Scaling microbial strain inference to thousands of metagenomes using fuzzy genotypes

Multiscale Microbial Communities
2022-02-21
Byron J. Smith

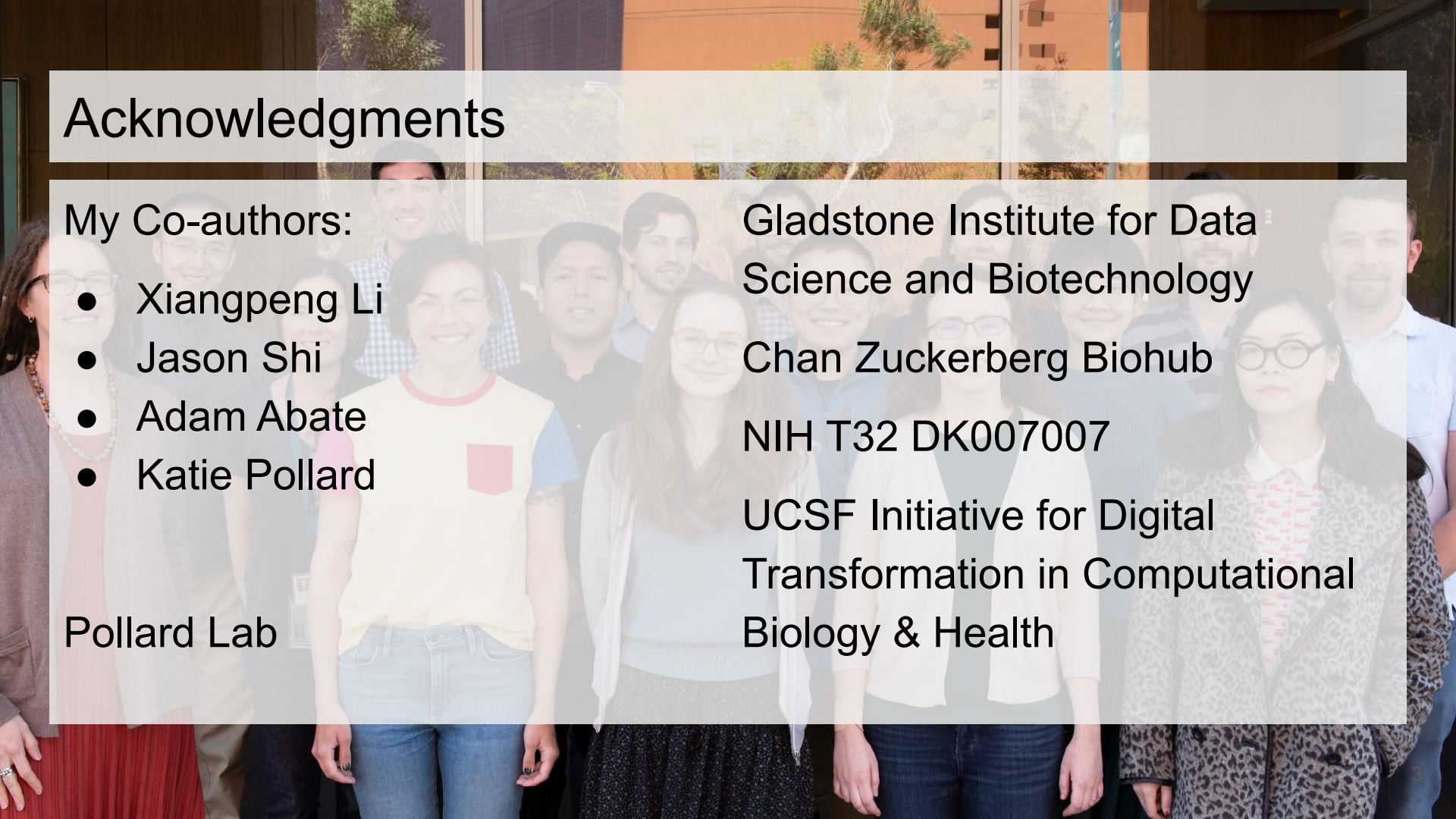
More information

bioRχiv

Scalable microbial strain inference in metagenomic data using StrainFacts. *bioRxiv* (2022)
doi: 10.1101/2022.02.01.478746

https://github.com/bsmith89/StrainFacts

@ByronJSmith



Acknowledgments

My Co-authors:

- Xiangpeng Li
- Jason Shi
- Adam Abate
- Katie Pollard

Pollard Lab

Gladstone Institute for Data Science and Biotechnology

Chan Zuckerberg Biohub

NIH T32 DK007007

UCSF Initiative for Digital Transformation in Computational Biology & Health

Outline

Intraspecific diversity in the microbiome

Strain inference

Metagenotype deconvolution

Application to large metagenome collections

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Intraspecific diversity in the microbiome

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Human associated microbes are diverse and important

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Important:

- Digestion
- Pathogen resistance
- Immune modulation

Diverse:

- Hundreds of bacterial species
- Also archaea, eukaryotes, and viruses
- High inter-individual variation

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Important:

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Diverse:

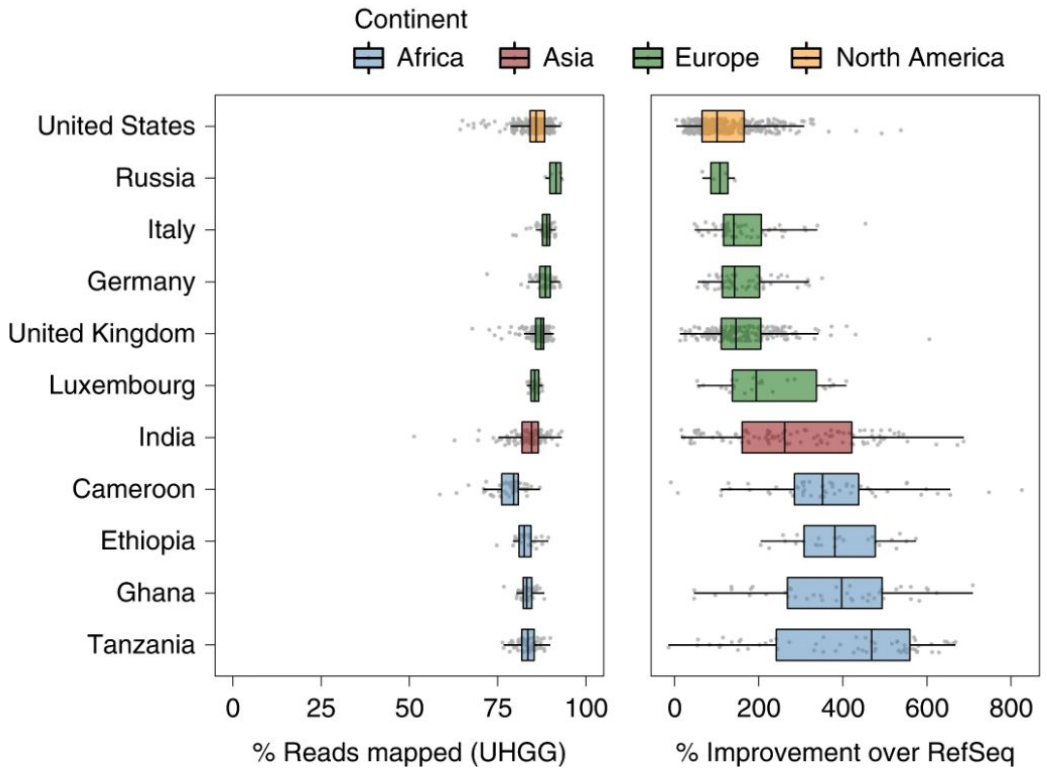
- Hundreds of bacterial species
- Also archaea, eukaryotes, and viruses
- High inter-individual variation
- **Huge (but under-explored) diversity *within* species**

Reference databases are approaching a complete catalog of species in the human gut

Key efforts include the Unified Human Gastrointestinal Genome (UHGG)

- Includes metagenome assembled genomes (MAGs)
- 204,938 genomes in 4,644 species

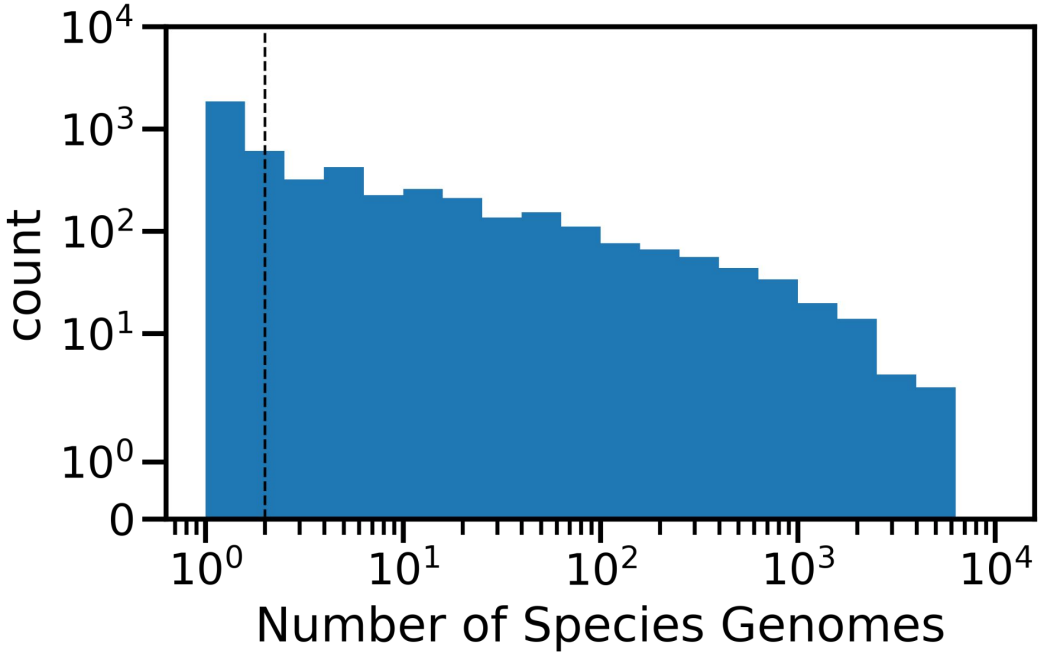
Remaining disparity from understudied human populations



Almeida, A. et al. *Nat Biotechnol* 39, 105–114 (2021).

Strain diversity is not well documented for vast majority

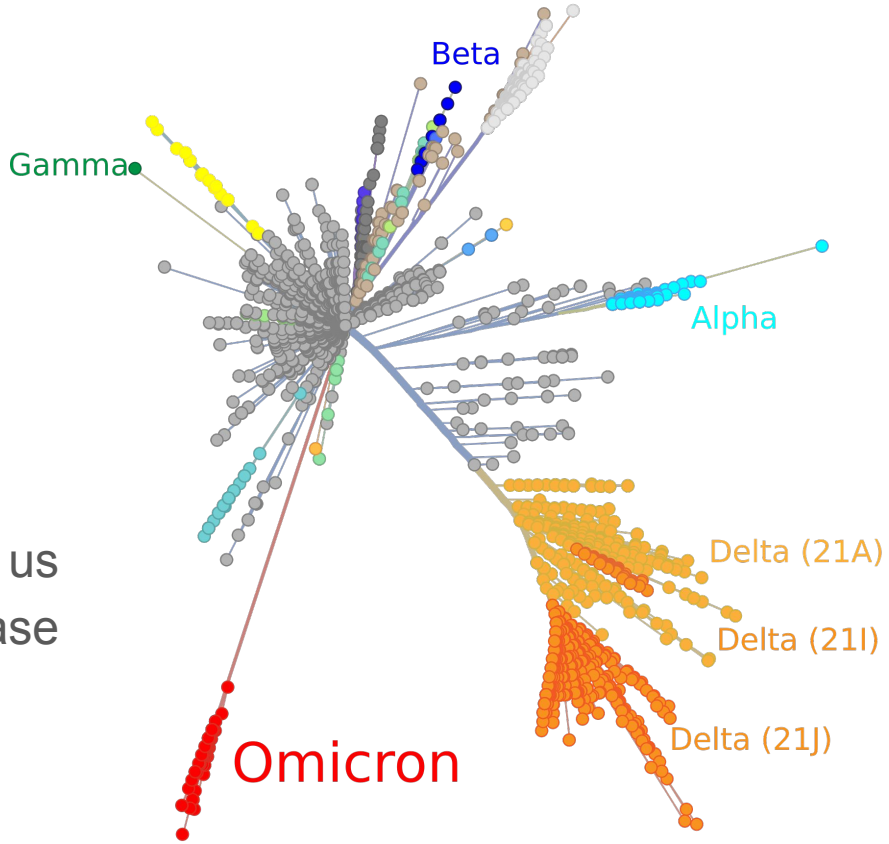
75% of species have fewer than 10 representative genomes



Strain diversity is both biologically important and scientifically informative

Differences between microbial strains can impact human health

Tracking strains between individuals, over time, or across global geography can help us to understand transmission patterns, disease associations, selection pressures, etc.



https://nextstrain.org

Strains of a species can have important differences

Intraspecific diversity has been appreciated for a long time

- Pathogenicity
- Antibiotic resistance
- Phage resistance
- Auxotrophy

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e.g. *E. coli*

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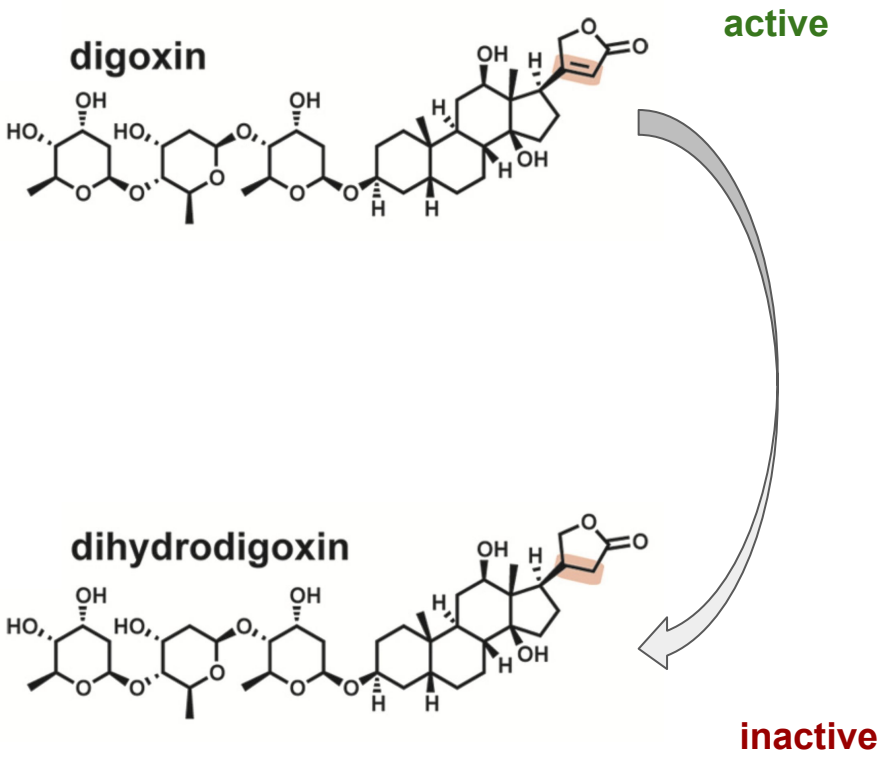

Haiser, H.J., et al. *Gut Microbes* 5, 233–238 (2014).

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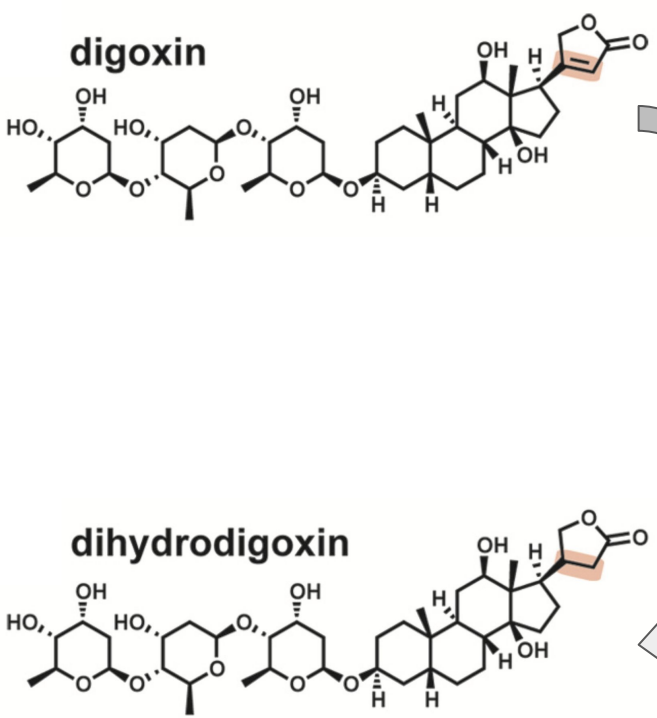

Eggerthella lenta strains encoding the *cgr*-operon result in reduction/inactivation of digoxin

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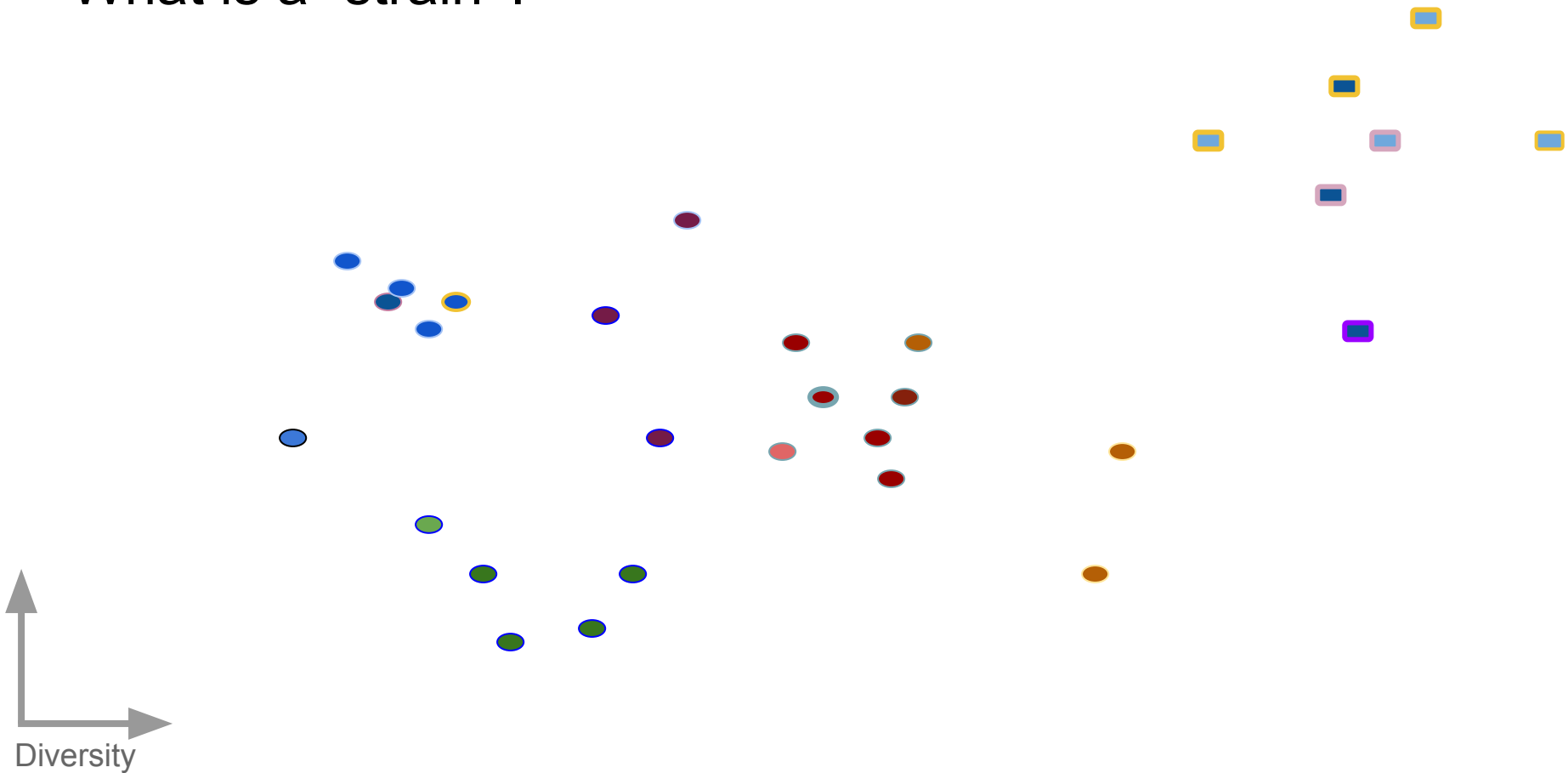

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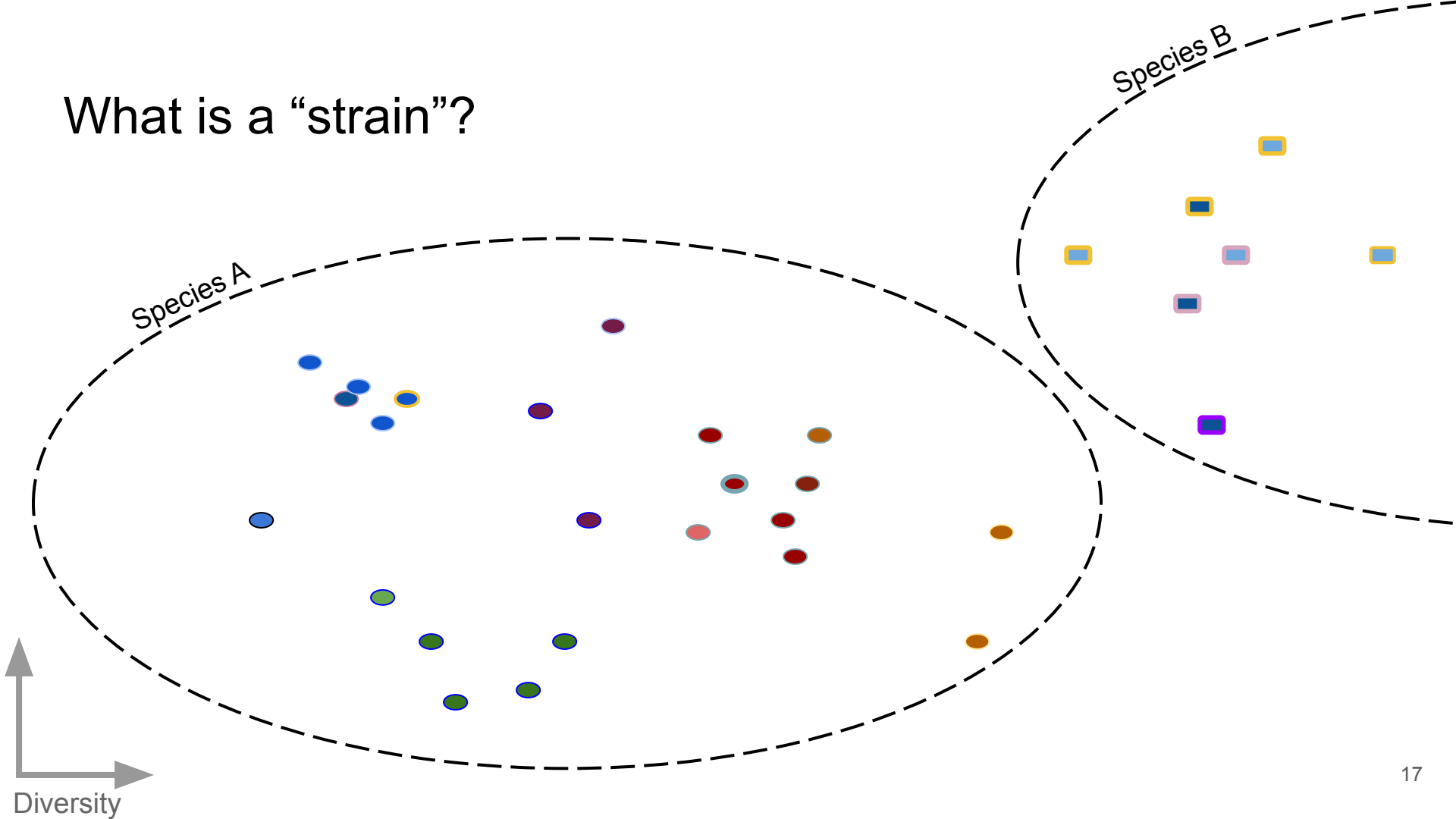

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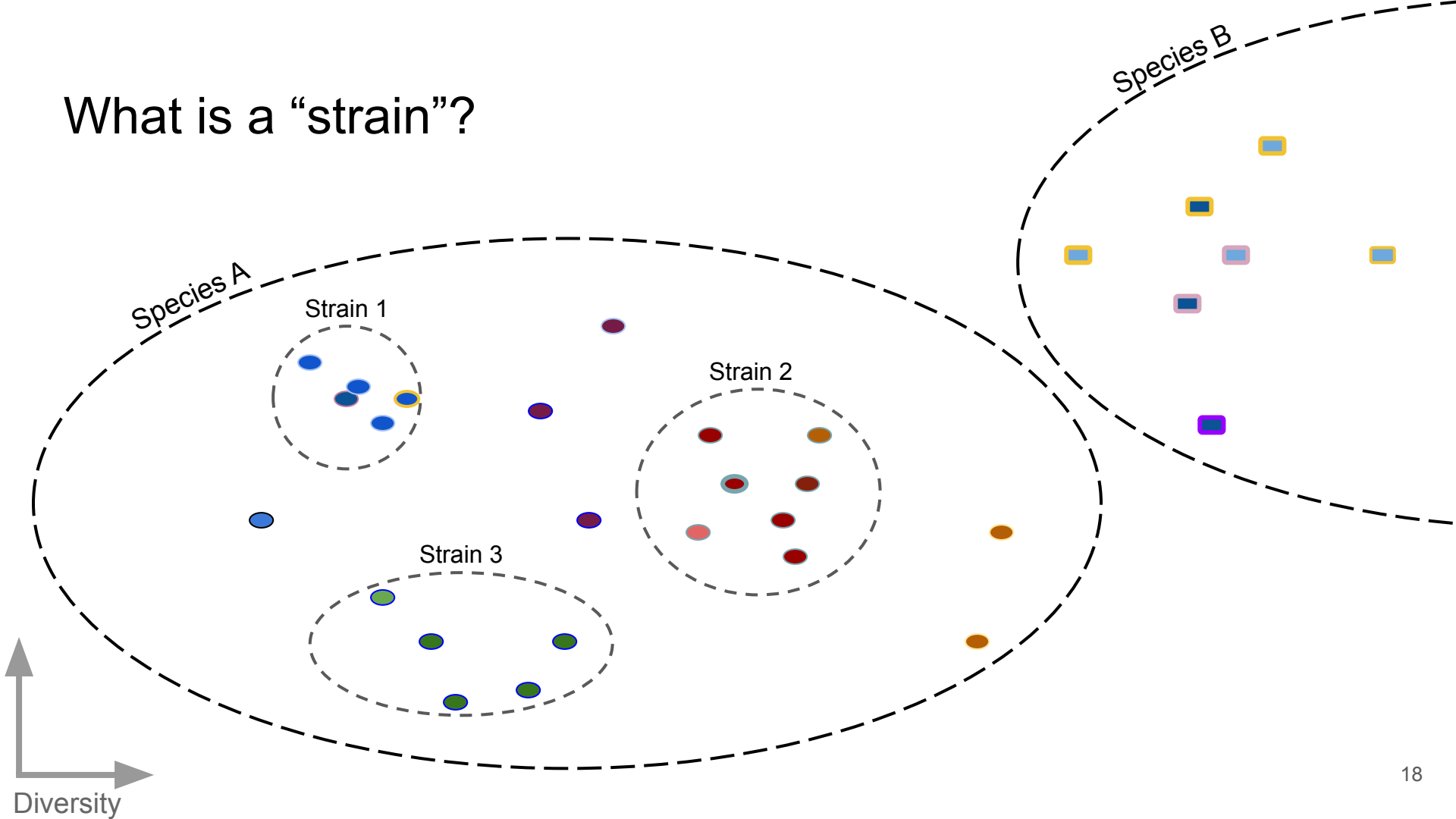
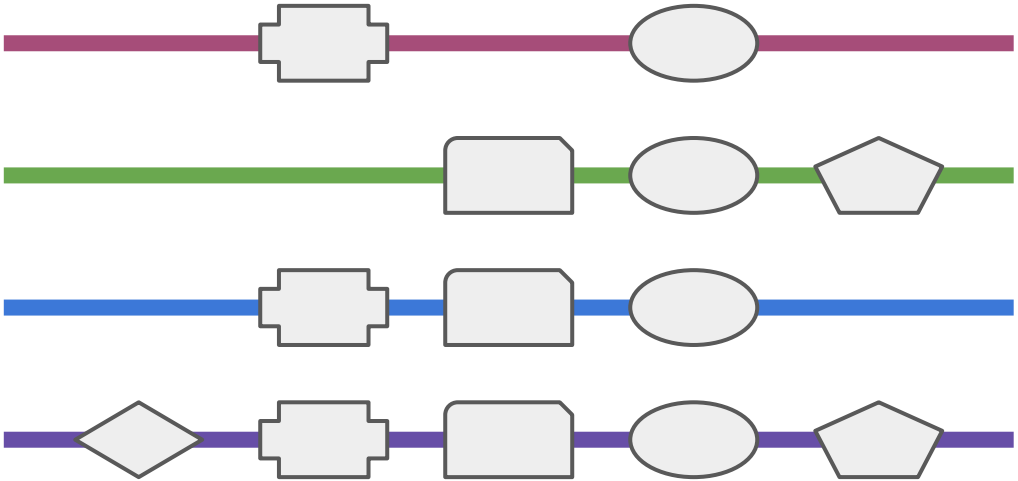
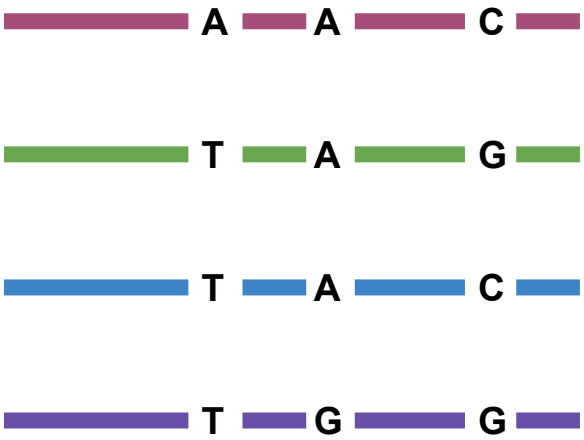


What differentiates strains?

Gene content



Genotype



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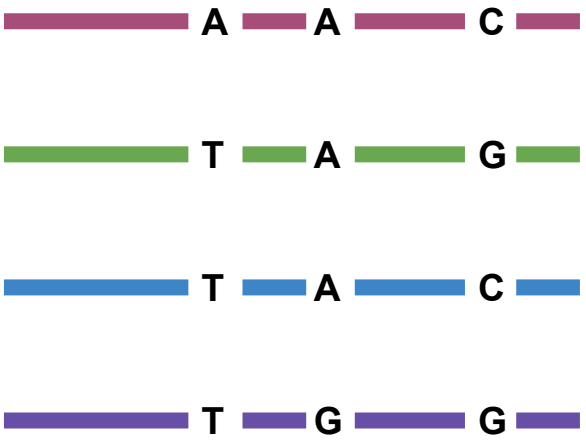

Outline

Intraspecific diversity in the microbiome

Strain inference

Metagenotype deconvolution

Application to large metagenome collections

Existing methods lack taxonomic resolution

Marker genes (e.g. 16S) are too conserved

Shotgun metagenomic data is increasingly available

Marker genes (e.g. 16S) are too conserved

Standard methods for taxonomic surveys assign reads to species based on read mapping

Metagenomic reads encode strain-level information

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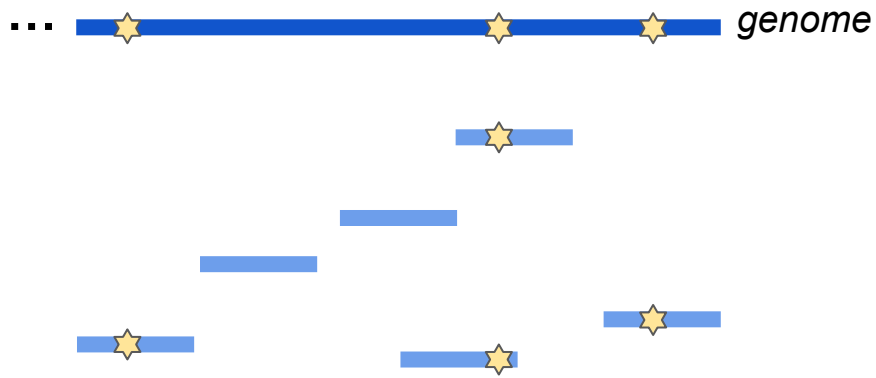
Standard methods for taxonomic surveys assign reads to species based on read mapping

But shotgun reads also cover single-nucleotide variants that encode much finer taxonomic detail



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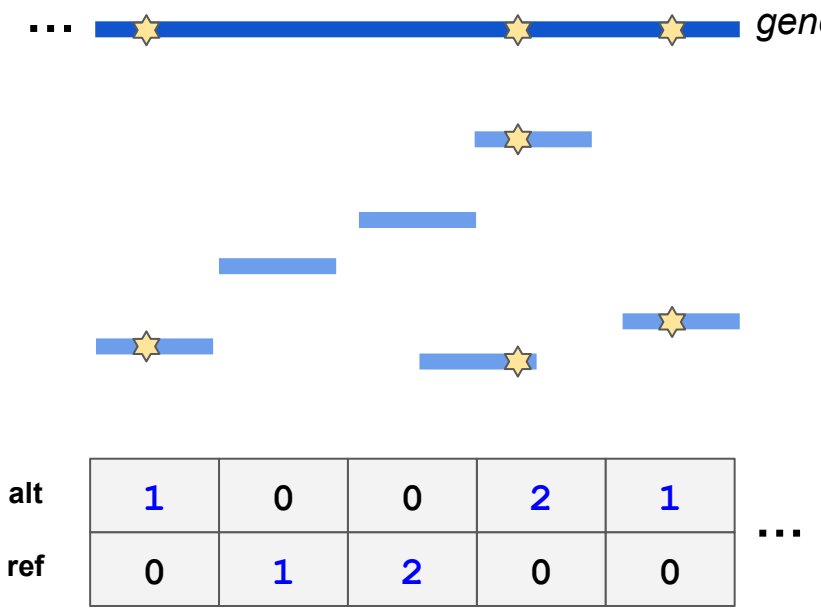
Metagenotypers tally variants at polymorphic positions (SNPs)



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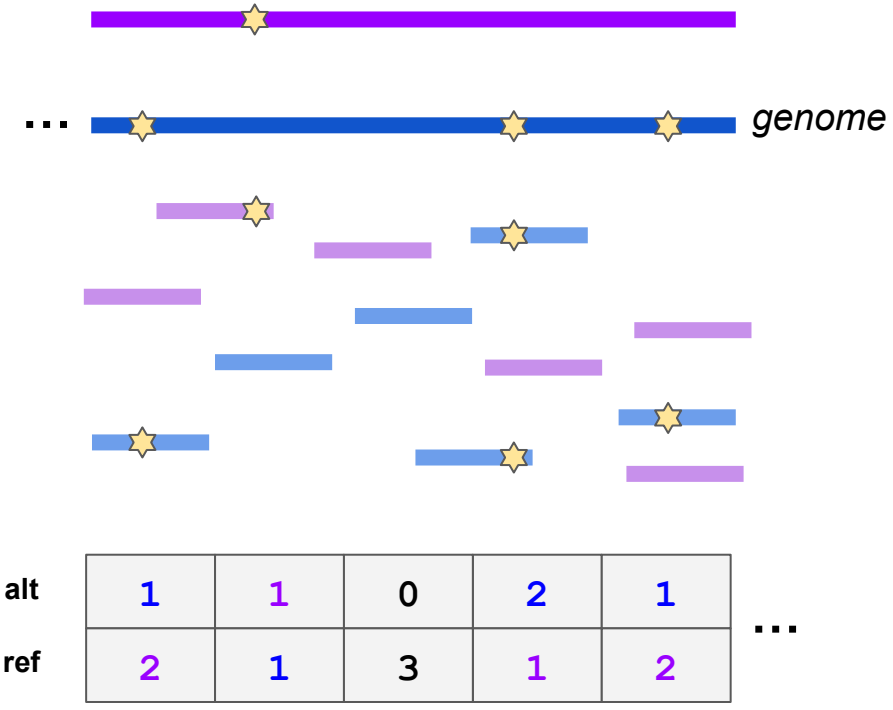
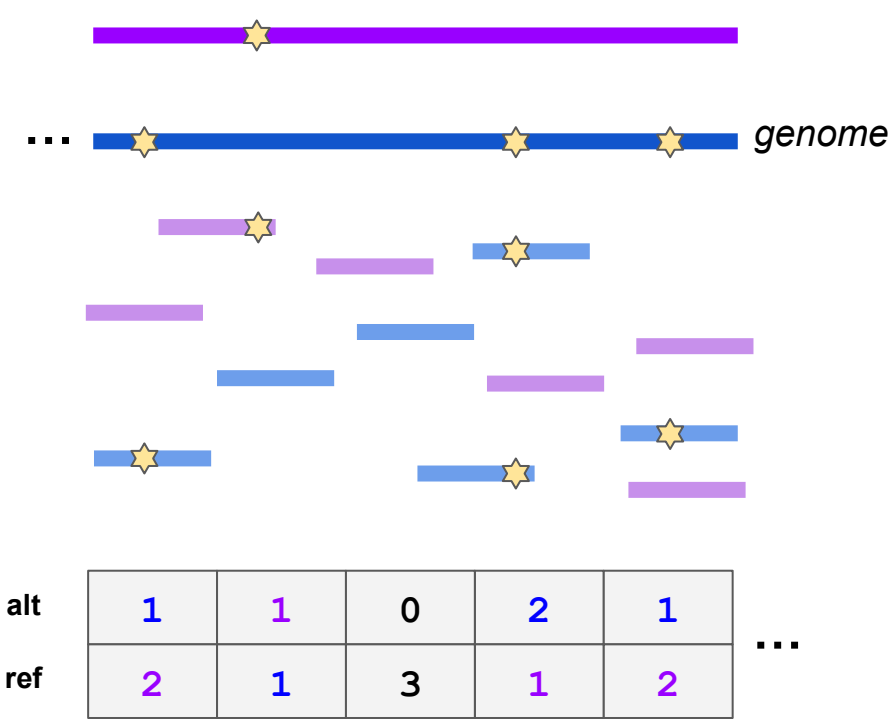


GT-Pro scales to tens-of-thousands of samples

Uses exact k-mer matching to accelerate metagenotyping



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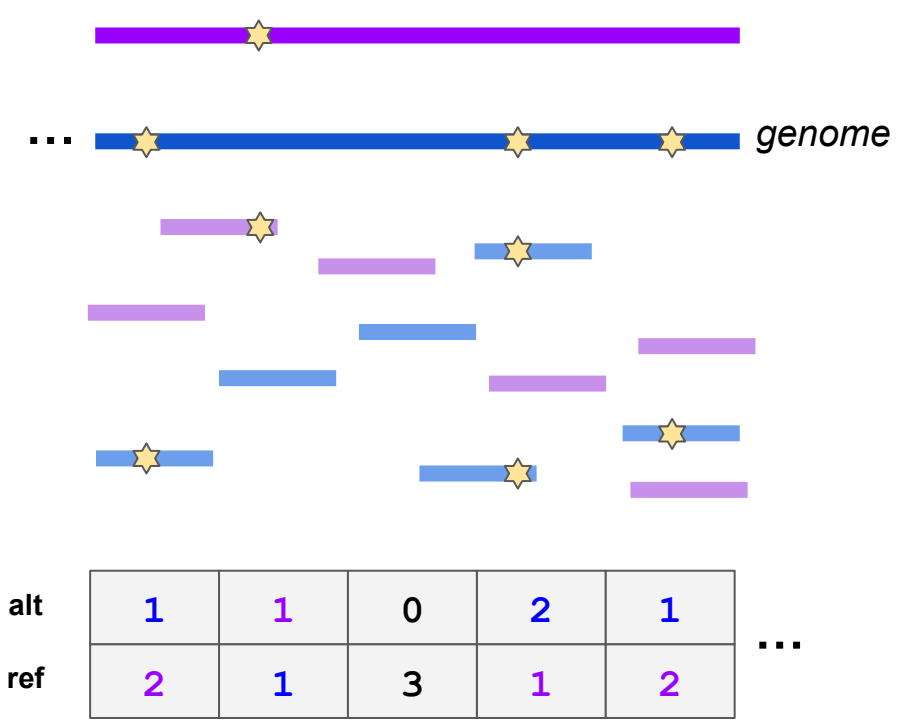
Tallies variants at known, bi-allelic SNPs in the core genome using a database of known SNVs



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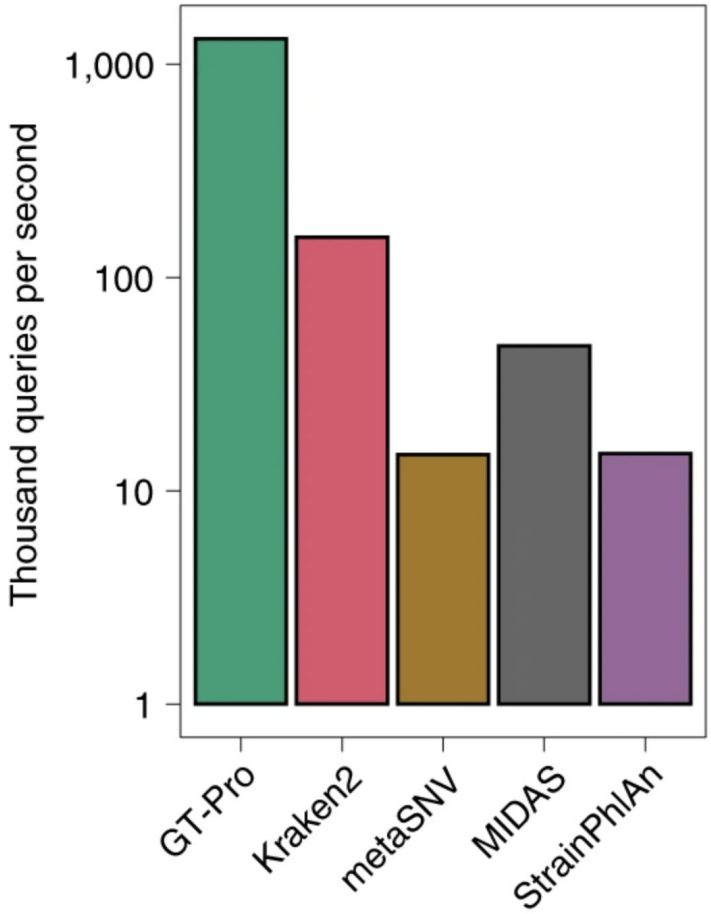

Shi, Z. J., *et al.* Nat Biotechnol (2021)

Low sequencing coverage

Mixtures of strains

Interpreting metagenotypes is challenging

Closely related strains

Novel strains

Low sequencing coverage

Consensus Genotypes

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Reference Database

Novel strains

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Dissimilarity Methods / Ordination

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Novel strains

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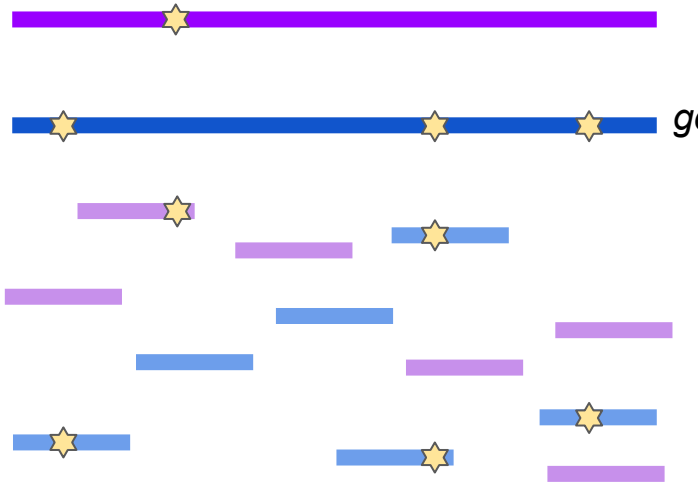
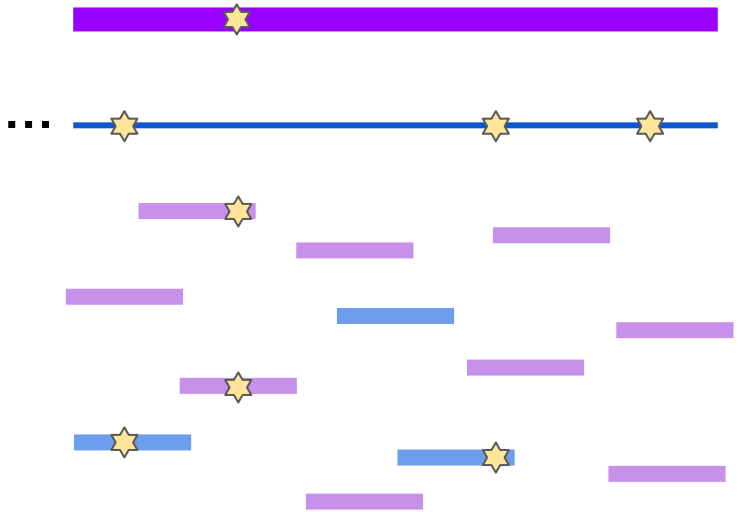
Strain deconvolution

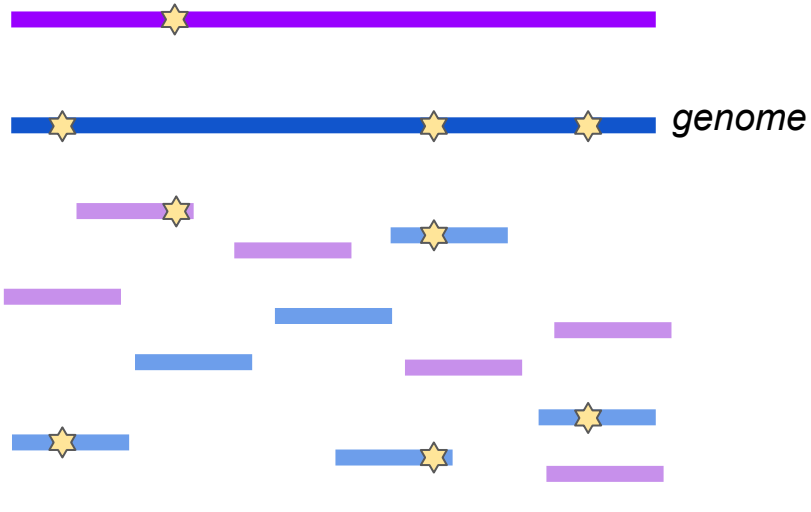


1	1	0	2	1
2	1	3	1	2

...

Strain deconvolution harnesses SNV covariance

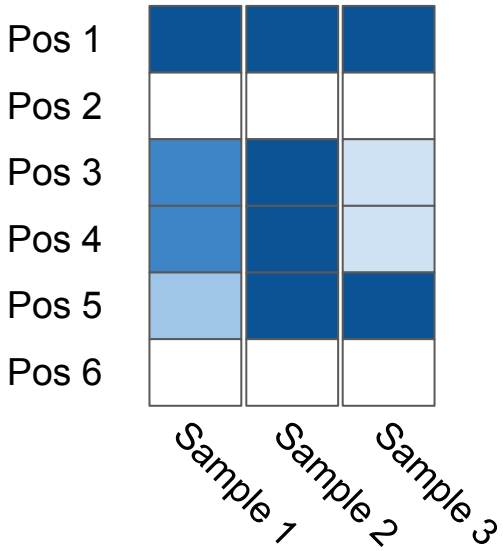




alt	1	2	0	1	0
ref	2	0	4	2	2

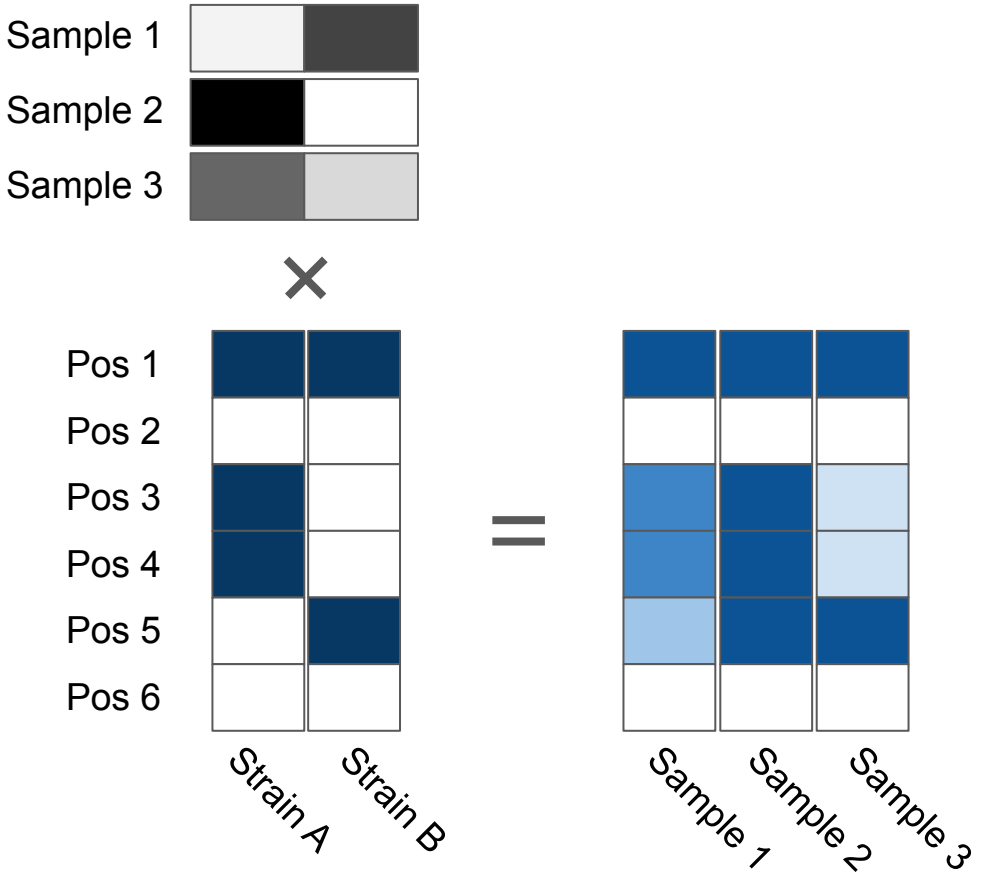
1	1	0	2	1	…
2	1	3	1	2	

Non-negative matrix factorization for metagenotypes



Non-negative matrix factorization for metagenotypes

Like NMF: model allele fractions as linear combinations of strain genotypes



Strain deconvolution as model-based inference

Like NMF: model allele fractions as linear combinations of strain genotypes

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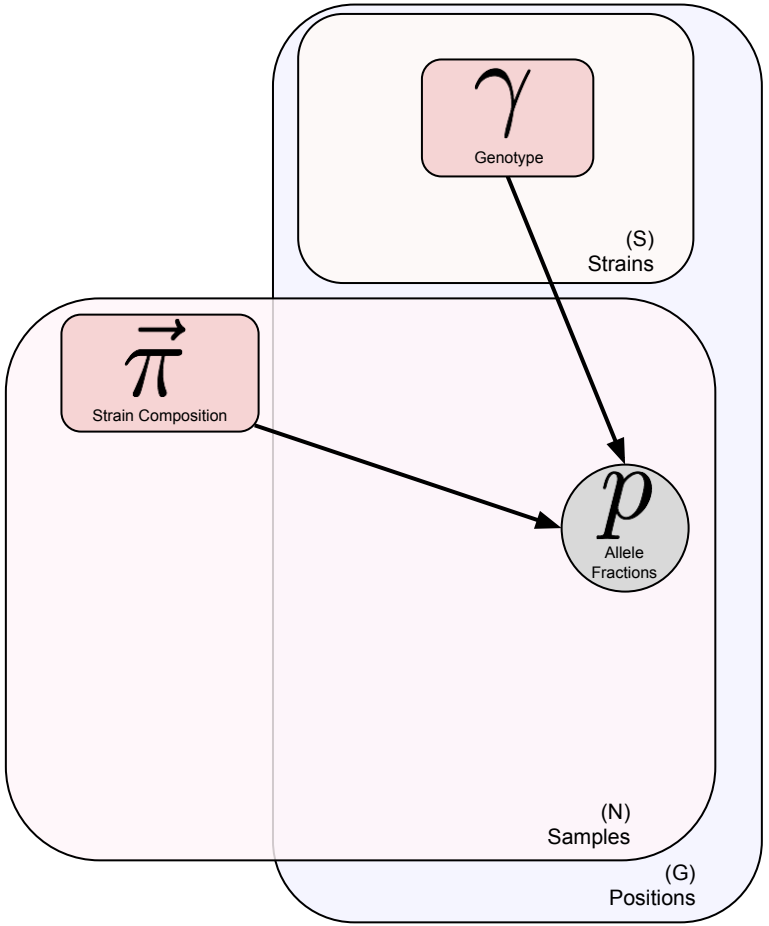

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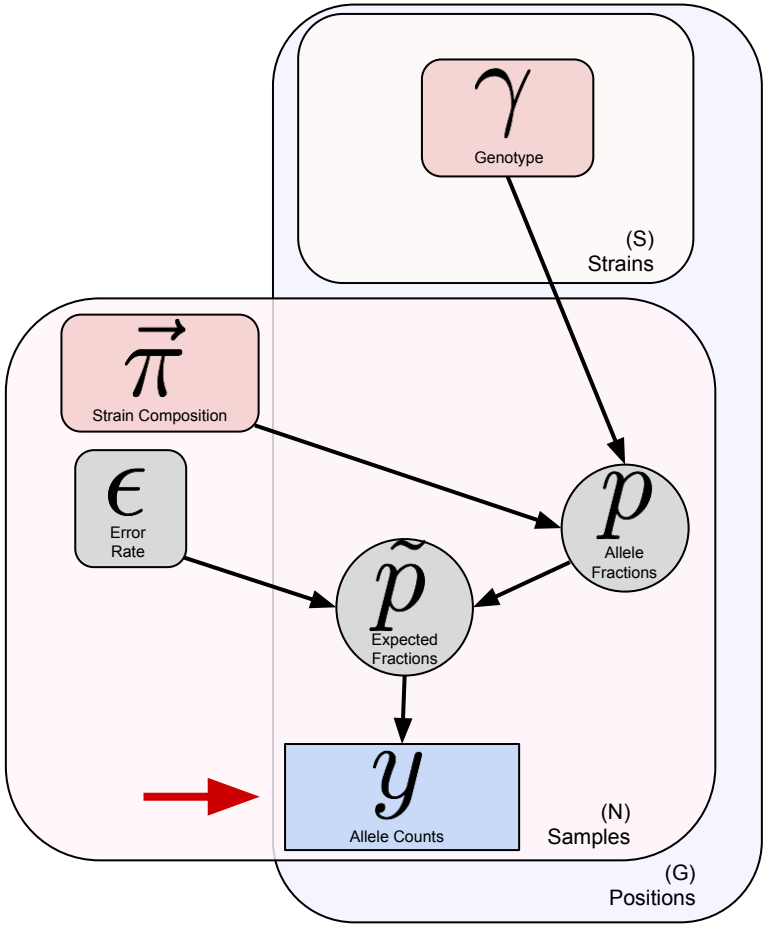

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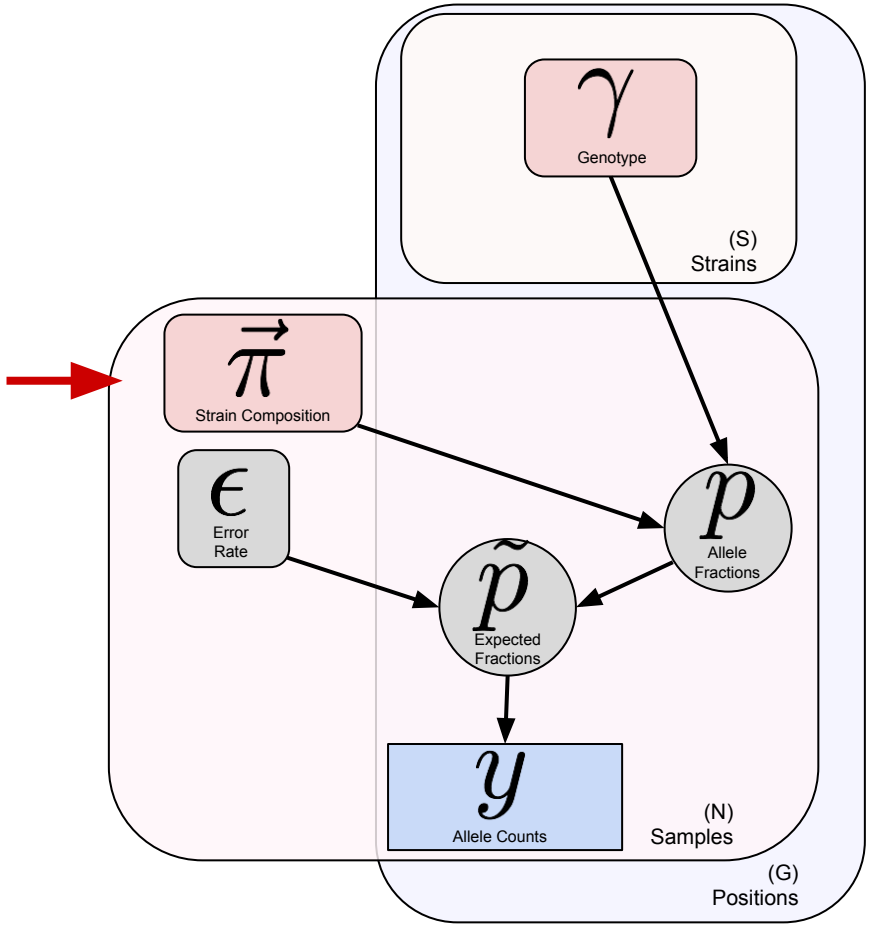

Strain deconvolution as model-based inference

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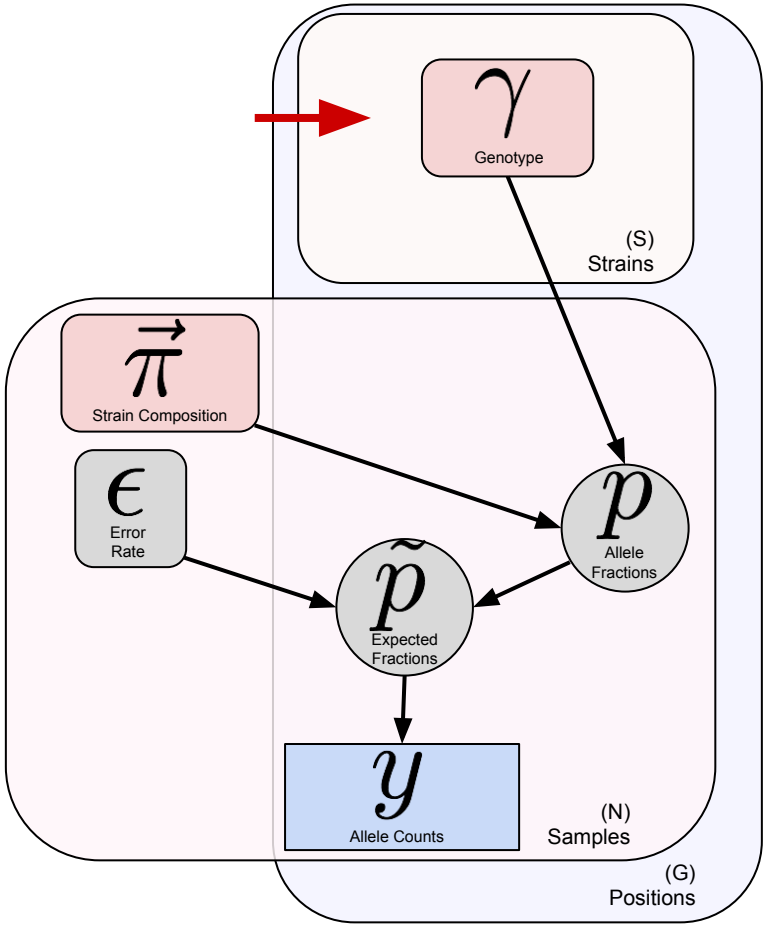
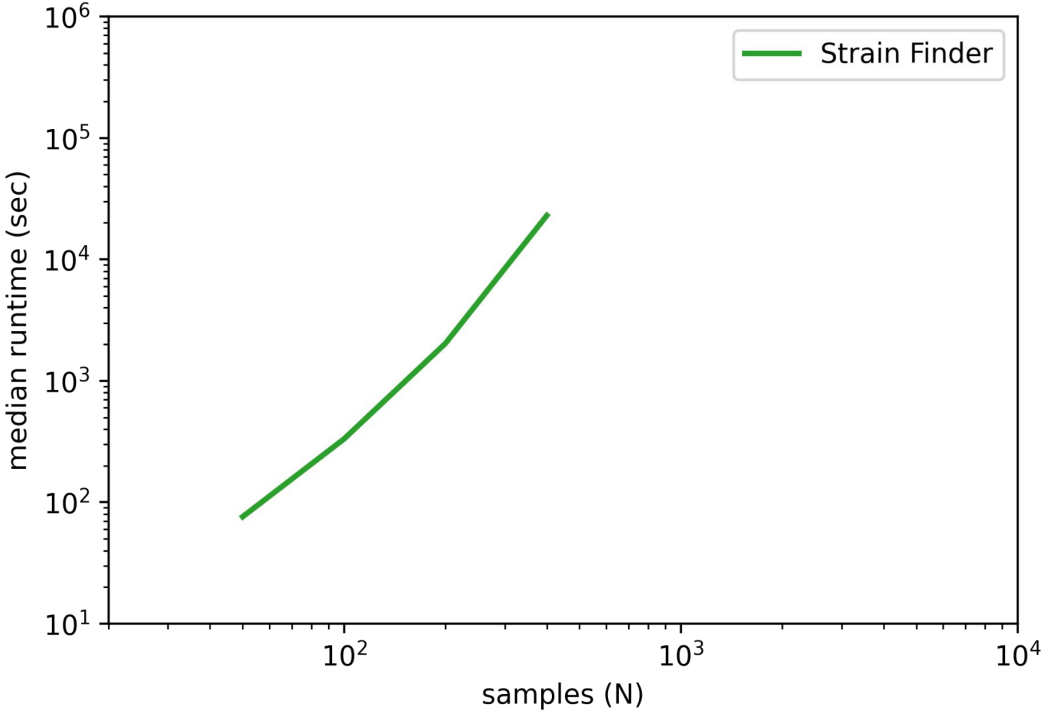
- Genotypes at each position are binary, either 0 (reference) or 1 (alternative) allele



Computational scalability of existing deconvolution tools



Discrete genotypes are computationally challenging

Like NMF: model allele fractions as linear combinations of strain genotypes

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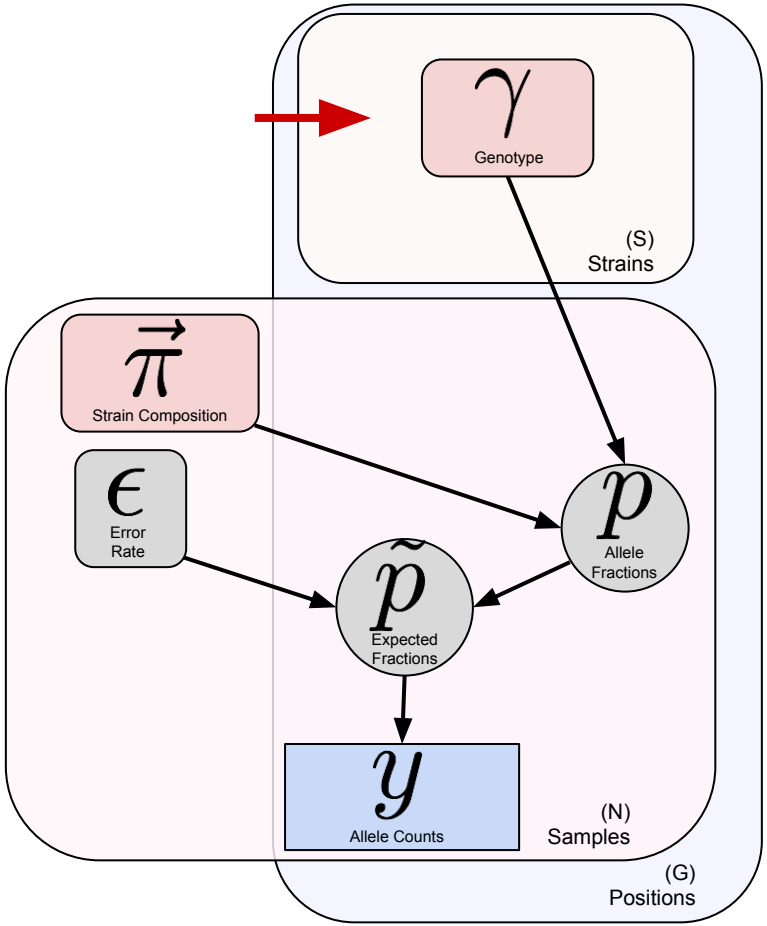

StrainFacts relaxes the discrete allele constraint

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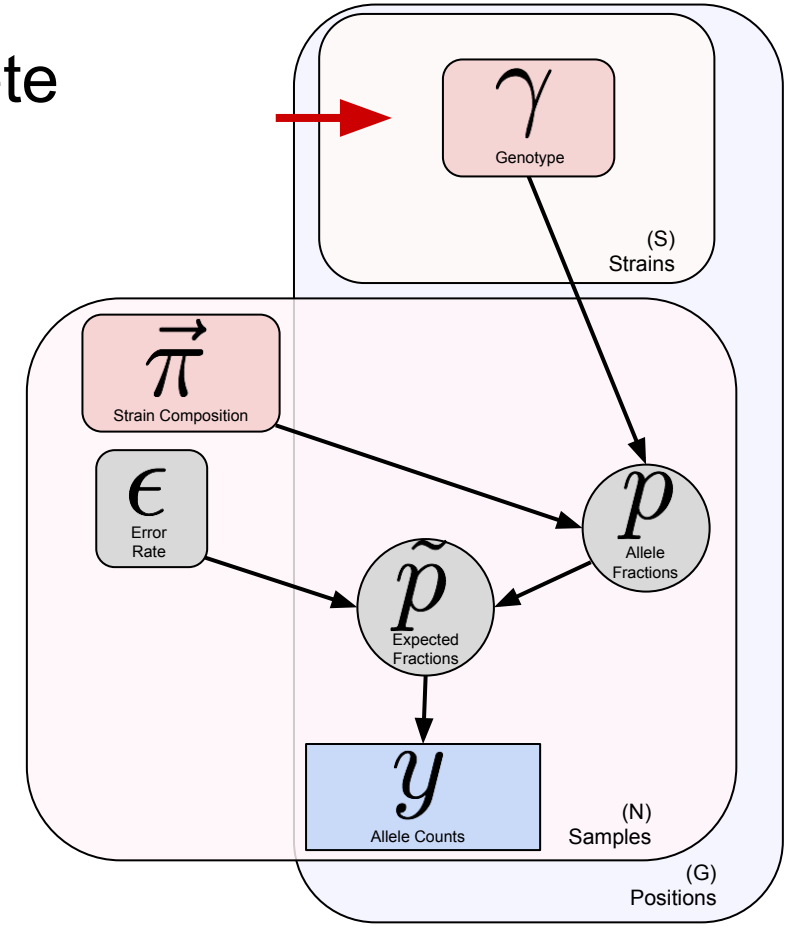
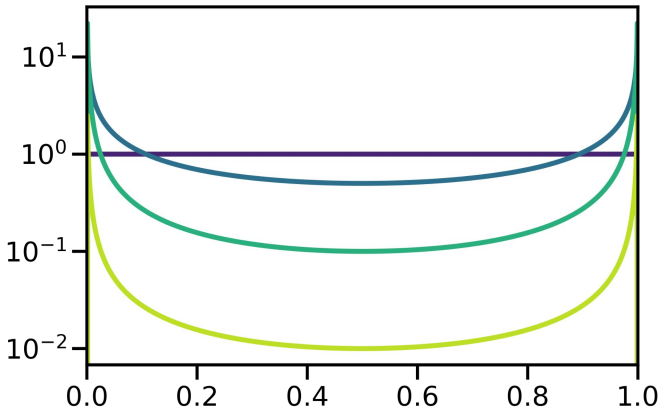
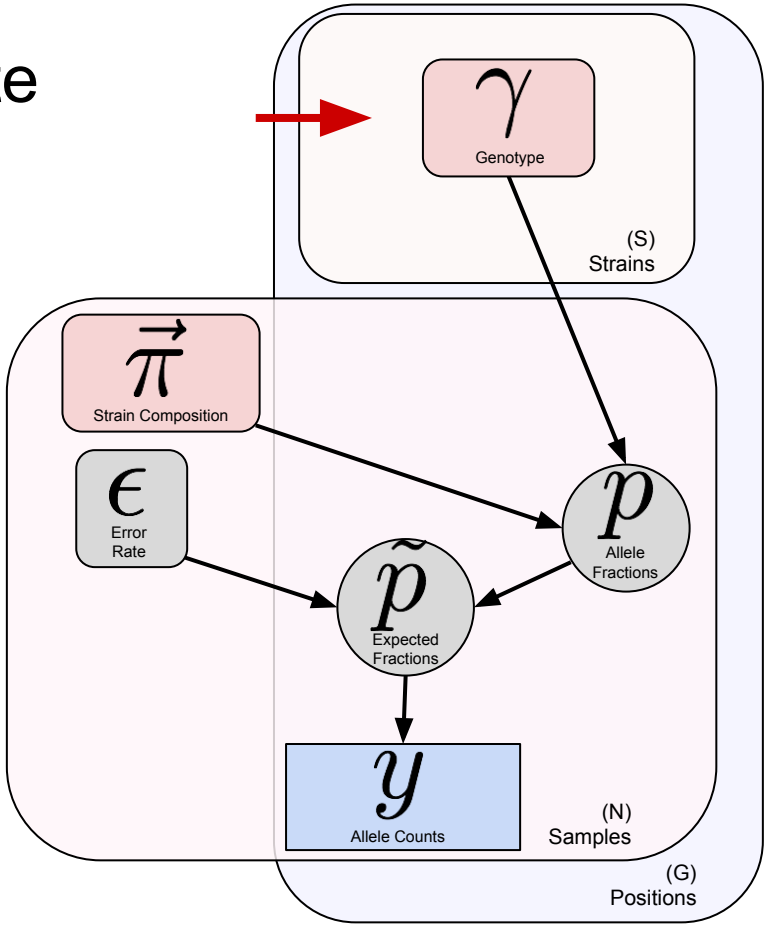


StrainFacts relaxes the discrete allele constraint

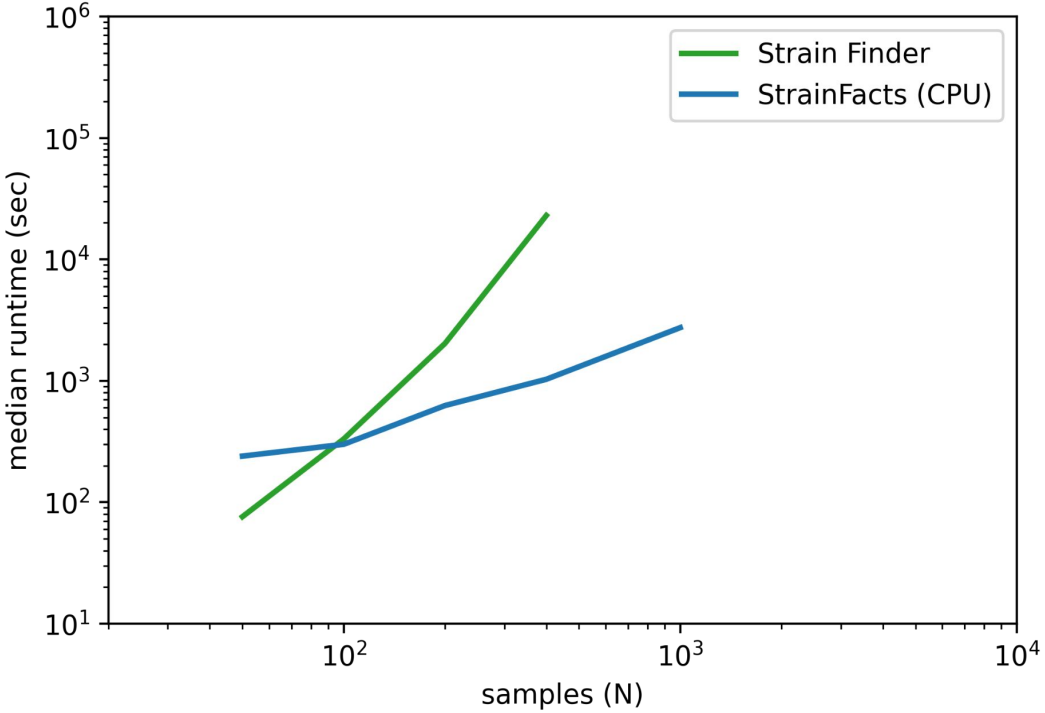
By putting a strong prior on γ, we encourage fuzzy genotypes to be closer to 0 or 1



Shifted-scaled Dirichlet distribution (SSD; similar to the Dirichlet/Beta)



Fuzzy genotypes and gradient descent can scale to thousands of samples



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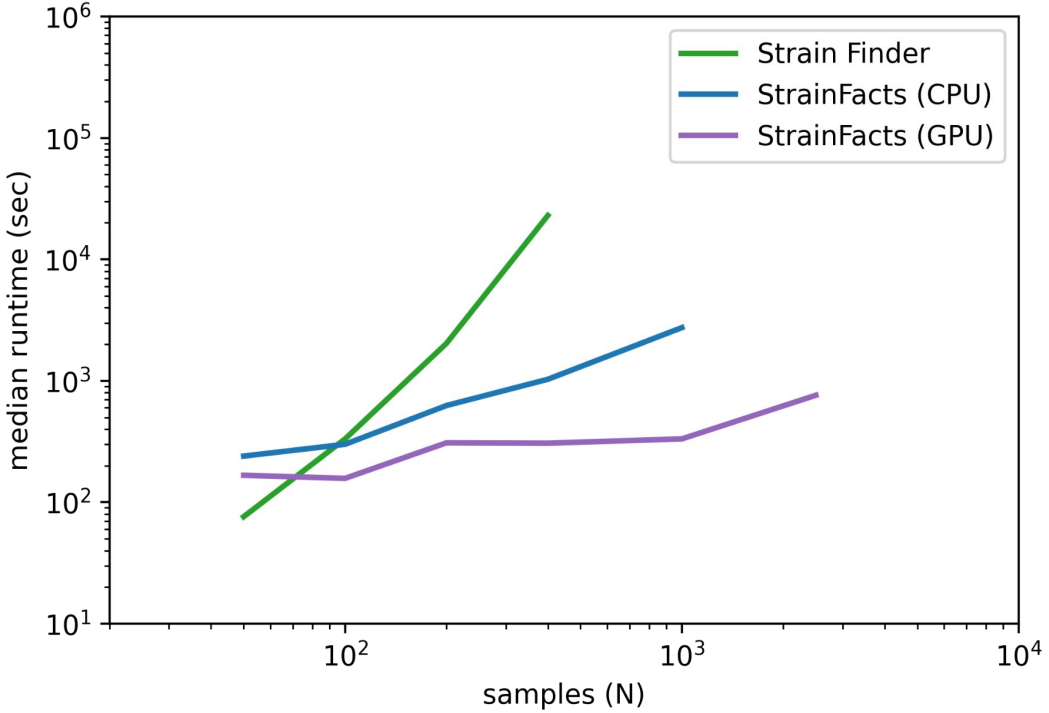
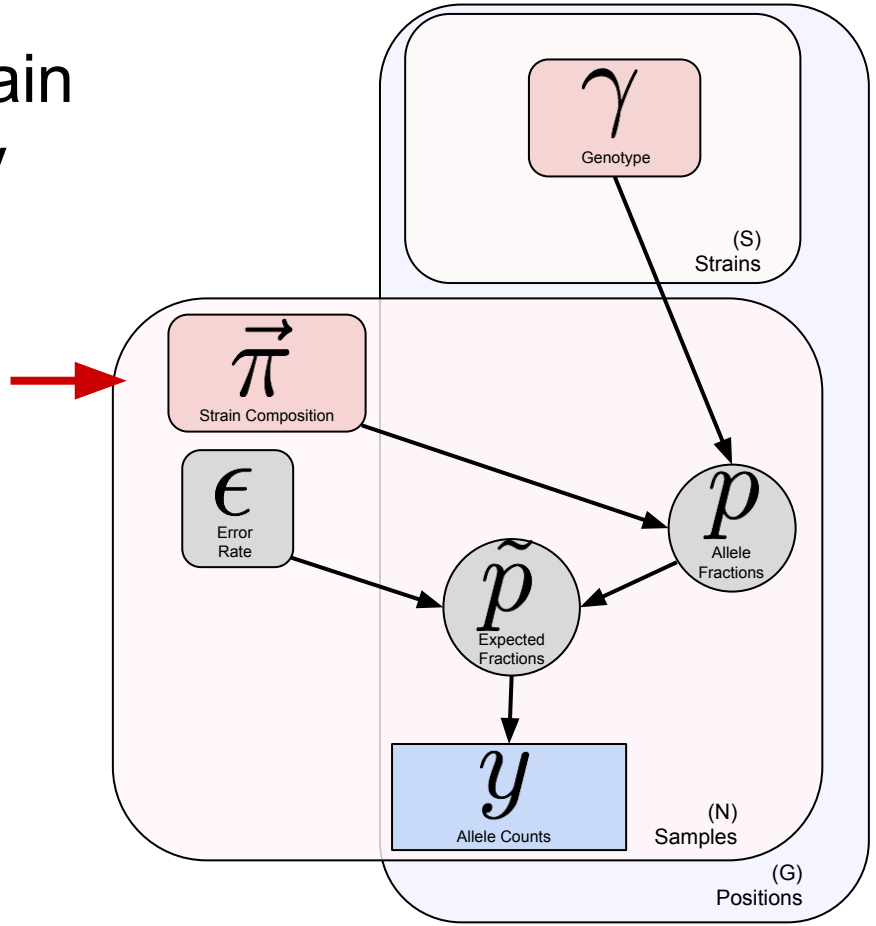


StrainFacts regularizes strain heterogeneity and diversity

Additionally, we put a hierarchical prior on π

- Strain heterogeneity regularization (strains per sample)



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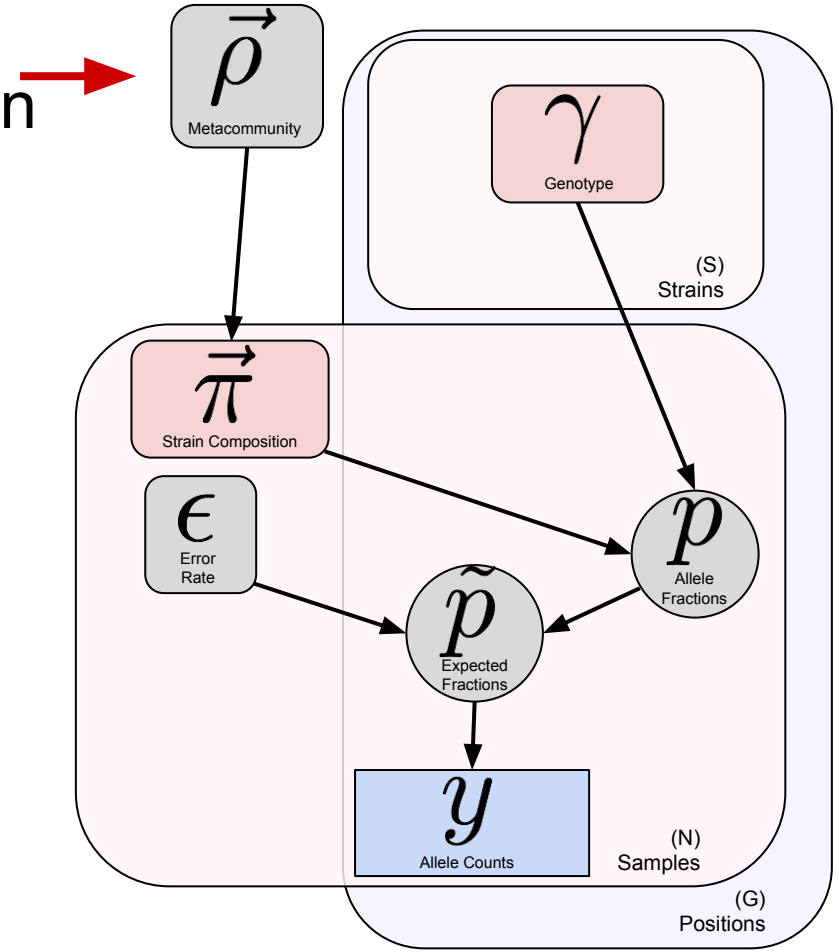
- Strain heterogeneity regularization (strains per sample)
- Overall strain diversity regularization



Strain deconvolution as model-based inference

Additionally, we put a hierarchical prior on π

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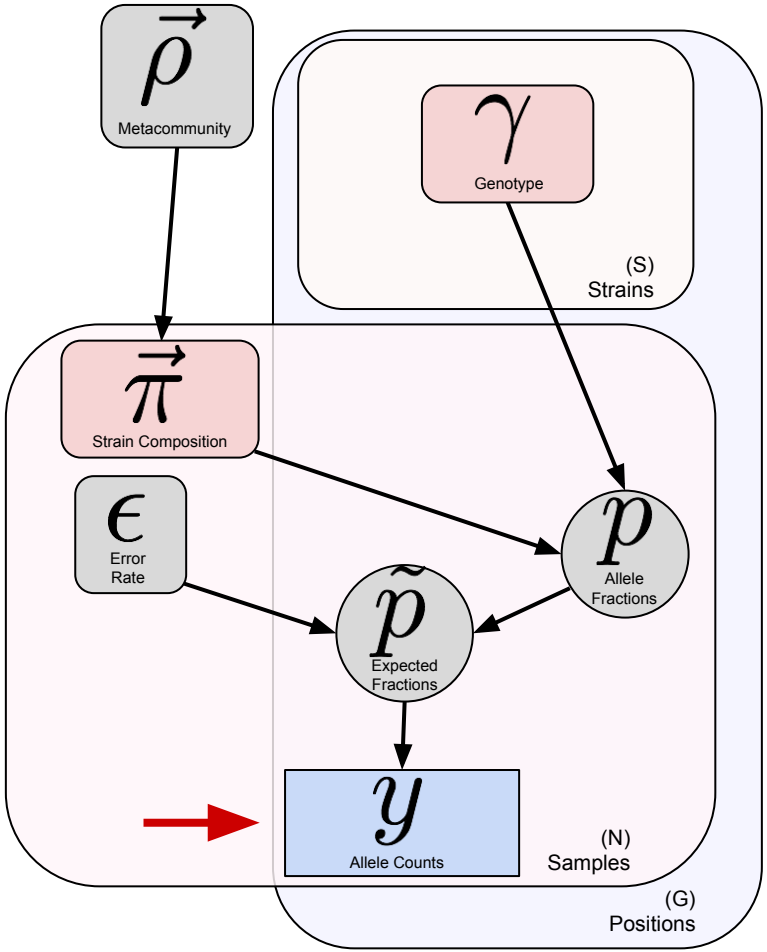
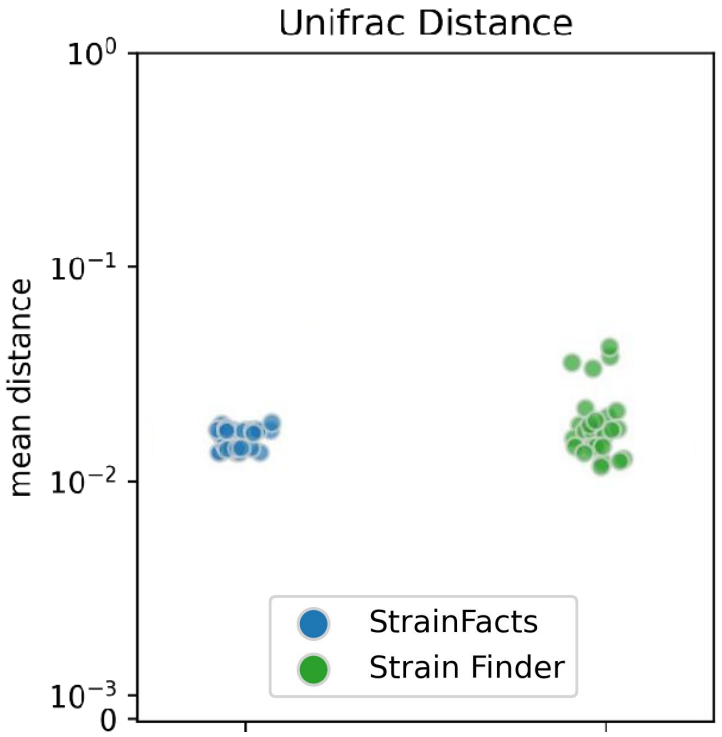
- Overall strain diversity regularization

Full model also includes count over-dispersion (Beta-Binomial likelihood)

Maximum a posteriori (MAP) estimation for inference



StrainFacts is accurate

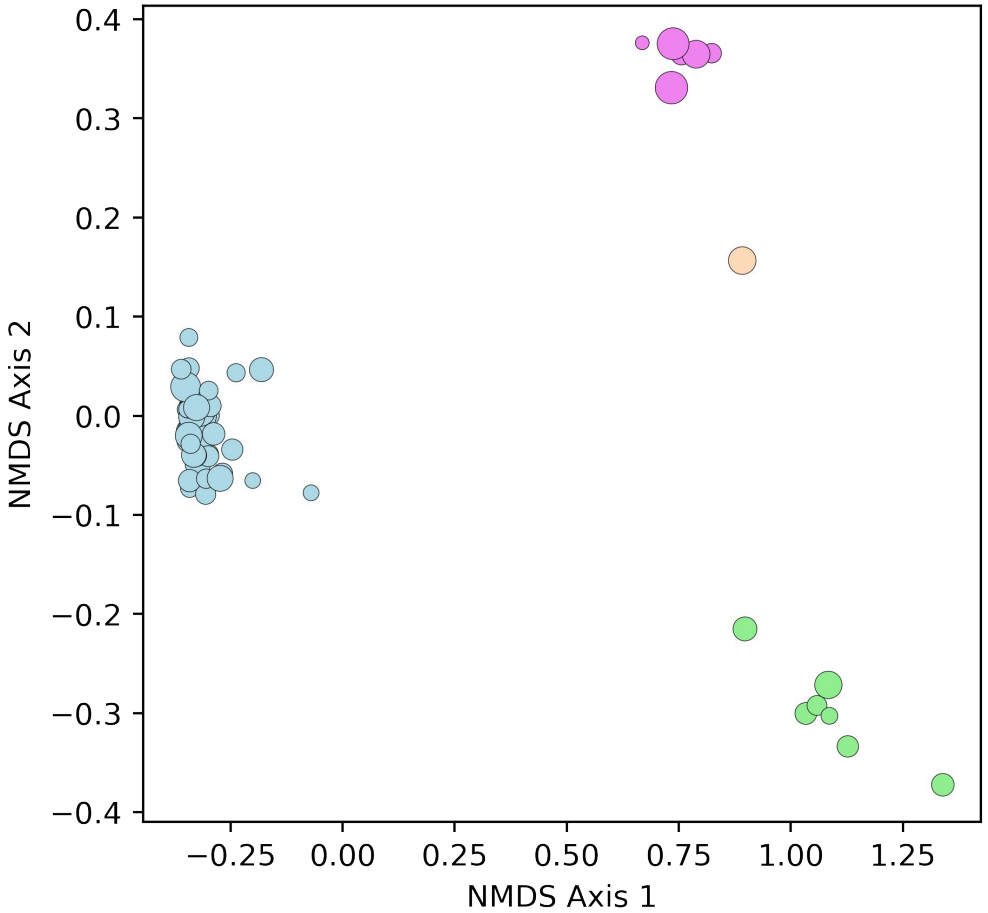


Based on simulations, StrainFacts is similarly accurate to an existing deconvolution tool

Several orders of magnitude faster

Single-cell genomics validates inferences in complex strain mixtures

Streptococcus thermophilus
single-cell genotypes cluster into four groups



Xiangpeng Li

Single-cell genomics validates inferences in complex strain mixtures

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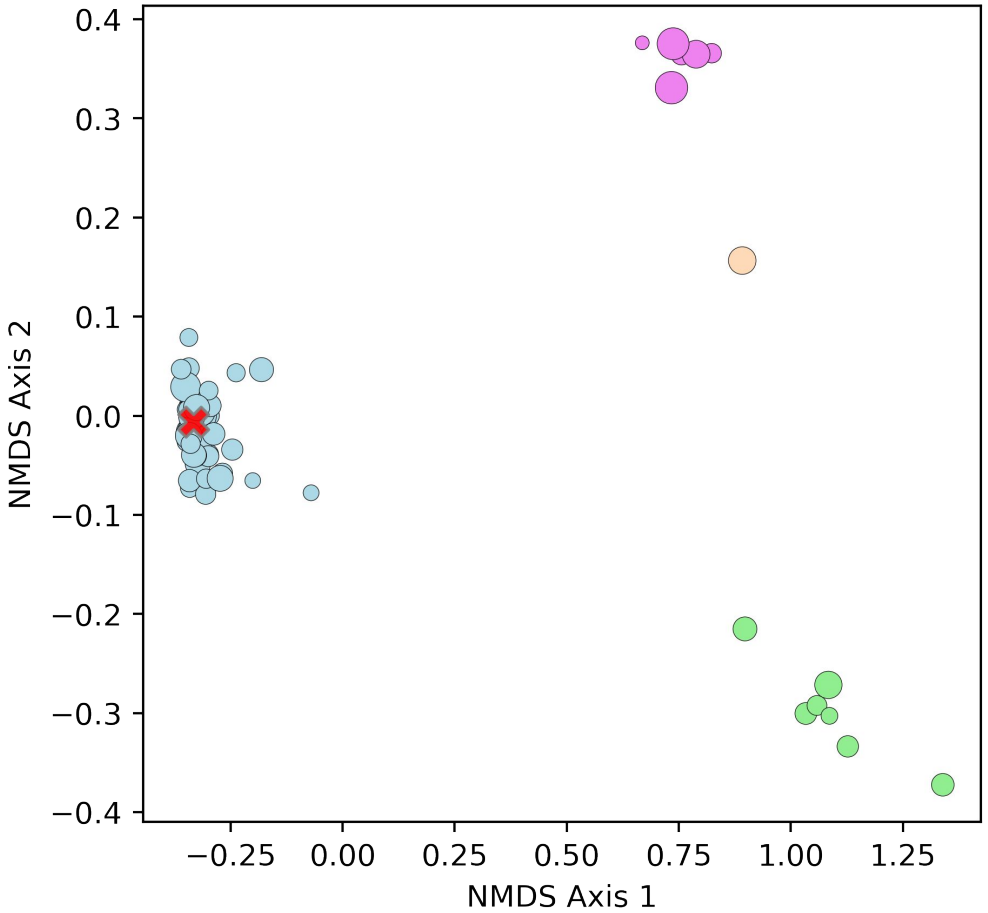
Consensus metagenotype only reflects dominant strain



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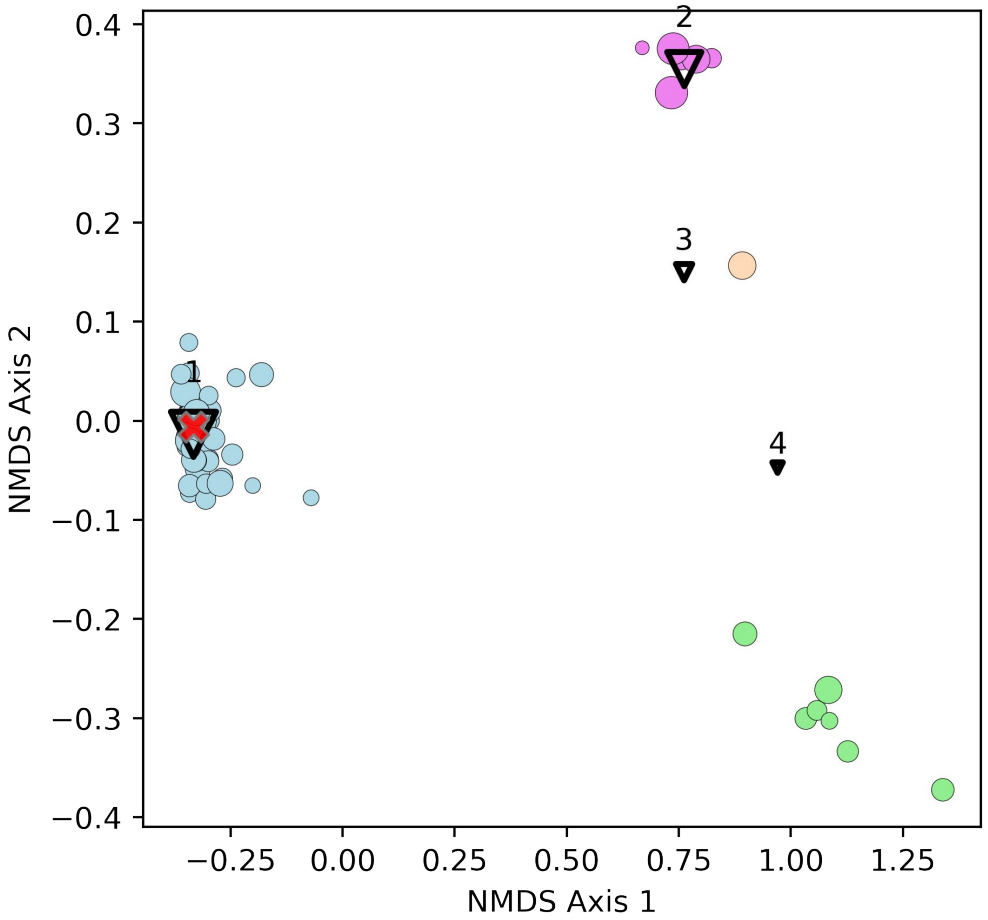
StrainFacts identifies four strains, three of which match the single-cell genotypes



Xiangpeng Li

Outline

Intraspecific diversity in the microbiome

Strain inference

Metagenotype deconvolution

Application to large metagenome collections

What can we do with strain inference across thousands of metagenomic samples?

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Two examples:

- Biogeography
- Population Genetics

Strain biogeography

Agathobacter rectalis is a prevalent and abundant gut bacterium

20 countries
→ 33 studies
→ 9,224 stool samples
→ 198 inferred strains

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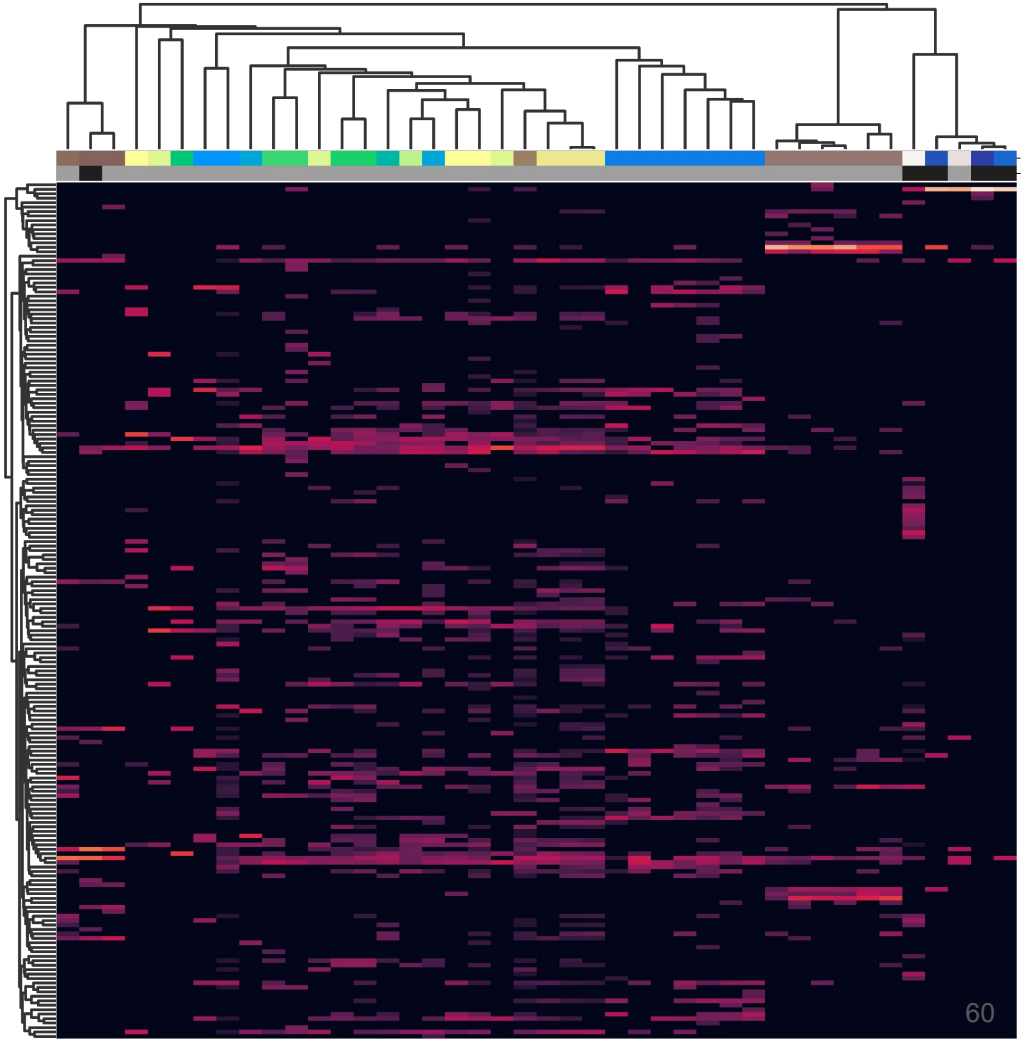

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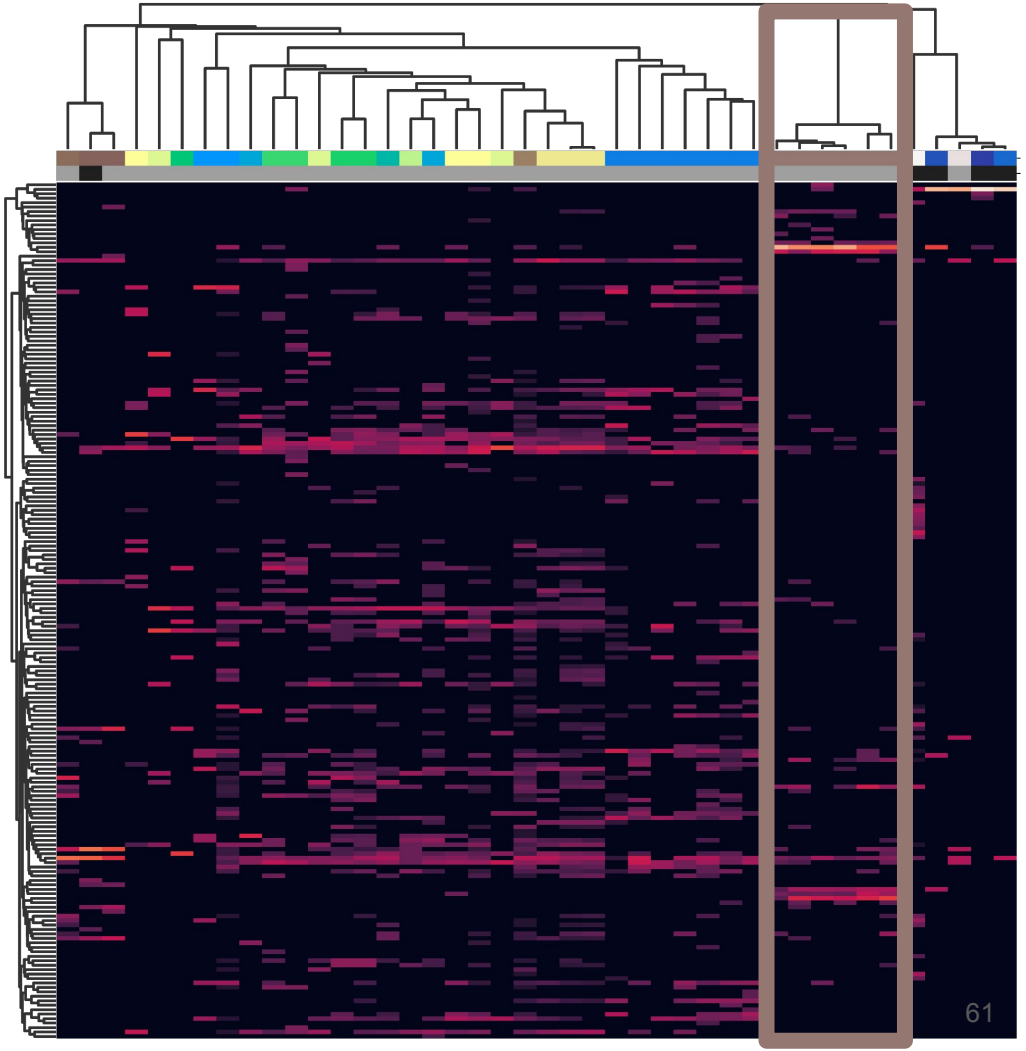

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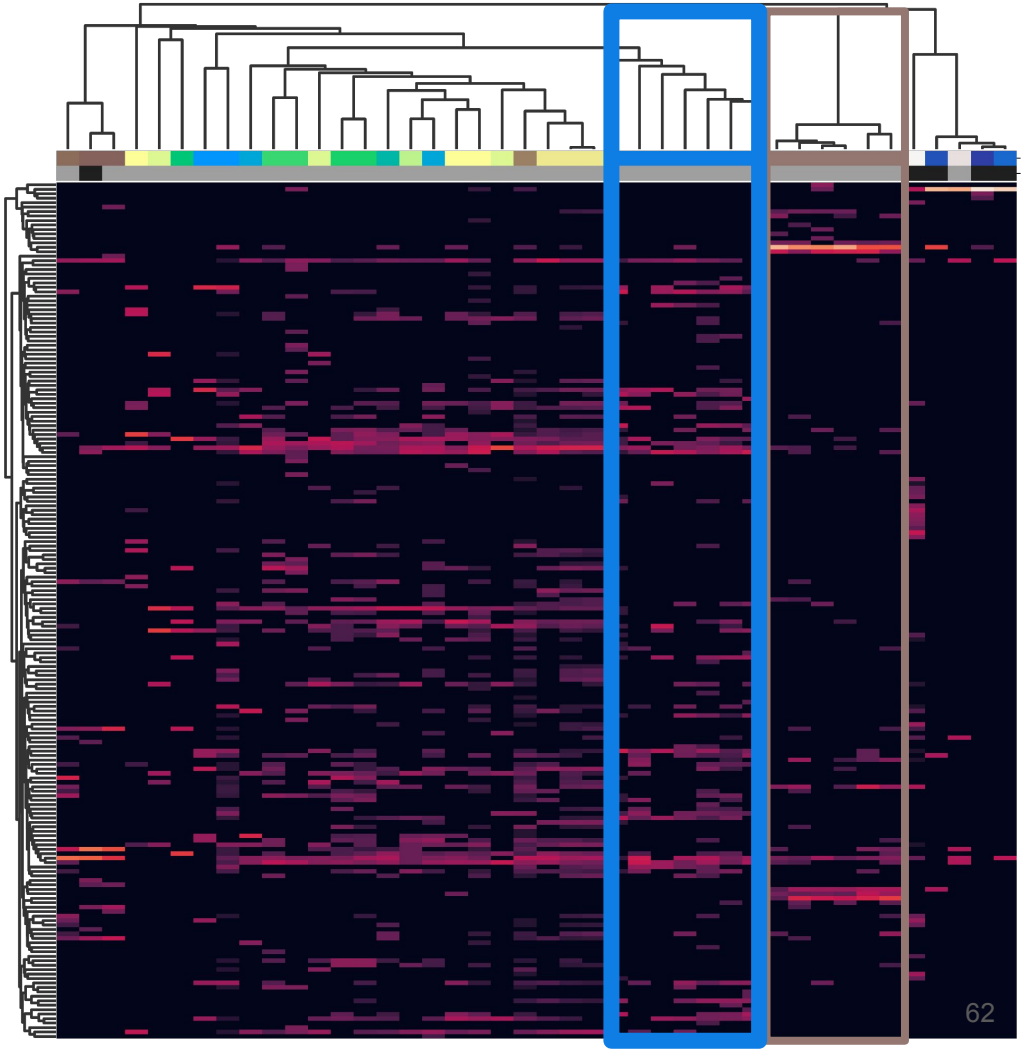

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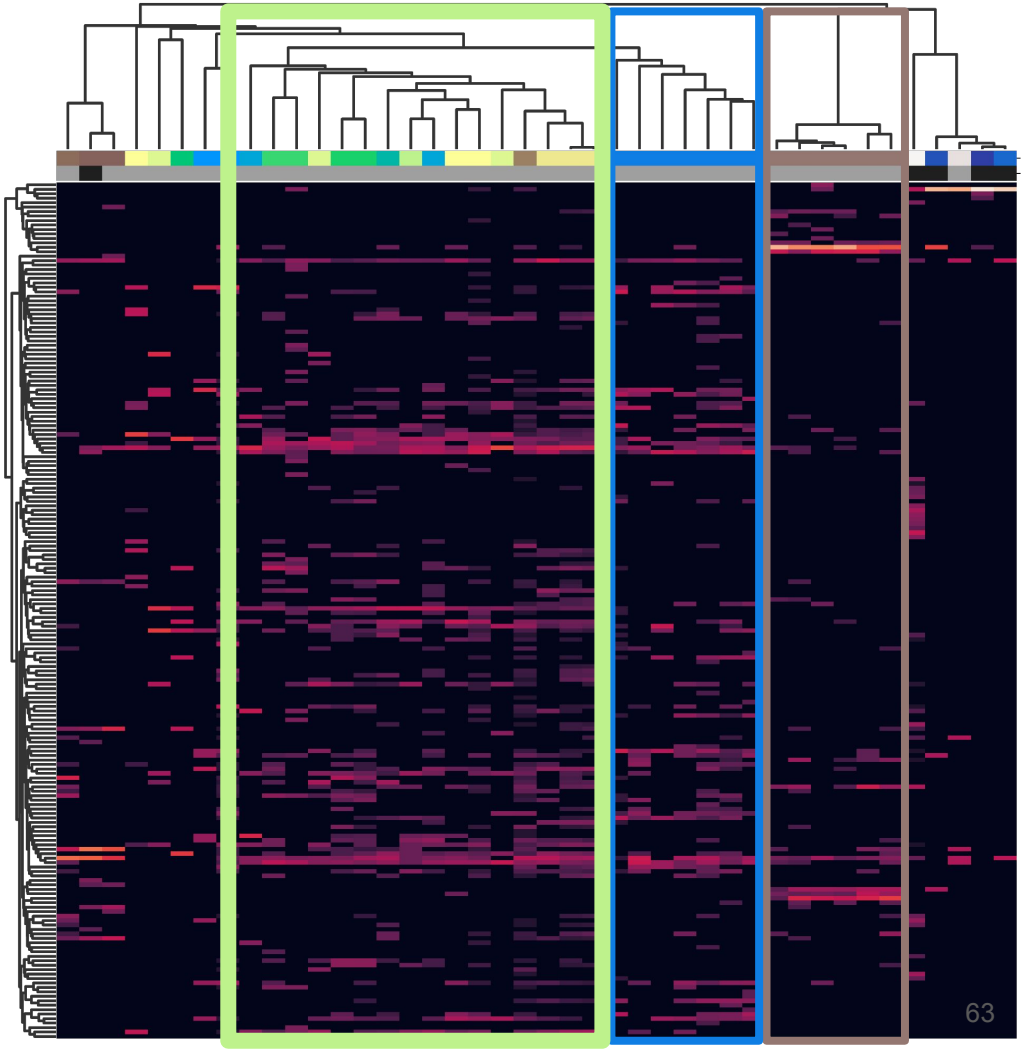

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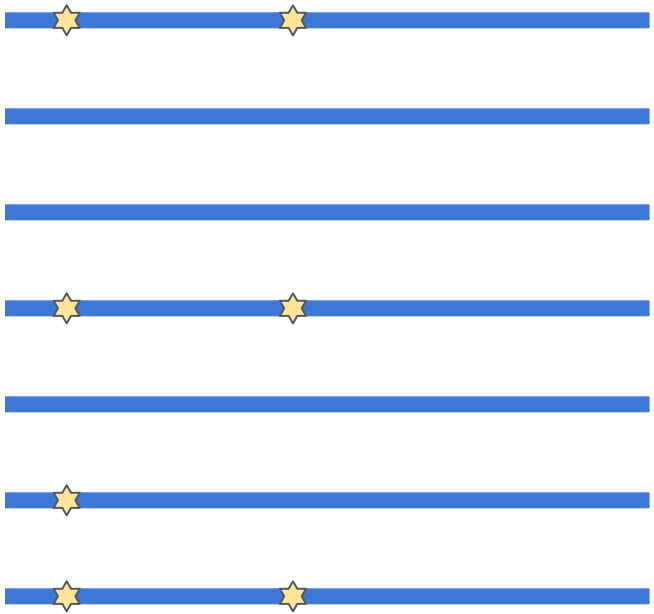
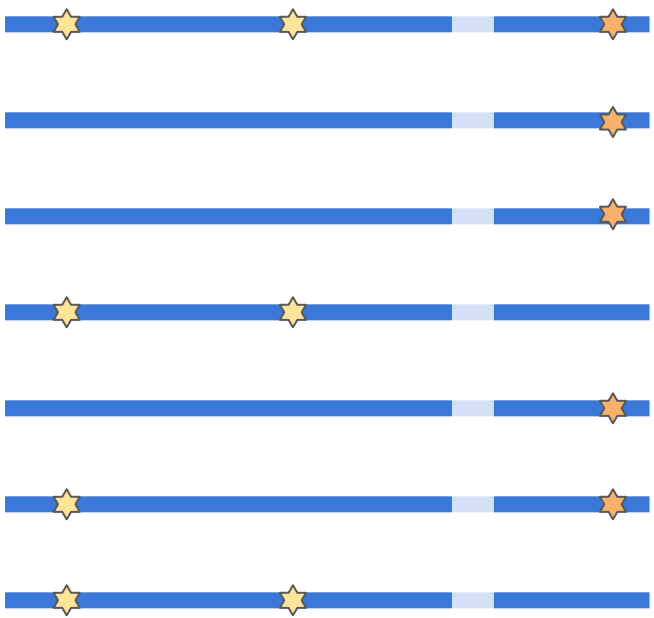


Linkage disequilibrium

Correlations between SNPs reflect shared descent

In asexual organisms, SNPs stay correlated

Population genetics



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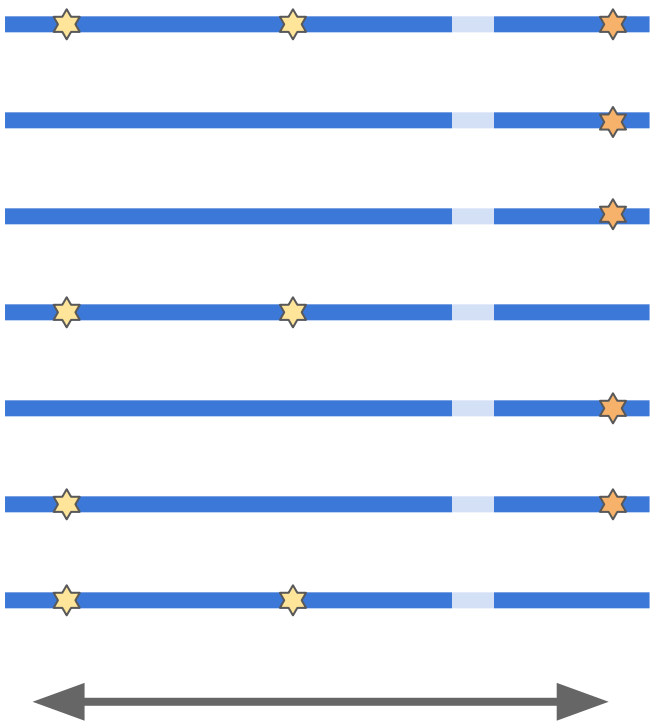

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We can see greater chance of recombination occurring between SNPs that are farther apart in the genome

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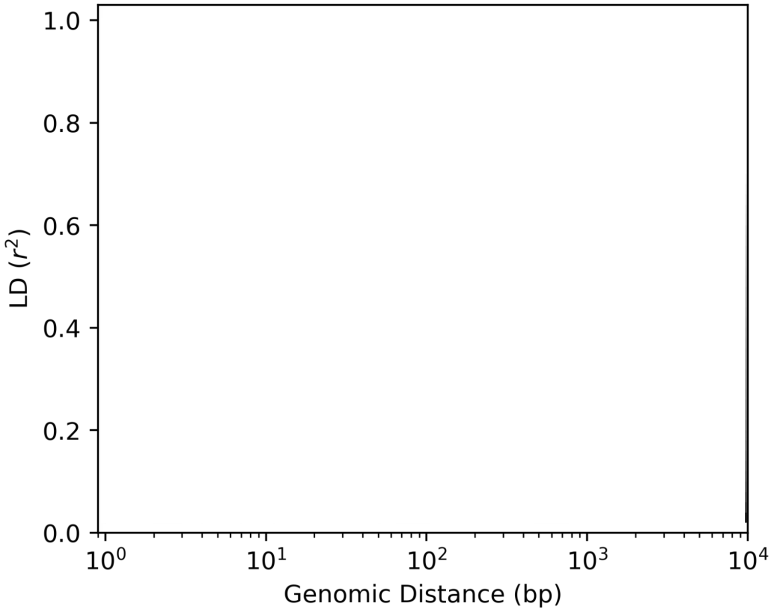

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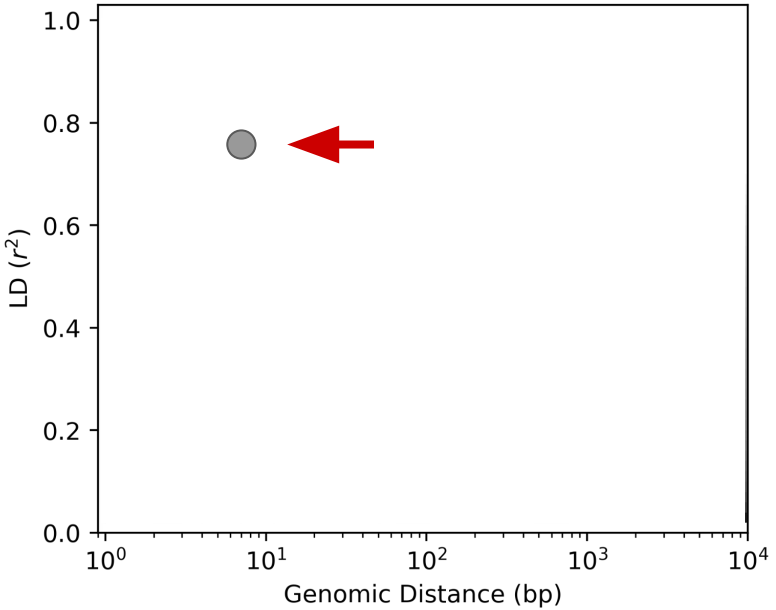
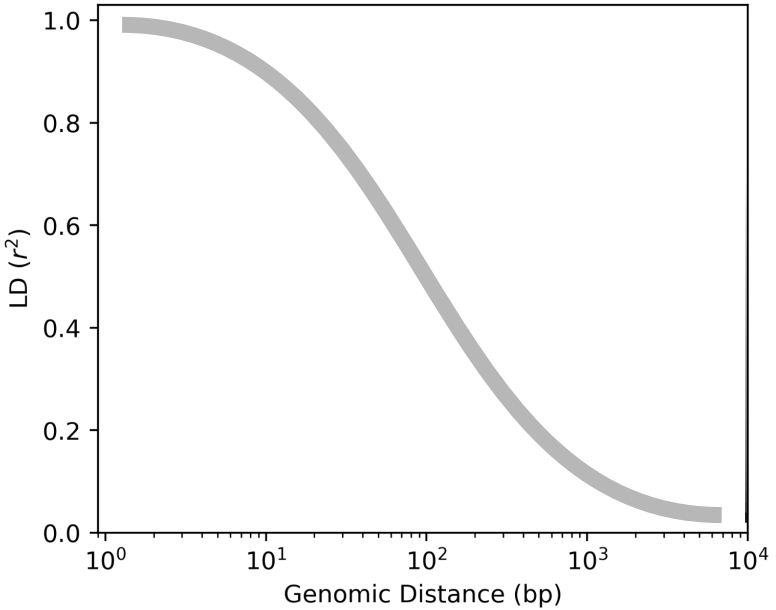


Population genetics

Decay of LD for pairs at larger distances reflects recombination



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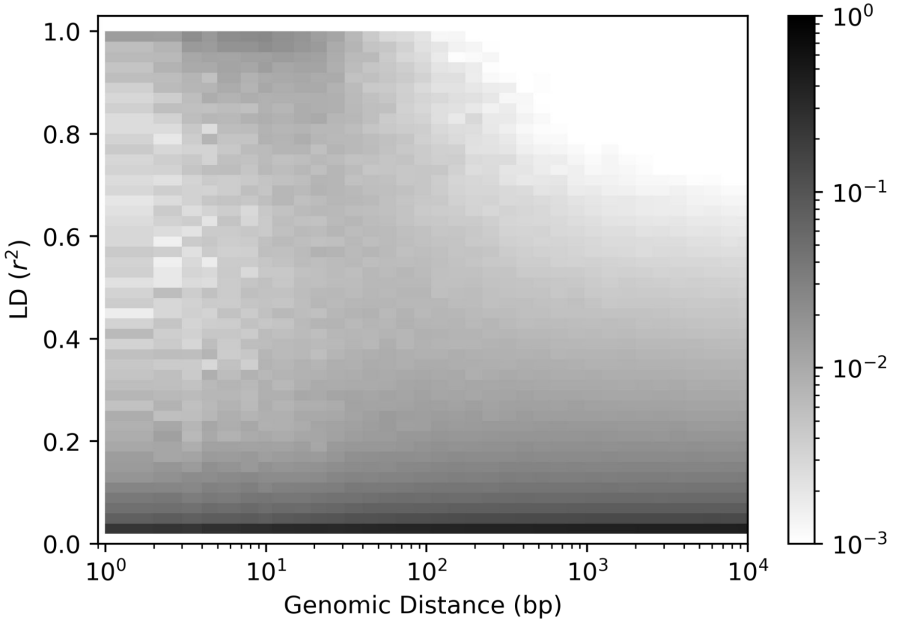
In *E. coli*, this is exactly what we see



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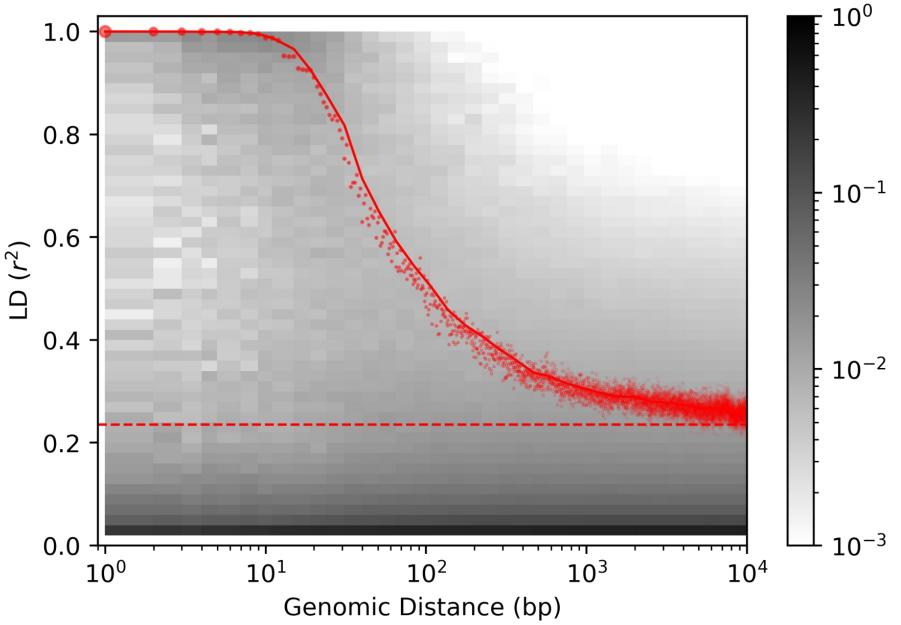

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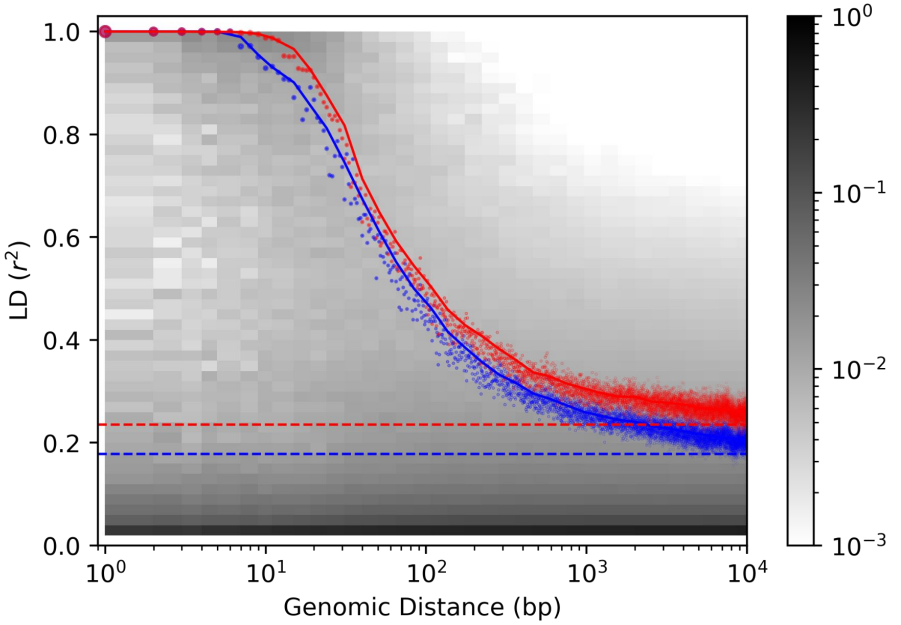

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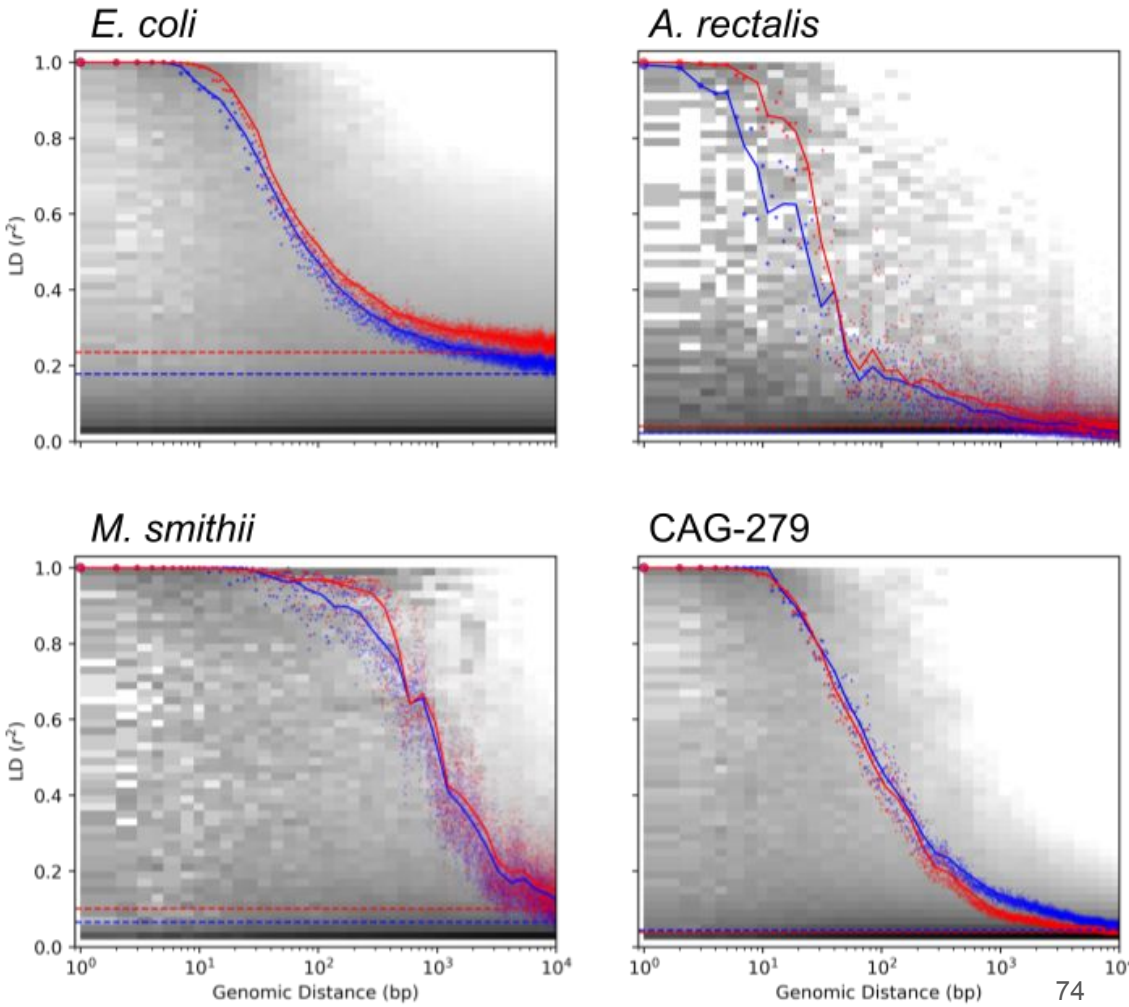

Summary and Conclusions

Deconvolution integrates strain quantification and genotype reconstruction

StrainFacts scales the approach to tens-of-thousands of metagenomes

Validated with simulations and single-cell genomics

Enables microbial biogeography, population genetics, and more at a global scale

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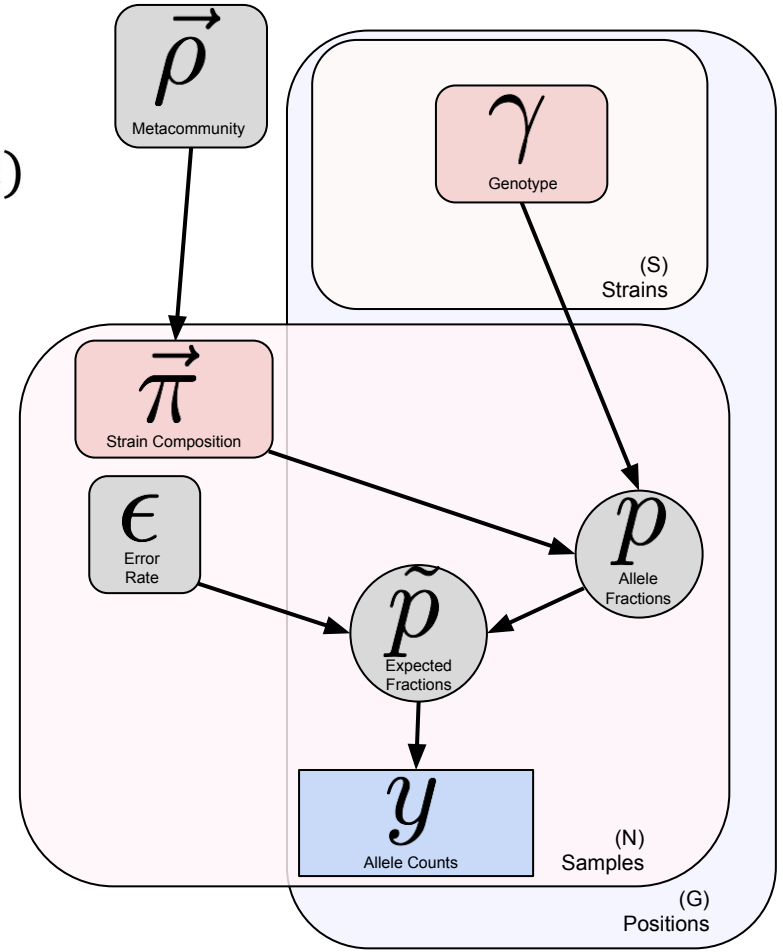
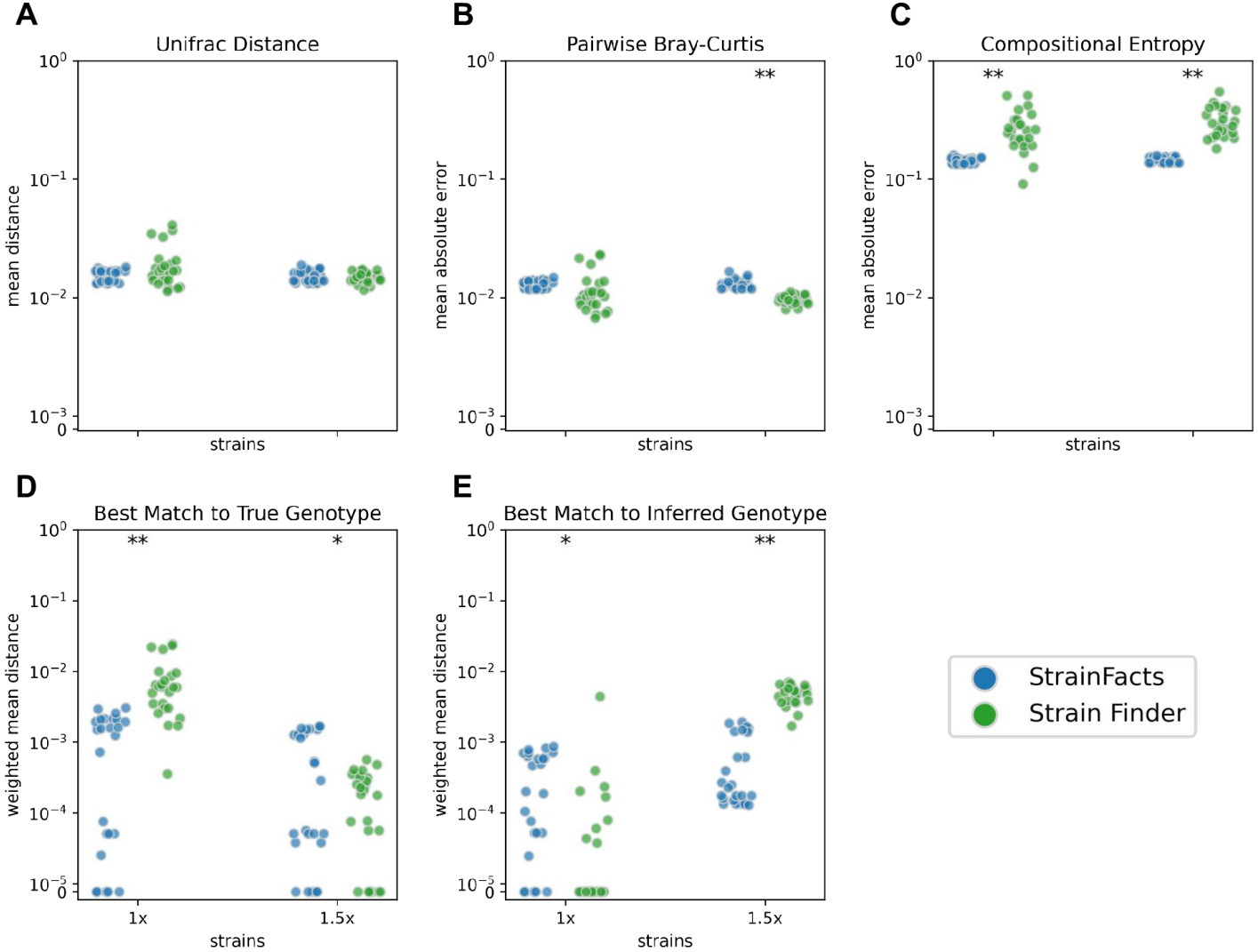
Questions?

Pocket Slides

$$
\begin{aligned}
y_{ig} &\sim \text{BetaBinom}\left(\tilde{p}_{ig}, \alpha^* \mid m_{ig}\right) \\
\tilde{p}_{ig} &= p_{ig}(1-\epsilon_i/2) + \left(1-p_{ig}\right)(\epsilon_i/2) \\
p_{ig} &= \sum_s \pi_{is}\,\gamma_{sg} \\
\gamma_{sg} &\sim \text{SSD}_0\left(\mathbf{1}, \mathbf{1}, \frac{1}{\gamma^*}\right) \\
\vec{\pi}_i &\sim \text{SSD}\left(\mathbf{1}, \vec{\rho}, \frac{1}{\pi^*}\right) \\
\vec{\rho} &\sim \text{SSD}\left(\mathbf{1}, \mathbf{1}, \frac{1}{\rho^*}\right) \\
\epsilon &\sim \text{Beta}\left(\epsilon_a^*, \frac{\epsilon_a^*}{\epsilon_b^*}\right)
\end{aligned}
$$




A
Unifrac Distance
mean distance
strains
B
Pairwise Bray-Curtis
mean absolute error
strains
**
C
Compositional Entropy
mean absolute error
strains
**
**
D
Best Match to True Genotype
weighted mean distance
strains
1x
1.5x
**
*
E
Best Match to Inferred Genotype
weighted mean distance
strains
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1.5x
*
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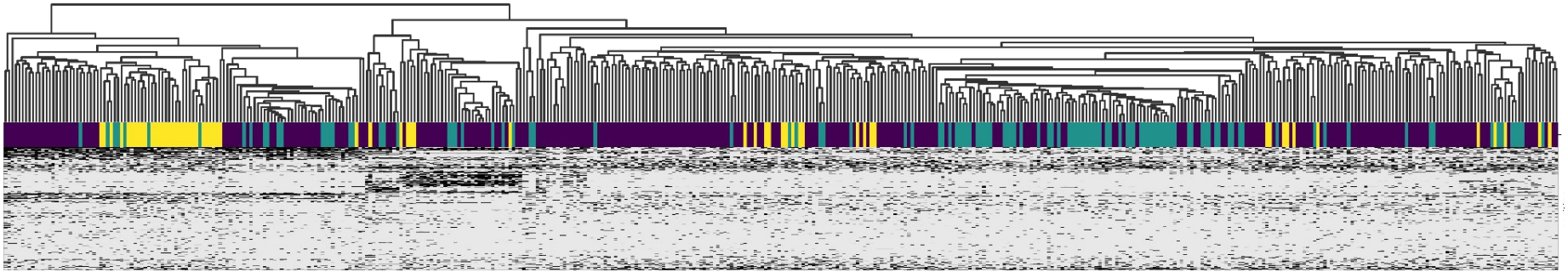
StrainFacts
Strain Finder

Cataloging strain diversity



Agathobacter rectalis is a prevalent and abundant gut bacterium

Dozens of studies → 11,860 samples → 198 inferred strains / 752 references

De novo strains reflect much of the diversity previously seen in references