Exact Amplicon Sequence Variants

Diversity of Life

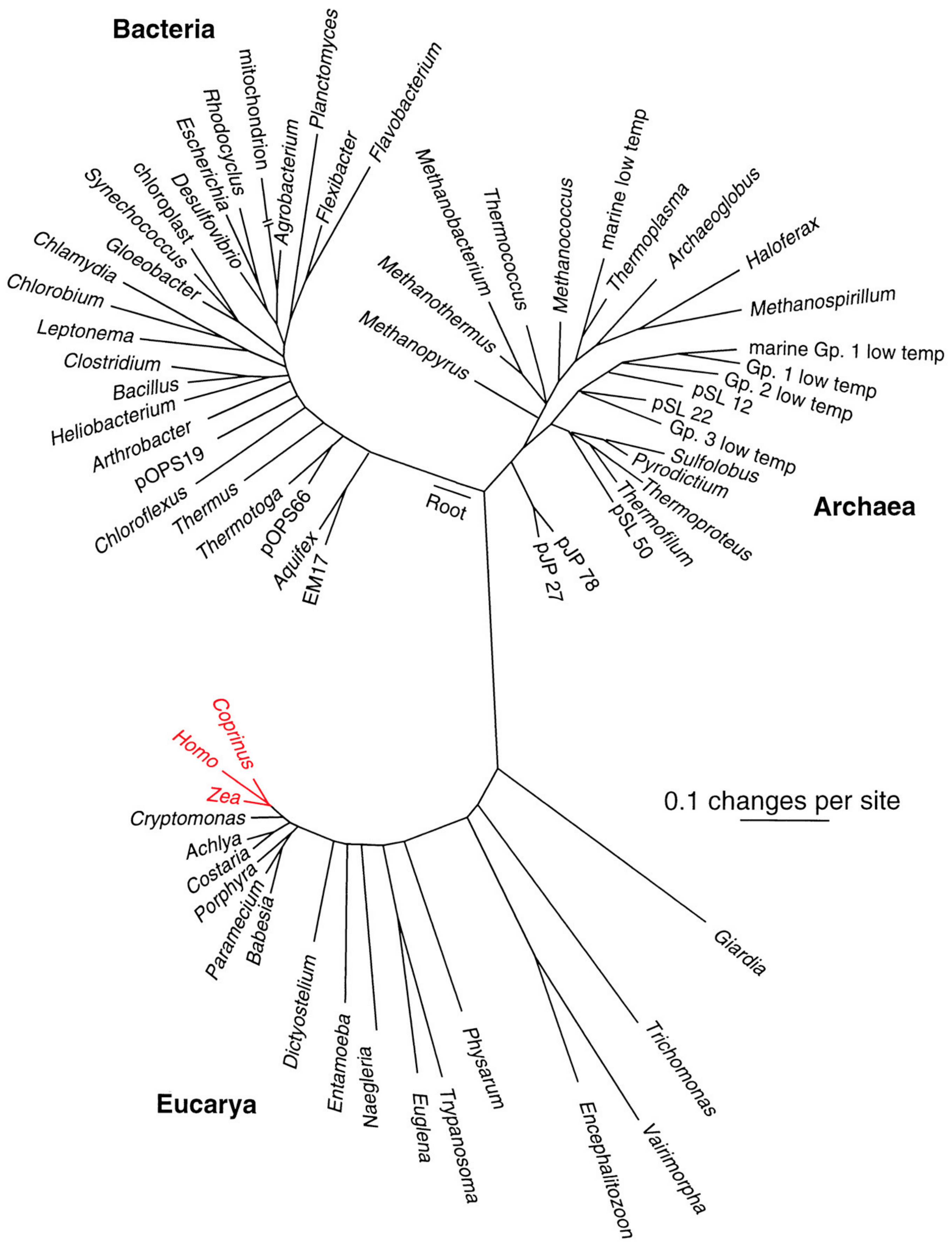
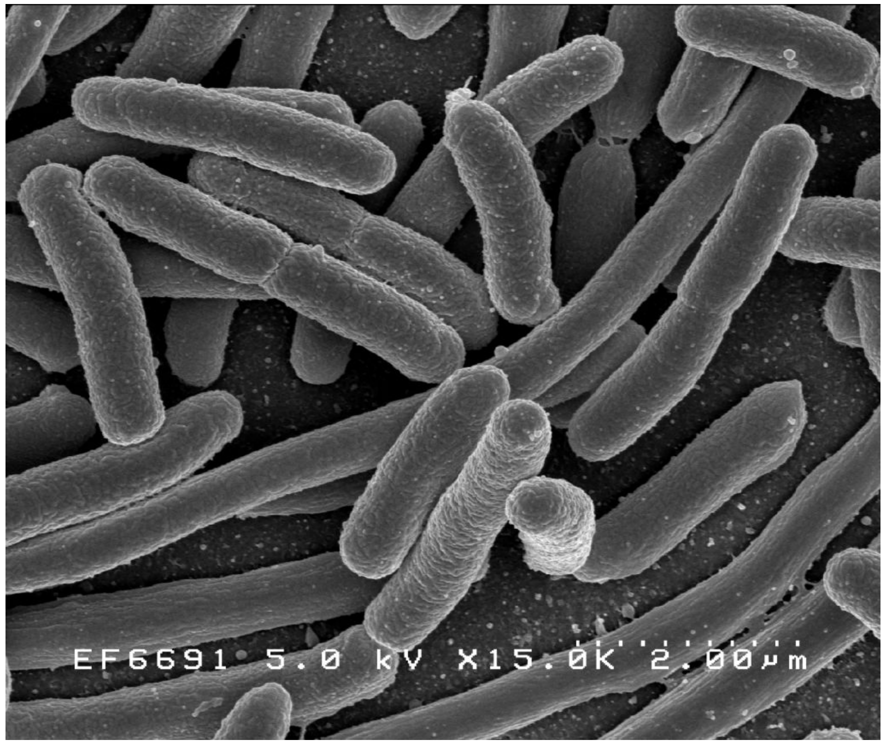


Image: Norman Pace

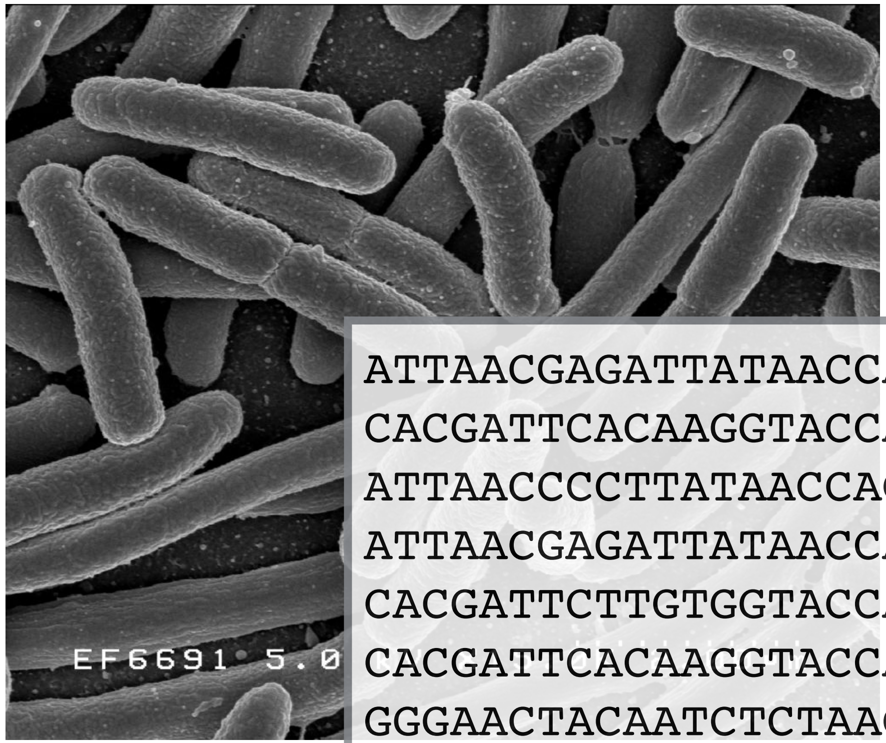
A Molecular Census

Metabarcoding or Marker-gene Sequencing



A Microbial Census

Metabarcoding or Marker-gene Sequencing



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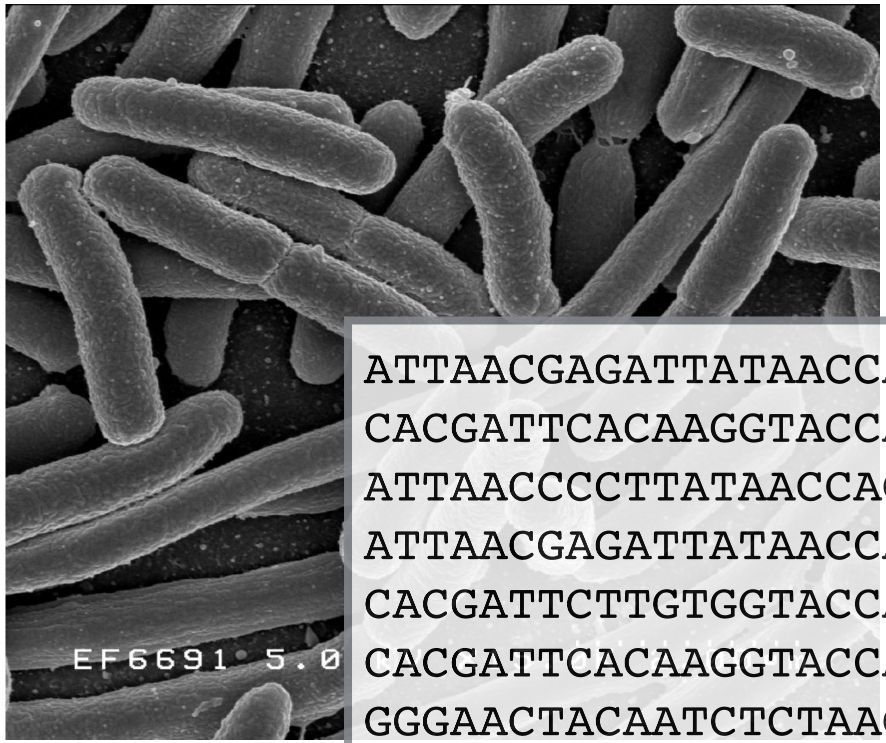
CACGATTCTTGTGGTACCACAAGGTAACATAGCTCC
CACGATTCACAAGGTACCACAAGGTAACATAGCTCC
GGGAACTACAATCTCTAAGGTGAAGTCTCAGTCTAT
ATTAACGAGATTATAACCAGAGTACGAATACCGAAC
CACGATTCACAAGGTACCACAAGGTAACATAGCTCC
ATTAACGAGATTATAACCAGAGTACGAATACCGAAC
```

A Microbial Census

Metabarcoding or Marker-gene Sequencing



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CACGATTCACAAGGTACCACAAGGTAACATAGCTCC
ATTAACCCCTTATAACCAGAGTACGAATACCGAACA
ATTAACGAGATTATAACCAGAGAGAGAATACCGAAC
CACGATTCTTGTGGTACCACAAGGTAACATAGCTCC
CACGATTCACAAGGTACCACAAGGTAACATAGCTCC
GGGAACTACAATCTCTAAGGTGAAGTCTCAGTCTAT
ATTAACGAGATTATAACCAGA
CACGATTCACAAGGTACCACA
ATTAACGAGATTATAACCAGA
```
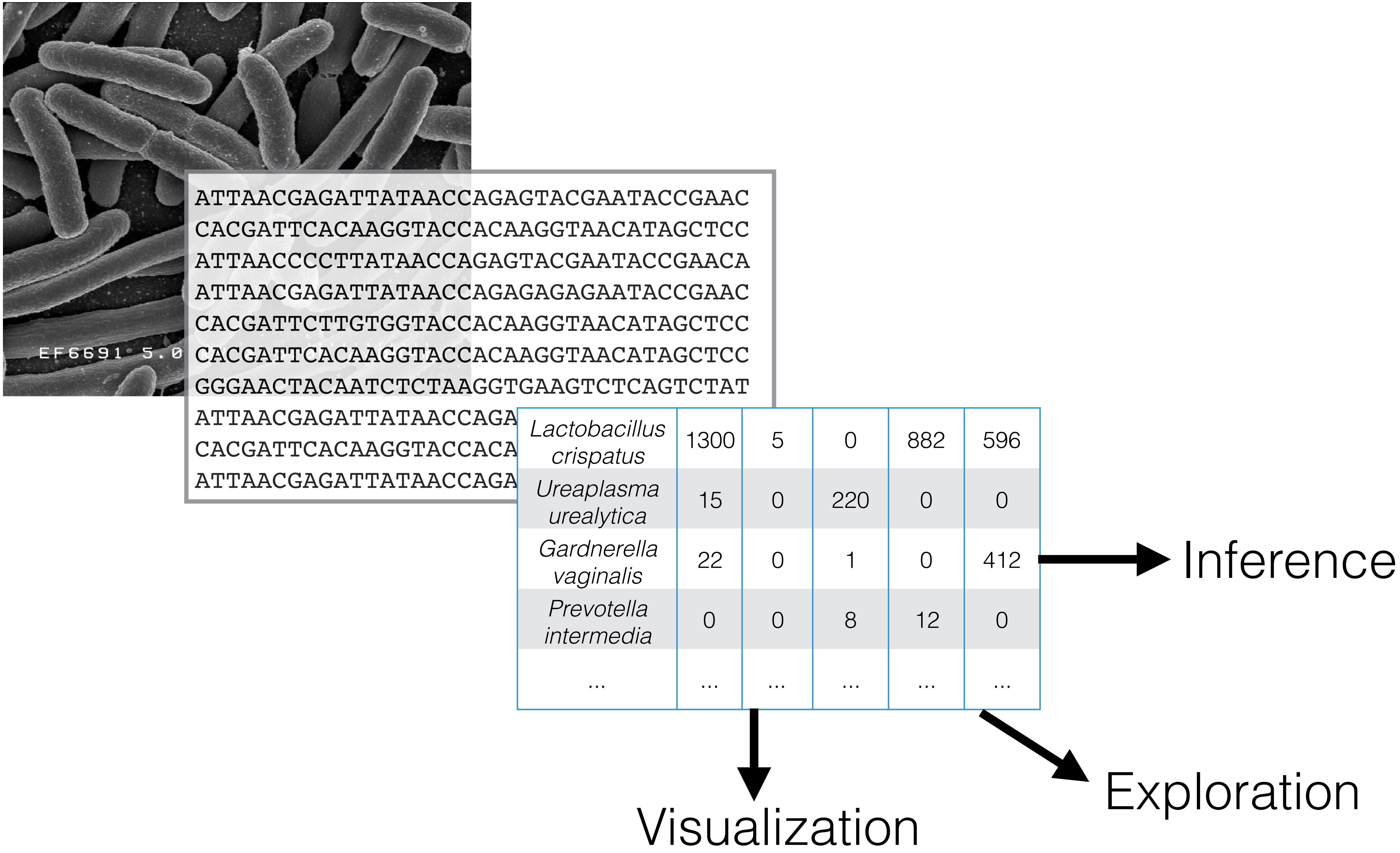

Lactobacillus crispatus	1300	5	0	882	596
Ureaplasma urealytica	15	0	220	0	0
Gardnerella vaginalis	22	0	1	0	412
Prevotella intermedia	0	0	8	12	0
...	...	...	...	...	...

A Microbial Census

Metabarcoding or Marker-gene Sequencing



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GGGAACTACAATCTCTAAGGTGAAGTCTCAGTCTAT
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...	...	...	...	...	...

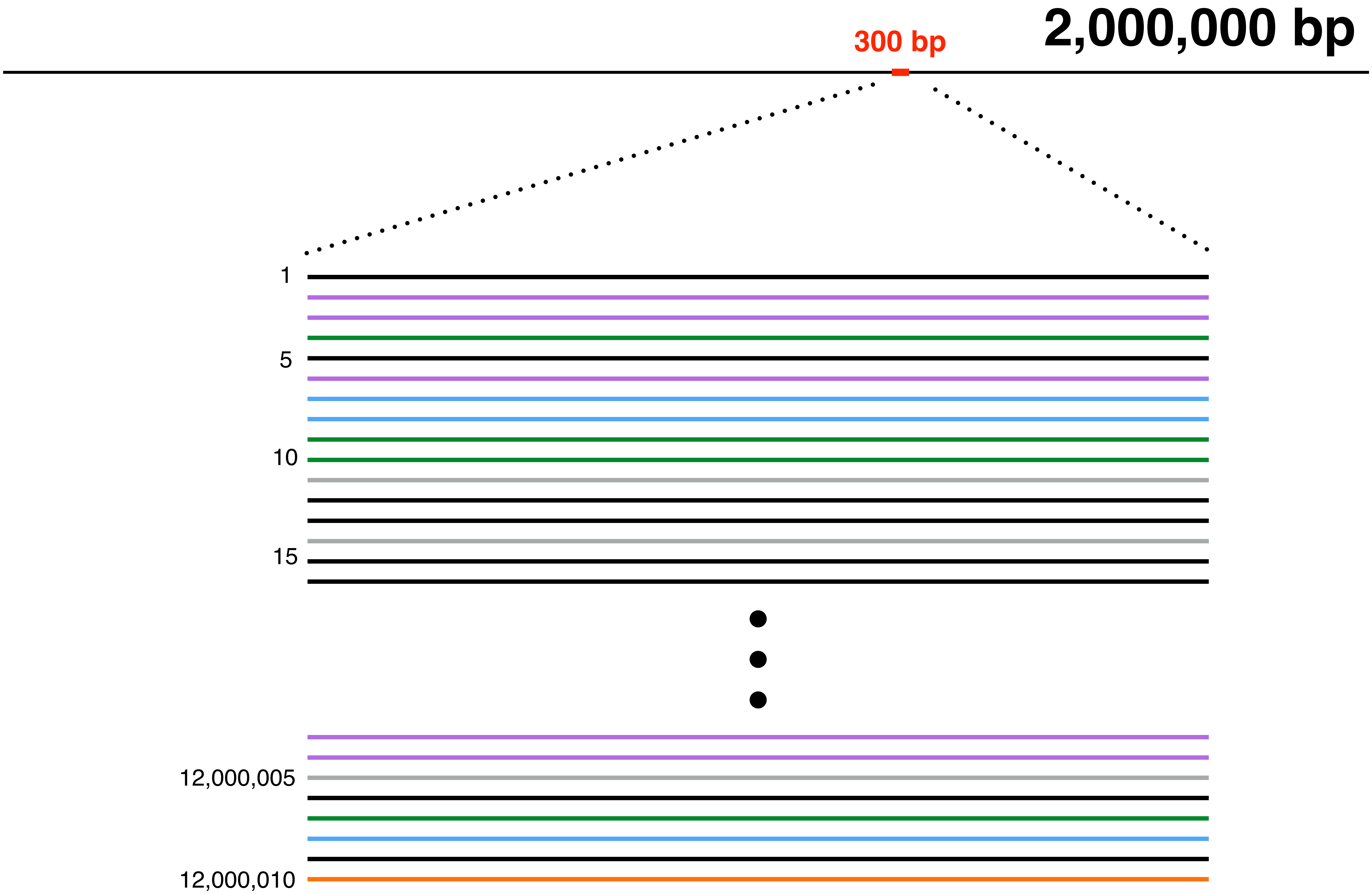
Inference

Visualization

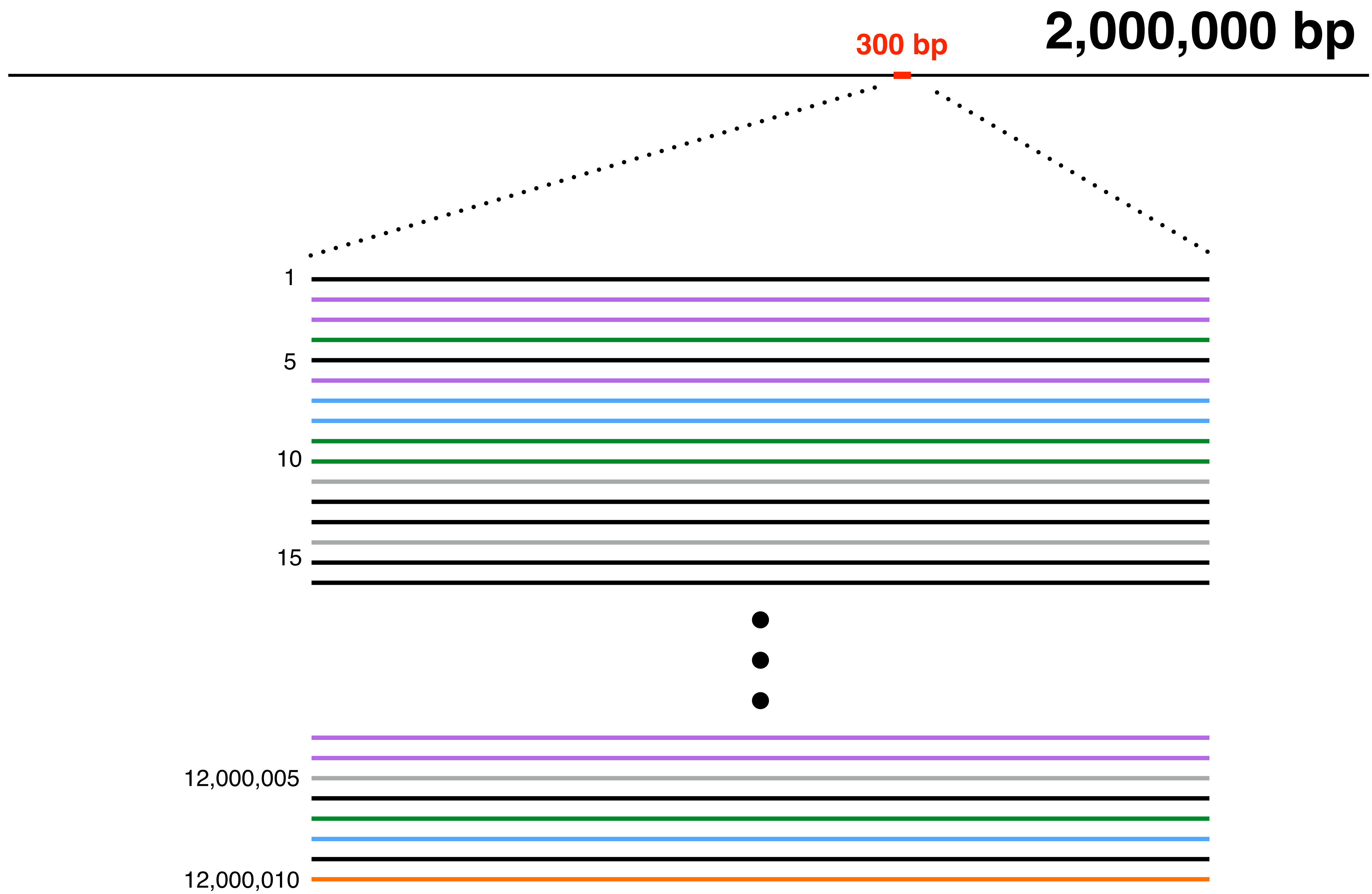
Exploration

Marker-gene Sequencing

Marker-gene Sequencing



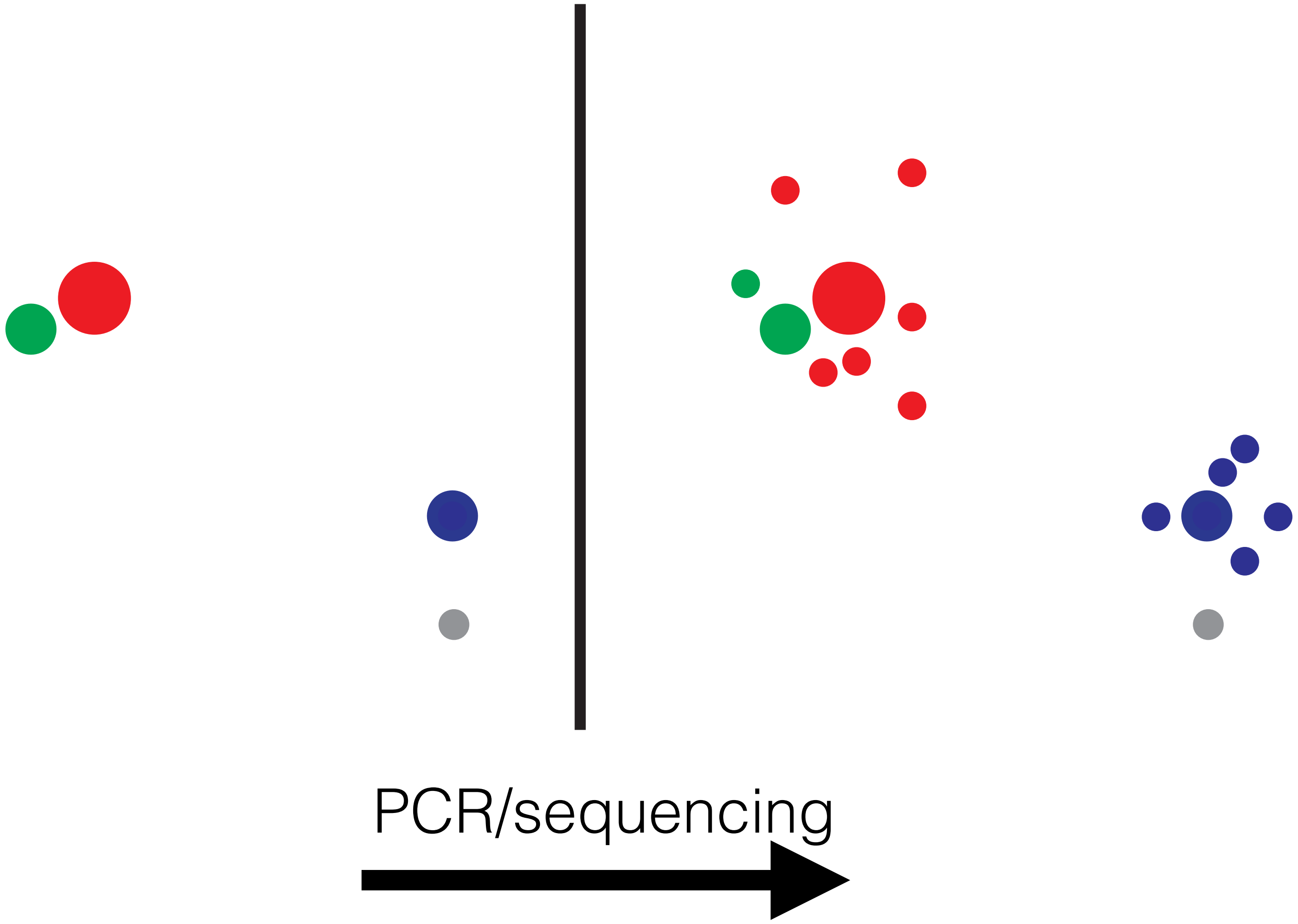
Marker-gene Sequencing



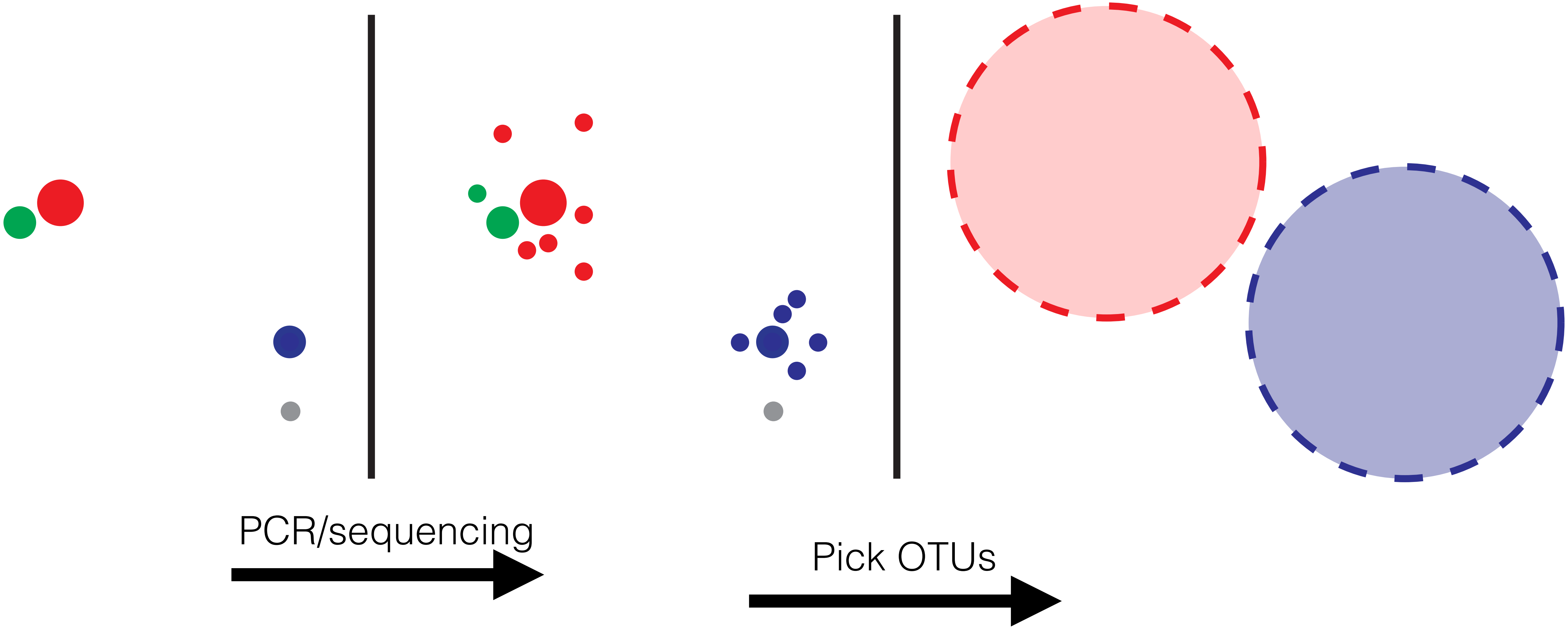
Thousands to Billions of “Barcodes”

Sample Sequences

Amplicon Reads



PCR/sequencing



Sample Sequences

Amplicon Reads

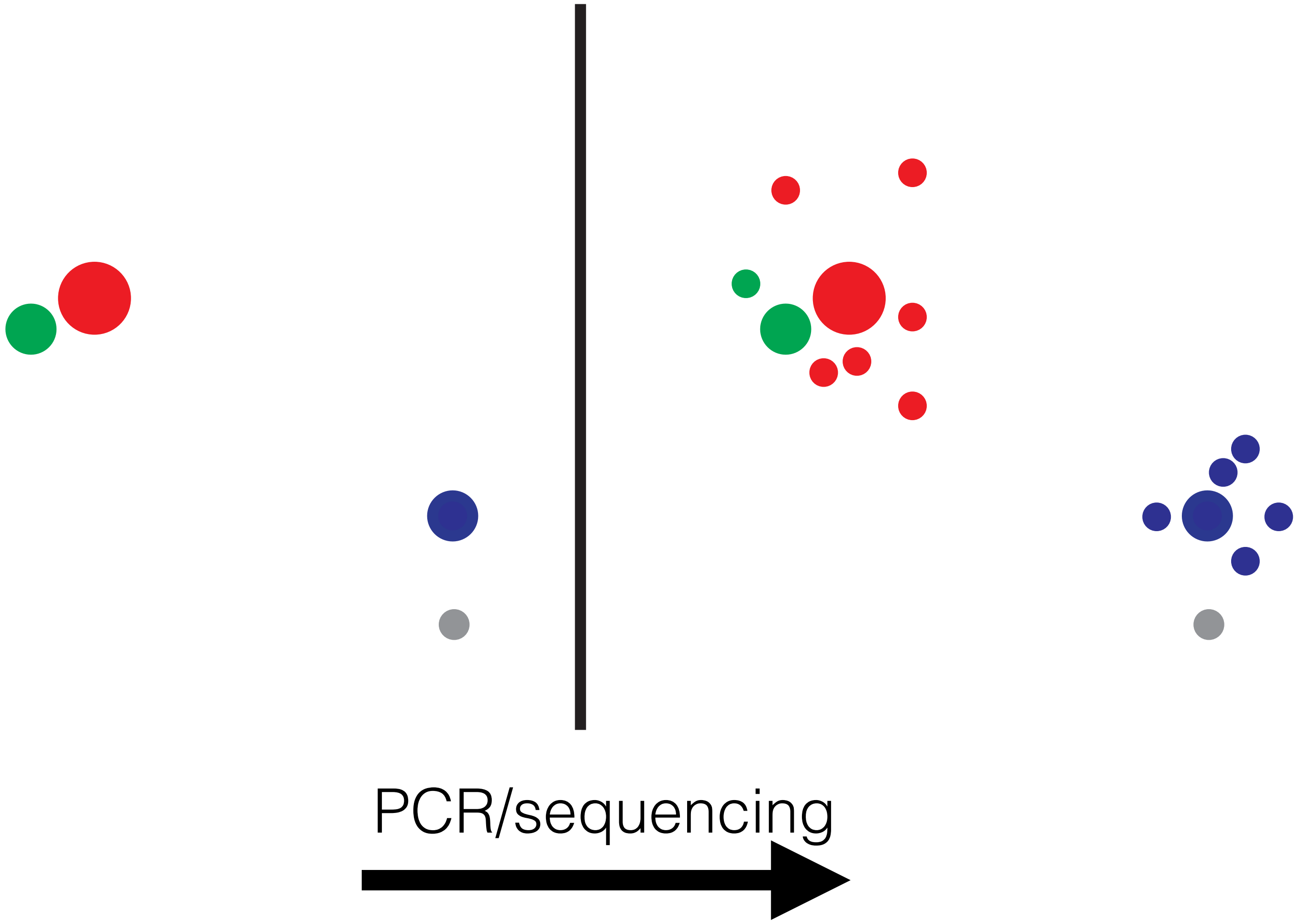
Operational Taxonomic Units

PCR/sequencing

Pick OTUs

Sample Sequences

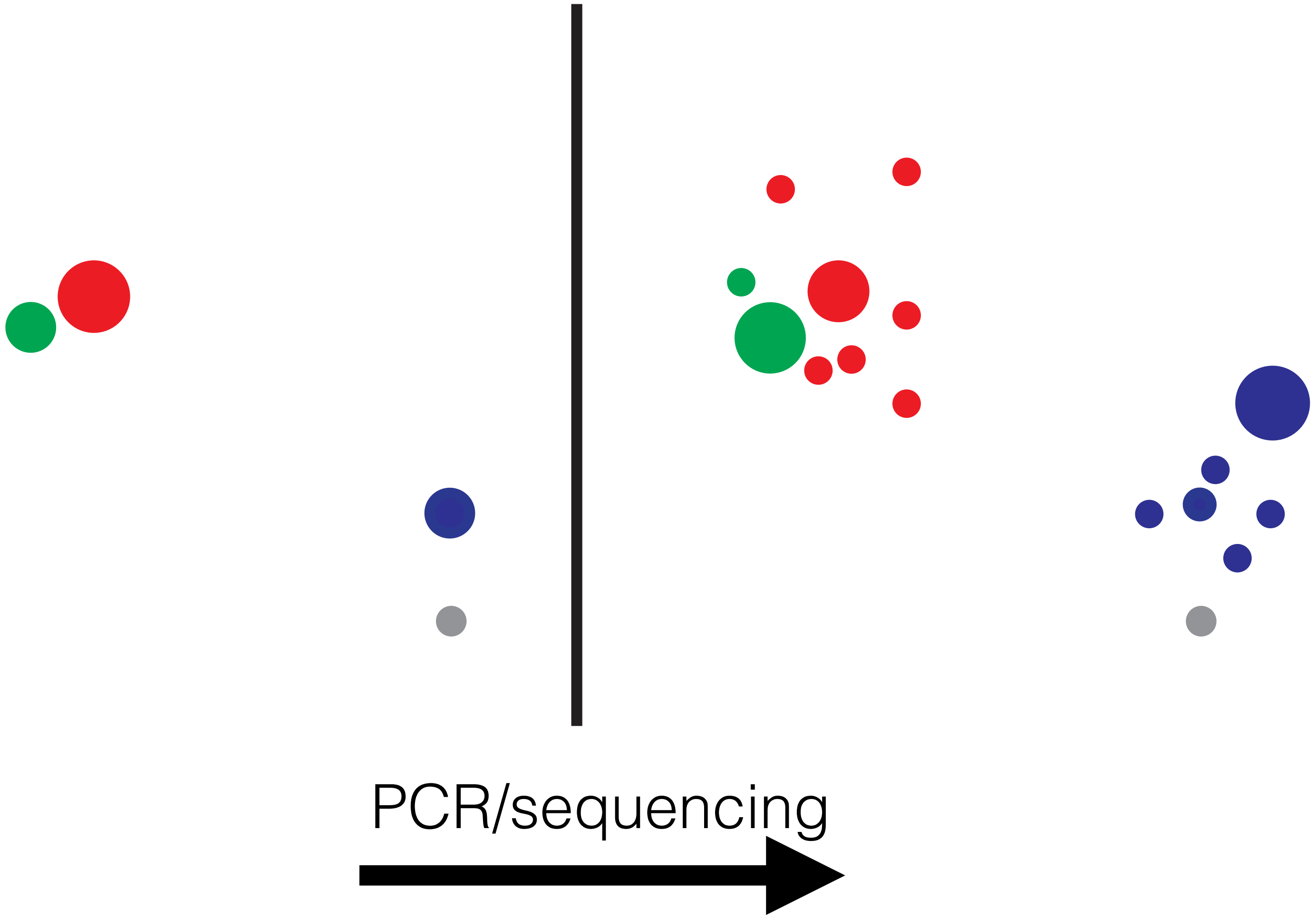
Amplicon Reads



PCR/sequencing

Sample Sequences

Amplicon Reads

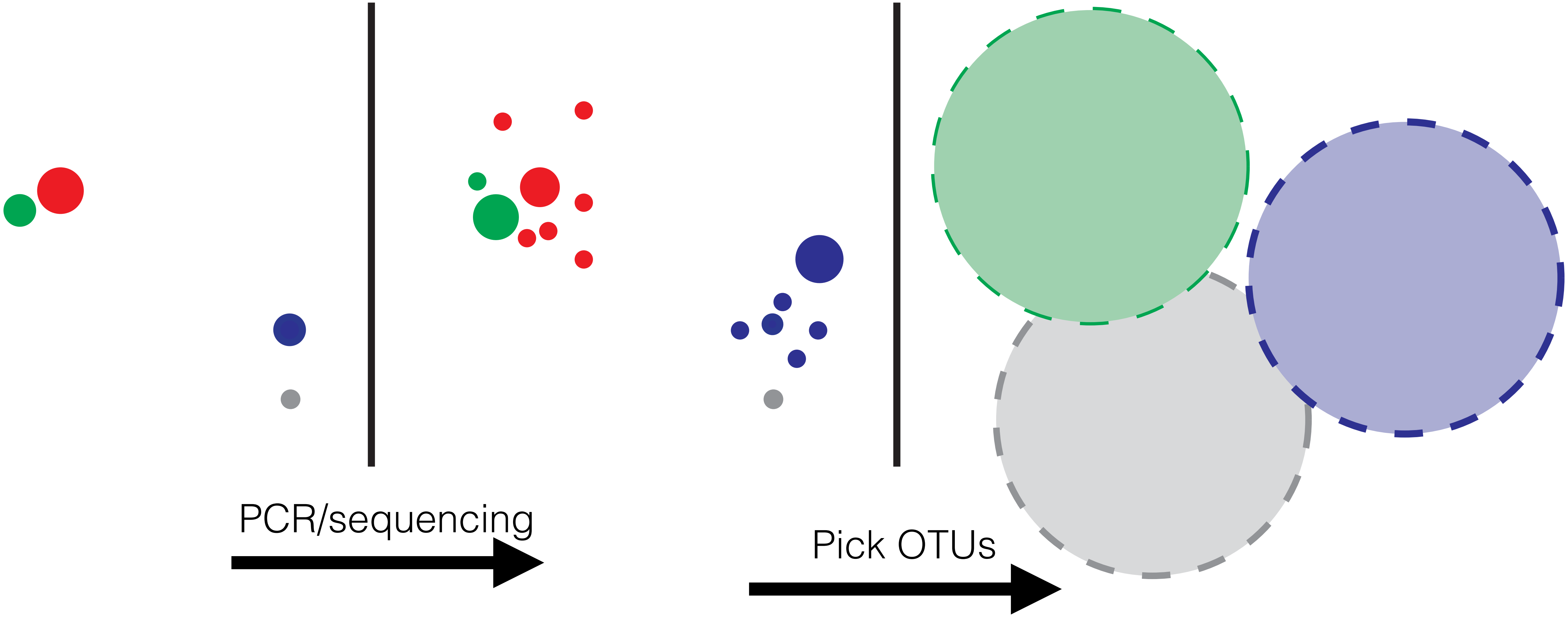


PCR/sequencing

Sample Sequences

Amplicon Reads

Different Operational Taxonomic Units



PCR/sequencing

Pick OTUs

Uh oh!

Exact Sequence Variants…

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

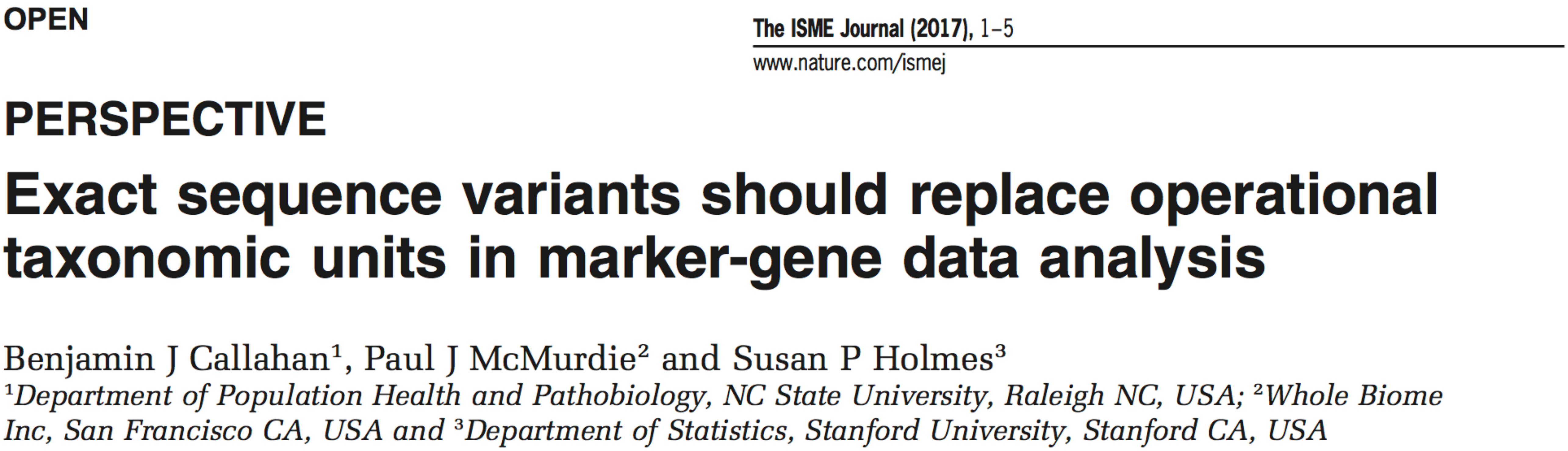
`OTU85` is *predictive of a disease?* **Not in future data!**

`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

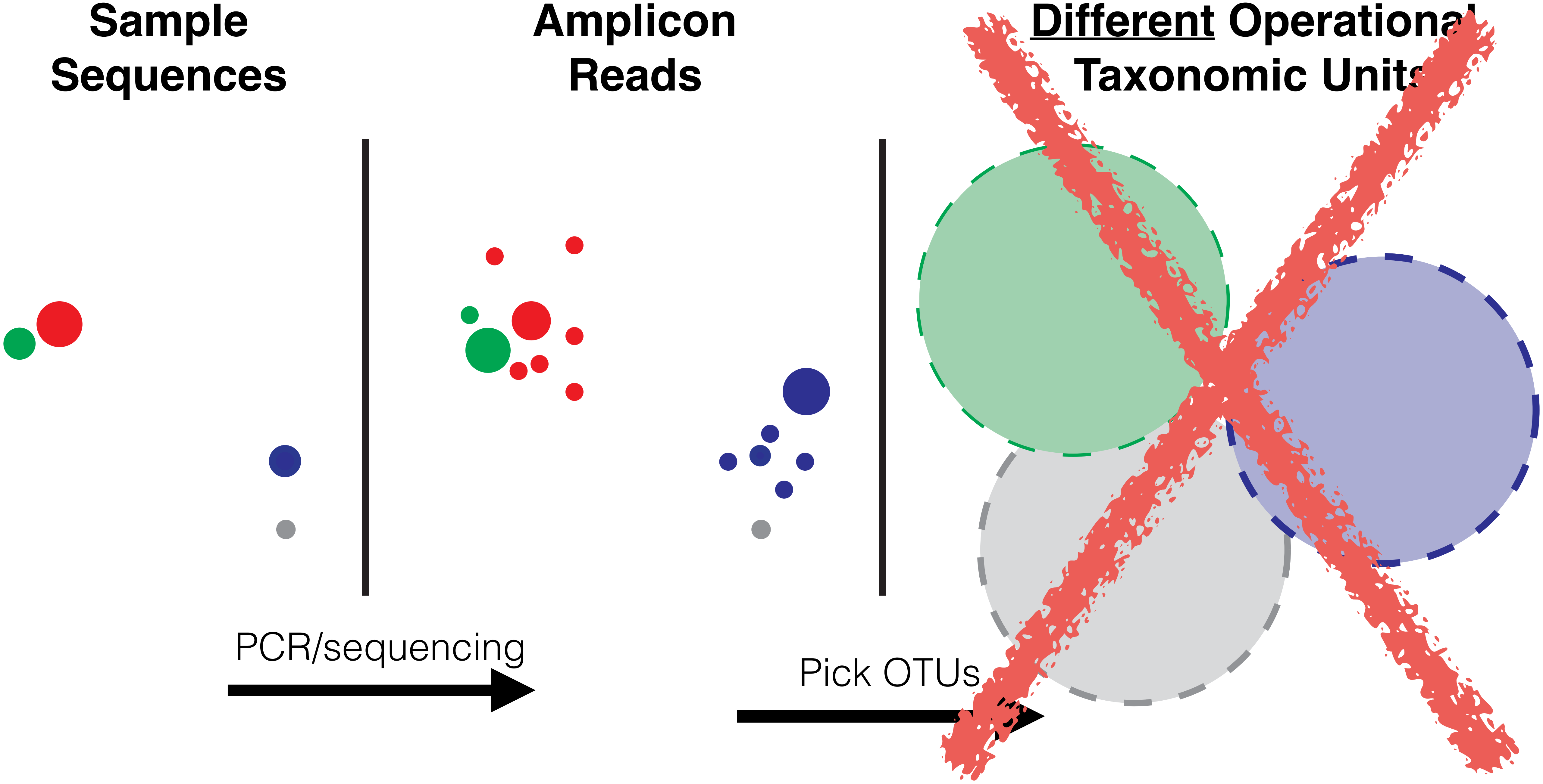
Exact sequence variants should replace operational taxonomic units in marker-gene data analysis

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

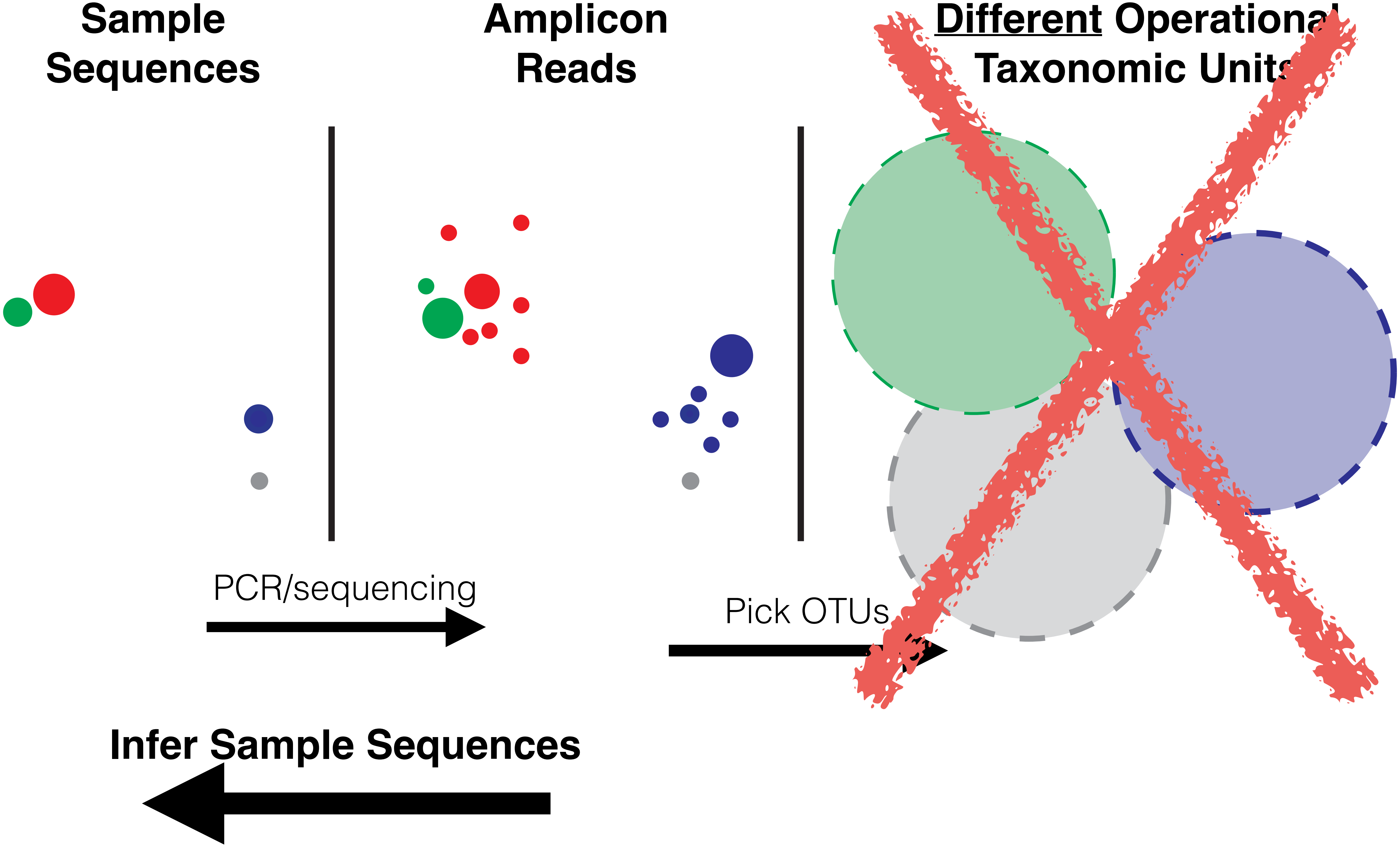
Amplicon Reads

~~**<u>Different</u> Operational Taxonomic Units**~~



PCR/sequencing

Pick OTUs



Sample Sequences

Amplicon Reads

~~**Different Operational Taxonomic Units**~~

PCR/sequencing

Pick OTUs

Infer Sample Sequences

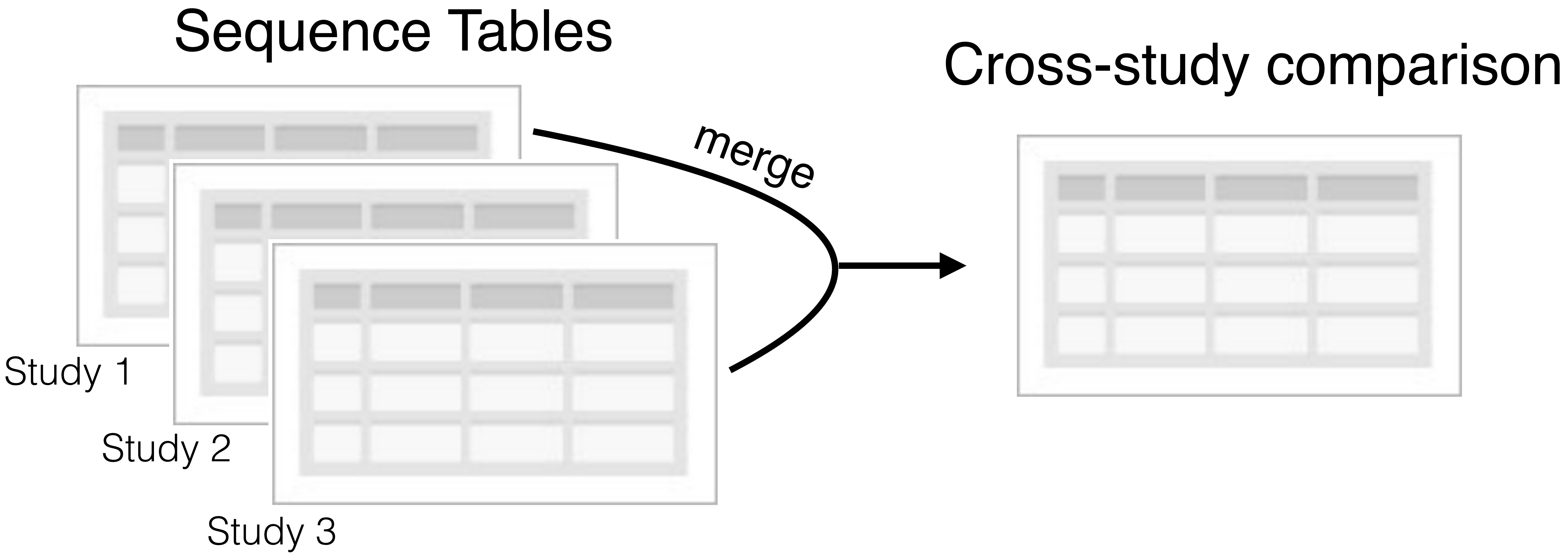
Exact Sequence Variants…

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

ATTAACGAGATTATAACCAGAGTACGAATA…

is consistent!

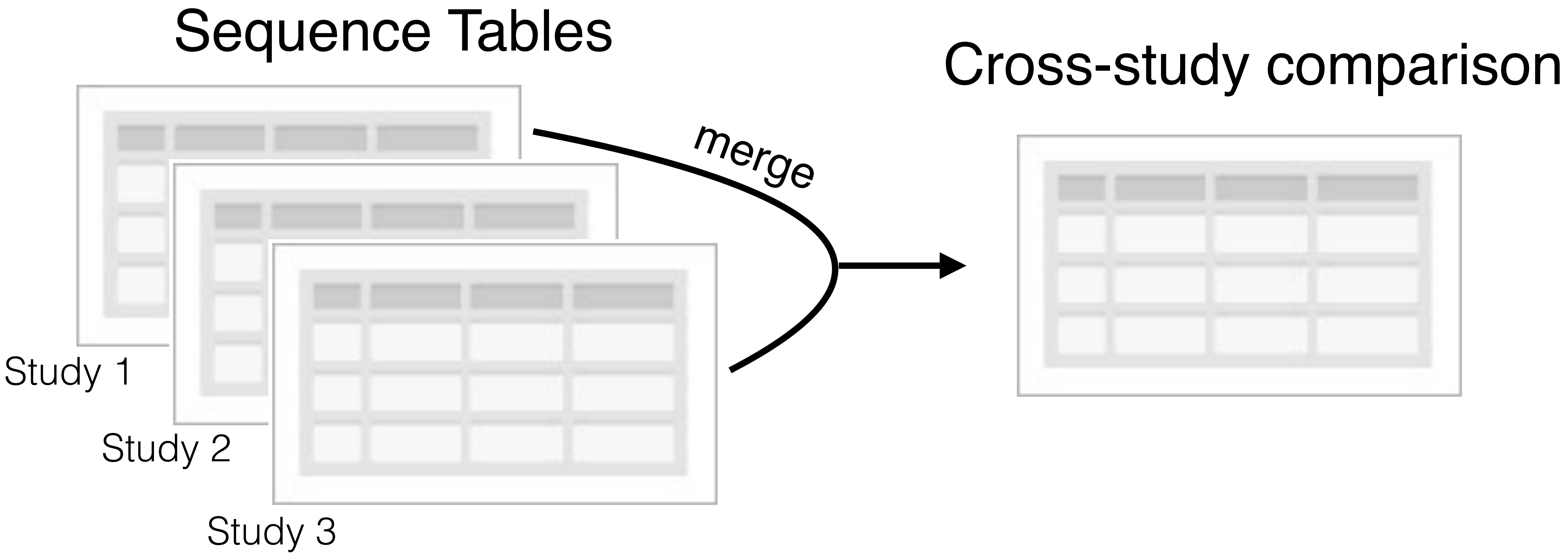
…the sequence is the label



Eliminates need for joint reprocessing of raw data.

Callahan, et al. ISMEJ, 2017.

…the sequence is the label



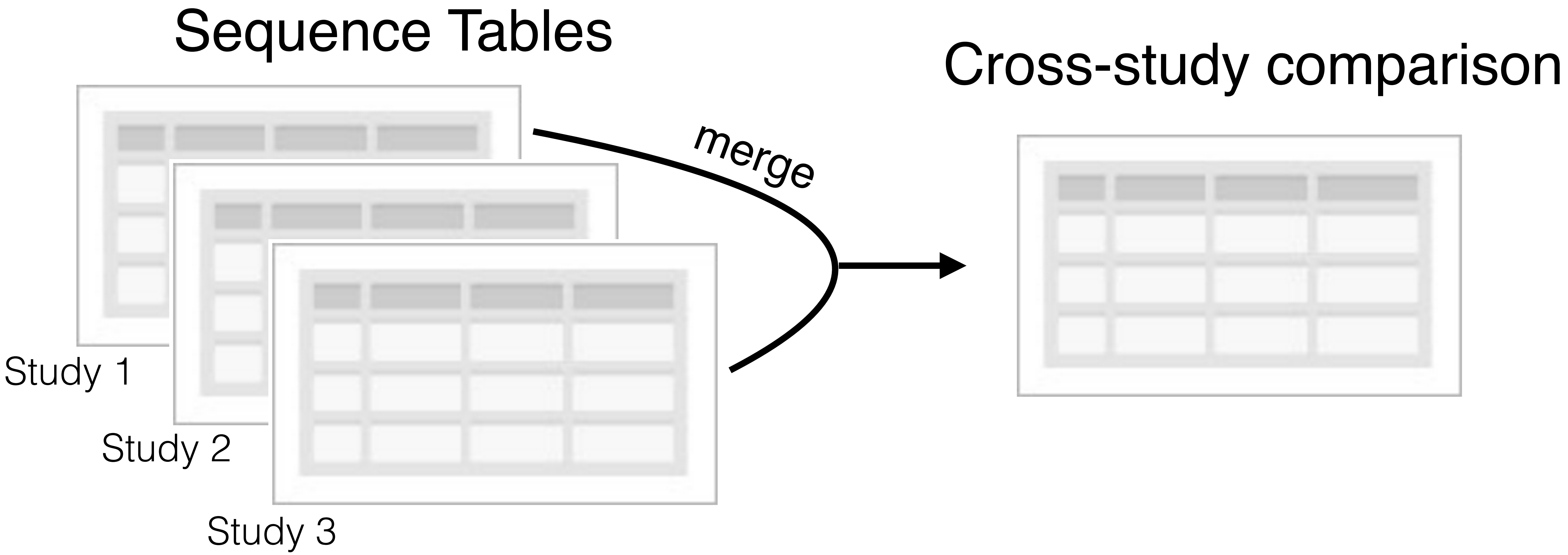
Sequence Tables

Cross-study comparison

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

Callahan, et al. ISMEJ, 2017.

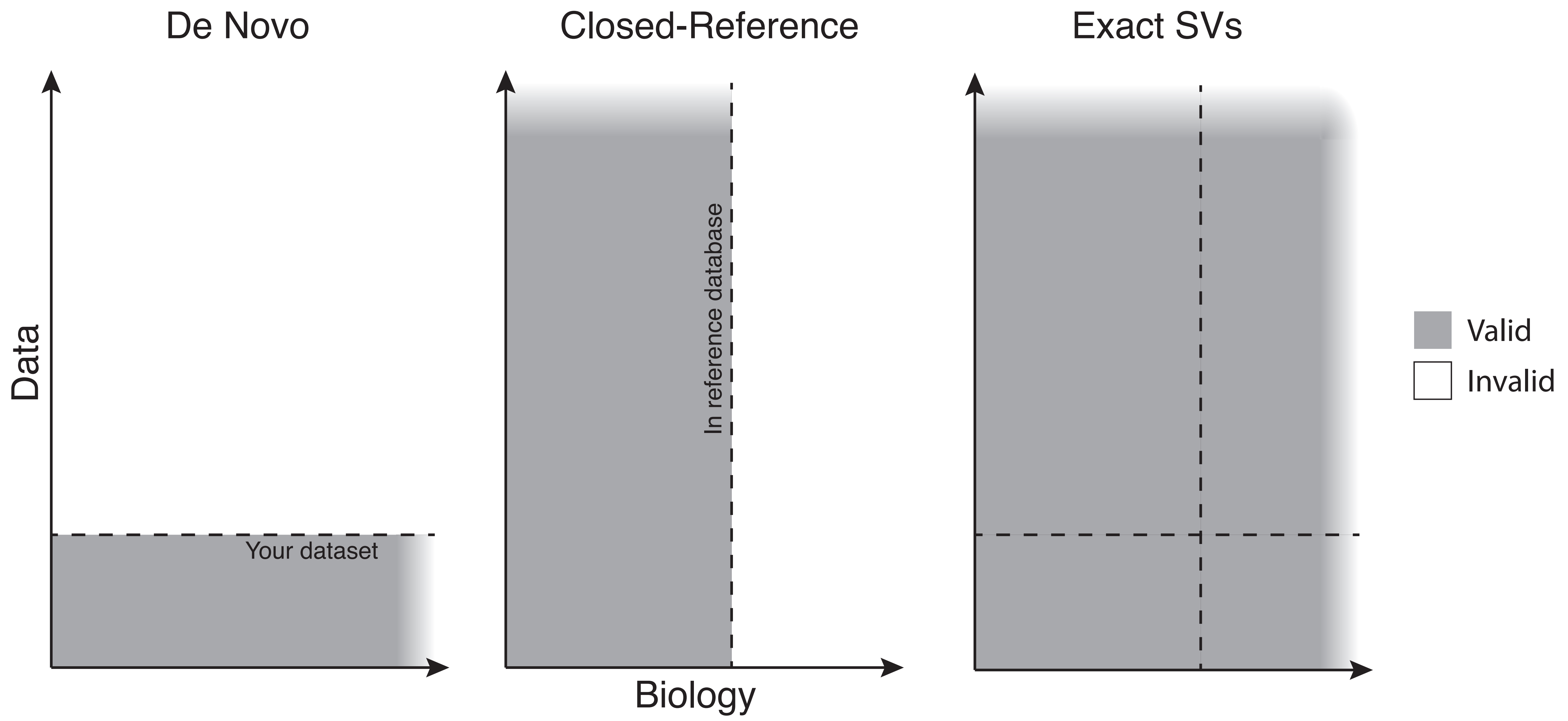
…the sequence is the label



Eliminates need for joint reprocessing of raw data.
Continuous data integration. Unlimited dataset size.
<u>You in 2020 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.

A rose by another name…

Amplicon Sequence Variants (ASVs)

- Needham et al. 2017
- Callahan et al. 2017

Exact Sequence Variants (ESVs)

- Callahan et al. 2017 (by accident)

sub-OTUs (sOTUs)

- Amir et al. 2017

Zero radius OTUs (zOTUs)

- Edgar 2017

Haplotypes, oligotypes, …

A rose by another name…

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Haplotypes, oligotypes, …

All the same thing! All the same (qualitative) benefits!

Exact Sequence Variants…

Precise

Tractable

Reproducible

Comprehensive

Callahan, et al. ISMEJ, 2017.

Exact Sequence Variants…

NATURE | ARTICLE OPEN

A communal catalogue reveals Earth's multiscale microbial diversity

Luke R. Thompson, Jon G. Sanders, Daniel McDonald, Amnon Amir, Joshua Ladau, *et al.*

Nature (2017) | doi:10.1038/nature24621

Received 13 March 2017 | Accepted 10 October 2017 | Published online 01 November 2017

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… are replacing OTUs

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*“the use of **exact sequences instead of clustered operational taxonomic units**, enable bacterial and archaeal ribosomal RNA gene sequences to be followed across multiple studies and allow us to explore patterns of diversity at an **unprecedented scale**…for many sample types, especially plant-associated and free-living communities, one-third of reads or more **could not be mapped** to [closed-reference OTUs]…Because exact sequences are stable identifiers, unlike OTUs, they can be compared to any 16S rRNA or genomic database now and in the future, thereby promoting **reusability** [22]… An advantage of using exact sequences is that they enable us to observe and analyse microbial distribution patterns at **finer resolution** than is possible with traditional OTUs…”*

Precise
Tractable
Reproducible
Comprehensive
Callahan, et al. ISMEJ, 2017.