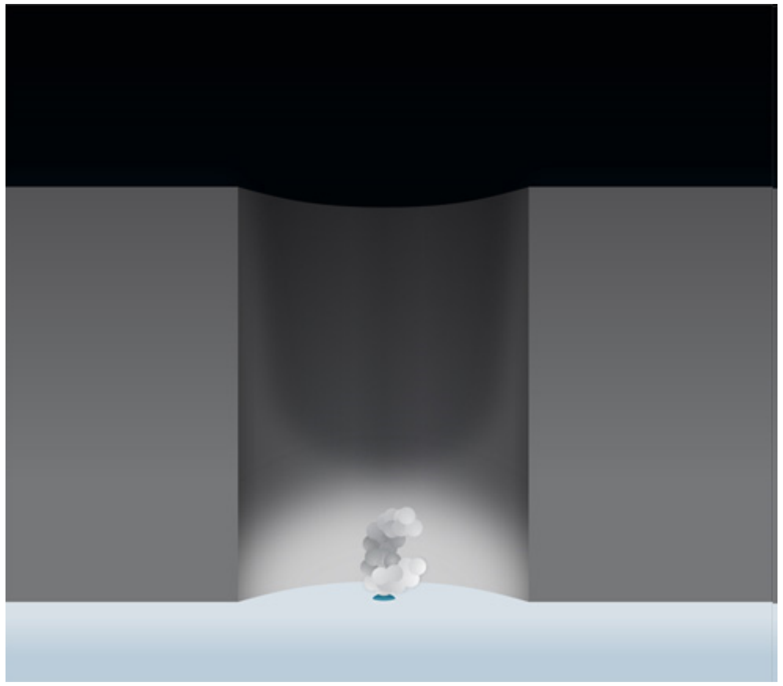


Laser light illuminates the ZMW

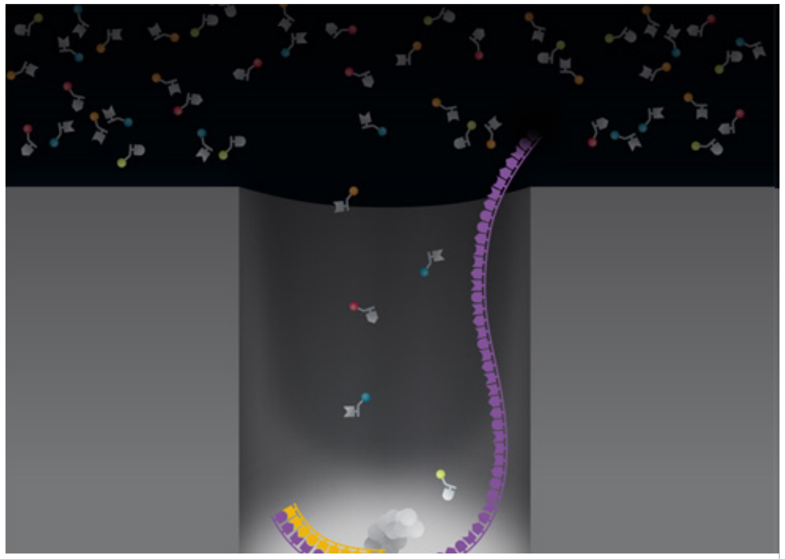
DNA Polymerase as a Sequencing Engine

A single DNA polymerase molecule is attached to the bottom of the ZMW

A single incorporation event can be identified against the background of fluorescently labeled nucleotides



ZMW with DNA polymerase

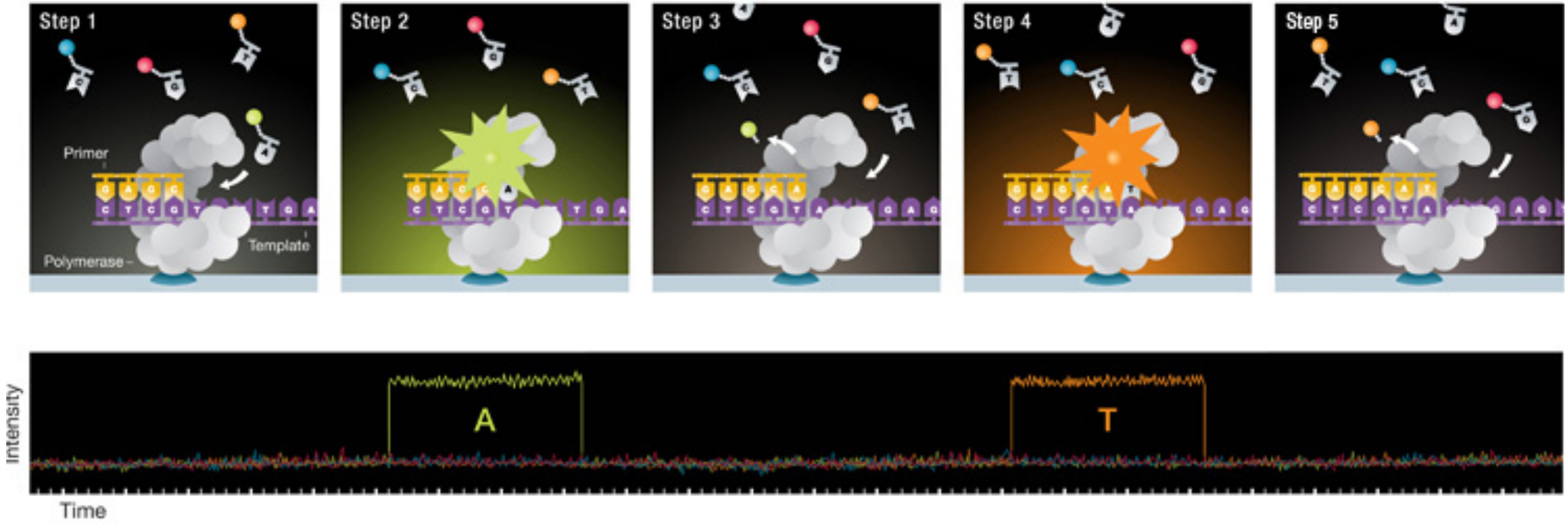


ZMW with DNA polymerase and phospholinked nucleotides

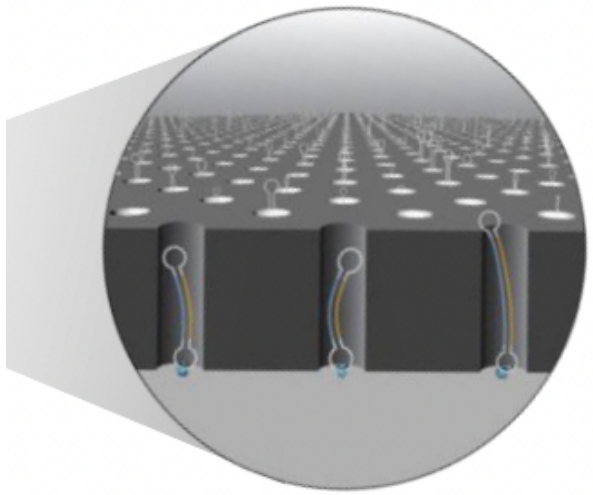
Processive Synthesis with Phospholinked Nucleotides

Enzymatic incorporation of the labeled nucleotide creates a flash of light, which is captured by the optics system and converted into a base call with associated quality metrics using optimized algorithms

To generate consensus sequence from the data, an assembly process aligns the different fragments based on common sequences



Sample Loading May Require Titration



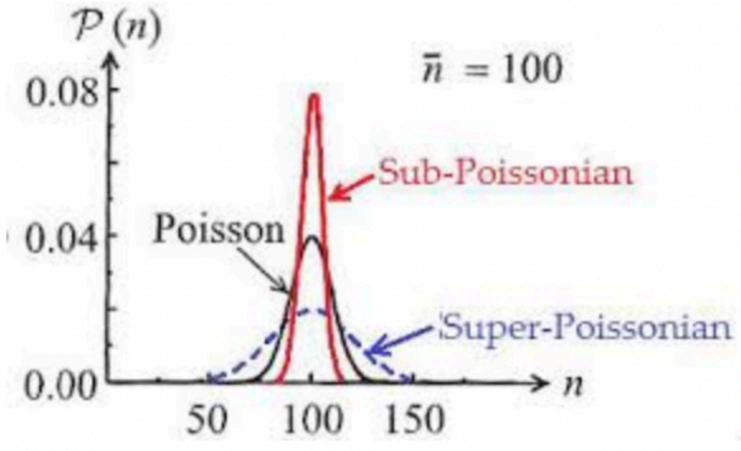
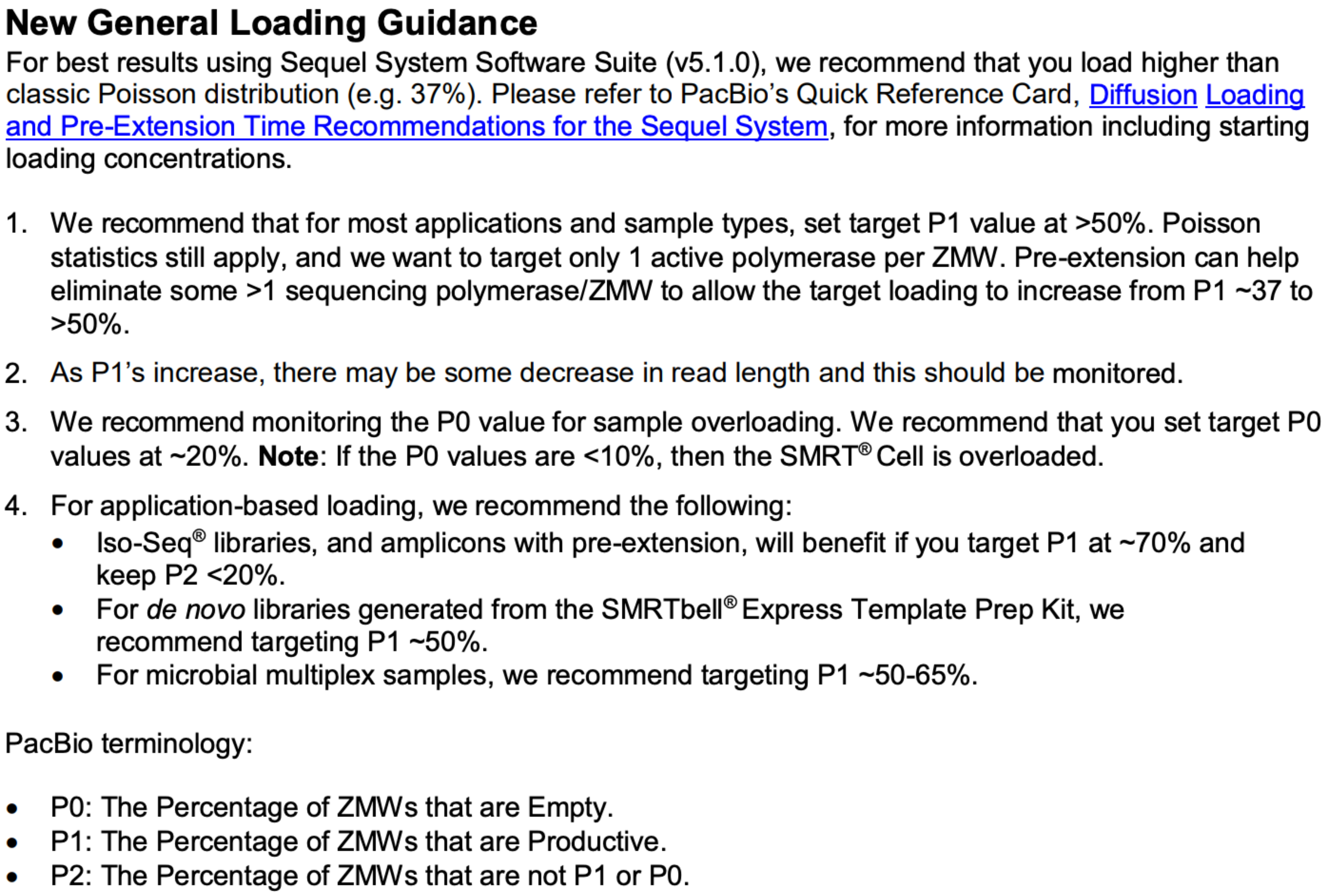


Figure 5.26 from Adrian Jeantet, Cavity quantum electrodynamics with carbon nanotubes, 2017.



New General Loading Guidance

For best results using Sequel System Software Suite (v5.1.0), we recommend that you load higher than classic Poisson distribution (e.g. 37%). Please refer to PacBio's Quick Reference Card, Diffusion Loading and Pre-Extension Time Recommendations for the Sequel System, for more information including starting loading concentrations.

1. We recommend that for most applications and sample types, set target P1 value at >50%. Poisson statistics still apply, and we want to target only 1 active polymerase per ZMW. Pre-extension can help eliminate some >1 sequencing polymerase/ZMW to allow the target loading to increase from P1 ~37 to >50%.
2. As P1's increase, there may be some decrease in read length and this should be monitored.
3. We recommend monitoring the P0 value for sample overloading. We recommend that you set target P0 values at ~20%. **Note**: If the P0 values are <10%, then the SMRT® Cell is overloaded.
4. For application-based loading, we recommend the following:
   - Iso-Seq® libraries, and amplicons with pre-extension, will benefit if you target P1 at ~70% and keep P2 <20%.
   - For *de novo* libraries generated from the SMRTbell® Express Template Prep Kit, we recommend targeting P1 ~50%.
   - For microbial multiplex samples, we recommend targeting P1 ~50-65%.

PacBio terminology:

- P0: The Percentage of ZMWs that are Empty.
- P1: The Percentage of ZMWs that are Productive.
- P2: The Percentage of ZMWs that are not P1 or P0.

NEW ADAPTIVE LOADING FEATURE FOR SEQUEL II AND IIe SYSTEMS

Adaptive Loading reduces sample overloading, allowing users to load higher with confidence

- Adaptive loading technology actively monitors polymerase complex binding to the bottom of ZMWs during the sample immobilization step.
- Detection of these active polymerase complexes allows the system to terminate the immobilization step when the desired loading target has been achieved.

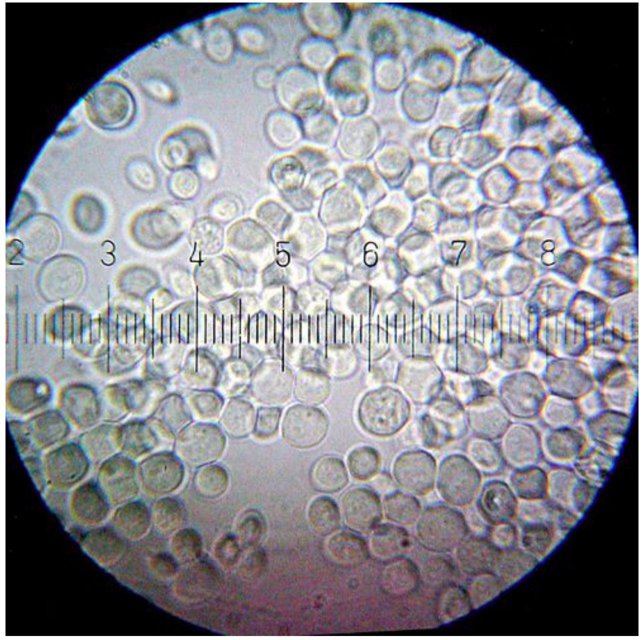
  → This approach can help reduce sample overloading and run-to-run yield variability

Adaptive Loading (AL) uses active monitoring of polymerase binding to the bottom of the ZMW during loading to reduce variability and the risk of overloading with high-concentration samples

LIGHTS ALL ASKEW IN THE HEAVENS; Men of Science More or Less Agog Over Results of Eclipse Observations. EINSTEIN THEORY TRIUMPHS Stars Not Where They Seemed or Were Calculated to be, but Nobody Need Worry. A BOOK FOR 12 WISE MEN No More in All the World Could Comprehend It, Said Einstein When His Daring Publishers Accepted It.

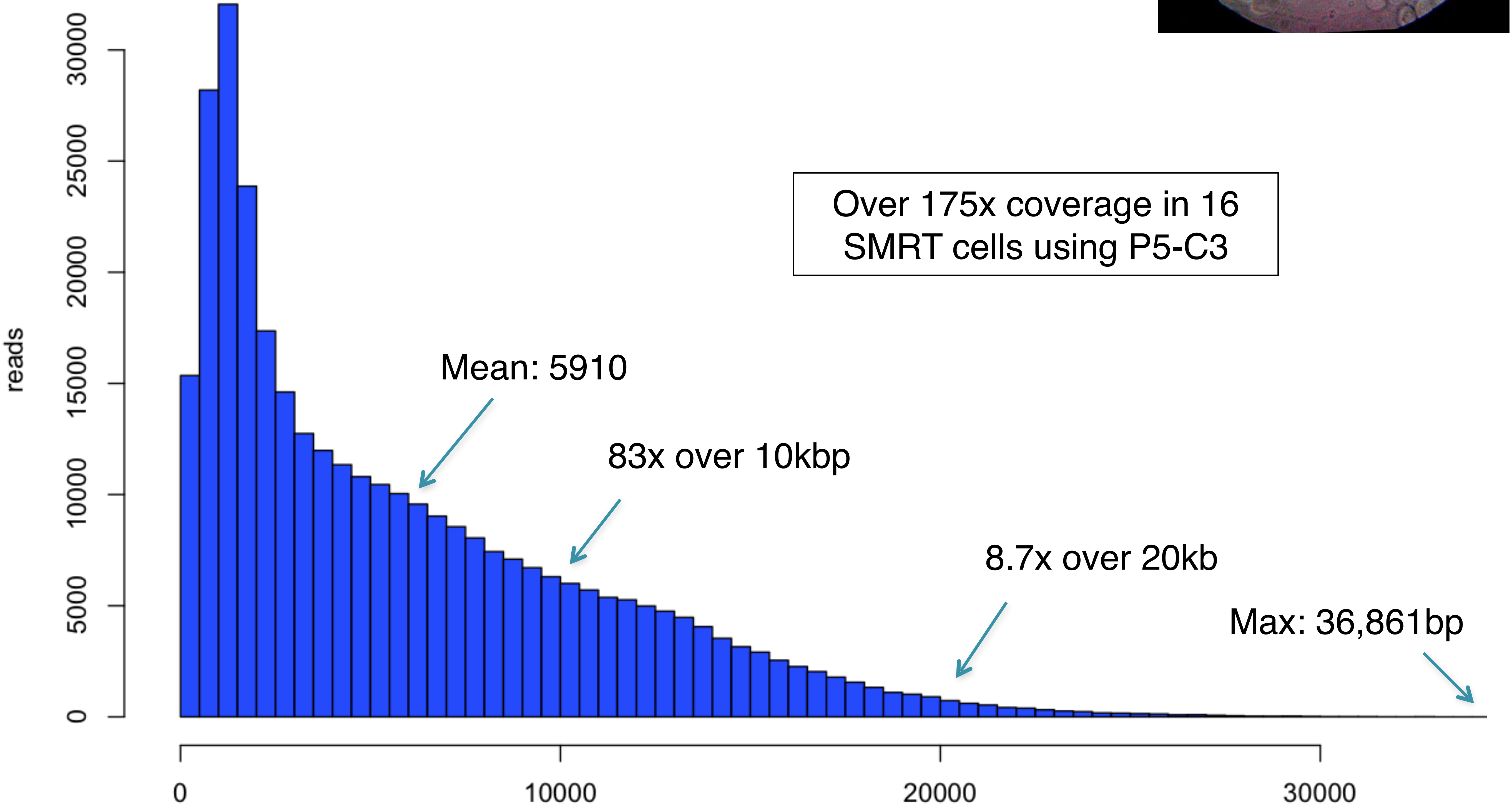
New York Times Nov. 9, 1919.

Yeast: S. cerevisiae W303



PacBio RS II *s*equencing at CSHL

Size selection using an 7 Kb elution window on a BluePippin™ device from Sage Science



Over 175x coverage in 16 SMRT cells using P5-C3

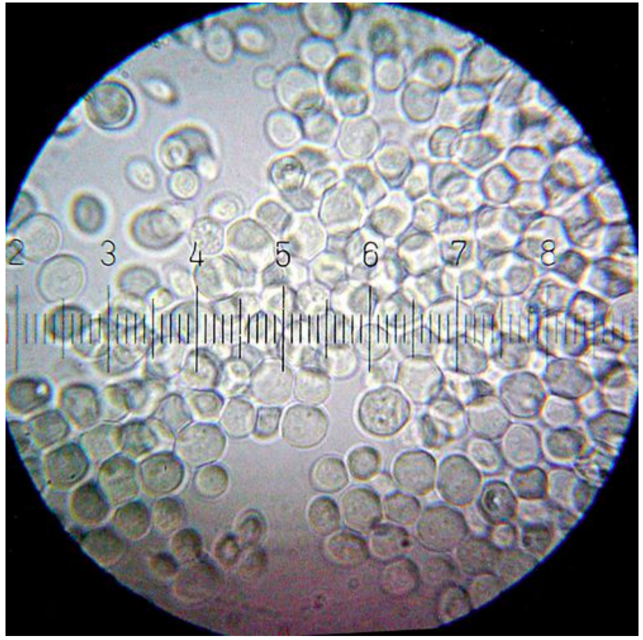
Mean: 5910

83x over 10kbp

8.7x over 20kb

Max: 36,861bp

S. cerevisiae W303

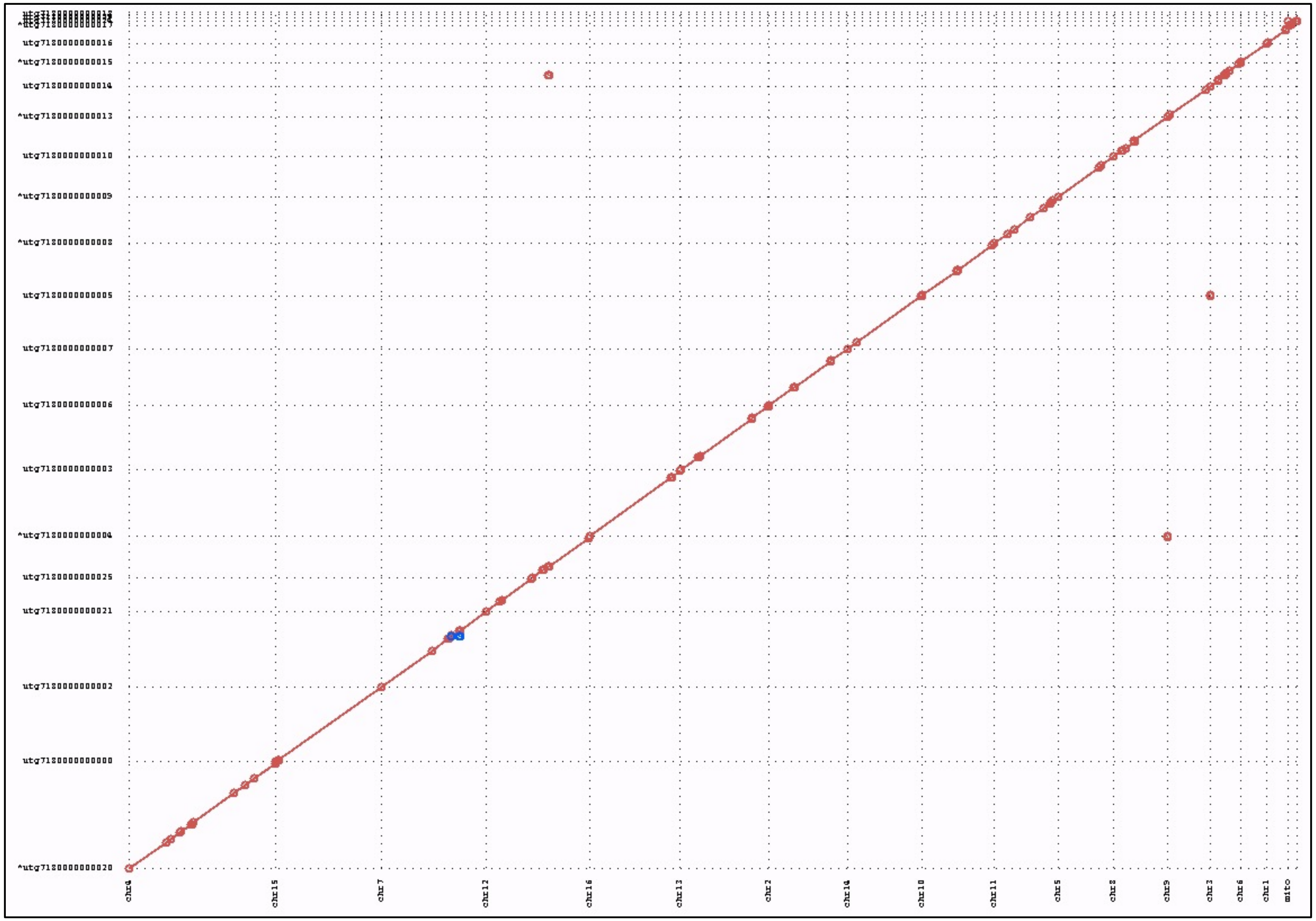


S288C Reference sequence

- 12.1Mbp; 16 chromo + mitochondria; N50: 924kbp

PacBio assembly using HGAP + Celera Assembler

- 12.4Mbp; 21 non-redundant contigs; N50: 811kbp; >99.8% id



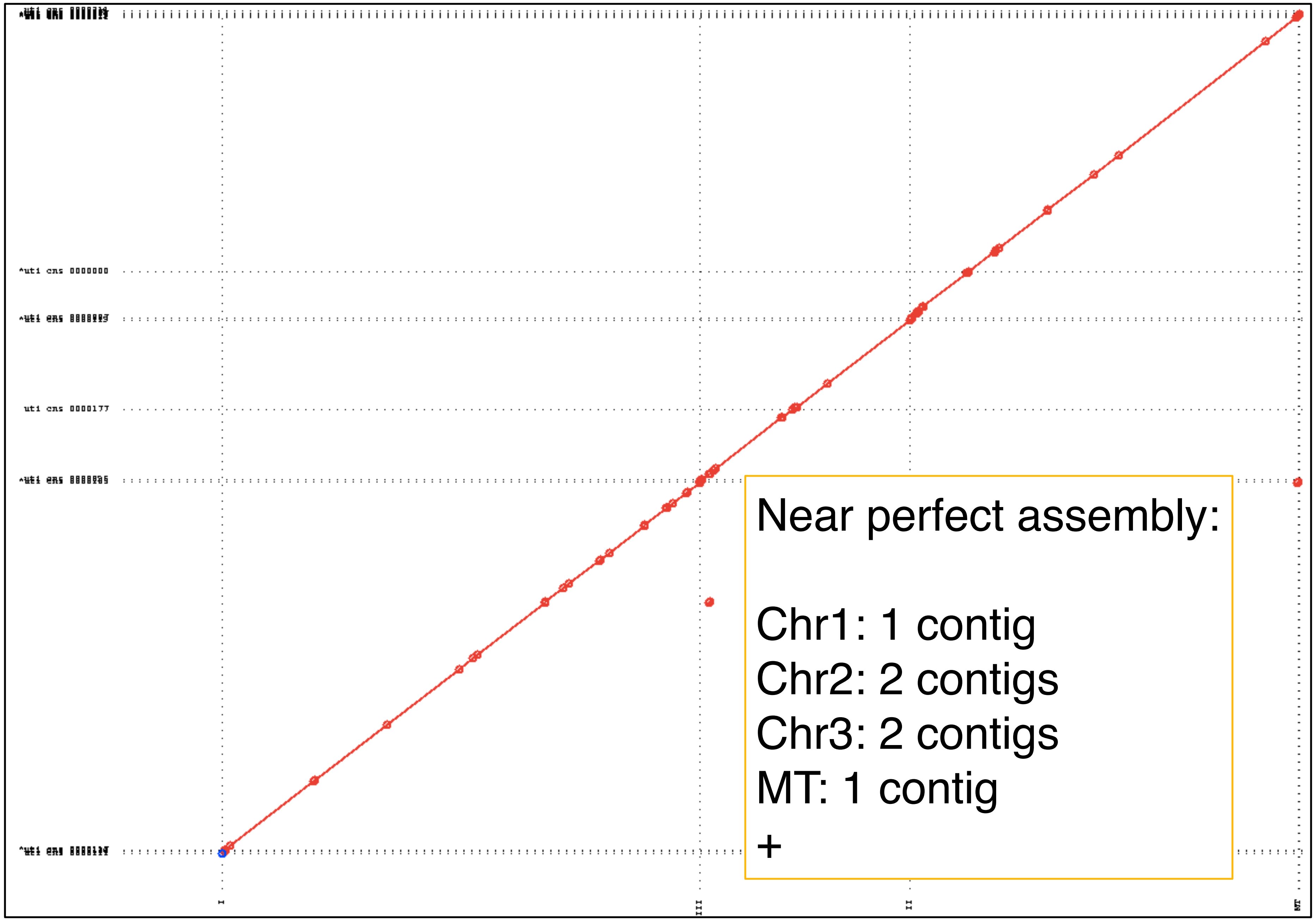
S. pombe dg21

ASM294 Reference sequence

- 12.6Mbp; 3 chromo + mitochondria; N50: 4.53Mbp

PacBio assembly using HGAP + Celera Assembler

- 12.7Mbp; 13 non-redundant contigs; N50: 3.83Mbp; >99.98% id



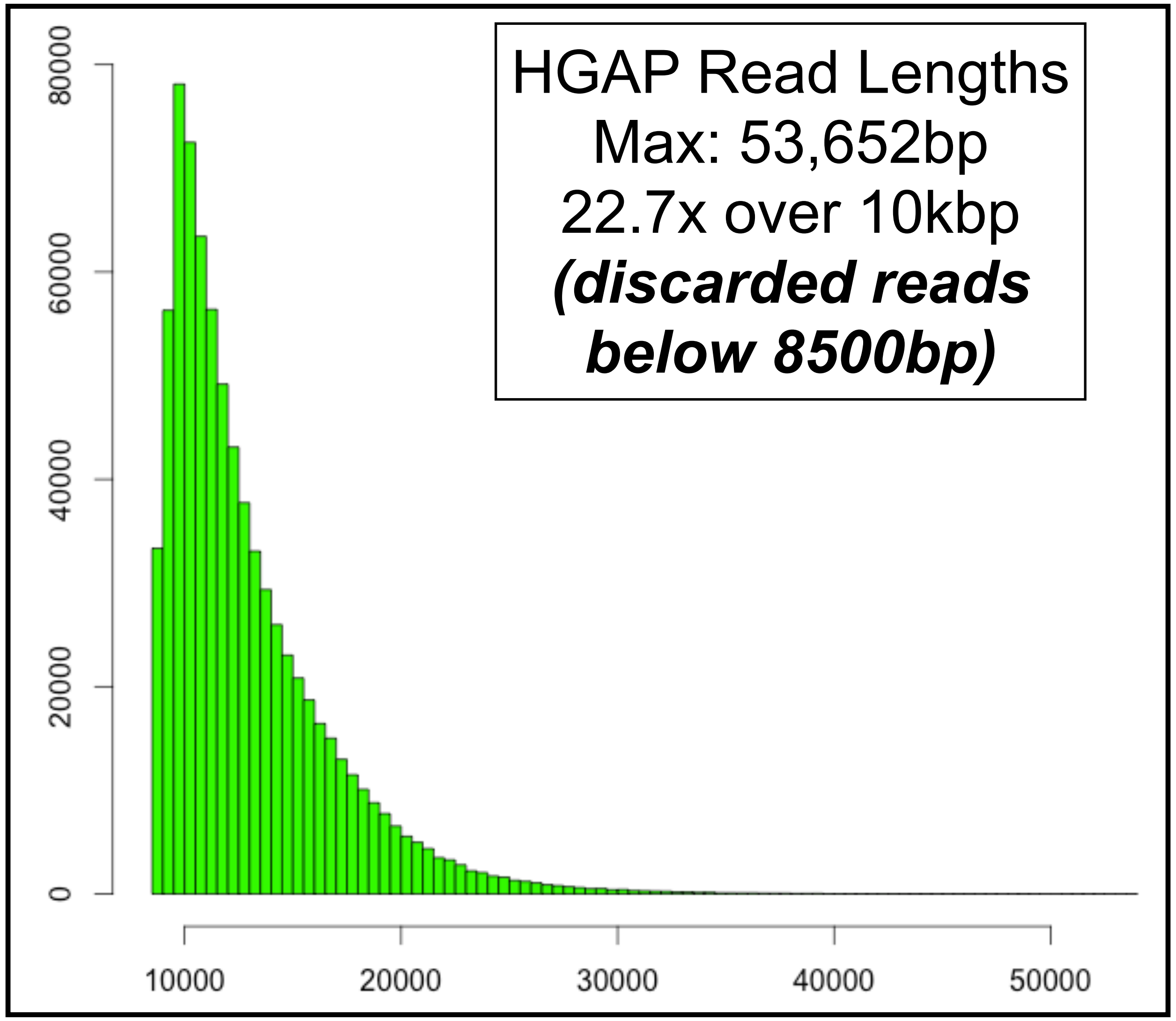
Near perfect assembly:

Chr1: 1 contig
Chr2: 2 contigs
Chr3: 2 contigs
MT: 1 contig
+

O. sativa pv Indica (IR64)

Genome size:	~370 Mb
Chromosome N50:	~29.7 Mbp

Assembly	Contig NG50
MiSeq Fragments 25x 456bp (3 runs 2x300 @ 450 FLASH)	19 kbp
"ALLPATHS-recipe" 50x 2x100bp @ 180 36x 2x50bp @ 2100 51x 2x50bp @ 4800	18 kbp
HGAP + CA 22.7x @ 10kbp	4.0 Mbp
Nipponbare BAC-by-BAC Assembly	5.1 Mbp

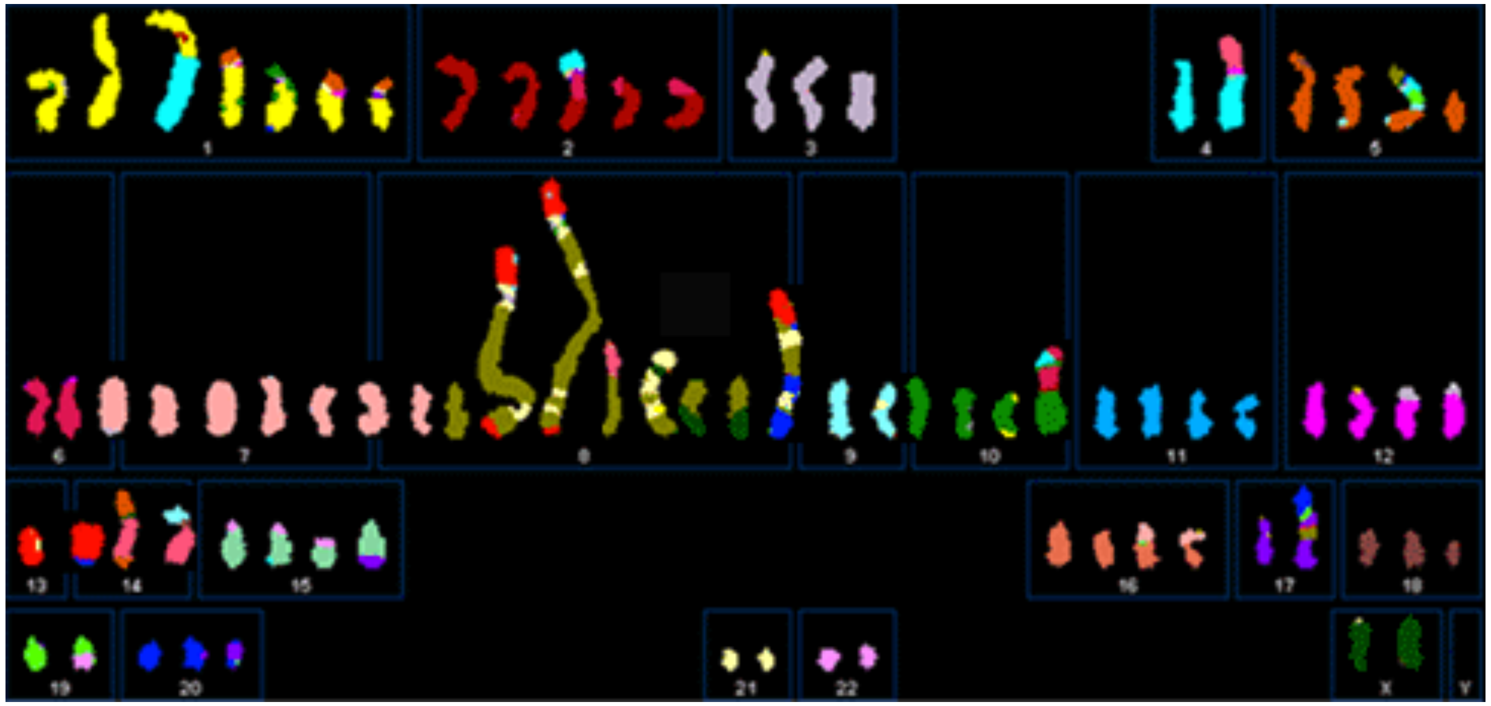

HGAP Read Lengths
Max: 53,652bp
22.7x over 10kbp
(discarded reads below 8500bp)

Structural Variations in SKBR3

SKRB3 cell line was derived by G. Trempe and L. J. Old in 1970 from pleural effusion cells of a patient, a white, Caucasian female

Most commonly used Her2-amplified breast cancer cell line

Often used for pre-clinical research on Her2-targeting therapeutics such as Herceptin (Trastuzumab) and resistance to these therapies.



(Davidson et al, 2000)

Nattestad, et al, Gen. Res. 2018

Assembly using PacBio yields far better contiguity

Number of sequences:
10,304

Total sequence length:
2.75 Gb

Mean: 266 kb

Max: 15 Mb

N50: 2.17 Mb

NG50: 1.86 Mb

PACIFIC BIOSCIENCES®

Number of sequences:
748,955

Total sequence length:
2.07 Gb

Mean: 2.8 kb

Max: 61 kb

N50: 3.3 kb

NG50: 1.9 kb

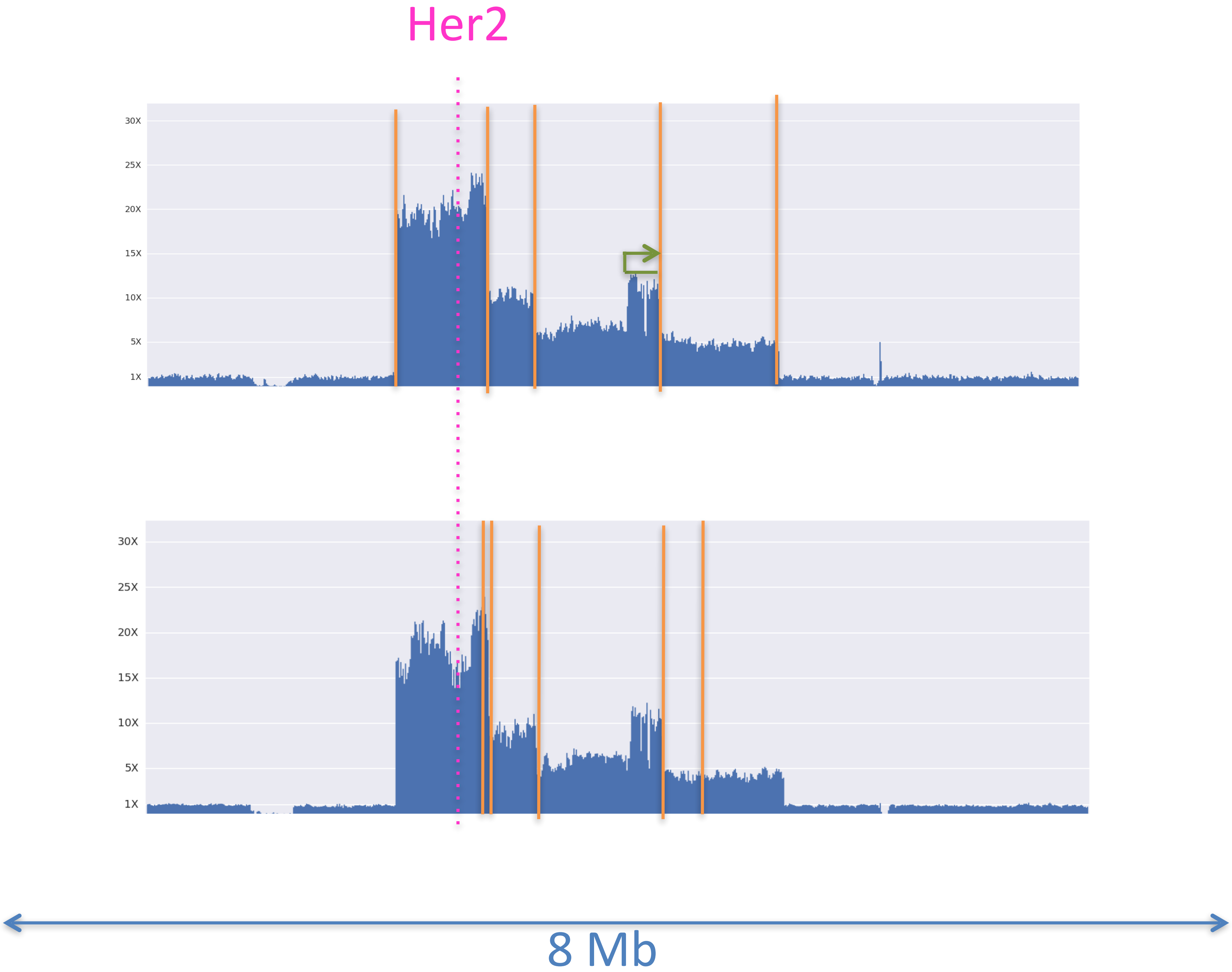
illumina®

PacBio
73X @ 10kb

# split reads

Illumina
120X @ 100bp

# split reads



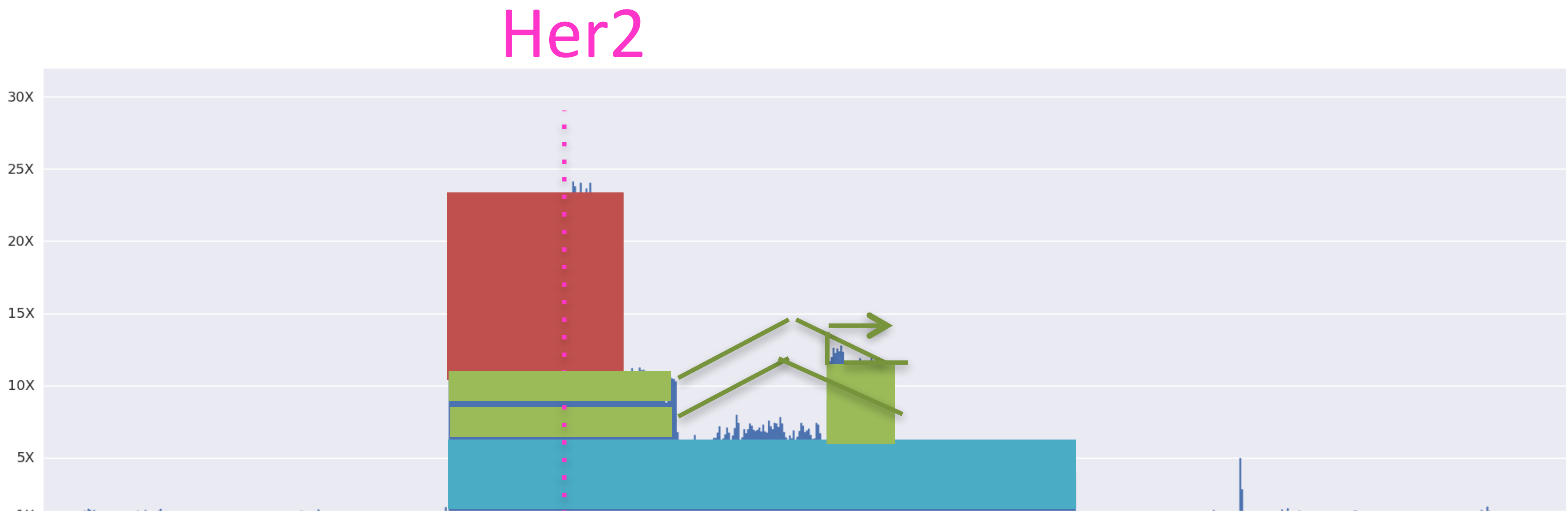
Green arrow indicates an inverted duplication.

False positive and missing Illumina calls due to mis-mapped reads (especially low complexity).

Cancer lesion reconstruction from genomic threads

PacBio

chr17



By comparing the proportion of reads that are spanning or split at breakpoints we can begin to infer the history of the genetic lesions.

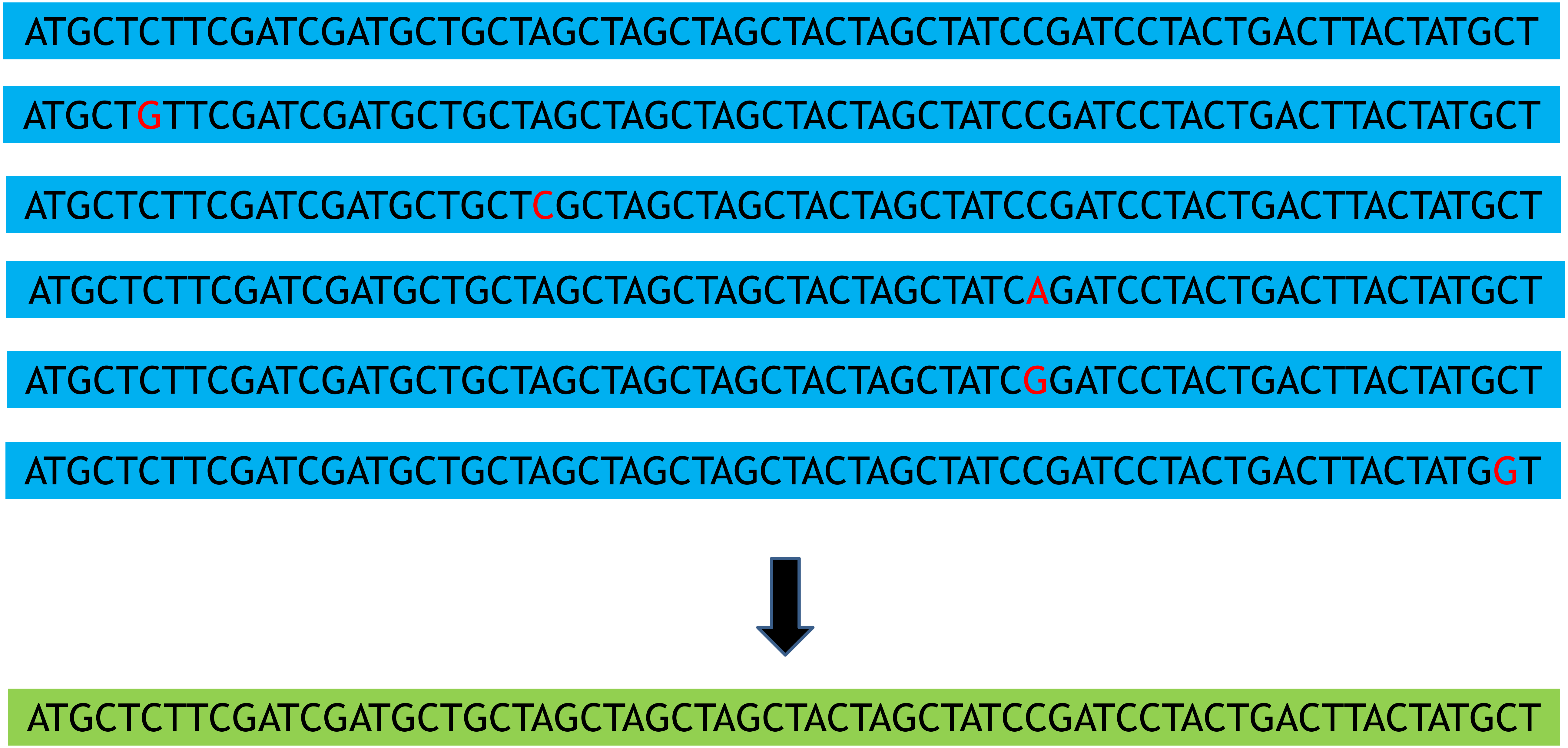
1. Healthy diploid genome

2. Original translocation into chromosome 8

3. Duplication, inversion, and inverted duplication within chromosome 8

4. Final duplication from within chromosome 8

PacBio errors are randomly distributed



ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTACTAGCTATCCGATCCTACTGACTTACTATGCT

ATGCTGTTCGATCGATGCTGCTAGCTAGCTAGCTACTAGCTATCCGATCCTACTGACTTACTATGCT

ATGCTCTTCGATCGATGCTGCTCGCTAGCTAGCTACTAGCTATCCGATCCTACTGACTTACTATGCT

ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTACTAGCTATCAGATCCTACTGACTTACTATGCT

ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTACTAGCTATCGGATCCTACTGACTTACTATGCT

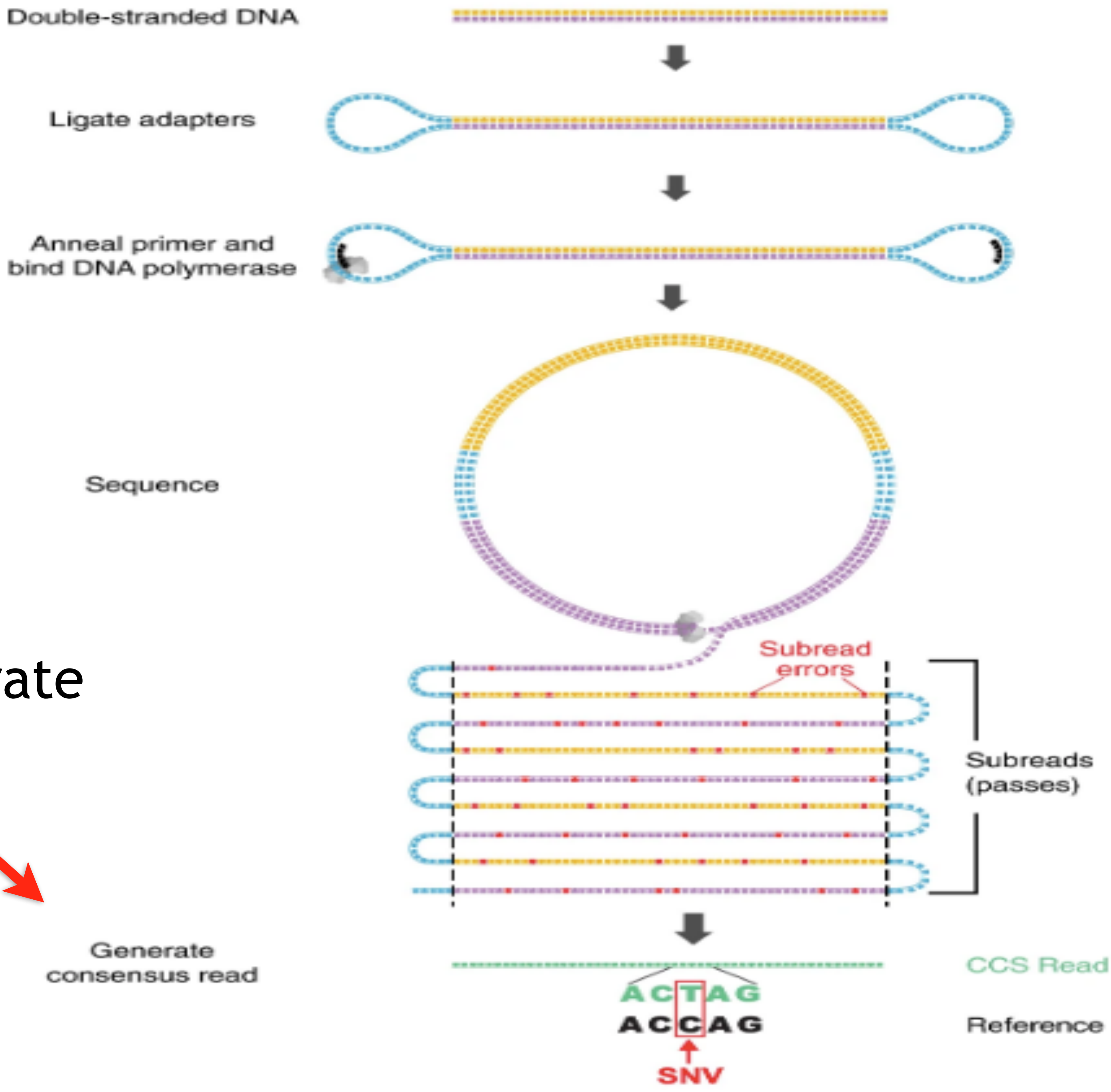
ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTACTAGCTATCCGATCCTACTGACTTACTATGGT

ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTACTAGCTATCCGATCCTACTGACTTACTATGCT

Enough coverage makes error drop out

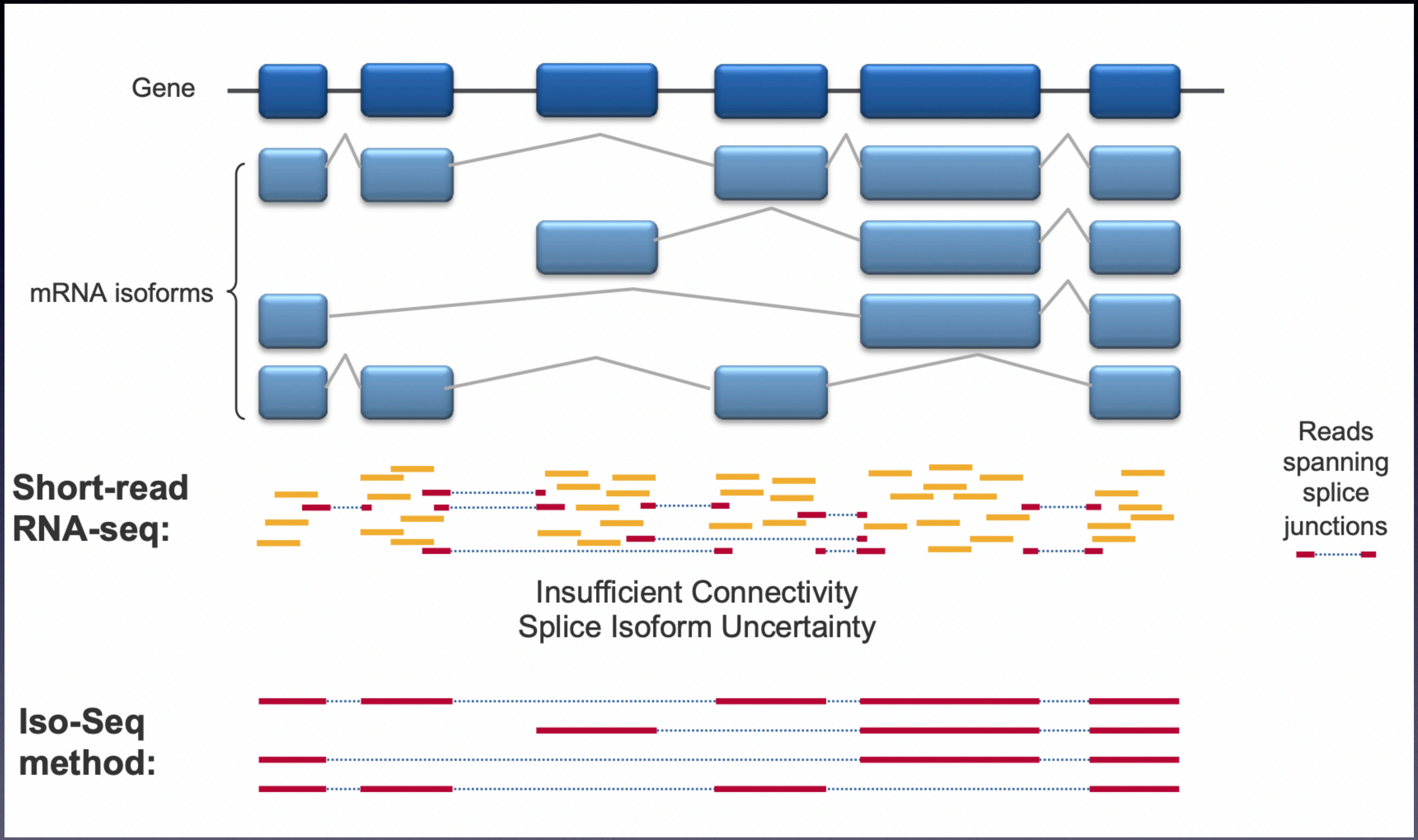
PacBio CCS
“HiFi” for longer
(~15kb)
fragments

99.99% Accurate



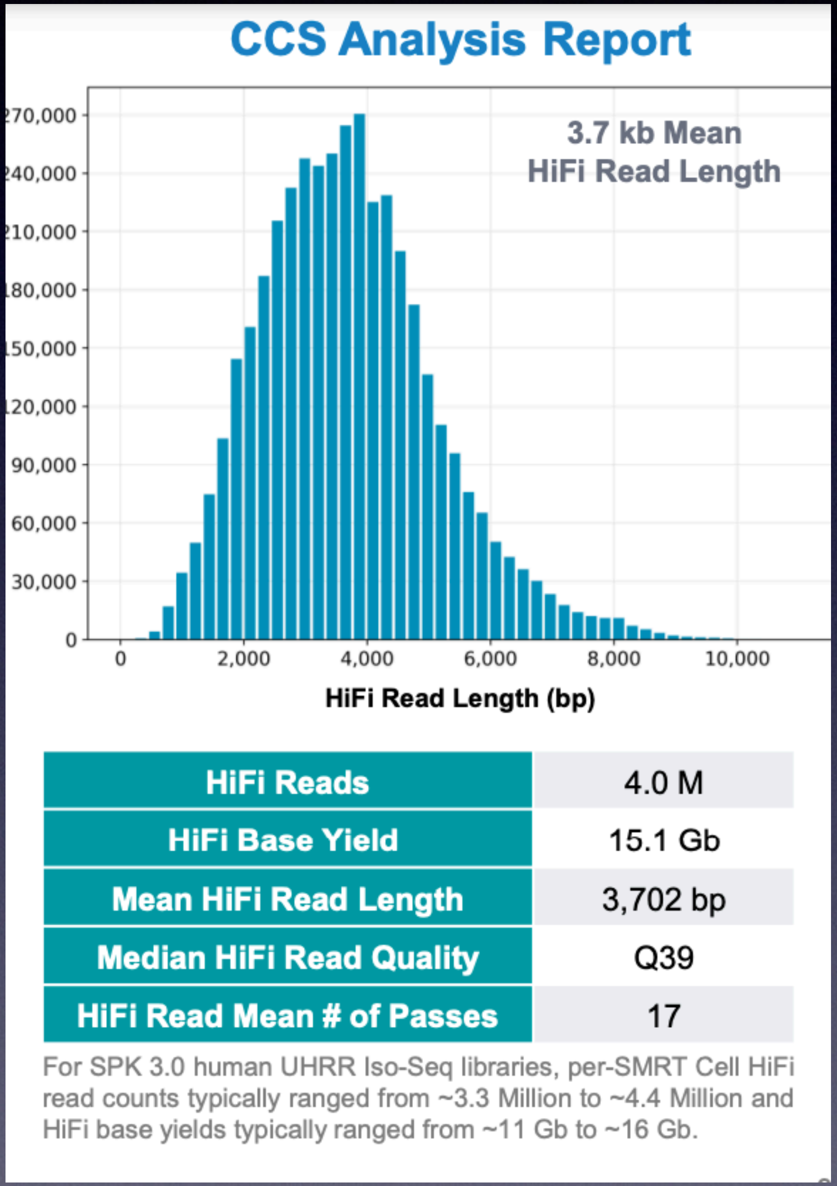
From Wenger et al (2019) Nature Biotechnology

Benefits of long read transcripts


Gene
mRNA isoforms
Short-read RNA-seq:
Reads spanning splice junctions
Insufficient Connectivity
Splice Isoform Uncertainty
Iso-Seq method:

Long read transcripts provide complete isoform information, enables identification of alternative splicing, fusion events, and allows for isoform level phasing

PacBio IsoSeq transcript sequencing



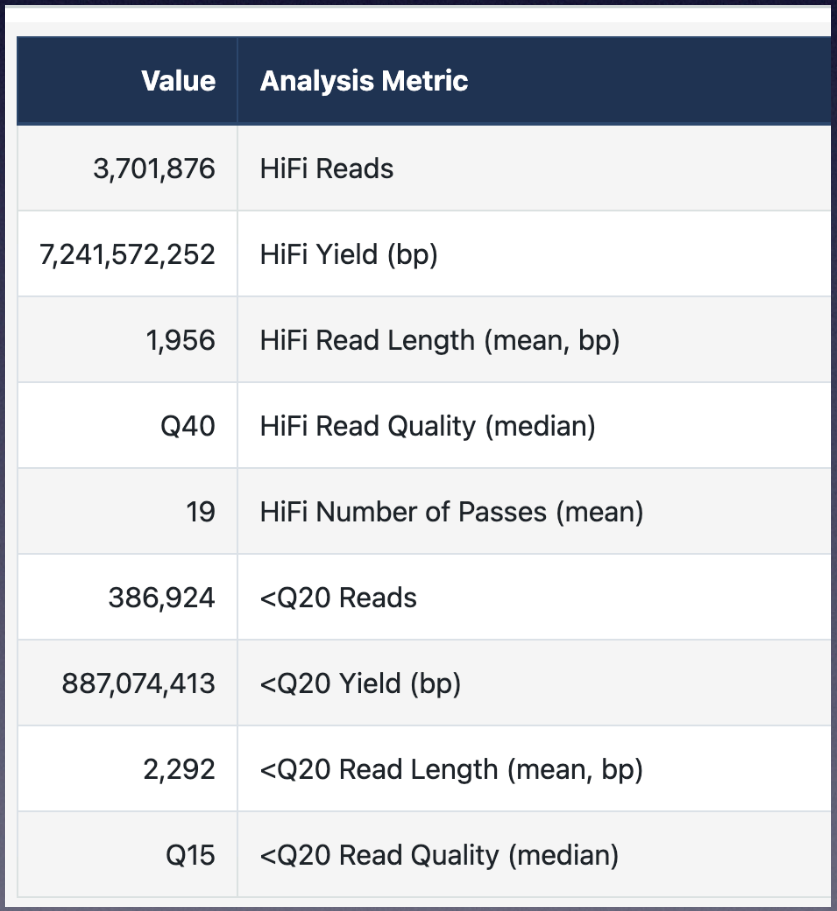
CCS Analysis Report

HiFi Reads	4.0 M
HiFi Base Yield	15.1 Gb
Mean HiFi Read Length	3,702 bp
Median HiFi Read Quality	Q39
HiFi Read Mean # of Passes	17

For SPK 3.0 human UHRR Iso-Seq libraries, per-SMRT Cell HiFi read counts typically ranged from ~3.3 Million to ~4.4 Million and HiFi base yields typically ranged from ~11 Gb to ~16 Gb.

High accuracy, low throughput

>300ng total RNA input



Value	Analysis Metric
3,701,876	HiFi Reads
7,241,572,252	HiFi Yield (bp)
1,956	HiFi Read Length (mean, bp)
Q40	HiFi Read Quality (median)
19	HiFi Number of Passes (mean)
386,924	<Q20 Reads
887,074,413	<Q20 Yield (bp)
2,292	<Q20 Read Length (mean, bp)
Q15	<Q20 Read Quality (median)

CSHL run

Oxford
NANOPORE
Technologies

PromethION

24 independent flowcells

500bp/s sequencing speed

3000 pores per flowcells = 144,000 pores (fully loaded) (MinION cells 512 pores)

On board single or duplex basecalling

>140Gb in CSHL hands

>100M cDNA reads

Up to ~5 Tb fully loaded in one week

Sequencing "flavors" include:

Ligation based - standard methods for gDNA

Q20 - enables higher accuracy including duplex

Barcoding - allows multiplexing up to 96 sample

16S - enables 16S metagenome sequencing

PCR sequencing - long-range PCR for low mass samples

Cas9 - enables Cas9 mediated target enrichment

Rapid - enables library prep <2hrs w/o mechanical shearing

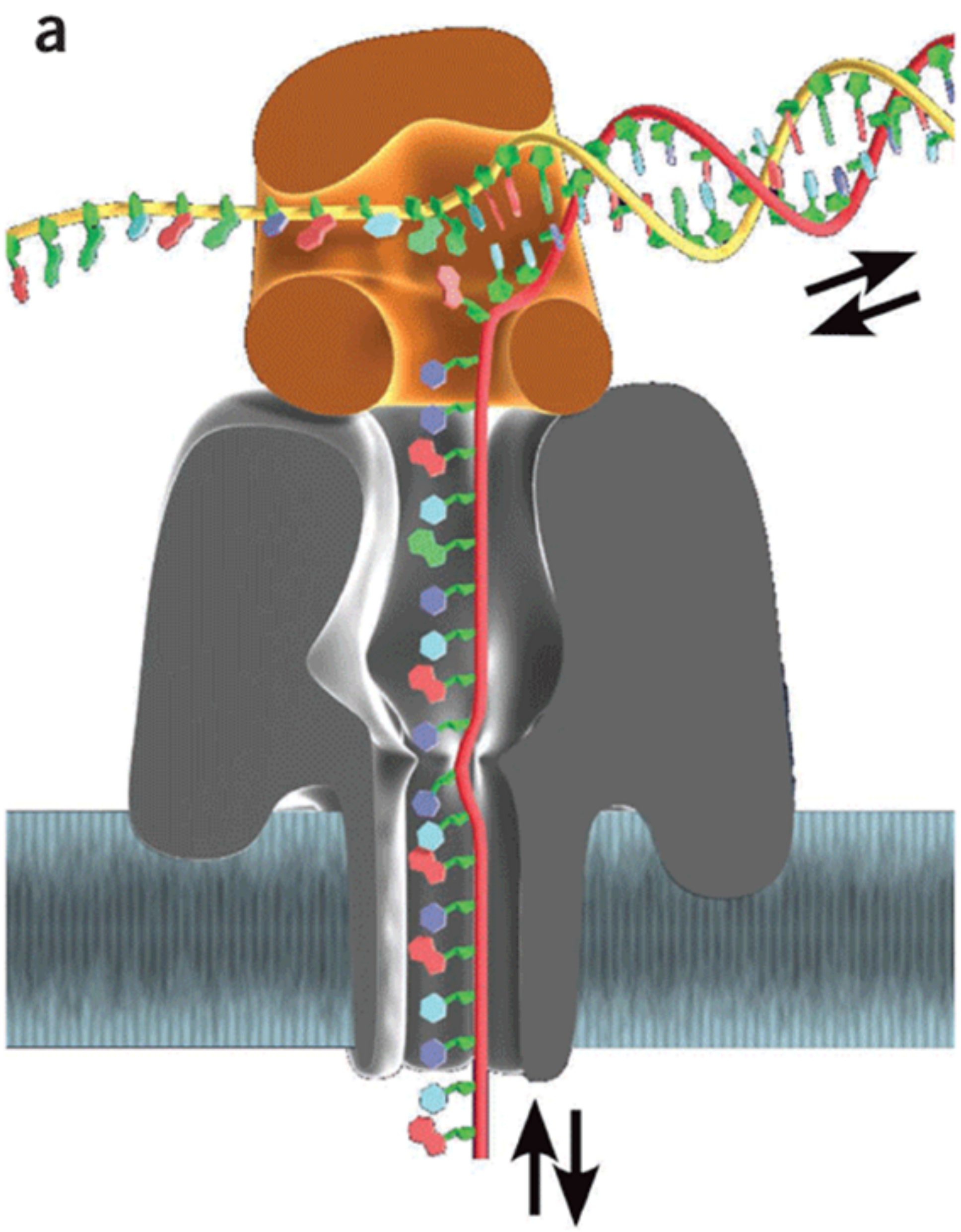
Ultra-long - enables N50s up to 100kb

Native barcoding - PCR-free barcoding to preserve epigenetic marks

Field kit - enables sequencing in the field w/o cold chain

Short fragment - enables sequencing of fragments <1000bp

Oxford Nanopore relies on CsgG and a non-destructive motor protein



Cis side voltage drives DNA through pore

Motor protein mediates DNA unwinding and translocation speed

Ions flow through the pore to change membrane potential

Small changes in measured voltage are translated into k-mers

Nature Biotechnology 30, 326–328 (2012) doi:10.1038/nbt.2181Published online 10 April 2012

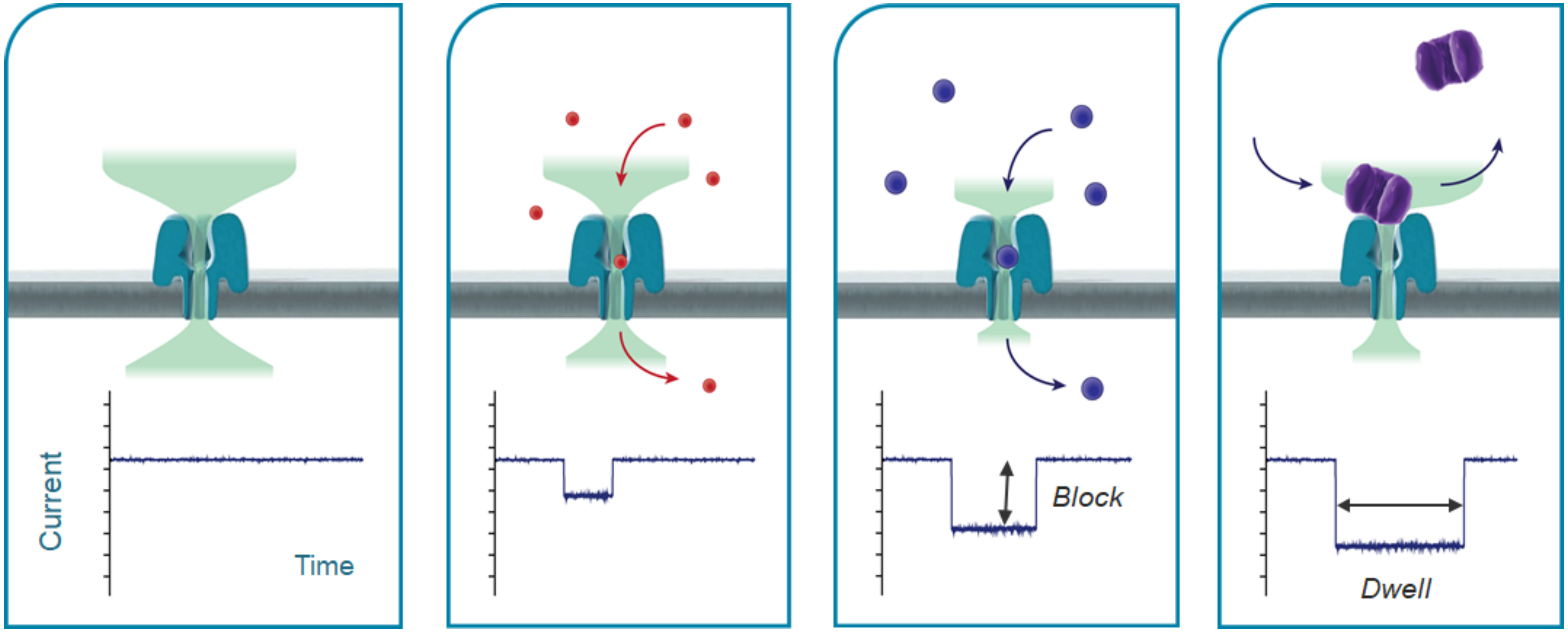
Nanopore Sensing Summary

Nanopore = ‘very small hole’

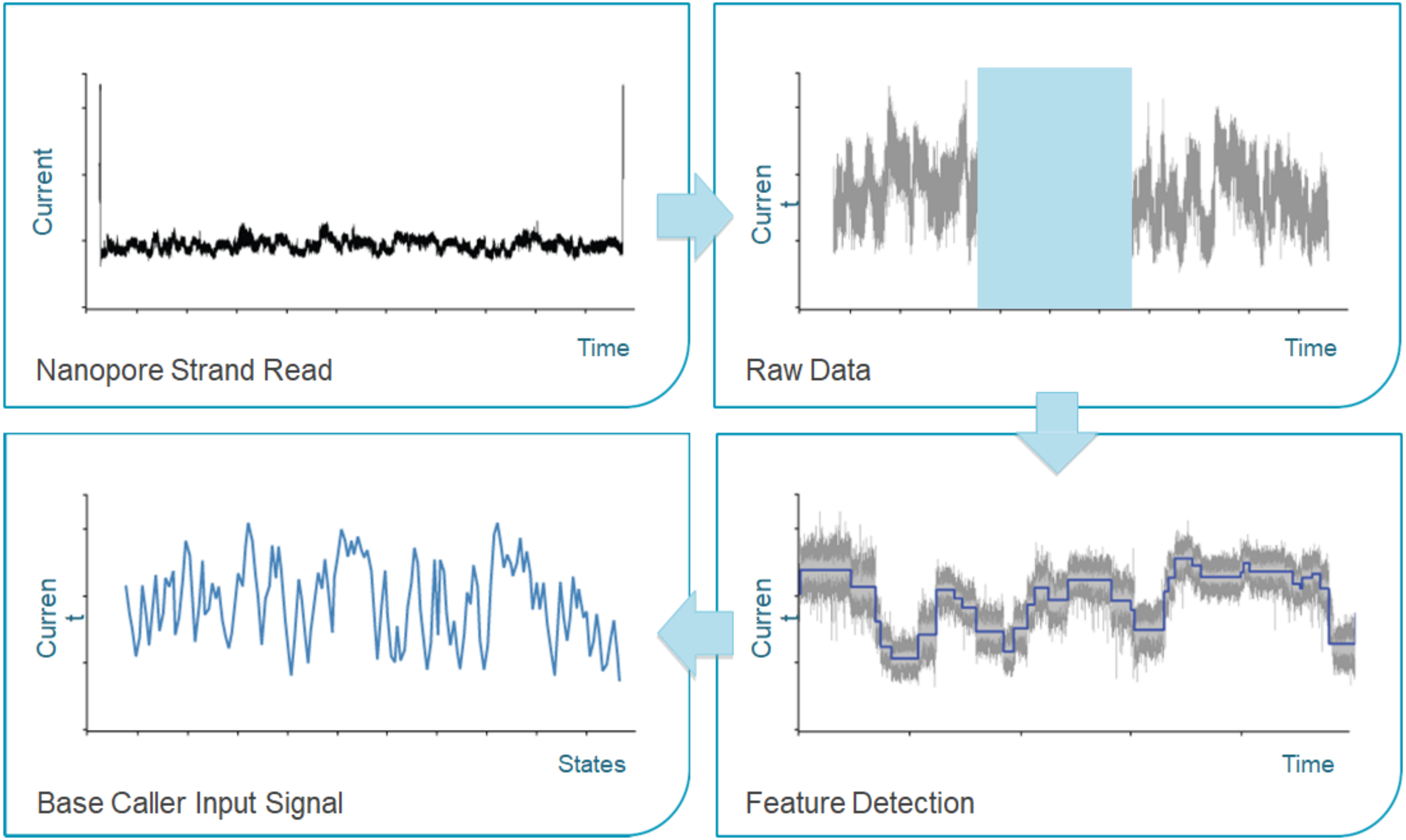
Ionic current flows through the pore Introduce analyte of interest into the pore

Identify target analyte by the characteristic disruption or block to the electrical current

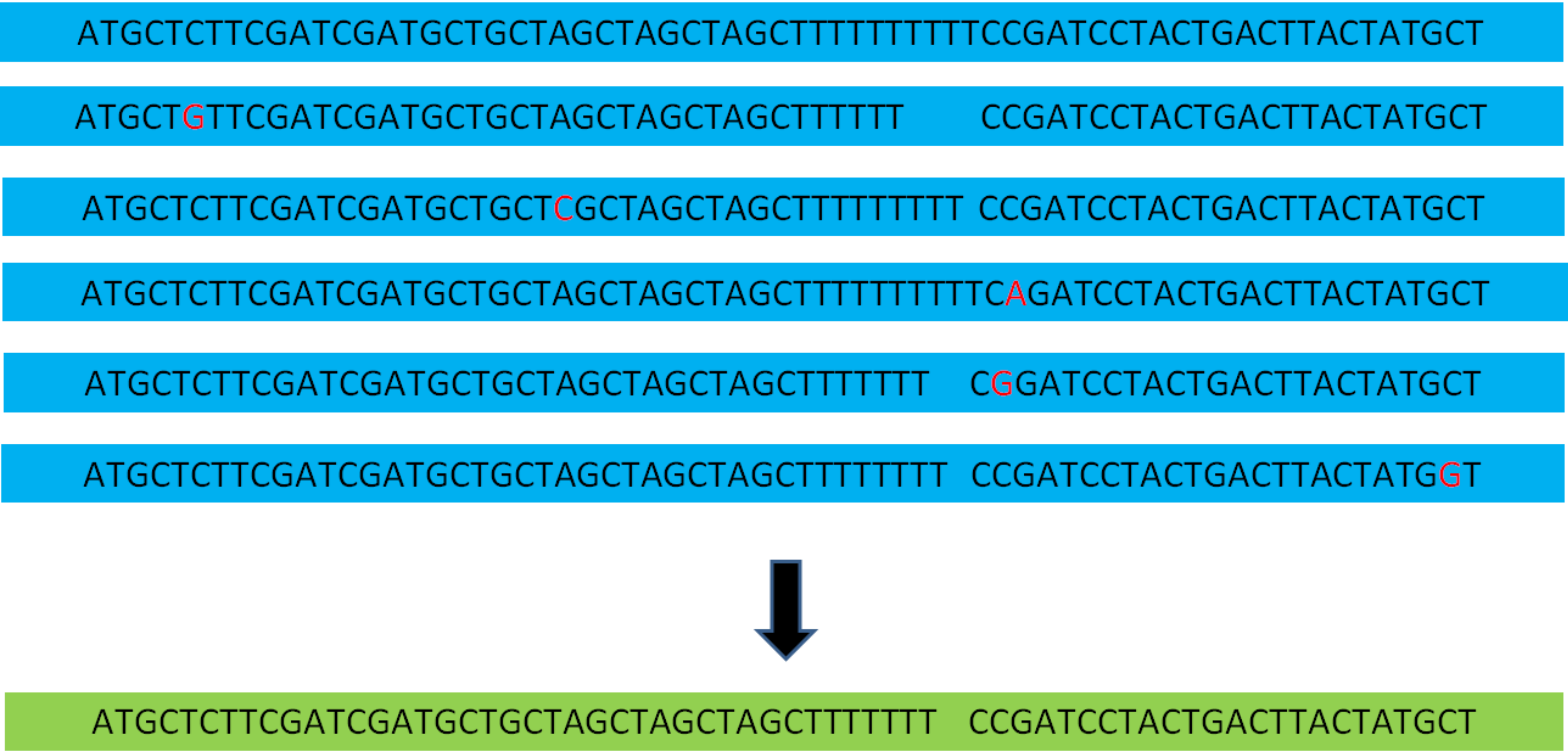
Block or ‘State’, Dwell, Noise



Raw Data and Data Reduction



Nanopore errors are (mostly) randomly distributed



ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTTTTTTTTTTCCGATCCTACTGACTTACTATGCT

ATGCTGTTCGATCGATGCTGCTAGCTAGCTAGCTTTTTT CCGATCCTACTGACTTACTATGCT

ATGCTCTTCGATCGATGCTGCTCGCTAGCTAGCTTTTTTTTT CCGATCCTACTGACTTACTATGCT

ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTTTTTTTTTTCAGATCCTACTGACTTACTATGCT

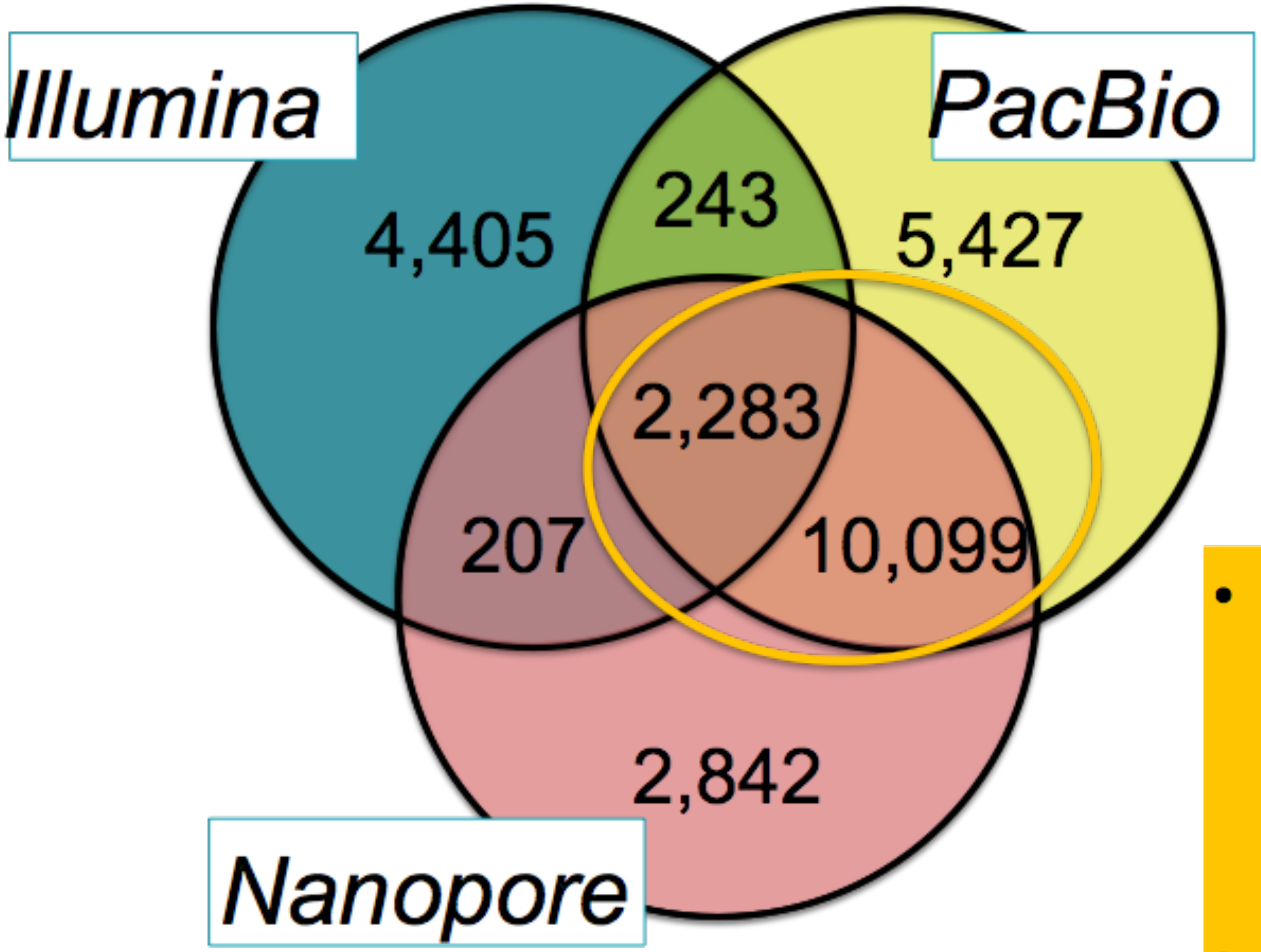
ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTTTTTTT CGGATCCTACTGACTTACTATGCT

ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTTTTTTTT CCGATCCTACTGACTTACTATGGT

ATGCTCTTCGATCGATGCTGCTAGCTAGCTAGCTTTTTTT CCGATCCTACTGACTTACTATGCT

Enough coverage makes error (mostly) drop out

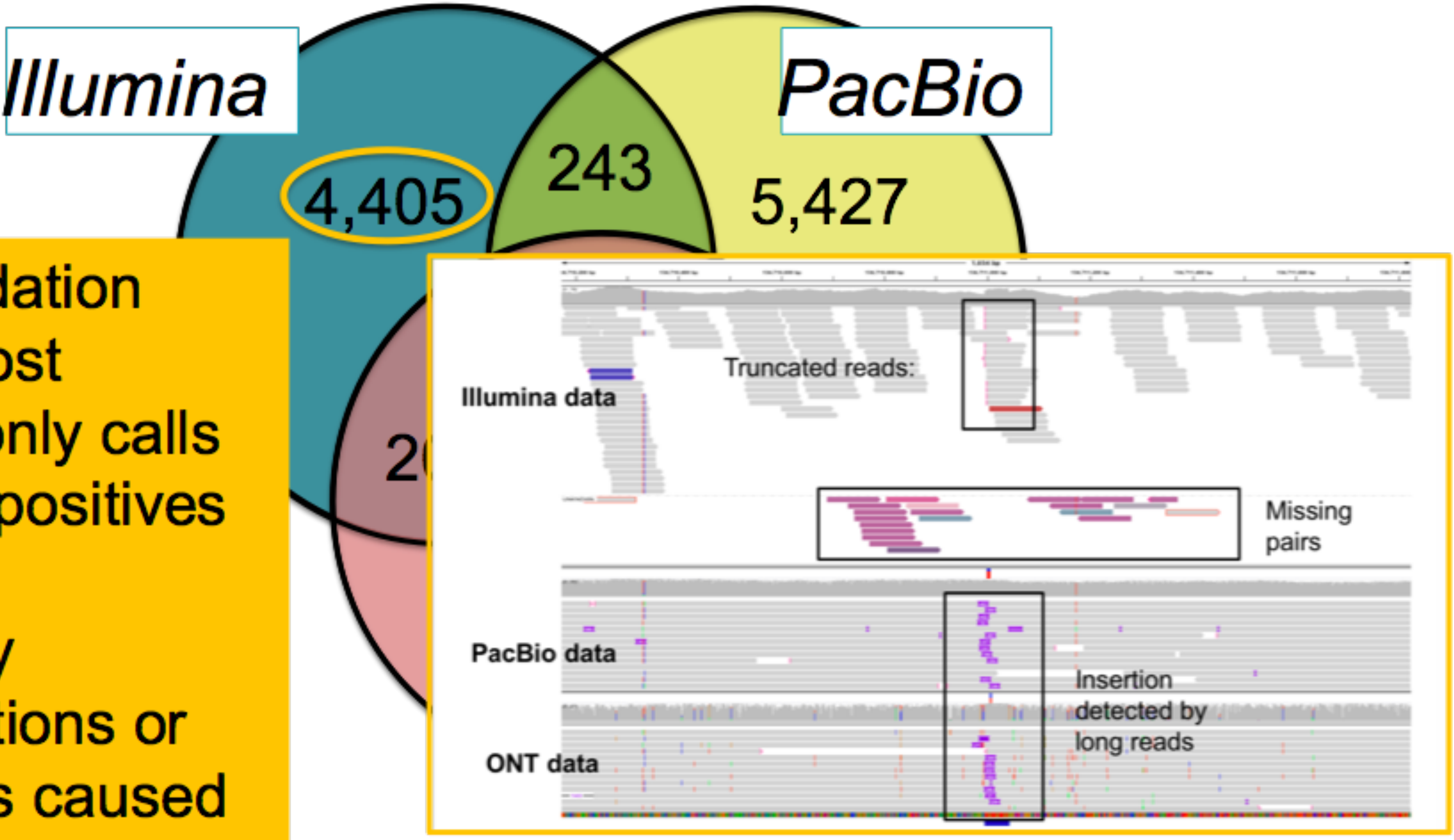
Structural Variant Comparison of SKBR3



(Hicks *et al*, 2006,

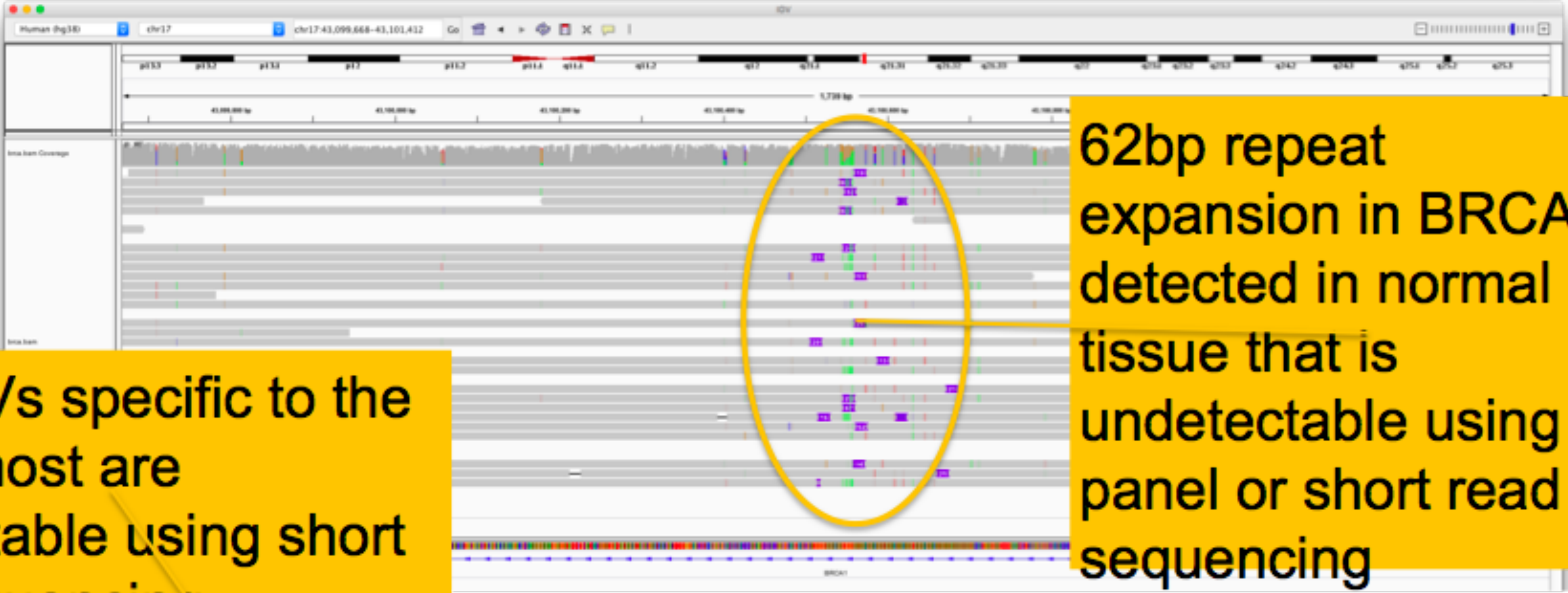
- Strong concordance between long read platforms
- Substantially more variants than detected by short reads

Structural Variant Comparison of SKBR3



- PCR validation shows most Illumina-only calls are false positives
- Especially translocations or inversions caused by smaller insertions or deletions

Preliminary Structural Variations Analysis



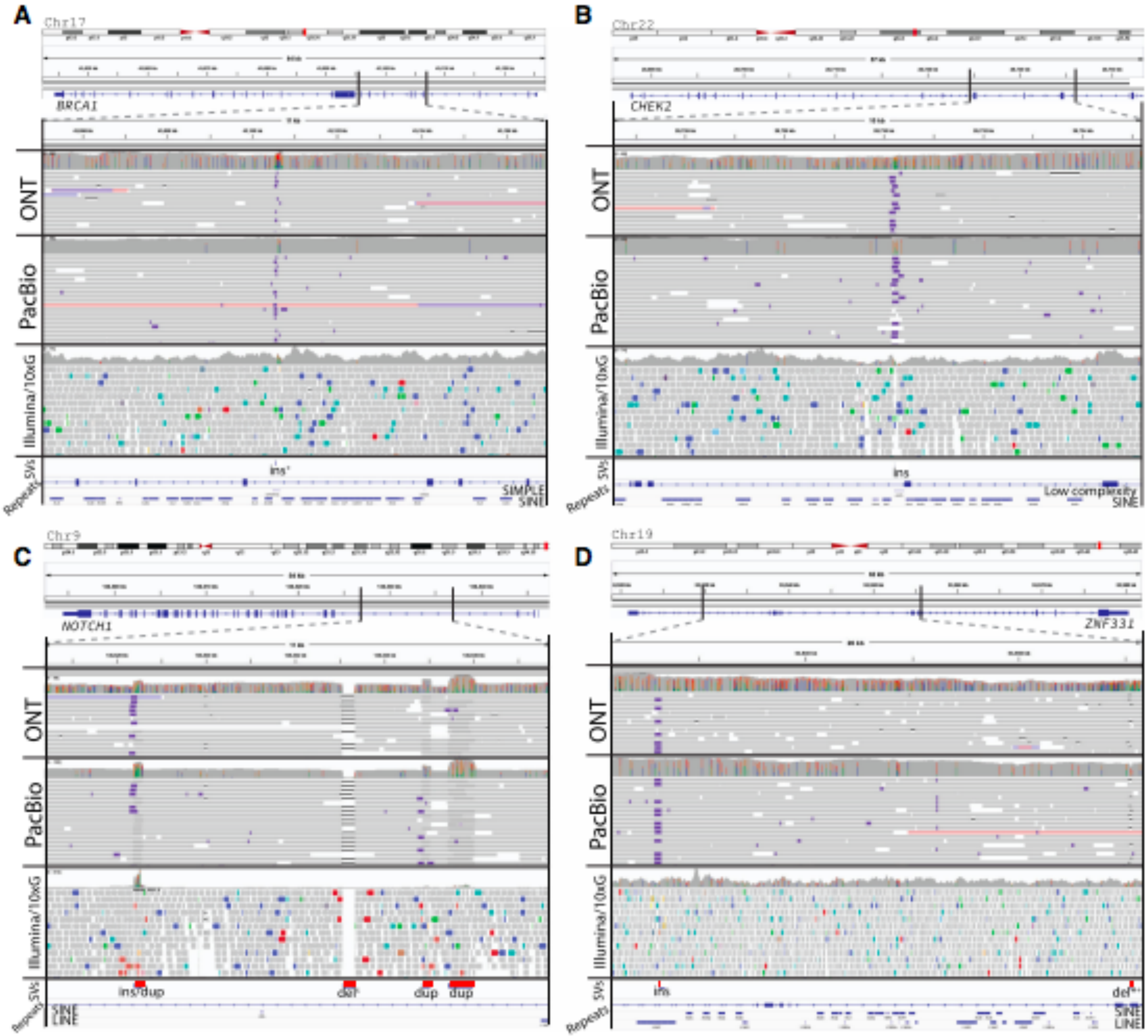
62bp repeat expansion in BRCA1 detected in normal tissue that is undetectable using a panel or short read sequencing

3,662 SVs specific to the tumor, most are undetectable using short read sequencing

	Total	Deletions	Duplications	Insertions	Inversions	Translocations
All SVs in normal	9816	5225	578	3727	130	156
All SVs in tumor	13737	7020	988	5292	202	235
SVs only in tumor (Also exclude NA12878)	3662	1805	420	1250	98	89

SVs in sample 51 not detected by short reads.

Insertions found in BRCA1 and CHEK2. Insertions and duplications found in NOTCH1.



Wollemia nobilis Genome Assembly

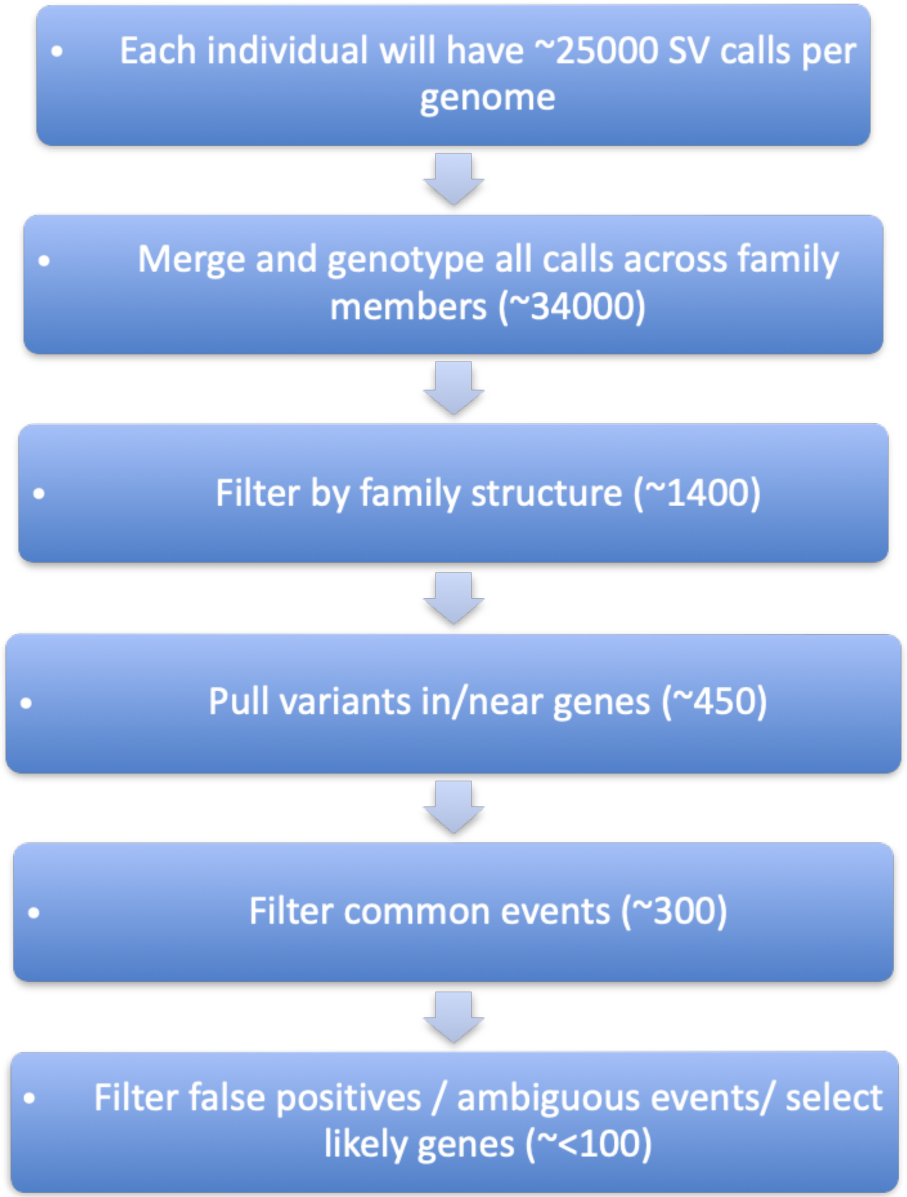
Previous Assembly with GuppyV3 and wtbg2 assembler		Current Assembly with GuppyV4 and Flye assembler	
Genome size	15.6 Gbp	Genome size	11.56 Gbp
No of Contigs	223,812	No of Contigs	17,294
N50 Contig-size	312 Kbp	N50 Contig-size	9.21 Mbp
Max Contig-size	7 Mbp	Max Contig-size	54.83 Mbp
Assembly Quality	Q20 (99%)	Assembly Quality	Q31 (99.9%)

Recently published 25Gb Chinese pine genome:
contig N50 of 2.6 Mb

Niu et al 2022 Cell
https://doi.org/10.1016/j.cell.2021.12.006

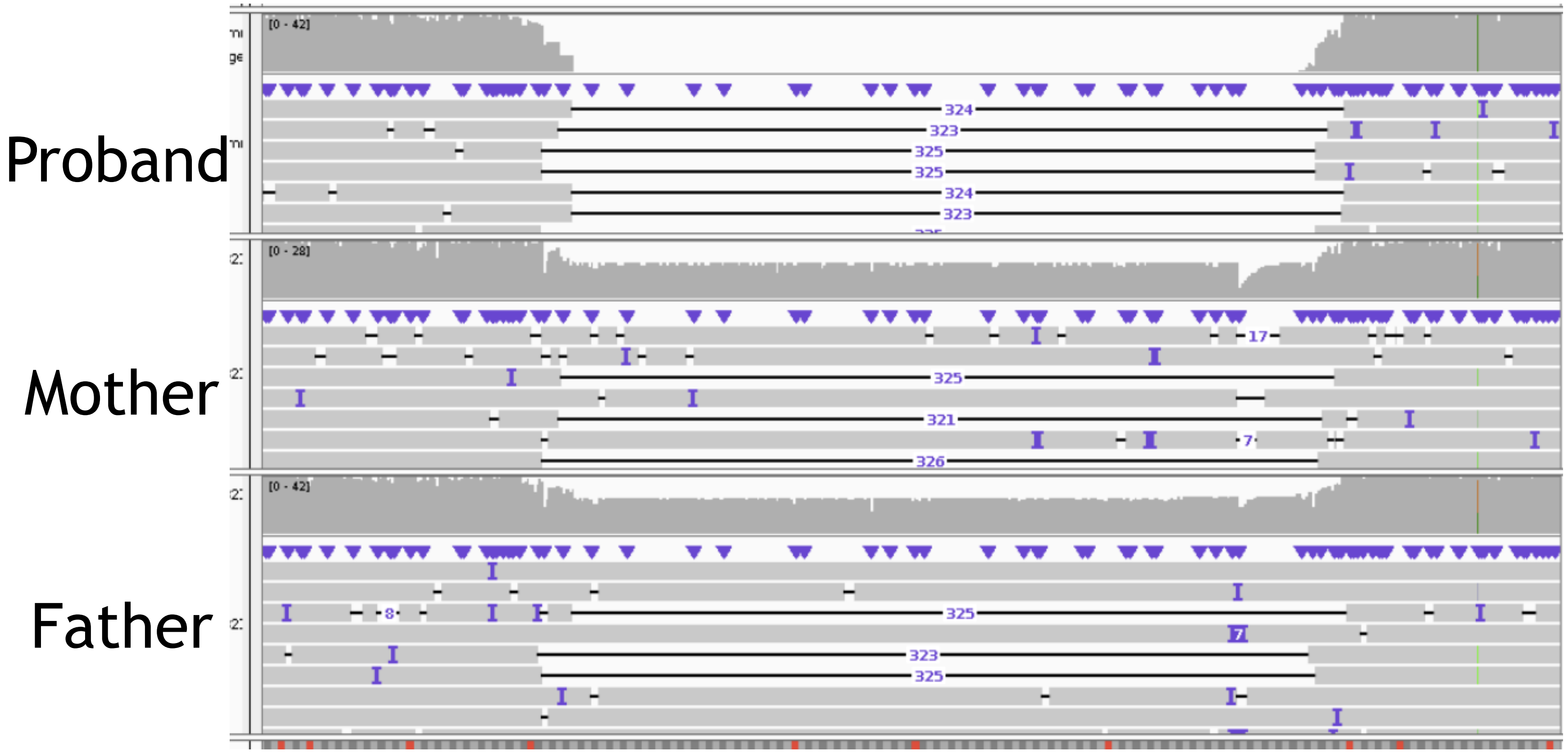
Long Read Sequencing of Early Onset Cancer Pedigrees

SV Filtering Workflow



- Each individual will have ~25000 SV calls per genome
- Merge and genotype all calls across family members (~34000)
- Filter by family structure (~1400)
- Pull variants in/near genes (~450)
- Filter common events (~300)
- Filter false positives / ambiguous events/ select likely genes (~<100)

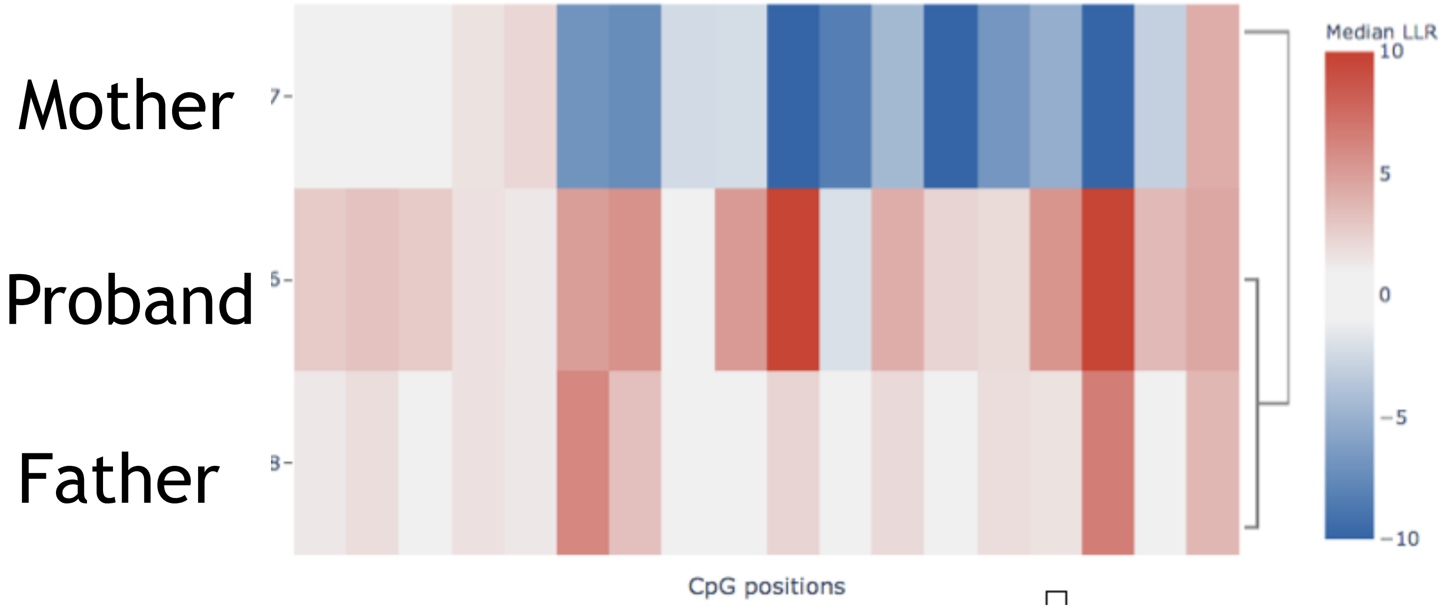
- No family history of cancer
- Standard IMPACT panel did not detect drivers



Homozygous intronic gene deletion in proband, heterozygous in healthy parents

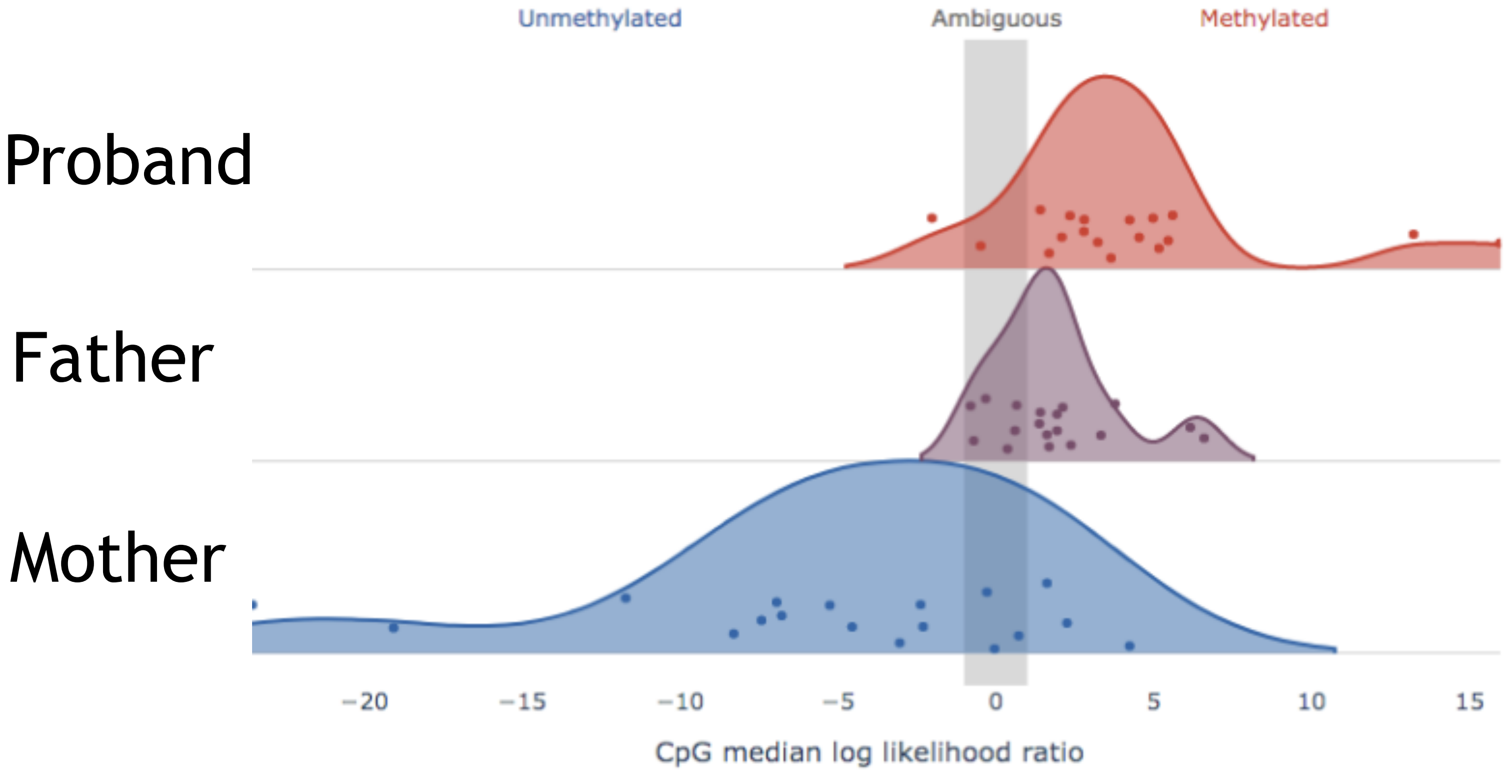
Collaboration with Zsofia Stadler MSKCC

Long Read Sequencing of Early Onset Cancer Pedigrees



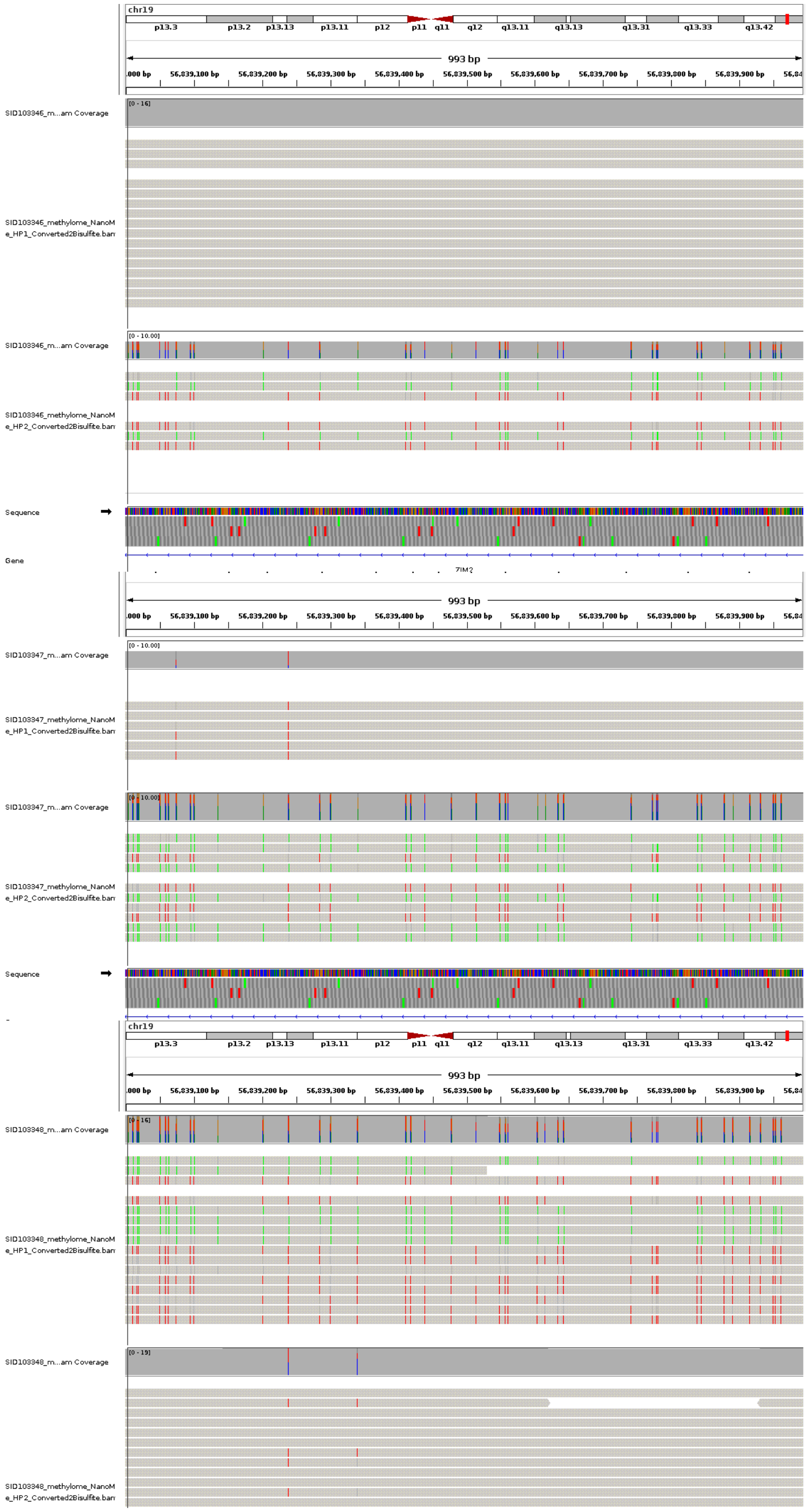
ONT signal data allows for direct detection of methylation state

Hypermethylation of promoter region of tumor suppressor in proband compared to healthy parents



Collaboration with Zsofia Stadler MSKCC

Phasing methylation provides allele specific context


chr19
993 bp
SID103346_m...am Coverage
SID103346_methylome_NanoMe_HP1_Converted2Bisulfite.bam
SID103346_methylome_NanoMe_HP2_Converted2Bisulfite.bam
SID103347_m...am Coverage
SID103347_methylome_NanoMe_HP1_Converted2Bisulfite.bam
SID103347_methylome_NanoMe_HP2_Converted2Bisulfite.bam
SID103348_m...am Coverage
SID103348_methylome_NanoMe_HP1_Converted2Bisulfite.bam
SID103348_methylome_NanoMe_HP2_Converted2Bisulfite.bam
Sequence
Gene
ZIM2

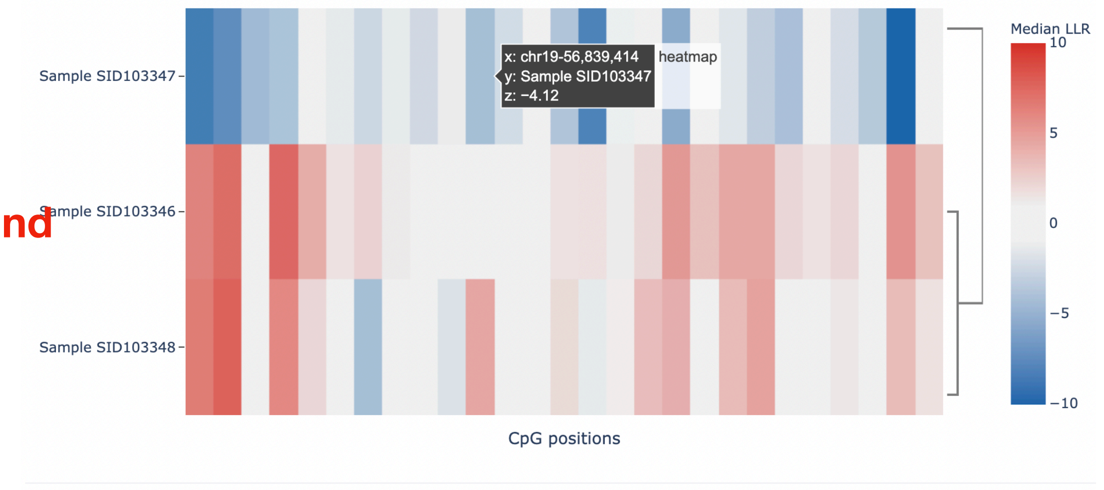
Proband

Unaffected parent

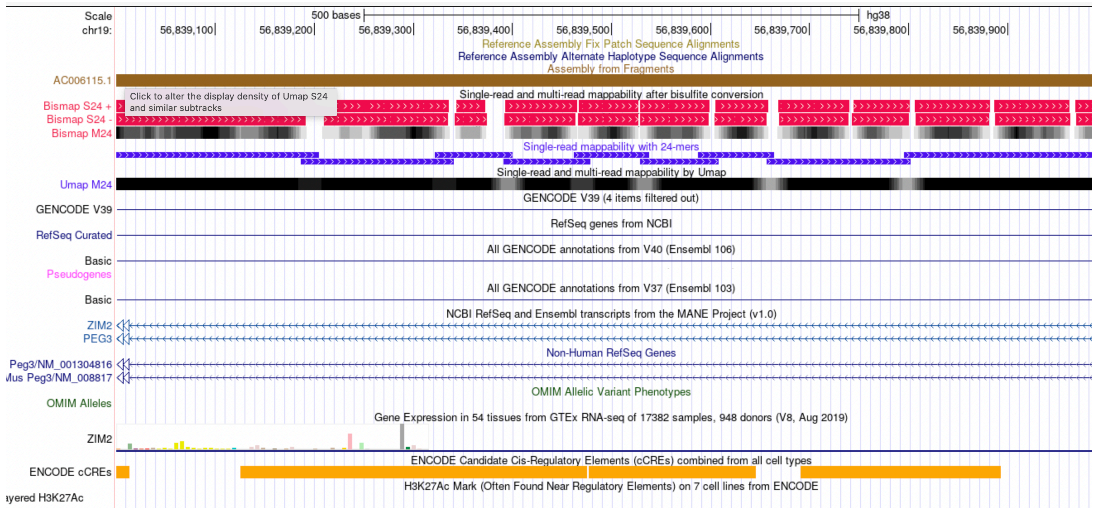
Unaffected parent

IGV showing increased methylation in proband (colored bases are "unprotected" converted bases)

Proband



PEG3 downregulated in colon cancer


Scale
chr19:
500 bases
hg38
56,839,100
56,839,200
56,839,300
56,839,400
56,839,500
56,839,600
56,839,700
56,839,800
56,839,900
Reference Assembly Fix Patch Sequence Alignments
Reference Assembly Alternate Haplotype Sequence Alignments
Assembly from Fragments
AC006115.1
Click to alter the display density of Umap S24 and similar subtracks
Single-read and multi-read mappability after bisulfite conversion
Bismap S24 +
Bismap S24 -
Bismap M24
Single-read mappability with 24-mers
Single-read and multi-read mappability by Umap
Umap M24
GENCODE V39 (4 items filtered out)
GENCODE V39
RefSeq genes from NCBI
RefSeq Curated
All GENCODE annotations from V40 (Ensembl 106)
Basic
Pseudogenes
All GENCODE annotations from V37 (Ensembl 103)
Basic
NCBI RefSeq and Ensembl transcripts from the MANE Project (v1.0)
ZIM2
PEG3
Non-Human RefSeq Genes
Peg3/NM_001304816
Mus Peg3/NM_008817
OMIM Allelic Variant Phenotypes
OMIM Alleles
Gene Expression in 54 tissues from GTEx RNA-seq of 17382 samples, 948 donors (V8, Aug 2019)
ZIM2
ENCODE Candidate Cis-Regulatory Elements (cCREs) combined from all cell types
ENCODE cCREs
H3K27Ac Mark (Often Found Near Regulatory Elements) on 7 cell lines from ENCODE
ayered H3K27Ac

Overlaps Enhancer region

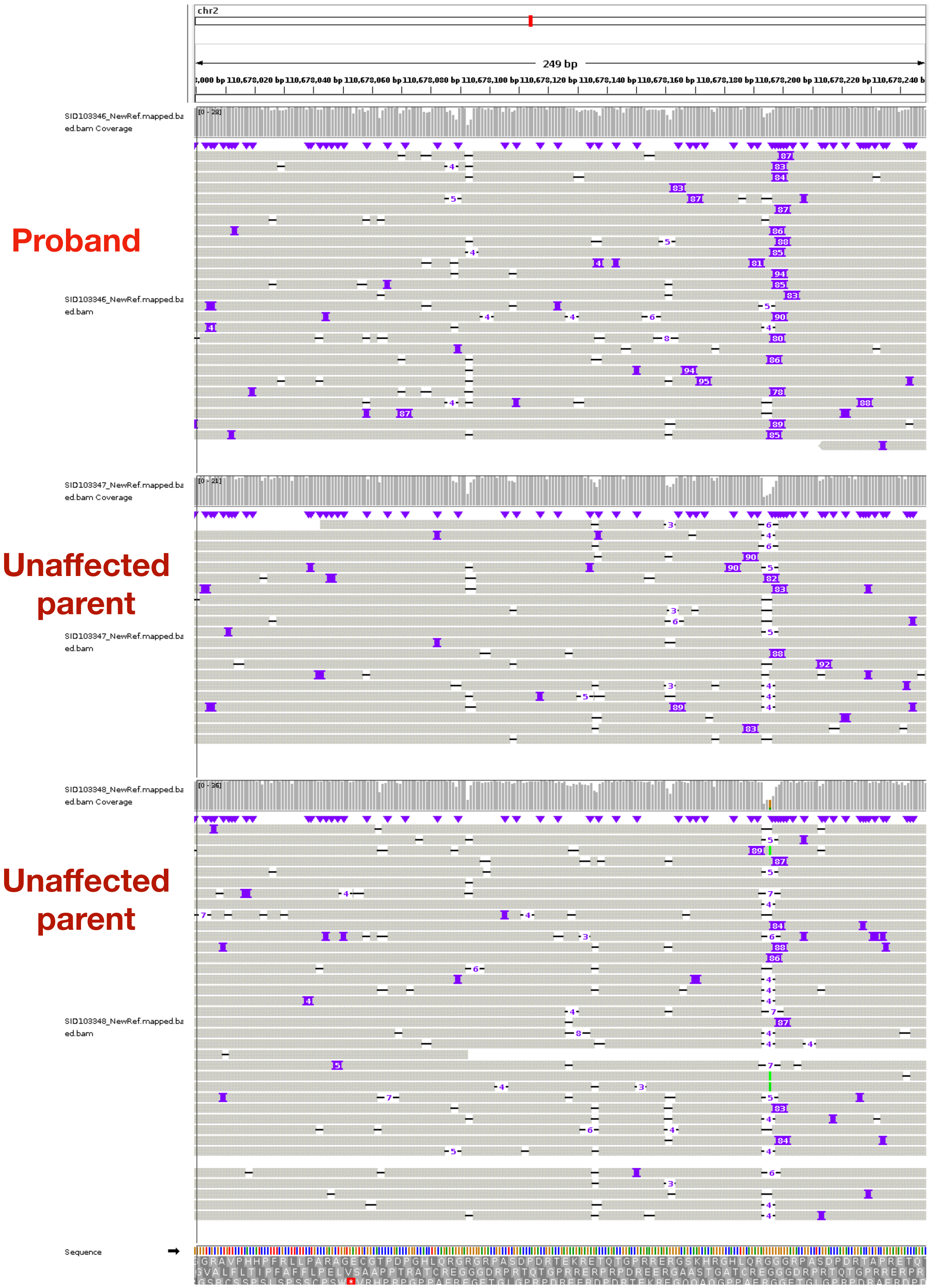
Improved T2T reference genome uncovers new variants

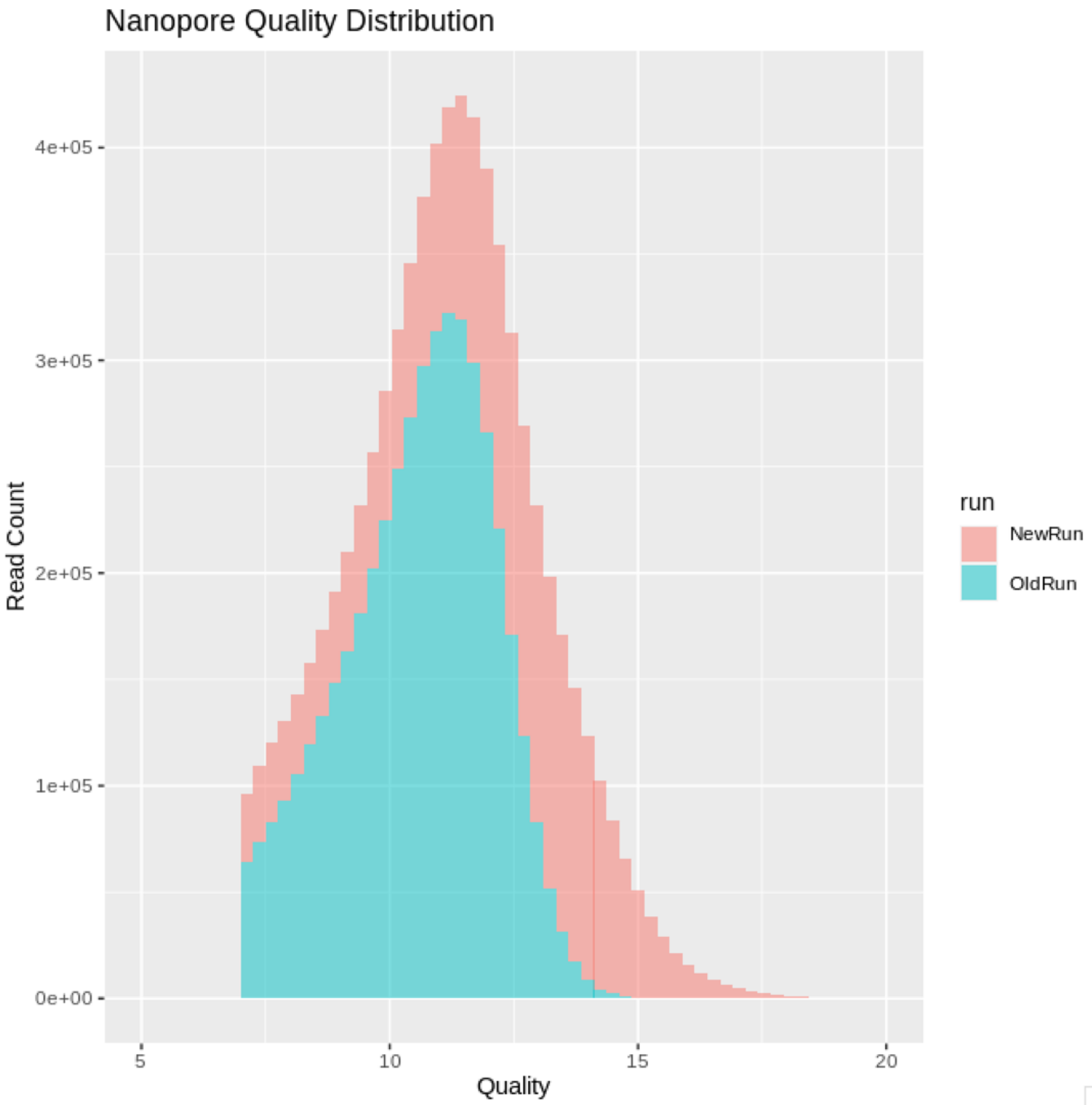
Early Onset ColorectalCancer Trio

Intronic insertion in MALL gene (homozygous in proband)

Region unique to CHM13 compared to hg38

MALL expression is reduced in colon tumor tissue



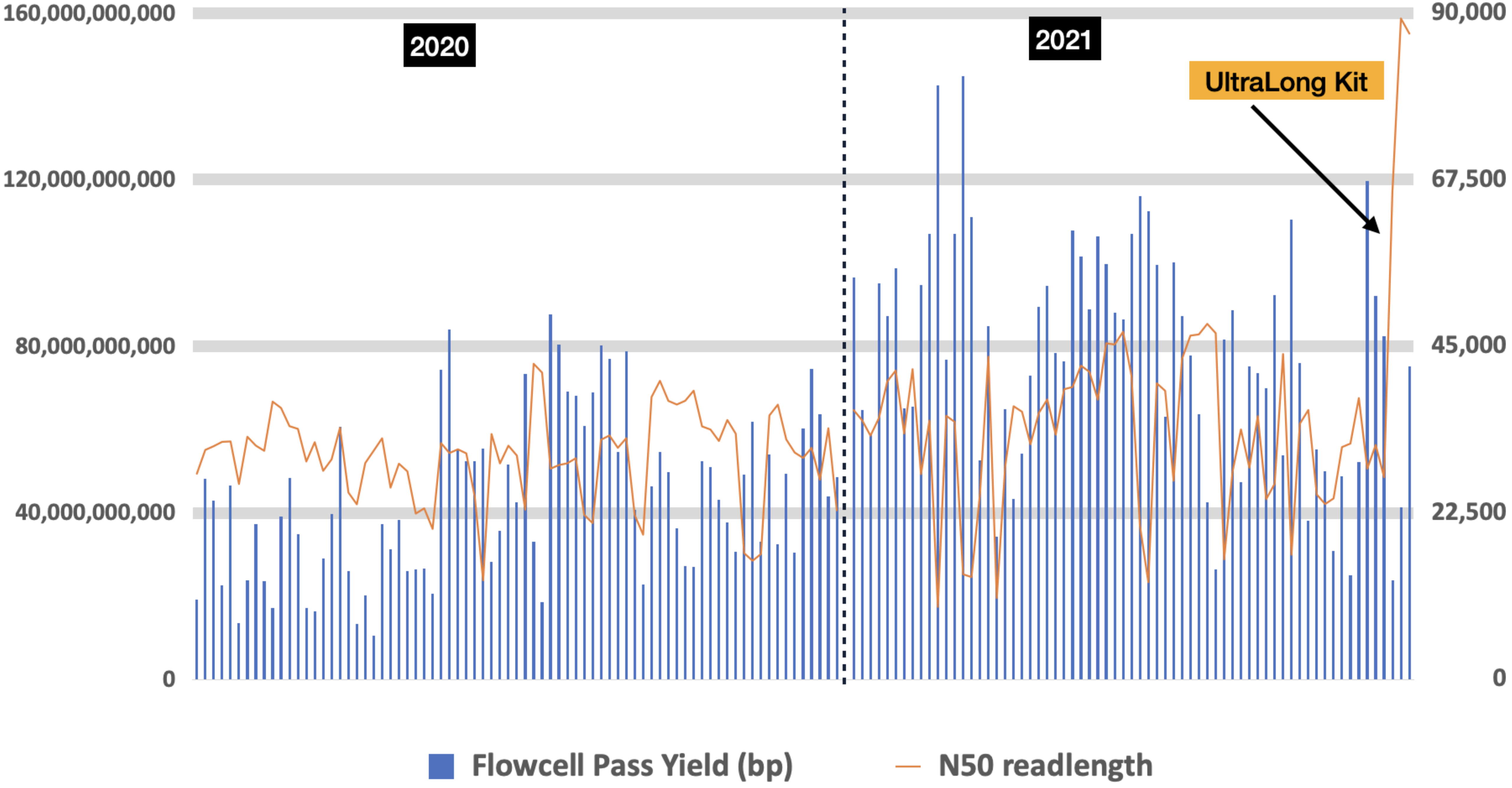


ONT software improvements are increasing base quality

"Guppy V5" sup accuracy model

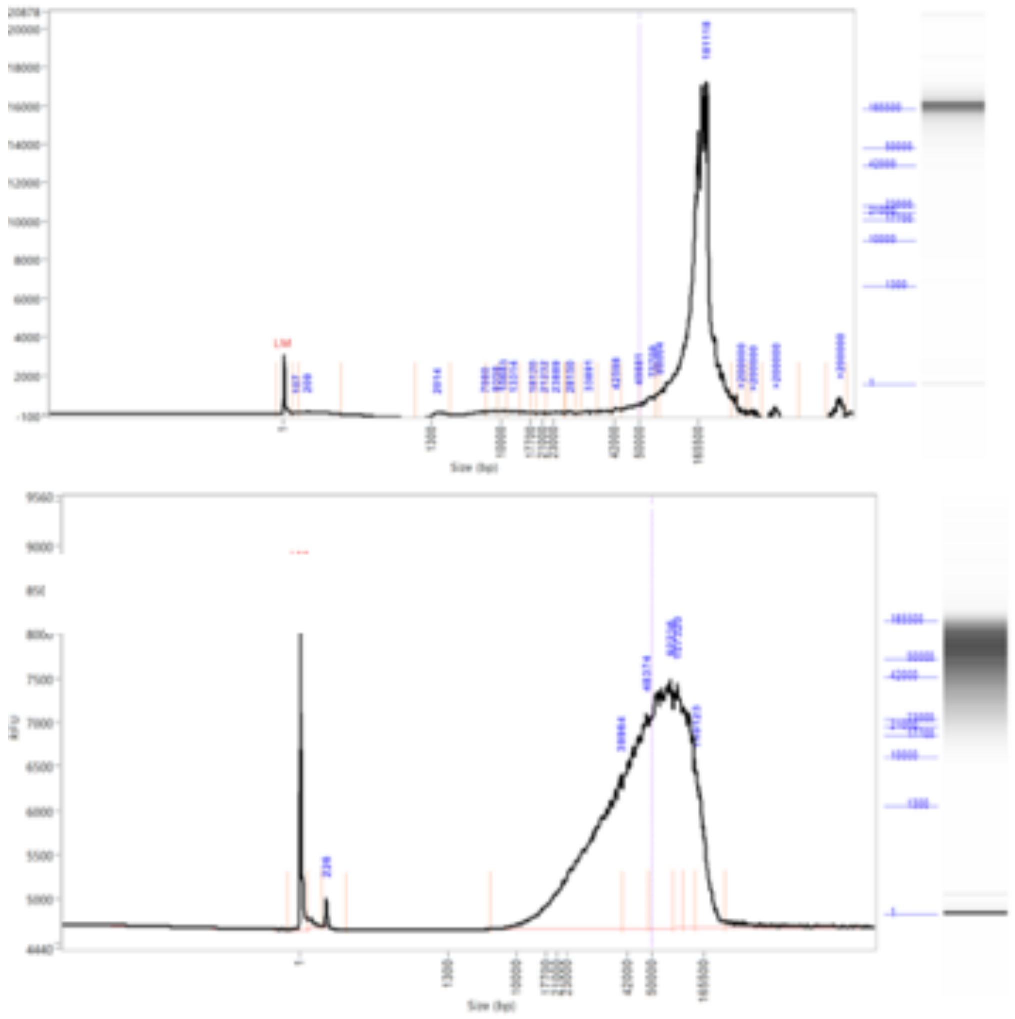
"Guppy V4" high accuracy model

Yield vs N50

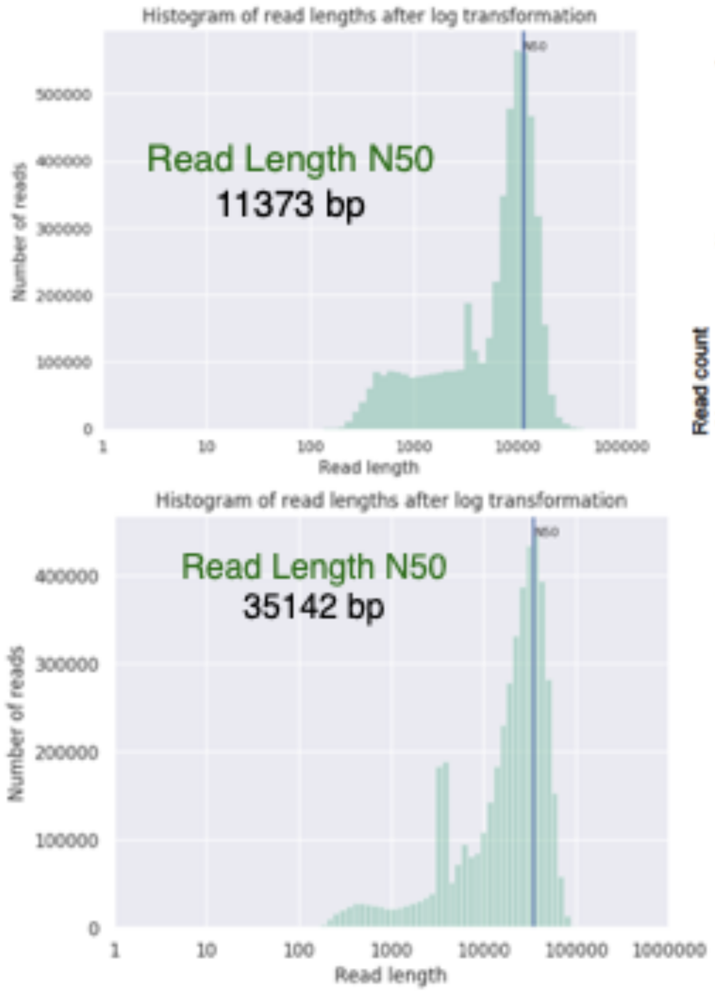

160,000,000,000
120,000,000,000
80,000,000,000
40,000,000,000
0
90,000
67,500
45,000
22,500
0
2020
2021
UltraLong Kit
Flowcell Pass Yield (bp)
N50 readlength

Optimization of long read sequencing on the PromethION

Femto Pulse Fragment Size Estimations before and after protocol adjustments for shearing and application of SRE



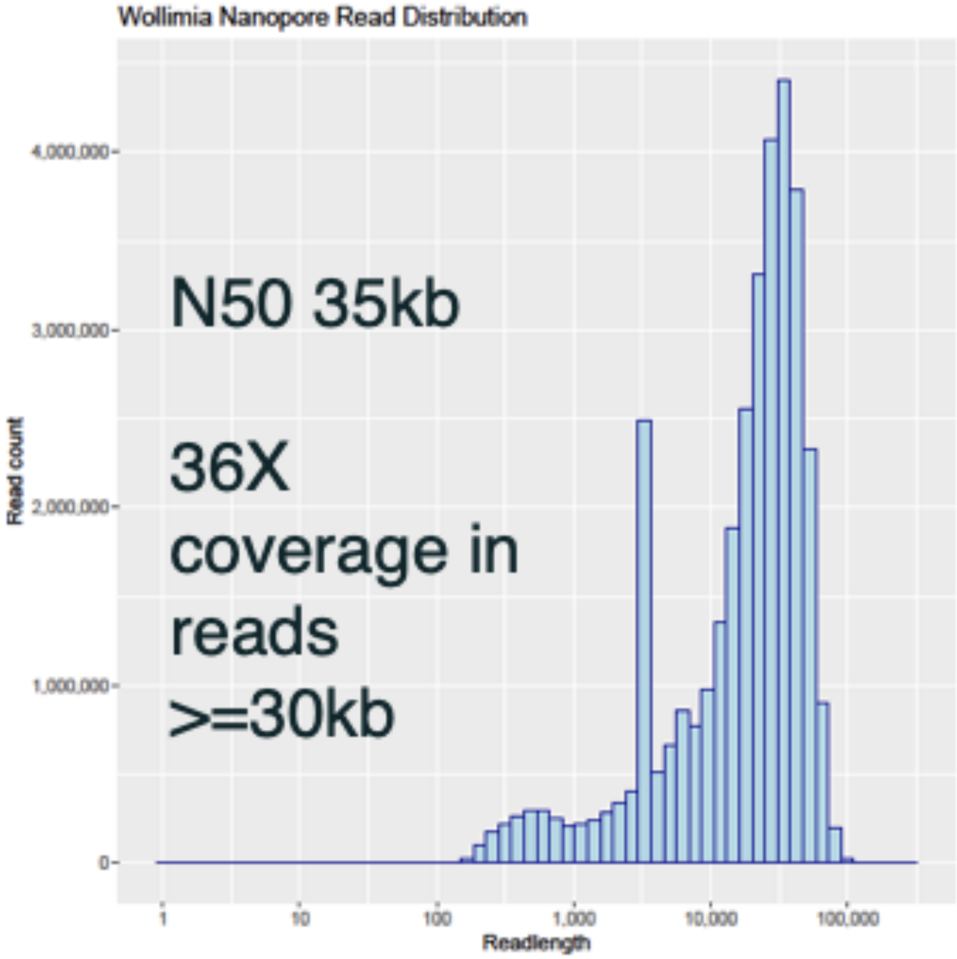
Read Length Distributions before and after protocol adjustments for shearing and application of SRE



Read Length N50
11373 bp

Read Length N50
35142 bp

Final ONT read length distribution

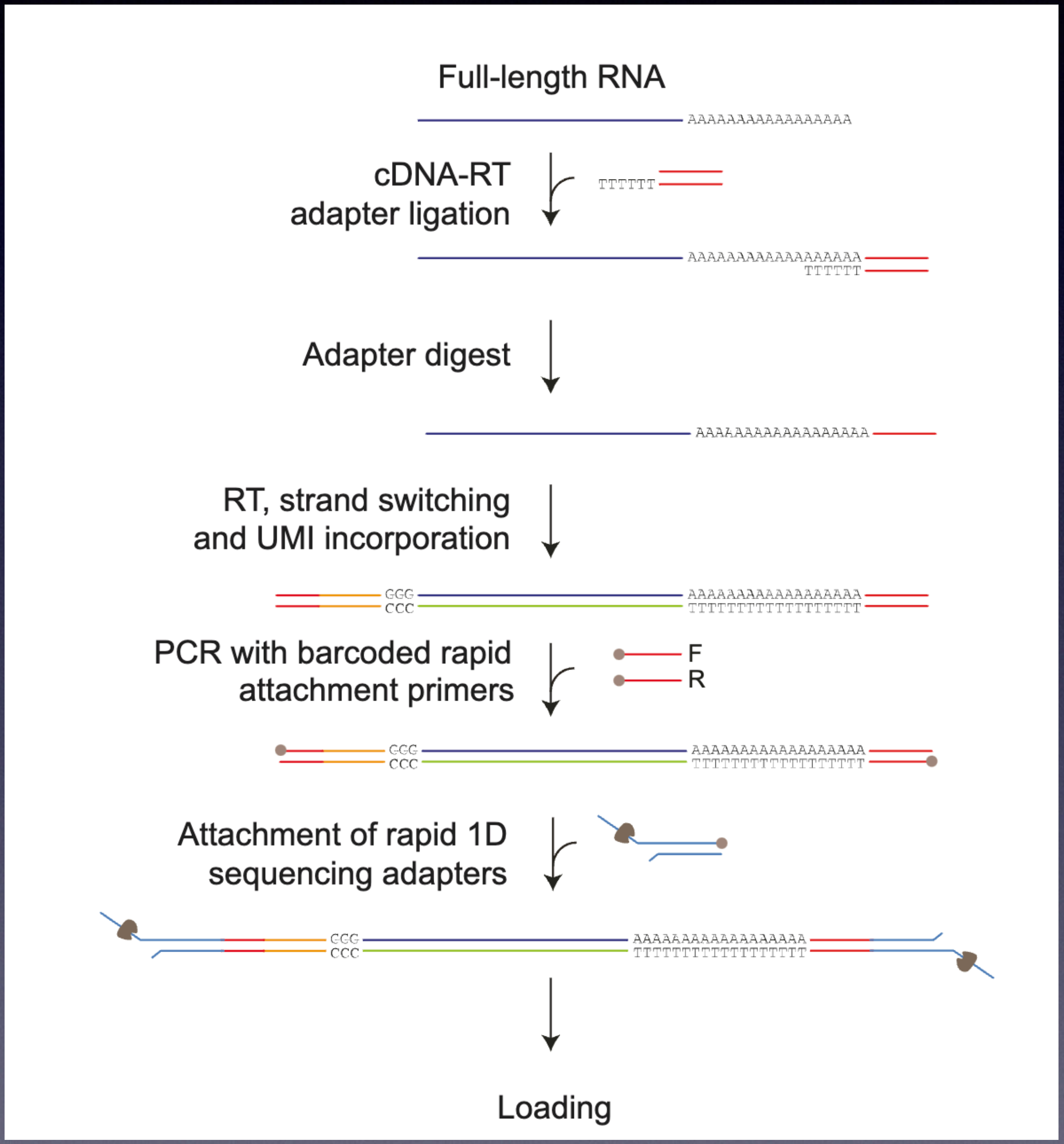


N50 35kb

36X coverage in reads >=30kb

Transcriptome Sequencing on Oxford Nanopore

ONT PCR cDNA



Low input (~1ng poly A+)

PCR may introduce biases

Enriched for full length cDNA (template switching)

Multiplex up to 24 samples

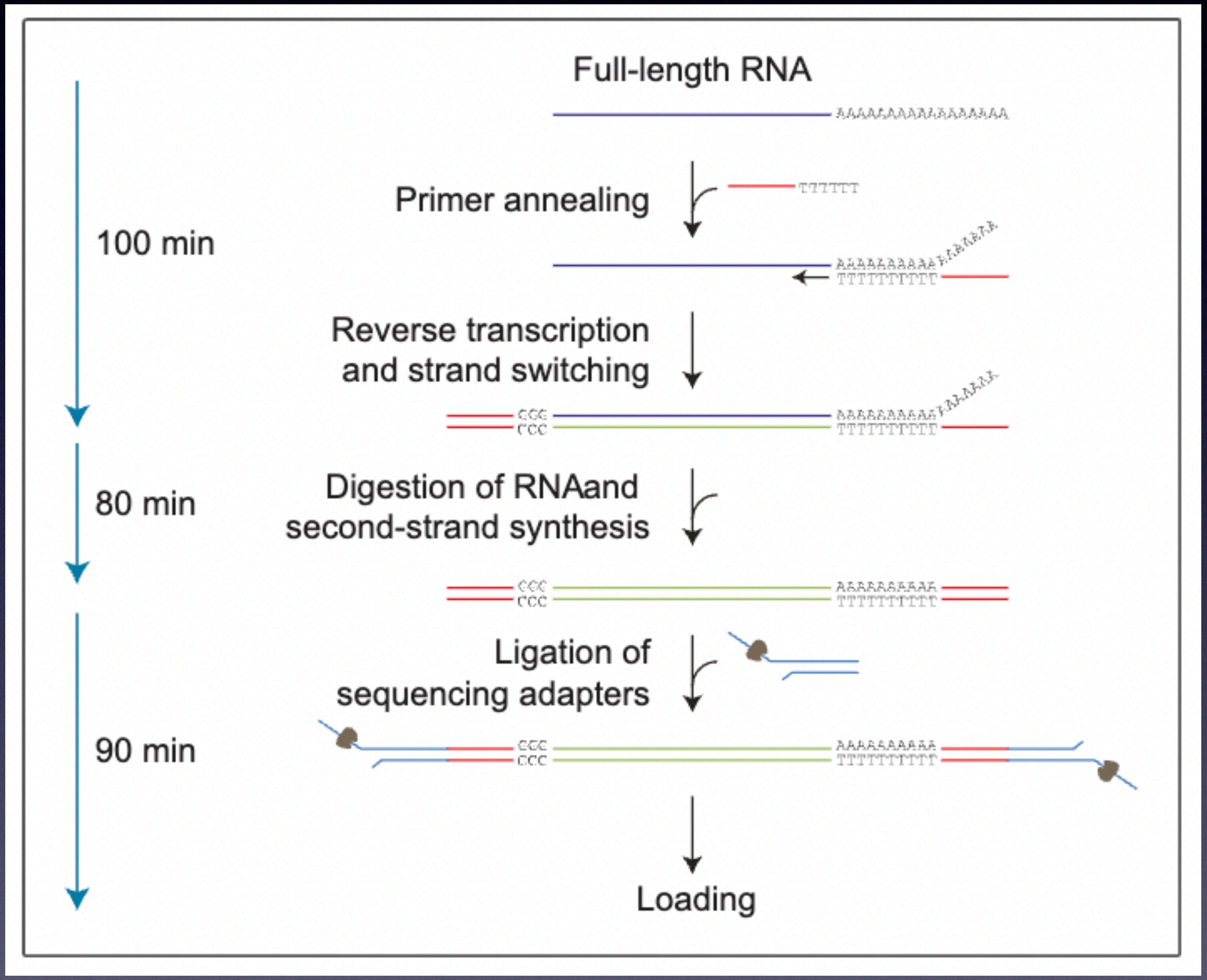
Much higher throughput (>60 million reads per PromethION cell, up to ~180M)

Lengths ~700bp

Recent paper shows 40 fold fewer long reads/8 fold fewer bases required to cover 6000 genes across 95%

Oikonomopoulos S, Bayega A, Fahiminiya S, Djambazian H, Berube P, Ragoussis J. Methodologies for Transcript Profiling Using Long-Read Technologies. Front Genet. 2020 Jul 7;11:606.

ONT direct cDNA



Requires more input (~100ng poly A+)

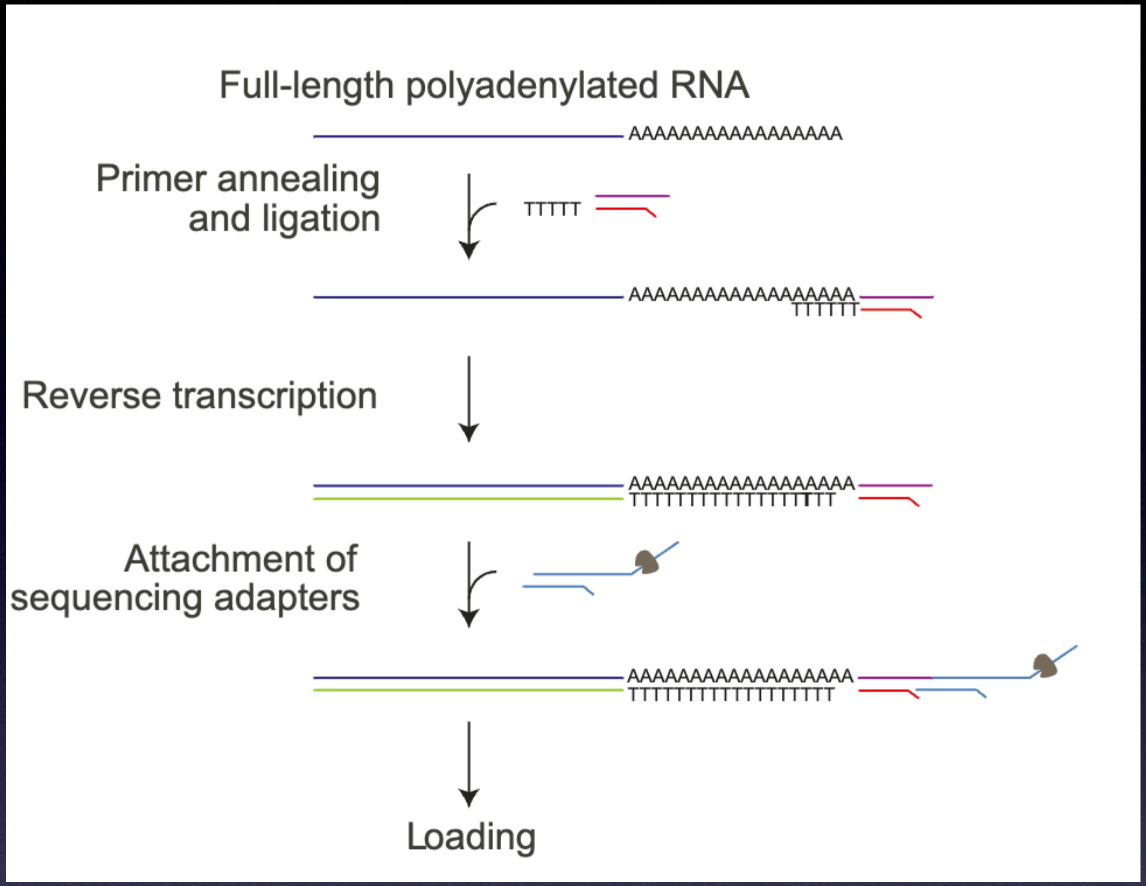
Does not use PCR

Enriched for full length cDNA (template switching)

Can multiplex with native barcoding kit

Less throughput (20-30 million reads per PromethION cell), lengths a bit longer ~1.5kb

ONT direct RNA



Input requirement (500 ng total RNA or 50 ng poly-A+ RNA)

RNA length preserved

No PCR, RT is optional

Can detect base modifications (6mA, 5mC)

Output is much lower, 6-8 million reads on PromethION

Lengths 1.5-2kb

Ribosomal RNA depletion is an issue

Many tools have been/are being developed to use the raw ONT signal data to detect modifications

Tombo (Stoiber et al 2017)
Nanocompore (Leger et al 2021)
xPore (Pratanwanich et al 2021)
nanoRMS (Begik et al 2021)

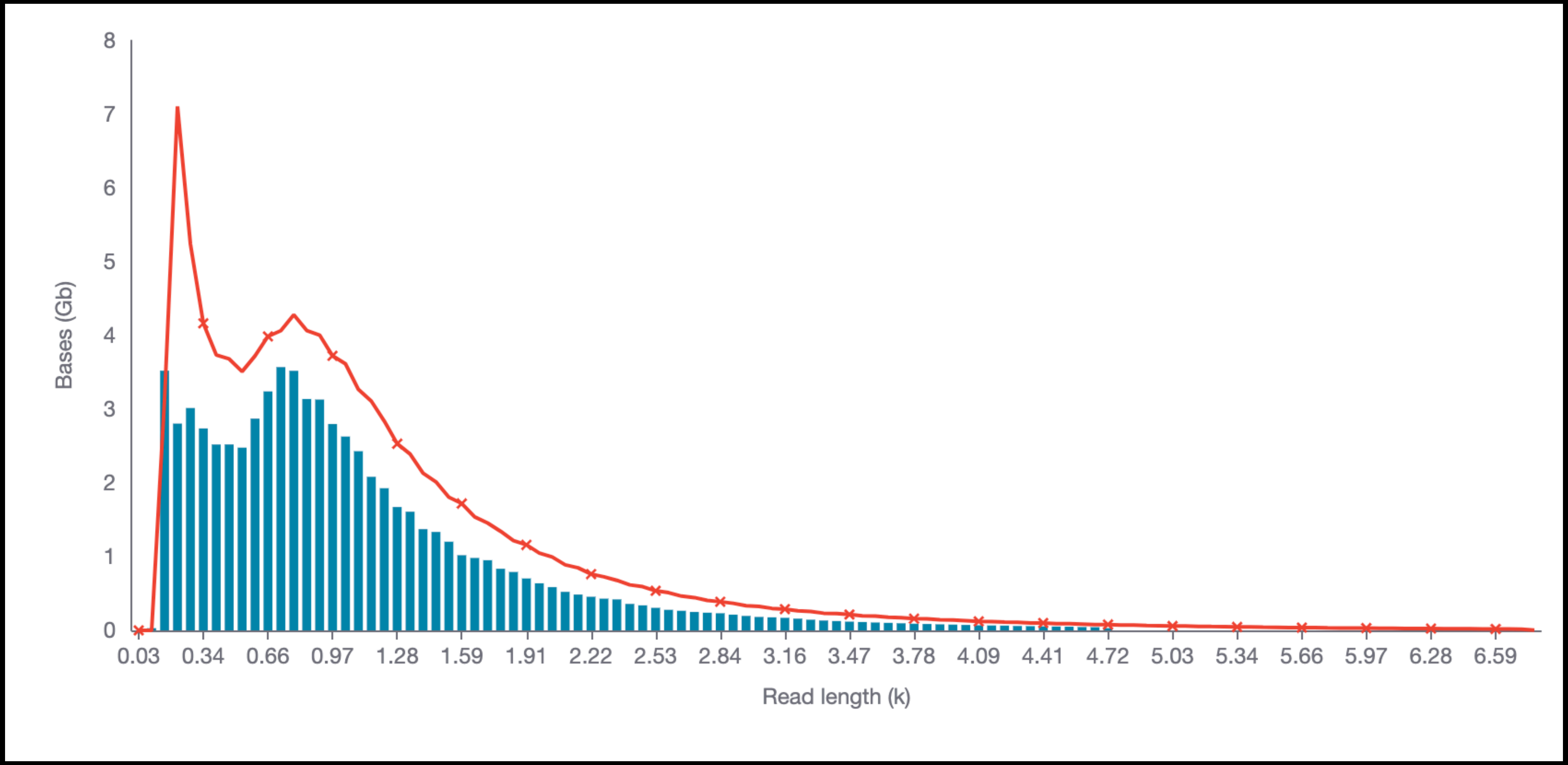
Recent PromethION sequencing metrics

PCR cDNA cell

169 million reads

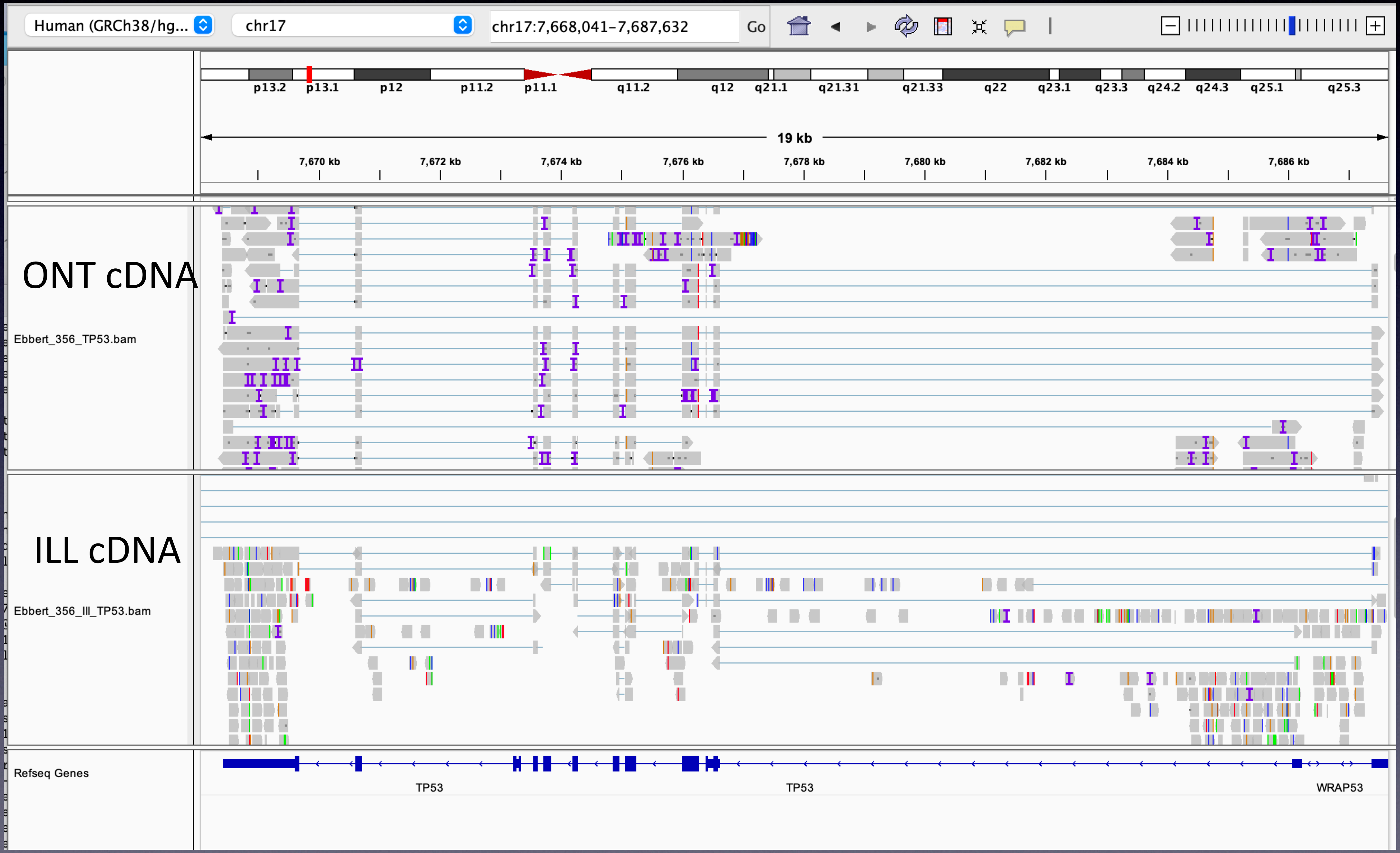
N50 readlength 899bp

108Gb total yield



Average human transcript ~2kb

Long reads span junctions and provide connection



Human (GRCh38/hg...

chr17

chr17:7,668,041-7,687,632

Go

Transcript Coverage

StringTie2 (Kovaka 2019) used to assemble transcripts and detect genes where transcripts were fully covered end to end by reads

Illumina data - 16,476 genes

ONT full data set - 25,478 genes

ONT 50pct downsample - 21,322 genes

ONT 75pct downsample - 19,088 genes

Transcriptome Sequencing Cost Comparisons Across Platforms

Illumina cost per million reads
$6

ONT PCR cDNA cost per million reads
$10

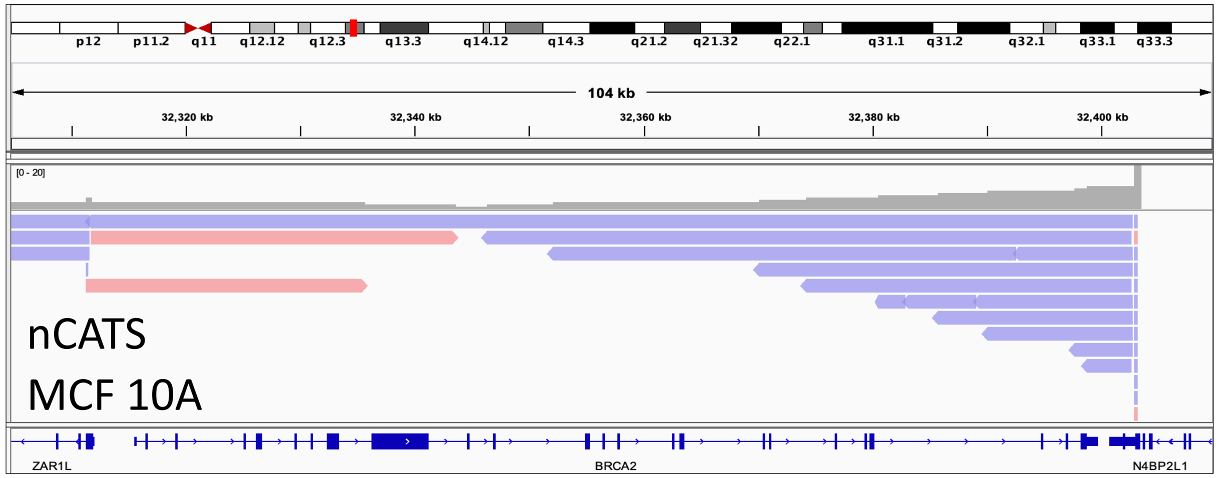
PacBio IsoSeq cost per million reads
$600

Tradeoffs on accuracy and length, so it is key to assess the method that will address critical questions of your experiment

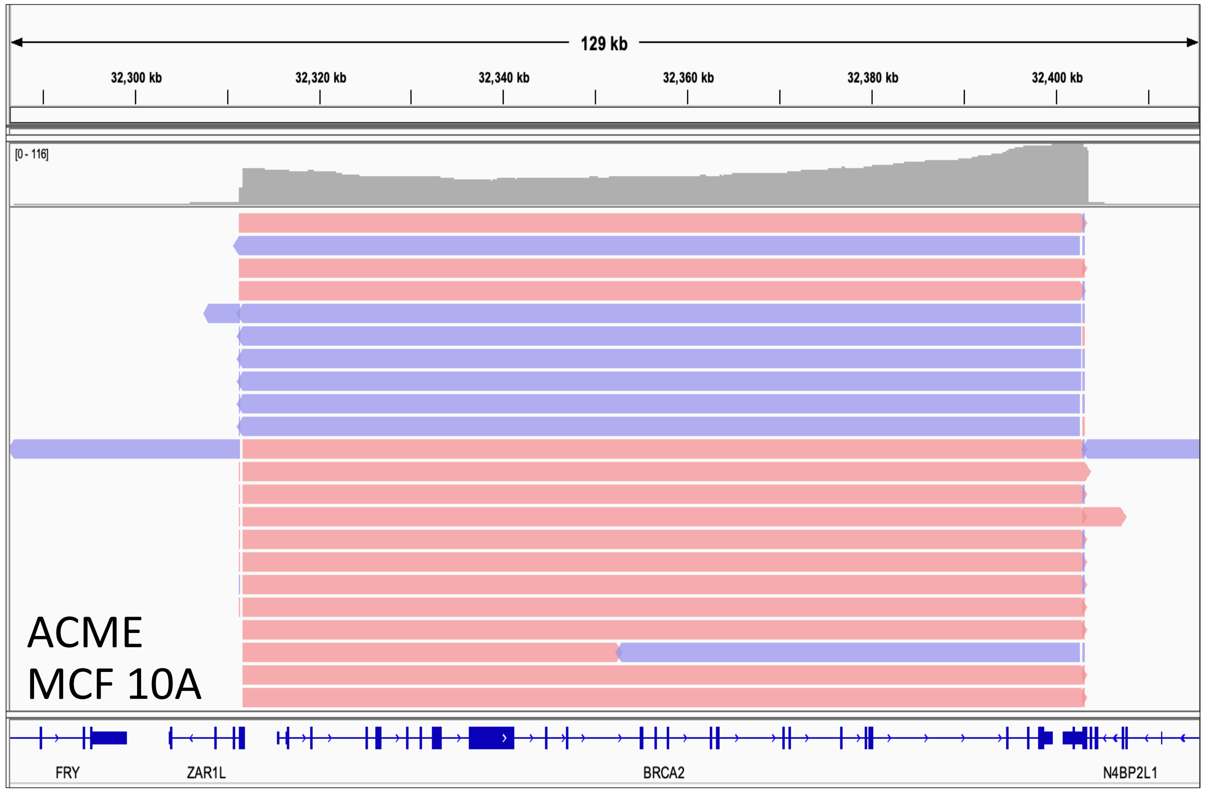
Long Read Sequence Capture - Shruti Iyer

- Original sequence capture with Illumina used hybridization methods to target exomes or other regions of the genome for Illumina sequencing with very short reads (Hodges et al, Nat Gen. 2007)
- Cancer cells within same sample can be heterogenous
- Malignant cells can be as low as 10%
  - Subpopulations exhibit different alleles / genomic features
- Detecting subpopulations difficult with 30x WGS
  - Targeted sequencing, exome capture -200 to 500 fold coverage is possible with Illumina sequencing
  - Relative coverage for same cost is higher
  -

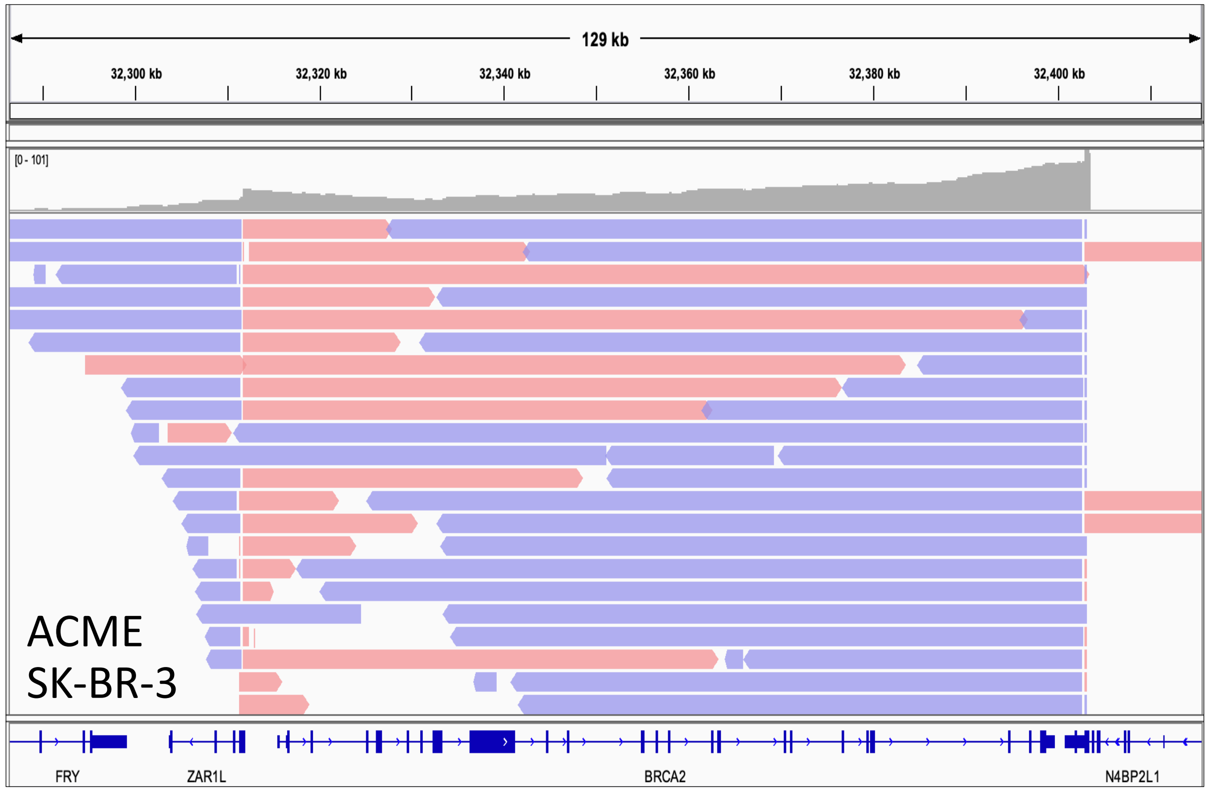
BRCA2 (~90 kb target size)



nCATS
MCF 10A



ACME
MCF 10A



ACME
SK-BR-3

Targeting Sequencing Methods for ONT

Molecular Method

- Enrich for regions of interest from each sample prior to sequencing
- ACME capture, nCATS, PCR

Computational Method

- Utilize “read until” capability to selectively eject molecules from the pore
- Reads are mapped to a reference in real time
- Enrich for targets or deplete unwanted regions

These methods may be used separately or combined for further enrichment

Summary

Long read platforms have matured significantly in the last few years

PacBio and Oxford Nanopore producing similar length distributions

Overcome high error sequencing with improved informatics

Oxford Nanopore exciting for methylation & direct RNA capabilities

Long reads are crucial for accurate SV calling

Finding thousands to tens of thousands of additional SVs over short reads

Resolves the false positives observed with short reads

Detecting potential cancer risk factors that would otherwise go unnoticed

Sample & DNA requirements one of the largest barriers for clinical application

Continue to advance protocols for extracting, preparing samples

Organoids (as opposed to primary tumors) enable large DNA amounts for long read sequencing, though it remains much more difficult then cell culture

Organoids also enable application and profiling of other molecular and pharmaceutical assays

Future goals

Reduce sample DNA input - tumors, single cell, targeting - Shruti Iyer

Analyse data from projects for relevant genome properties

Improve long read sequencing efficiency - read length, yield, combination of input data types

Optimum cost benefit analyses of different long read approaches and coverage

Optimize long read transcriptome sequencing

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