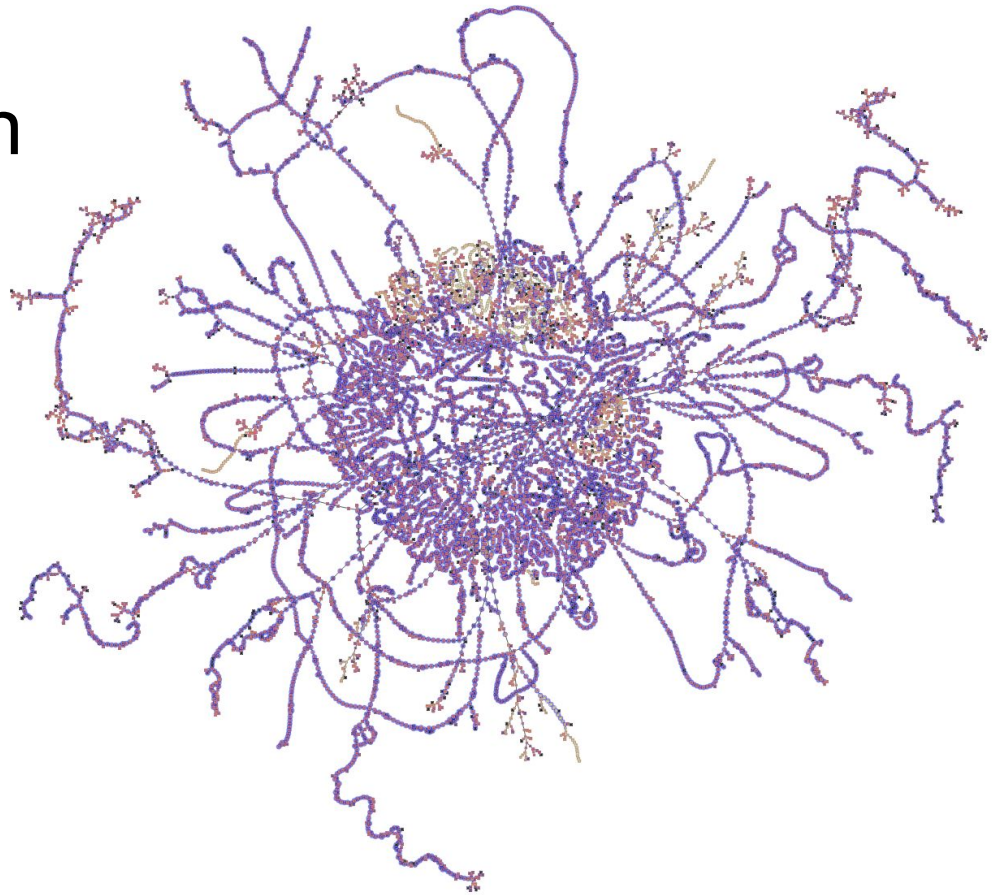
Disentangling depth on the De Bruijn graph:

Combining quantification and *de novo* assembly

Byron J. Smith
IGGSy
2024-07-04



Acknowledgments

Acknowledgments

Pollard Lab

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- Veronika Dubinkina
- et al.

Engelhardt Lab

- *Archit Verma*
- Dylan Cable

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TODO: Add QR Code to PDF

Outline of the presentation

- **Motivation:**

  Profiling the microbiome with metagenomics

- **Method:**

  Assembly graph deconvolution with StrainZip

- **Demonstration:**

  Application to a complex, ground-truthed dataset

Motivation

Human associated microbes are diverse and important

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Important:

- Digestion
- Pathogen resistance
- Immune modulation
- Drug metabolism

Diverse:

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

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Important:

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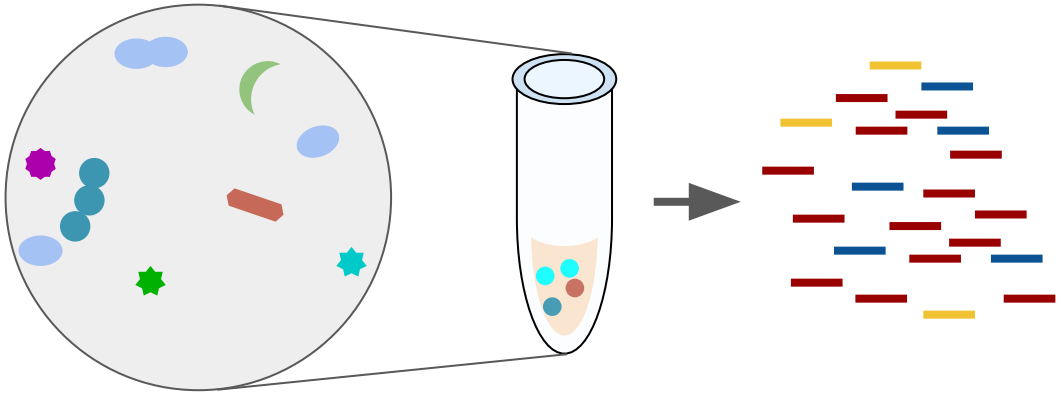
Diverse:

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

Metagenomics enables modern microbiome science



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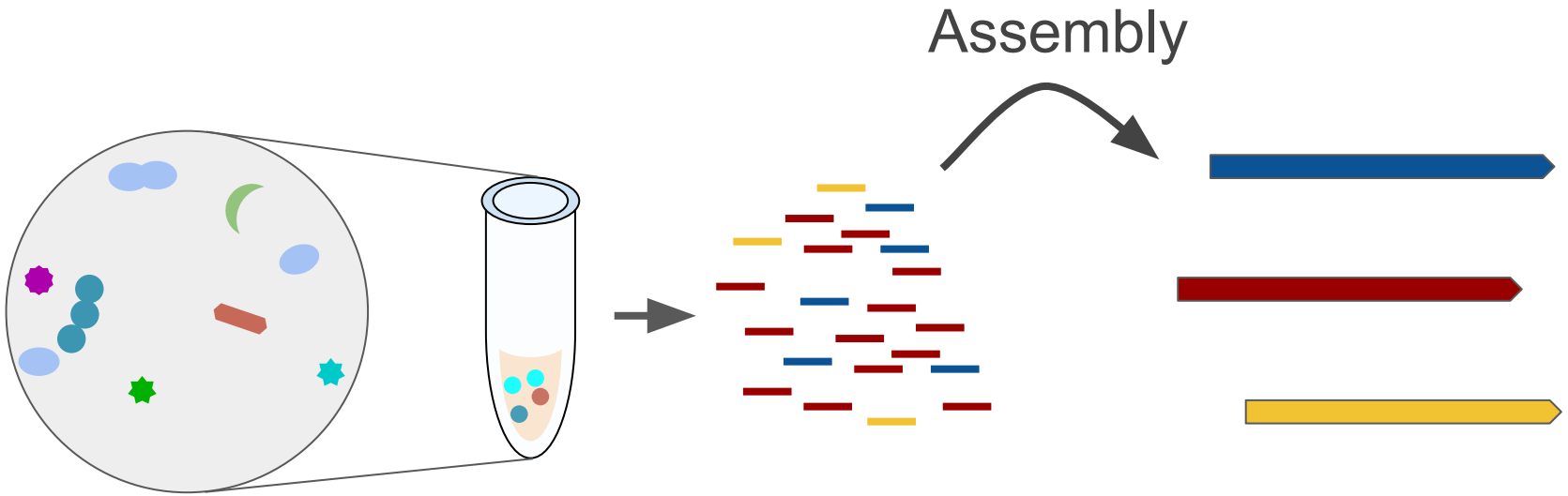
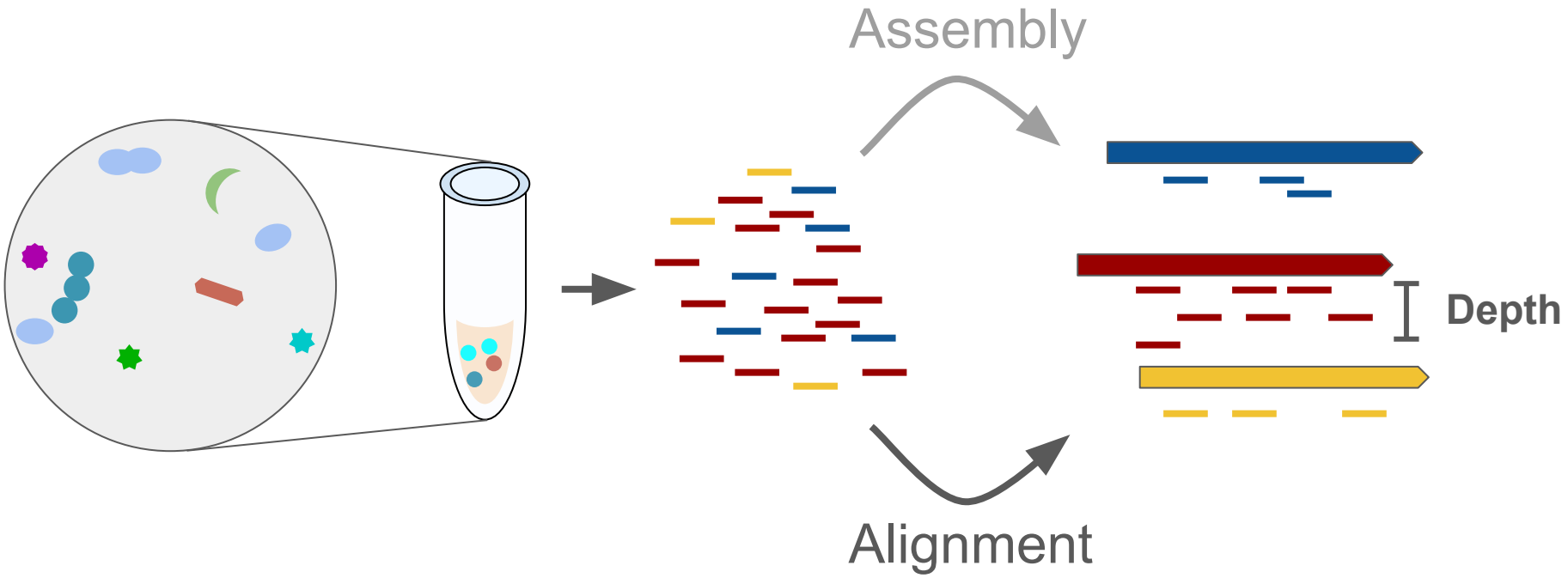
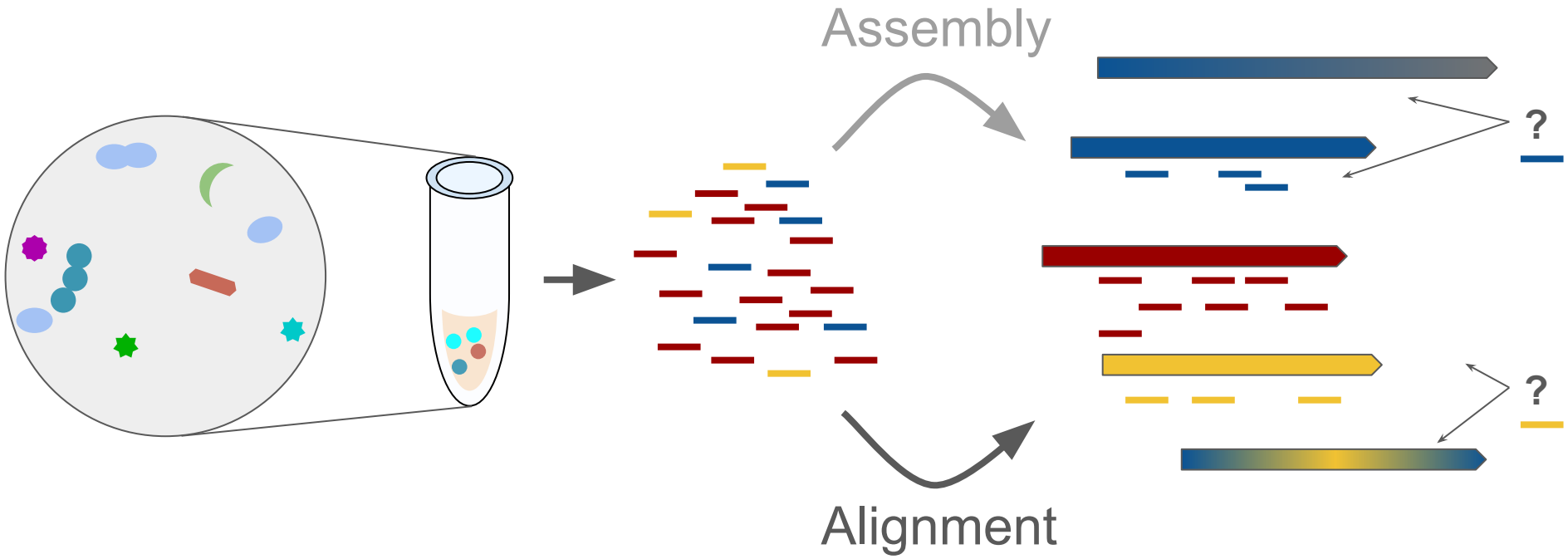


Assembly and depth quantification are complementary



Closely related sequences are a major challenge for alignment



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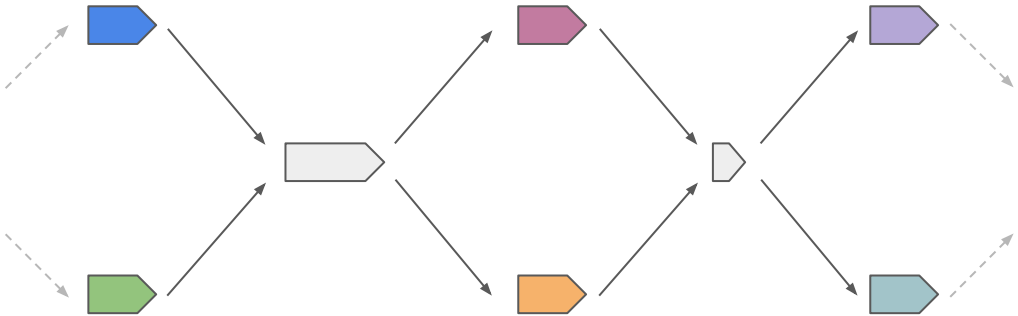
Complex graph structure



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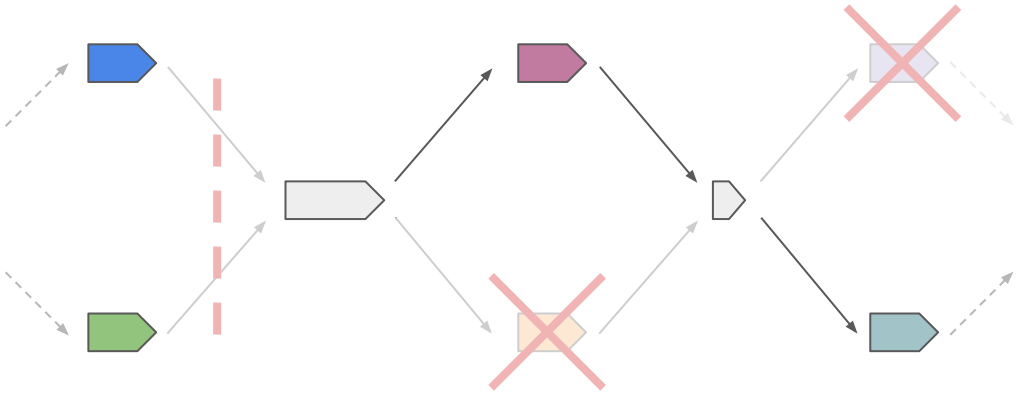

Complex graph structure
leads to low-quality assembly

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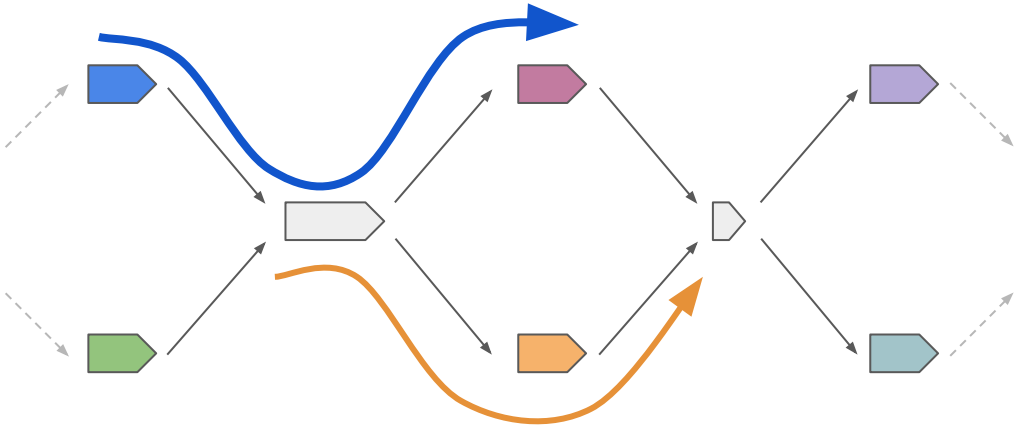

Complex graph structure leads to low-quality assembly

Graph-pangenome approaches account for this variability better than linear references

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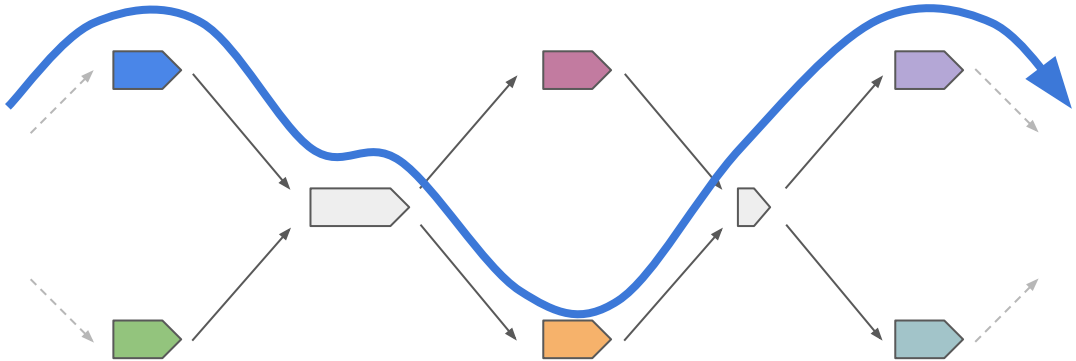

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But long reads too expensive for profiling multiple samples

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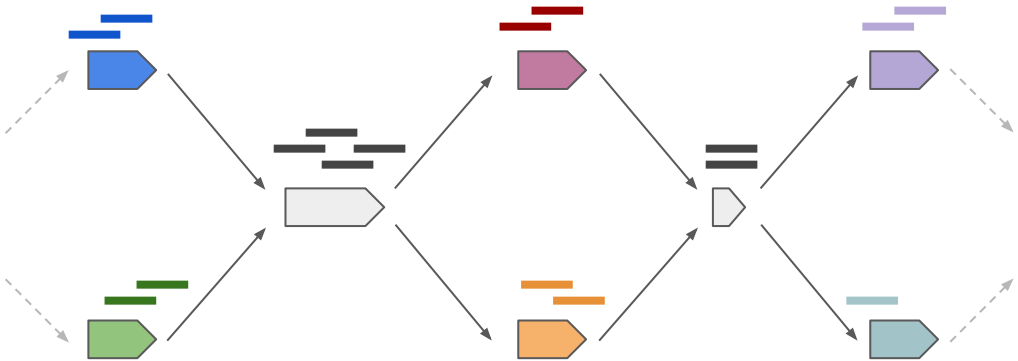


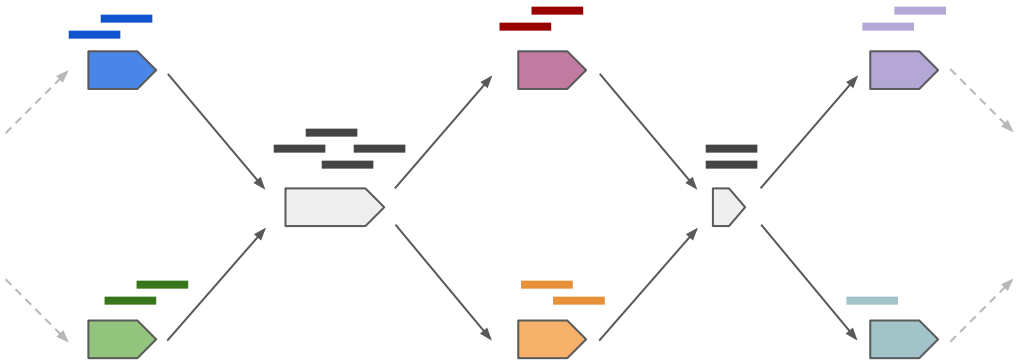
Complex graph structure leads to low-quality assembly

Graph-pangenome approaches account for this variability better than linear references

But long reads too expensive for profiling multiple samples

And short-reads are inherently ambiguous

KEY IDEA: The expected depth of a k-mer is the sum of the paths that include that k-mer



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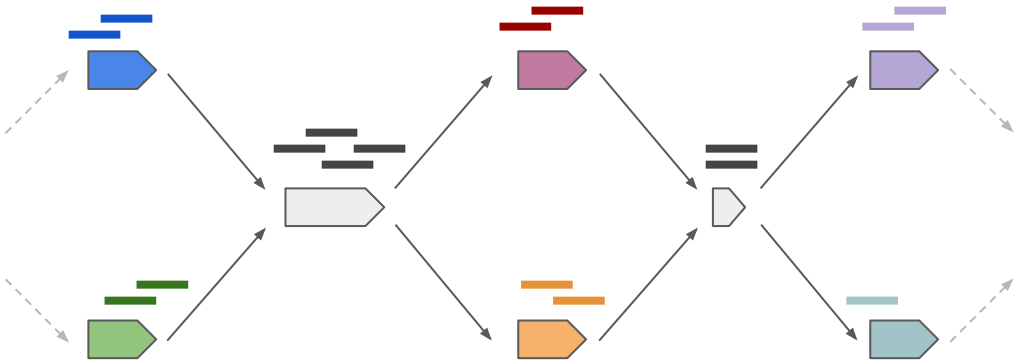

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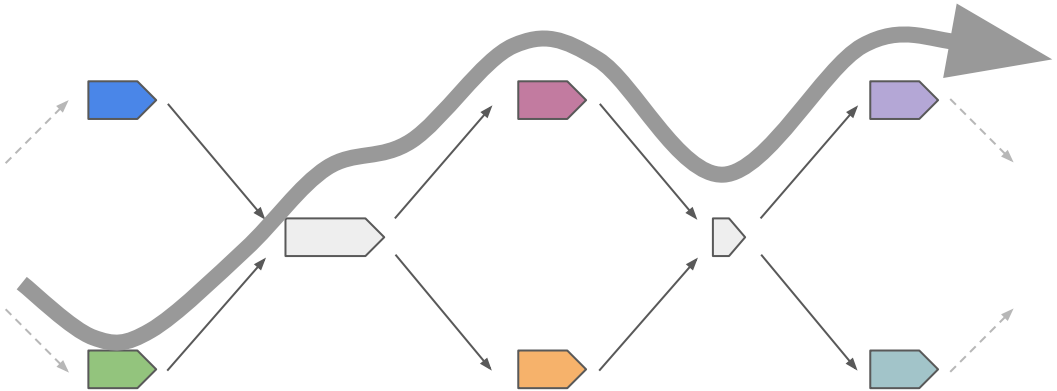

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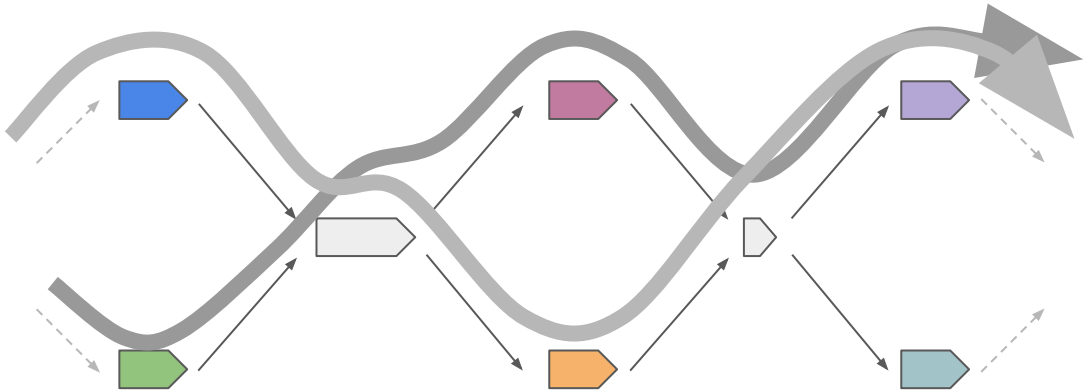

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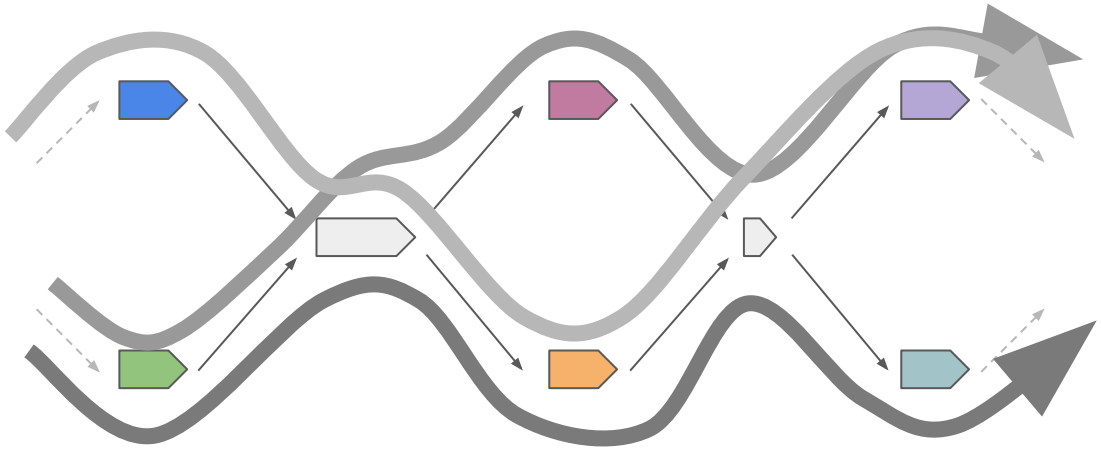

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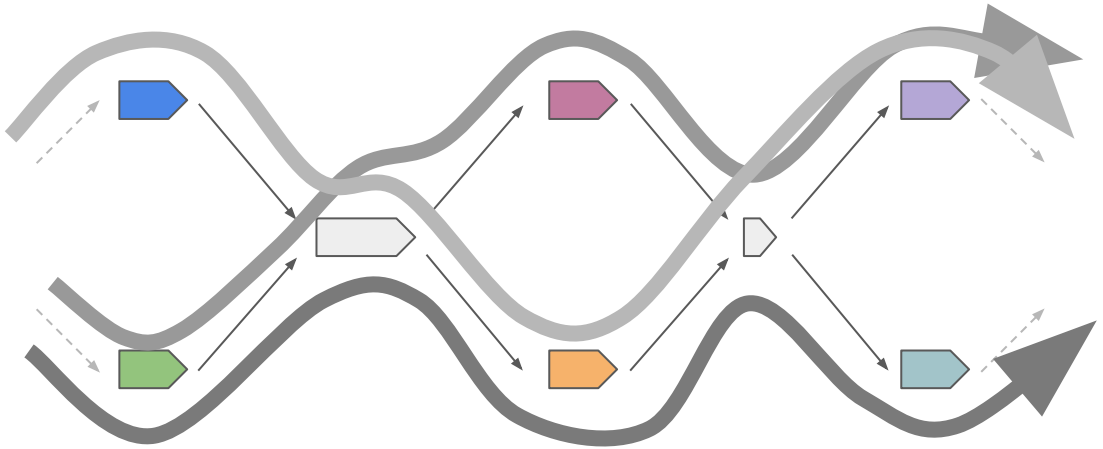


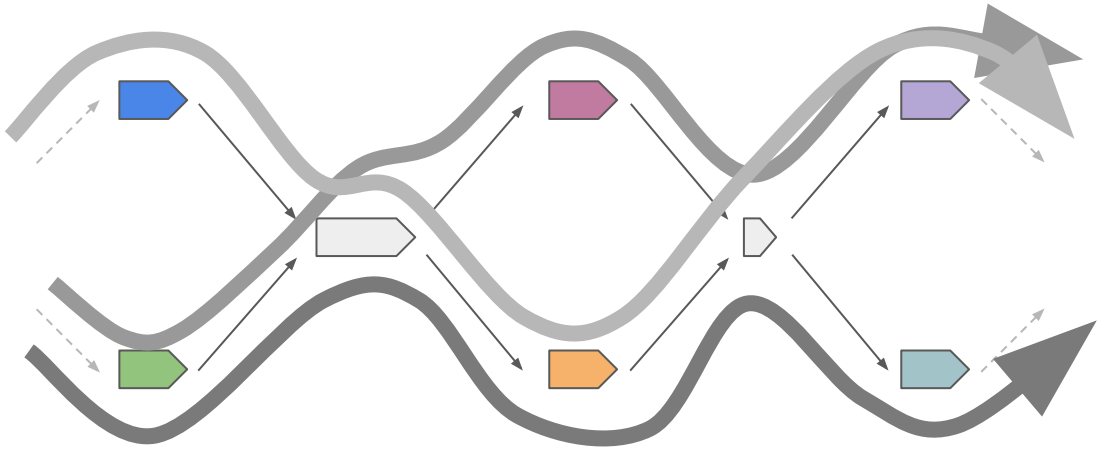
Path depths (unknown)

Indicator: k-mer in path

Observed depths

$$\sum_{p} x_{pk}\beta_p \approx Y_k$$

KEY IDEA: The expected depth of a k-mer is the sum of the paths that include that k-mer



Path depths (unknown)

Path encoding

Observed depths

$$X\beta \approx Y$$

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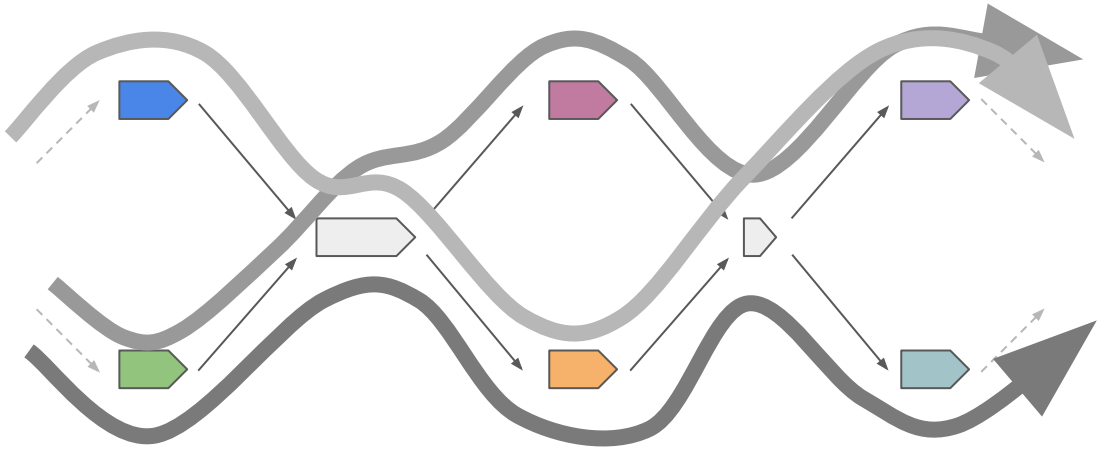
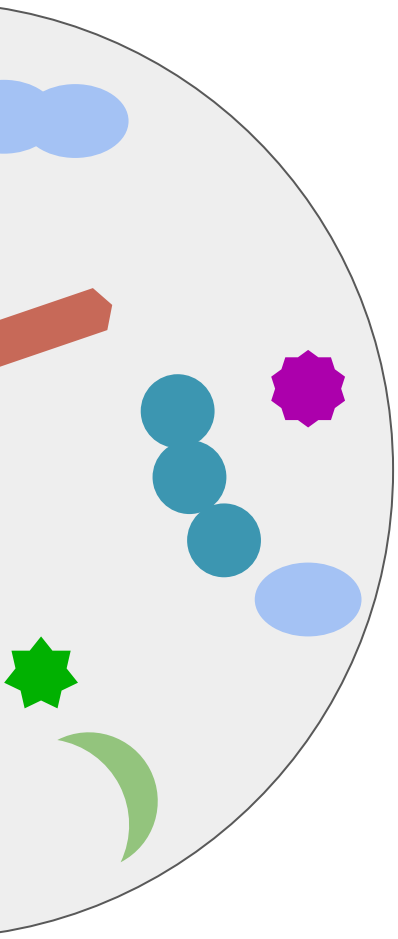


Deconvolution: Inferring the depth of these latent paths based on observed k-mer depths

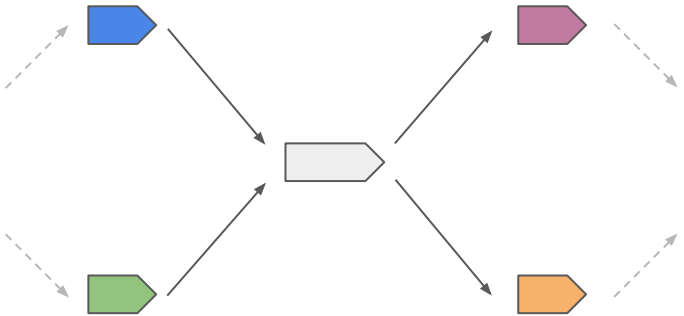
$$\hat{\beta} = \text{argmin}\, L(X\beta \mid Y)$$



How can we extend the deconvolution approach to de novo assembly graphs?

Method

We can enumerate all possible paths on our assembly graph



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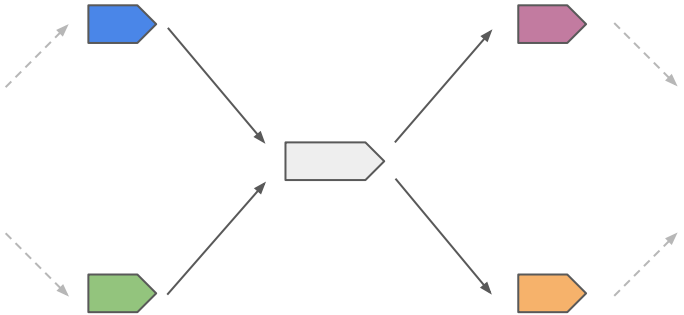

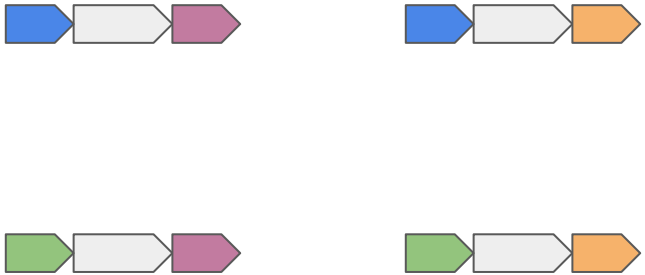

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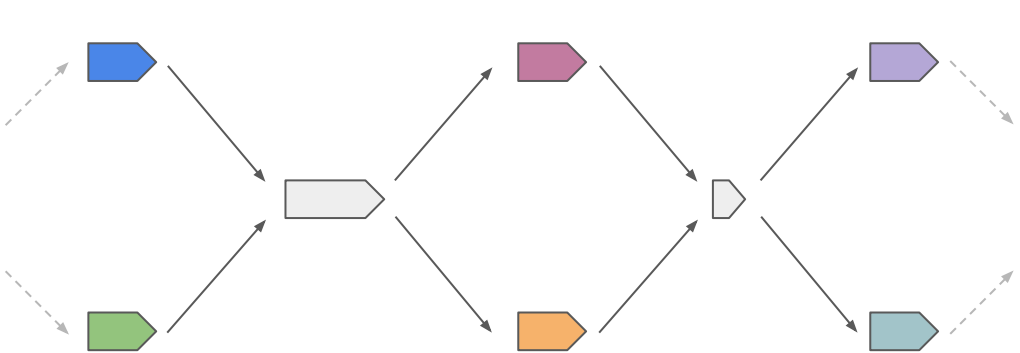
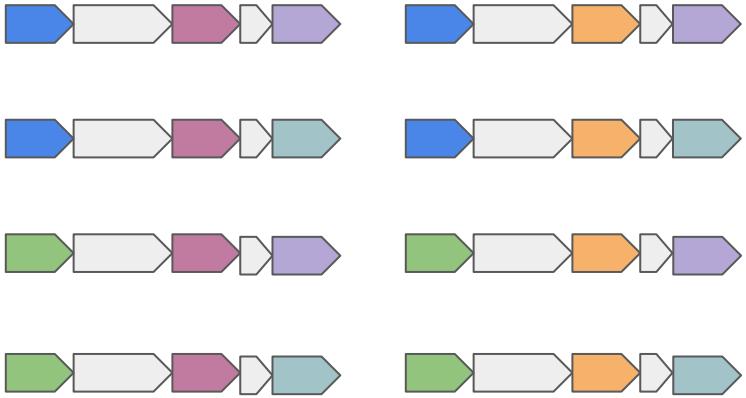
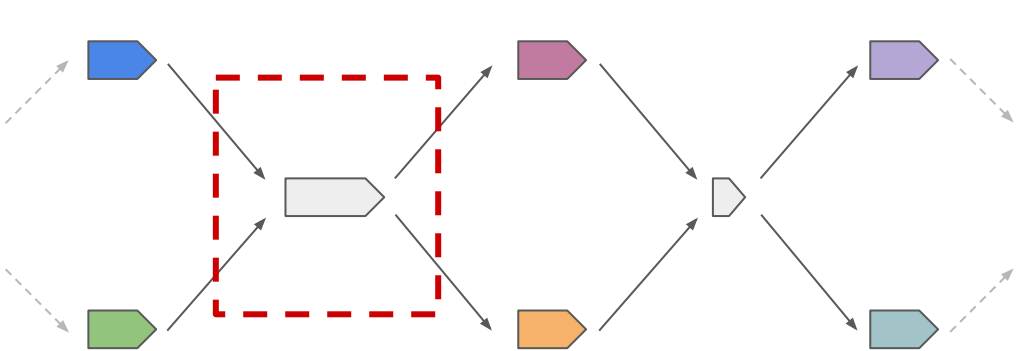
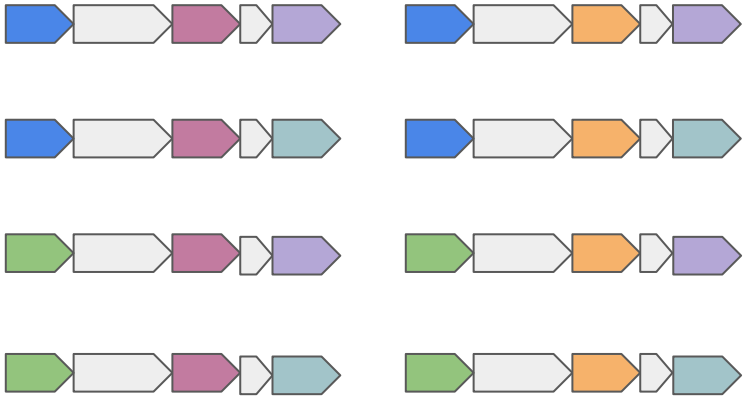




…but this grows exponentially with graph complexity

Divide and conquer: a single "junction" is the minimum unit of deconvolution





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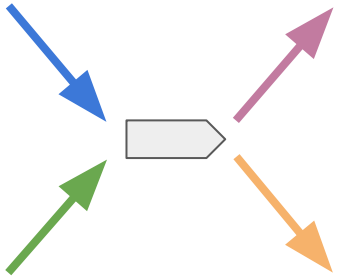
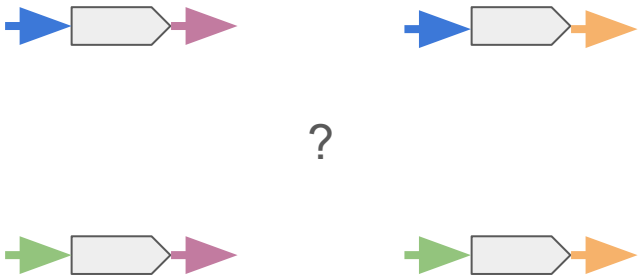
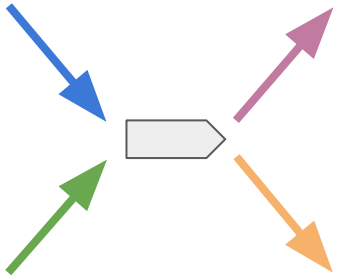
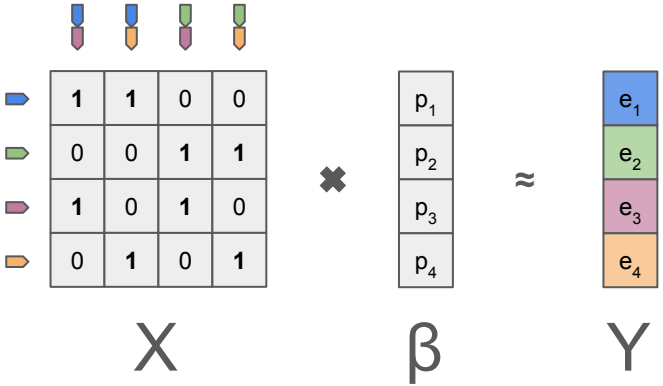


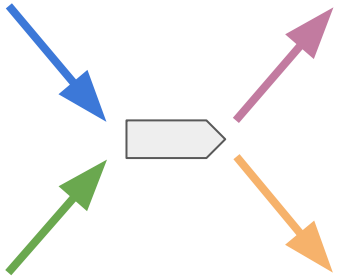


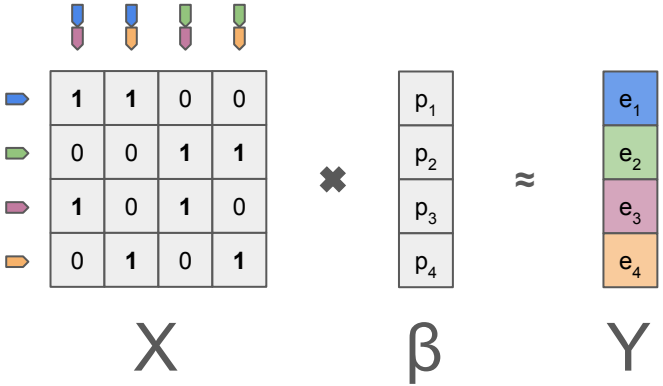
Linear model of path depths





But not all paths exist: picking active paths is model selection





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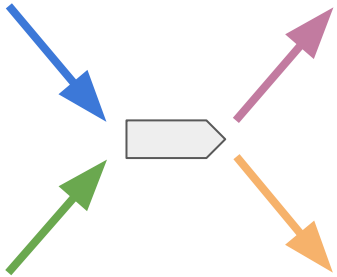

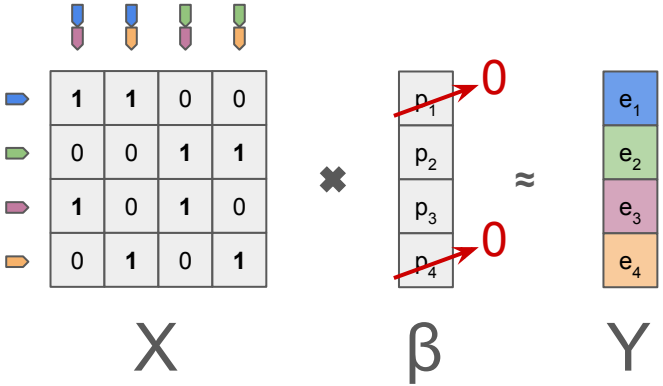

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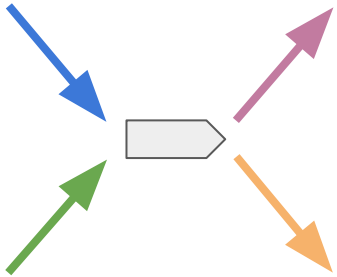
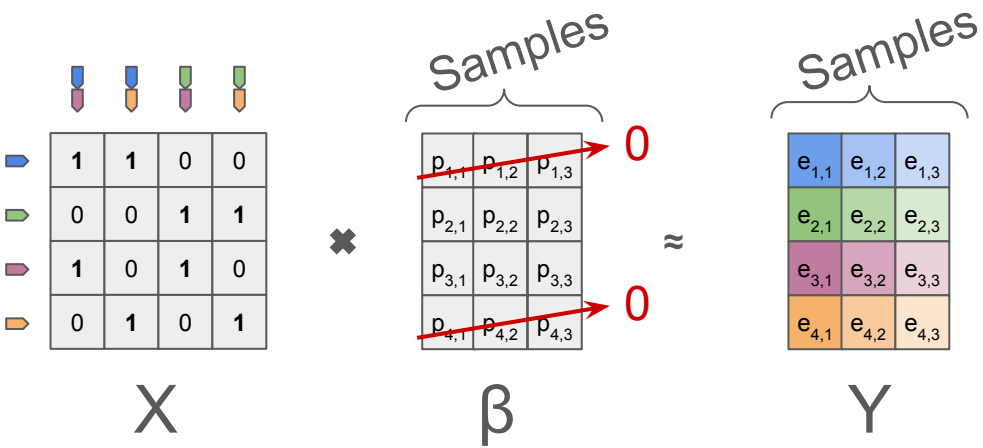
Last trick: To increase our power to pick paths, combine multiple samples





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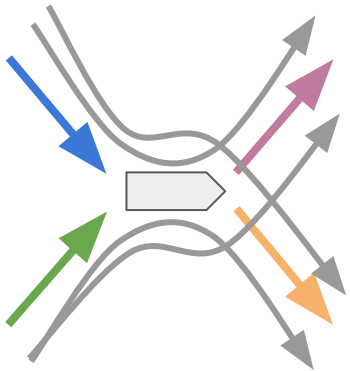
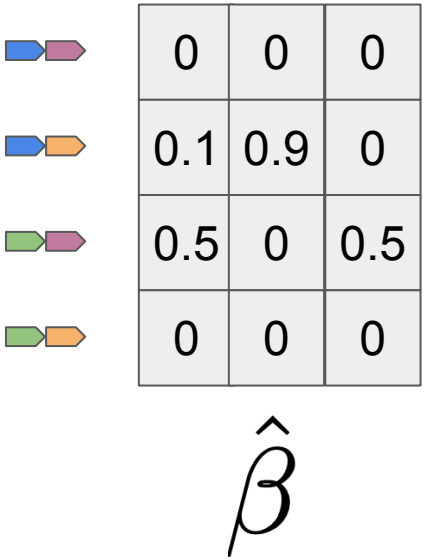
Drop paths with no depth in any sample





Used statistical linkage to resolve ambiguity about which of possible paths are "real"

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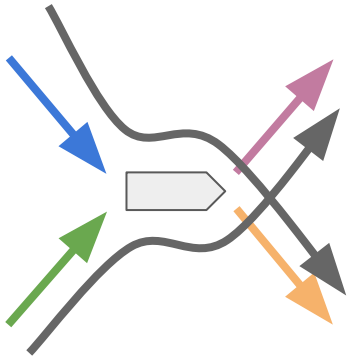

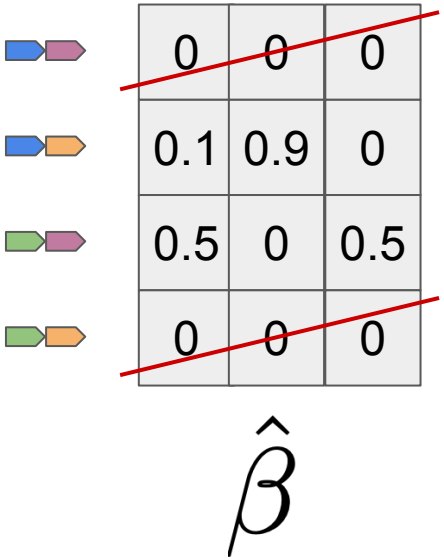

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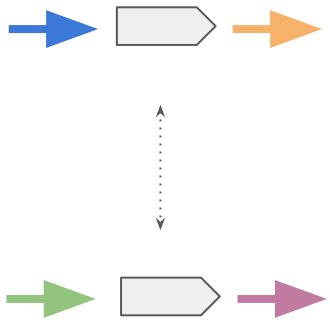
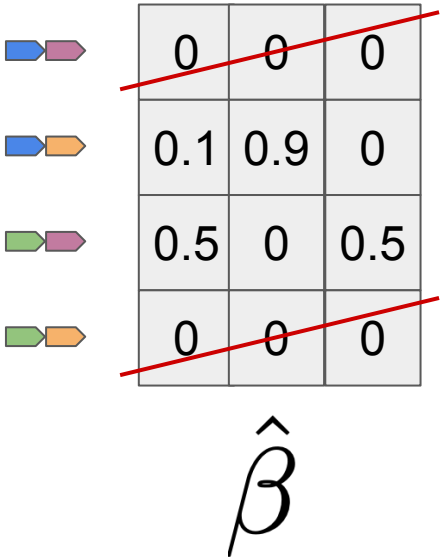
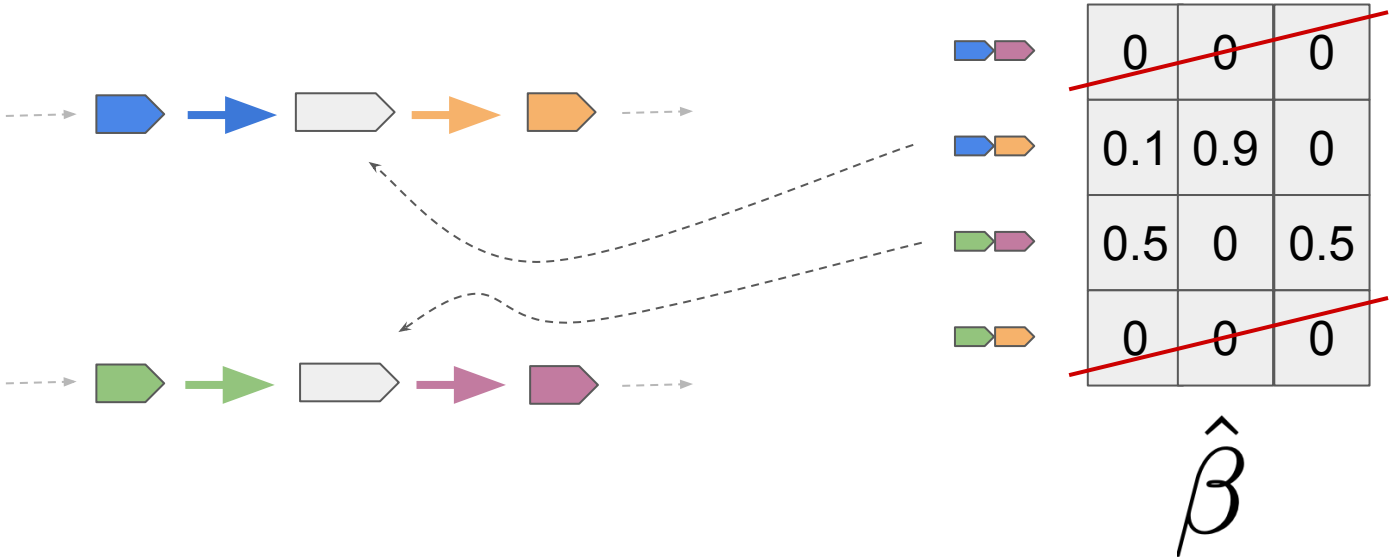
Resolve ambiguity, longer linear sequences





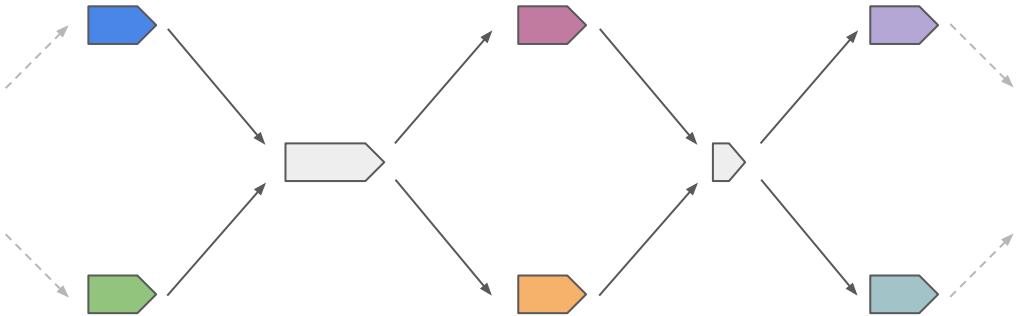
Can "unzip" this unitig into two paths

Resolve ambiguity, longer linear sequences



Newly split unitigs already have depths estimated across samples

Iteratively unzipping local junctions



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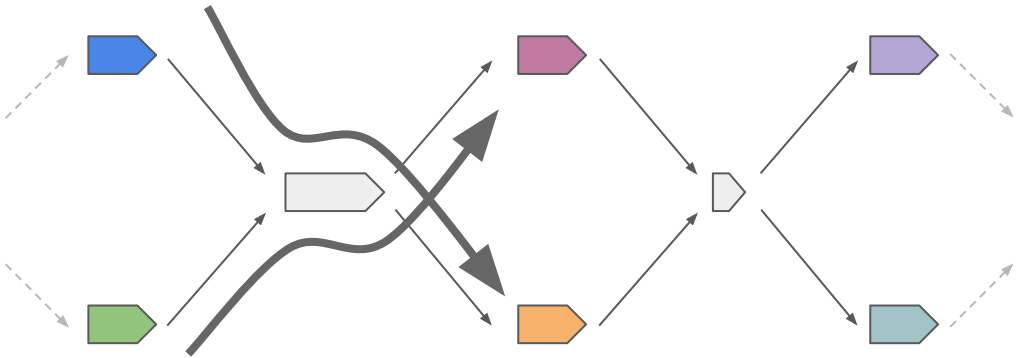
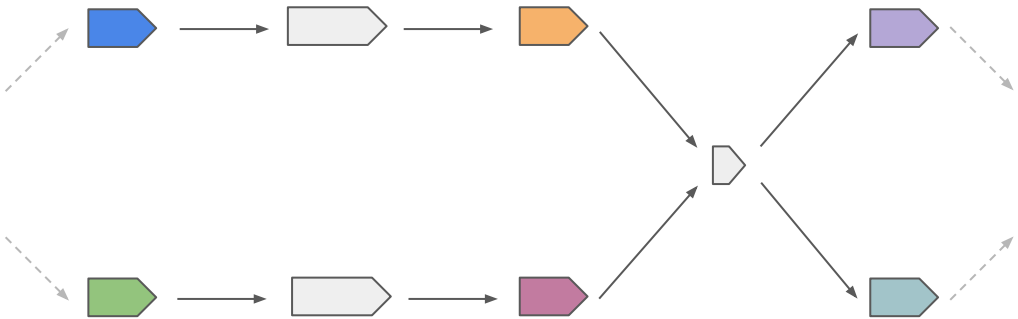
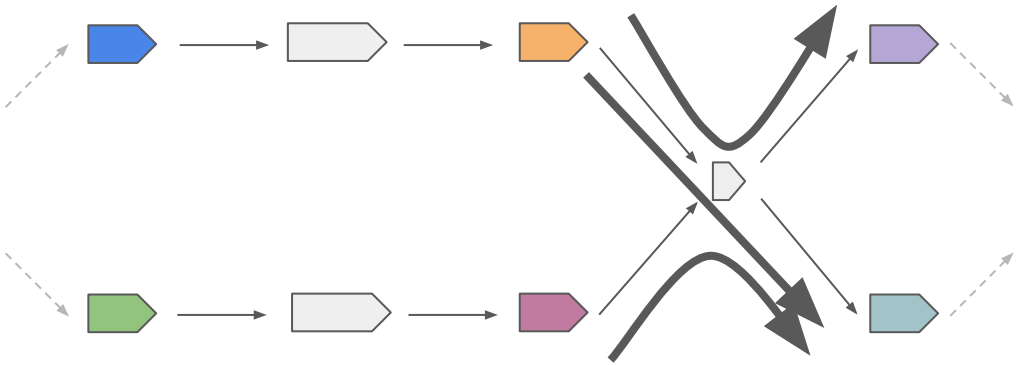


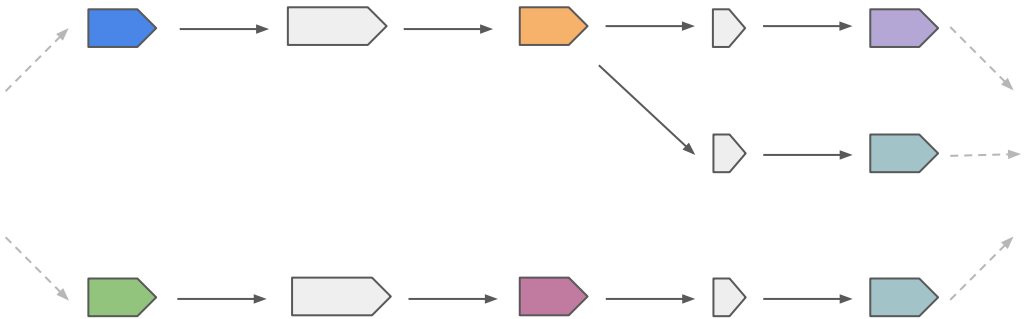
Iteratively unzipping local junctions



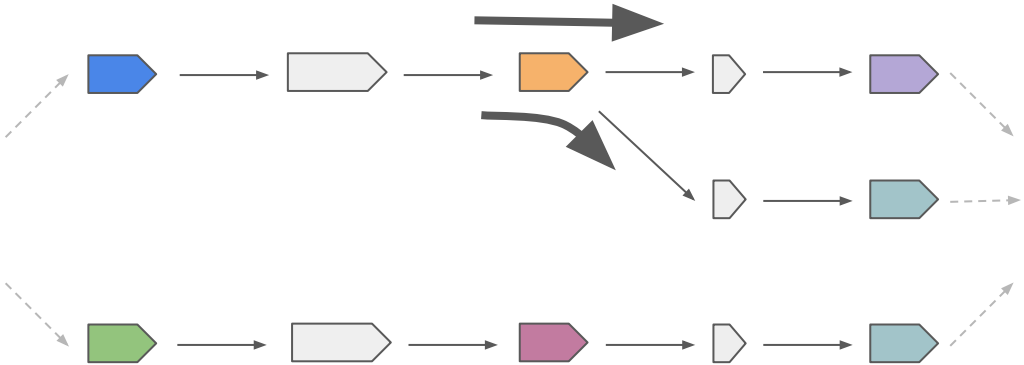
Iteratively unzipping local junctions



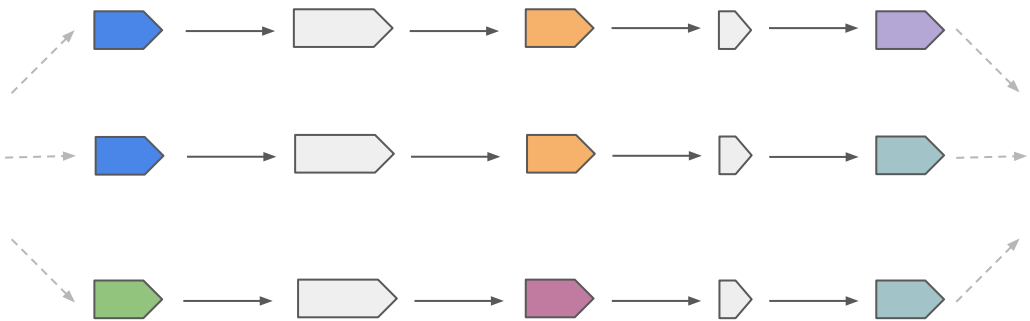
Iteratively unzipping local junctions



Iteratively unzipping local junctions



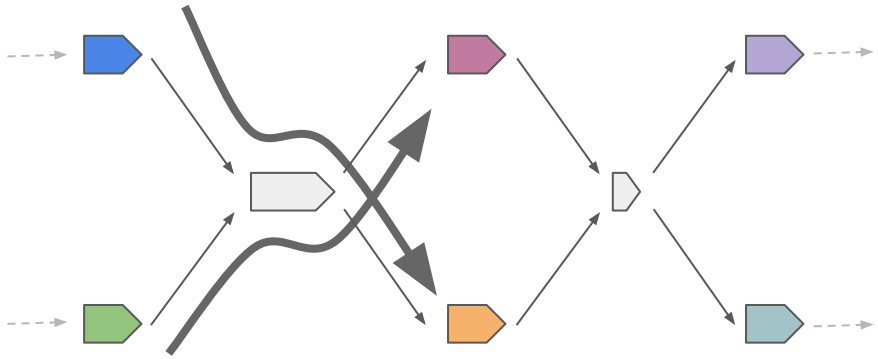
Iteratively unzipping local junctions

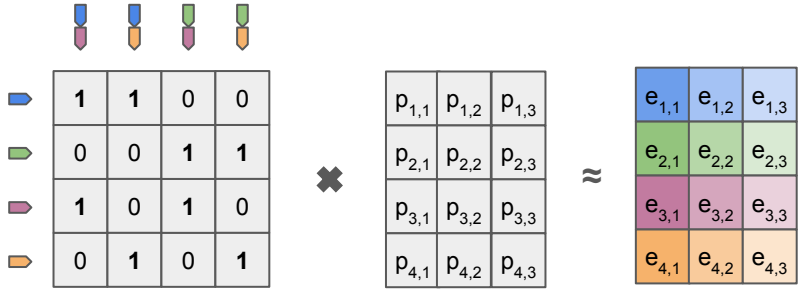


StrainZip

Assembly Graph Deconvolution for Quantification of Strain-Specific Sequences across Metagenomes

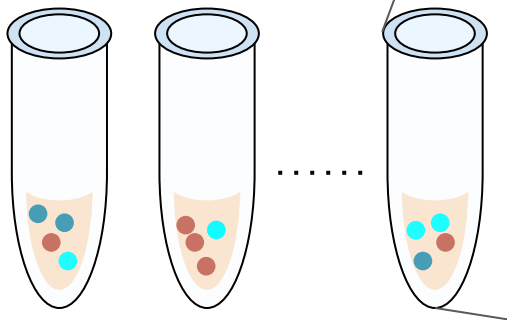
https://github.com/bsmith89/StrainZip



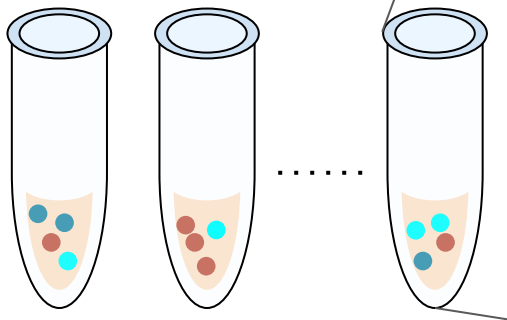


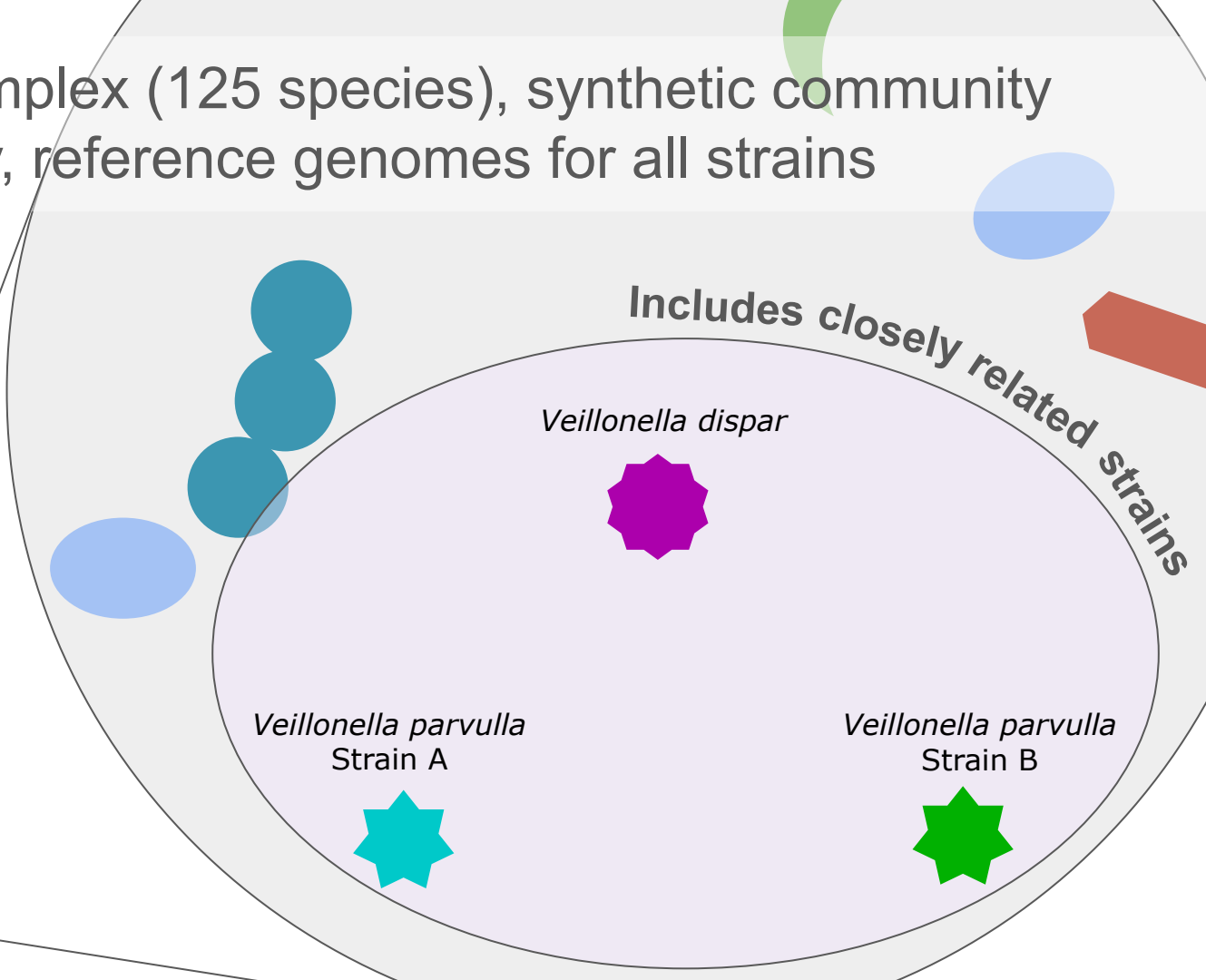
Demonstration

hCOM2 is a complex (125 species), synthetic community with high-quality, reference genomes for all strains

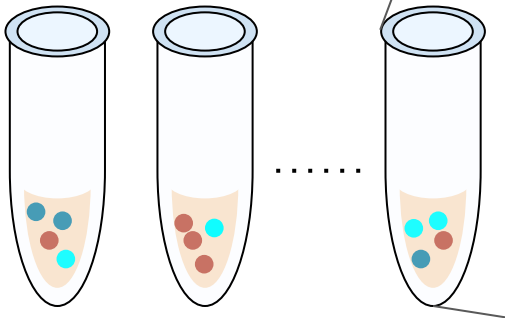


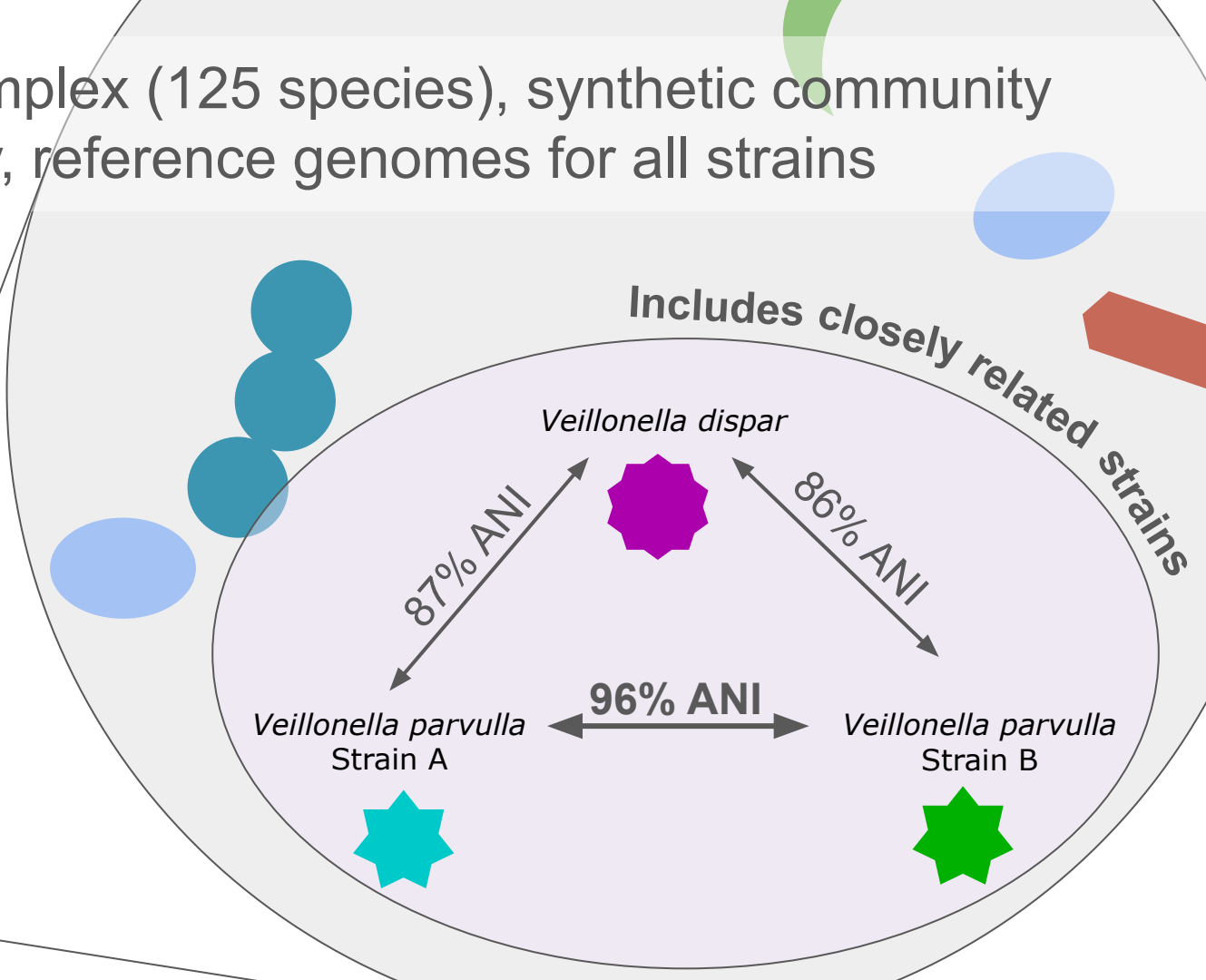
hCOM2 is a complex (125 species), synthetic community with high-quality, reference genomes for all strains

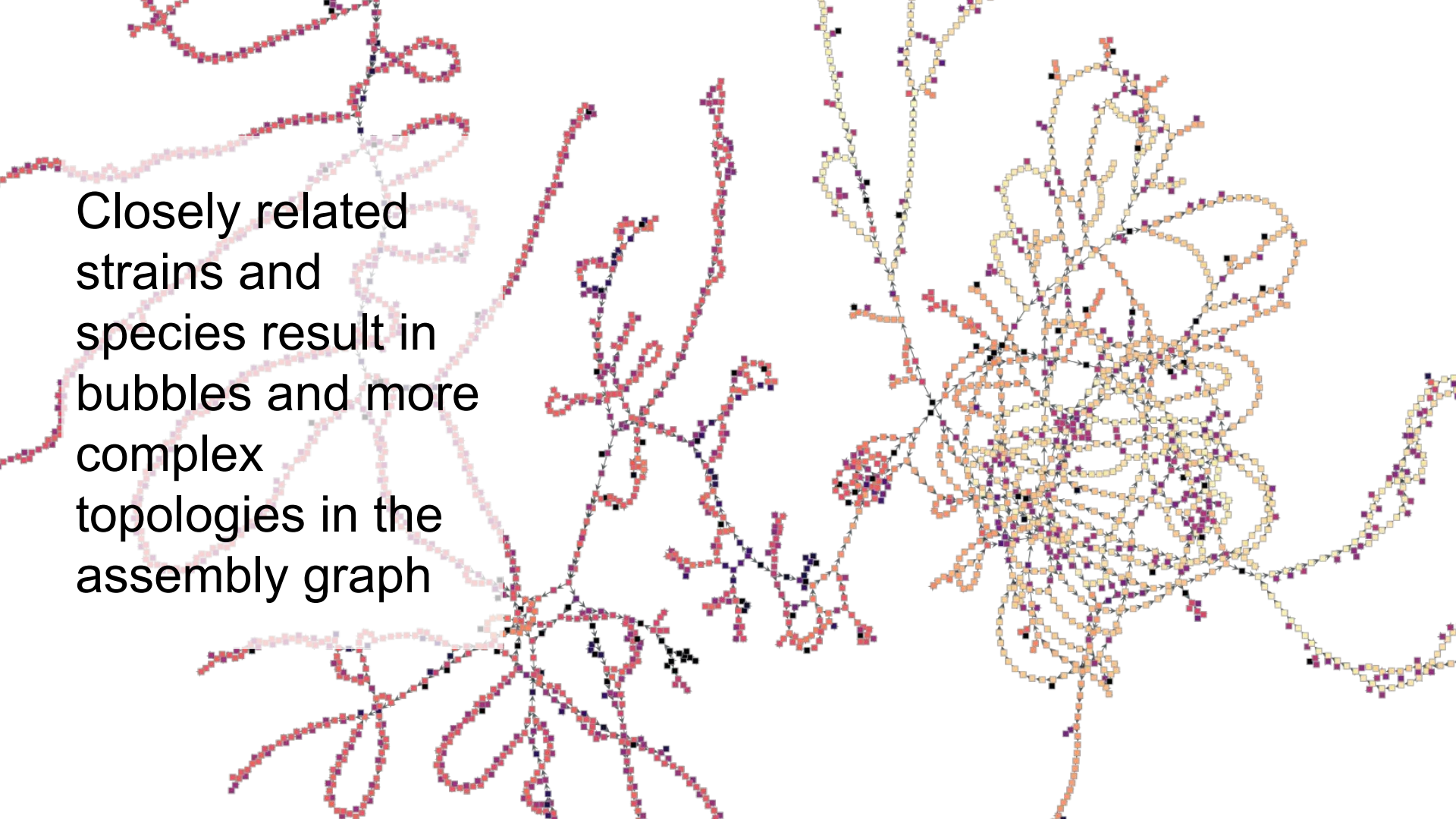


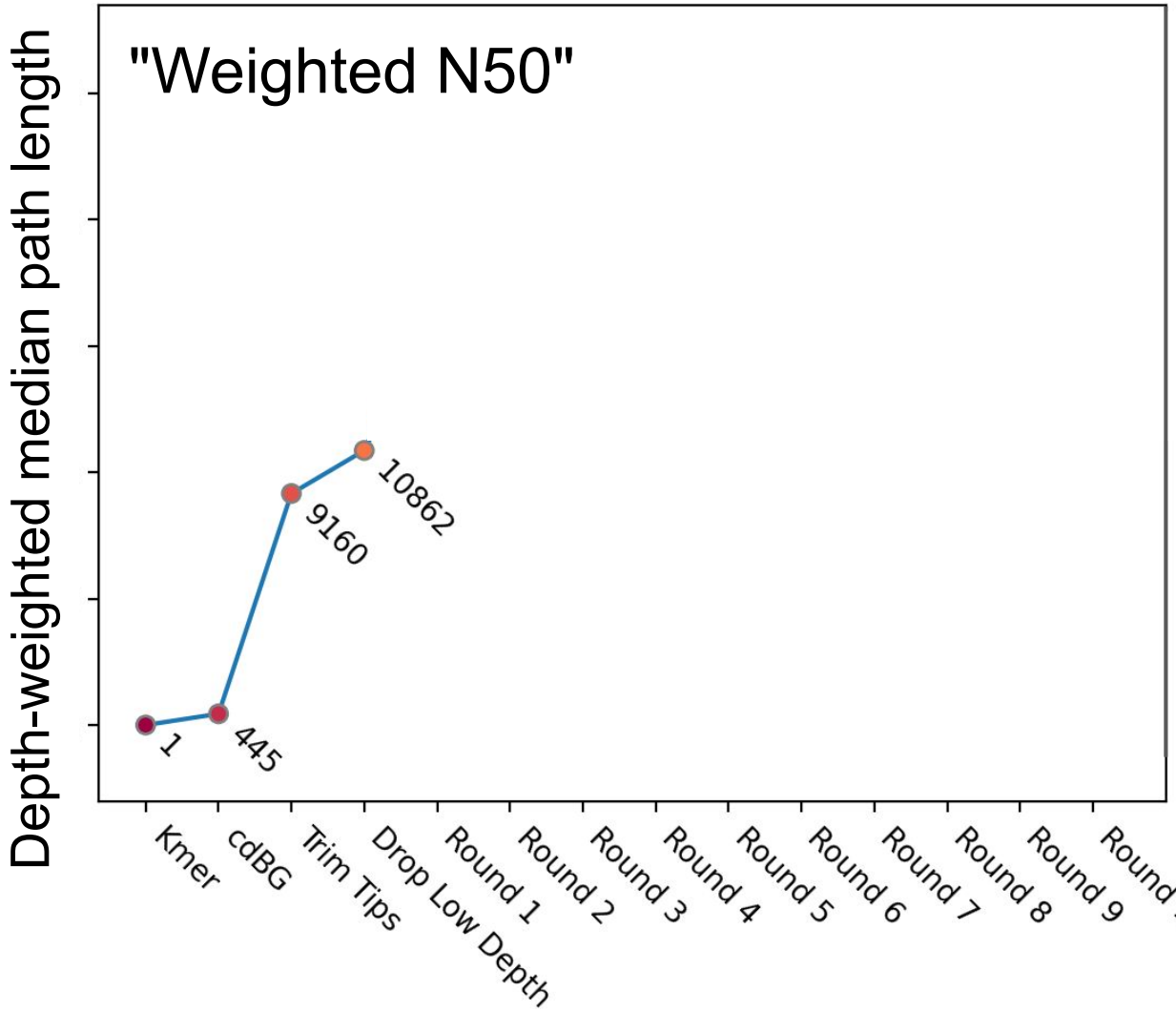


hCOM2 is a complex (125 species), synthetic community with high-quality, reference genomes for all strains

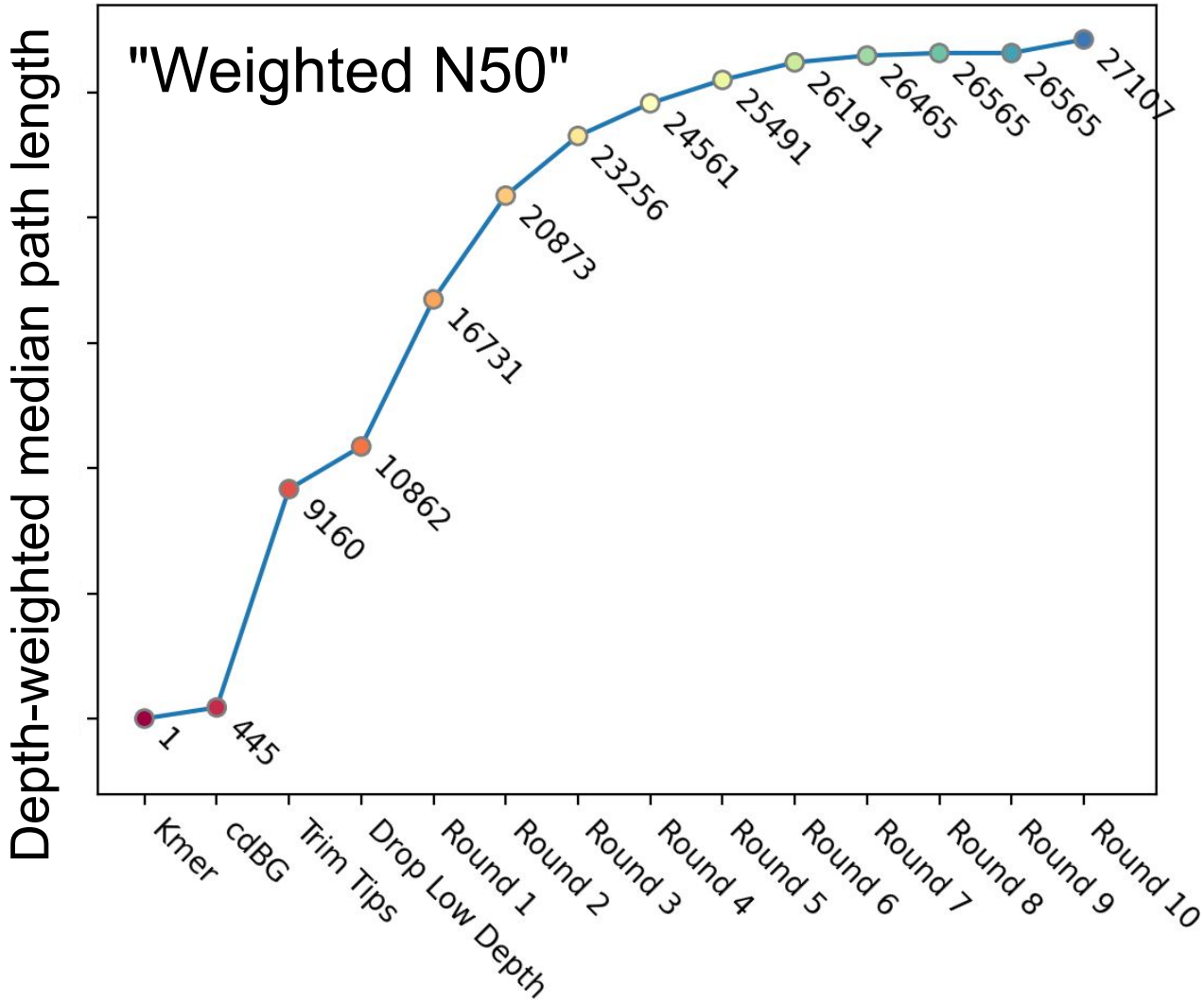





Closely related strains and species result in bubbles and more complex topologies in the assembly graph

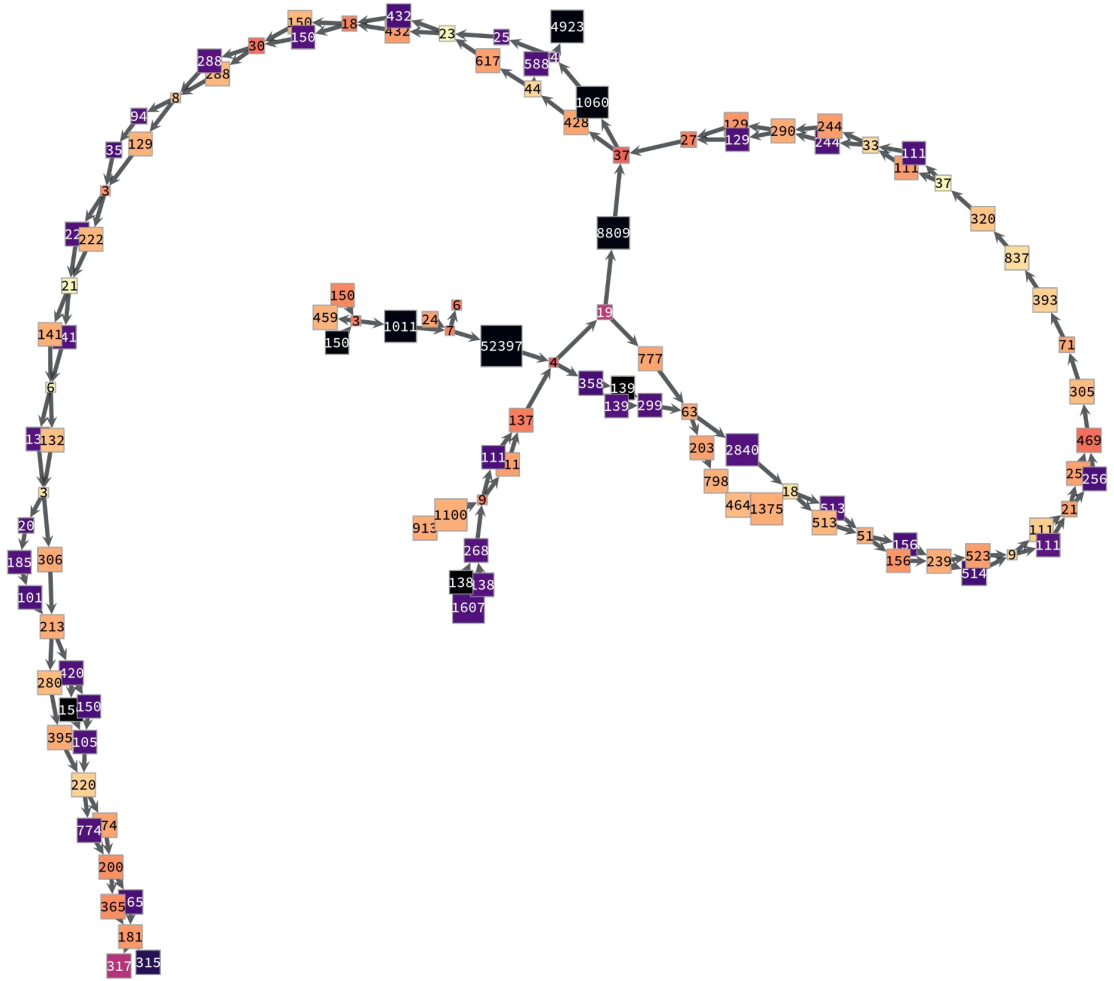


Complex assembly graph results in short path lengths

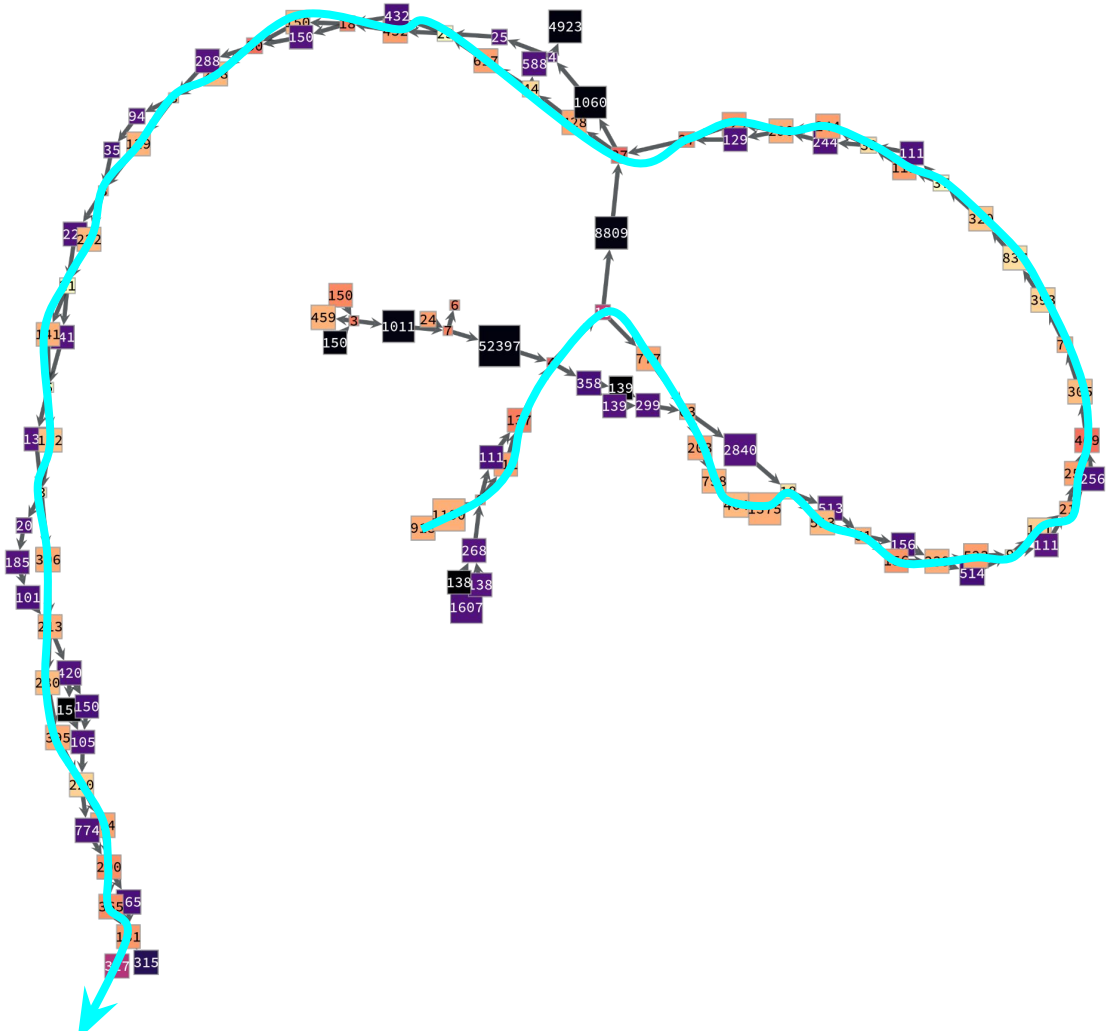


Path lengths increase over successive rounds of deconvolution

Closely related strains are interspersed in the assembly graph

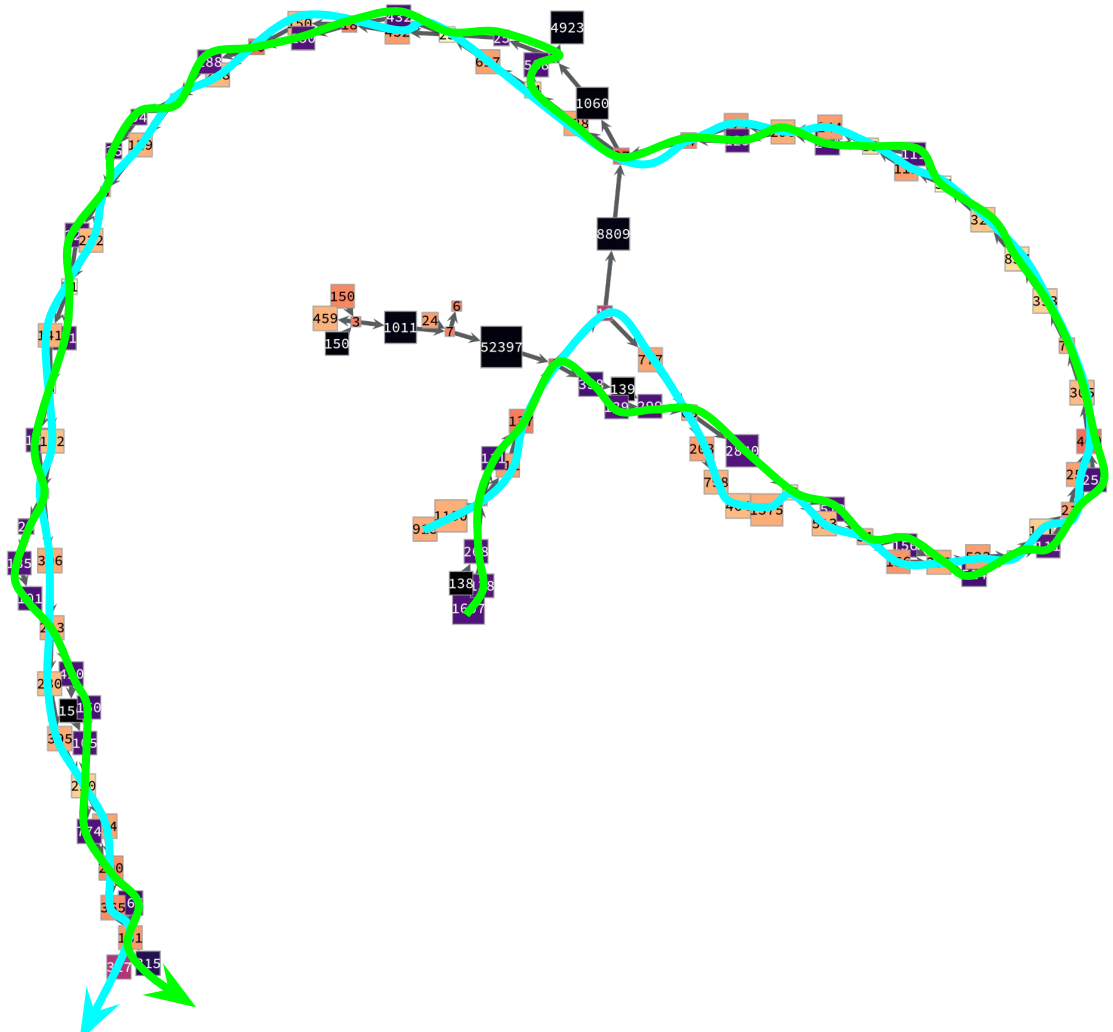


Deconvolution recovers longer, strain-specific sequences



Deconvolution recovers longer, strain-specific sequences

…including lower-abundance strains



Deconvolution recovers longer, strain-specific sequences

…including lower-abundance strains

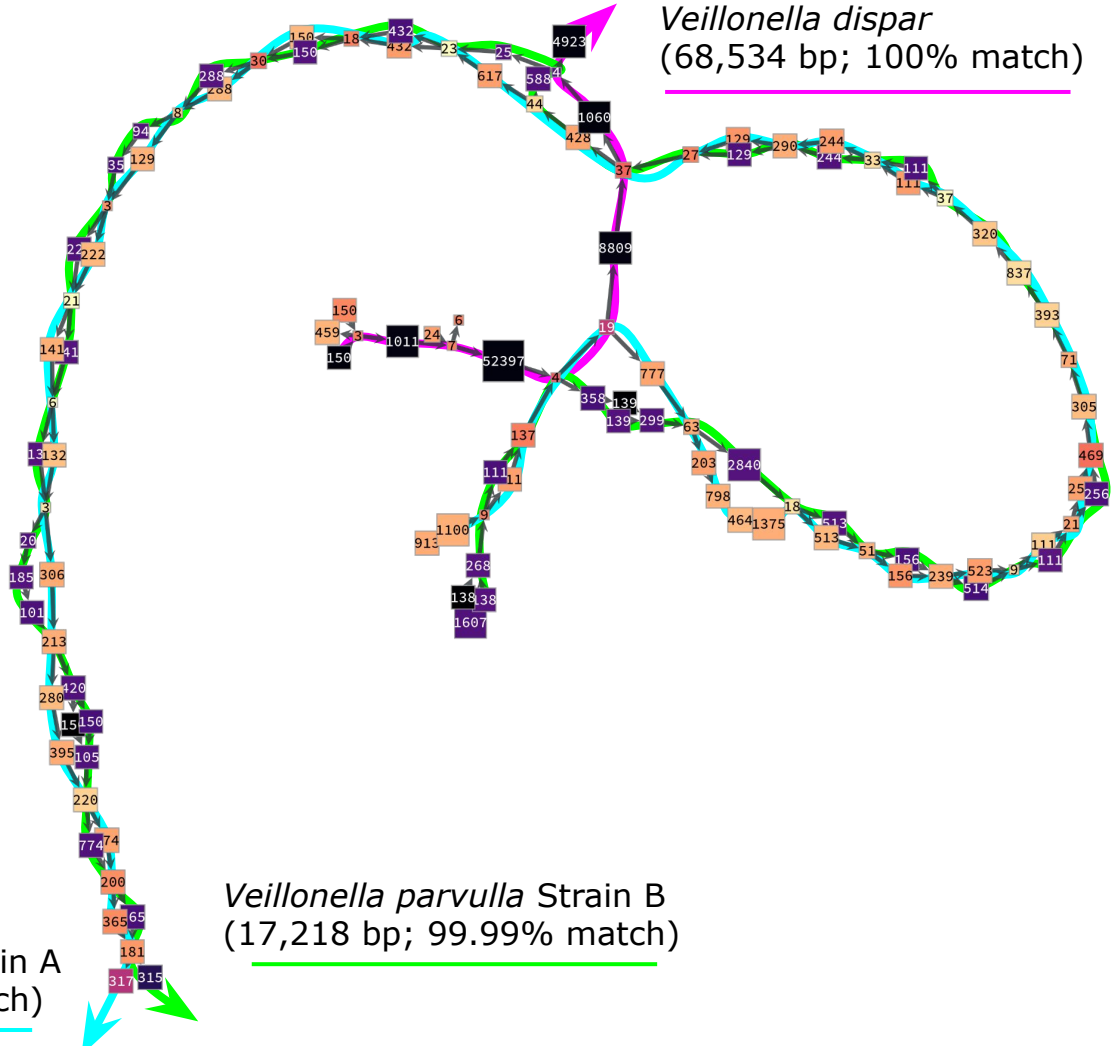
…and species

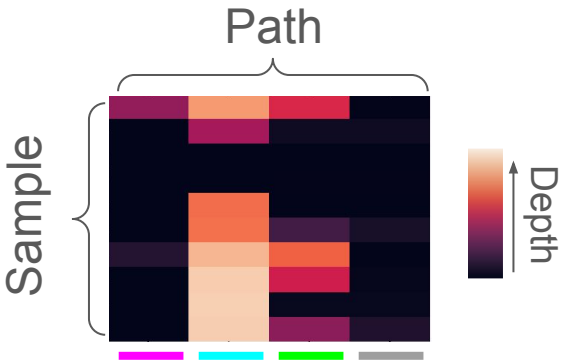
Deconvolution recovers longer, strain-specific sequences

…including lower-abundance strains

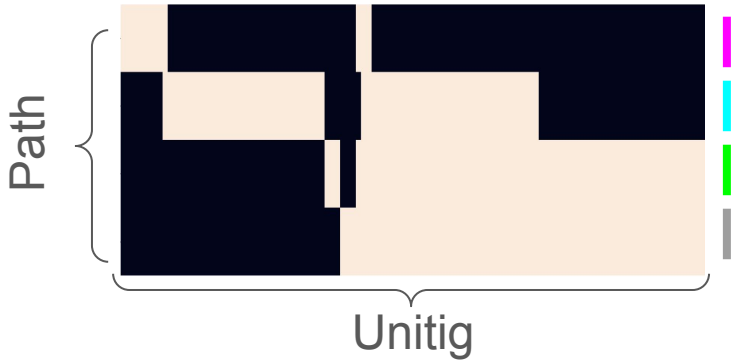
…and species

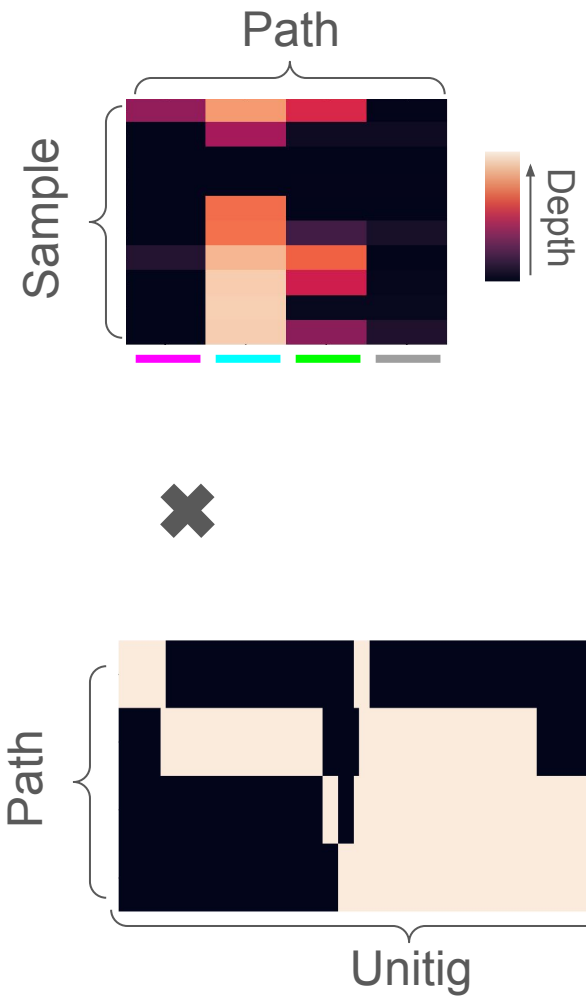
…accurately



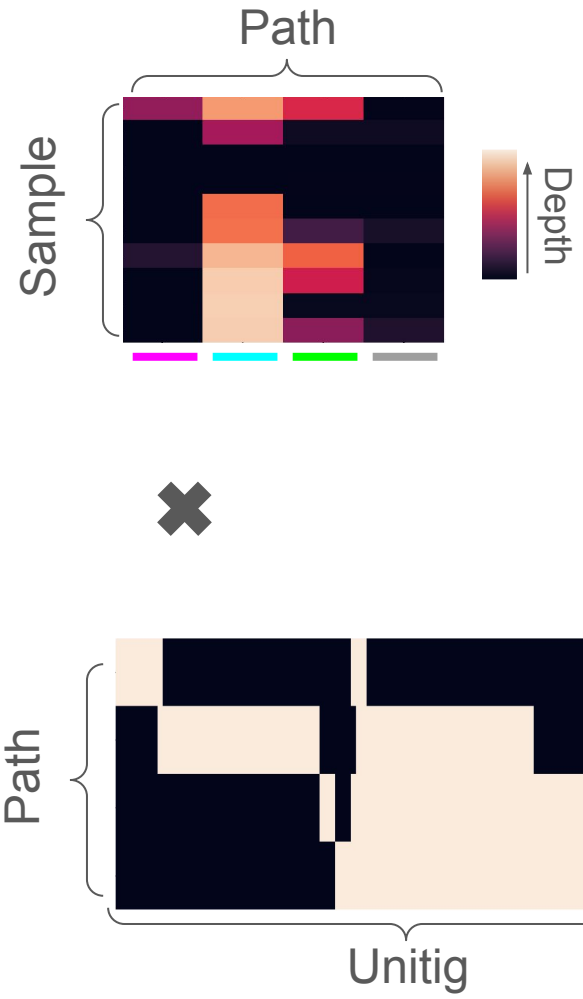


Result: both paths, and path depths across samples (without read mapping)

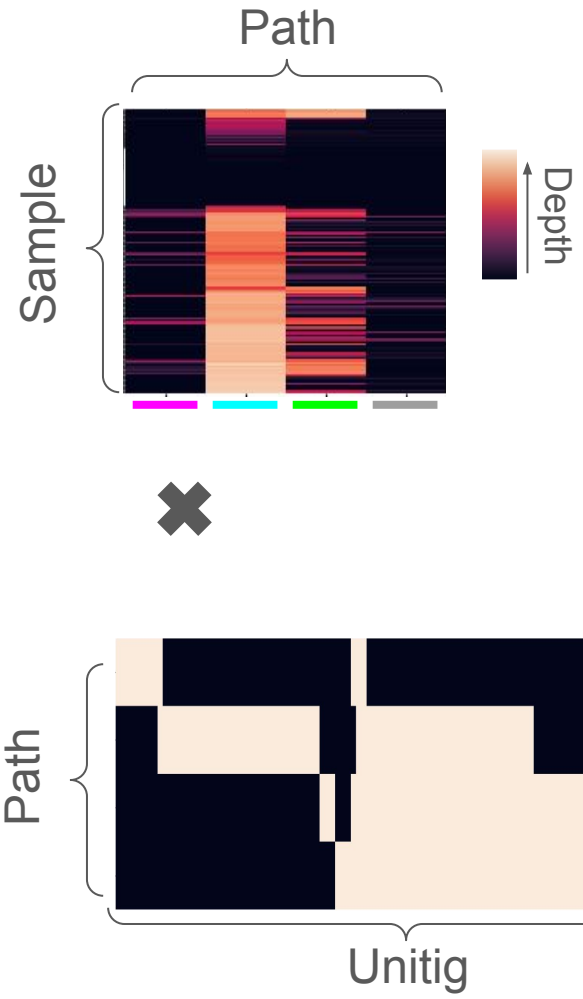




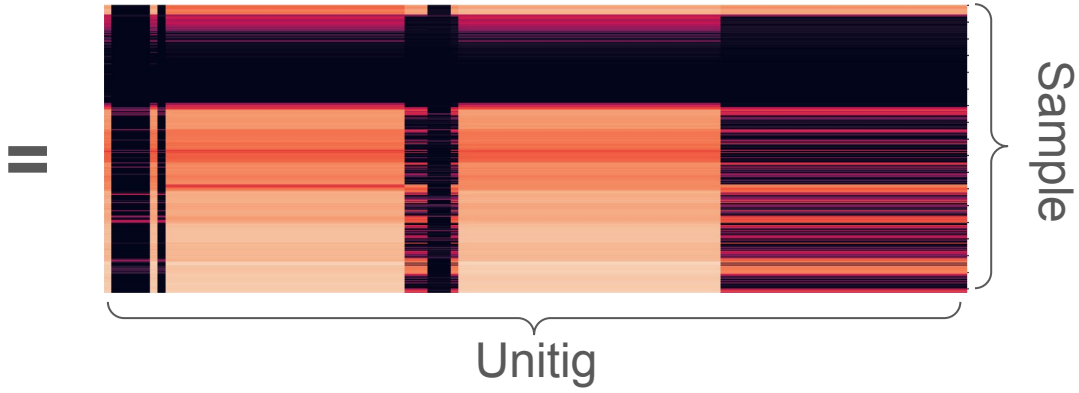
Result: both paths, and path depths across samples (without read mapping)

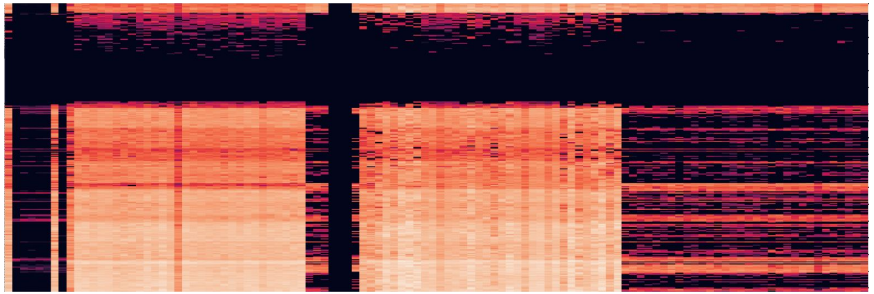


Result: both paths, and path depths across samples (without read mapping)

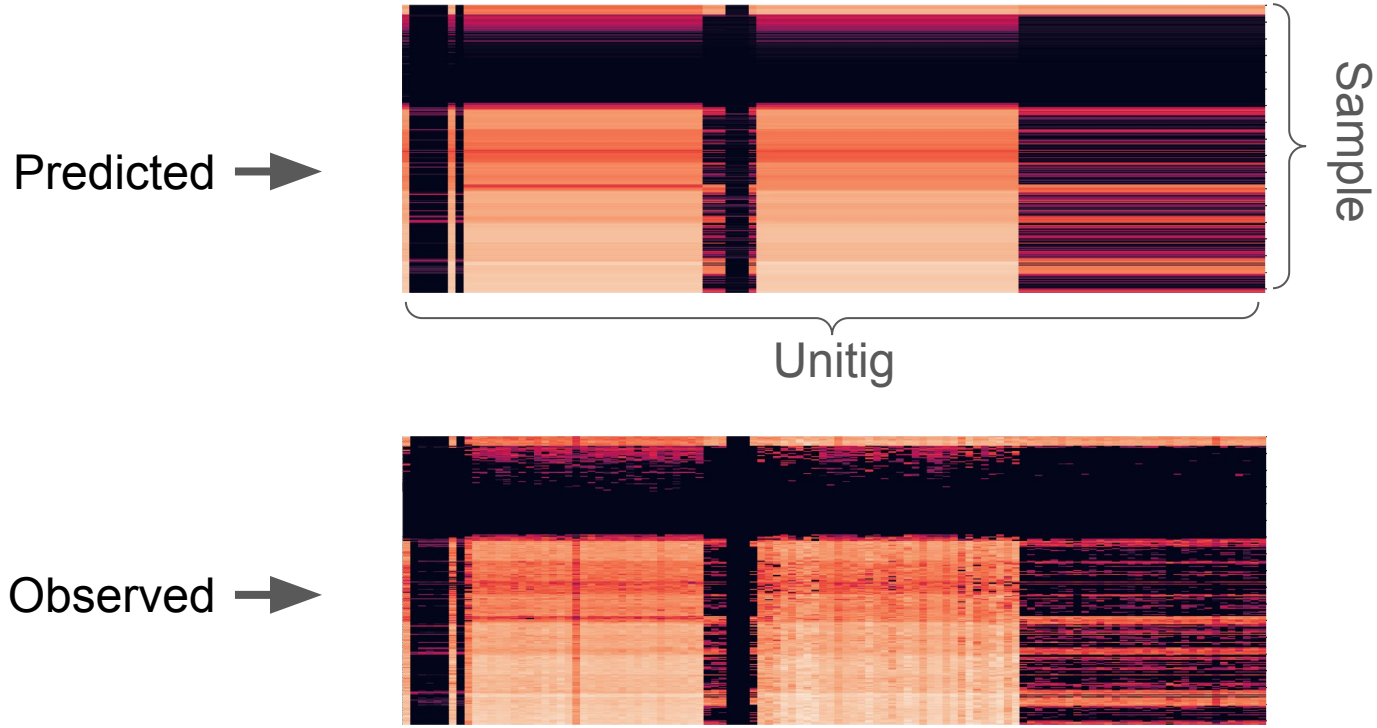


Result: both paths, and path depths across samples (without read mapping)





Estimated unitig depths closely match observed depths



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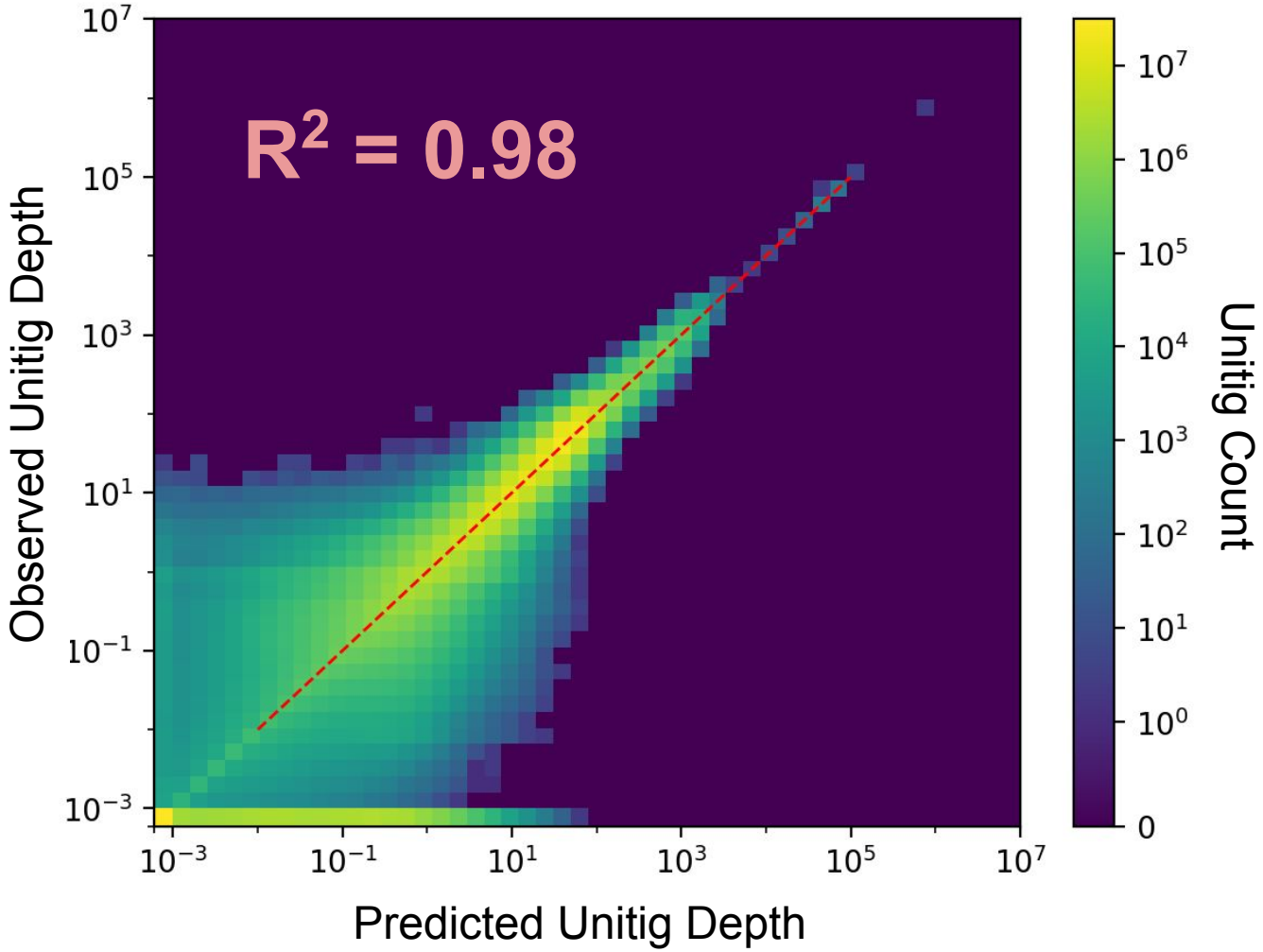
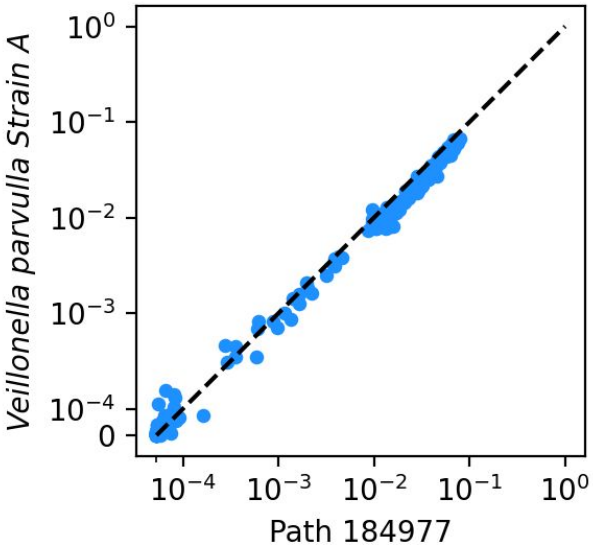
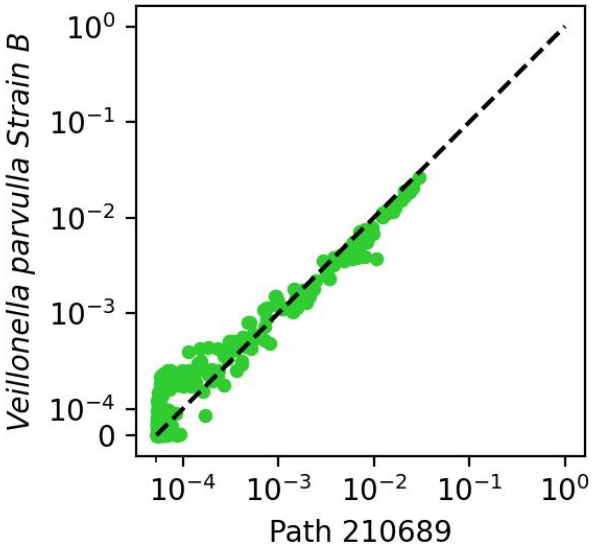
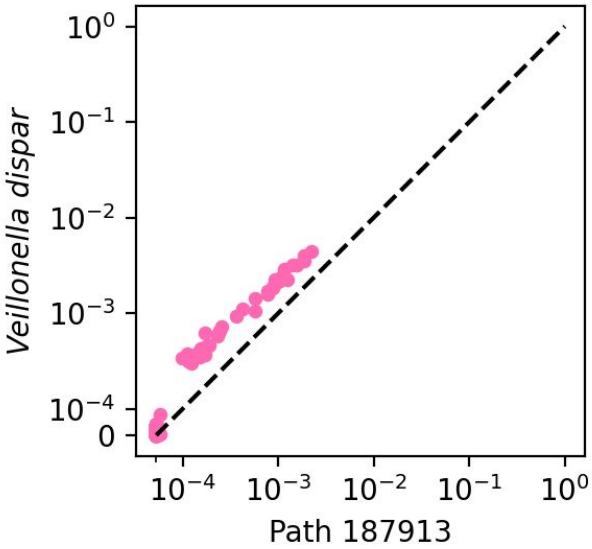


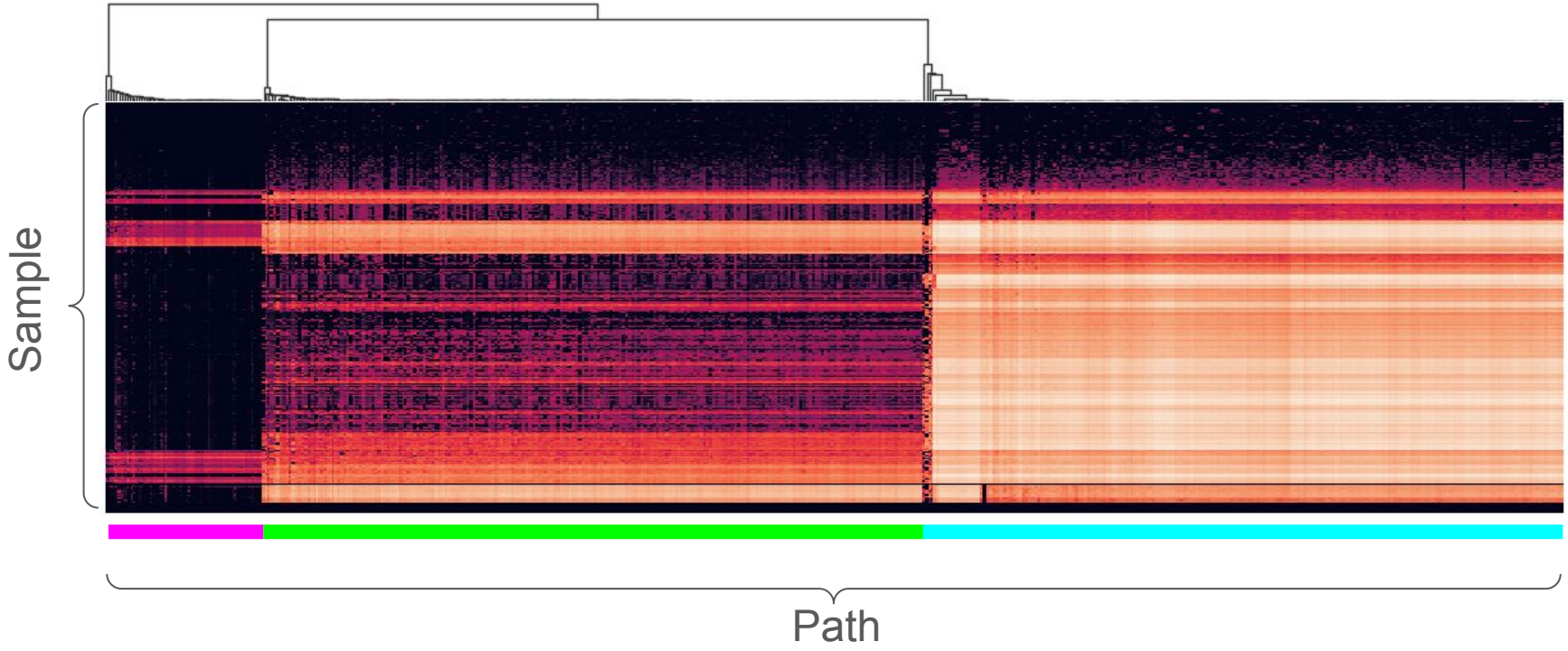
Path depths match reference-based strain depth estimates

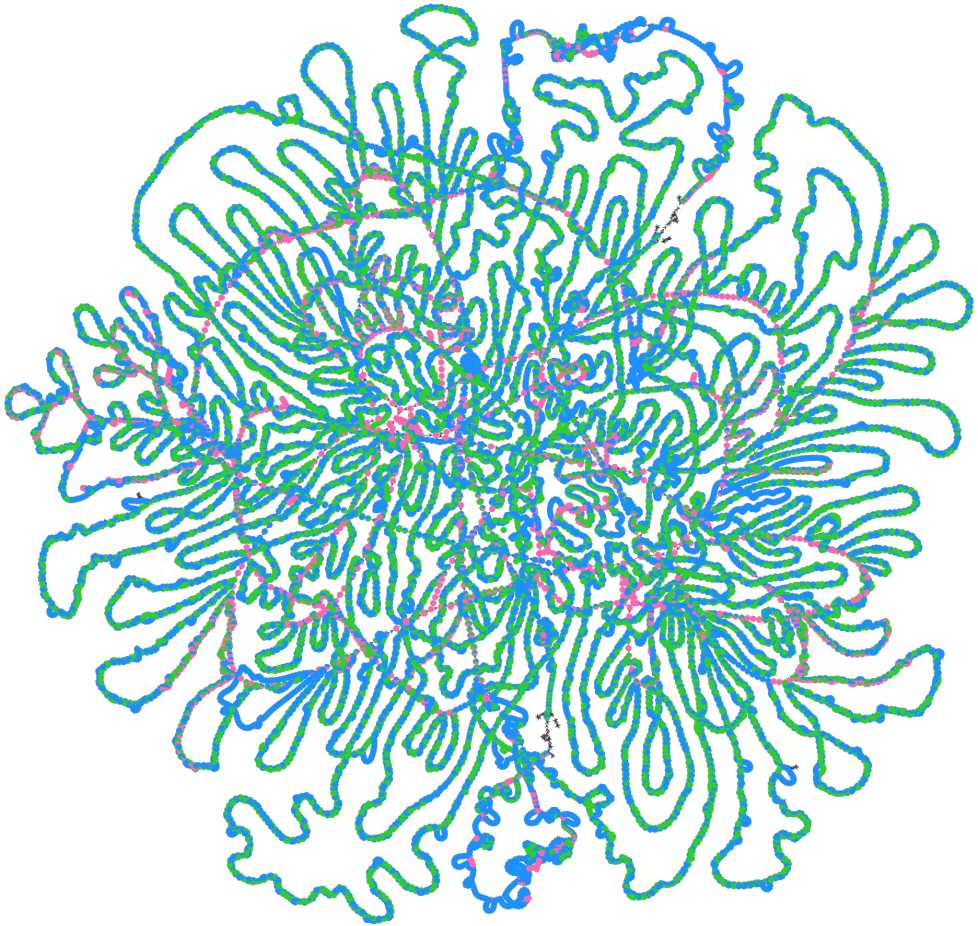






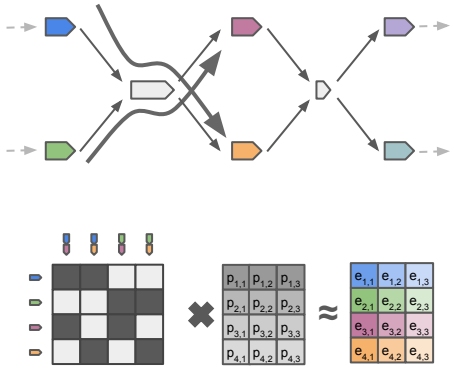
Clustering paths by depth combines multiple sequences from the same strain



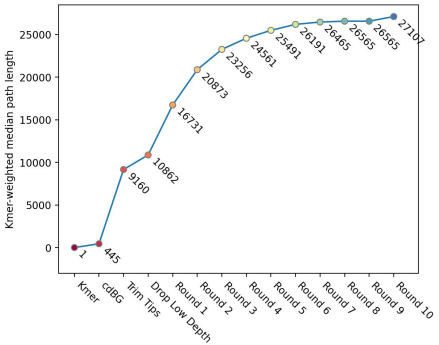


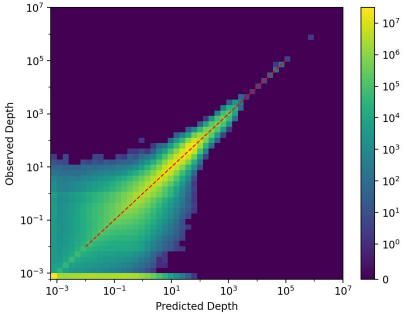
Enables
strain-resolved
genome assembly
from metagenomes

Iterative Junction Deconvolution

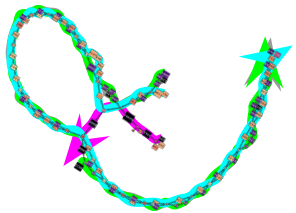


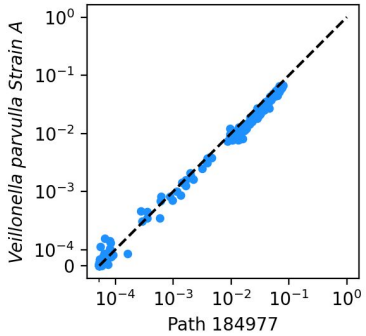
Combines Assembly, Depth Estimation



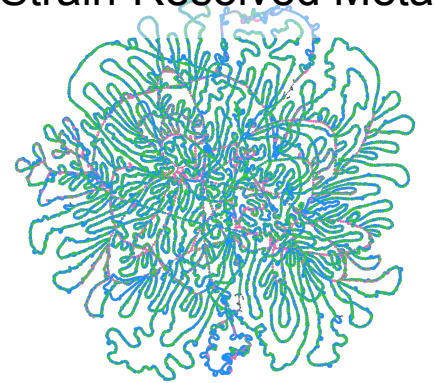


Recovers Closely Related Genomes

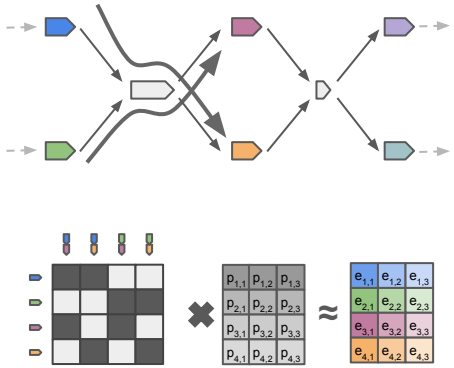




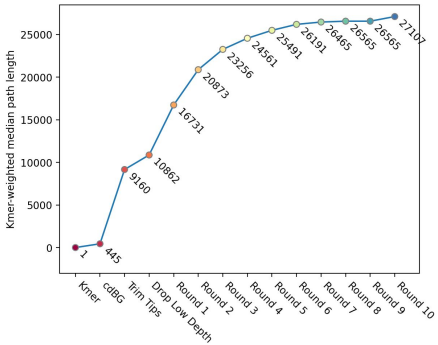
Enables Strain-Resolved Metagenomics

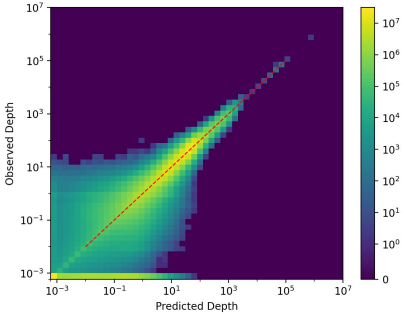


Iterative Junction Deconvolution

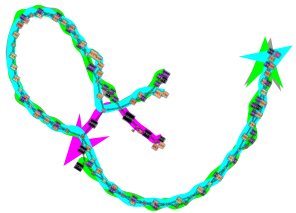


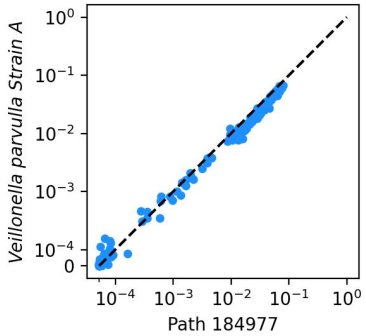
Combines Assembly, Depth Estimation



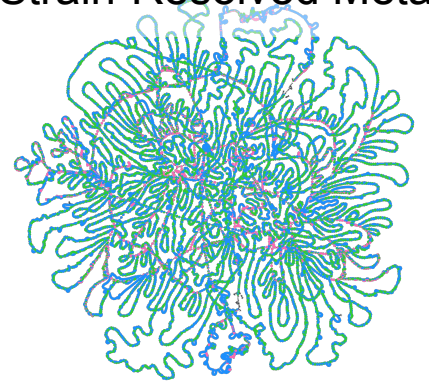


Recovers Closely Related Genomes



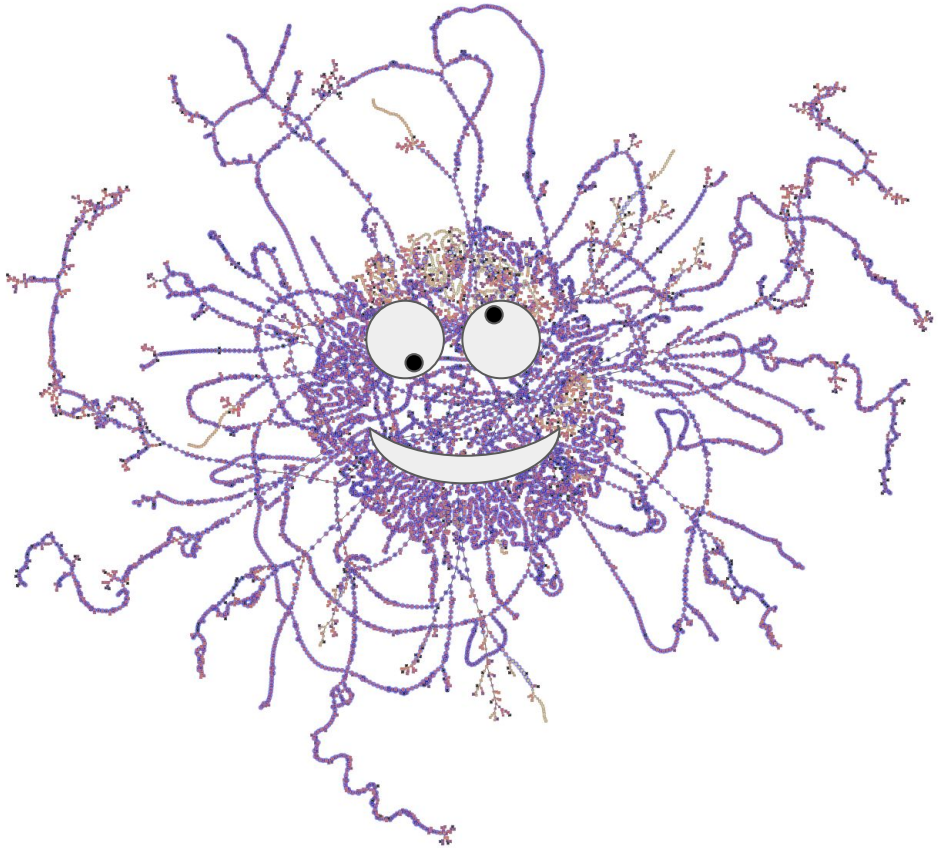


Enables Strain-Resolved Metagenomics



TODO: Add QR Code to PDF

Thank You!



Pocket Slides