PacBio

# Sequencing by binding (SBB®) delivers unprecedented NGS accuracy

June 8, 2022 | AGBT

Jonas Korlach | CSO

# The last 18 months have been transformational for PacBio

| | |
|---|---|
| Genomes | Foundation for T2T, calling all variants |
| Epigenomes | Simultaneous 5mC calling |
| Transcriptomes | MAS-Iso-Seq |
| Chromatin architecture & dynamics | Fiber-Seq/SAMOSA |
| Metagenomics | Full-length 16S, complete MAGs |
| Gene therapy | Complete AAV sequencing solution |
| SARS-CoV-2 | HiFiViral |

# The last 18 months have been transformational for PacBio

Released new binding kits

Drove DNA input down >5×

Increased average yield >30%

Released new prep kits

Released automated protocols

Released HiFiViral kit


Enabled 5mC calling on instrument

Released AAV workflow

Enabled demultiplex on instrument

Empowered high-throughput processing

Consolidated workflows and protocols

And much more…

# Accuracy matters — it's the hallmark of who we are

| |
|---|
| Human genetics — Neuroscience |
| Human genetics — Immunology |
| Rare + inherited disease research |
| Plant + animal sciences |
| Infectious disease / microbiology |
| Potential for early-stage cancer screening |
| Potential for cancer recurrence monitoring |
| Enabling therapy selection |
| Targeted clinical panels |
| Potential for noninvasive prenatal screening |


## HiFi sequencing

Delivers long reads with the highest accuracy — even in hard-to-sequence regions

## SBB sequencing

Promises significant accuracy improvements over conventional NGS approaches

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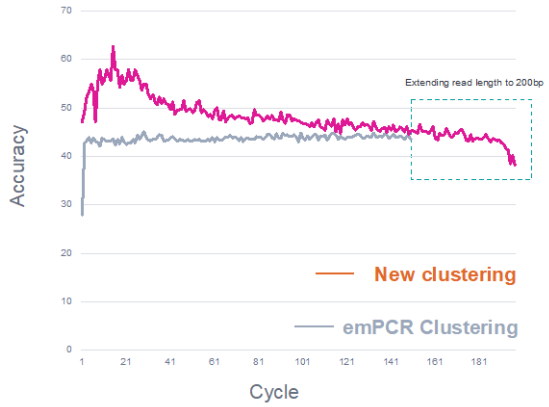
# Going beyond what we shared at JP Morgan

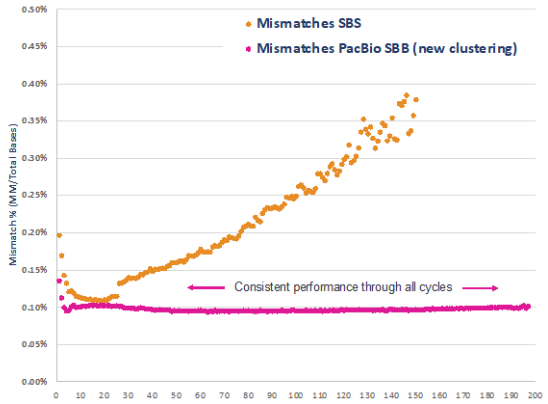
## Implemented novel clustering method to vastly improve SBB™ performance




Even lower error rate than original emulsion PCR method

PacBio Observed quality scores for internal run - benchmark against lambda

## SBB demonstrates greater accuracy & consistency vs current technologies



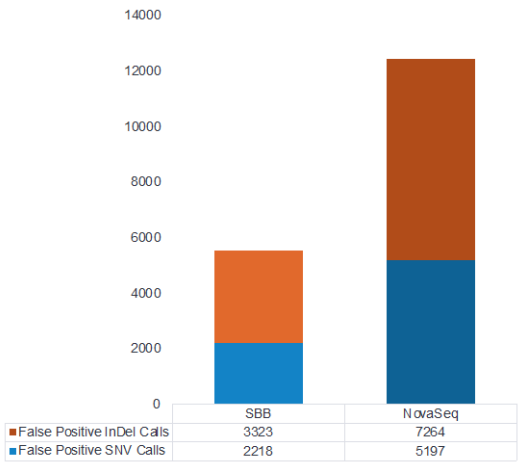

Mismatch rate by cycle

Even lower error rate than original emulsion PCR method

Reaches 0.1% "true variation level" between the sample and reference

PacBio Whole genome sequence of HG002 against HG38 reference
SBS sequencing data derived from Precision FDA Challenge: https://precision.fda.gov/challenges/10

## Higher accuracy translates to lower false positive base calls




| | SBB | NovaSeq |
|---|---|---|
| False Positive InDel Calls | 3323 | 7264 |
| False Positive SNV Calls | 2218 | 5197 |

Even lower error rate than original emulsion PCR method

Reaches 0.1% "true variation level" between the sample and reference

SBB Over 2x lower false positives than SBS

PacBio NovaSeq data from: https://precision.fda.gov/challenges/10

PacBio confidential 7

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# Sequencing by binding (SBB)

1 Technology

2 Benchmark

3 Application

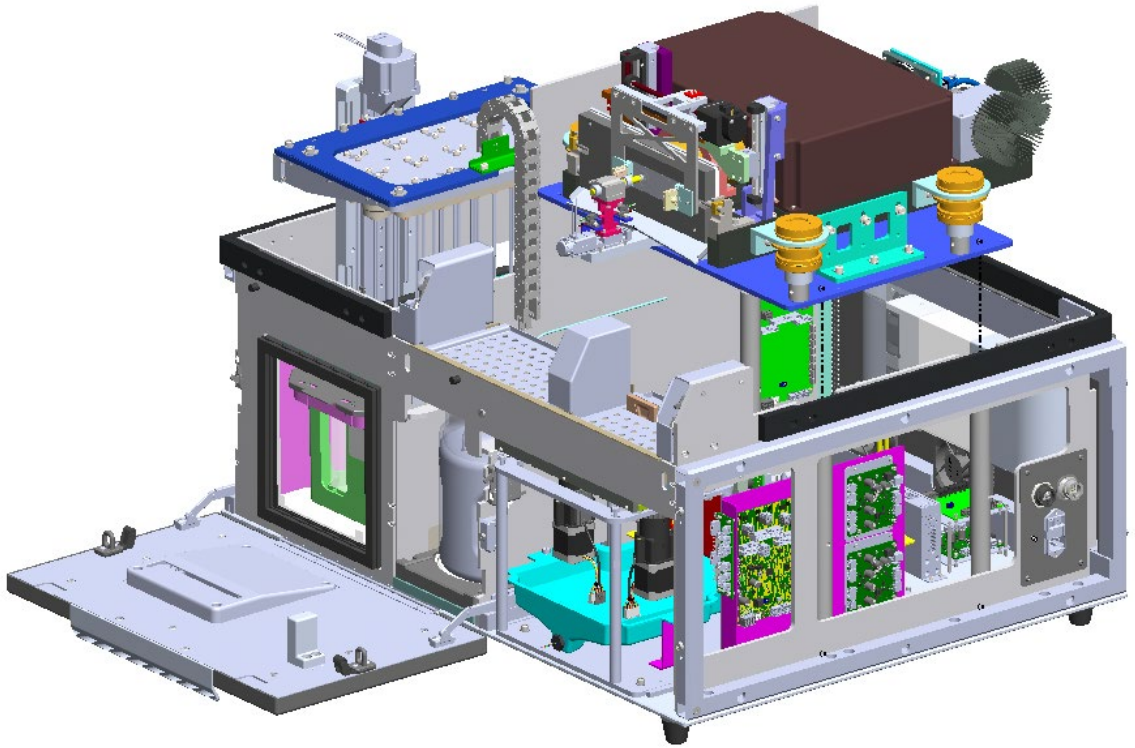
# Developing an innovative platform to house SBB

## Novel and state-of-the-art inventions

**4** core focus areas

**140+** patents pending

**~50** patents allowed/issued



Proprietary chemistry

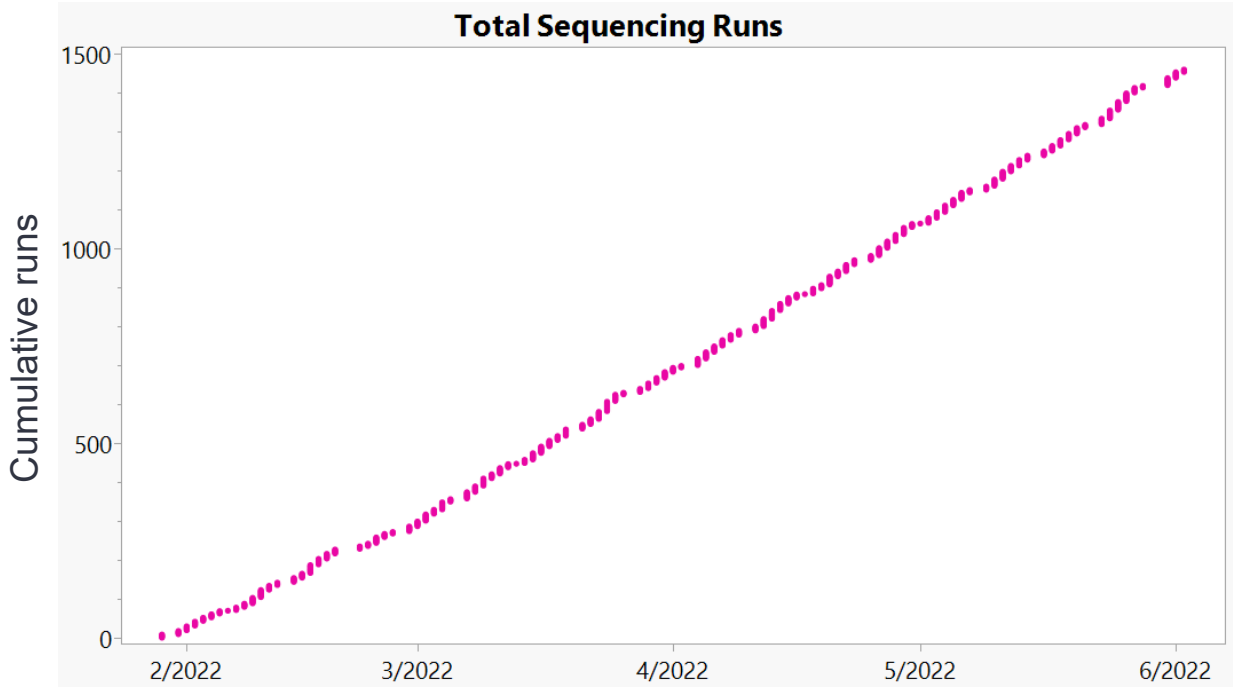
Consumables design

Instrument engineering

Software algorithms

# Sequencing around the clock

>1500 runs completed in 2022 alone




| Success: | 70% | 89% | 92% | 92% | 95% |
|---|---|---|---|---|---|

# Breakthrough short-read sequencing

## Key design principles and goals

- Mid- to high-throughput NGS platform
- Optical and mechanical innovations
- Scalable, flexible, and cost-optimized
- Unparalleled accuracy from SBB

# SBB is fundamentally designed to maximize accuracy

**SBB chemistry separates *interrogation* and *incorporation* steps**

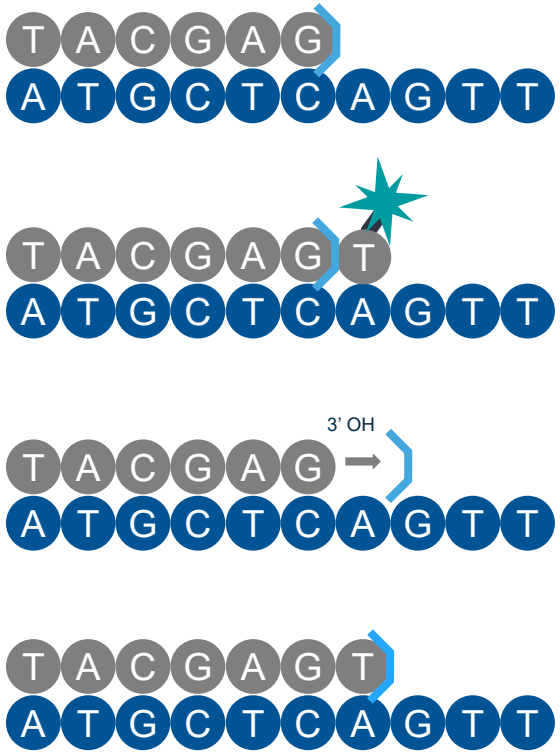
**Multiple optimization points increase accuracy and flexibility**

Blocked 3’ end

Interrogate
Flow nucleotides, image, wash

Activate
Remove 3’ RT

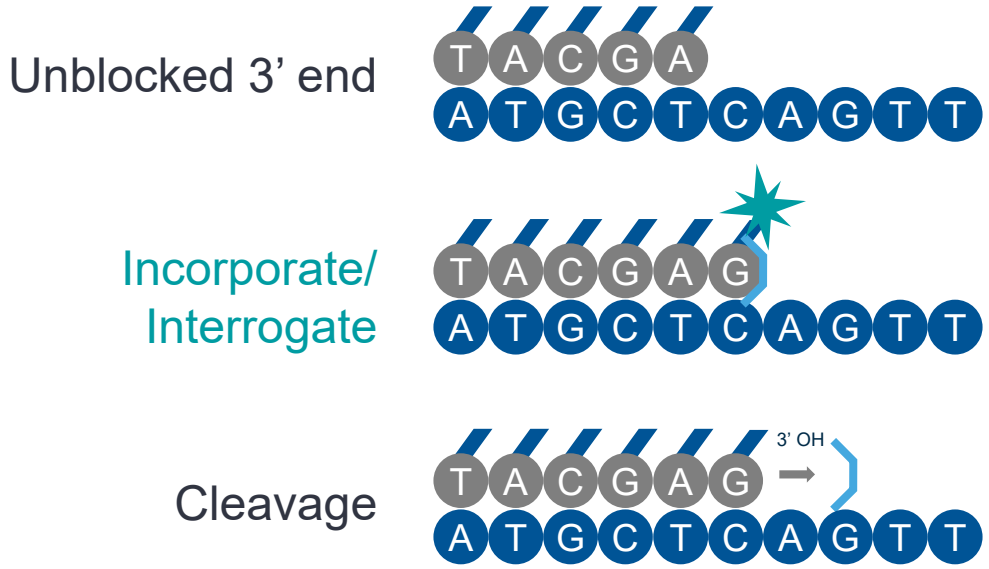
Incorporate
Flow blocked nucleotides



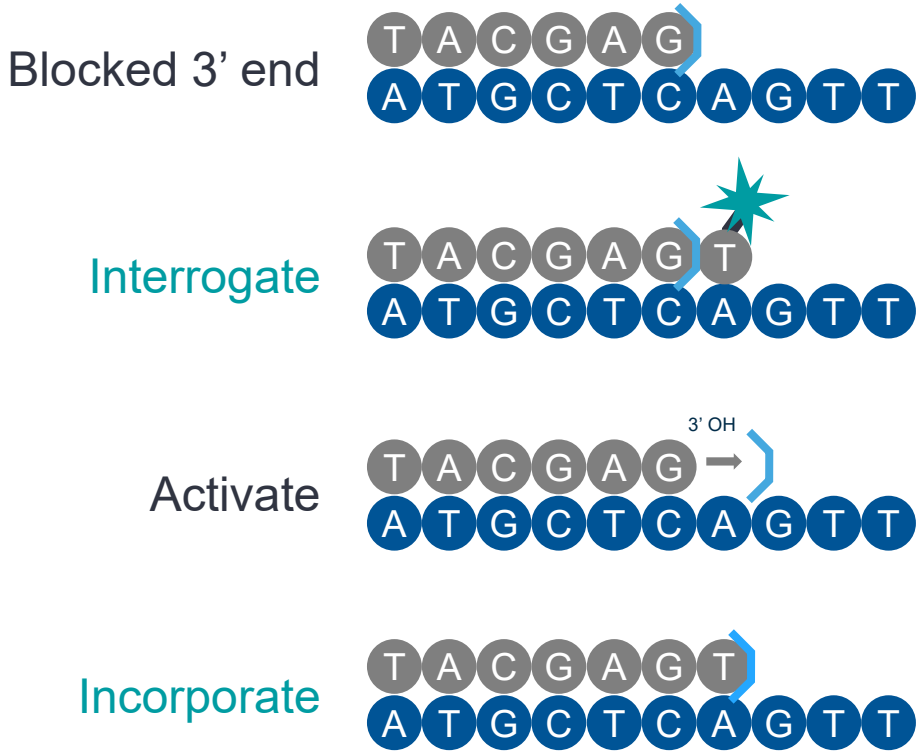
# SBB advantages: Incorporates native nucleotides, produces unmodified DNA

**No base modifications, no molecular scarring**

## Sequencing by synthesis (SBS)




## Sequencing by binding (SBB)



# Benefits of SBB over traditional short read NGS

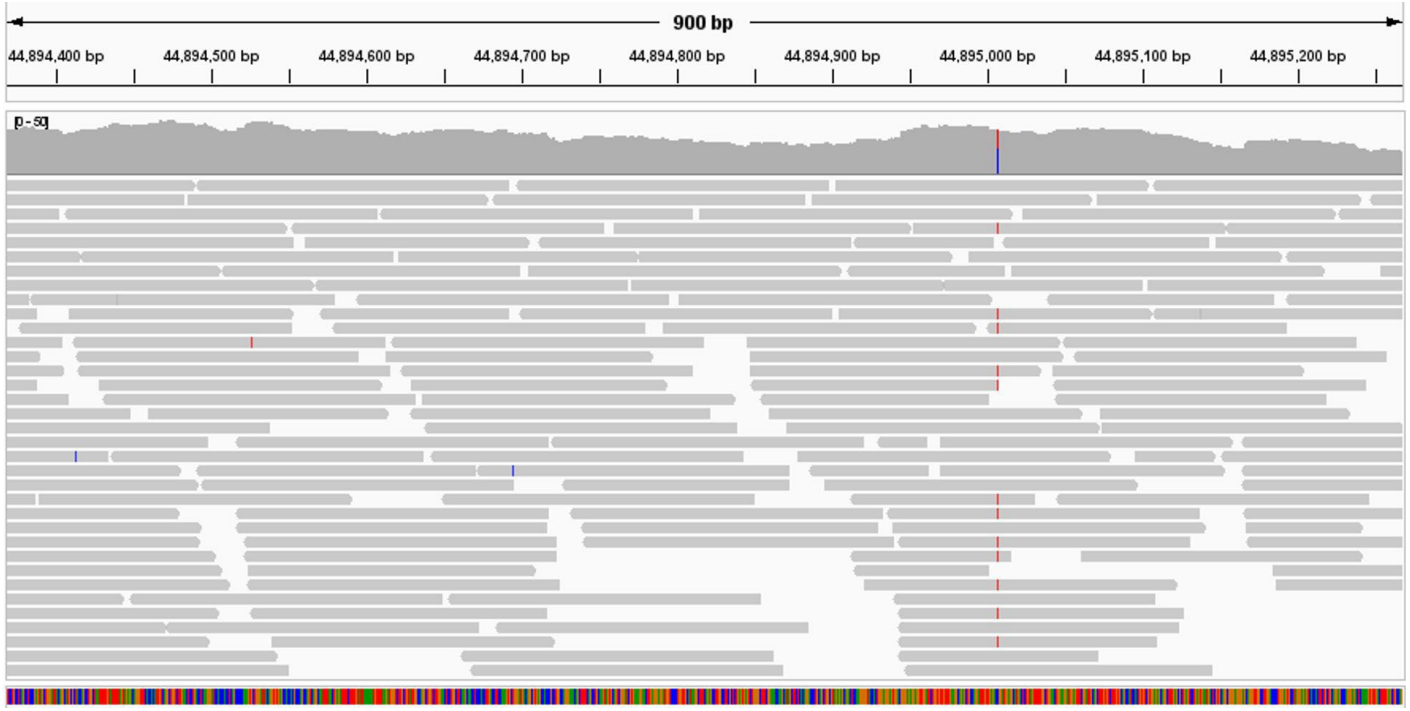
**>90% bases at Q40+**

**Low duplications rate**

**No index hopping**

**Sequence through difficult / repetitive regions**

## SBB offers near “perfect” reads



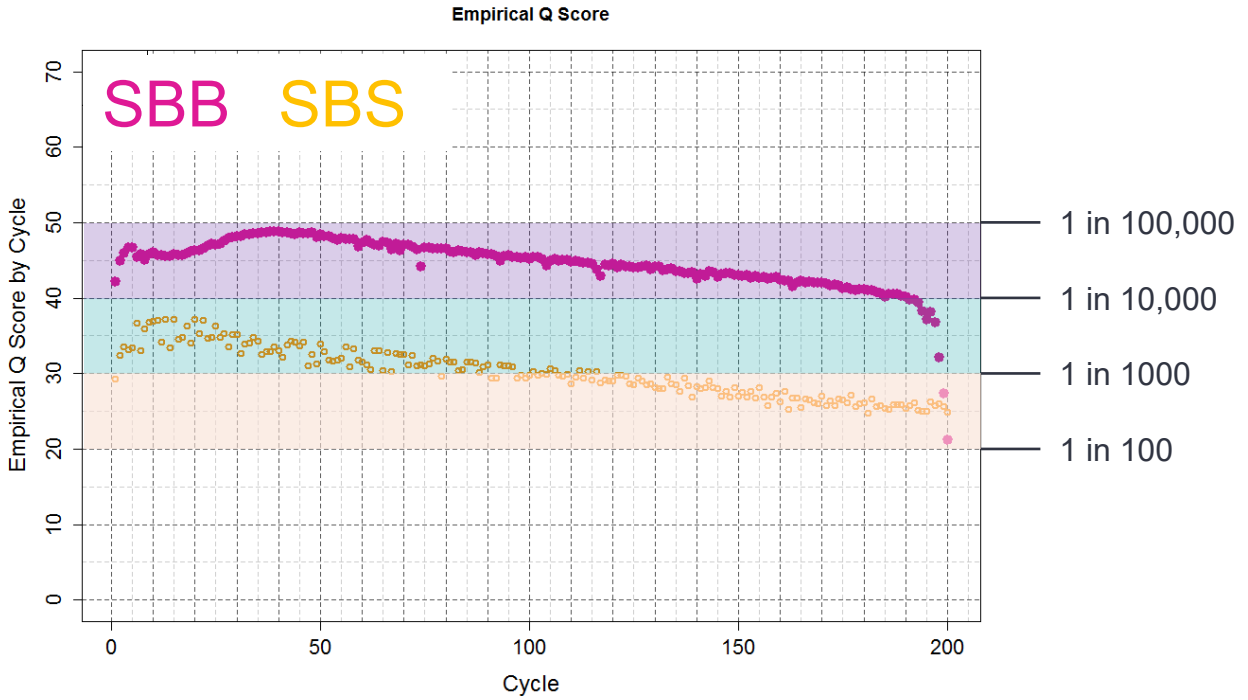

HG002; chr19:44,894,368-44,895,266

# SBB offers best-in-class accuracy

## SBB error rates ~15× lower at any given cycle, between 1:10,000 to 1:100,000

### The complete sequence of a human genome

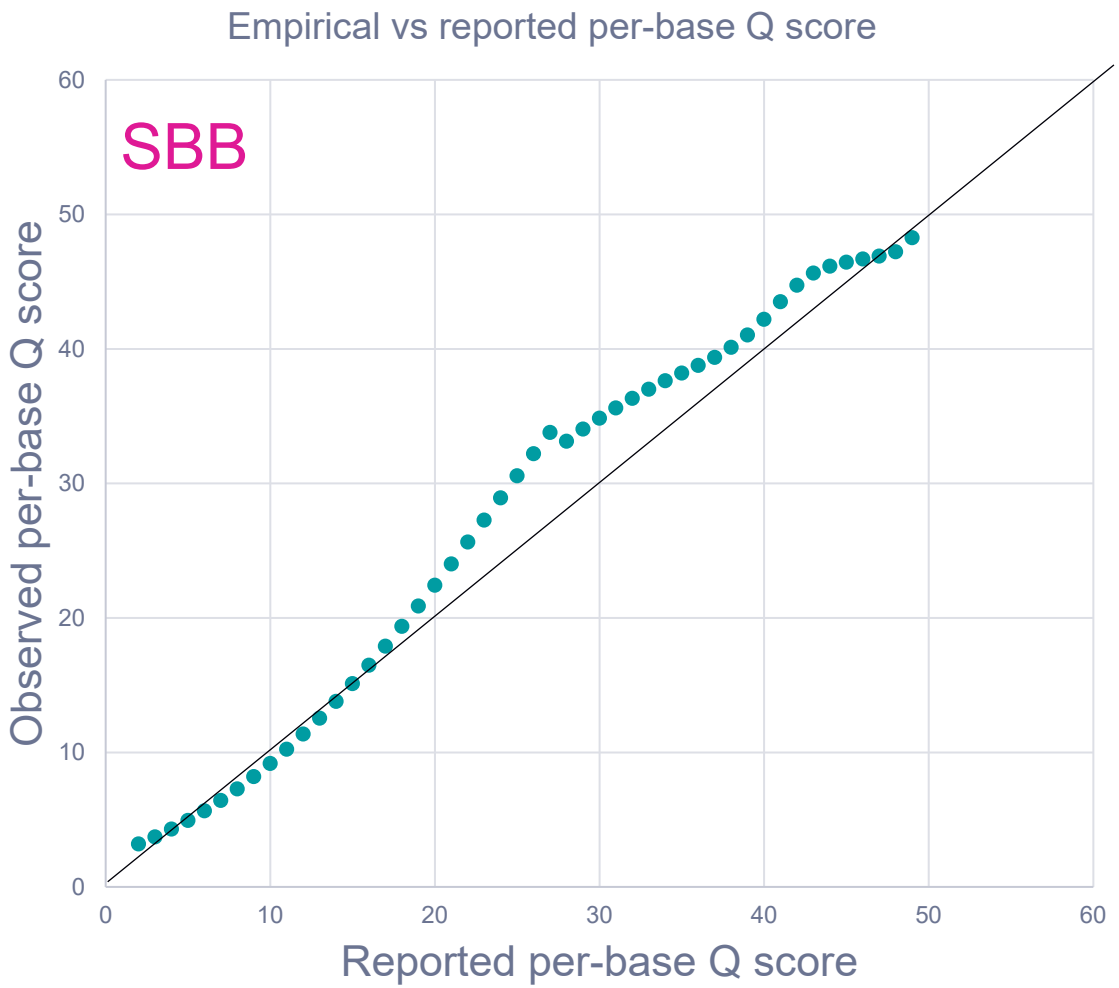
Sergey Nurk[1,*], Sergey Koren[1,*], Arang Rhie[1,*], Mikko Rautiainen[1,*], Andrey V. Bzikadze[2], Alla Mikheenko[3], Mitchell R. Vollger[4], Nicolas Altemose[5], Lev Uralsky[6,7], Ariel Gershman[8], Sergey Aganezov[9], Savannah J. Hoyt[10], Mark Diekhans[11], Glennis A. Logsdon[4], Michael Alonge[9], Stylianos E. Antonarakis[12], Matthew Borchers[13], Gerard G. Bouffard[14], Shelise Y. Brooks[14], Gina V. Caldas[15], Haoyu Cheng[16,17], Chen-Shan Chin[18], William Chow[19], Leonardo G. de Lima[13], Philip C. Dishuck[4], Richard Durbin[21], Tatiana Dvorkina[3], Ian T. Fiddes[22], Giulio Formenti[23,24], Robert S. Fulton[25], Arkarachai Fungtammasan[18], Erik Garrison[11,26], Patrick G.S. Grady[10], Tina A. Graves-Lindsay[27], Ira M. Hall[28], Nancy F. Hansen[29], Gabrielle A. Hartley[10], Marina Haukness[11], Kerstin Howe[19], Michael W. Hunkapiller[30], Chirag Jain[1,31], Miten Jain[11], Erich D. Jarvis[23,24], Peter Kerpedjiev[32], Melanie Kirsche[9], Mikhail Kolmogorov[33], Jonas Korlach[30], Milinn Kremitzki[27], Heng Li[16,17], Valerie V. Maduro[34], Tobias Marschall[35], Ann M. McCartney[1], Jennifer McDaniel[36], Danny E. Miller[4,37], James C. Mullikin[14,29], Eugene W. Myers[38], Nathan D. Olson[36], Benedict Paten[11], Paul Peluso[30], Pavel A. Pevzner[33], David Porubsky[4], Tamara Potapova[13], Evgeny I. Rogaev[6,7,39,40], Jeffrey A. Rosenfeld[41], Steven L. Salzberg[9,42], Valerie A. Schneider[43], Fritz J. Sedlazeck[44], Kishwar Shafin[11], Colin J. Shew[20], Alaina Shumate[42], Yumi Sims[19], Arian F. A. Smit[45], Daniela C. Soto[20], Ivan Sović[30,46], Jessica M. Storer[45], Aaron Streets[5,47], Beth A. Sullivan[48], Françoise Thibaud-Nissen[43], James Torrance[19], Justin Wagner[36], Brian P. Walenz[1], Aaron Wenger[30], Jonathan M. D. Wood[19], Chunlin Xiao[43], Stephanie M. Yan[49], Alice C. Young[14], Samantha Zarate[9], Urvashi Surti[50], Rajiv C. McCoy[49], Megan Y. Dennis[20], Ivan A. Alexandrov[3,7,51], Jennifer L. Gerton[13], Rachel J. O'Neill[10], Winston Timp[8,42], Justin M. Zook[36], Michael C. Schatz[9,49], Evan E. Eichler[4,24,†], Karen H. Miga[11,†], Adam M. Phillippy[1,†]

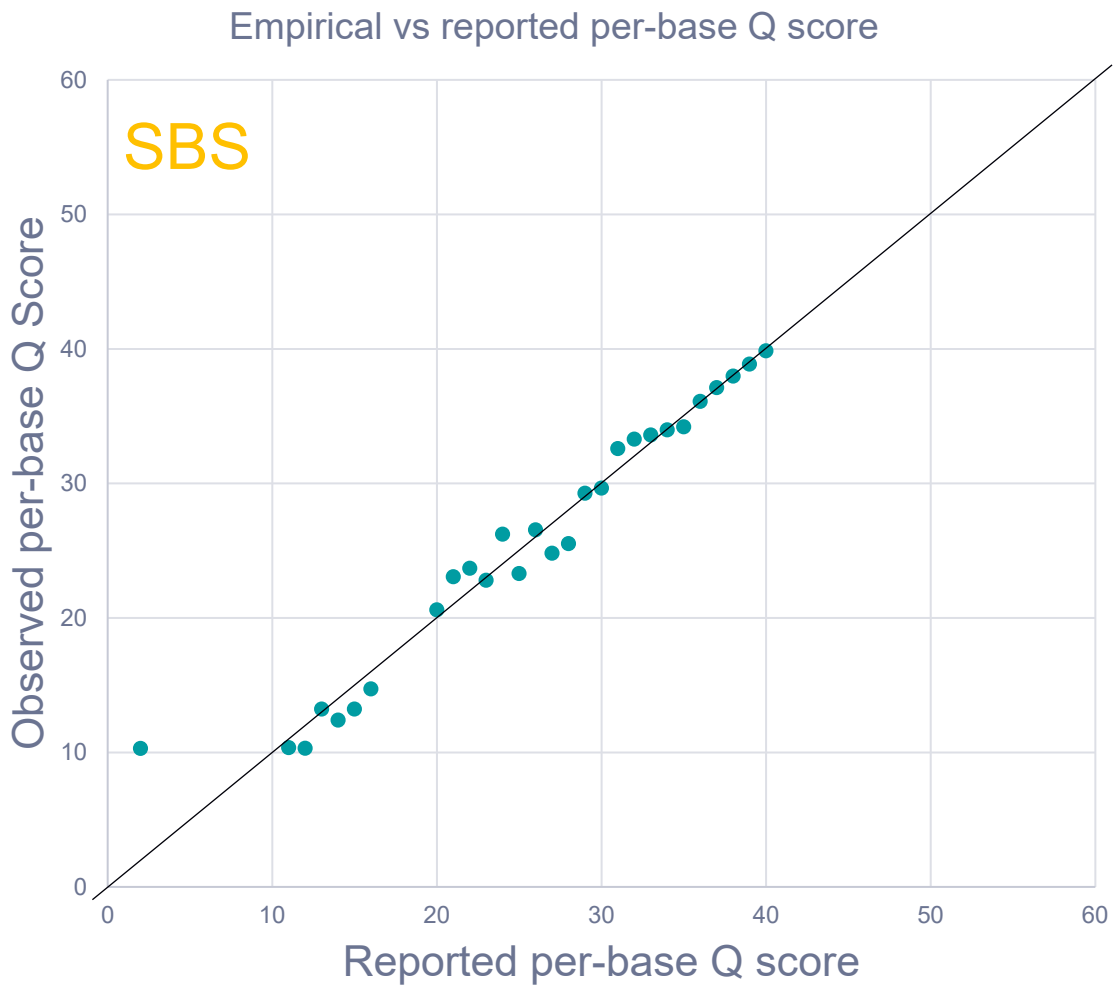



Chm13 sequencing. Empirical Q score evaluated on 99% of bases for each cycle.

# SBB vs SBS empirical vs reported per-base Q score for CHM13

## Uncalibrated SBB Q score correlates well with observed errors

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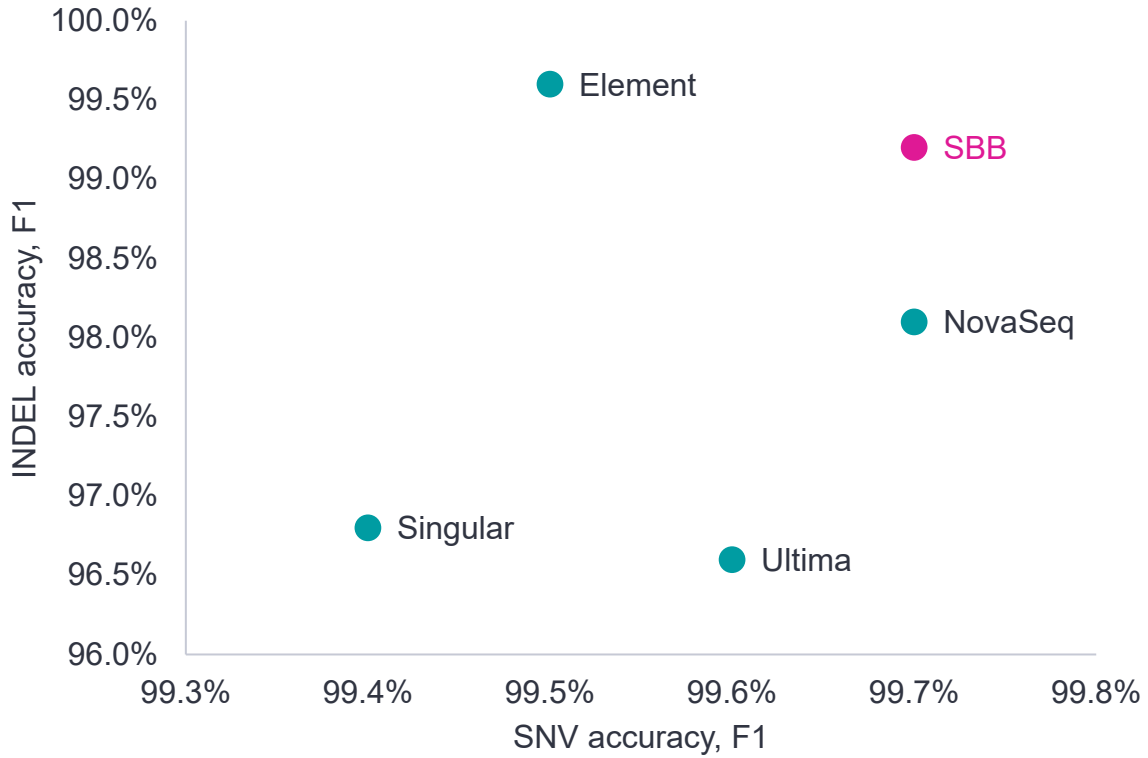
Chm13 sequencing. Empirical Q score evaluated on 99% of bases for each cycle.

# Excellent variant calling performance for SBB

| | | SBB | NovaSeq | Element | Ultima | Singular |
|---|---|---|---|---|---|---|
| SNV | Recall | 99.6% | 99.9% | 99.1% | 99.6% | 99.2% |
| | Prec. | 99.7% | 99.5% | 99.8% | 99.6% | 99.7% |
| | **F1** | **99.7%** | **99.7%** | **99.5%** | **99.6%** | **99.4%** |
| INDEL | Recall | 98.9% | 97.9% | 99.3% | 96.4% | 96.4% |
| | Prec. | 99.4% | 98.4% | 99.8% | 96.8% | 97.1% |
| | **F1** | **99.2%** | **98.1%** | **99.6%** | **96.6%** | **96.8%** |




- Expecting further improvements through variant caller training

SBB / NVSQ: HG002 GIAB benchmark v4.2, DeepVariant 1.3.0 with the Illumina WGS model
Element: https://go.elementbiosciences.com/access-app-note-roche-human-microbial
Ultima: https://doi.org/10.1101/2022.05.29.493900 HG002 40x coverage
Singular: G4 Human Genome Sequencing Technical Report 1.0. NA12878 40x coverage

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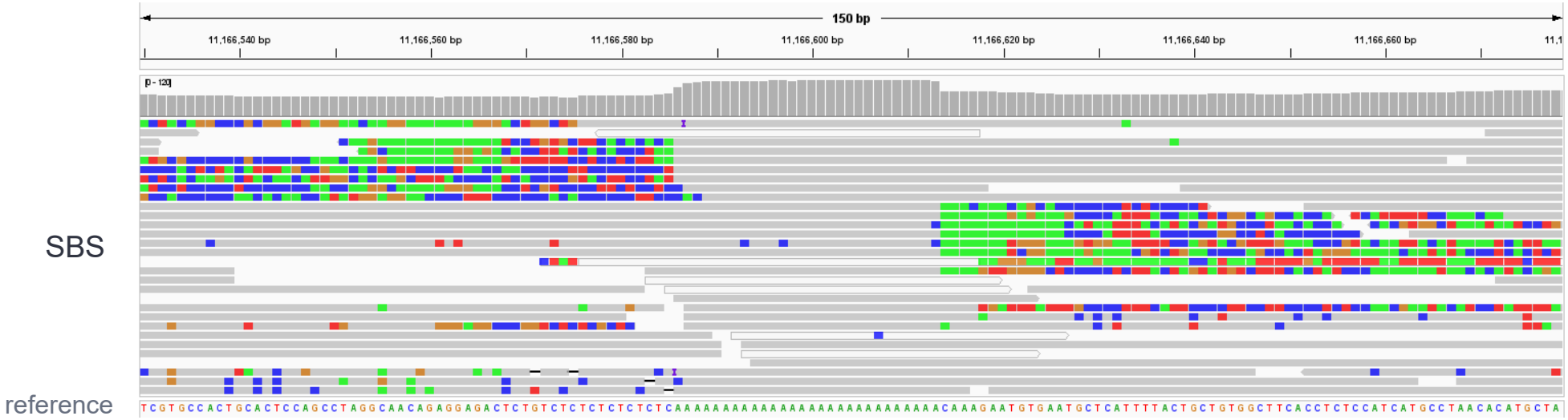
# What does unprecedented accuracy look like?

## Low-complexity region (28 As in the reference)

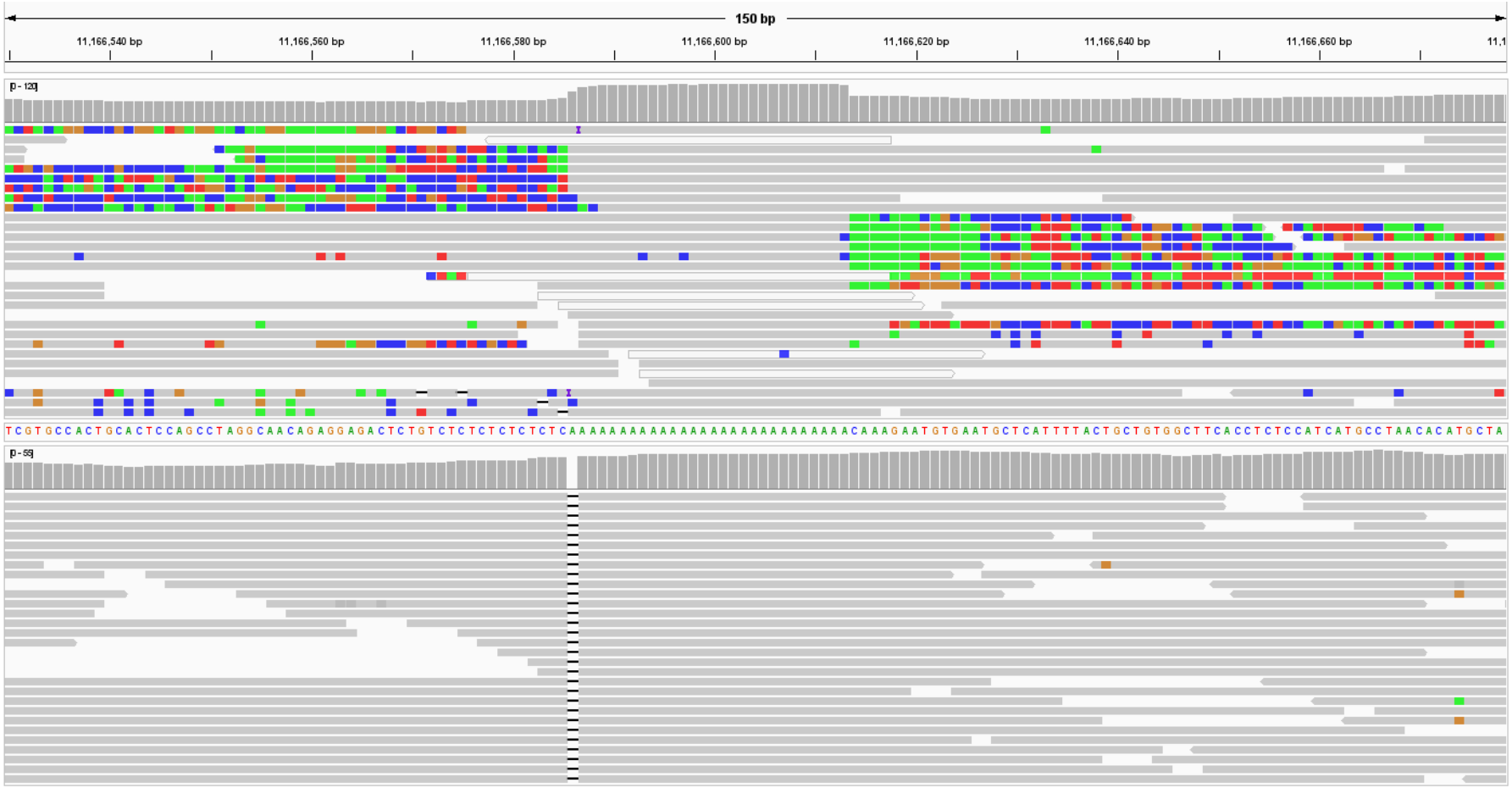



No high-quality reads

No variants called

# What does unprecedented accuracy look like?

## SBB cleanly sequences through 27 bp poly A (28 bp in the reference)




No high-quality reads

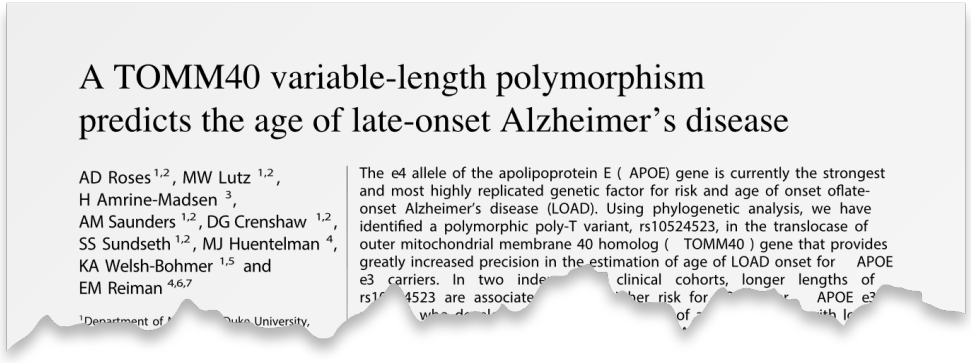
No variants called

**Full resolution**

**1 bp deletion (27 As)**

CHM13; chr5:11,166,530-11,166,679. PacBio SBB 1x200; HiSeq 2x250

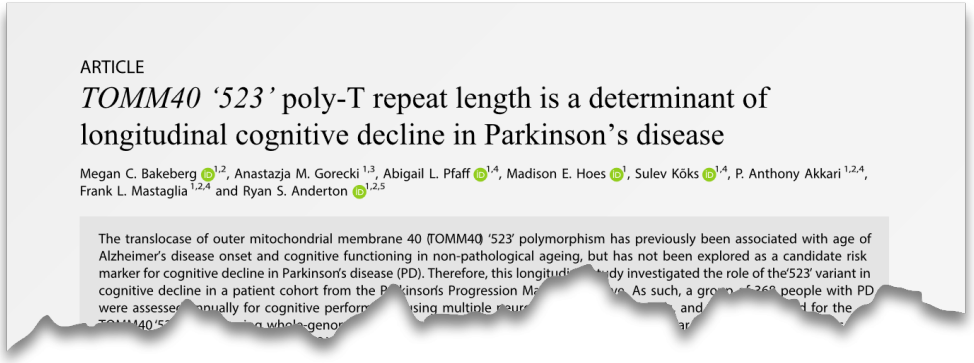
# Example of a ‘difficult’ region – *TOMM40*

A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer’s disease

AD Roses[1,2], MW Lutz[1,2], H Amrine-Madsen[3], AM Saunders[1,2], DG Crenshaw[1,2], SS Sundseth[1,2], MJ Huentelman[4], KA Welsh-Bohmer[1,5] and EM Reiman[4,6,7]

The e4 allele of the apolipoprotein E ( APOE) gene is currently the strongest and most highly replicated genetic factor for risk and age of onset oflate-onset Alzheimer’s disease (LOAD). Using phylogenetic analysis, we have identified a polymorphic poly-T variant, rs10524523, in the translocase of outer mitochondrial membrane 40 homolog ( TOMM40 ) gene that provides greatly increased precision in the estimation of age of LOAD onset for APOE e3 carriers. In two inde clinical cohorts, longer lengths of rs1 4523 are associate her risk for APOE e3



ARTICLE

*TOMM40 ‘523’* poly-T repeat length is a determinant of longitudinal cognitive decline in Parkinson’s disease

Megan C. Bakeberg[1,2], Anastazja M. Gorecki[1,3], Abigail L. Pfaff[1,4], Madison E. Hoes[1], Sulev Kõks[1,4], P. Anthony Akkari[1,2,4], Frank L. Mastaglia[1,2,4] and Ryan S. Anderton[1,2,5]

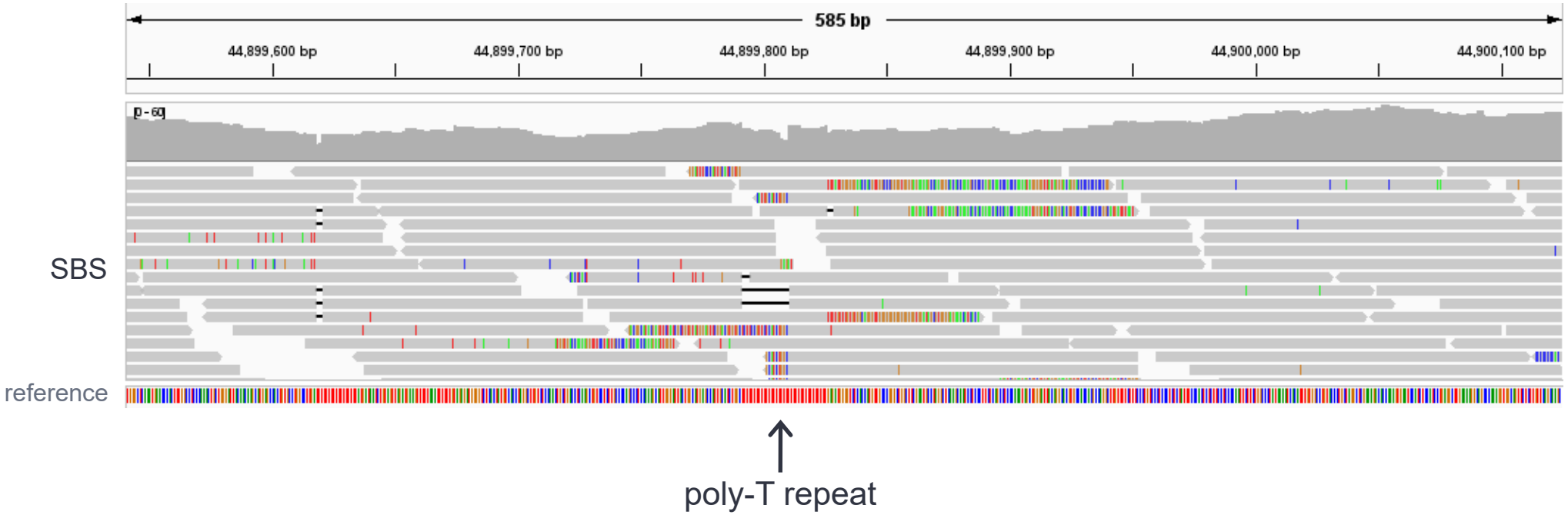
The translocase of outer mitochondrial membrane 40 (TOMM40) ‘523’ polymorphism has previously been associated with age of Alzheimer’s disease onset and cognitive functioning in non-pathological ageing, but has not been explored as a candidate risk marker for cognitive decline in Parkinson’s disease (PD). Therefore, this longitudi dy investigated the role of the‘523’ variant in cognitive decline in a patient cohort from the P insons Progression Ma ve. As such, a gro 368 people with PD were assesse nually for cognitive perform sing multiple neur and for the TOMM40 ‘5 g whole-genom

***“Assay development for the TOMM40 ‘523’ variant is generally considered to be difficult, as poly-T variants are challenging to sequence.”*** [1]



Adapted from Larsen *et al.* (2017) https://alz-journals.onlinelibrary.wiley.com/doi/10.1016/j.jalz.2017.01.017; https://www.nature.com/articles/s41531-021-00200-y; https://pubmed.ncbi.nlm.nih.gov/20029386/ [1]https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7973542/

# Example of a ‘difficult’ region – *TOMM40*

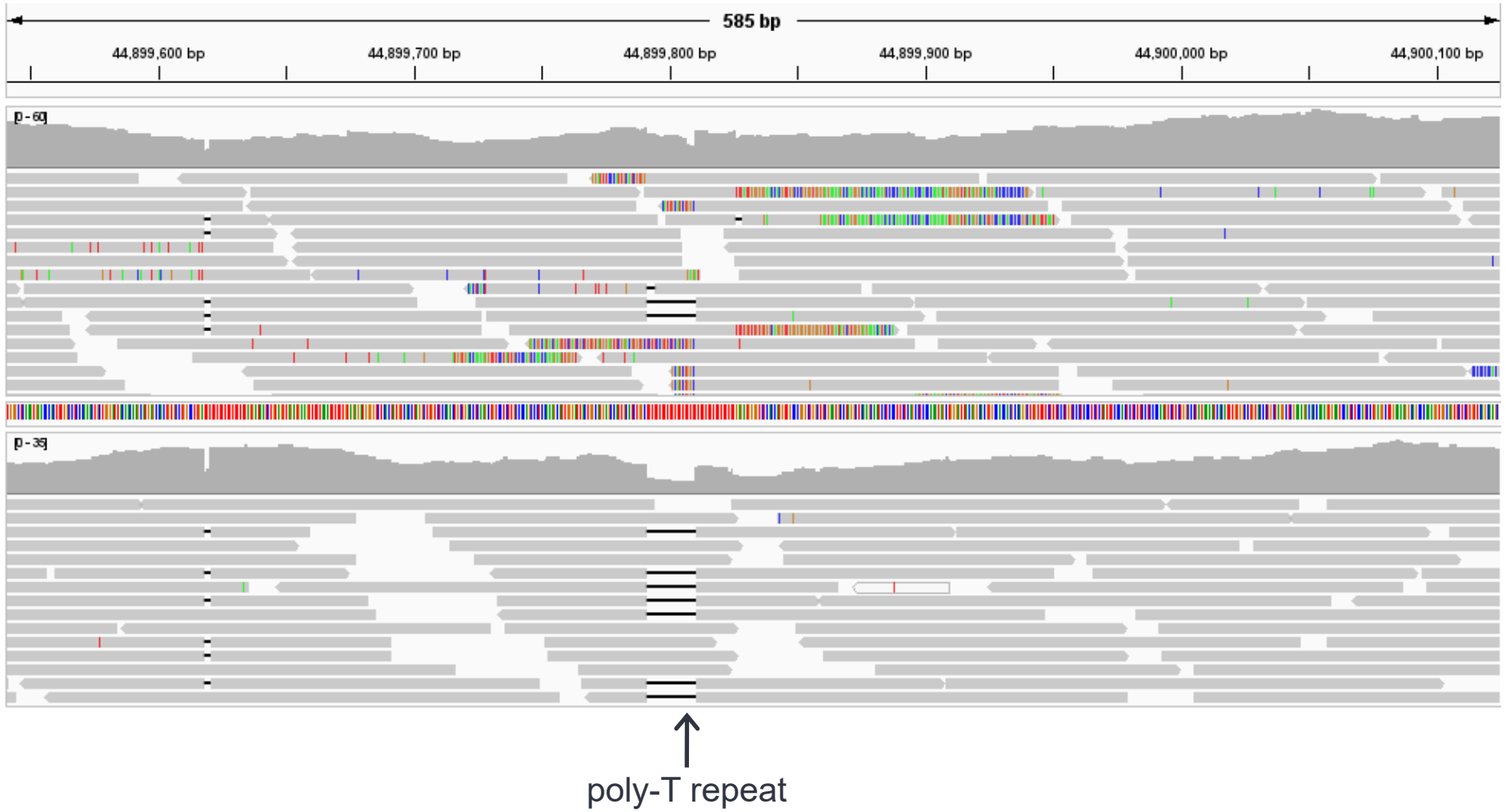



No high-quality reads

poly-T repeat unresolved

# Example of a ‘difficult’ region – *TOMM40*

## SBB cleanly sequences through poly-T repeat locus

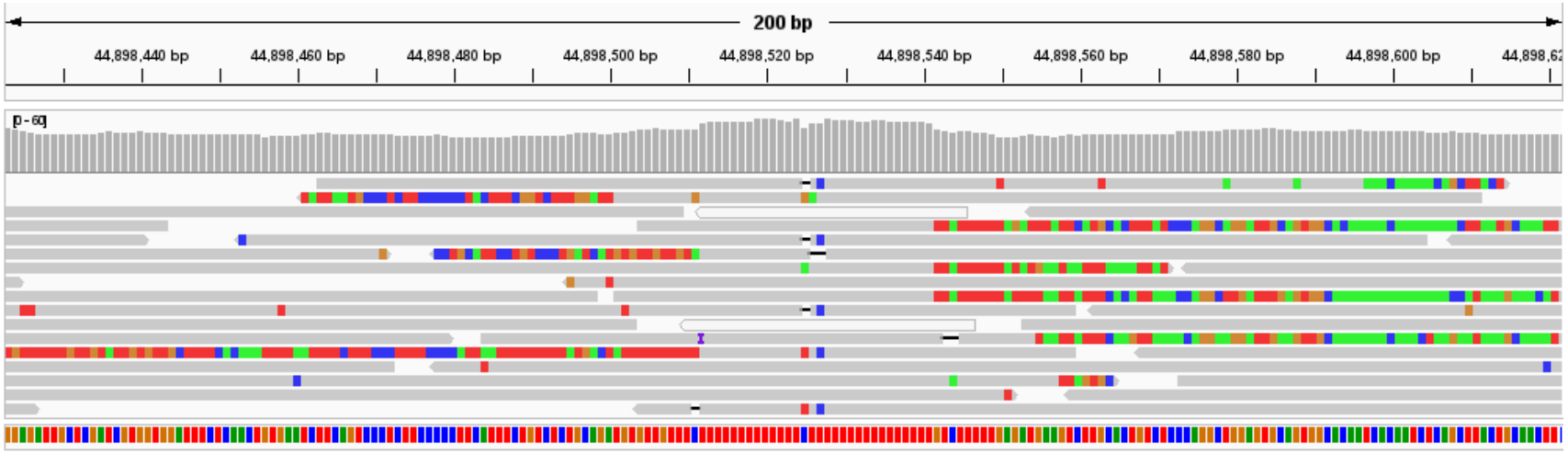



No high-quality reads
poly-T repeat unresolved

**poly-T repeat fully resolved: $T_{16}$ / $T_{35}$**

HG002; chr19:44,899,541-44,900,124; PacBio SBB 1x200 bp; NovaSeq 2x151 bp

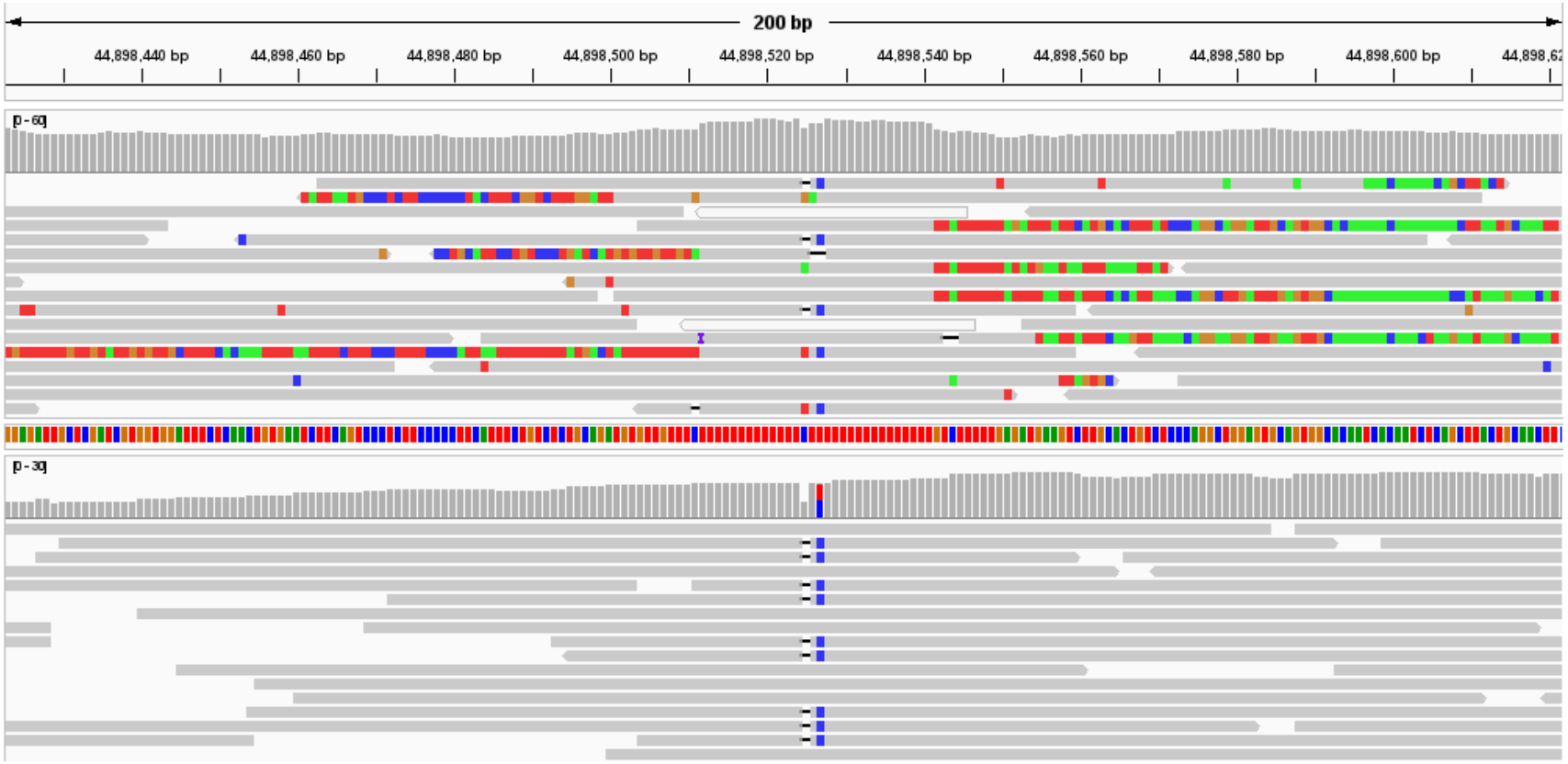
# Another similar region nearby




No high-quality reads

No variants called

# Another similar region nearby




No high-quality reads

No variants called

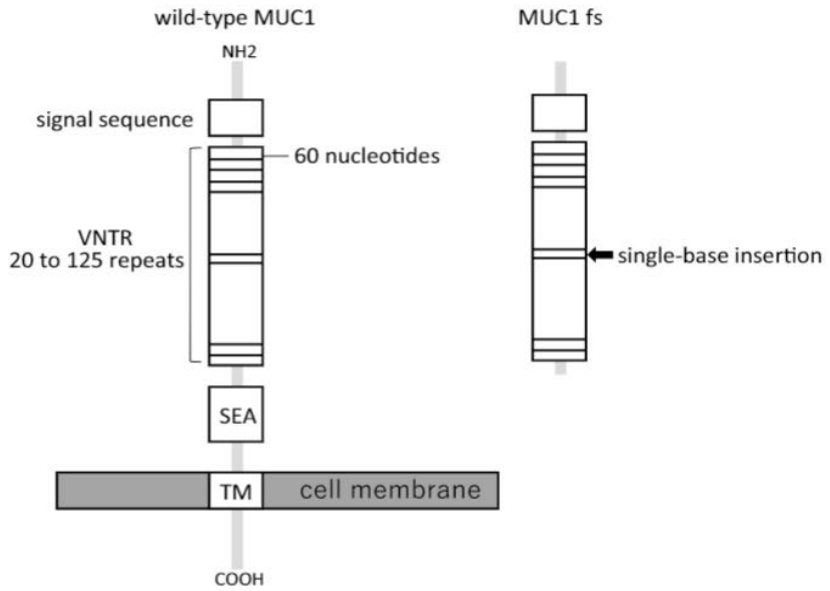
**Two het variants called**

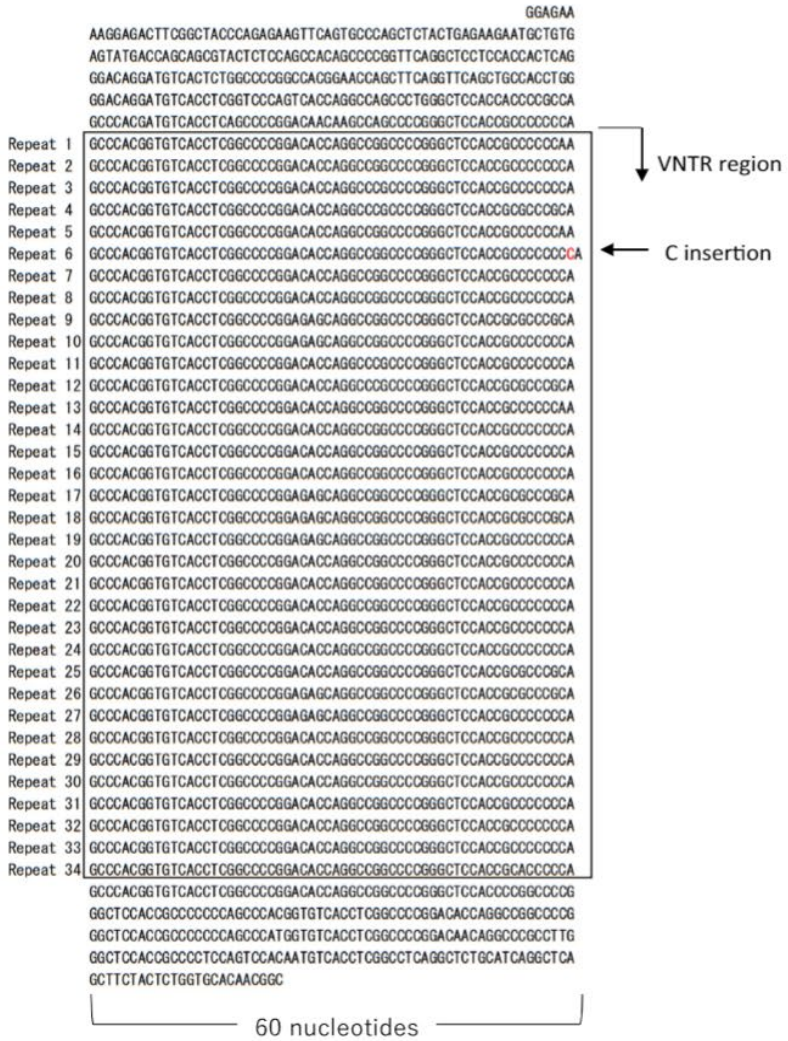
**Full resolution & phasing:**

```
TTTTTTTTTTTTTCTTTTTTTTTTTTTTTT
TTTTTTTTTTTTT-TCTTTTTTTTTTTTTT
```

# SBB correctly sequences through lengthy C/G repeats

## Example Mucin 1 (*MUC1*) kidney disease



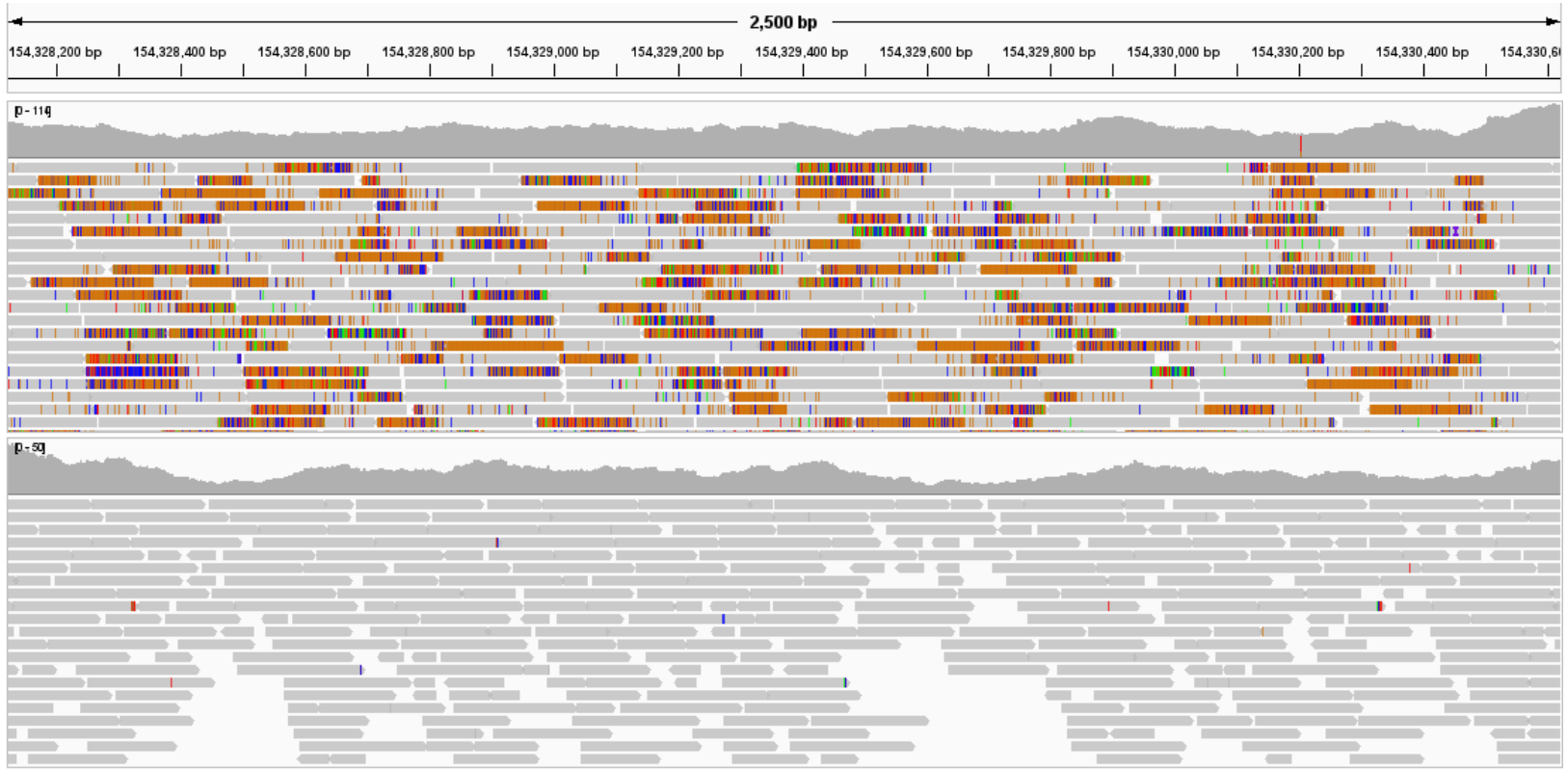






From Okada *et al.* (2022): https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9039432/

# Sequencing performance on a healthy control sample

## SBB did not observe spurious mutant variants in CHM13



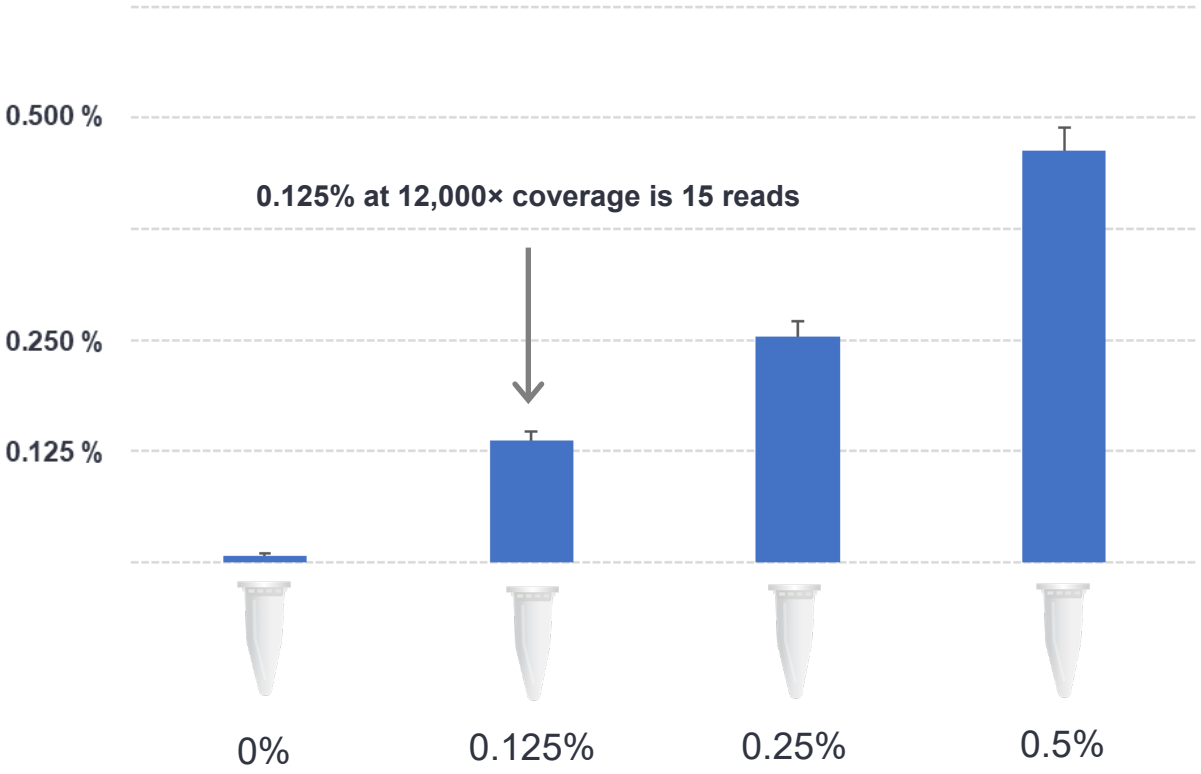

| | Observed wildtype CCCCCCC | Observed pathogenic variant CCCCCCCC |
|---|---|---|
| SBS | 1280 | 68 |
| PacBio SBB | 494 | 0 |

CHM13 chr1:154,328,121-154,330,619; PacBio SBB 1x200 bp; HiSeq 1x250 bp

# Observed *vs* expected ctDNA at low variant allele frequency

## Variant allele percent shows good linearity, even without use of UMIs

High sensitivity and specificity down to
0.125%, even with modest (<12,000×) coverage



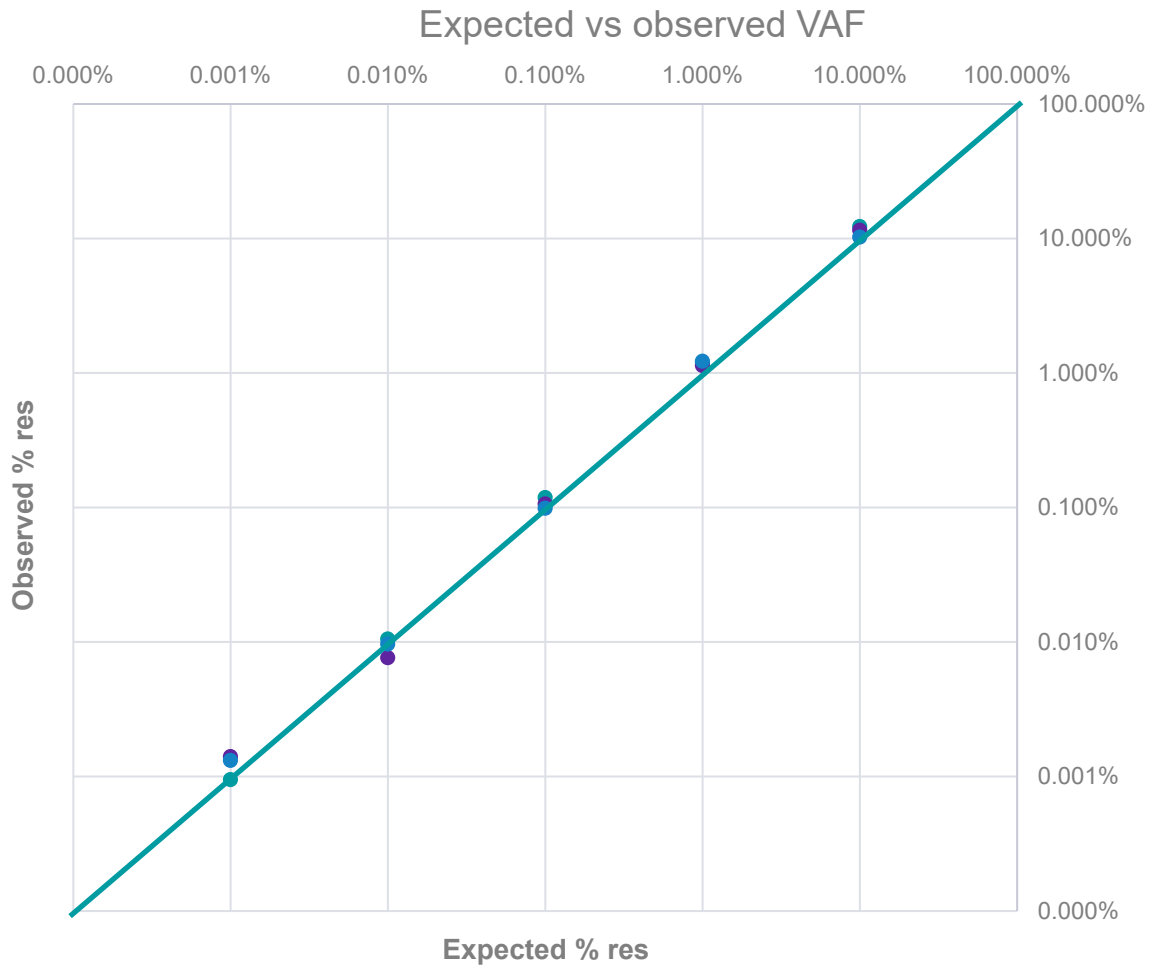


Controls and reference materials
**Seraseq® ctDNA Mutation Mix v2**

# How low can SBB go without UMIs?

## Tuberculosis amplicon shows near perfect linearity from 10% to 0.001%

| | Res (C) counts | WT (G) counts | Total # counts | Observed % res |
|---|---|---|---|---|
| 10%_rep1 | 2580549 | 18479388 | 21,059,937 | 12.2534% |
| 10%_rep2 | 2998687 | 23062382 | 26,061,069 | 11.5064% |
| 10%_rep3 | 743168 | 6508219 | 7,251,387 | 10.2486% |
| 1%_rep1 | 76912 | 6388097 | 6,465,009 | 1.1897% |
| 1%_rep2 | 46152 | 4025690 | 4,071,842 | 1.1334% |
| 1%_rep3 | 65964 | 5334716 | 5,400,680 | 1.2214% |
| 0.1%_rep1 | 22836 | 19283363 | 19,306,199 | 0.1183% |
| 0.1%_rep2 | 8121 | 7646655 | 7,654,776 | 0.1061% |
| 0.1%_rep3 | 6505 | 6587930 | 6,594,435 | 0.0986% |
| 0.01%_rep1 | 1164 | 11078726 | 11,079,890 | 0.0105% |
| 0.01%_rep2 | 197 | 2579126 | 2,579,323 | 0.0076% |
| 0.01%_rep3 | 1030 | 10684737 | 10,685,767 | 0.0096% |
| 0.001%_rep1 | 171 | 18047826 | 18,047,997 | 0.0009% |
| 0.001%_rep2 | 117 | 8328474 | 8,328,591 | 0.0014% |
| 0.001%_rep3 | 786 | 59804418 | 59,805,204 | 0.0013% |



# Where to from here for SBB?

## Today

- Taking applications for collaboration; run your samples in our lab on SBB
- Visit our suite or www.pacb.com/sbb

## Tomorrow

- Thurs, June 9, 8:00–8:30 am: *Advancing NGS accuracy by an order of magnitude*
  Jennifer Stone, PhD, Vice President, Segment Marketing

## Late Q3

- Formal external beta commences; more information to be shared at ASHG

## 1H 23

- On track for platform commercial availability


PacBio