Strain-resolved inference of microbial gene content in large metagenomic datasets

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CSHL Microbiome (2022-10-27)

Acknowledgments

Acknowledgments

Pollard Lab

- Katie Pollard
- Chunyu Zhao
- Jason Shi

GLADSTONE INSTITUTES

UCSF

CZ BIOHUB

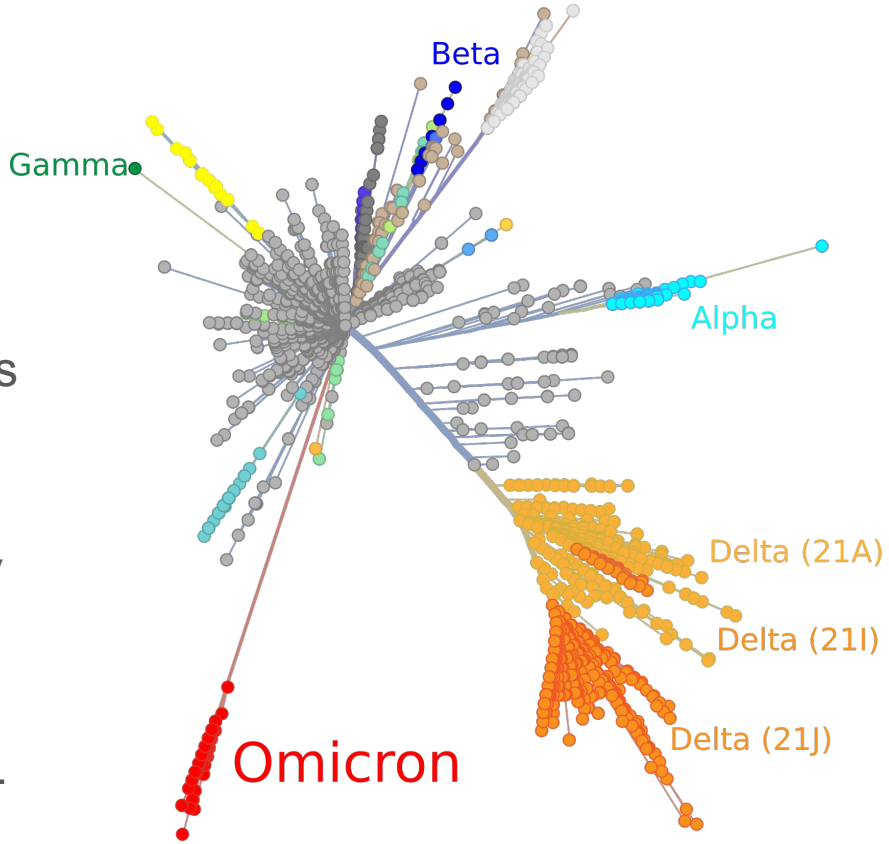
NIH National Institutes of Health

UC Noyce Initiative for Digital Transformation in Computational Biology & Health

@ByronJSmith

Strain diversity is both biologically important and scientifically informative

- Functional differences between strains
- Tracking strains between individuals, over time, or across global geography
- Transmission patterns, disease associations, selection pressures, etc.



https://nextstrain.org

Reference based methods for metagenomic profiling

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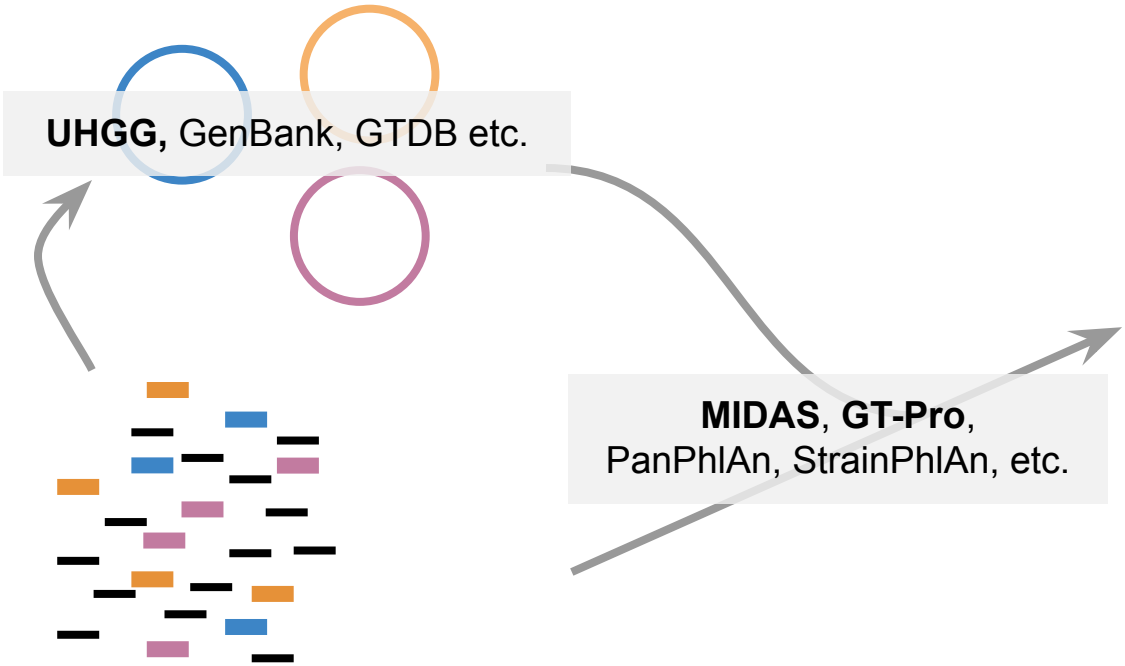
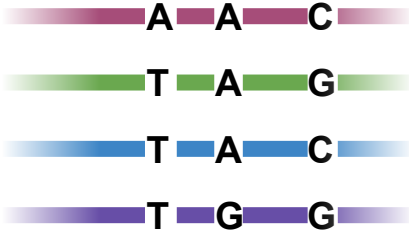
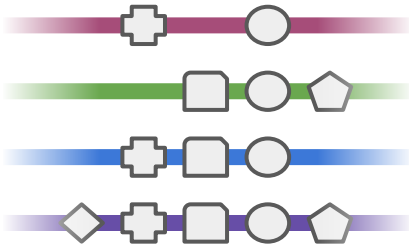


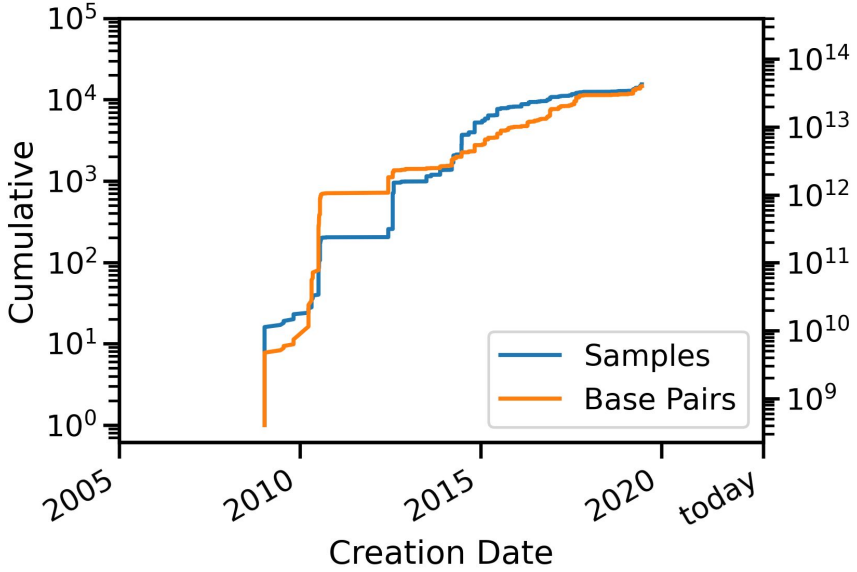
SNP Profiles



Gene Profiles

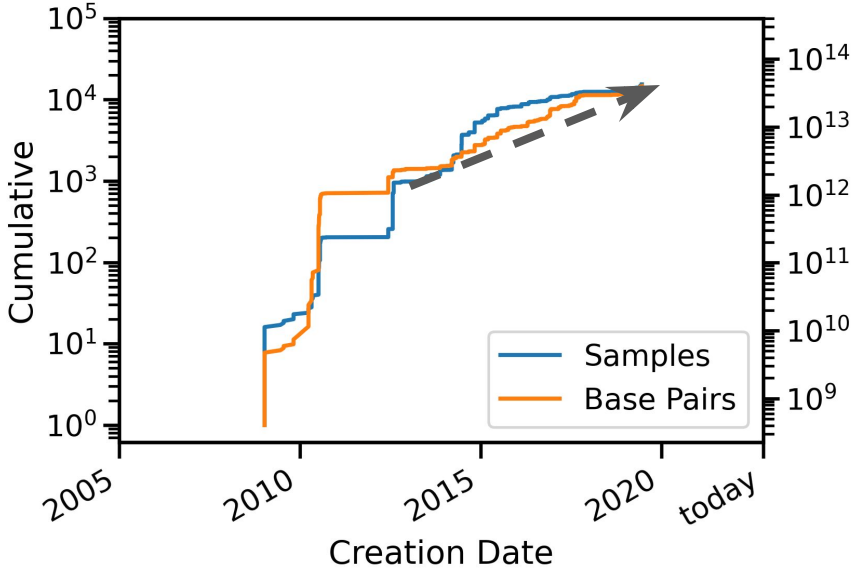


Metagenomic datasets are growing rapidly



Kasmanas et al., HumanMetagenomeDB, *Nucleic Acids Res*. 2021

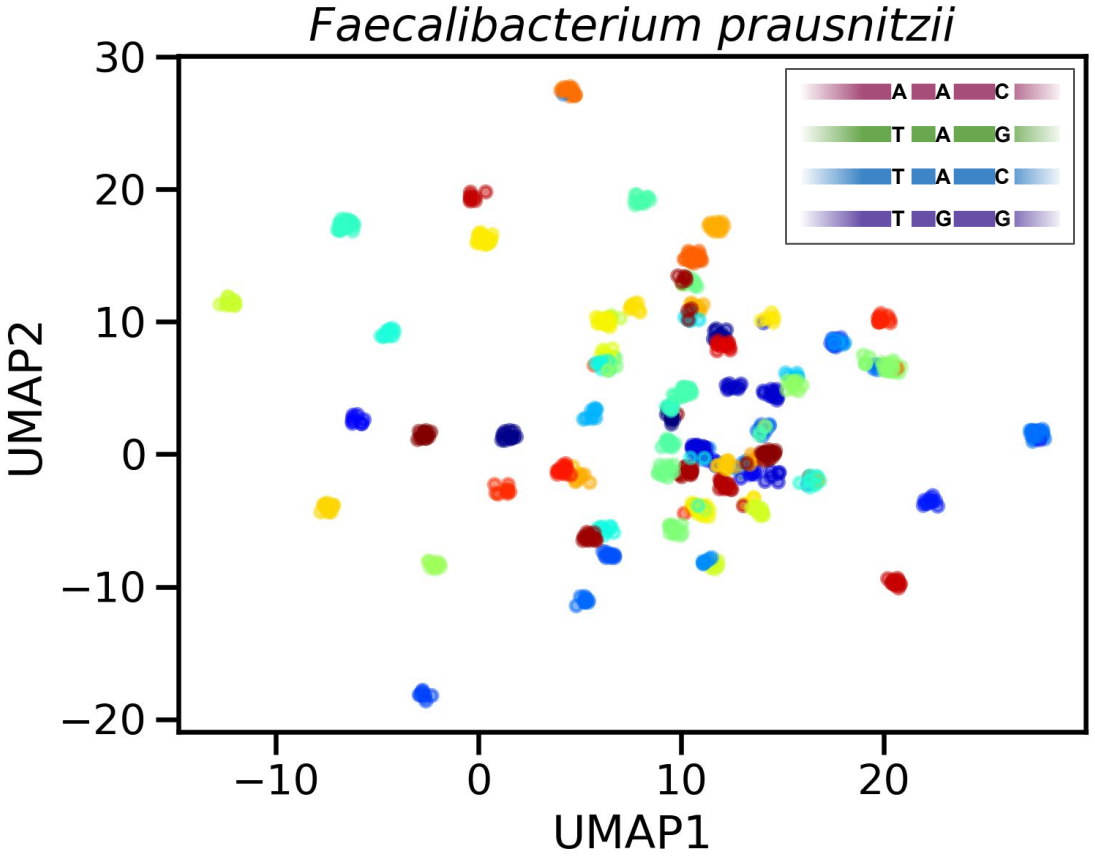
Metagenomic datasets are growing rapidly

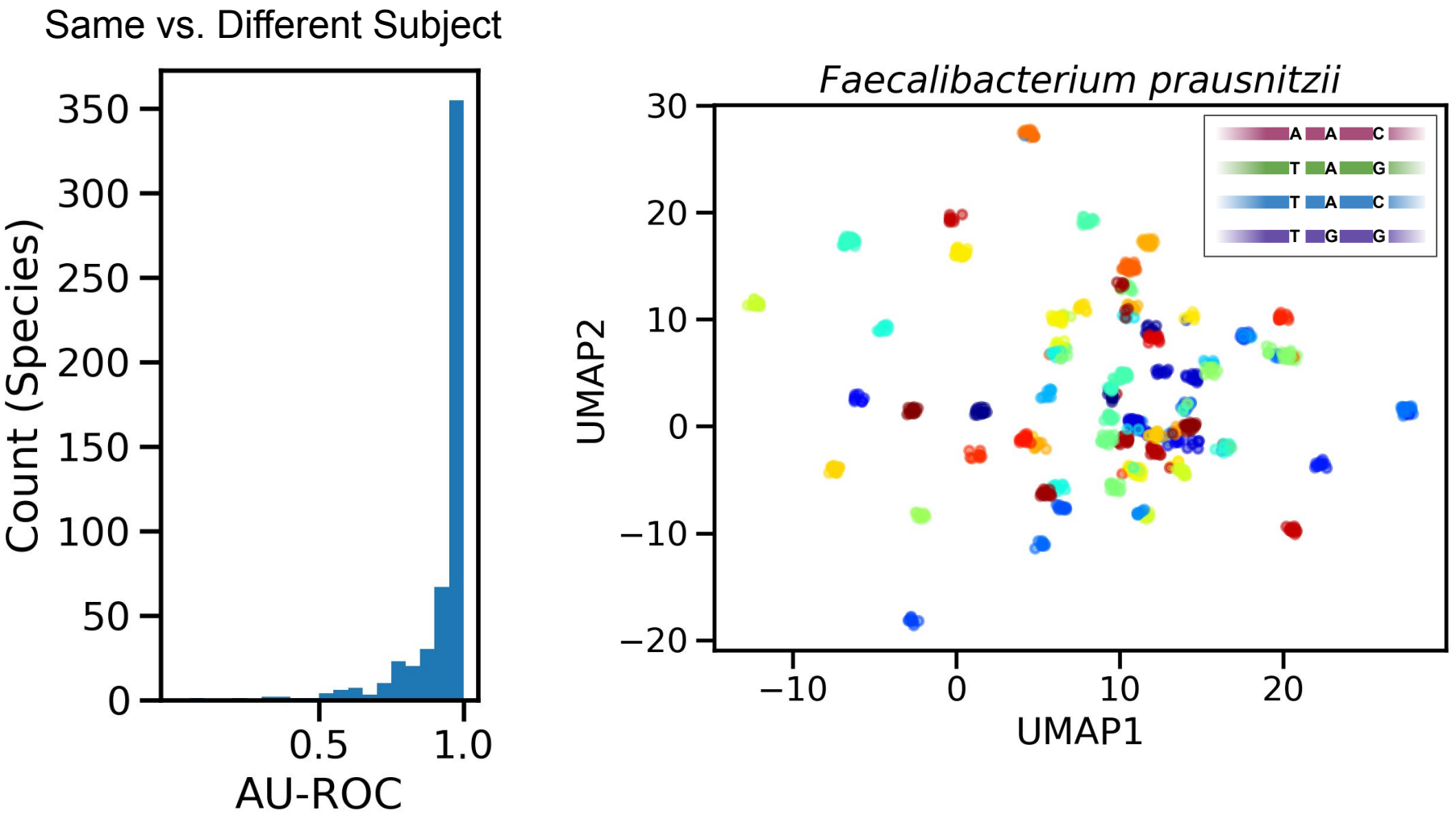


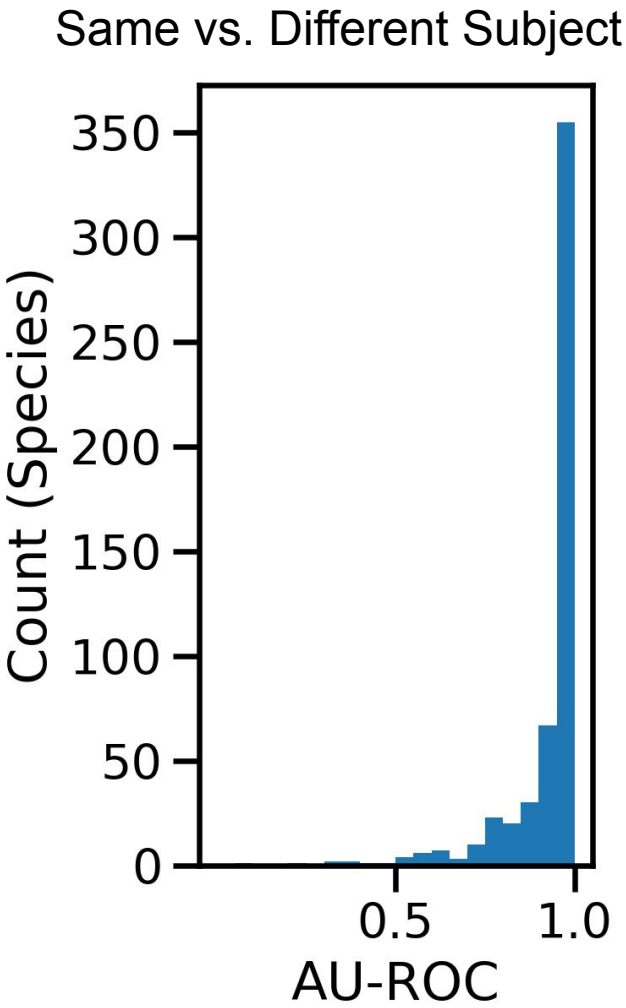
- 10^5 stool metagenomes publicly available (or soon)
- Median depth of ~10M reads
- One notable example: HMP2 composed of
  - ~1300 samples
  - ~100 subjects
  - Longitudinal sampling

Kasmanas et al., HumanMetagenomeDB, *Nucleic Acids Res*. 2021

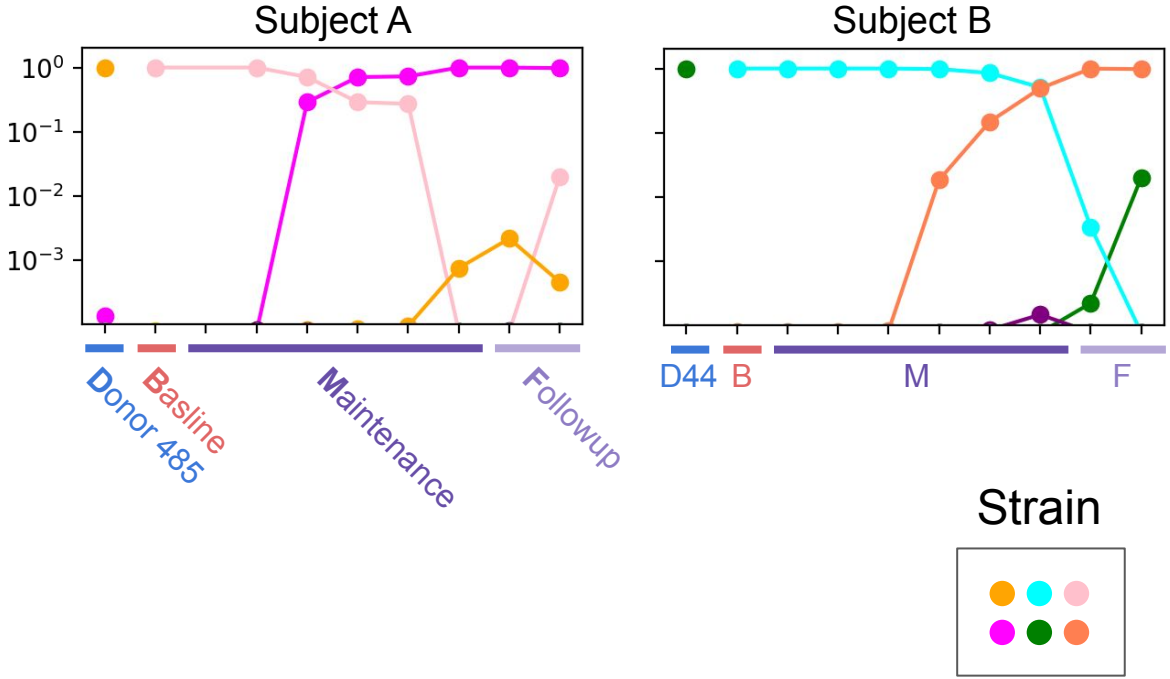
Beyond species diversity:
100 or 1000s of distinct strains across subjects



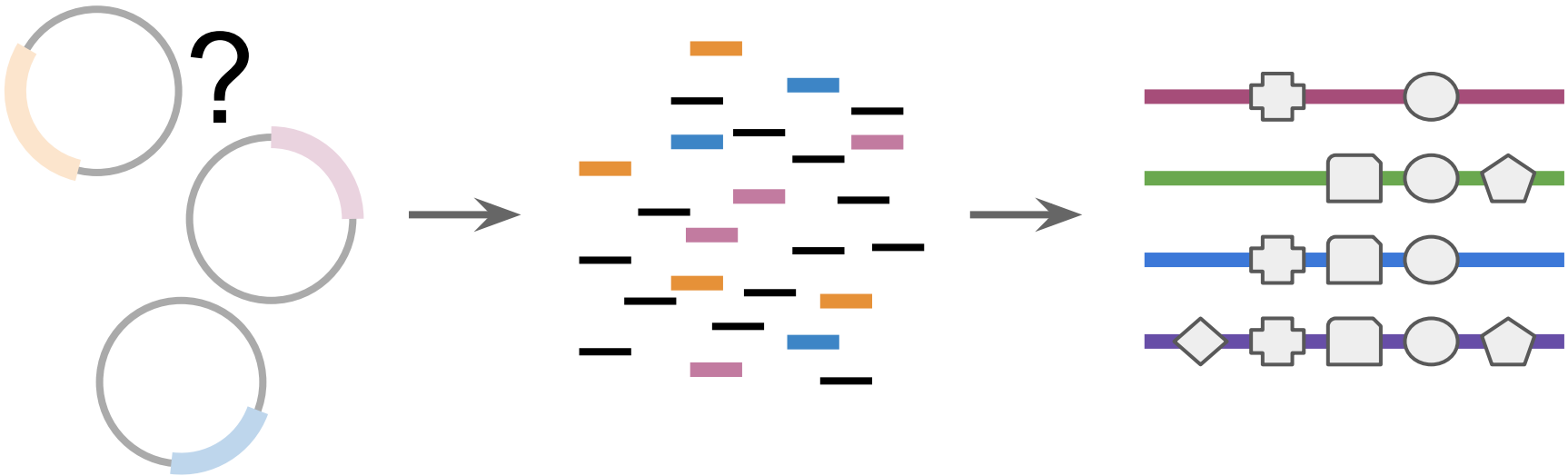

Same vs. Different Subject
Count (Species)
AU-ROC
0
50
100
150
200
250
300
350
0.5
1.0
Faecalibacterium prausnitzii
UMAP2
UMAP1
30
20
10
0
−10
−20
−10
0
10
20
A A C
T A G
T A C
T G G



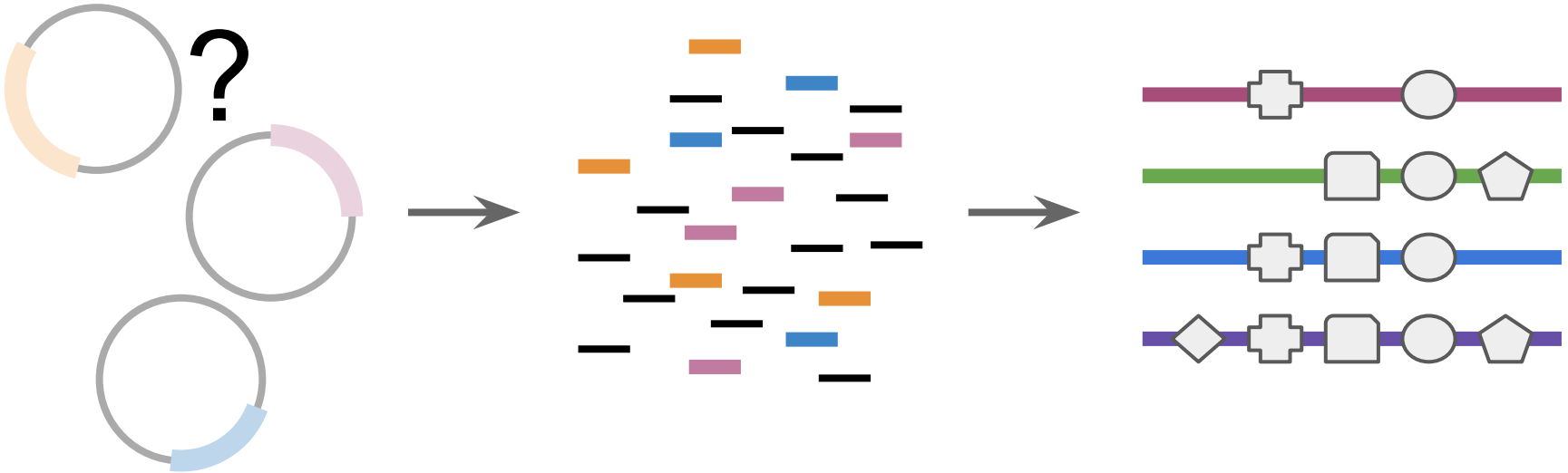
Strain diversity enables tracking of transmission between microbiomes



Smith et al., FMT for UC, *Scientific Reports* (2022)
Smith et al., StrainFacts *Frontiers in Bioinformatics* (2022)

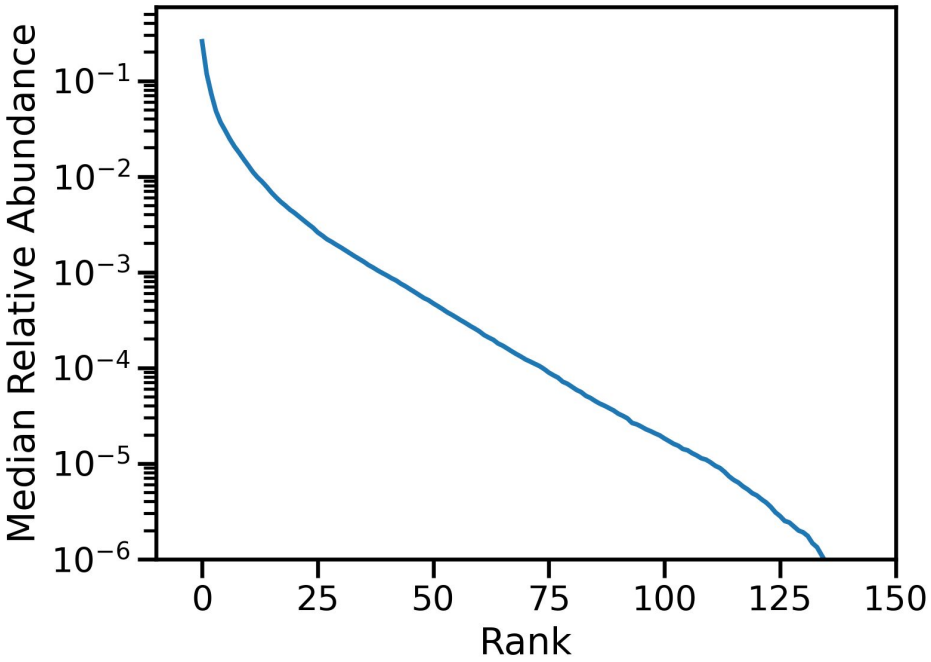


How do we accurately reconstruct strains from metagenomes?



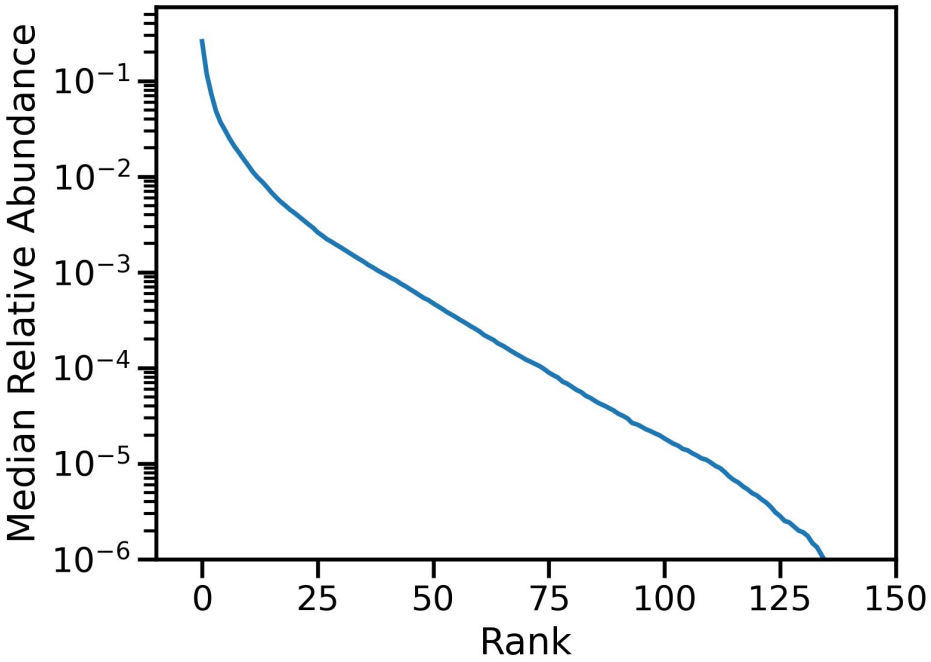
Inferring gene content accurately is difficult.

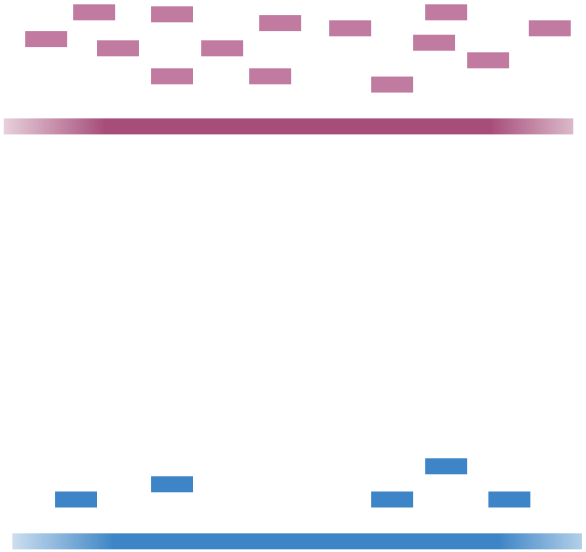
Challenge: Long tail of species abundance



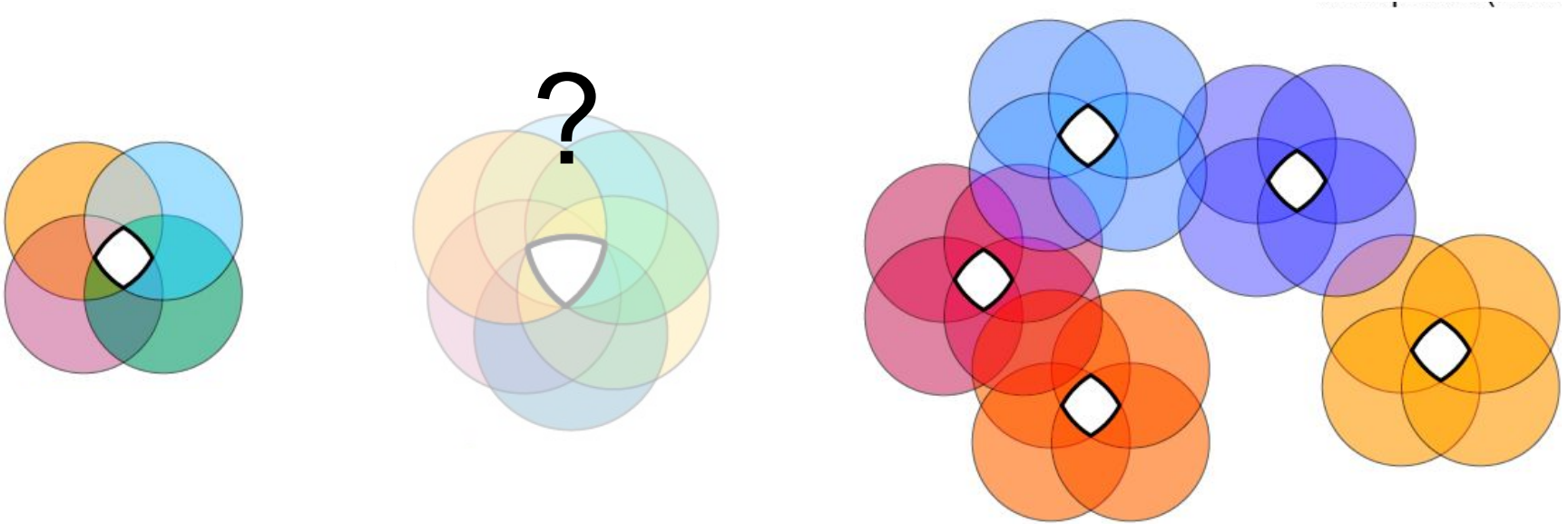
Inferring gene content accurately is difficult.

High levels of diversity results in insufficient sequencing depth for low-abundance species





Challenge: Pangenomes are large, incomplete, and overlapping



wikipedia.org

Strain-resolved gene content reconstruction: major challenges

- Low abundance (sparsity)

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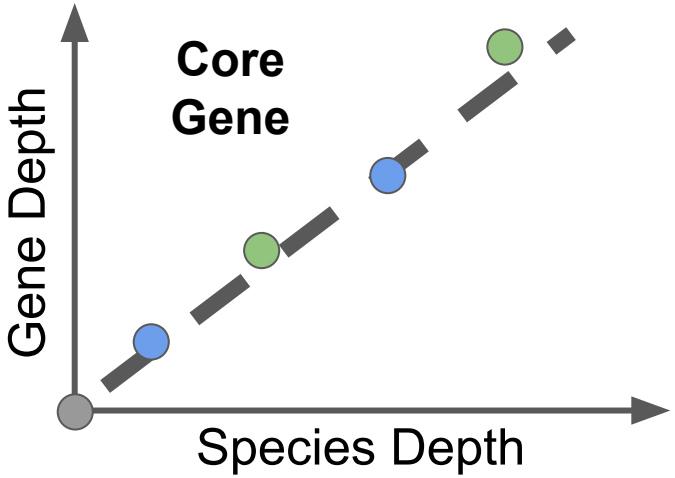
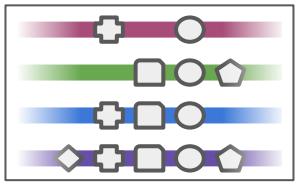
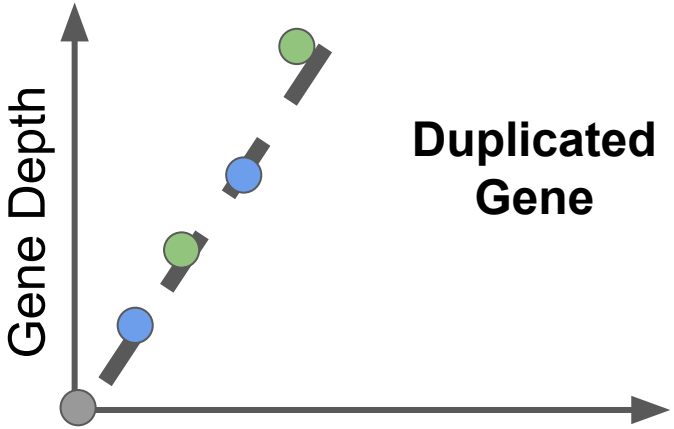
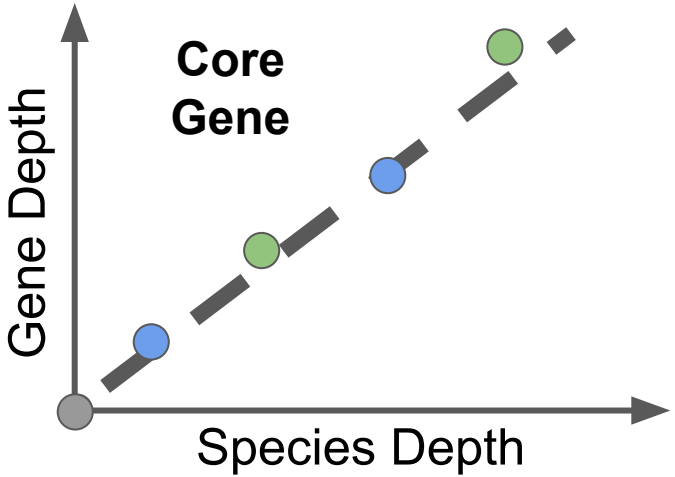
How to overcome these limitations?

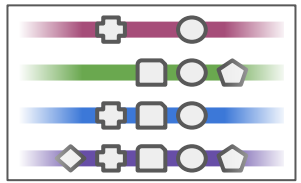
Solution: Look for correlations across multiple samples, instead of depth alone

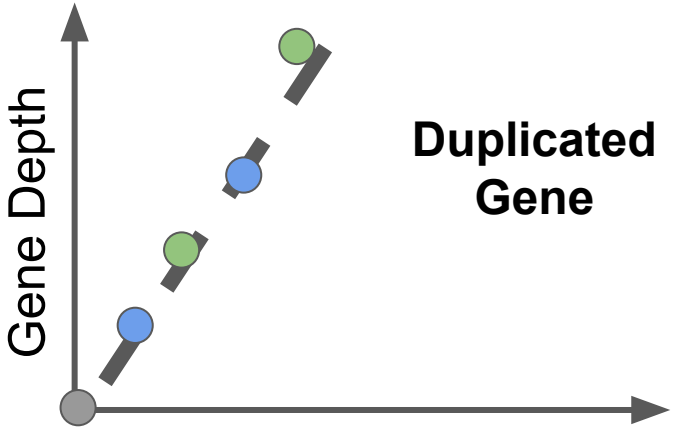


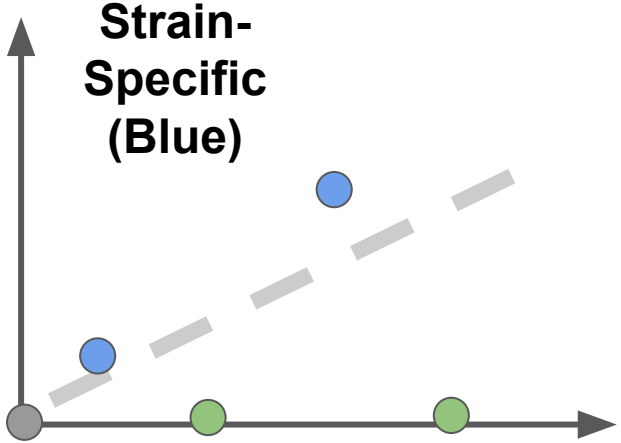


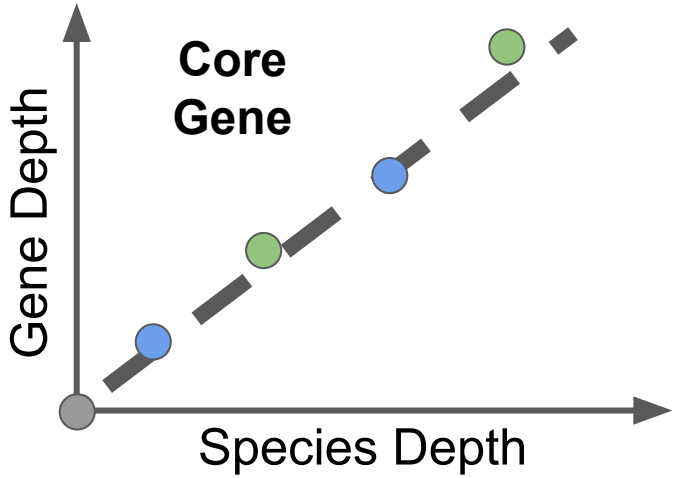

Gene Depth
Duplicated
Gene

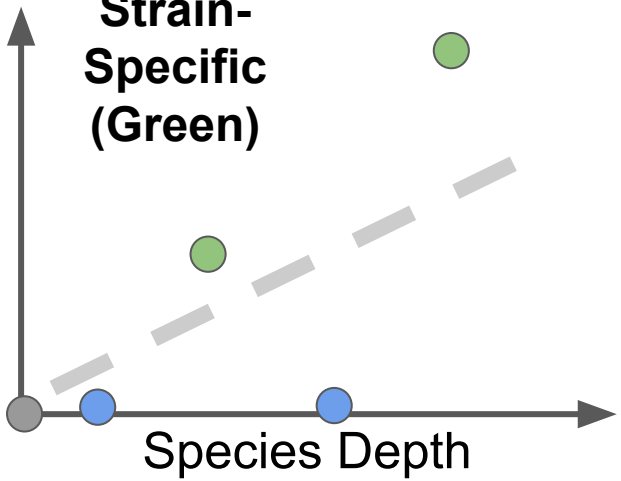

Core
Gene
Gene Depth
Species Depth





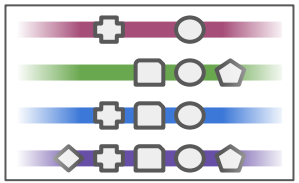






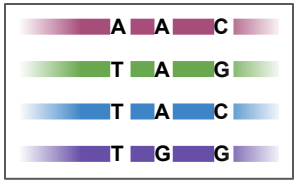
Challenge:
Strain variation

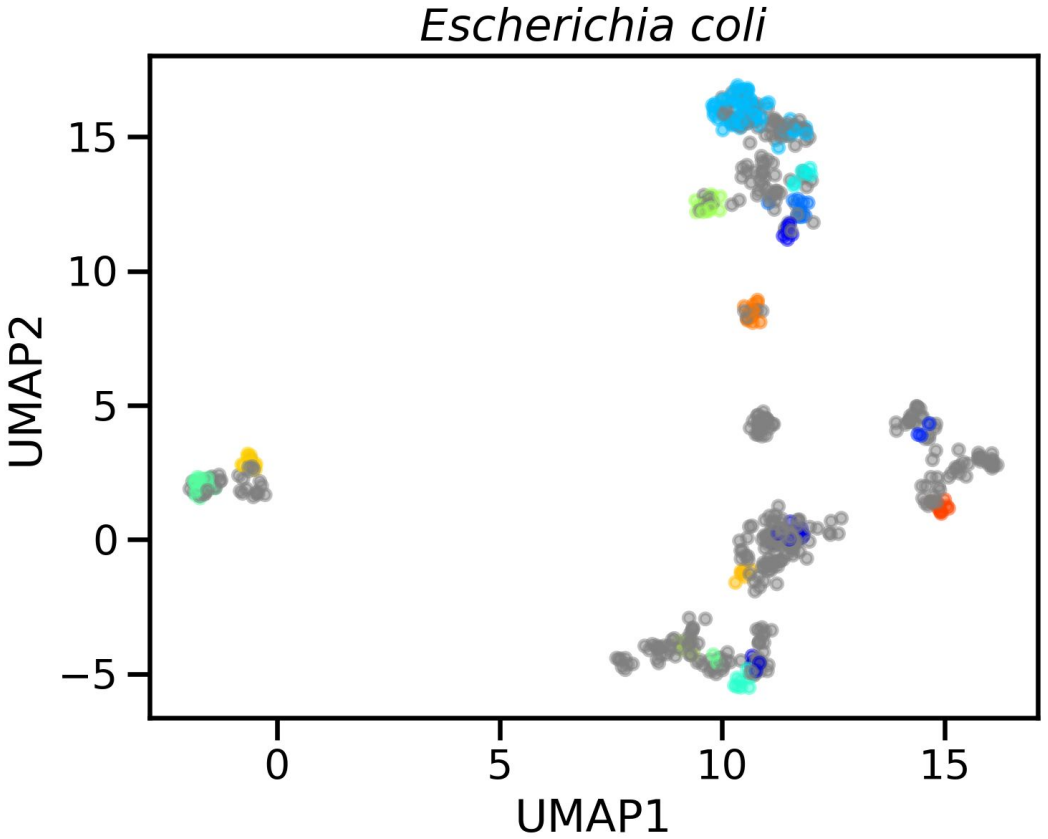
Correlations are weakened and strain-specific genes are lost due to inconsistent depth.

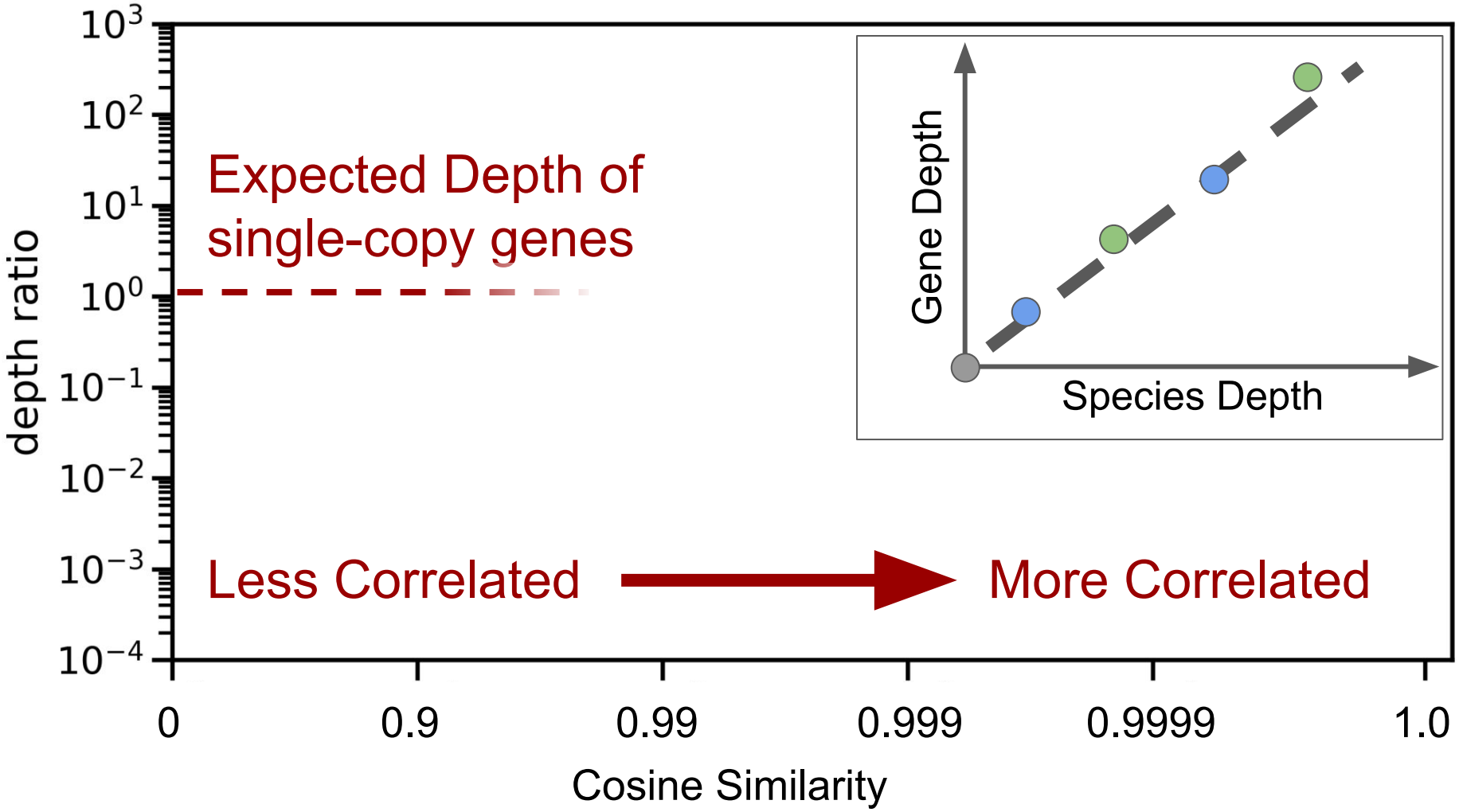


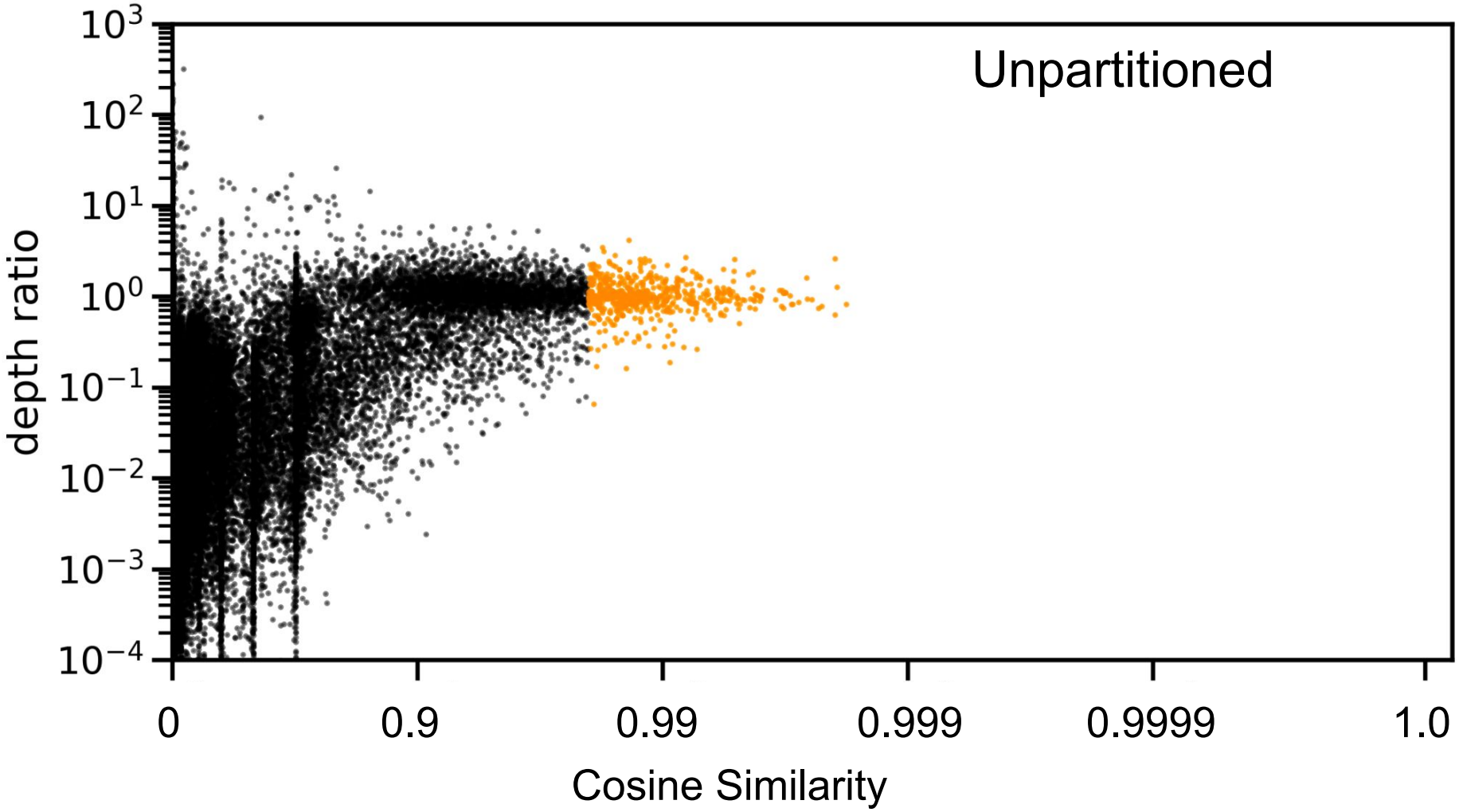
Solution: Partition samples by strain

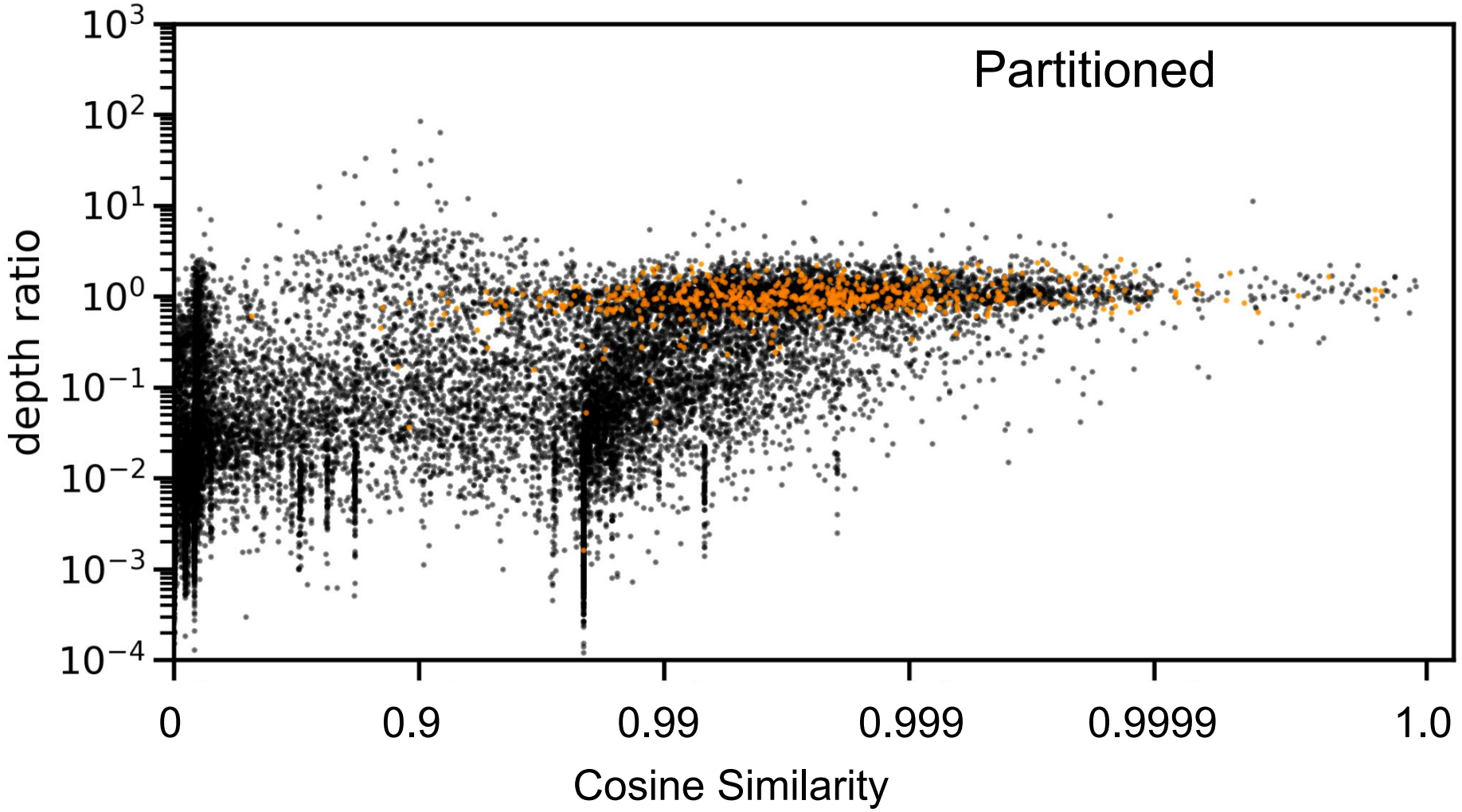
Use strain SNP profiles to select pure samples of each strain.

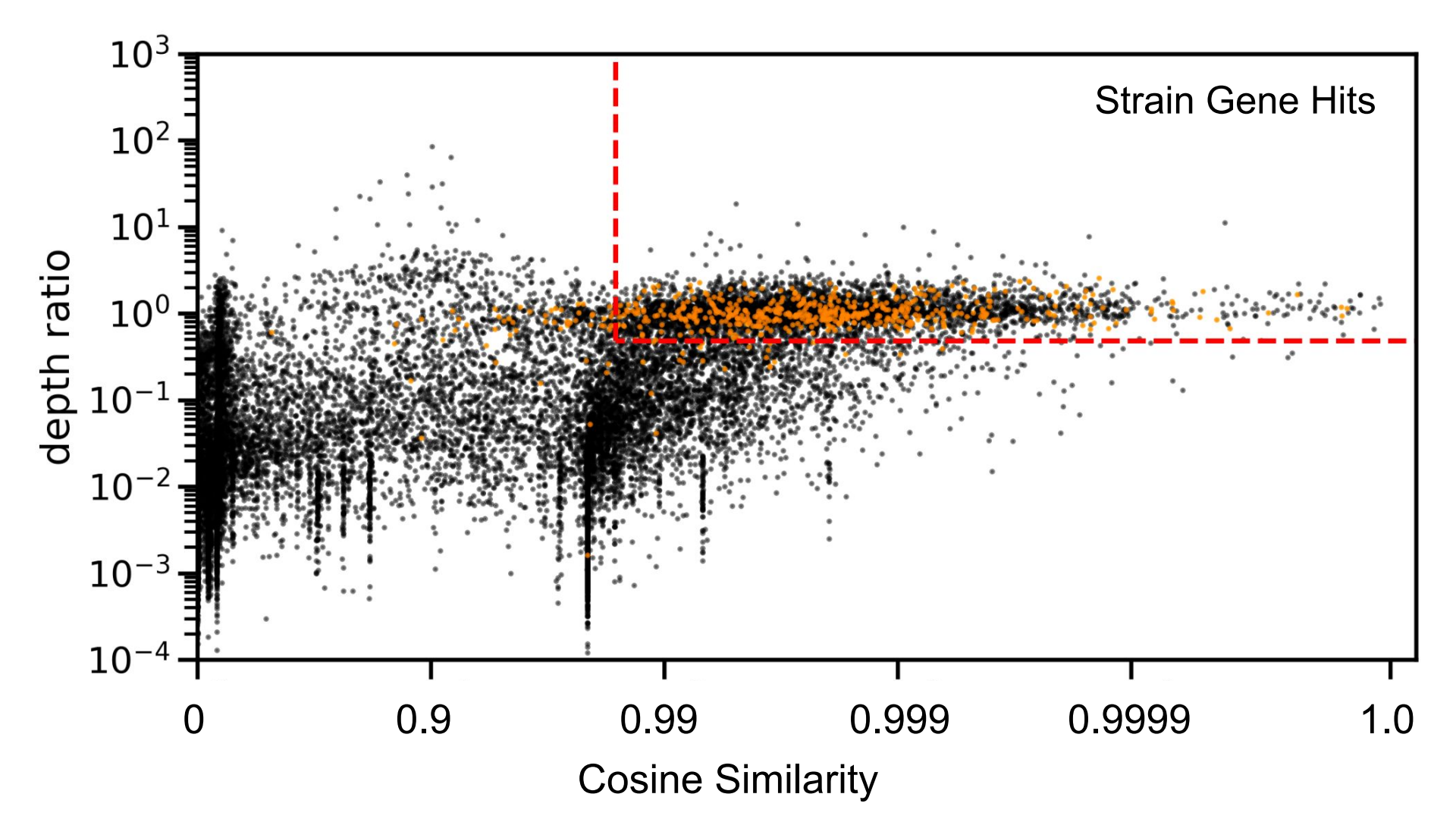




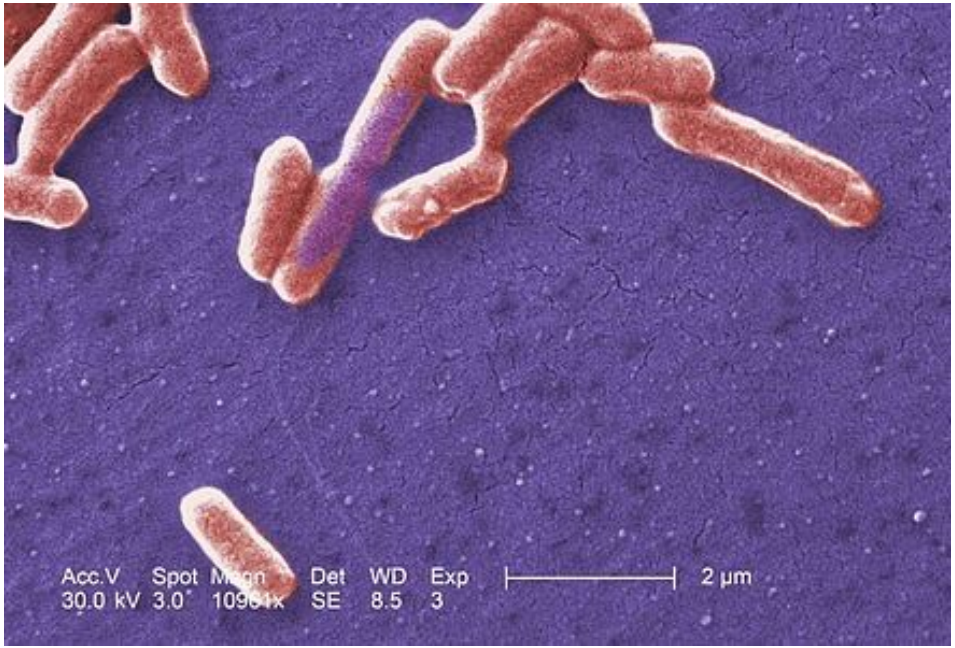

depth ratio
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
Expected Depth of single-copy genes
Less Correlated
More Correlated
Gene Depth
Species Depth
0
0.9
0.99
0.999
0.9999
1.0
Cosine Similarity


Unpartitioned
depth ratio
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
0.9
0.99
0.999
0.9999
1.0
Cosine Similarity


Partitioned
depth ratio
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
0.9
0.99
0.999
0.9999
1.0
Cosine Similarity


Strain Gene Hits
depth ratio
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
0.9
0.99
0.999
0.9999
1.0
Cosine Similarity

Genes inferred for 16 distinct *E. coli* strains



wikipedia.org

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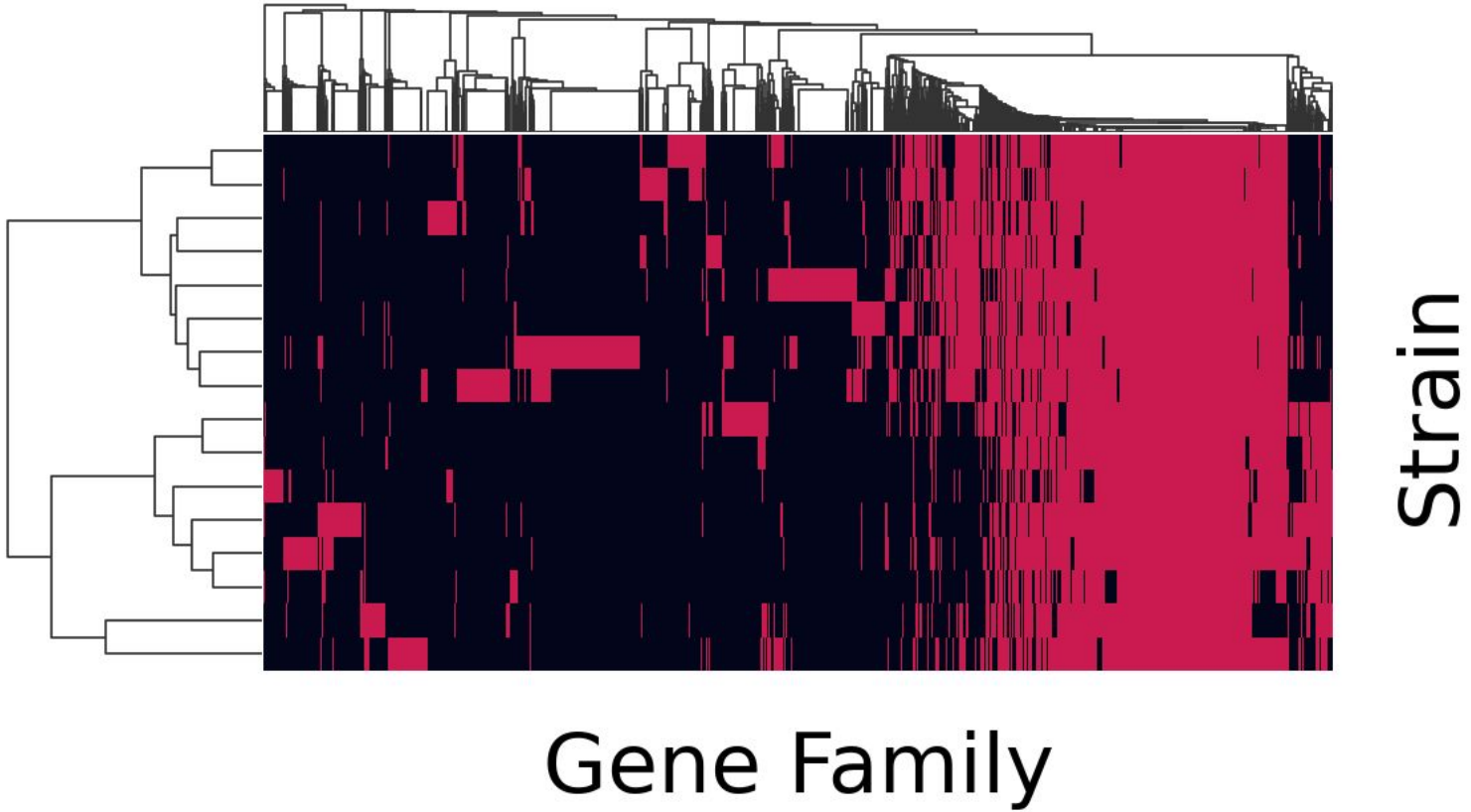
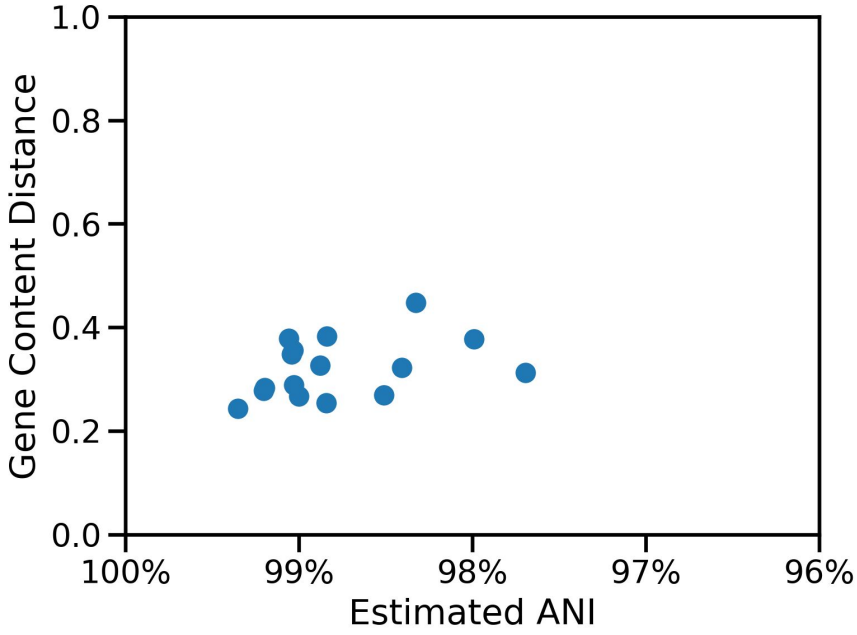
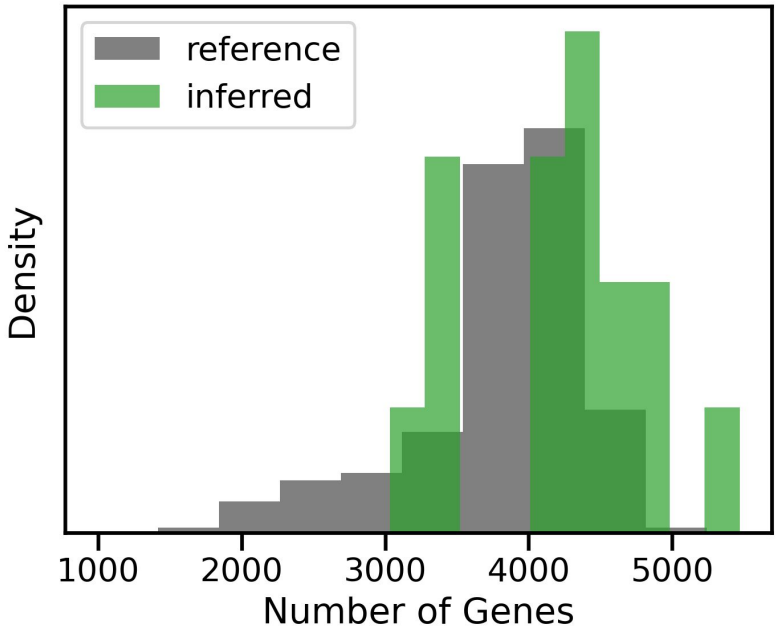
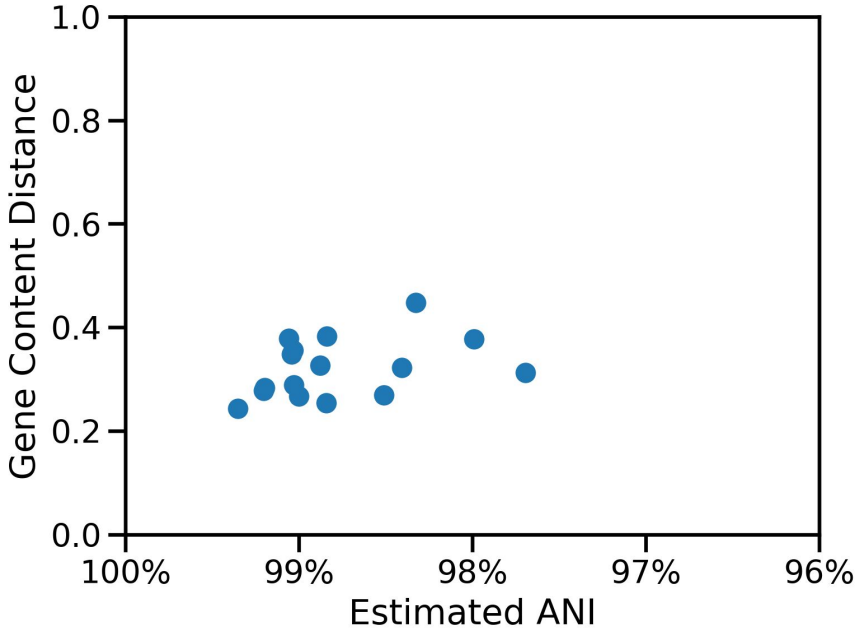


Strain genotypes and gene content are both different from existing references



Genome size and gene prevalence are consistent with reference databases





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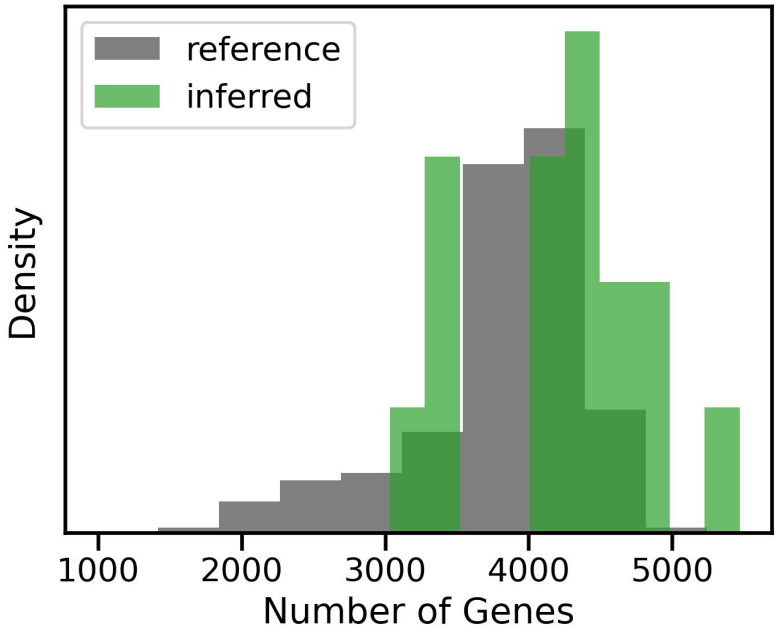

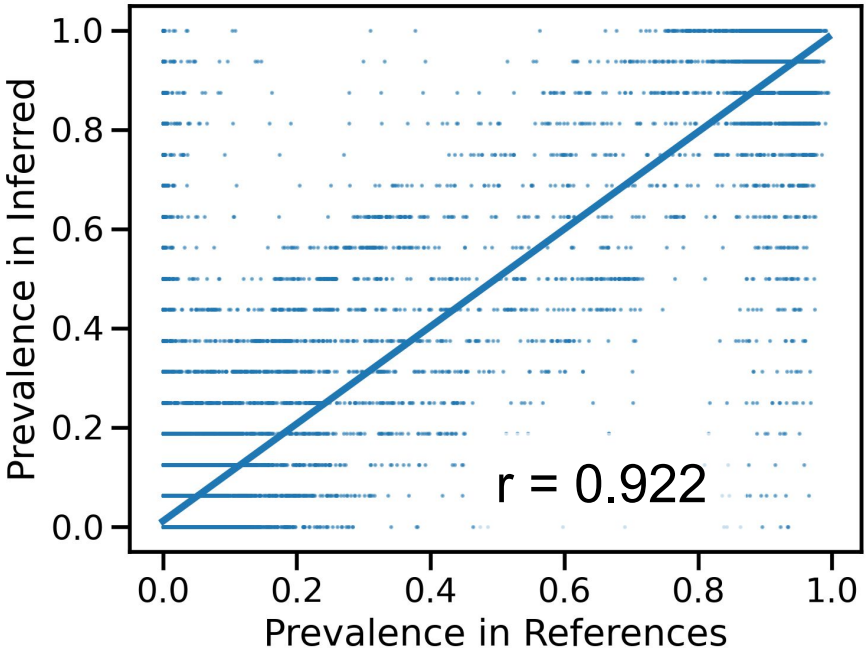

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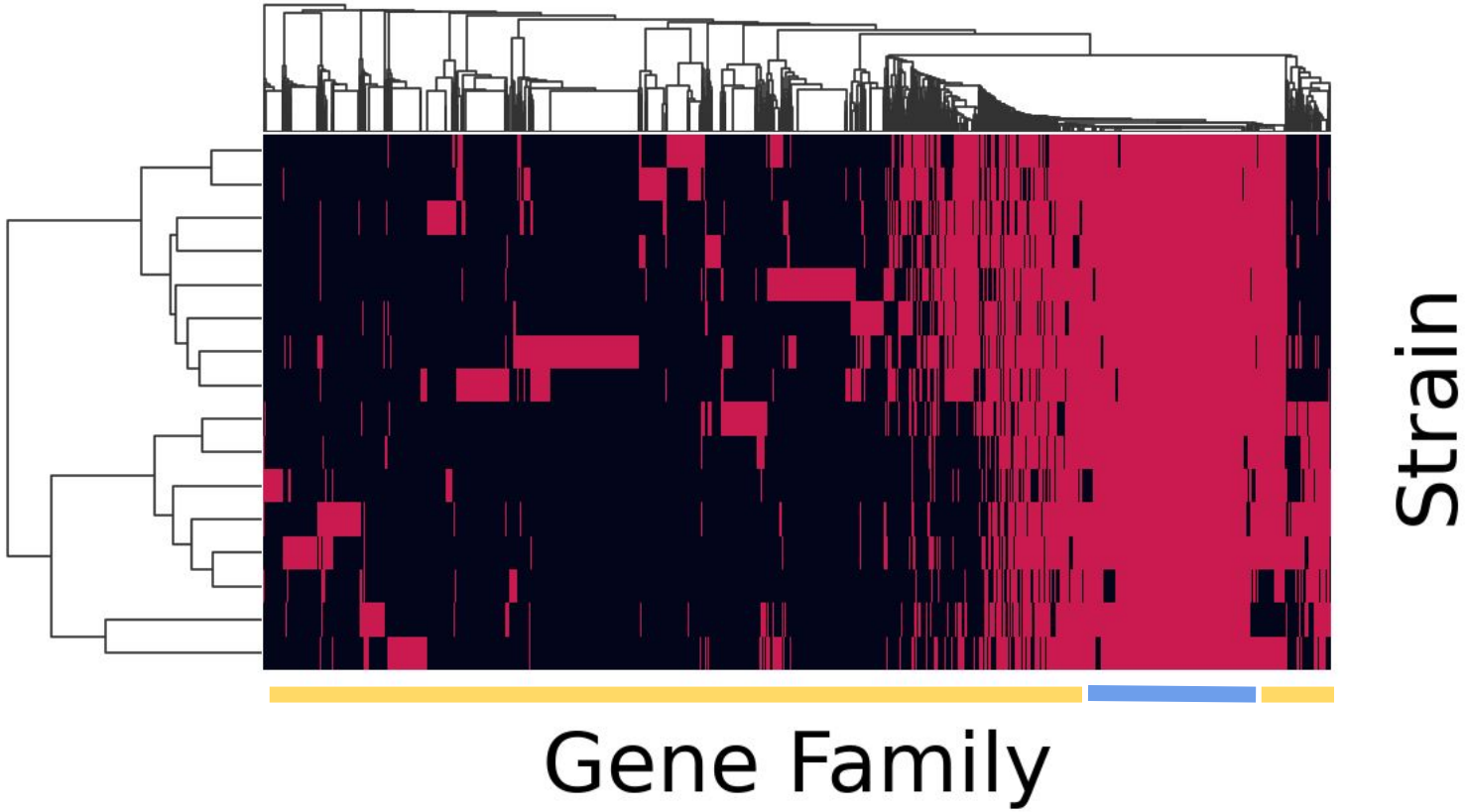
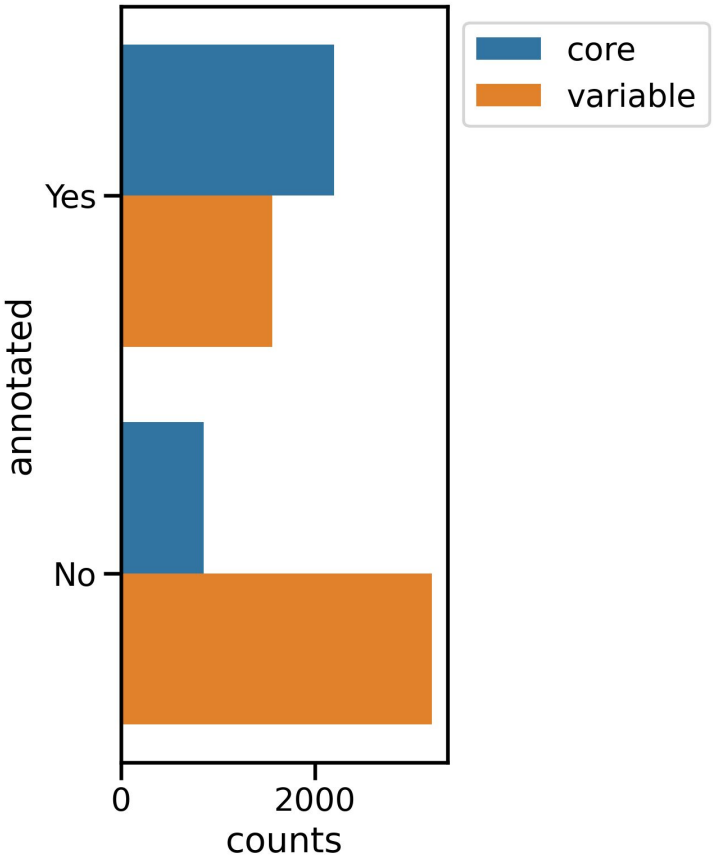
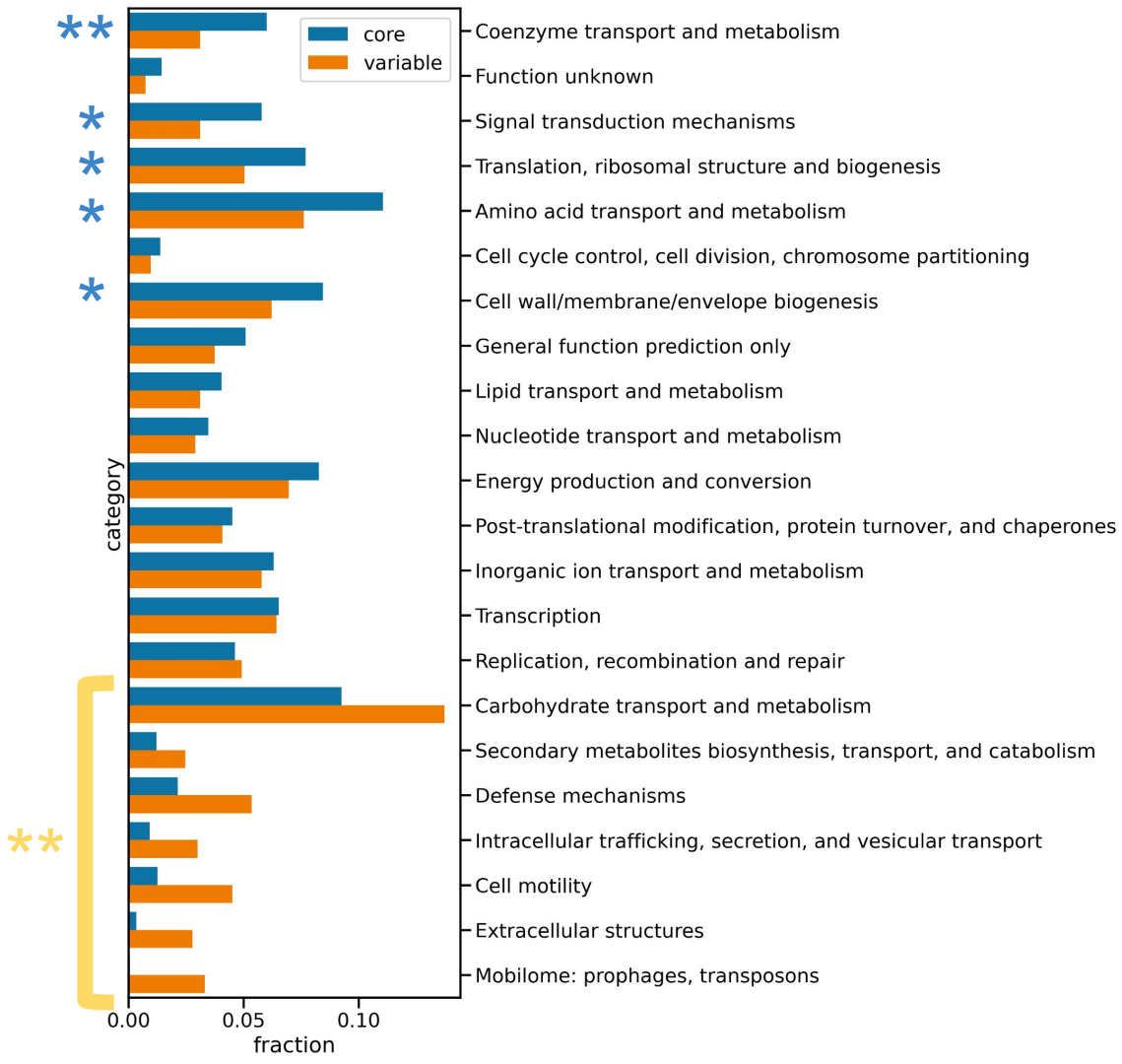


The variable fraction is enriched with un-annotated genes.



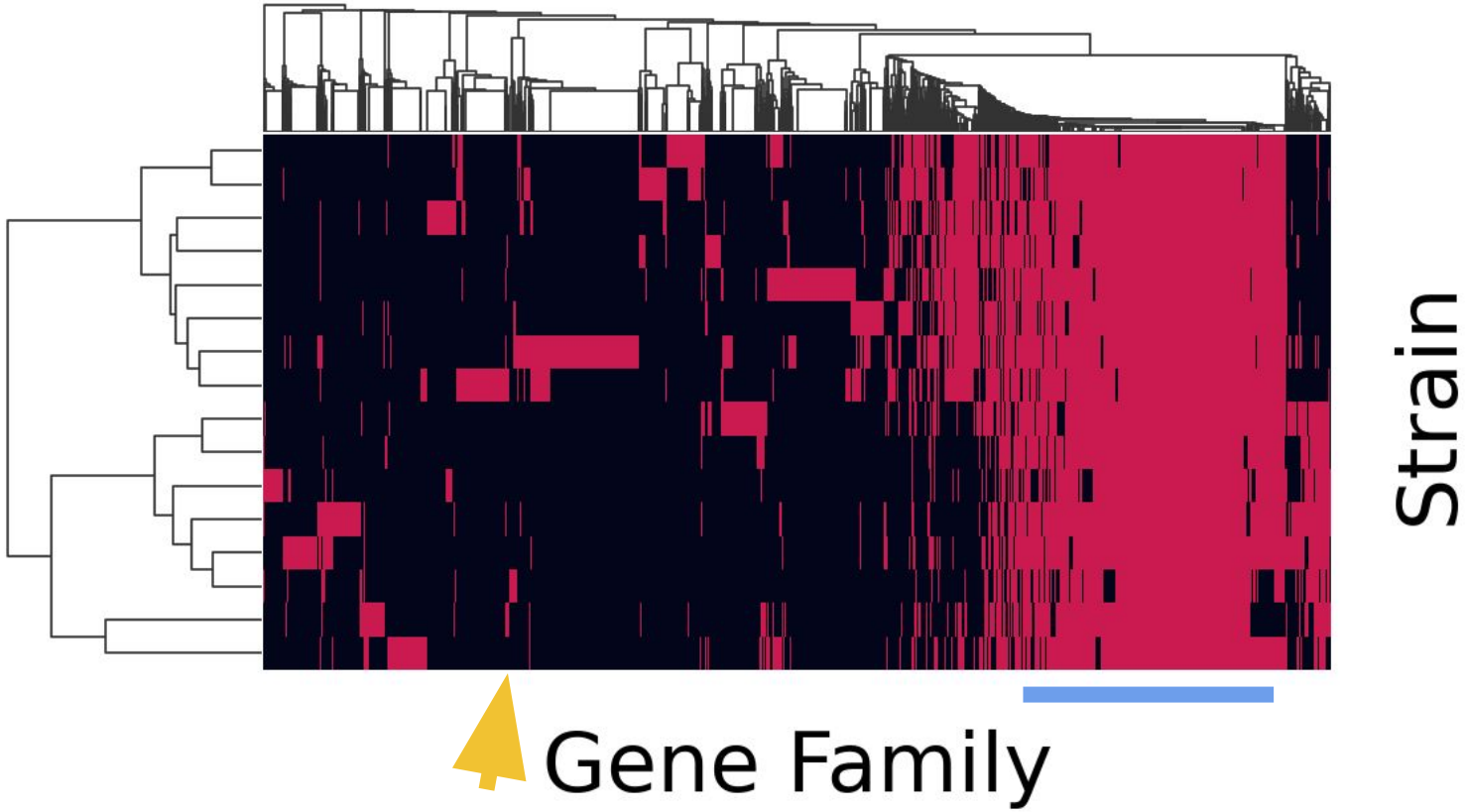
Model lab strains and other isolates may be insufficient for understanding physiology in the gut microbiome.



Among COG-annotated genes, variable genome is enriched with important functional categories, e.g.:

- Motility
- Carbohydrate and secondary metabolism
- Defense
- Etc.

Distantly related strains can share an entire suite of genes



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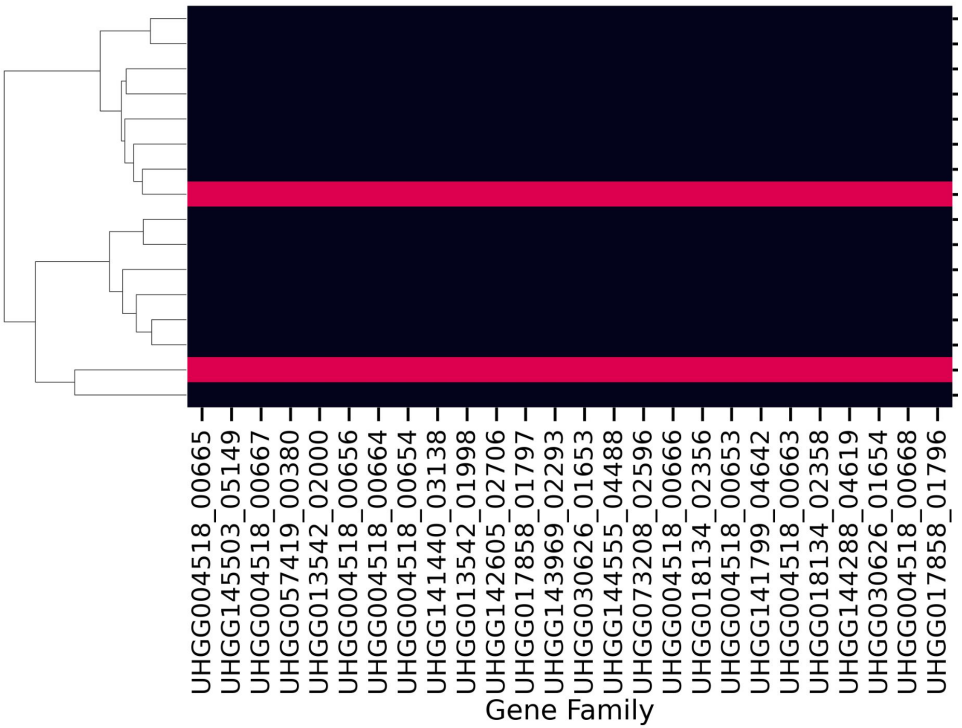


Transporter for capsular polysaccharide:

- kpsD/M
  (COG1596, COG1682)

Rhamnose synthesis (component of O-antigen)

- rfbB/C/D
  (COG1088, COG1898, COG1091)
- rmlA (COG1209)

S-layer glycoprotein synthesis

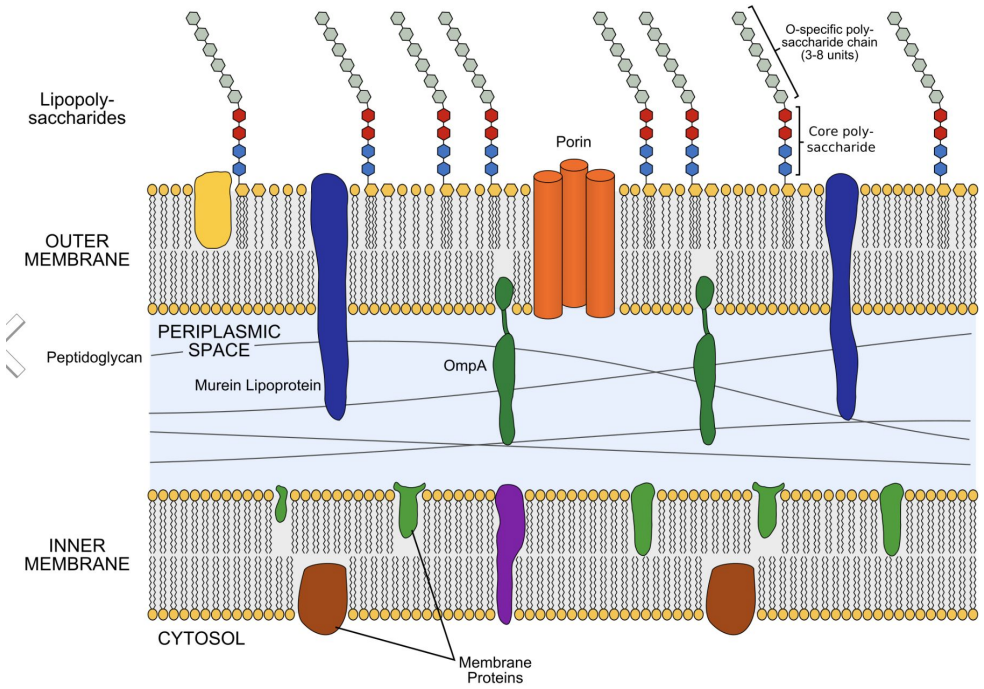
- fdtC

Prophage integrase

- intA (COG0582)

18 un-annotated proteins

Distantly related strains can share an entire suite of genes



Prophage integrase

- intA (COG0582)

Transporter for capsular polysaccharide:

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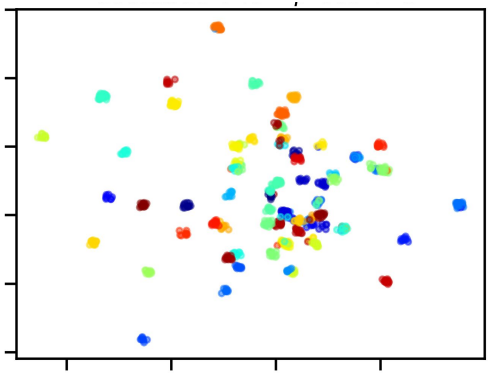
- rfbB/C/D (COG1088, COG1898, COG1091)
- rmlA (COG1209)

S-layer glycoprotein synthesis

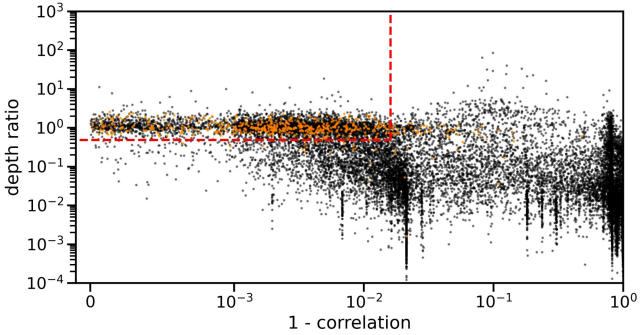
- fdtC

18 un-annotated proteins

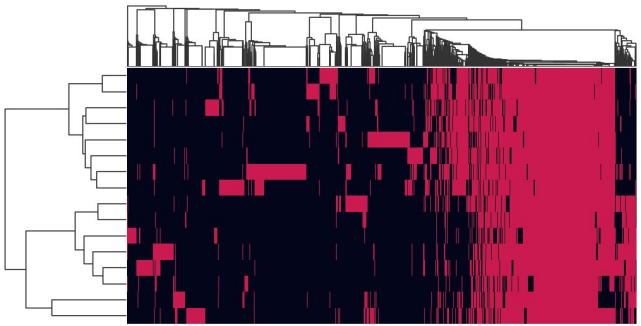
Enormous Strain Diversity



Strain-informed Gene Inference



Core and Variable Gene Content



Functional Enrichment in Variable Fraction