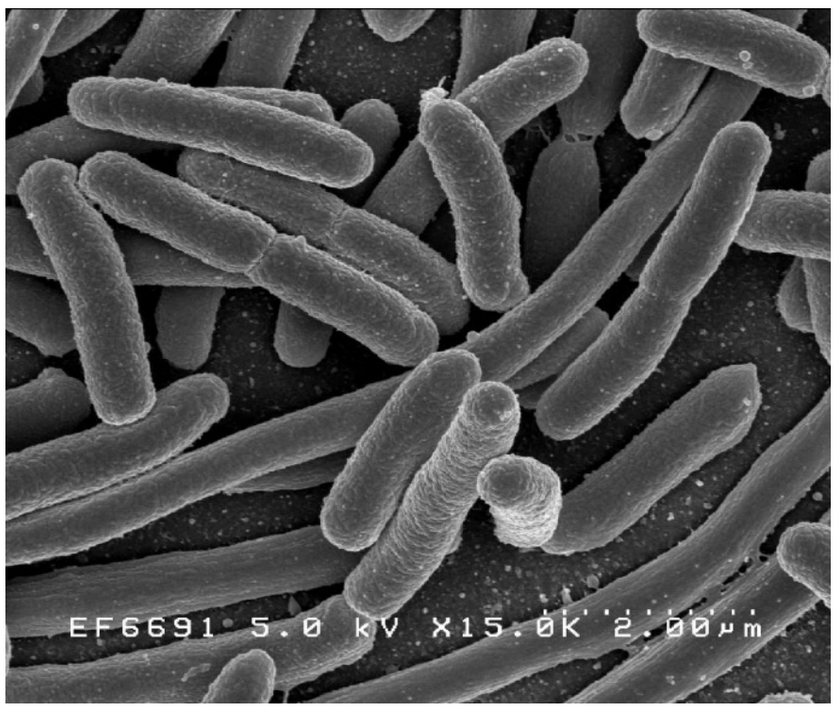
Amplicon Sequencing

OTUs and ASVs

A Microbial Census

Metabarcoding or Marker-gene Sequencing



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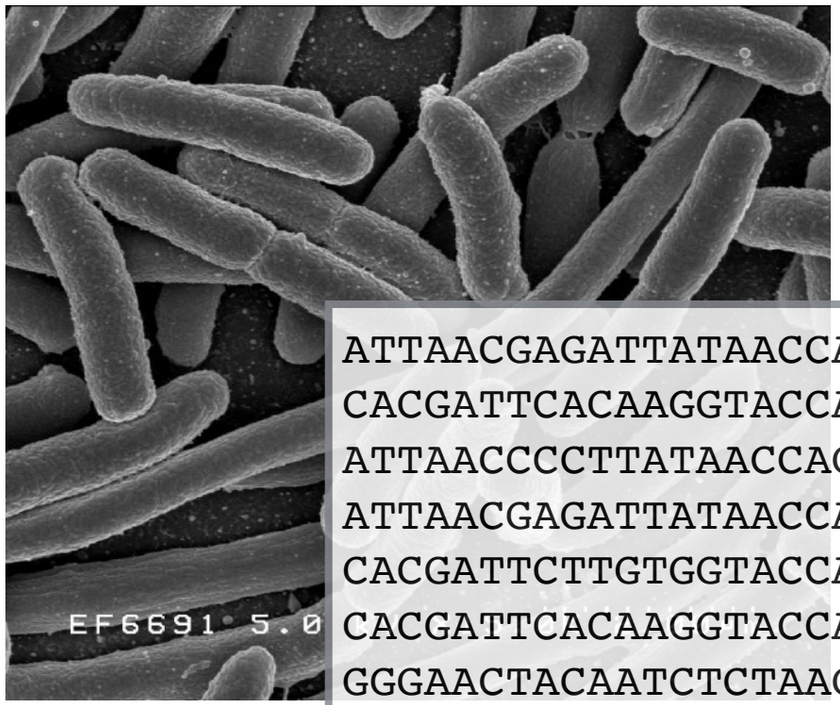


```
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CACGATTCACAAGGTACCACAAGGTAACATAGCTCC
ATTAACCCCTTATAACCAGAGTACGAATACCGAACA
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CACGATTCTTGTGGTACCACAAGGTAACATAGCTCC
CACGATTCACAAGGTACCACAAGGTAACATAGCTCC
GGGAACTACAATCTCTAAGGTGAAGTCTCAGTCTAT
ATTAACGAGATTATAACCAGAGTACGAATACCGAAC
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```

A Microbial Census

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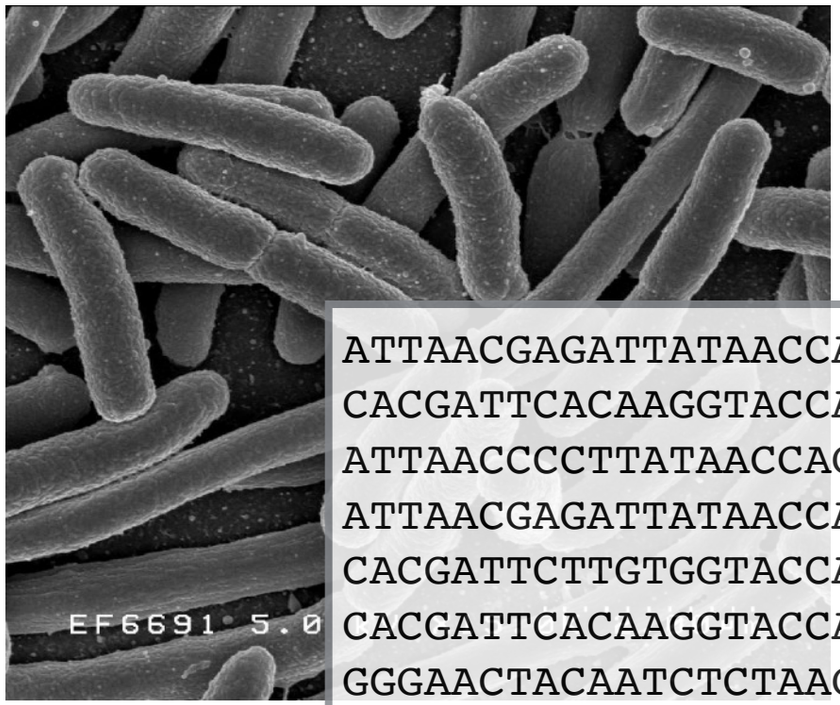


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CACGATTCTTGTGGTACCACAAGGTAACATAGCTCC
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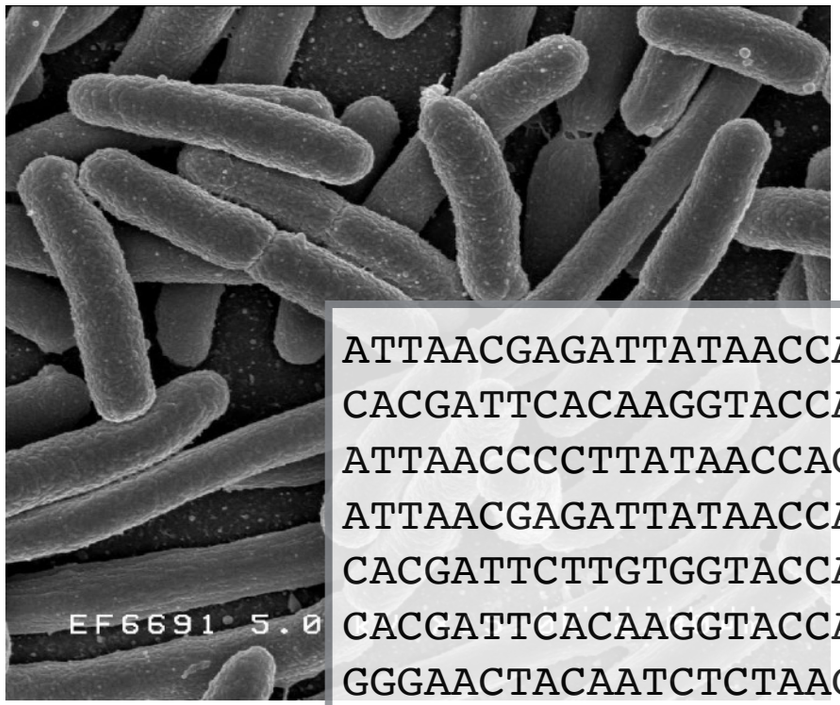
GGGAACTACAATCTCTAAGGTGAAGTCTCAGTCTAT
ATTAACGAGATTATAACCAGA
CACGATTCACAAGGTACCACA
ATTAACGAGATTATAACCAGA
```

Lactobacillus crispatus	1300	5	0	882	596
Ureaplasma urealytica	15	0	220	0	0
Gardnerella vaginalis	22	0	1	0	412
Prevotella intermedia	0	0	8	12	0
...	...	...	...	...	...

A Microbial Census

Metabarcoding or Marker-gene Sequencing

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...	...	...	...	...	...

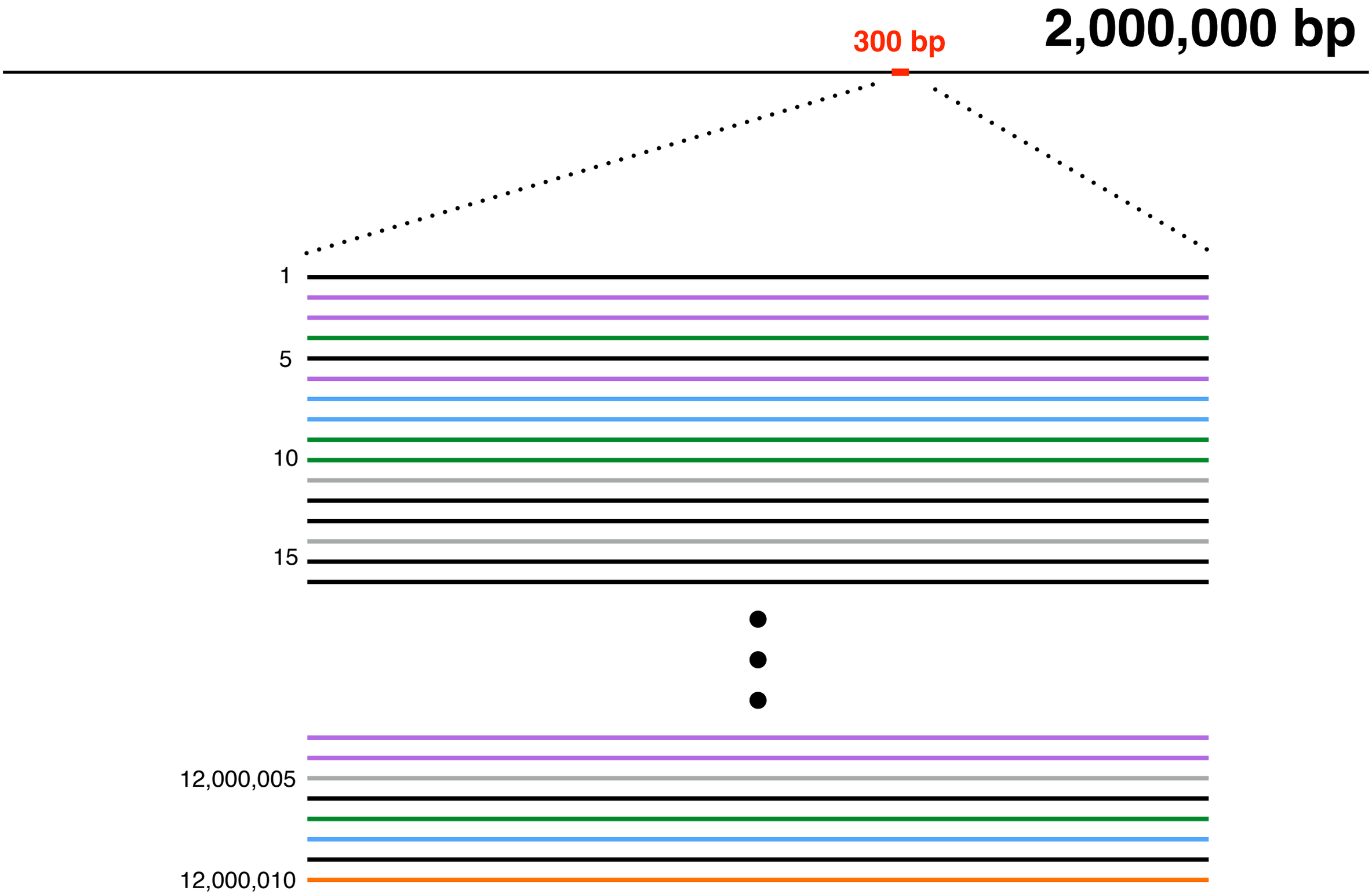
Inference

Visualization

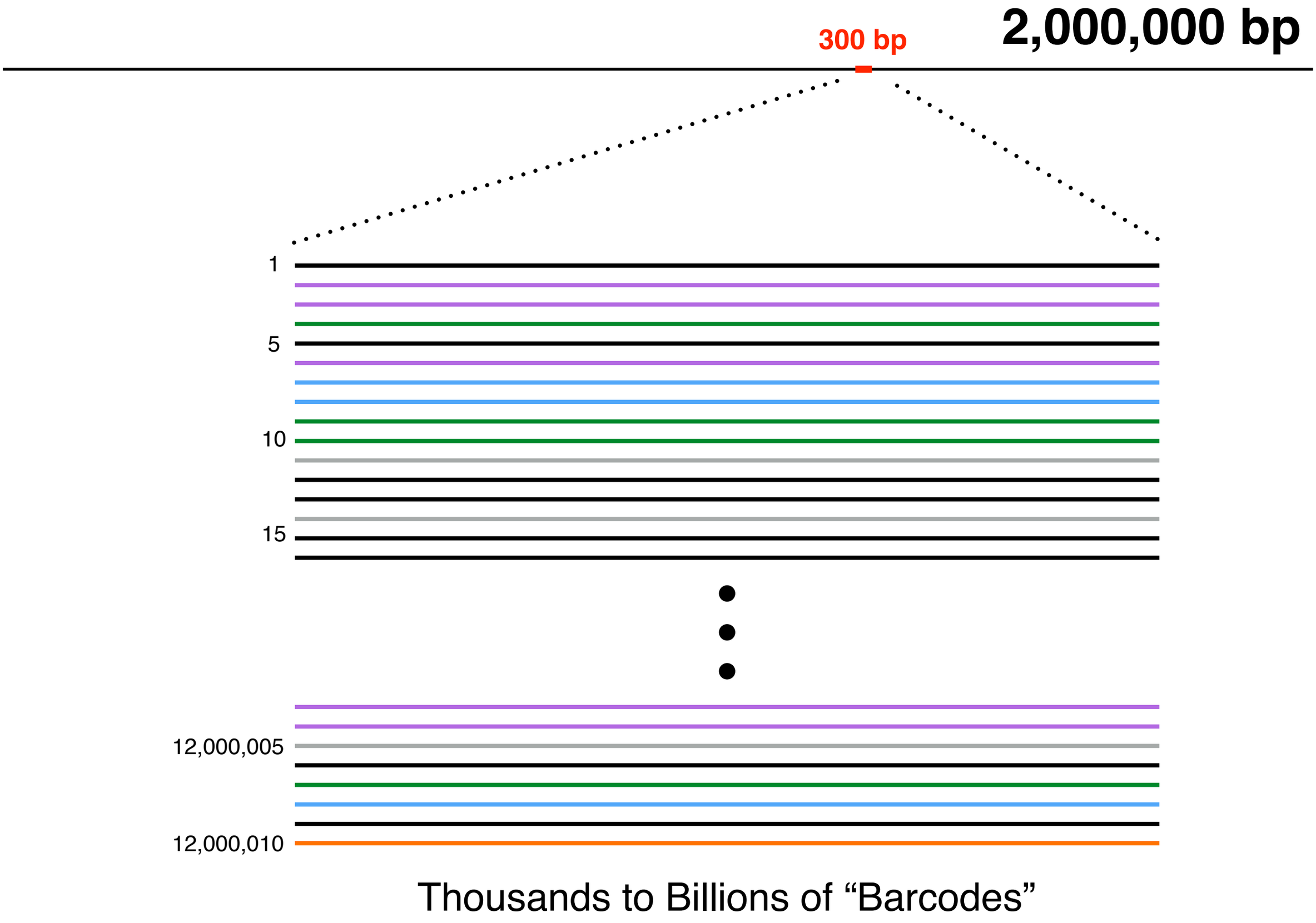
Exploration

Marker-gene Sequencing

Marker-gene Sequencing

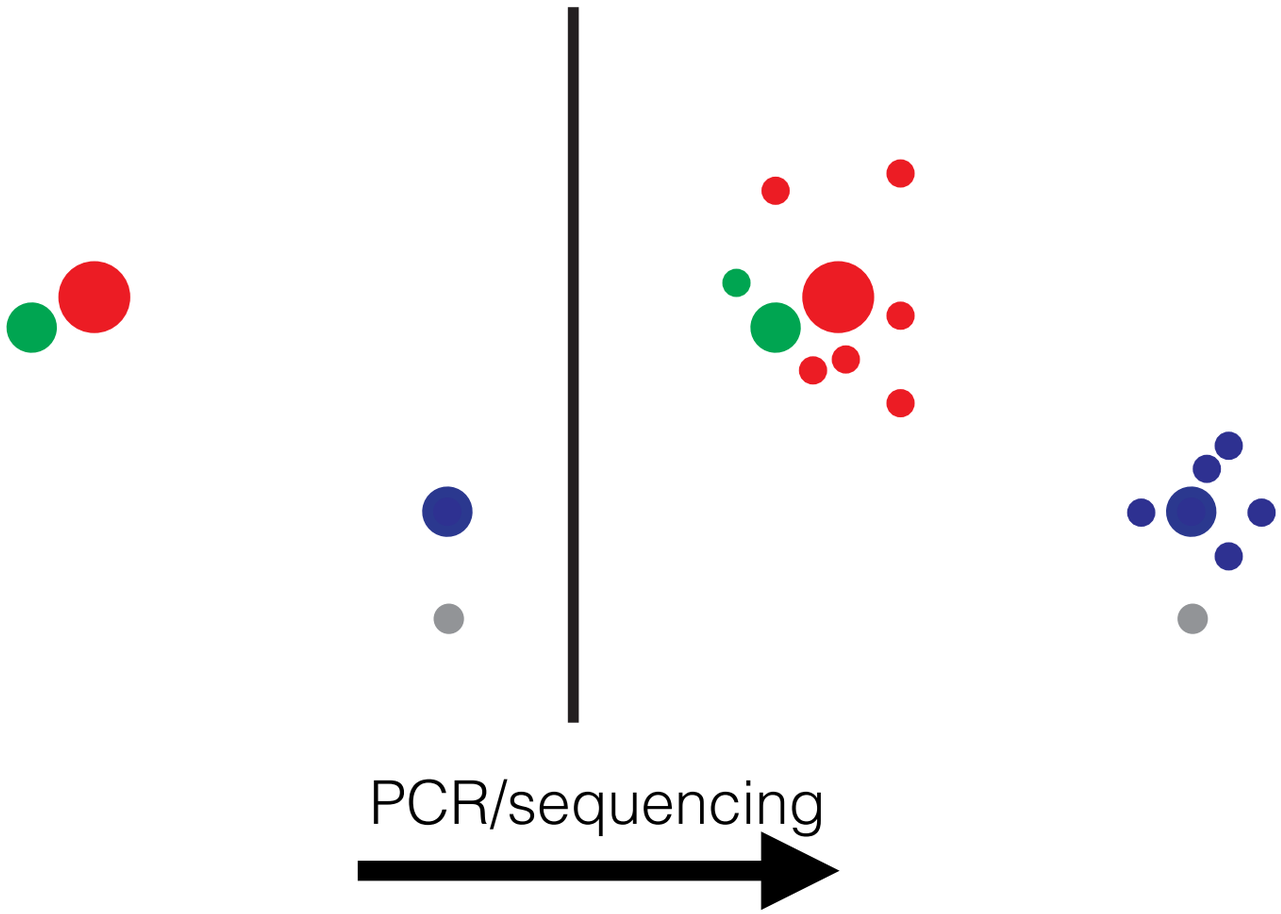


Marker-gene Sequencing

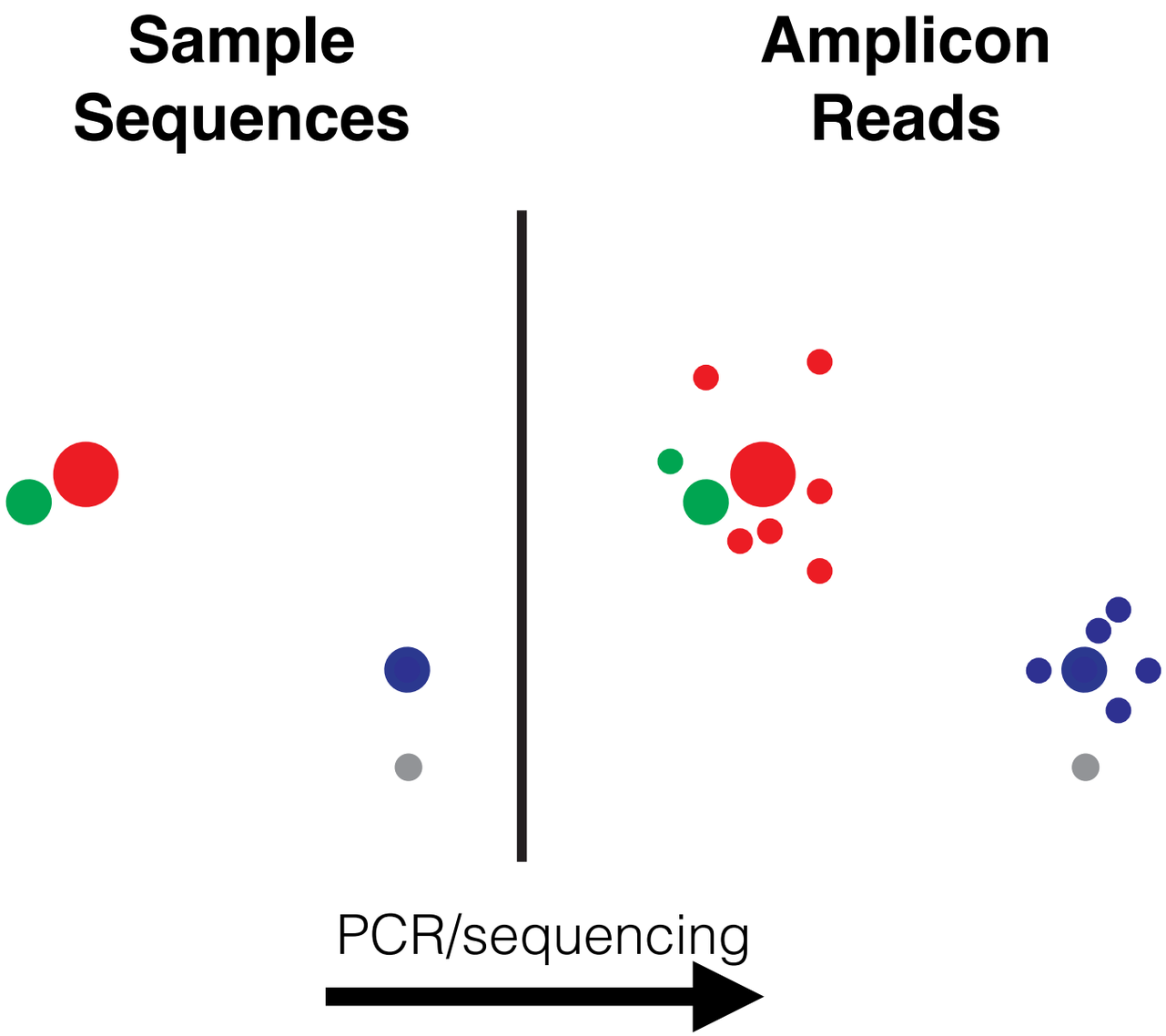


Sample Sequences

Amplicon Reads



PCR/sequencing

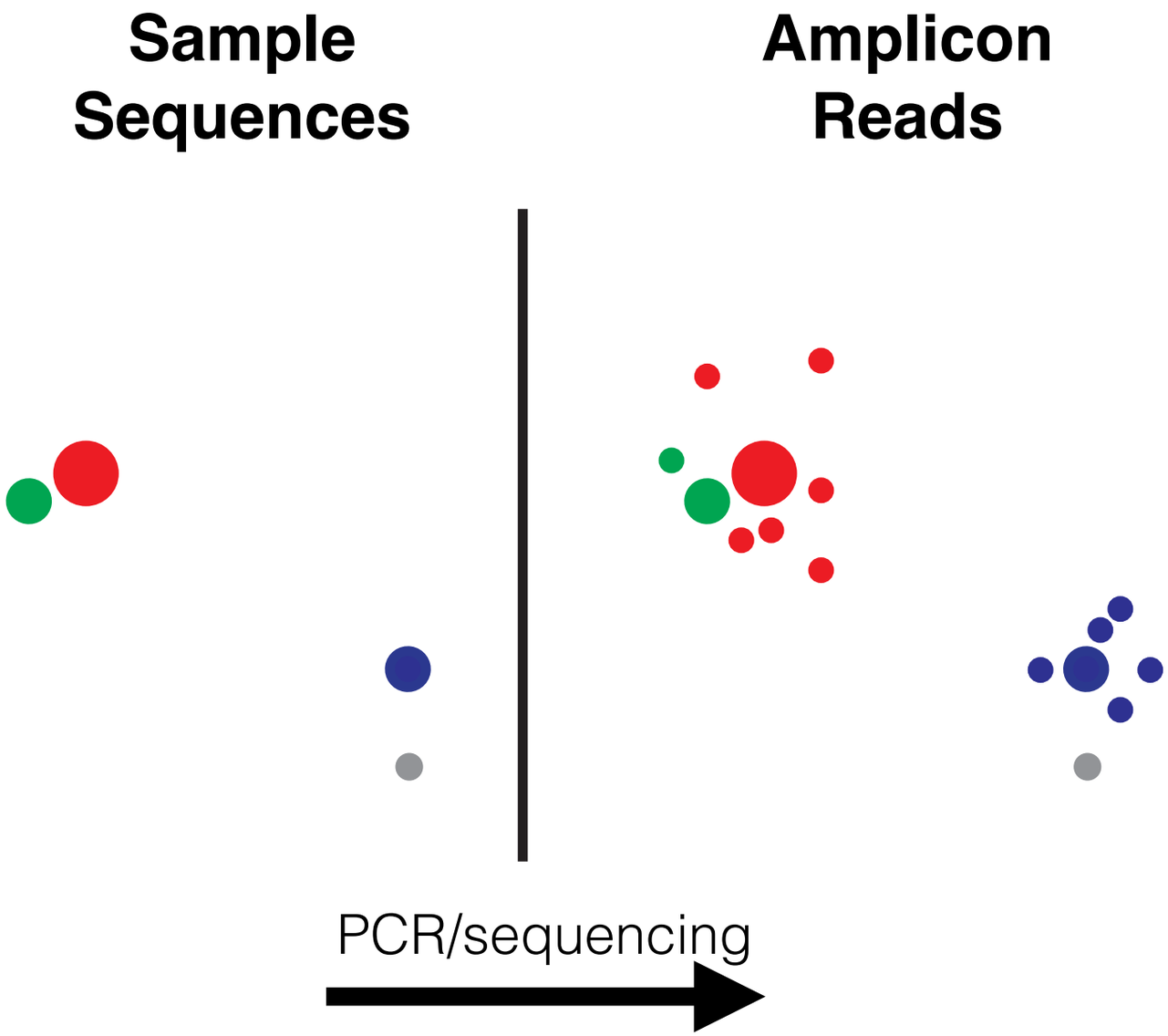


Two Challenges

How do we deal with errors?

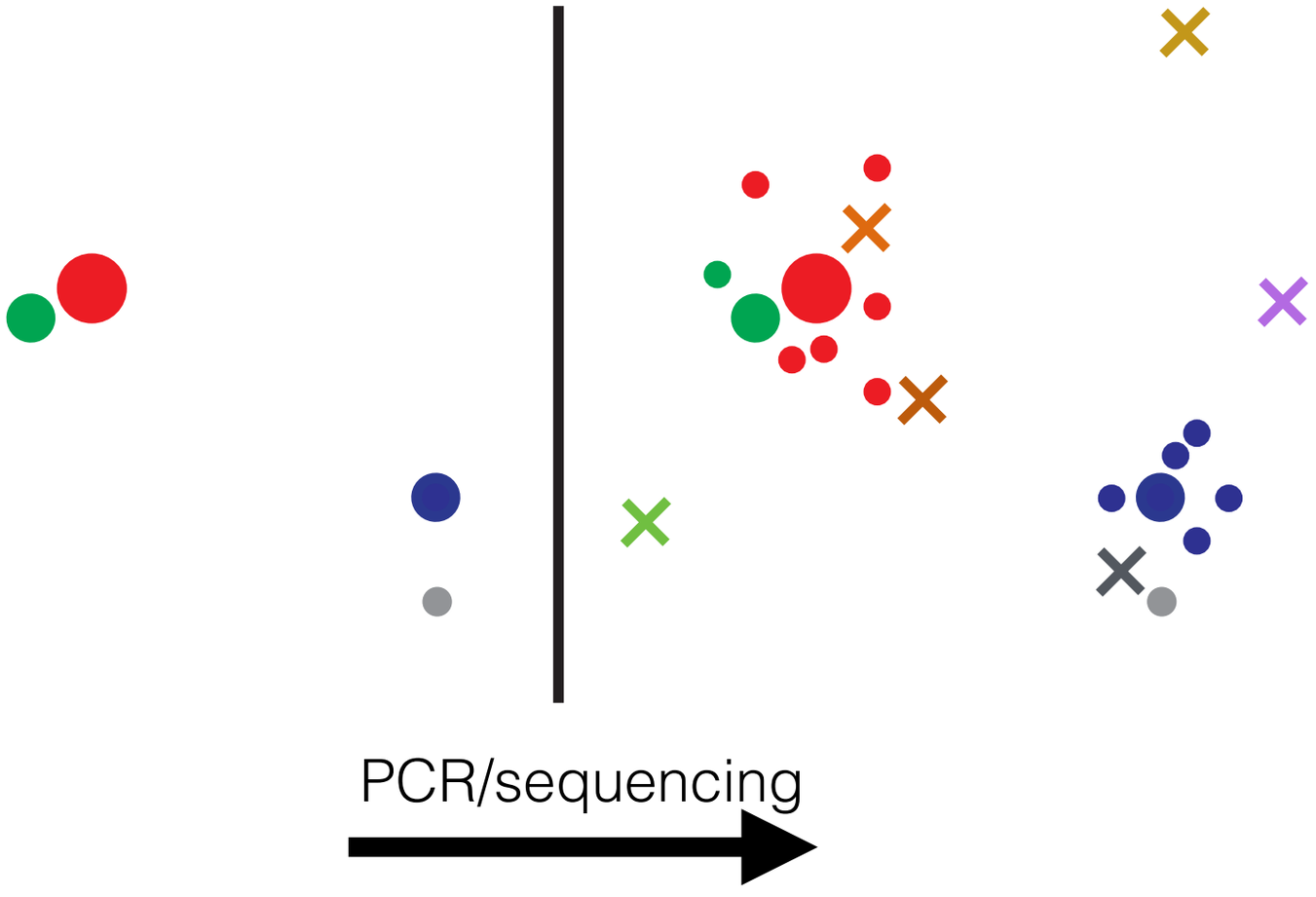
How do we define the units of our analysis?

Closed reference OTUs



Sample Sequences

Amplicon Reads

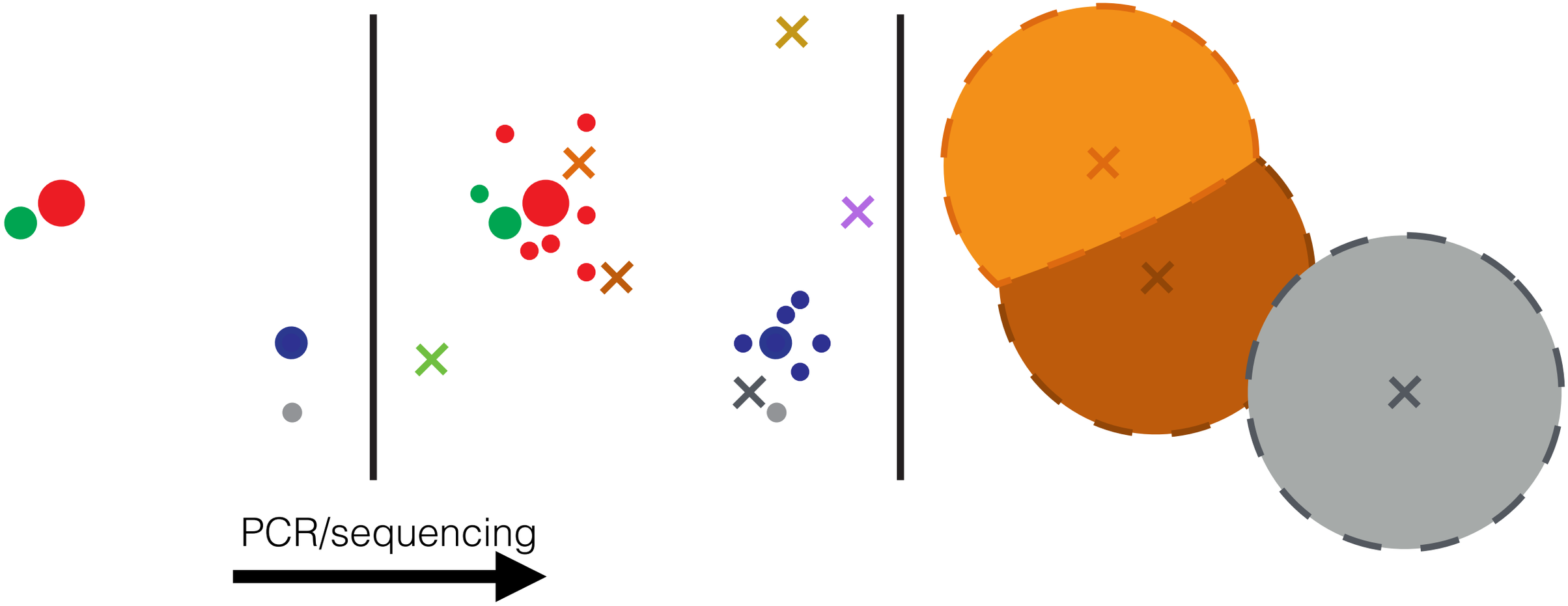


PCR/sequencing

Sample Sequences

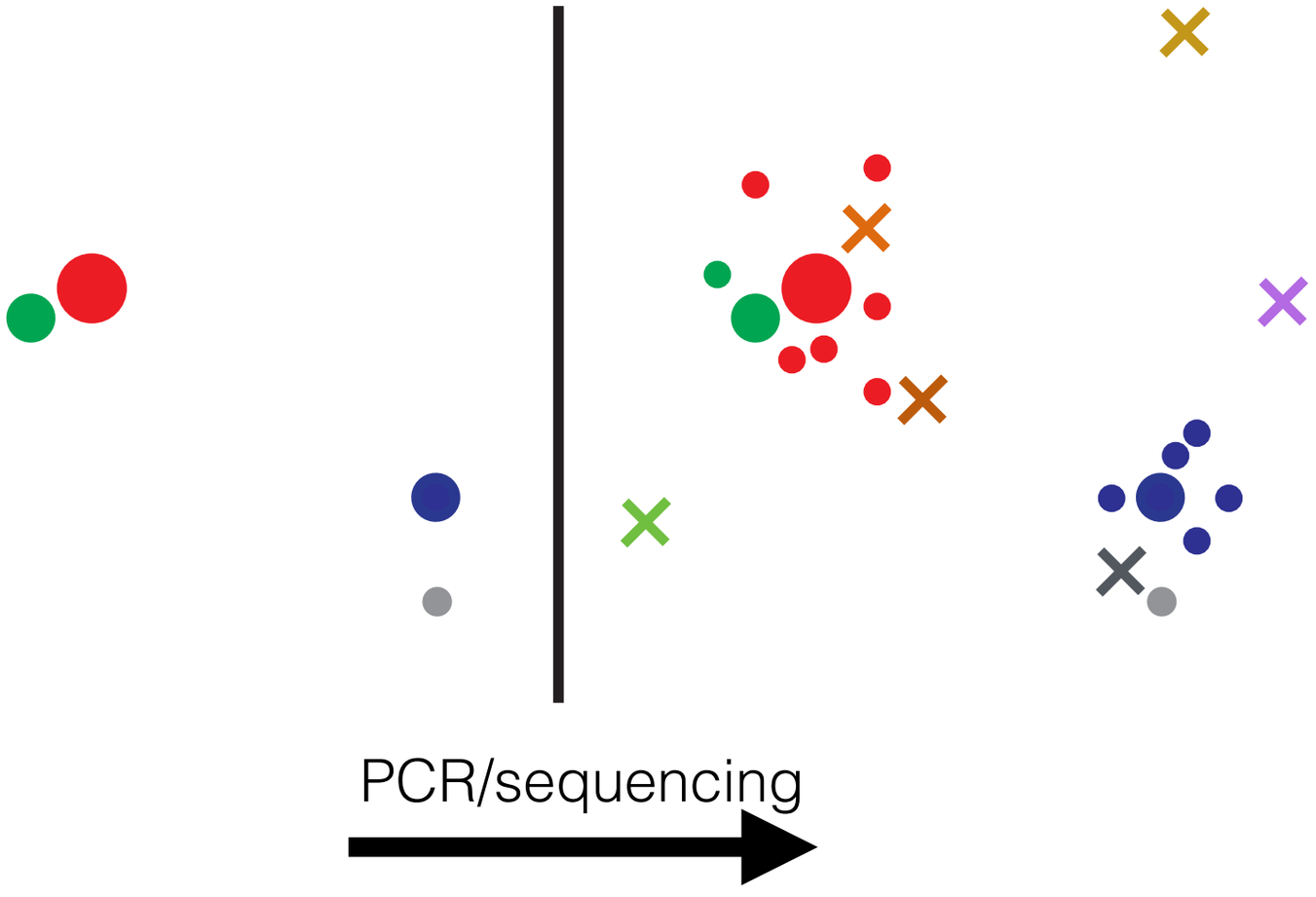
Amplicon Reads

Operational Taxonomic Units (closed reference)



Sample Sequences

Amplicon Reads

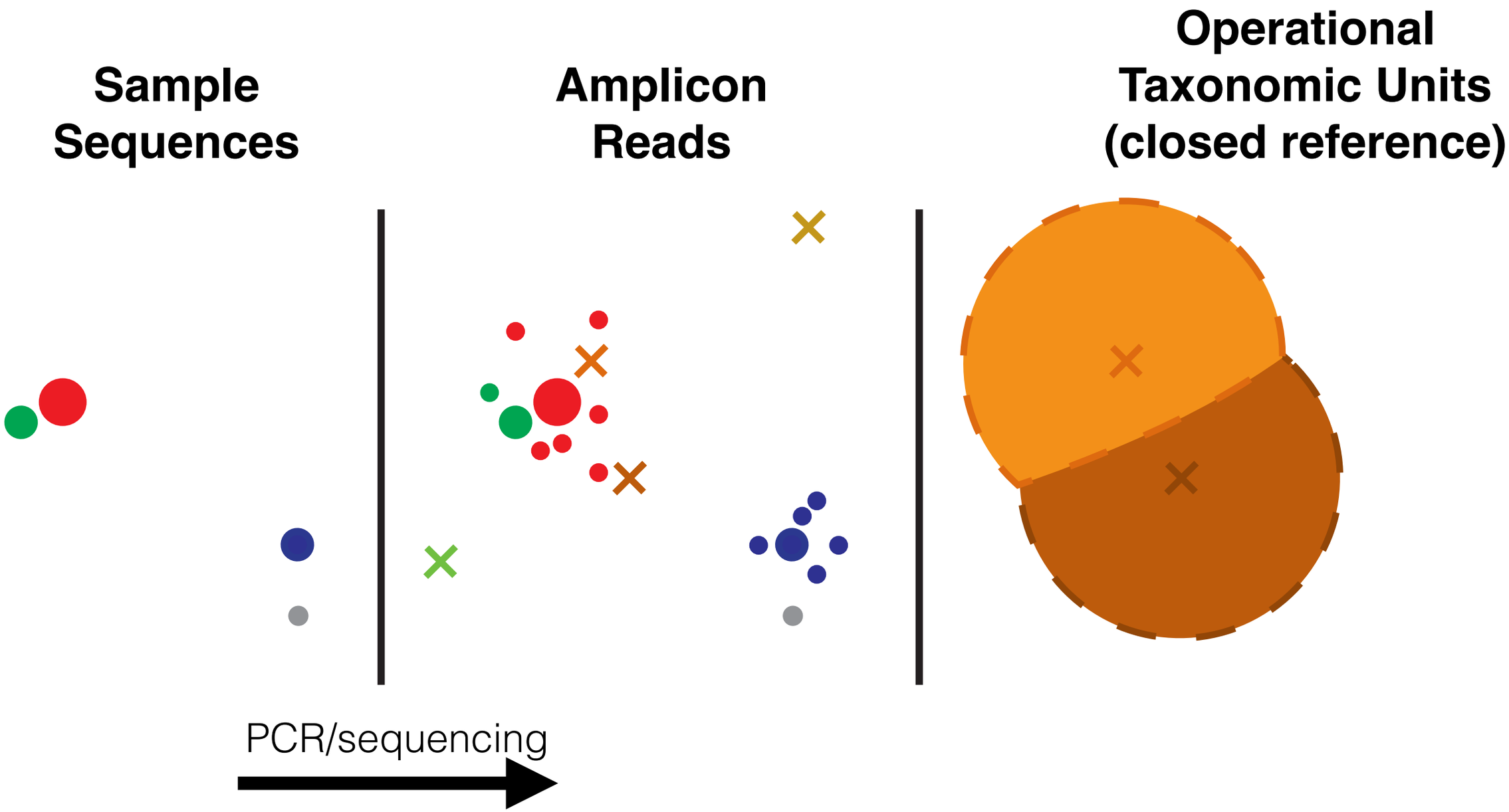


PCR/sequencing

Sample Sequences

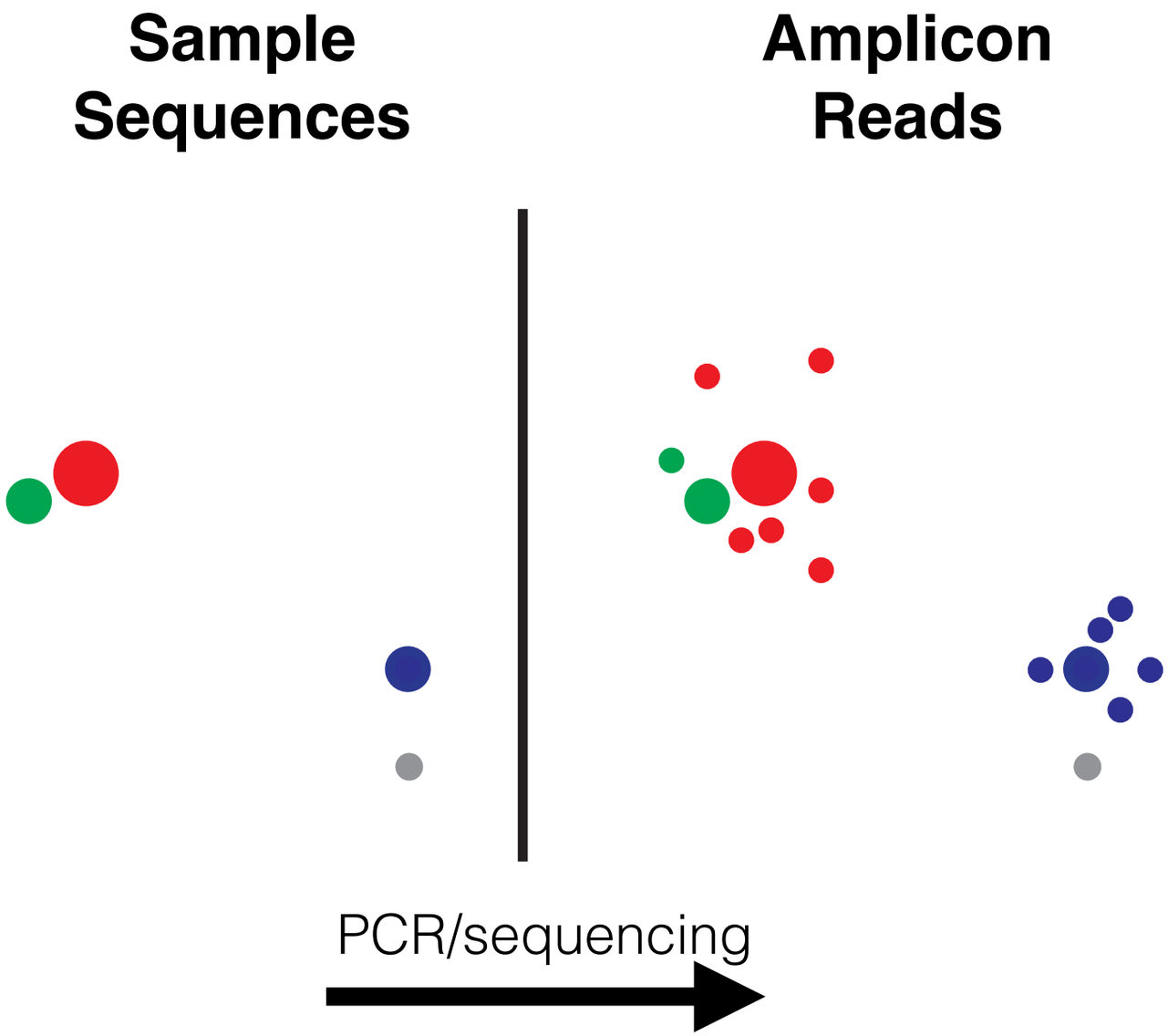
Amplicon Reads

PCR/sequencing



Incomplete OTU table!

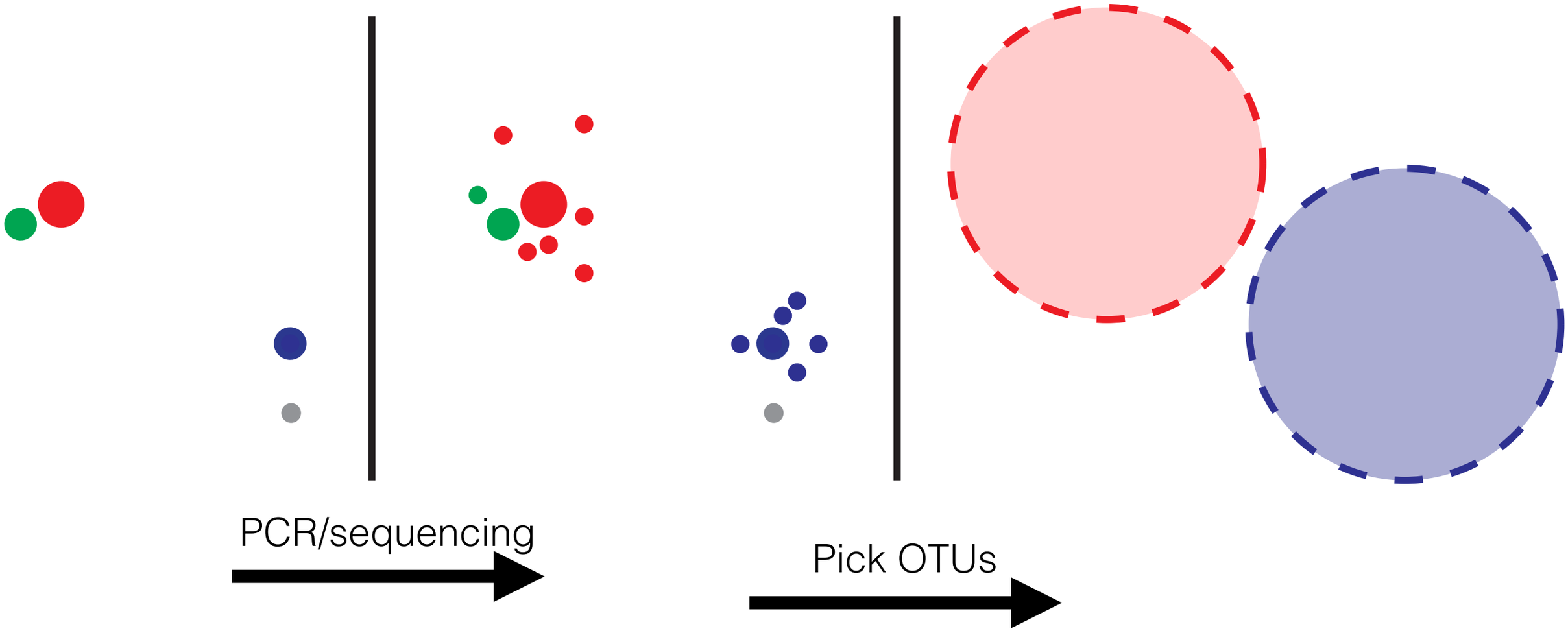
de novo OTUs



Sample Sequences

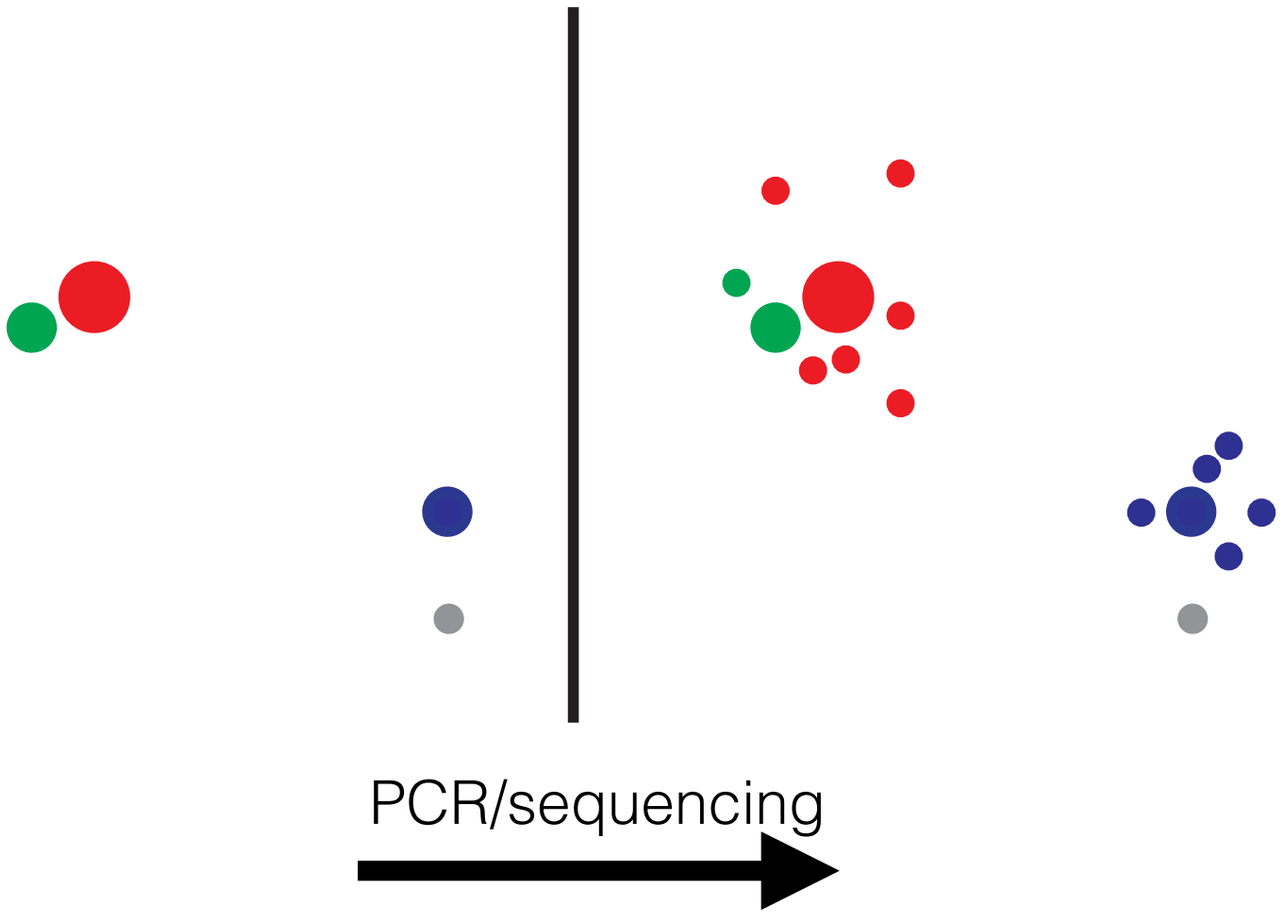
Amplicon Reads

Operational Taxonomic Units (de novo)



Sample Sequences

Amplicon Reads



PCR/sequencing

Sample Sequences

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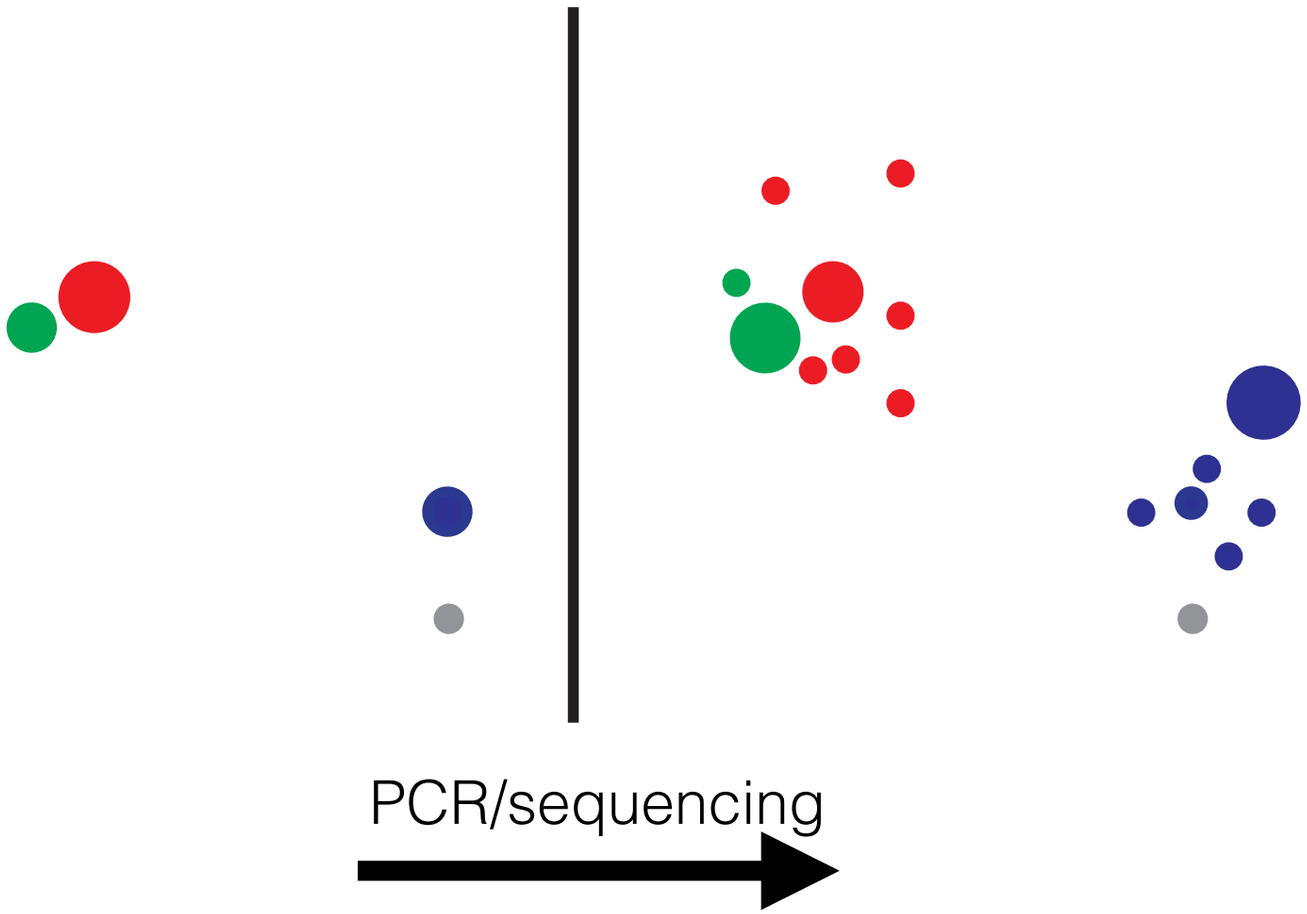

PCR/sequencing

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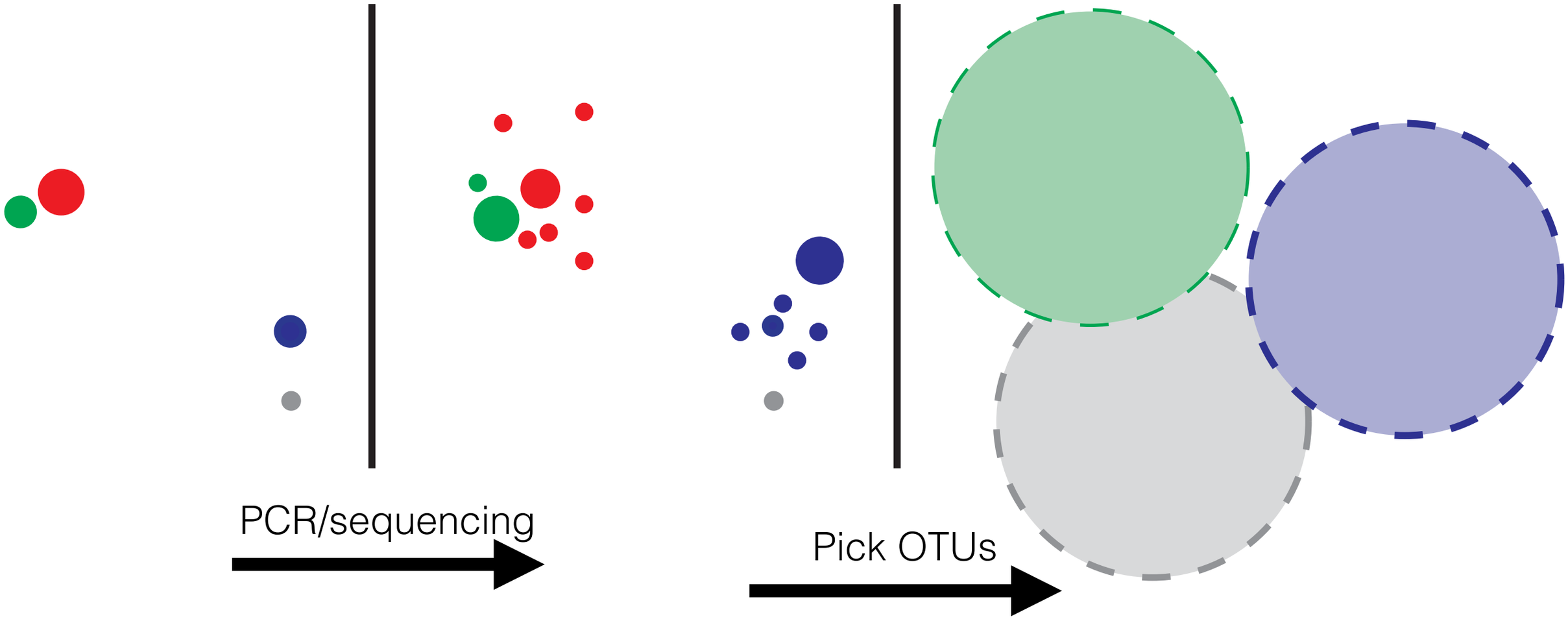


Different OTUs!

(In)consistent labels

`OTU85` is ***not a consistent label***

`OTU85` is *predictive of a disease?* **Not in future data!**

`OTU85` is *associated w/ X and Y?* **Can't be tested!**

`OTU85` is *in this community?* **OTUs don't exist in nature!**

McLaren & Callahan, mBio, 2018.

Exact Sequence Variants…

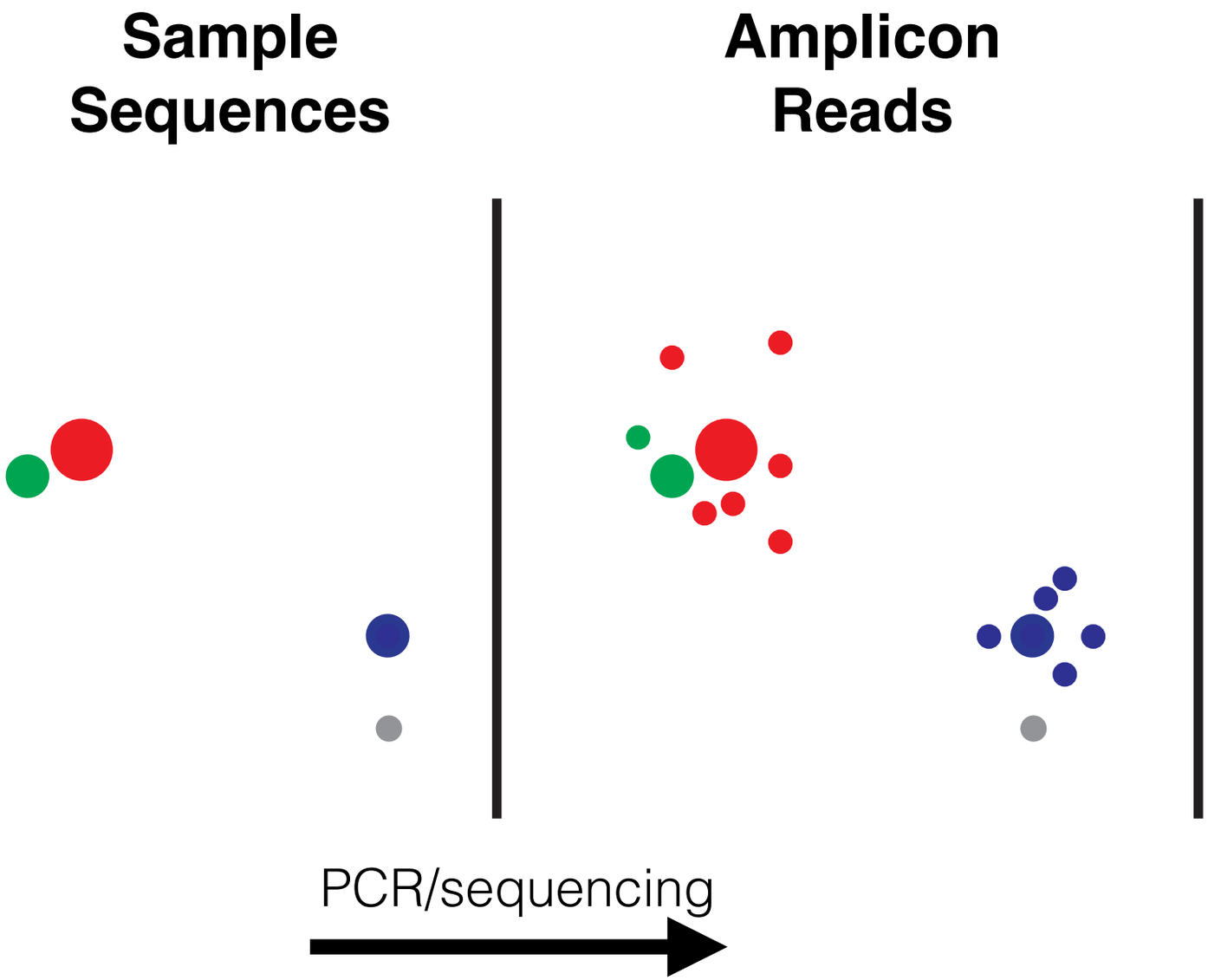
OPEN

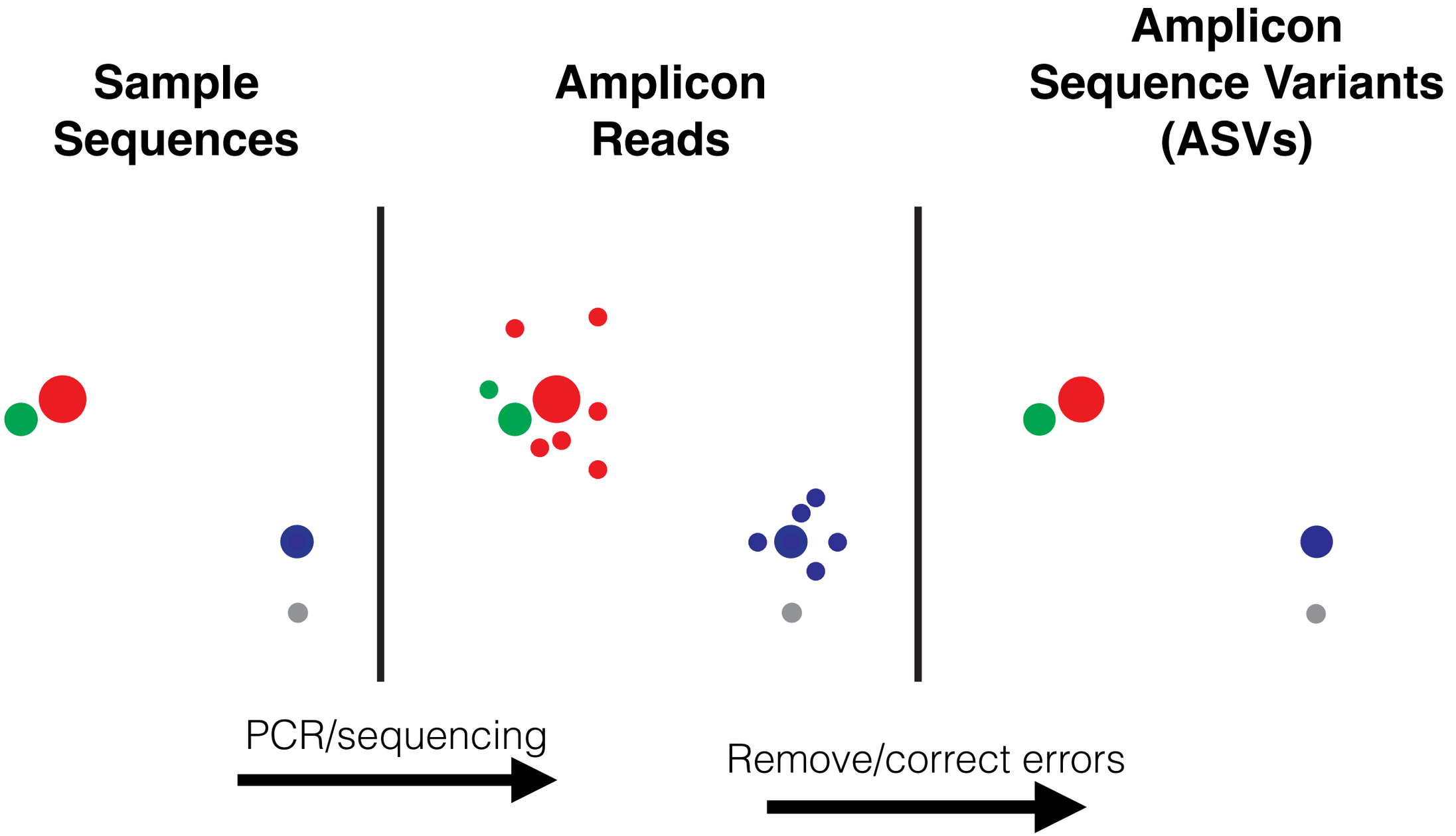
The ISME Journal (2017), 1–5
www.nature.com/ismej

PERSPECTIVE

Exact sequence variants should replace operational taxonomic units in marker-gene data analysis

Benjamin J Callahan[1], Paul J McMurdie[2] and Susan P Holmes[3]
[1]*Department of Population Health and Pathobiology, NC State University, Raleigh NC, USA;* [2]*Whole Biome Inc, San Francisco CA, USA and* [3]*Department of Statistics, Stanford University, Stanford CA, USA*


Sample
Sequences
Amplicon
Reads
PCR/sequencing


Sample Sequences
Amplicon Reads
Amplicon Sequence Variants (ASVs)
PCR/sequencing
Remove/correct errors

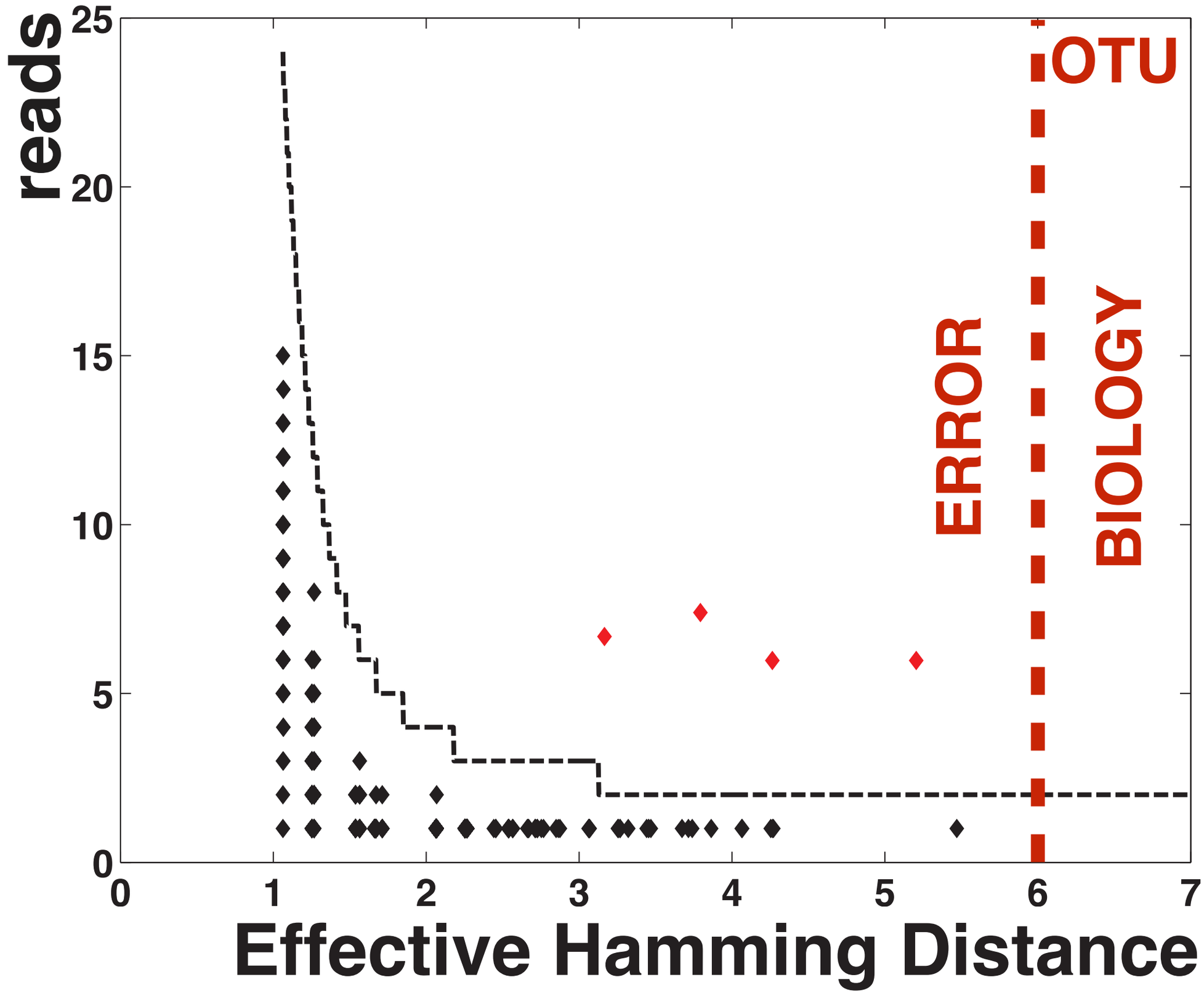
Exact Sequence Variants

OTU85 is ***not*** ***a consistent label,*** *but…*

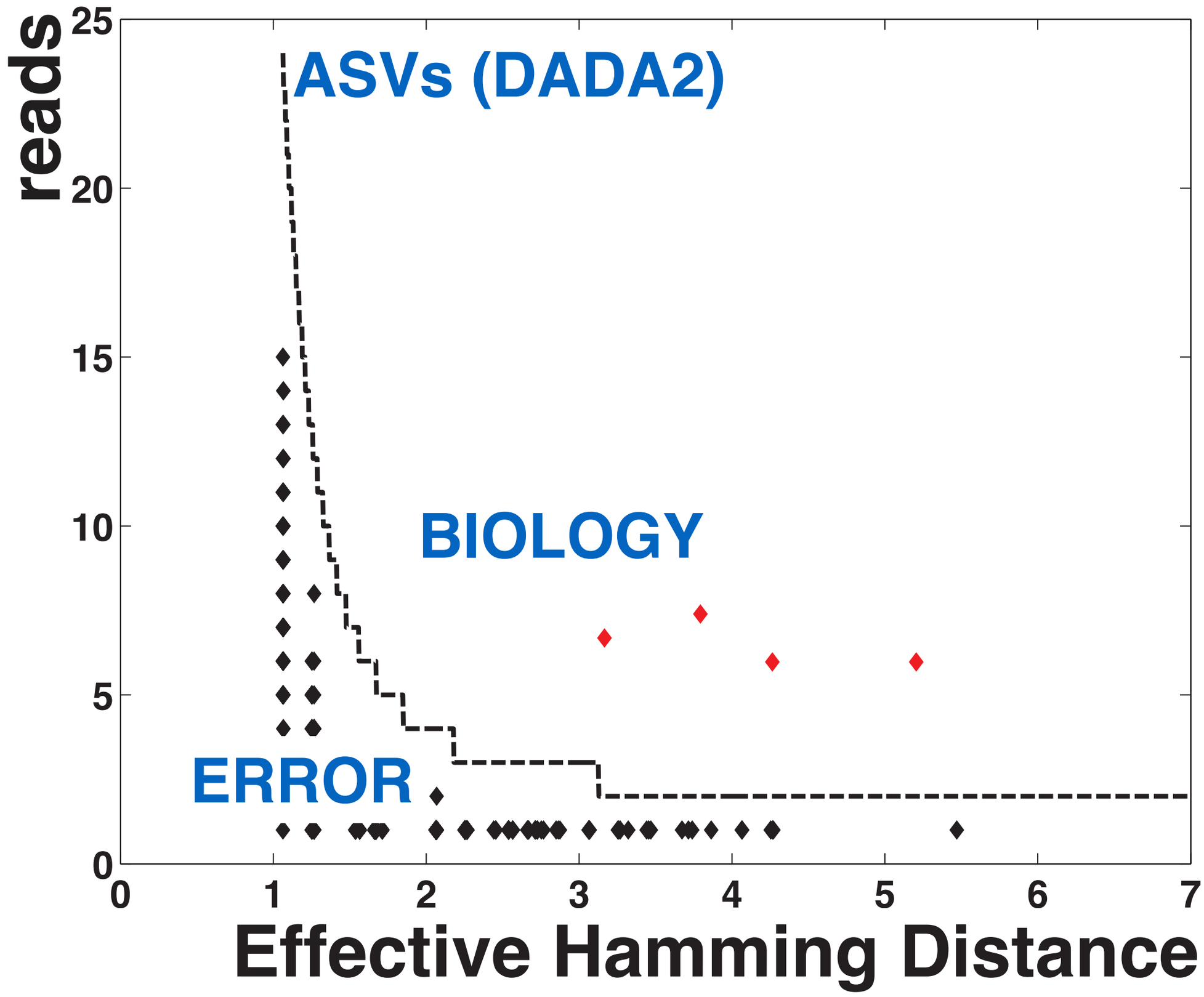
ATTAACGAGATTATAACCAGAGTACGAATA…

is consistent!

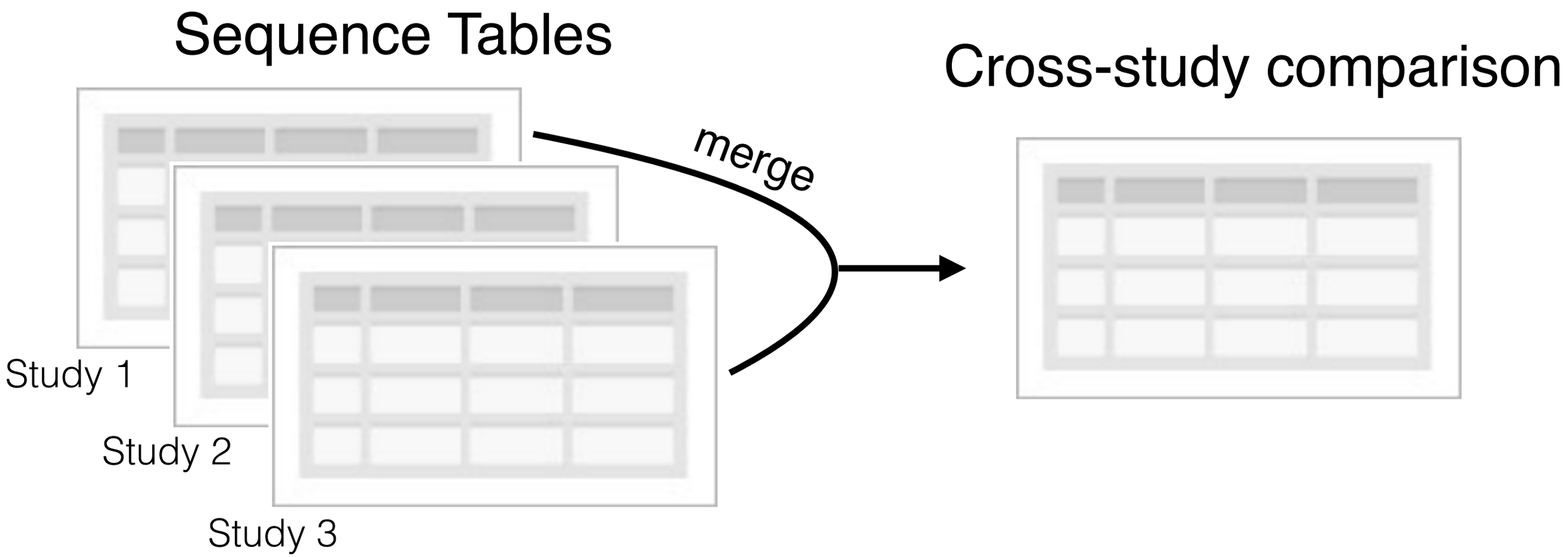
Signal from Noise: OTUs



Signal from Noise: ASVs



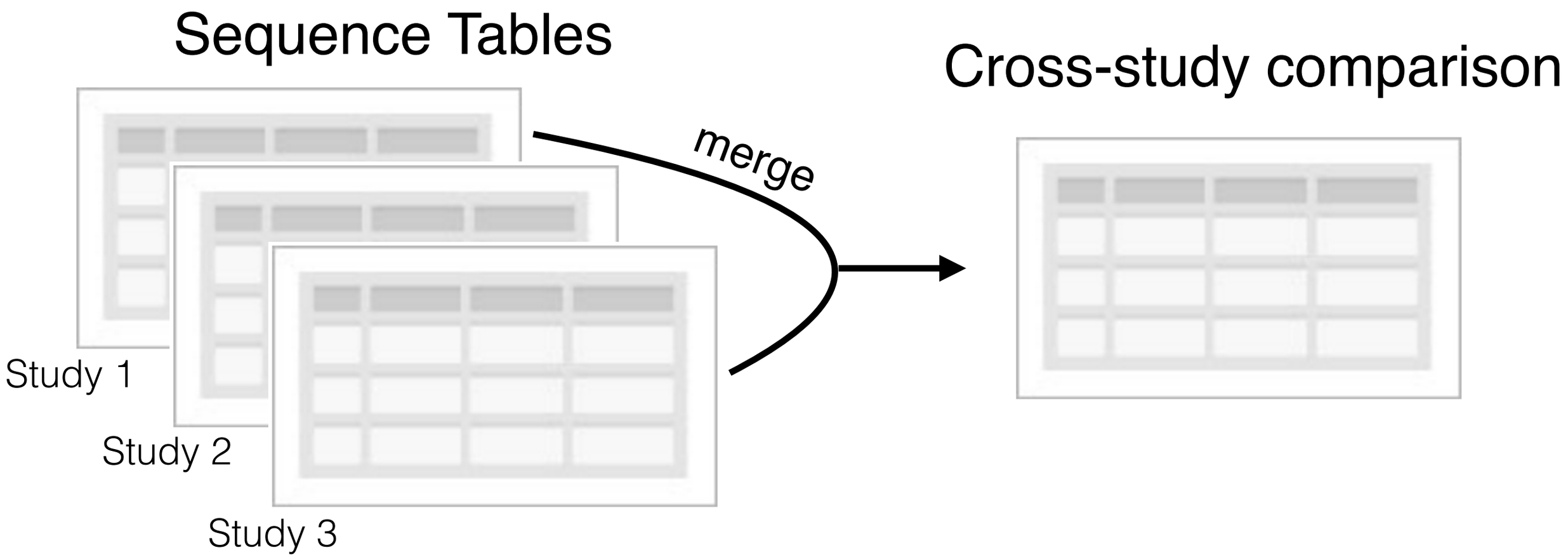
The Sequence is the Label



Eliminates need for joint reprocessing of raw data.

Callahan, et al. ISMEJ, 2017.

The Sequence is the Label



Eliminates need for joint reprocessing of raw data.
Continuous data integration. Unlimited dataset size.

Callahan, et al. ISMEJ, 2017.

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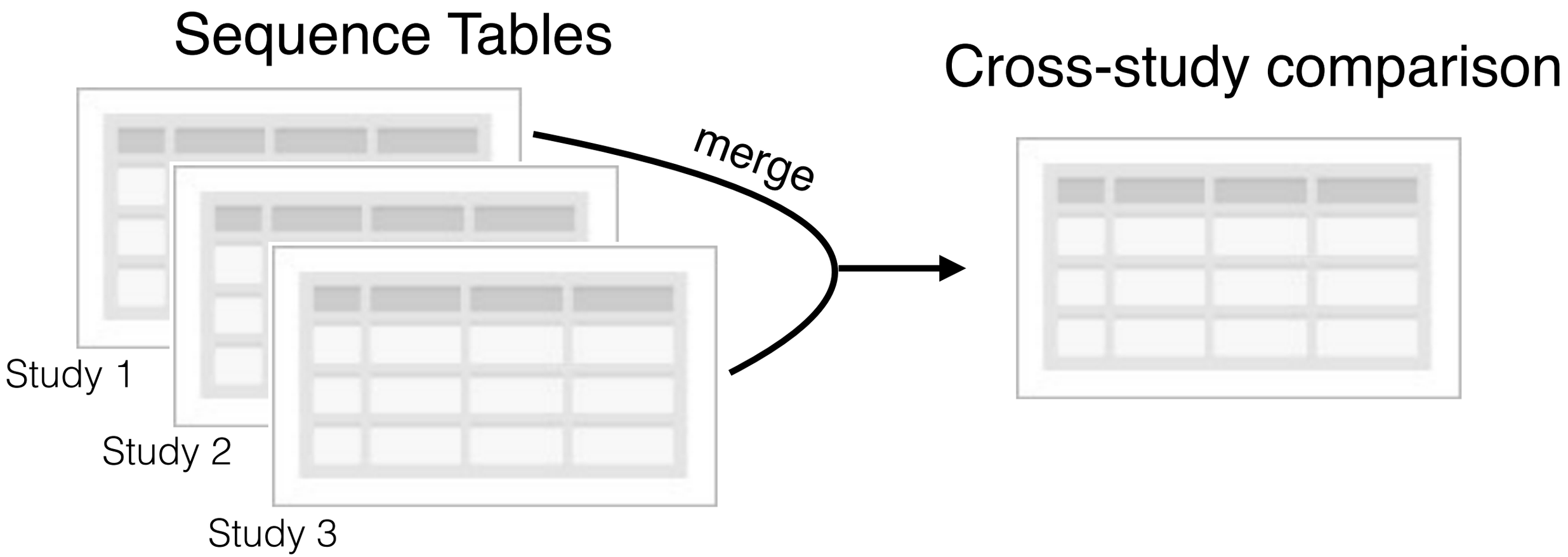
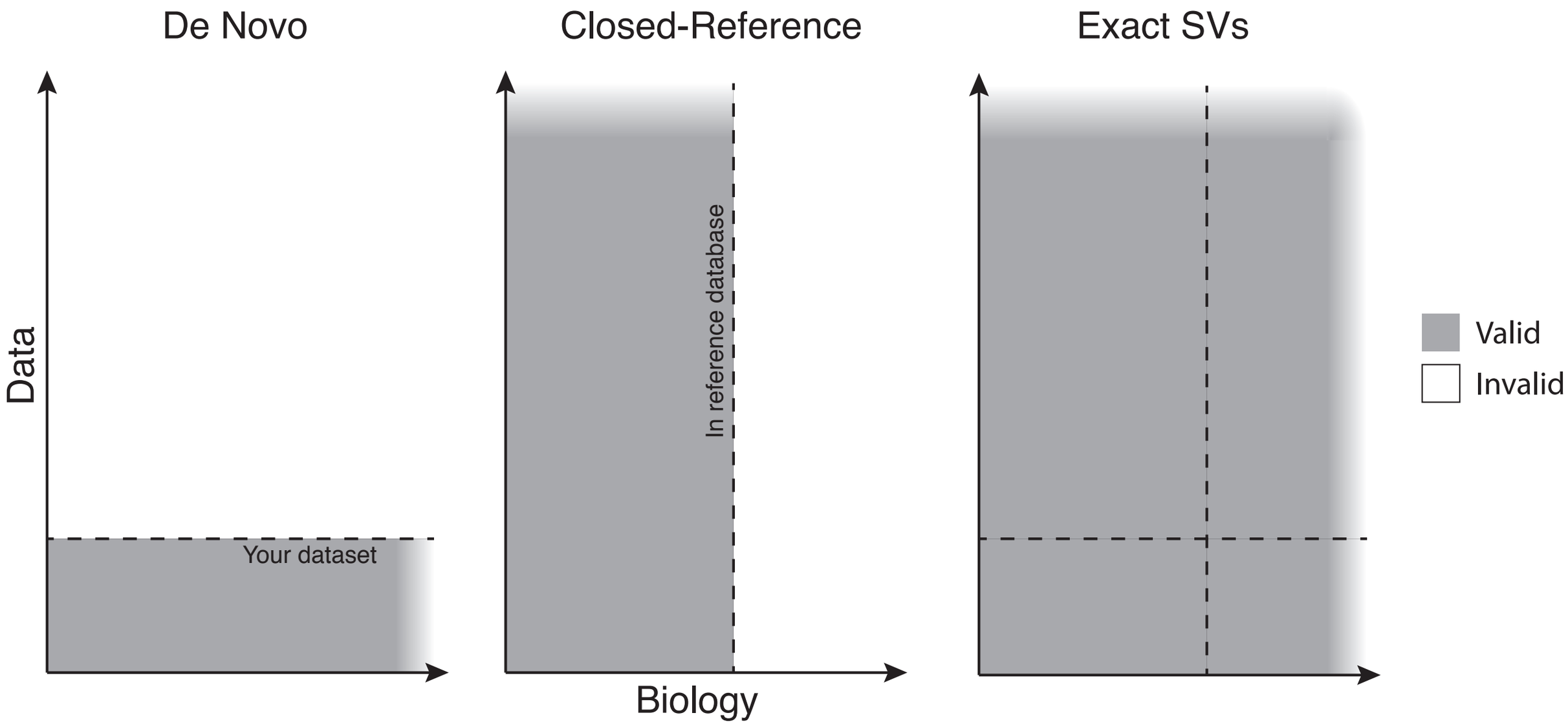


Eliminates need for joint reprocessing of raw data.
Continuous data integration. Unlimited dataset size.
<u>You in 2 years can work directly with you today.</u>

Callahan, et al. ISMEJ, 2017.

The Sequence is the Label



"Replacing OTUs with ASVs makes marker-gene sequencing more precise, reusable, reproducible and comprehensive."

Callahan, et al. ISMEJ, 2017.

ASV Methods and Terminology

ASV Methods

- DADA2
- Deblur
- Unoise2
- Oligotyping/MED
- AmpliCI
- . . .

ASV Methods and Terminology

ASV Methods

- DADA2
- Deblur
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- …

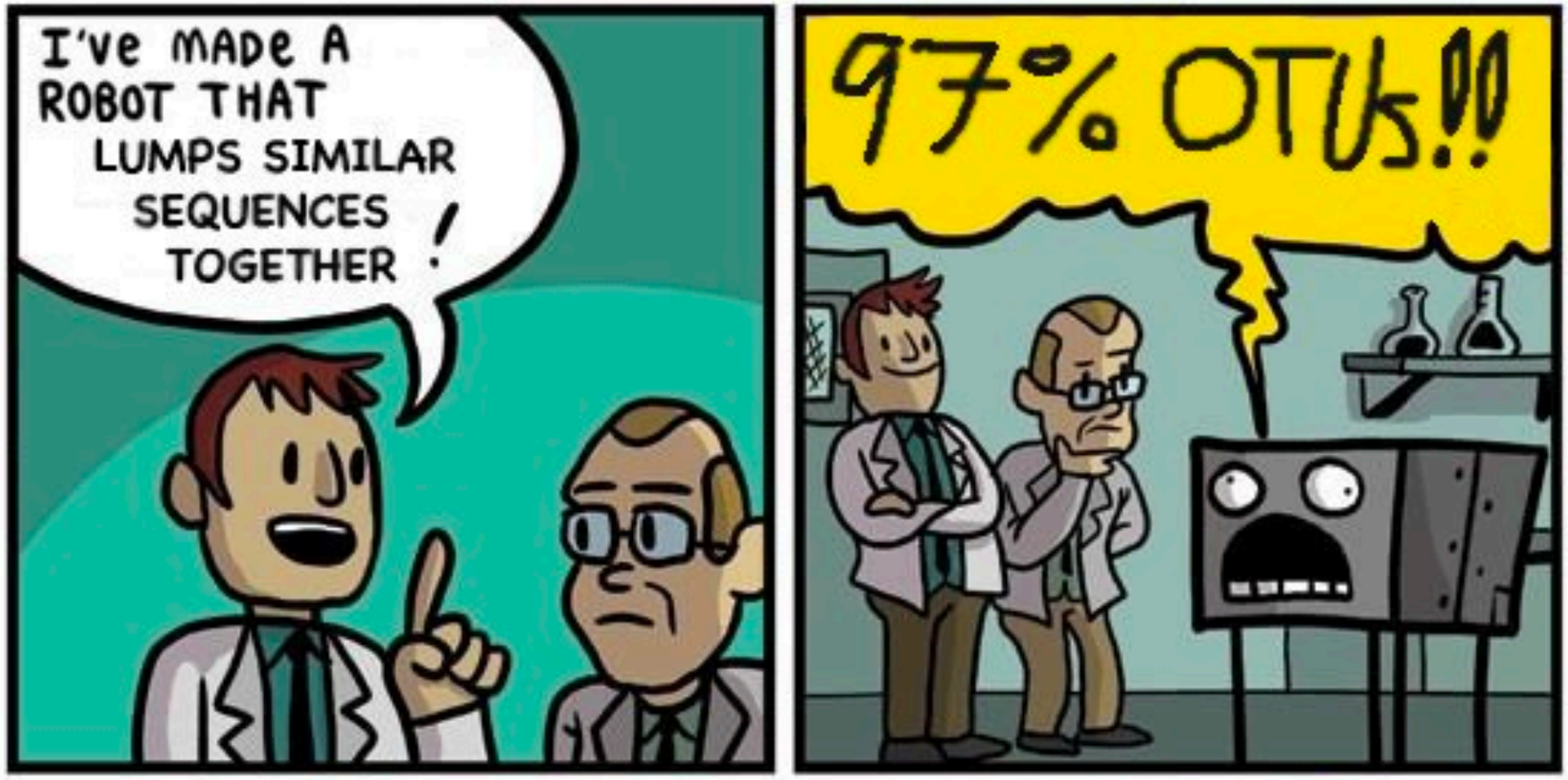
Synonym acronyms

- Amplicon Sequence Variant (ASV)
- Exact Sequence Variant (ESV)
- subOTU (sOTU)
- Zero-radius OTU (zOTU)
- Haplotypes, oligotypes, …

ASVs vs. OTUs

	ASVs	De novo	Closed-ref
Precise	✓	~	~
Tractable	✓	~	✓
Reproducible	✓	X	✓
Comprehensive	✓	✓	X

Callahan, et al. ISMEJ, 2017.


I've made a robot that lumps similar sequences together!
97% OTUs!!

OTU
WHY?

ASVs vs. OTUs

	ASVs	De novo	Closed-ref
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Callahan, et al. ISMEJ, 2017.

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Are OTUs *wrong*?

Callahan, et al. ISMEJ, 2017.

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Are ASVs *always* the best unit of analysis?

Callahan, et al. ISMEJ, 2017.

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Callahan, et al. ISMEJ, 2017.

Phylogenetic Scale

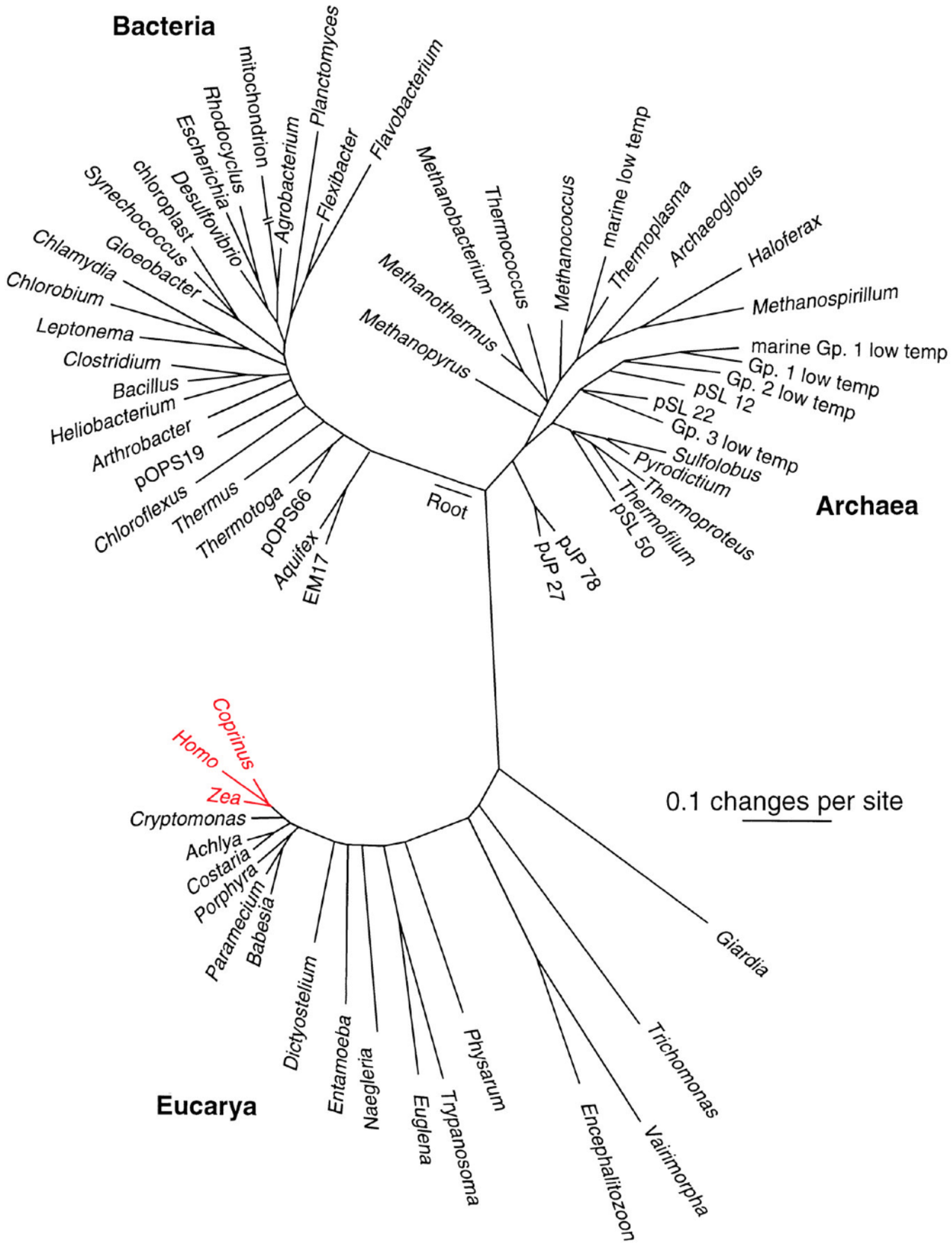
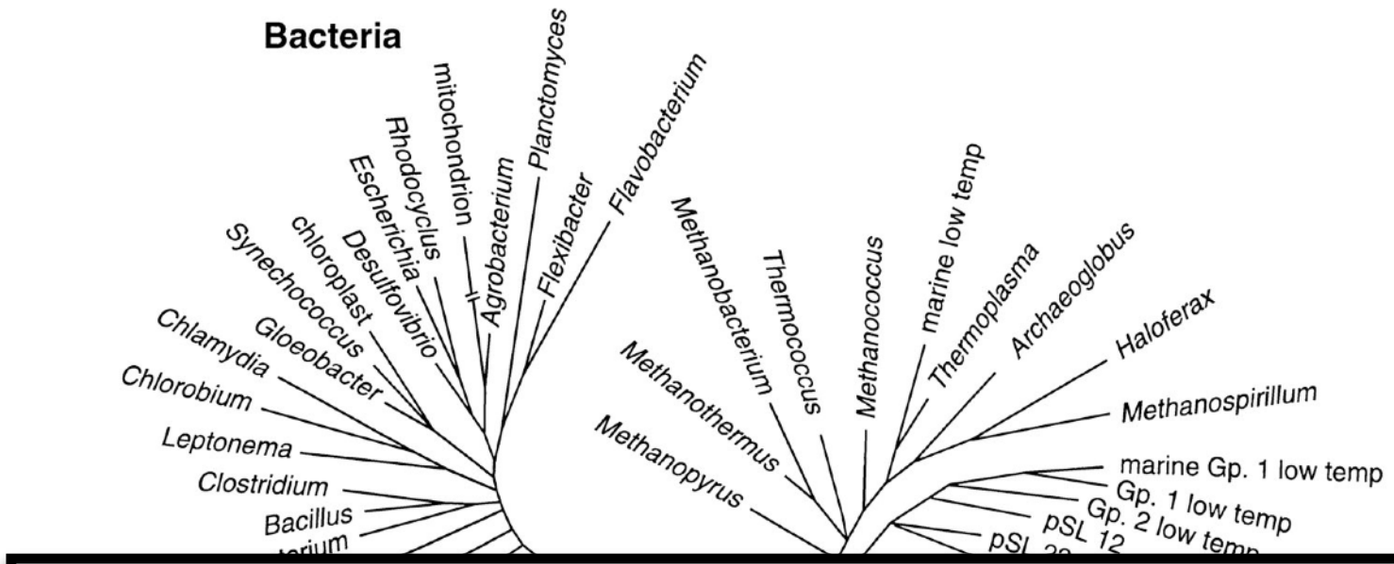


Image: Norman Pace

Phylogenetic Scale



What"taxonomic unit" best captures the phylogenetic scale at which the function relevant to a scientific question varies?

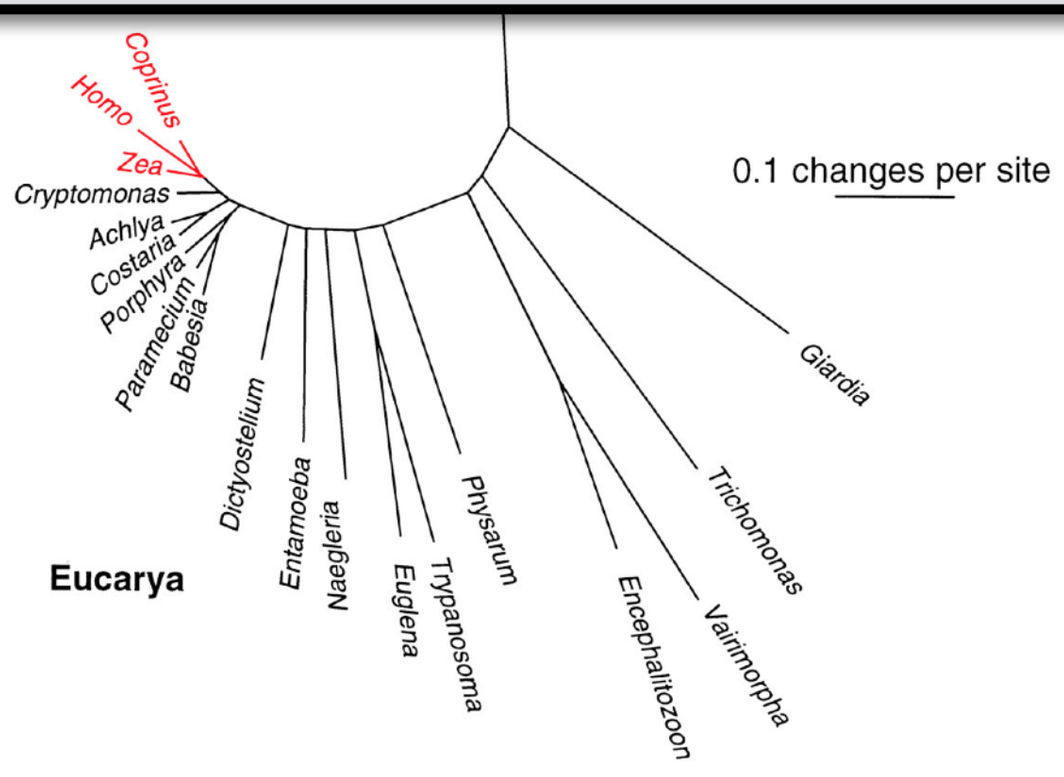
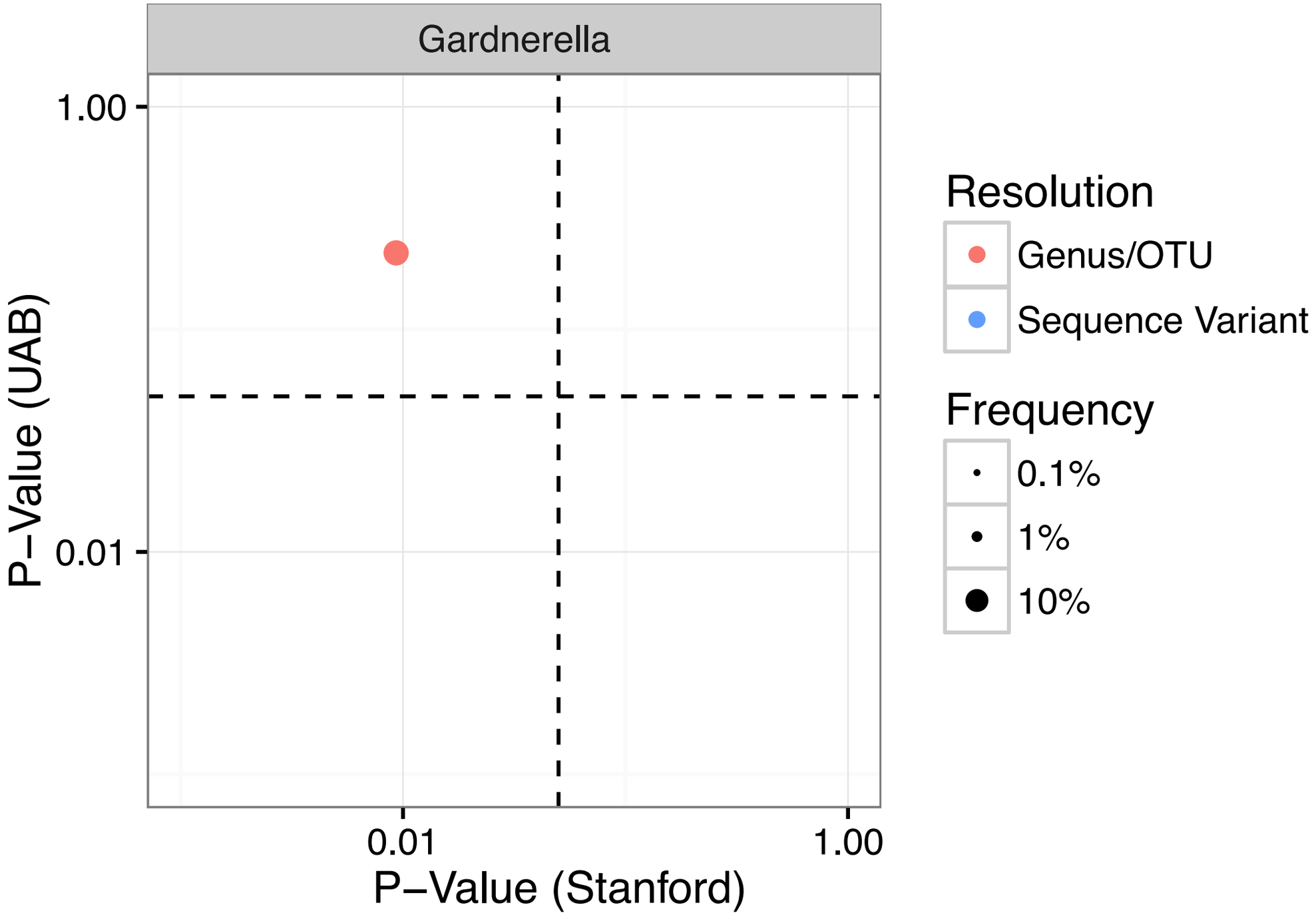


Image: Norman Pace

Resolution: Preterm Birth



Callahan et al. PNAS, 2017.

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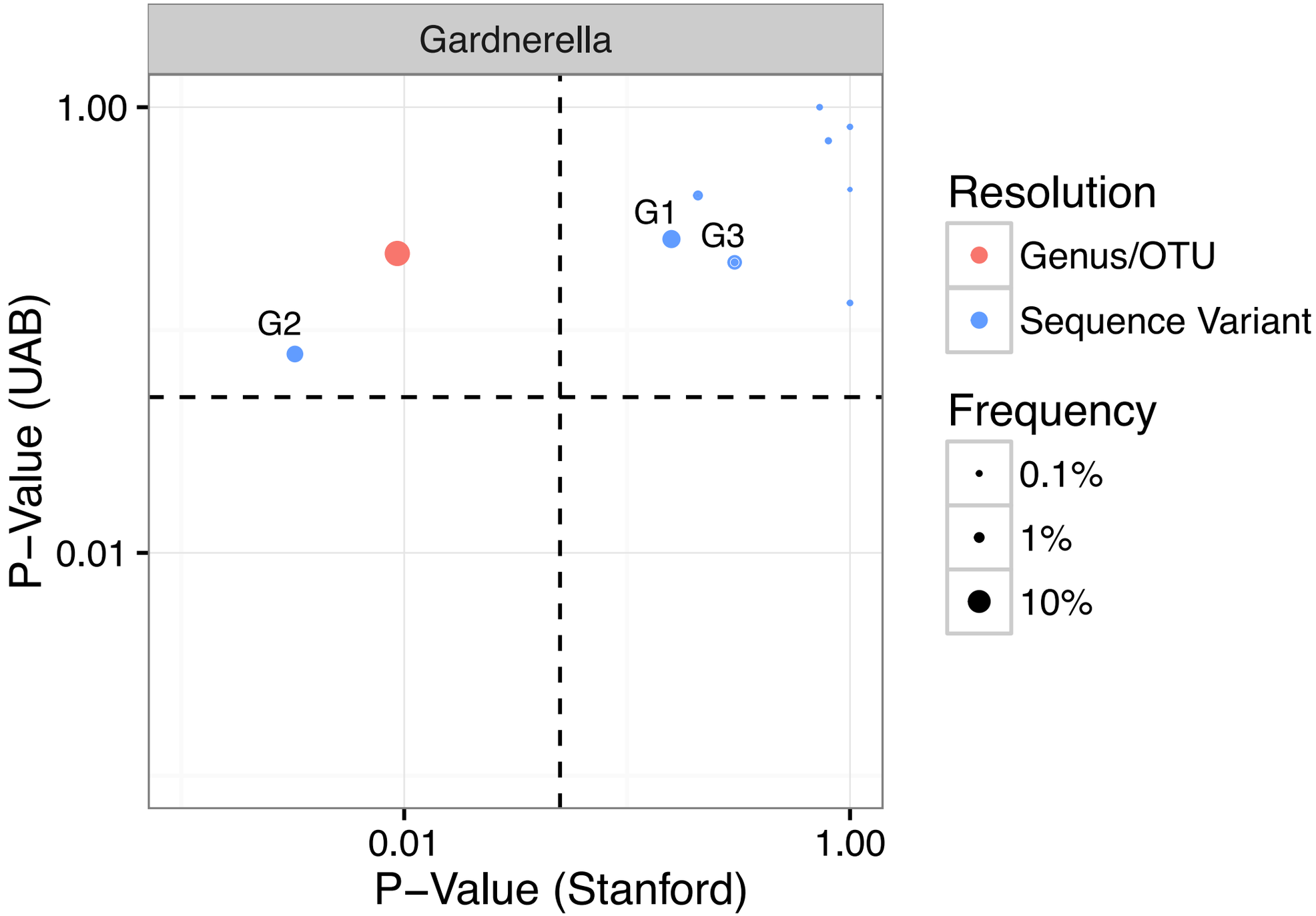

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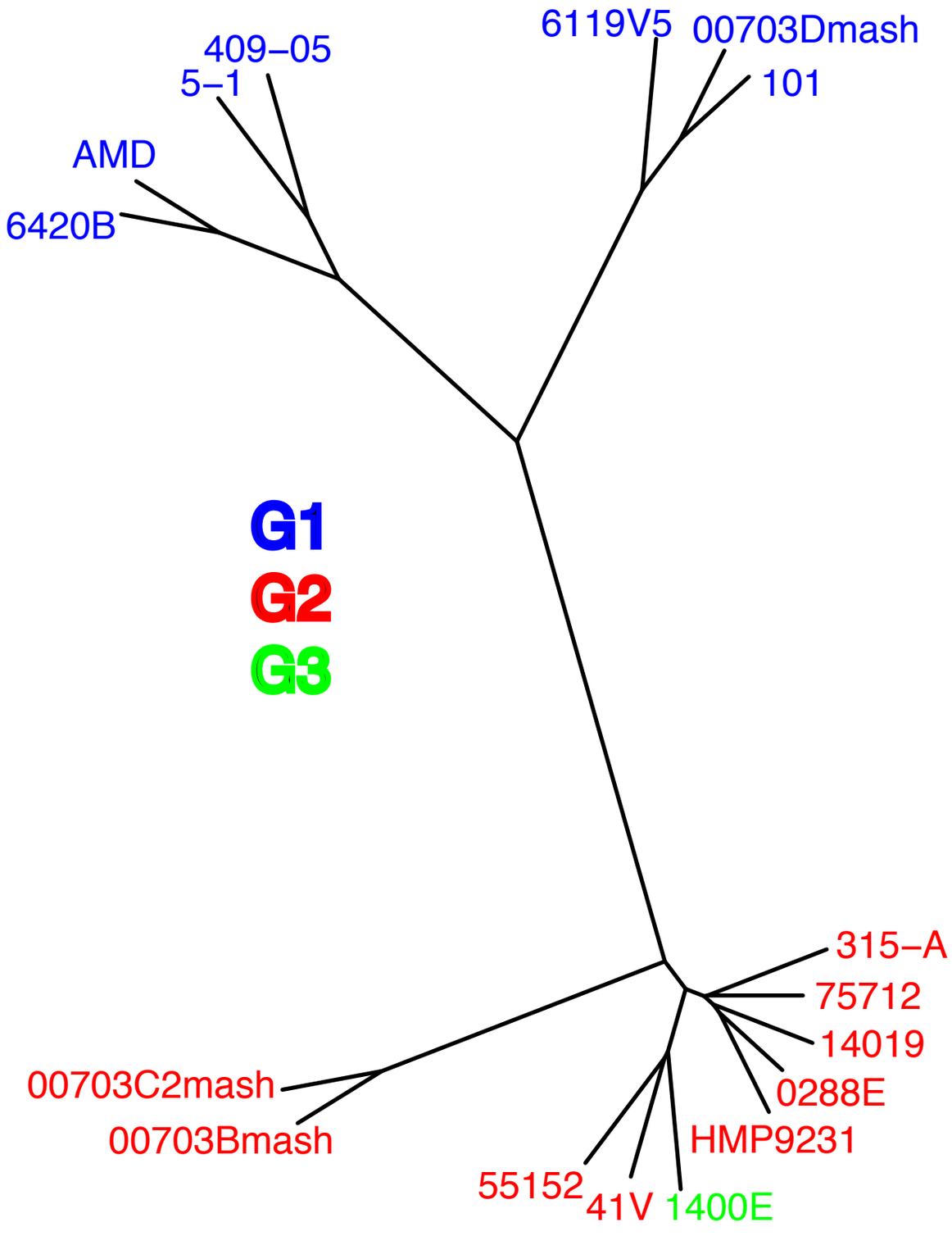
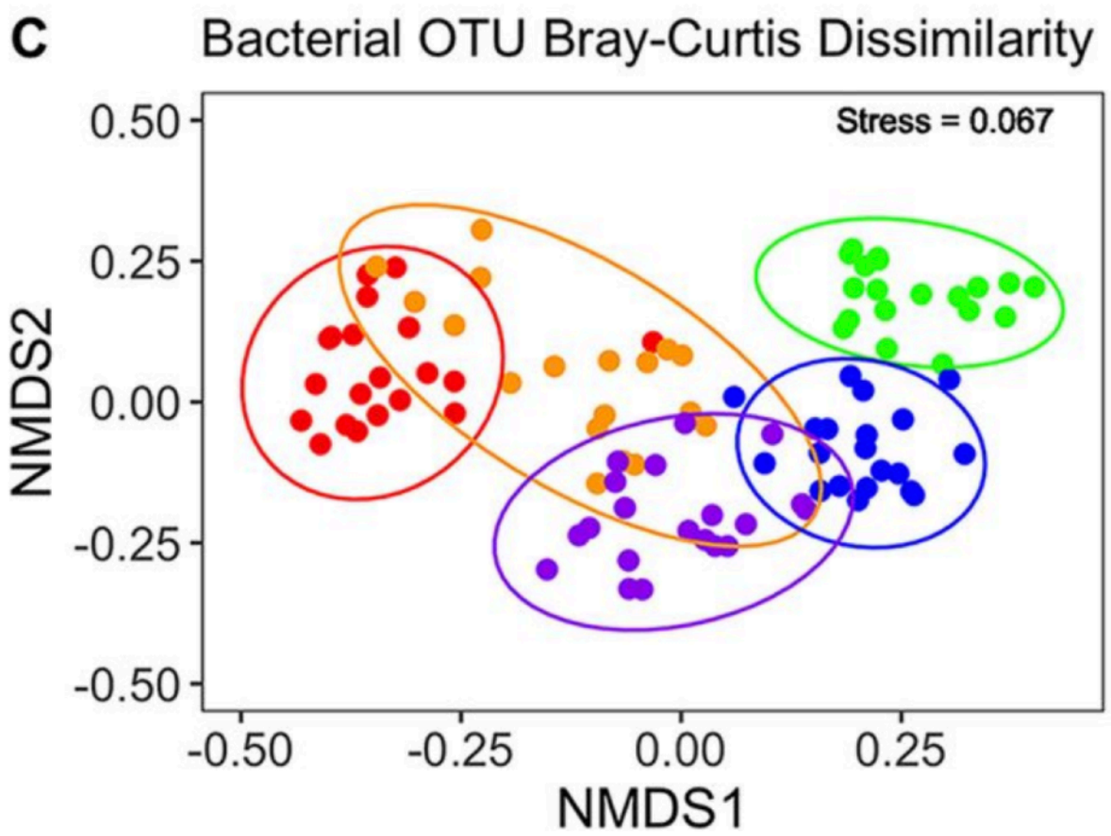


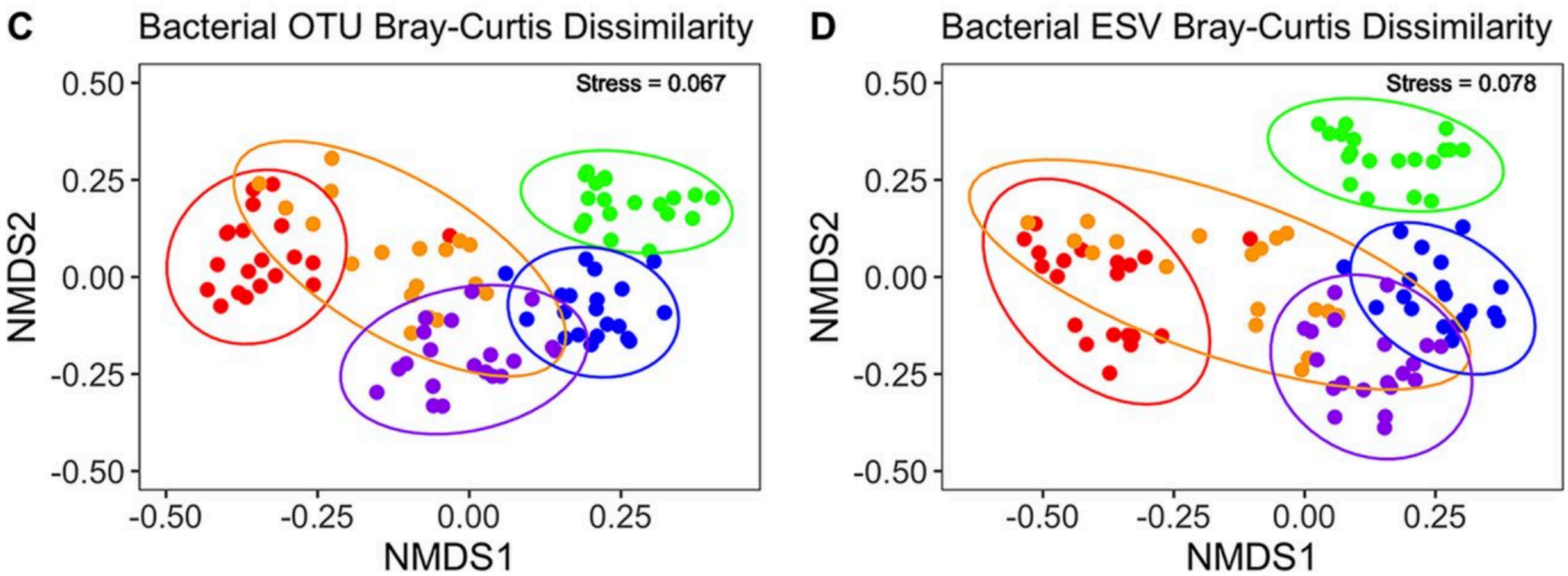
Tree from whole genomes

Callahan et al. PNAS, 2017.

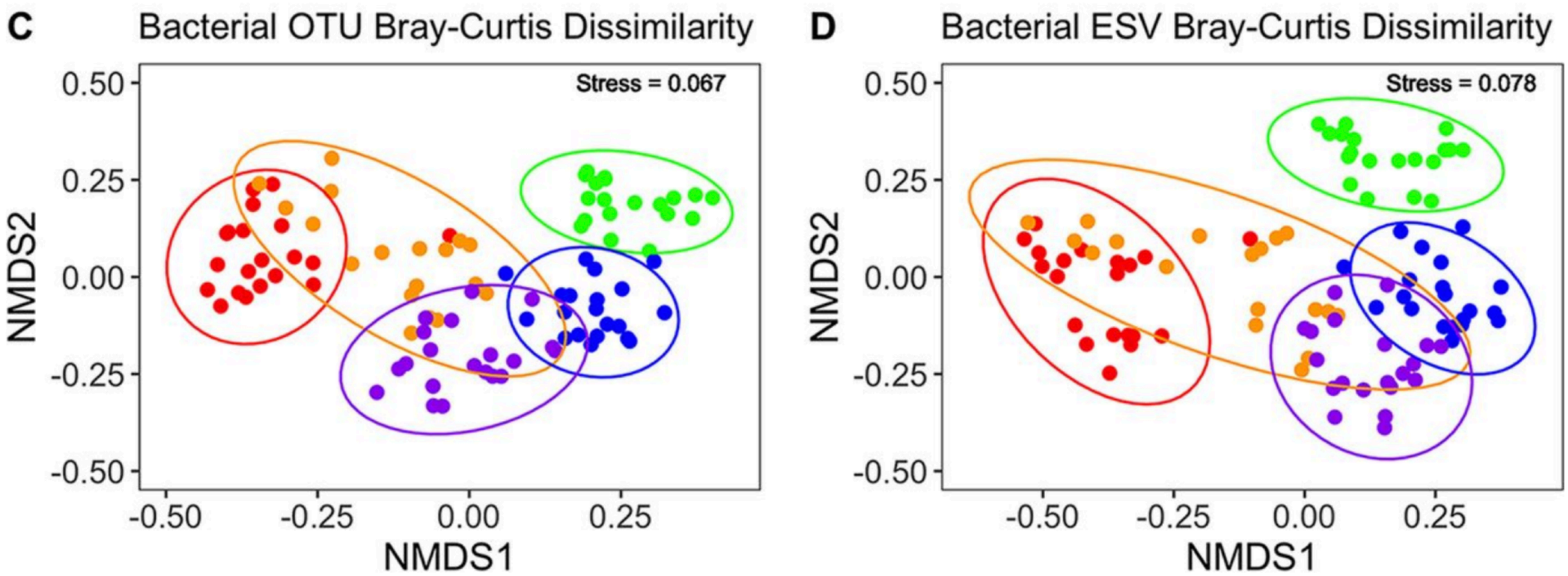
Resolution: Broadscale Patterns



Resolution: Broadscale Patterns



Resolution: Broadscale Patterns



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Ecological and Evolutionary Science

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Broadscale Ecological Patterns Are Robust to Use of Exact Sequence Variants versus Operational Taxonomic Units

Sydney I. Glassman,[a,b] Jennifer B. H. Martiny[a]

To species and beyond?

Short-read 16S ASVs

Typically will discriminate pairs of species in a genus.
Typically can't unambiguously identify a species.

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Long-read 16S/+ ASVs

Almost always discriminate pairs of species in a genus.
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To species and beyond?

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Other phylogenetic marker ASVs

It depends! Some markers can go below species.
Often a resolution/taxonomic-breadth tradeoff.

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Often a resolution/taxonomic-breadth tradeoff.

** All of this assumes a comprehensive reference database.*

This speaker recommends…

- **You should start with ASVs**
- Consider analysis at ***multiple*** taxonomic levels
- **Reproducible**: ASVs, taxonomy. **Not**: *de novo* OTUs.
- **Comprehensive**: ASVs, *de novo* OTUs. **Not**: taxonomy.
- Use domain knowledge on relevant phylogenetic scale
- Deposit and share your ASV table

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- Use domain knowledge on relevant phylogenetic scale
- Deposit and share your ASV table

Can agglomerate up, but can't divide down.

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Amplicon Sequence Variants …for ITS

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Minimum Entropy Decomposition

USEARCH
Ultra-fast sequence analysis

Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns

NIH National Institutes of Health

BIO-X

NSF

NC STATE UNIVERSITY Plant Soil Microbial Community Consortium