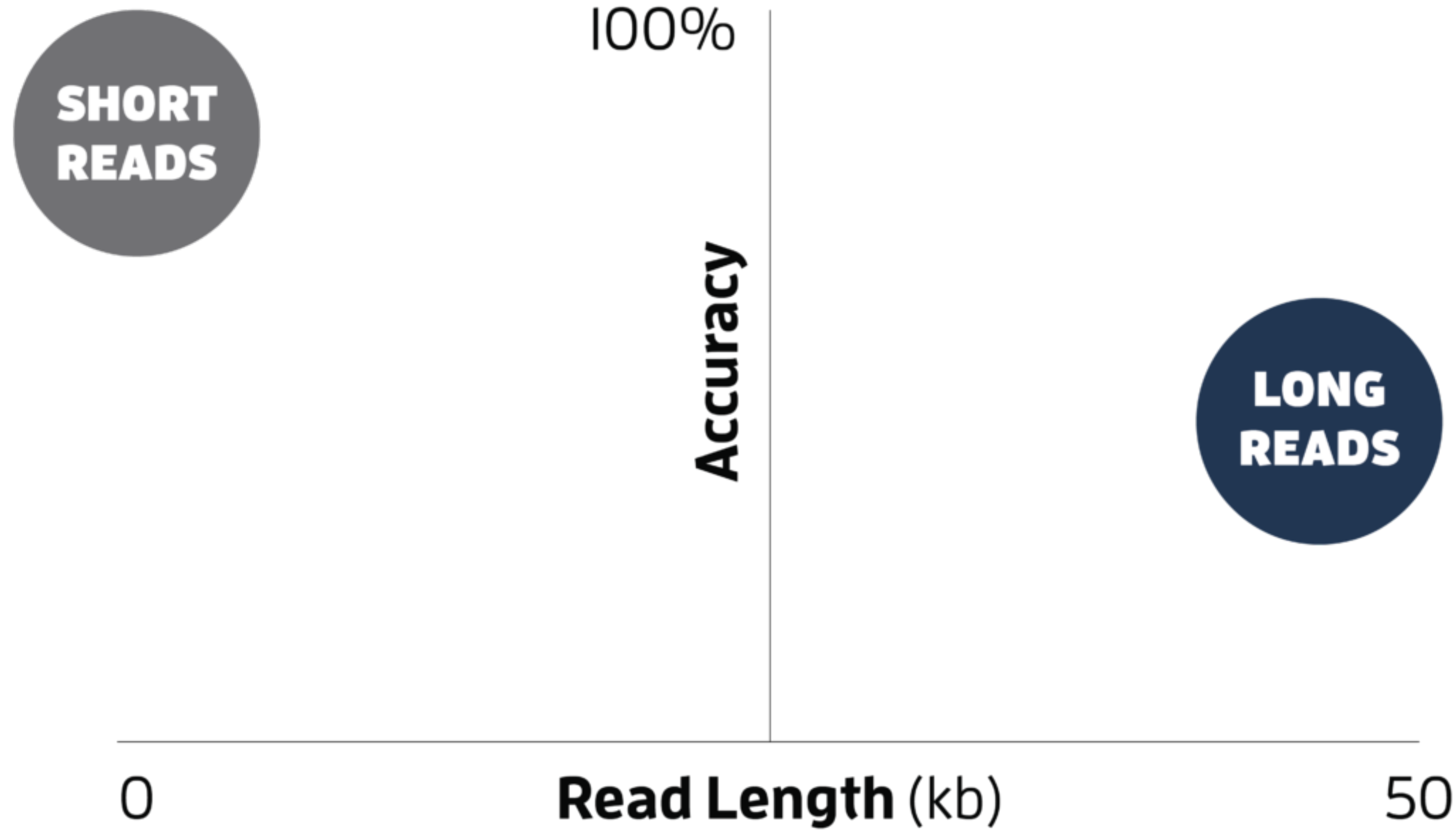
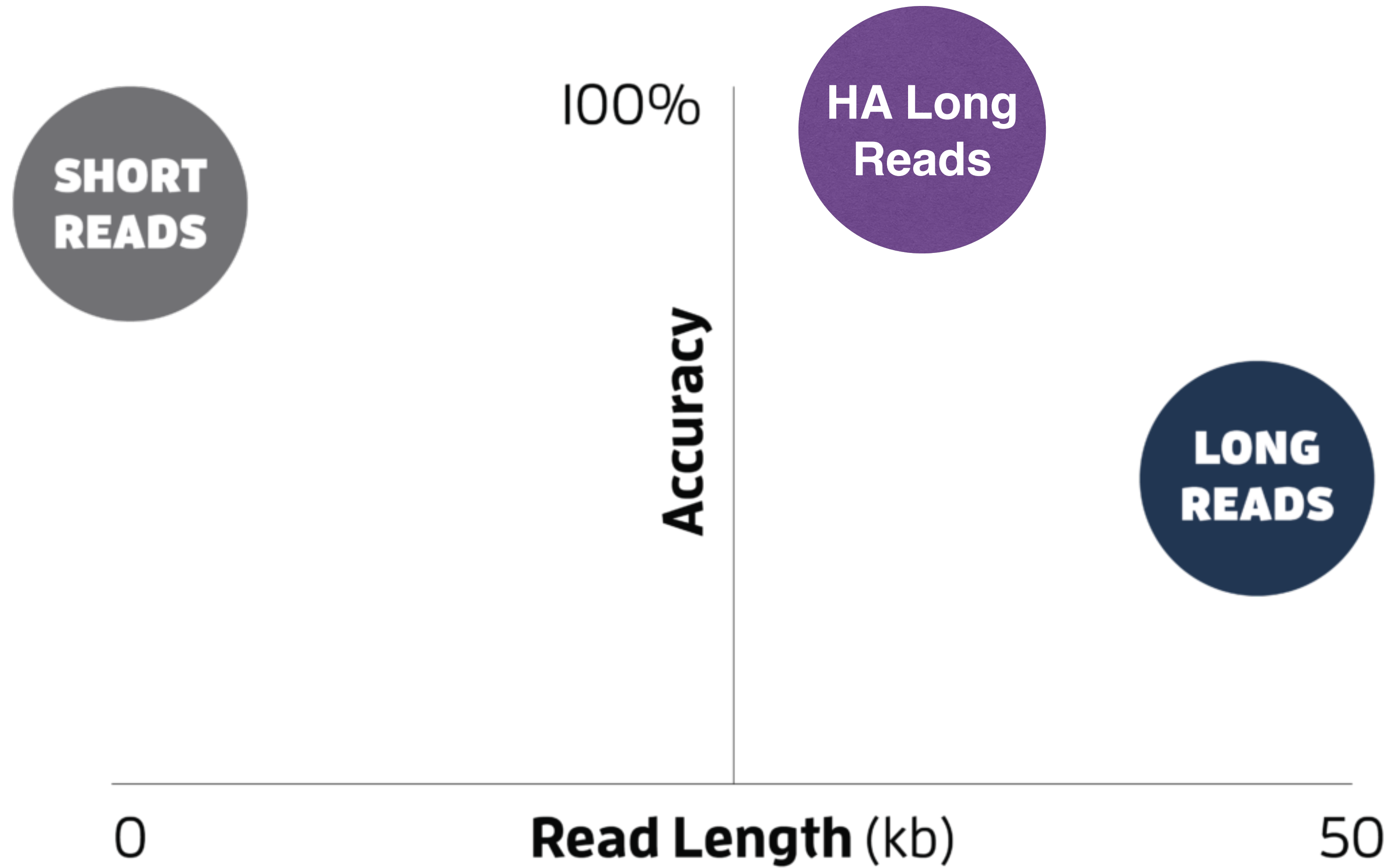


Maximizing Resolution with  
DADA2  
and Long-read Amplicon Sequencing

# Highly-Accurate Long Reads



# Highly-Accurate Long Reads



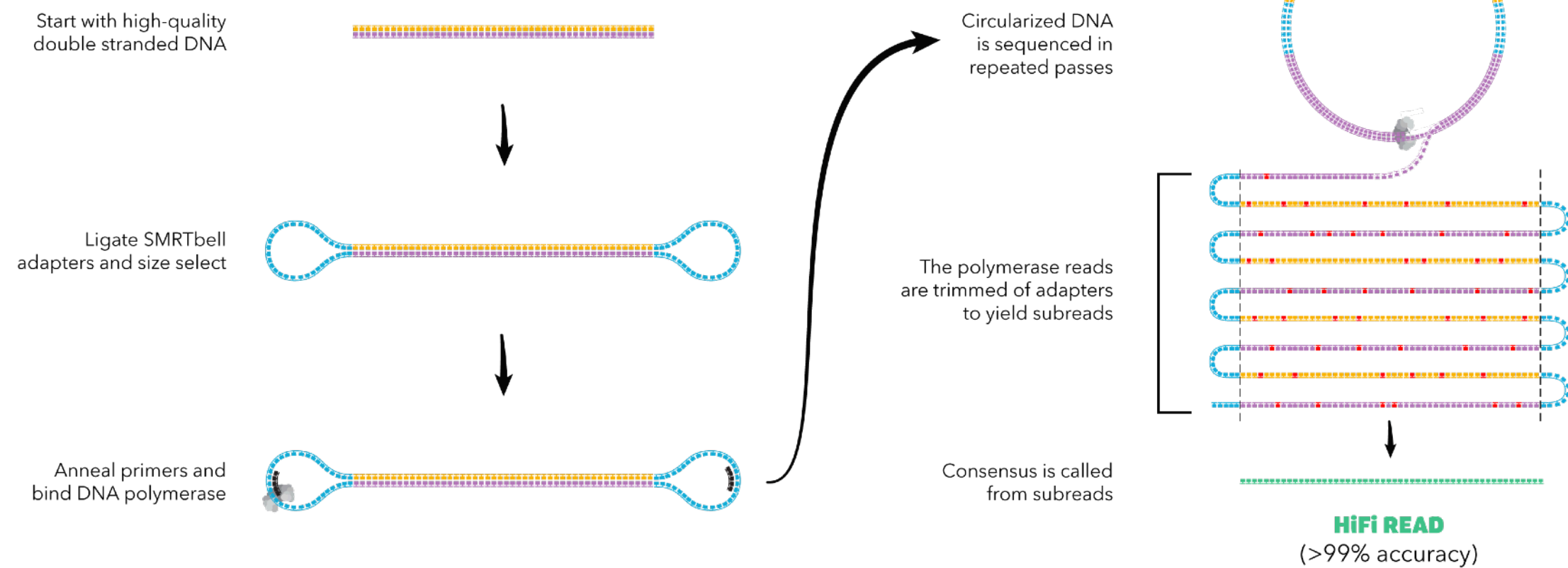
# Highly-Accurate Long Reads

**Strategy:** Build Consensus from Multiple Reads of a Long DNA Molecule

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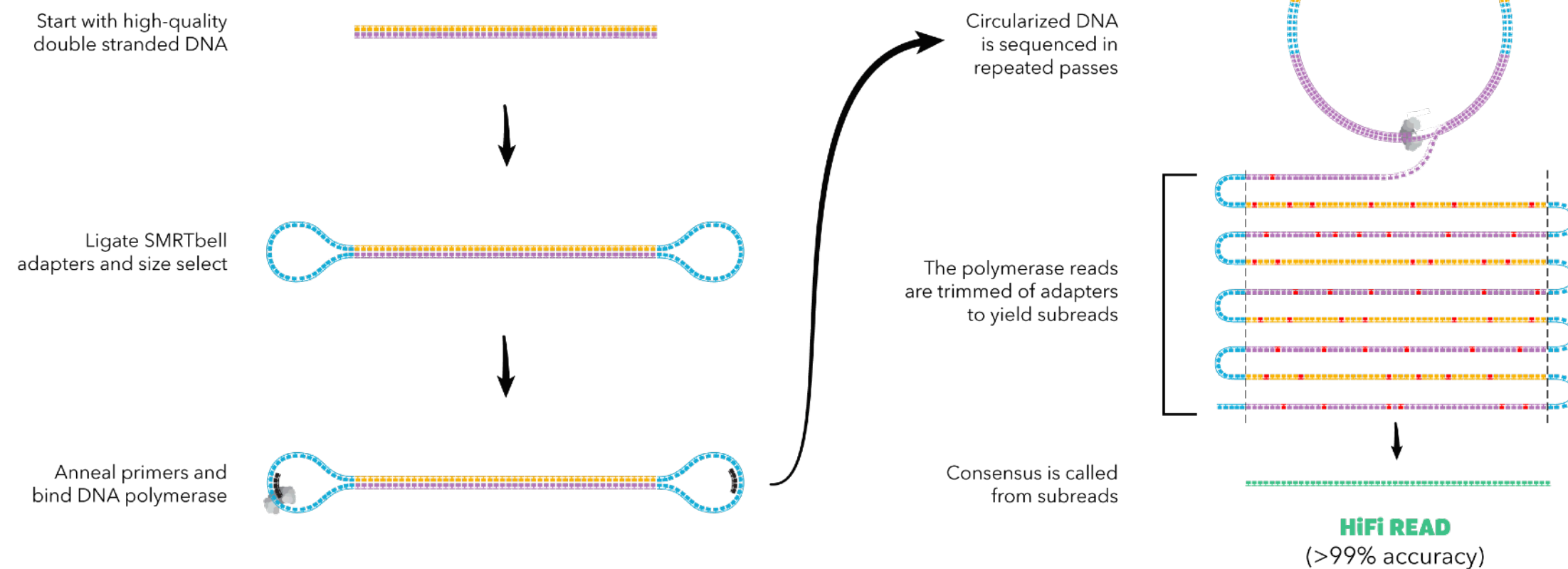
## PacBio HiFi Sequencing



# Highly-Accurate Long Reads

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## PacBio HiFi Sequencing

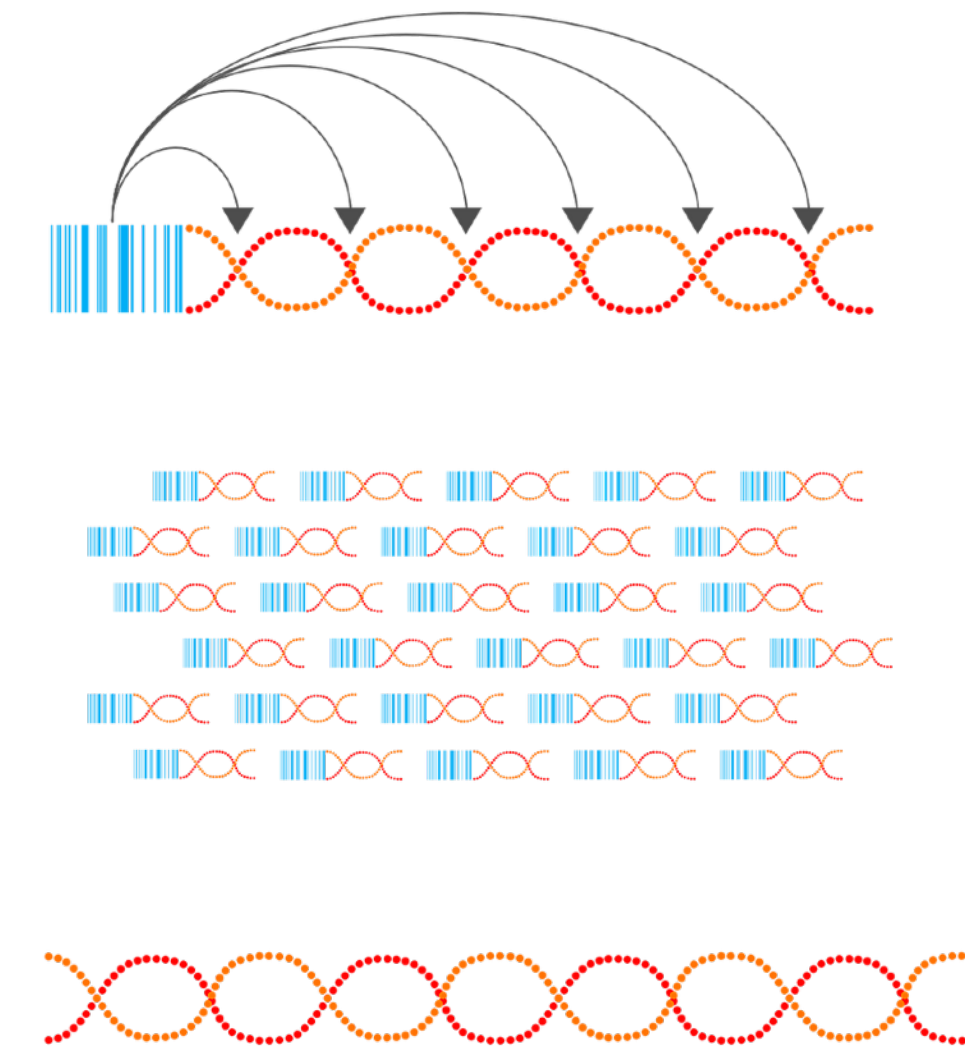


## Loop Genomics Synthetic Long Reads (SLRs)

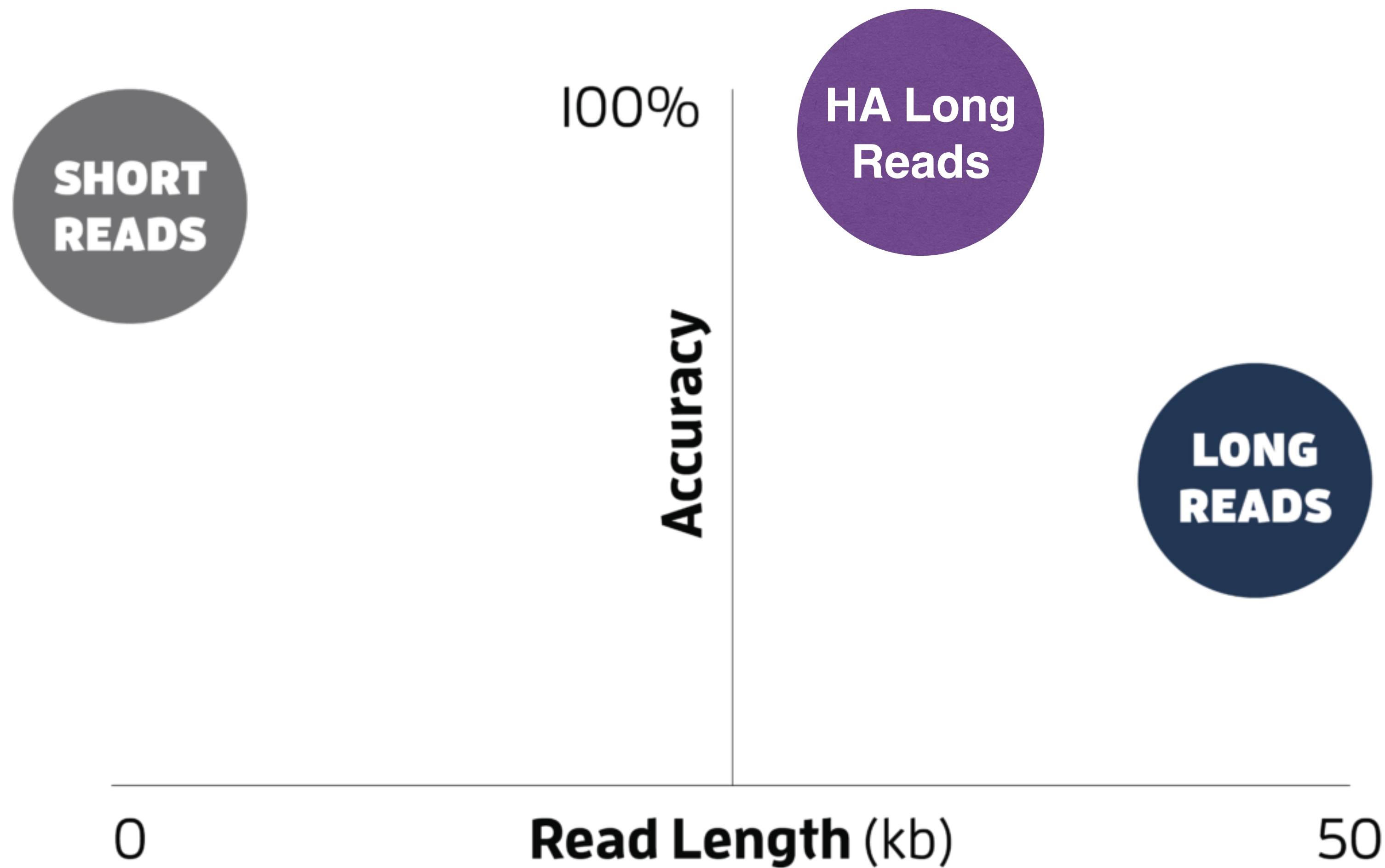
Distribute UMI throughout long DNA molecule

Short-read sequencing

Consensus assembly



# Highly-Accurate Long Reads



## Short Reads (Illumina)

Read length: ~**250 nts**

Per-base error-rate: **0.1 - 0.5%**

## Long Reads (PacBio, Oxford)

Read length: **1 - 200 kilobases**

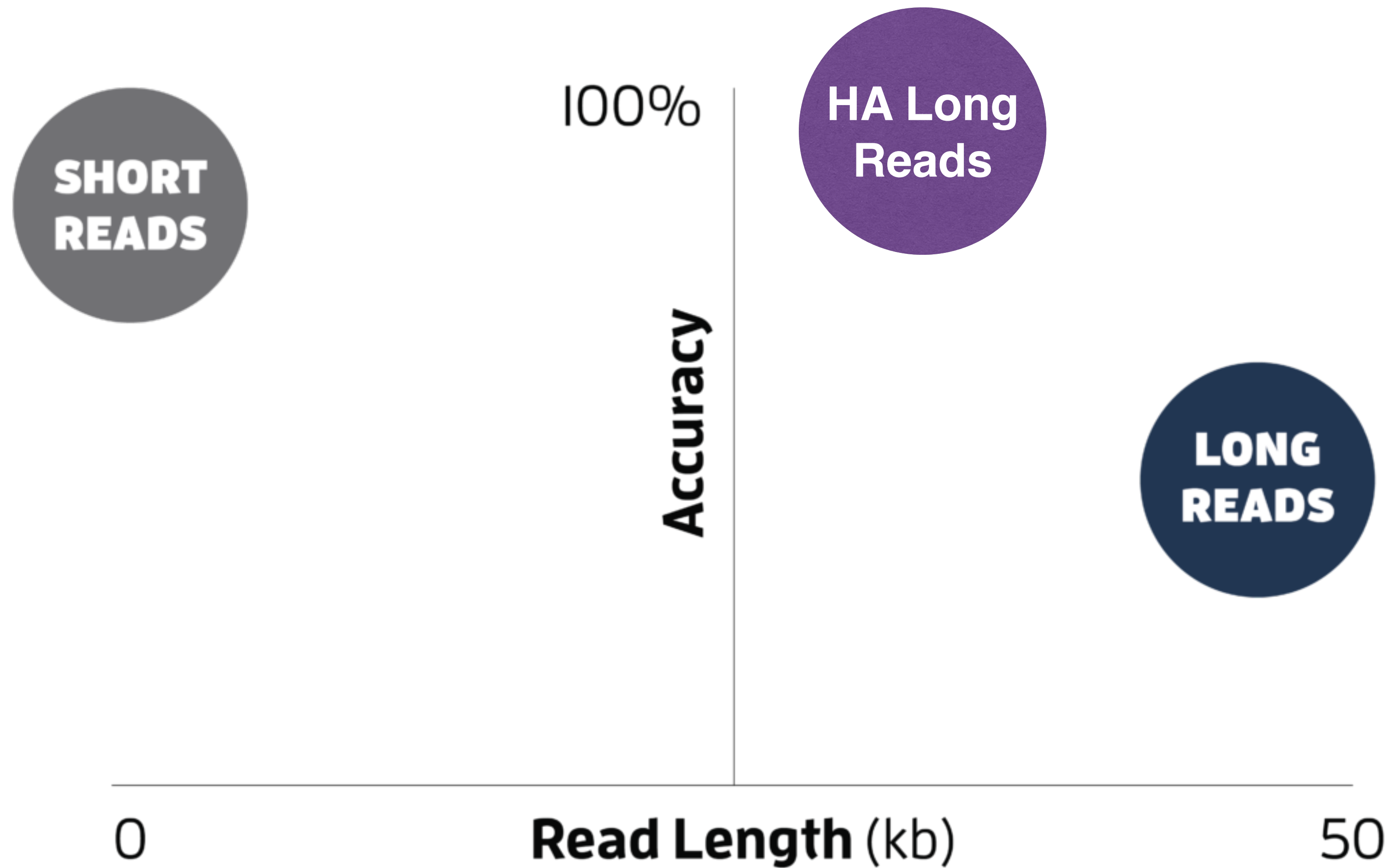
Per-base error-rate: **2 - 10%**

## HA Long Reads (HiFi, LoopSeq)

Read length: **1 - 20 kilobases**

Per-base error-rate: **< 0.1%**

# Highly-Accurate Long Reads



**Highly-accurate long reads are effectively a new sequencing technology.**

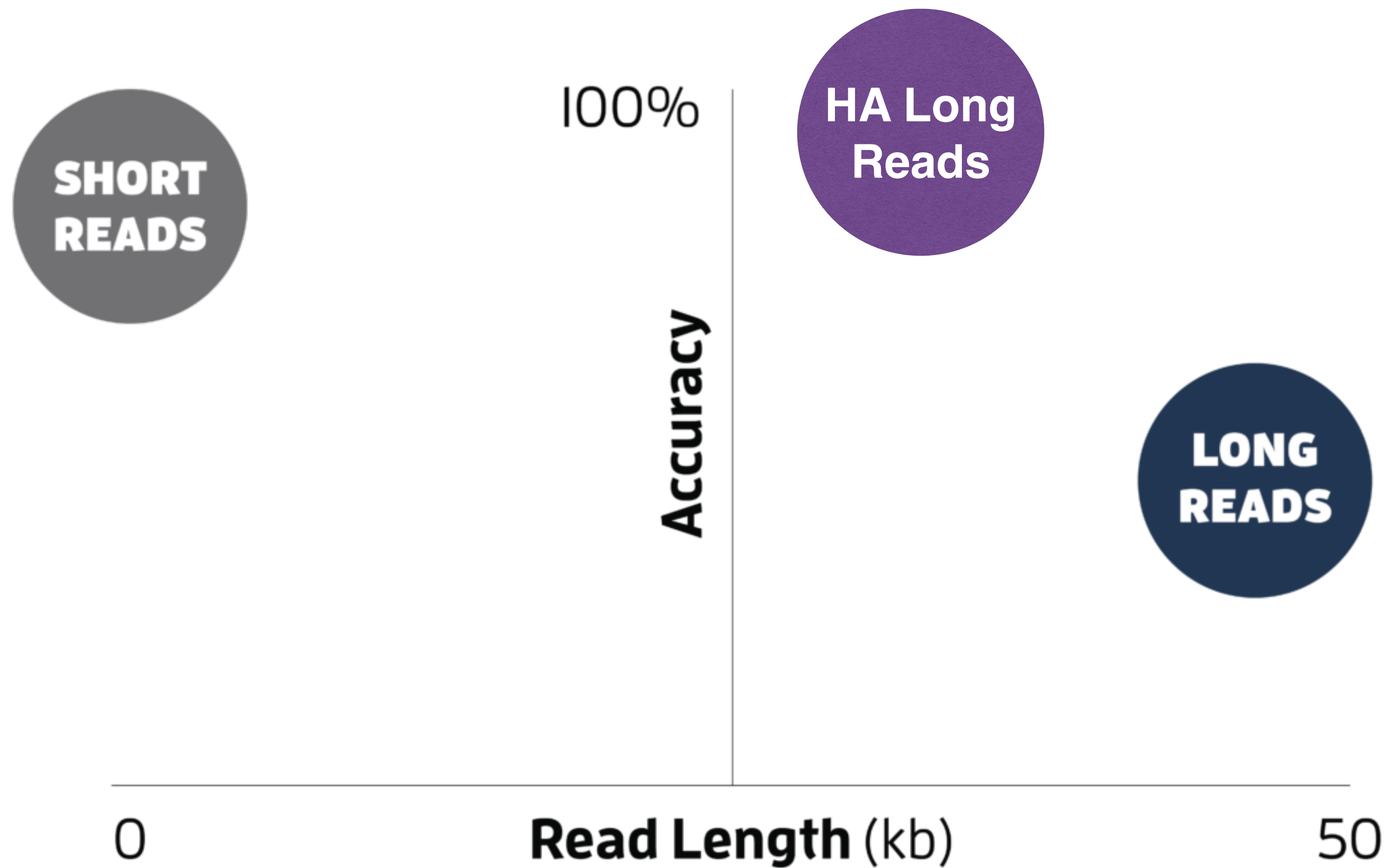
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# Highly-Accurate Long Reads



Highly-accurate long reads are effectively a new sequencing technology. **But do they really work for amplicon sequencing?**

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## Zymo Mock Community

8 Bacterial Strains at  
nominally equal concentrations

- *Pseudomonas aeruginosa*
- *Escherichia coli*
- *Salmonella enterica*
- *Lactobacillus fermentum*
- *Enterococcus faecalis*
- *Staphylococcus aureus*
- *Listeria monocytogenes*
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**PacBio  
HiFi +  
DADA2**

# Full-Length, Full-Complement 16S

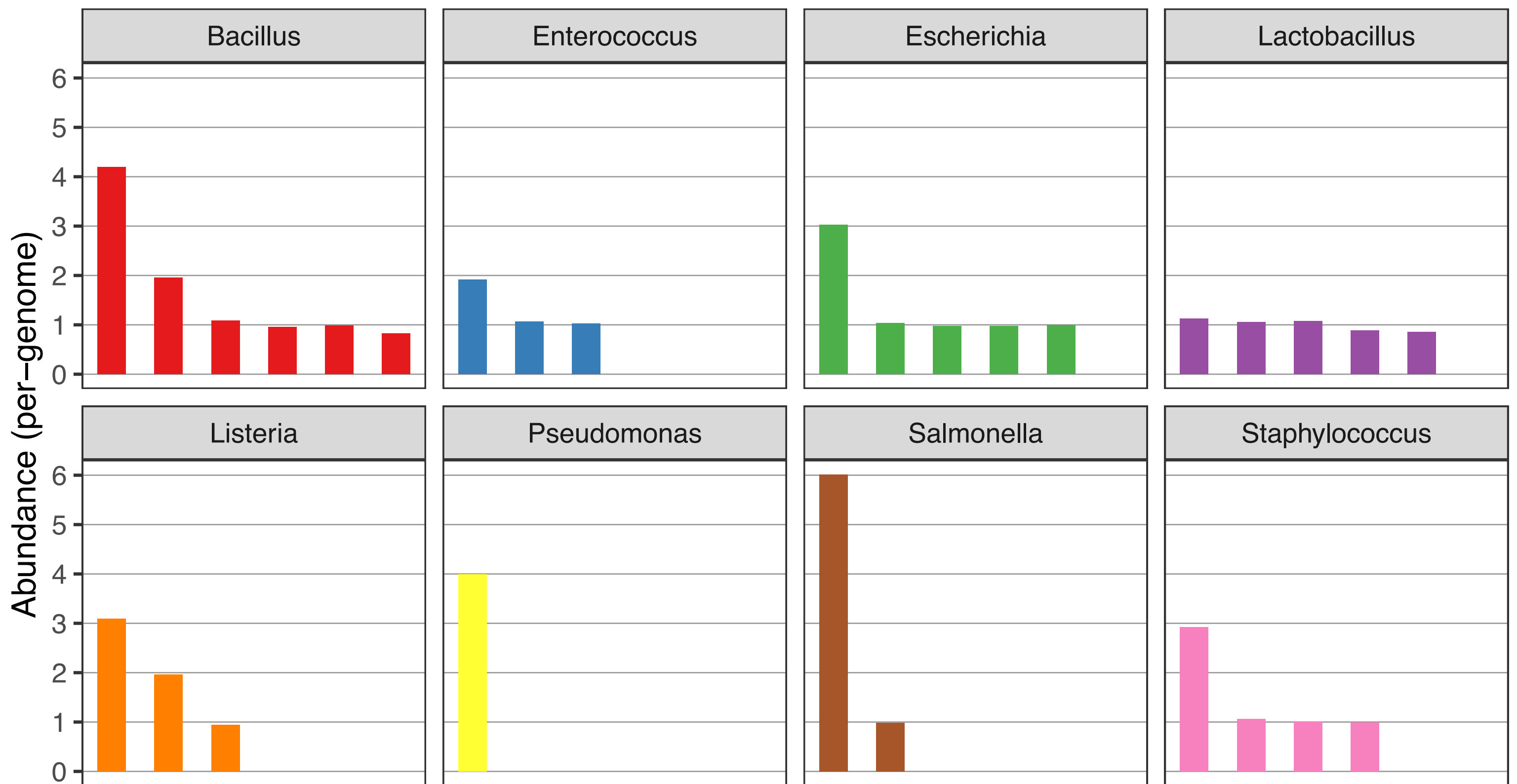
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**PacBio  
HiFi +  
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Full-length 16S Sequence Variants

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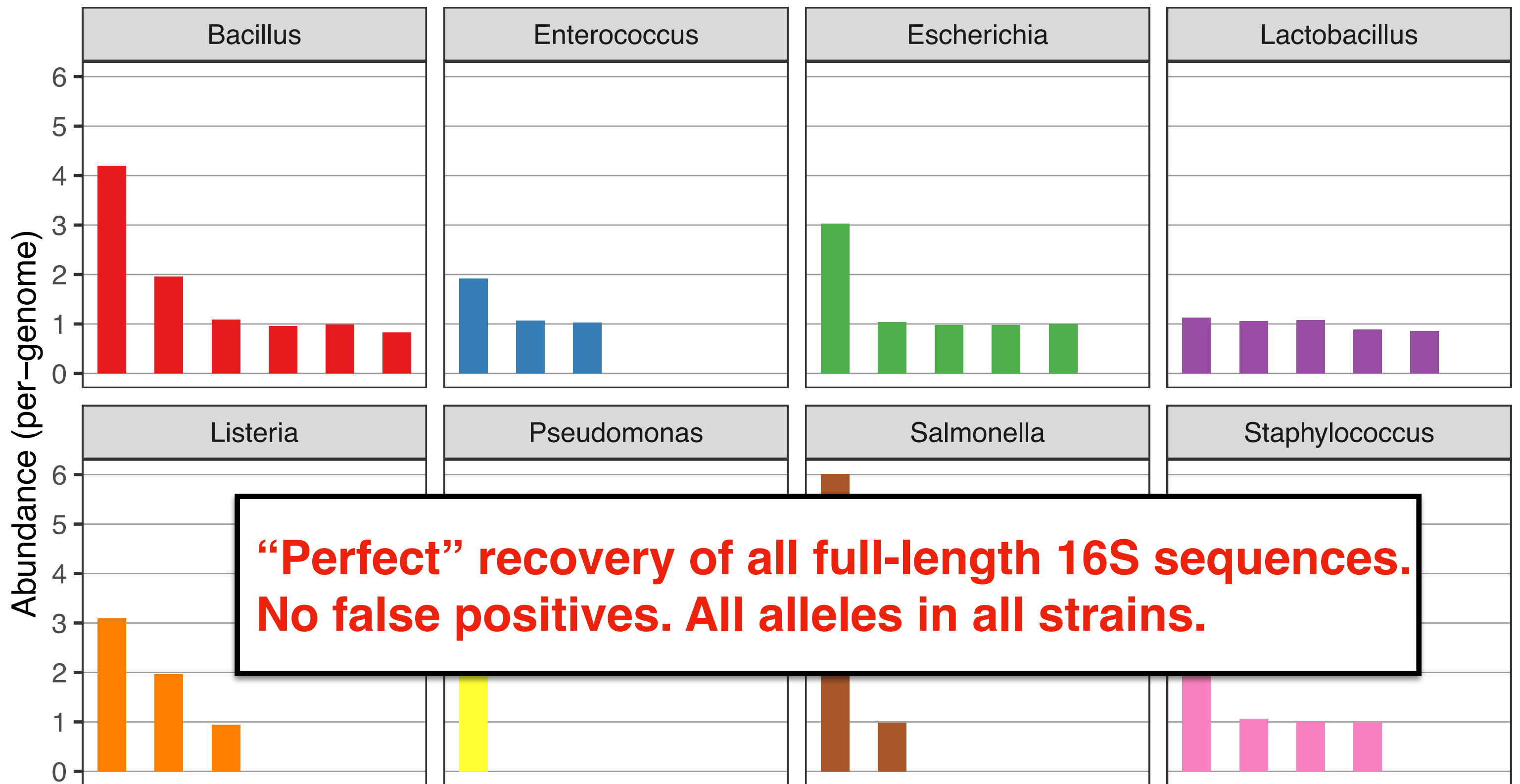
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**PacBio  
HiFi +  
DADA2**



**“Perfect” recovery of all full-length 16S sequences.  
No false positives. All alleles in all strains.**

Full-length 16S Sequence Variants

# Full-Length, Full-Complement 16S

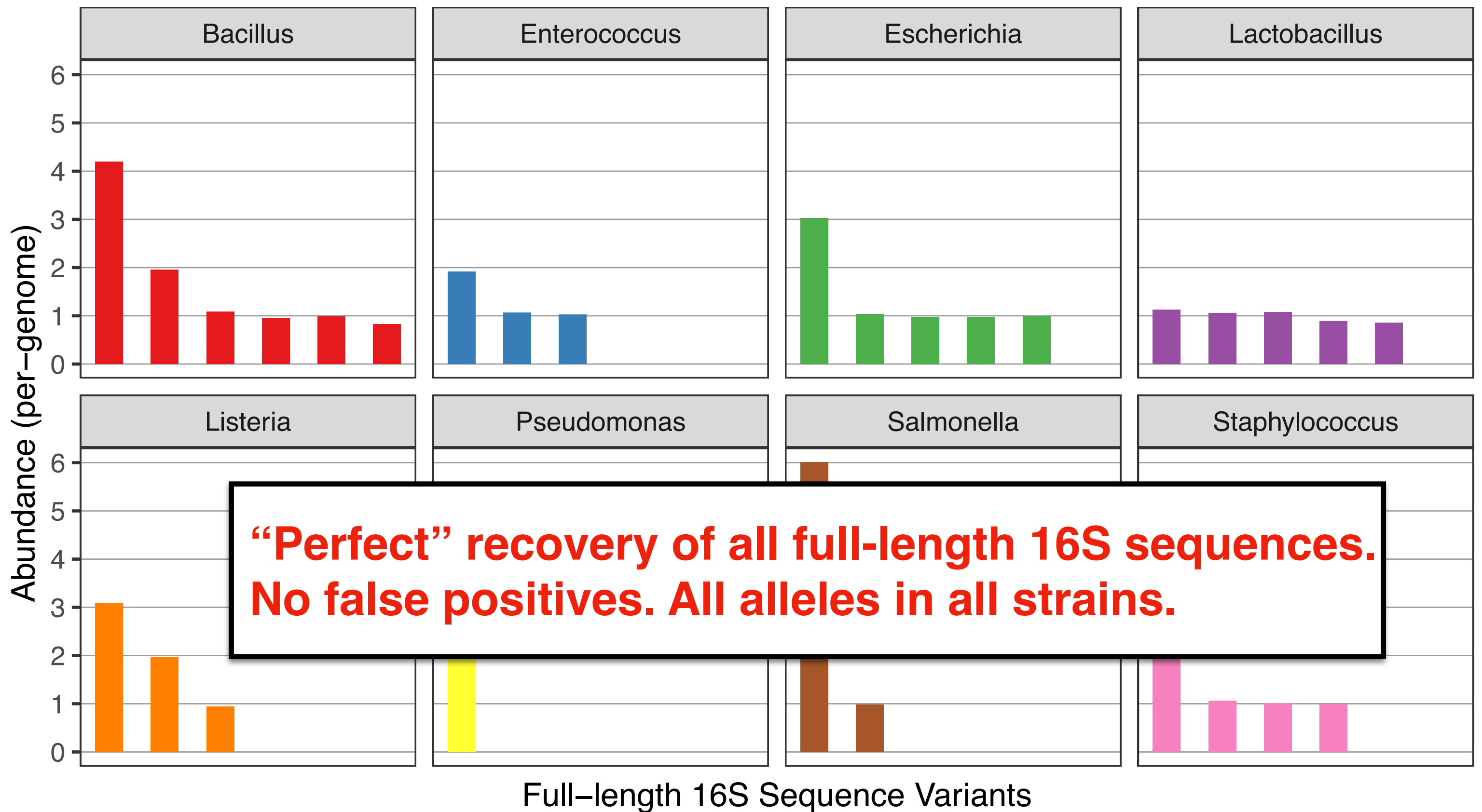
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**PacBio  
HiFi +  
DADA2\***



\* Modestly modified workflow for long-read amplicon sequencing.

## PacBio HiFi

### High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution

[Benjamin J Callahan](#) , [Joan Wong](#), [Cheryl Heiner](#), [Steve Oh](#), [Casey M Theriot](#), [Ajay S Gulati](#), [Sarah K McGill](#), [Michael K Dougherty](#)

*Nucleic Acids Research*, Volume 47, Issue 18, 10 October 2019, Page e103,

<https://doi.org/10.1093/nar/gkz569>

**Published:** 03 July 2019 **Article history** ▼

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**Highly-accurate long reads  
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But do they really  
work for amplicon sequencing?**

**Yes!**  
Multiple technologies  
Out to 6+ kilo bases  
>99.9% accuracy

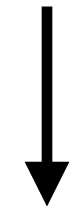


*We support long-read amplicon sequencing!*



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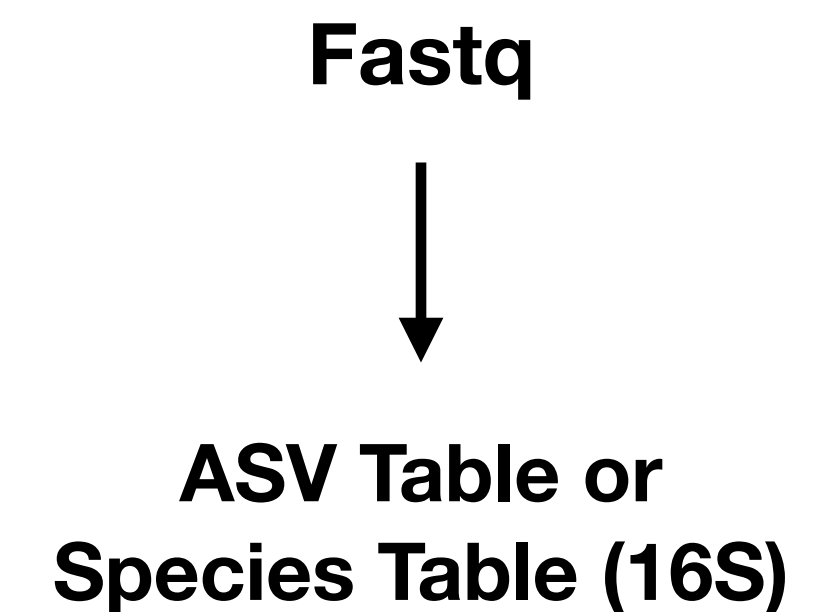
Fastq



**ASV Table or  
Species Table (16S)**



*We support long-read amplicon sequencing!*



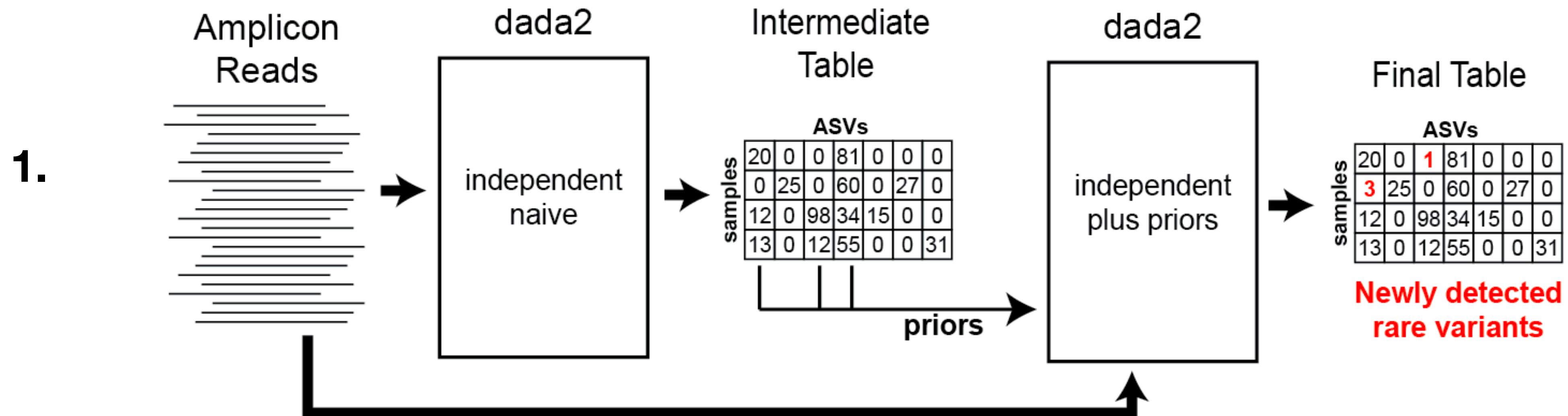
---

## Long-read specific documentation

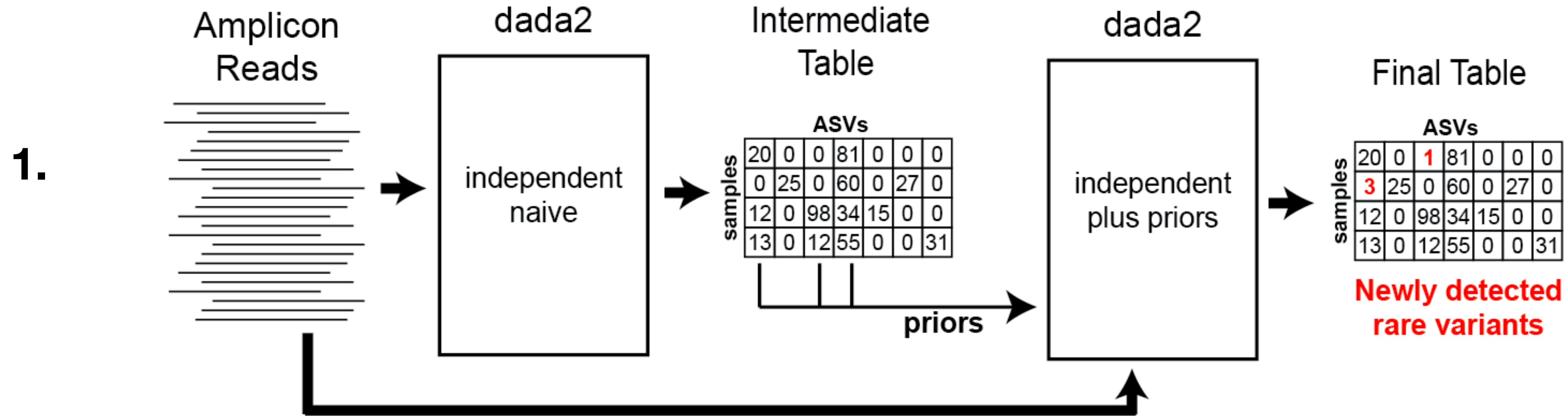
**PacBio HiFi:** <https://github.com/benjjneb/LRASmanuscript>

**LoopSeq:** <https://github.com/benjjneb/LoopManuscript>

## Pseudo-Pooling

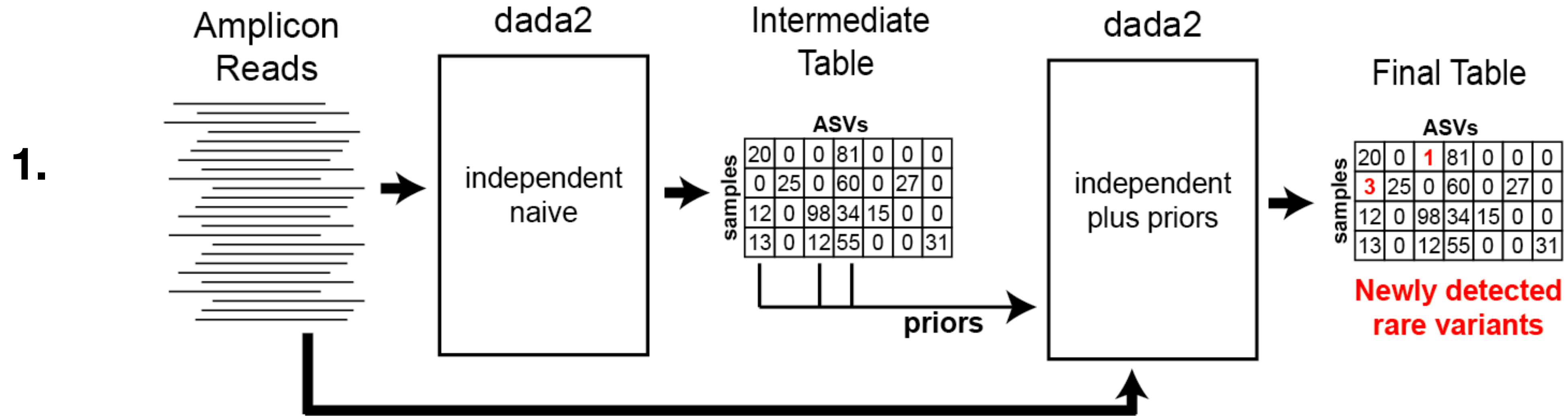


## Pseudo-Pooling



2. DETECT\_SINGLETONS=TRUE

## Pseudo-Pooling

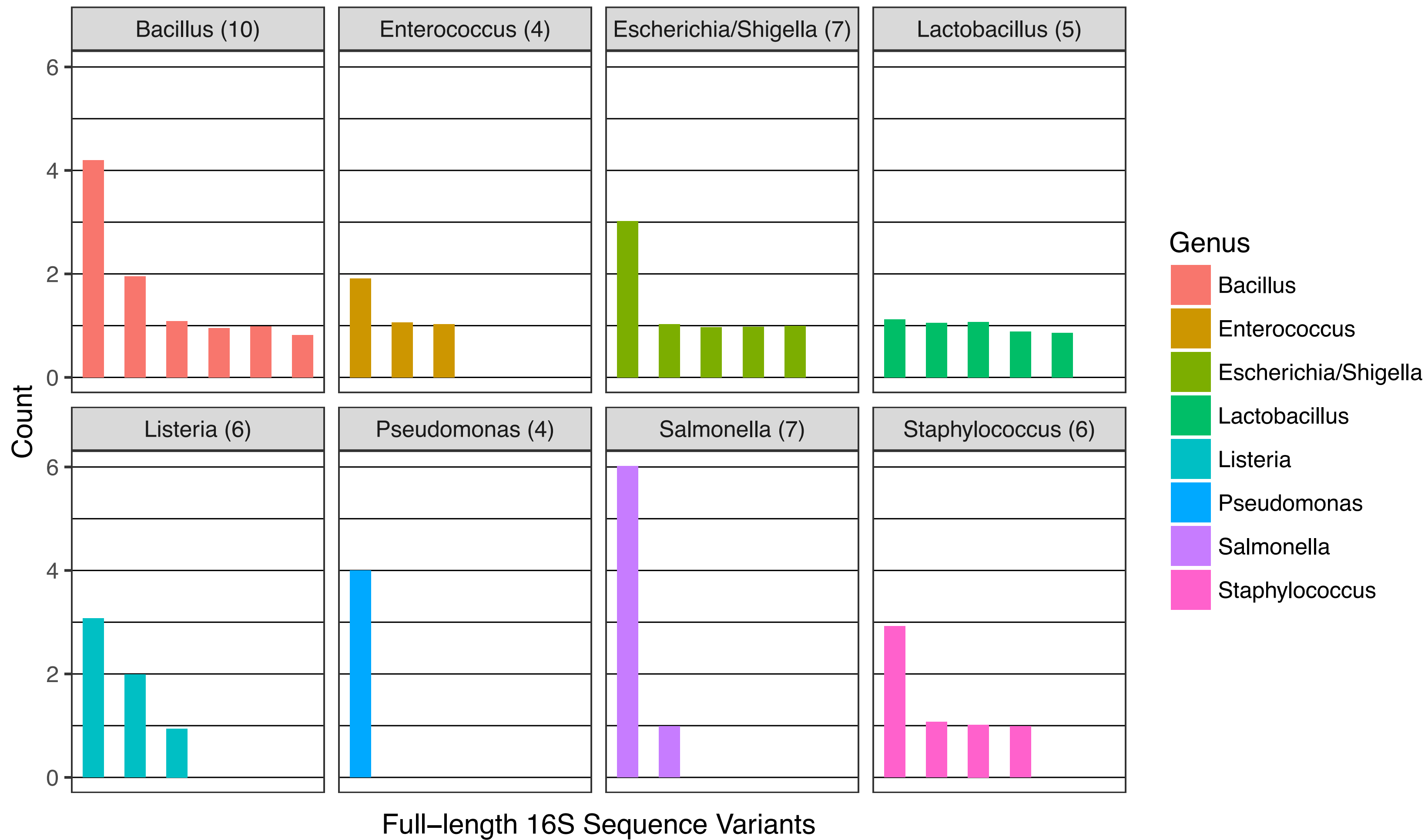


2. DETECT\_SINGLETONS=TRUE

3. Long-read specific parameters.

# Sub-species bacterial profiling

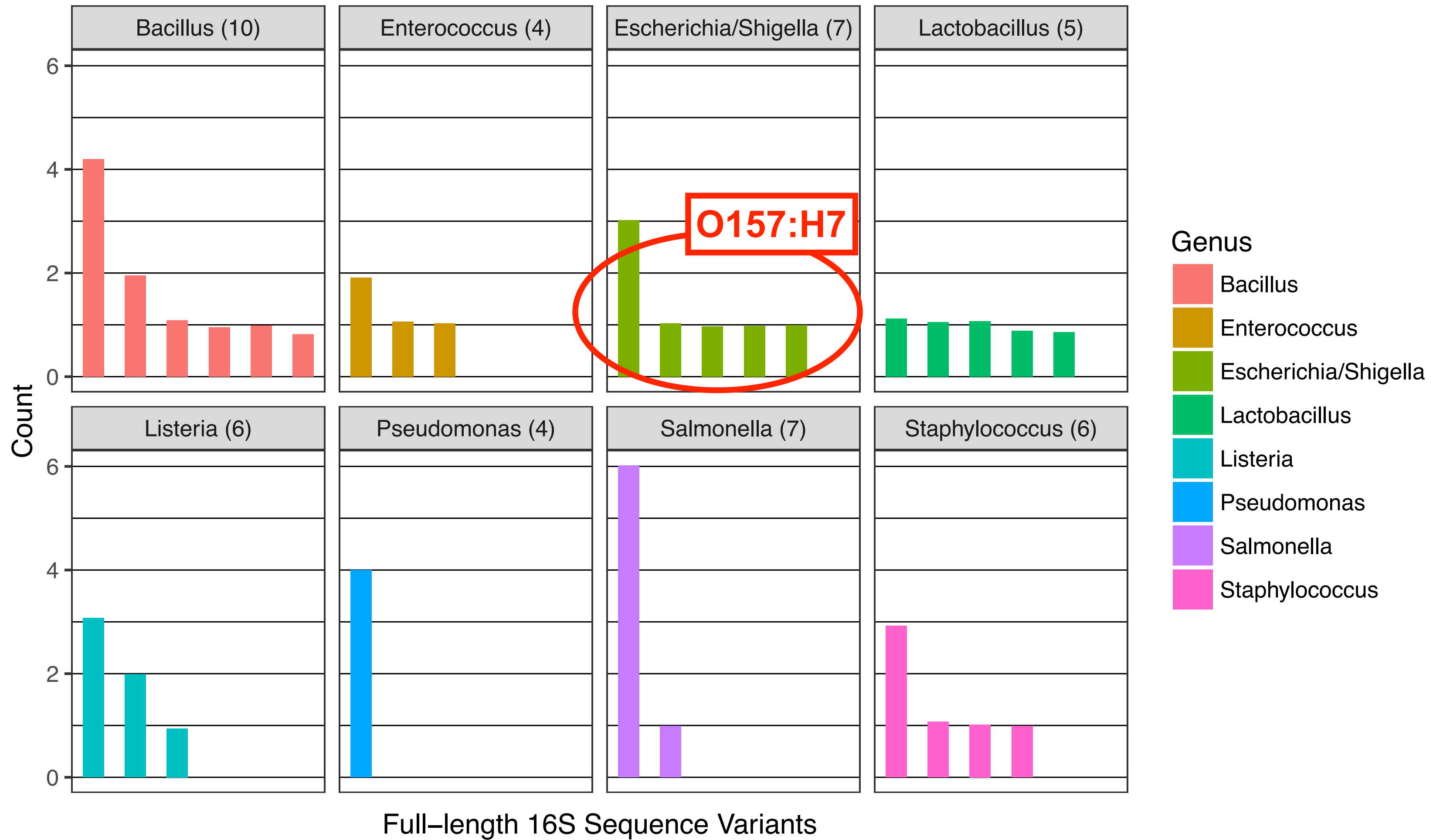
*Full-length 16S from Zymo mock community*





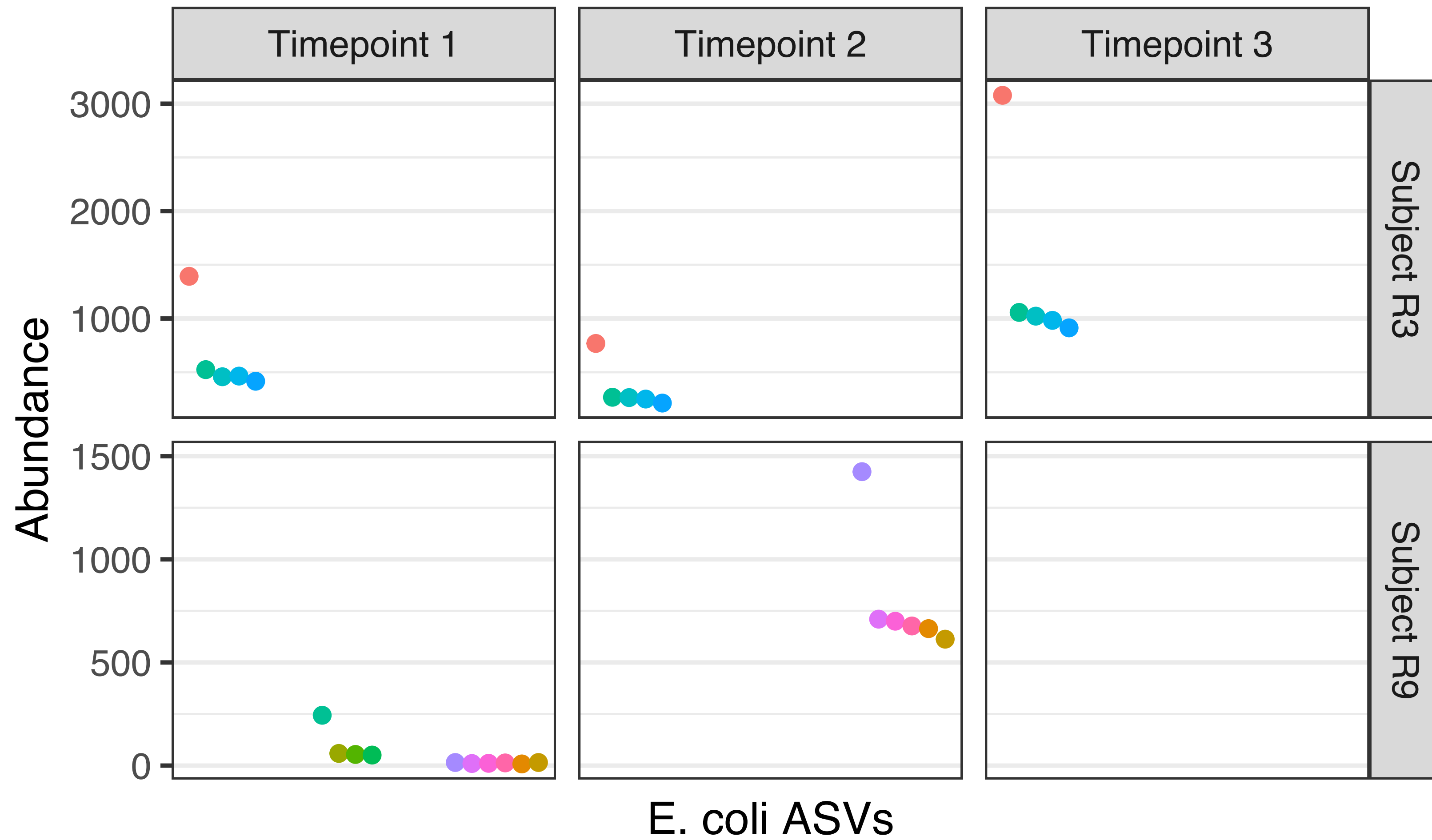
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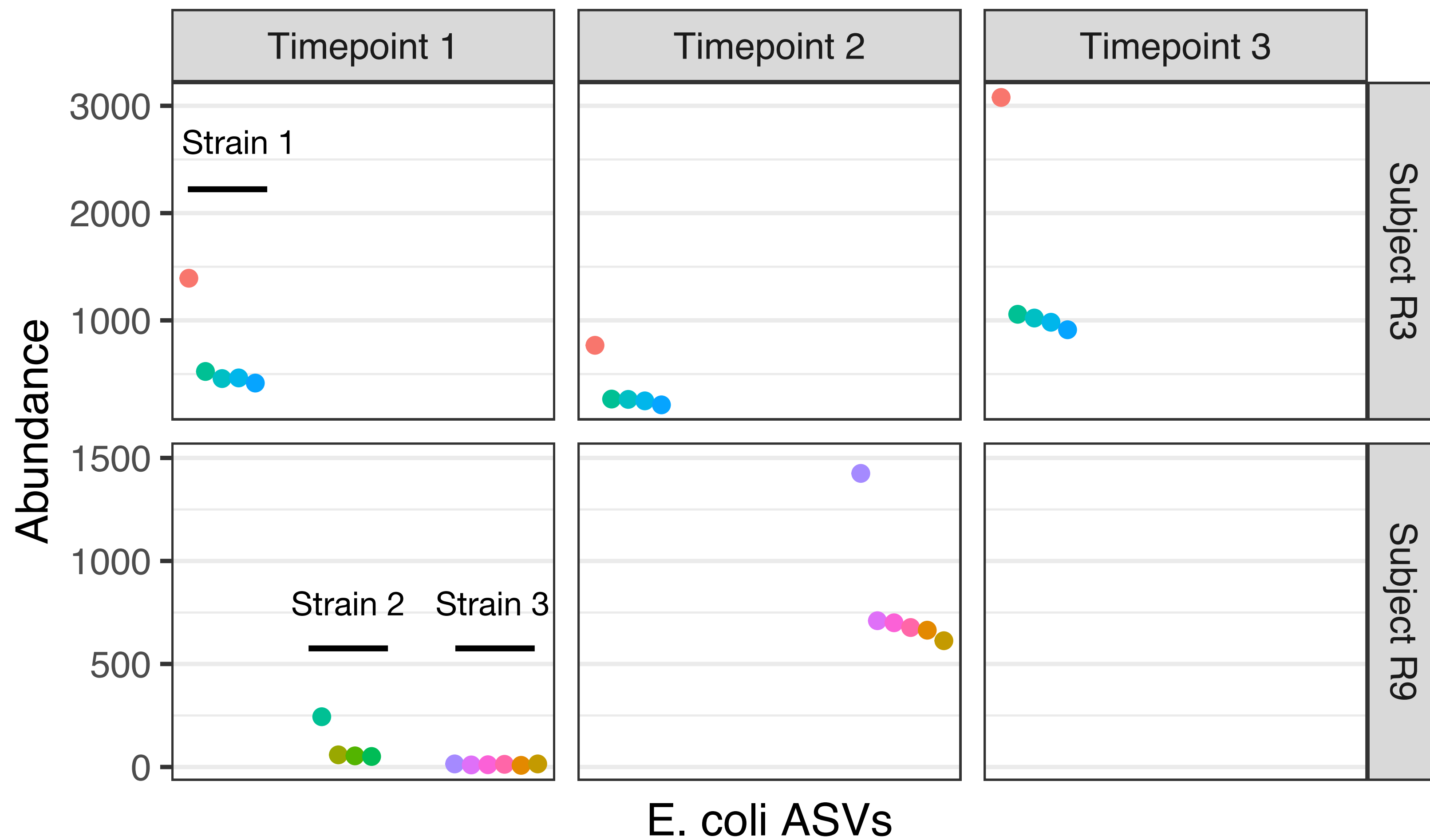
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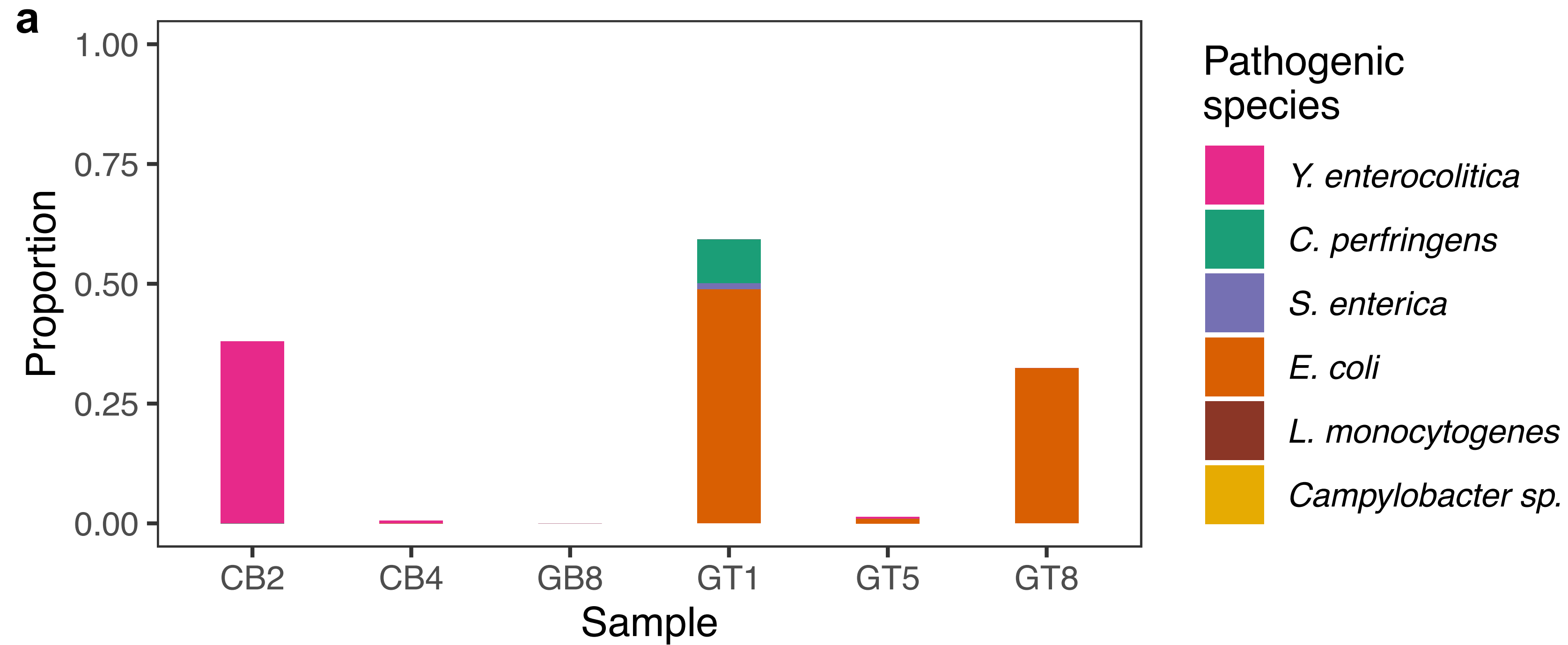
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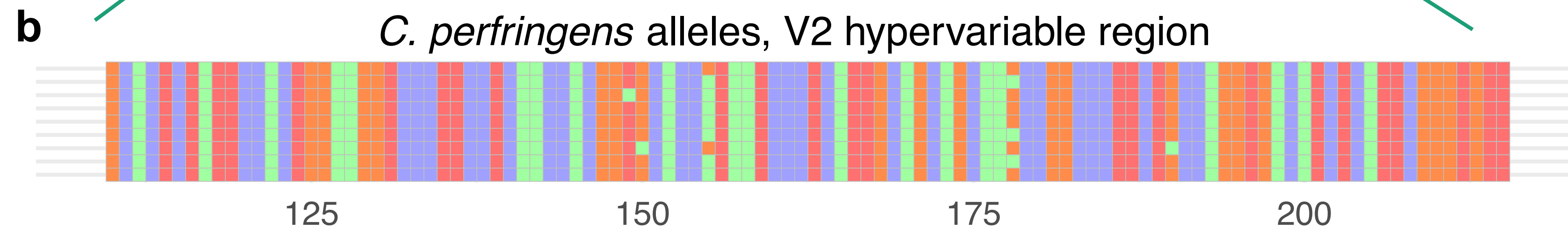
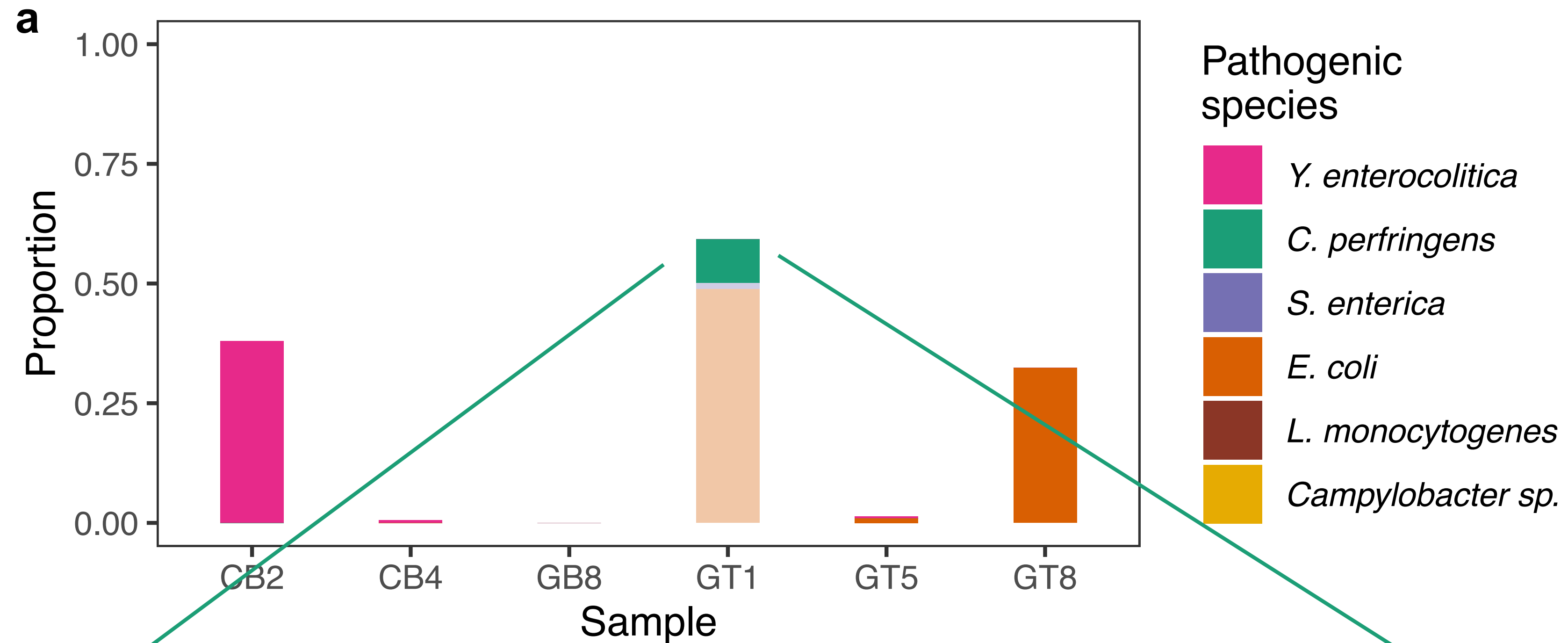
# Sub-species bacterial profiling

*Full-length 16S from retail meat samples*



# Sub-species bacterial profiling

Full-length 16S from retail meat samples

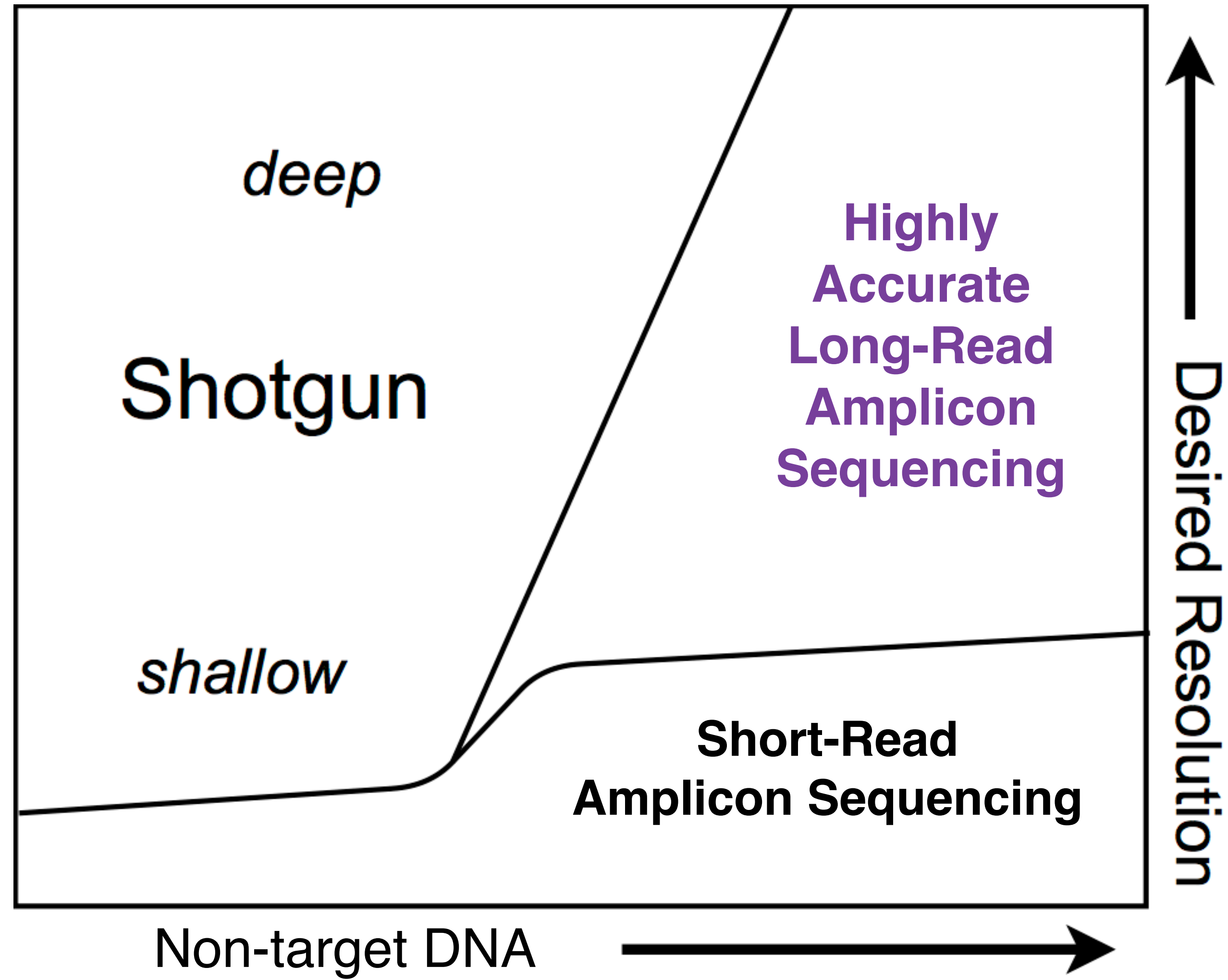


Full genomic complement of 16S alleles

## Limitations

- PCR Biases
- PCR Length Scaling (past ~5 kilo bases)
- Cost-per-base
- Chimeras
- Tooling (especially sub-species assignment)
- ...

# Highly Accurate Long-Read Amplicon Seq



## Limitations

- PCR Biases
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- Chimeras
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- ...



## “Serovar-level Detection of Bacterial Foodborne Pathogens Using Long-read Amplicon Sequencing”



**Dmitry Grinevich**



Sid Thakur and lab



National Institute of  
Food and Agriculture

**Coming soon!**

## **Bioinformatics**

Susan Holmes  
Joey McMurdie  
Michael Rosen  
Dmitry Grinevich

## **Applications**

Dmitry Grinevich  
Casey Theriot  
Siddhartha Thakur



## **Technology (PacBio)**

Joan Wong  
Cheryl Heiner  
Steve Oh



## **Technology (Loop Genomics)**

Tuval Ben Yehezkel  
Michael Balamotis

