Gladstone Scientific Retreat 2024

Unzipping the metagenome:

strain-level discovery in the gut microbiome

Byron J. Smith

Bioinformatics Fellow

First Thing: Thank You!

Pollard Lab

Katie Pollard
Veronika Dubinkina
and *everyone*

Collaborators

Archit Verma
Dylan Cable

Funders

Gladstone Institutes
NIH
CZ Biohub
UC Noyce Initiative
Helmsley Charitable Trust

CHAN ZUCKERBERG BIOHUB

NIH National Institutes of Health

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Gladstone Scientific Retreat 2024

Introduction:

The gut microbiome and shotgun metagenomics

The Gut Microbiome is Challenging

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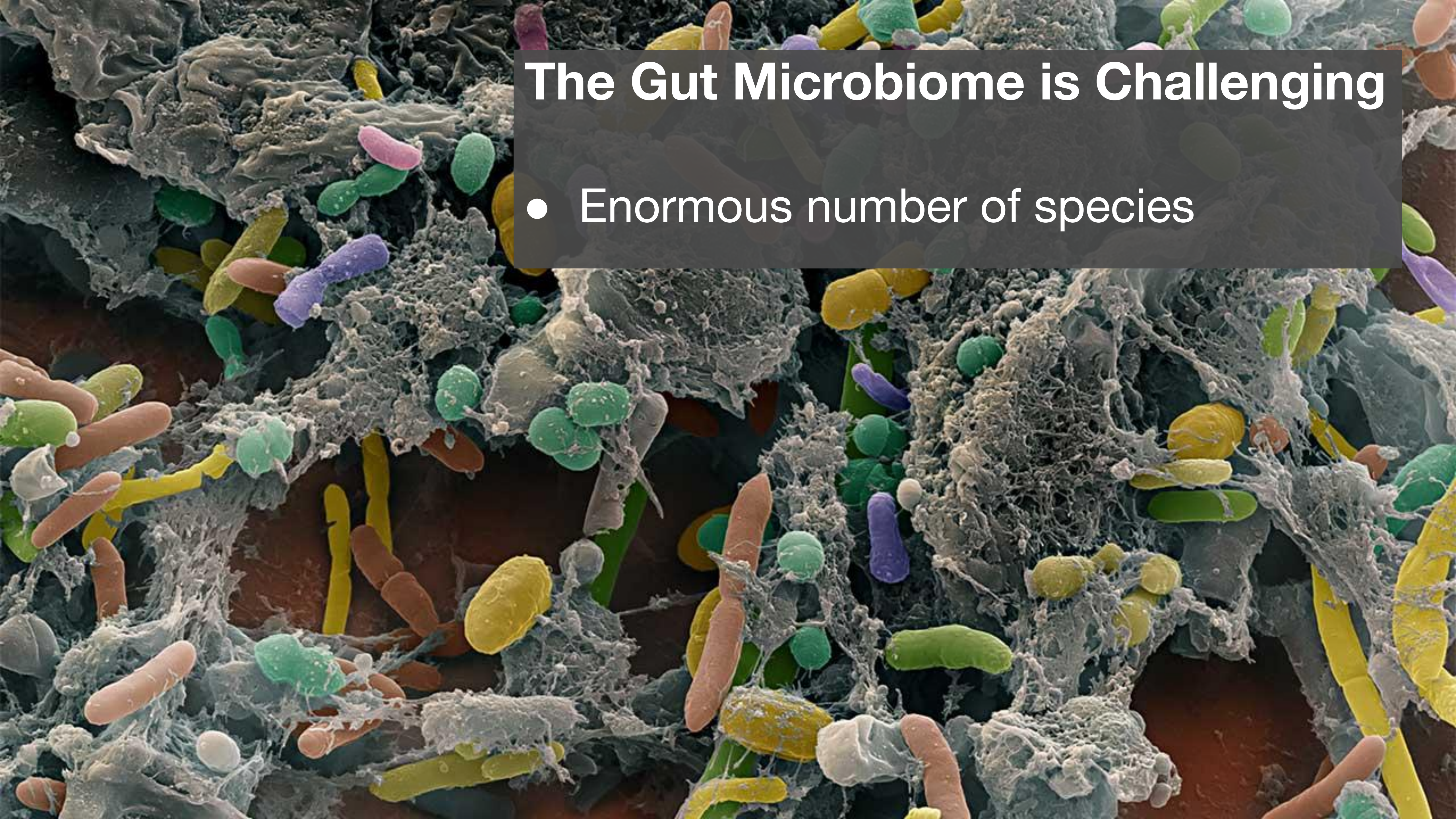
- Enormous number of species



The Gut Microbiome is Challenging

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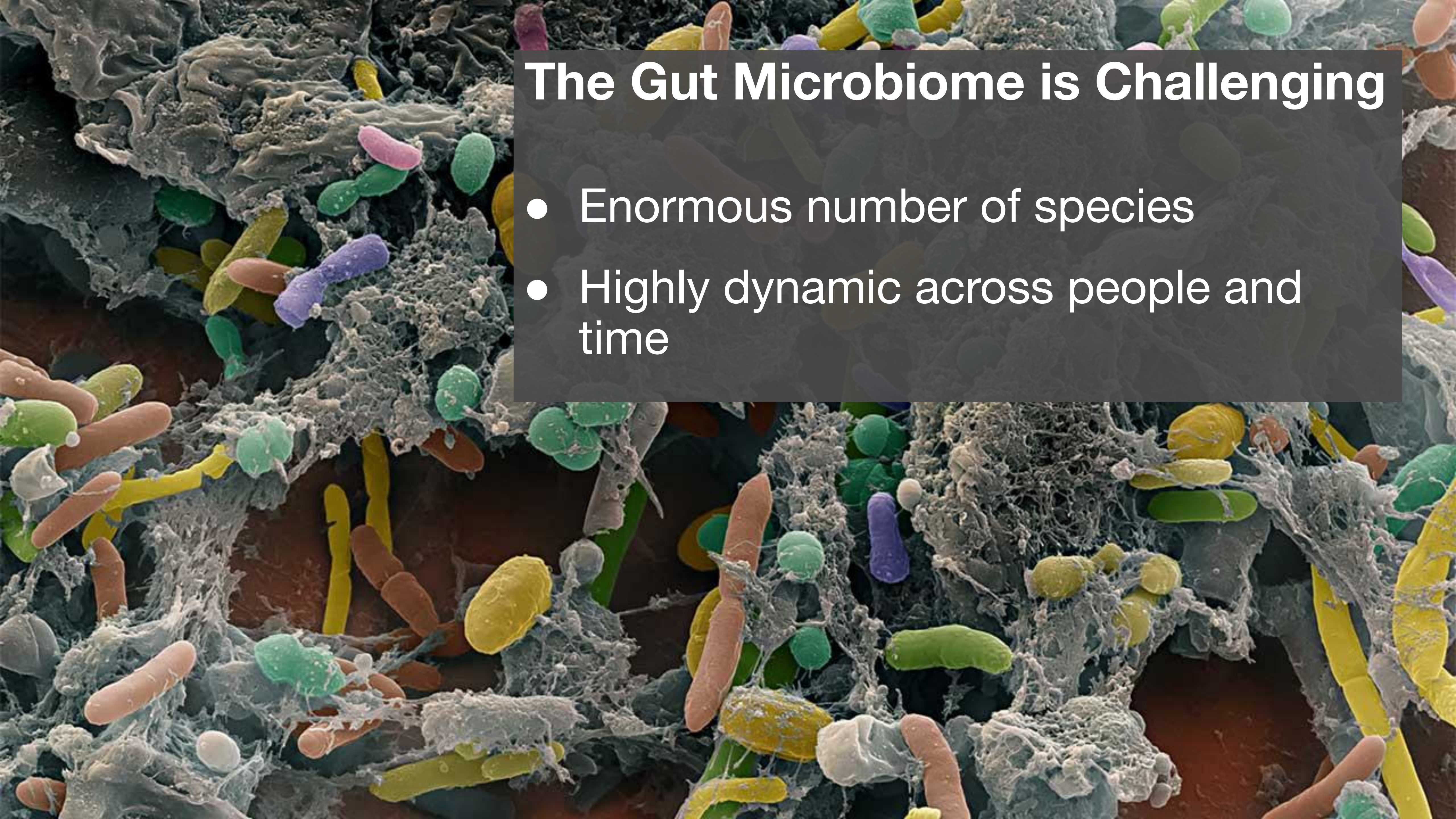

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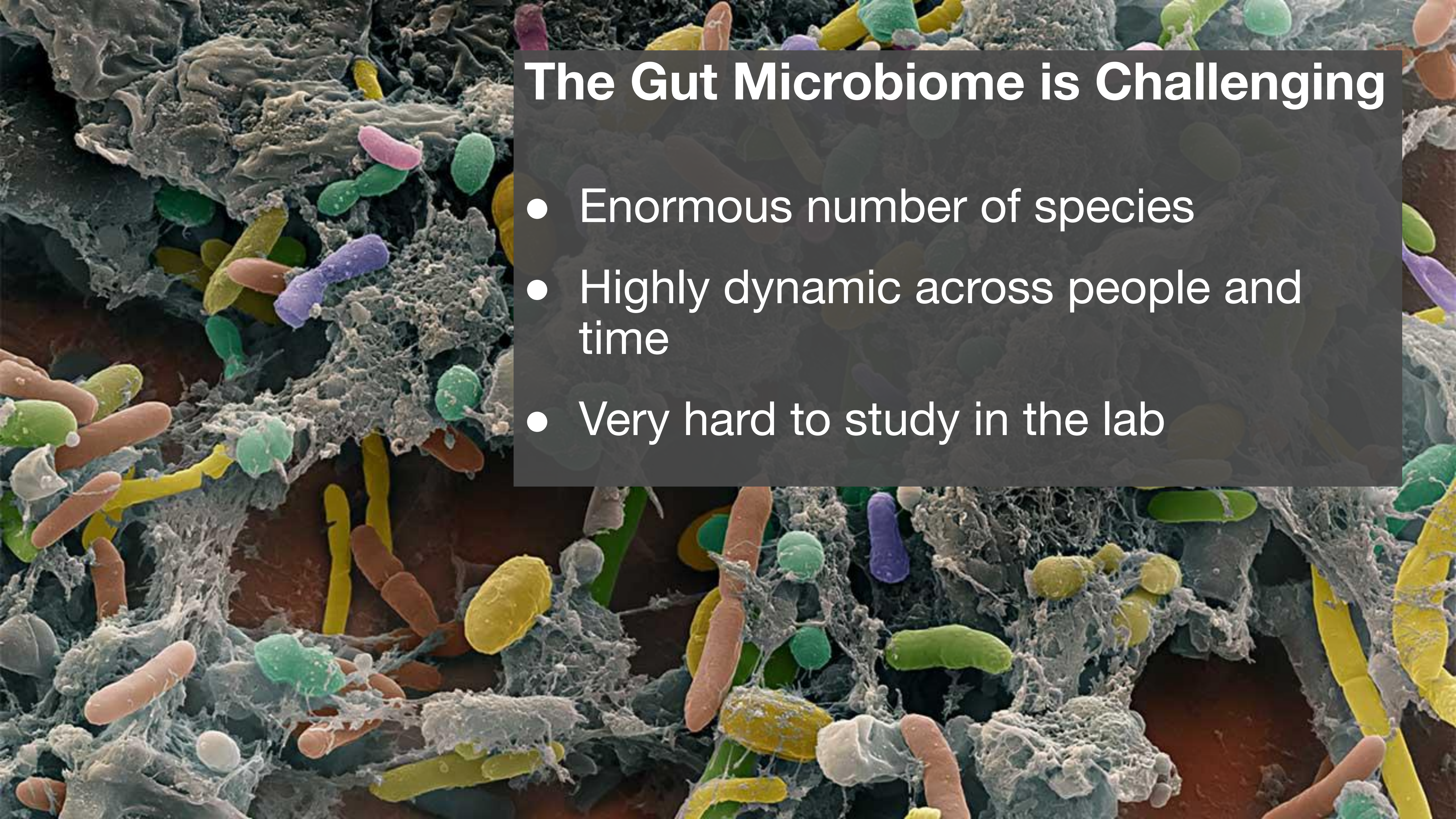

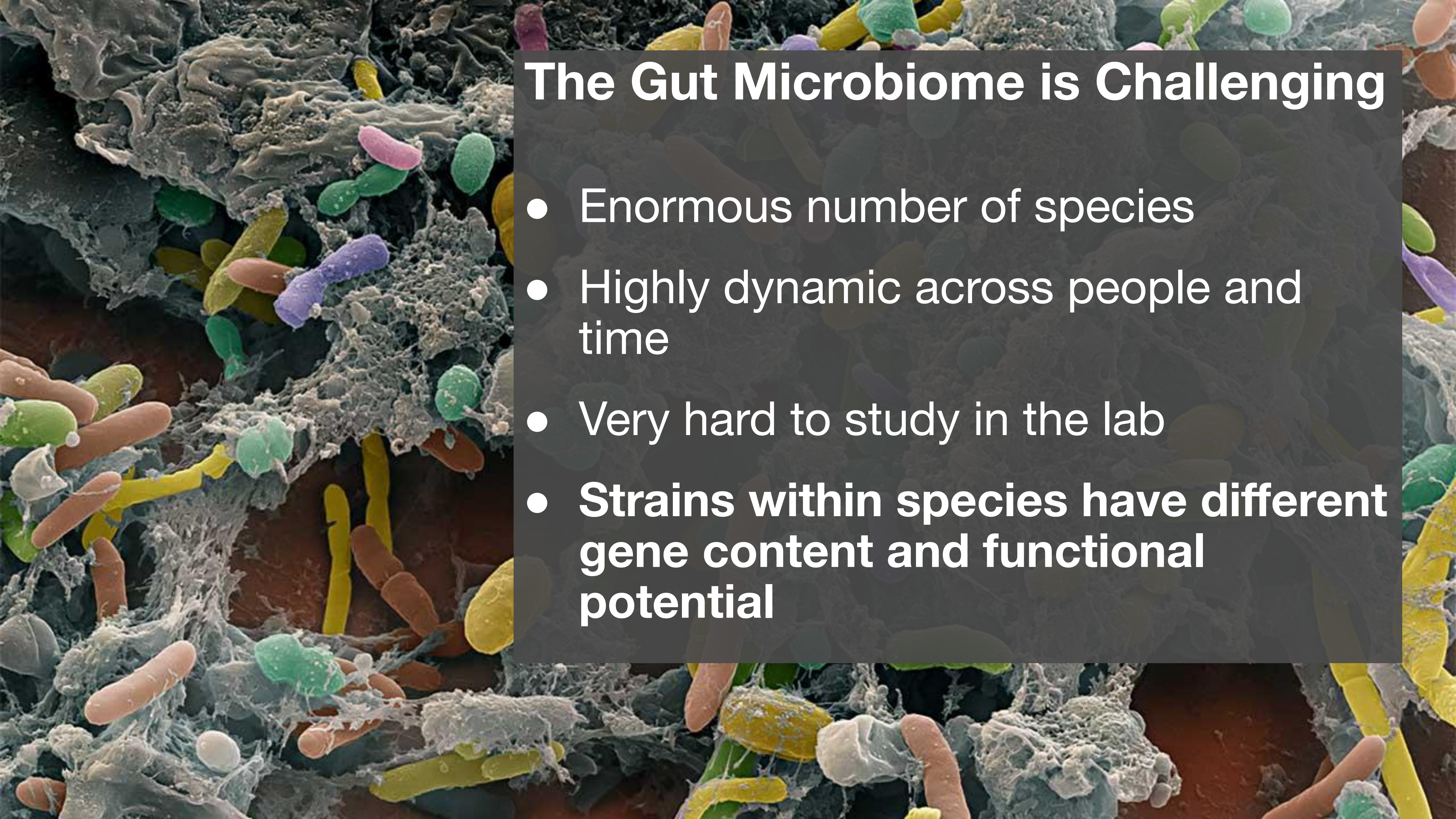

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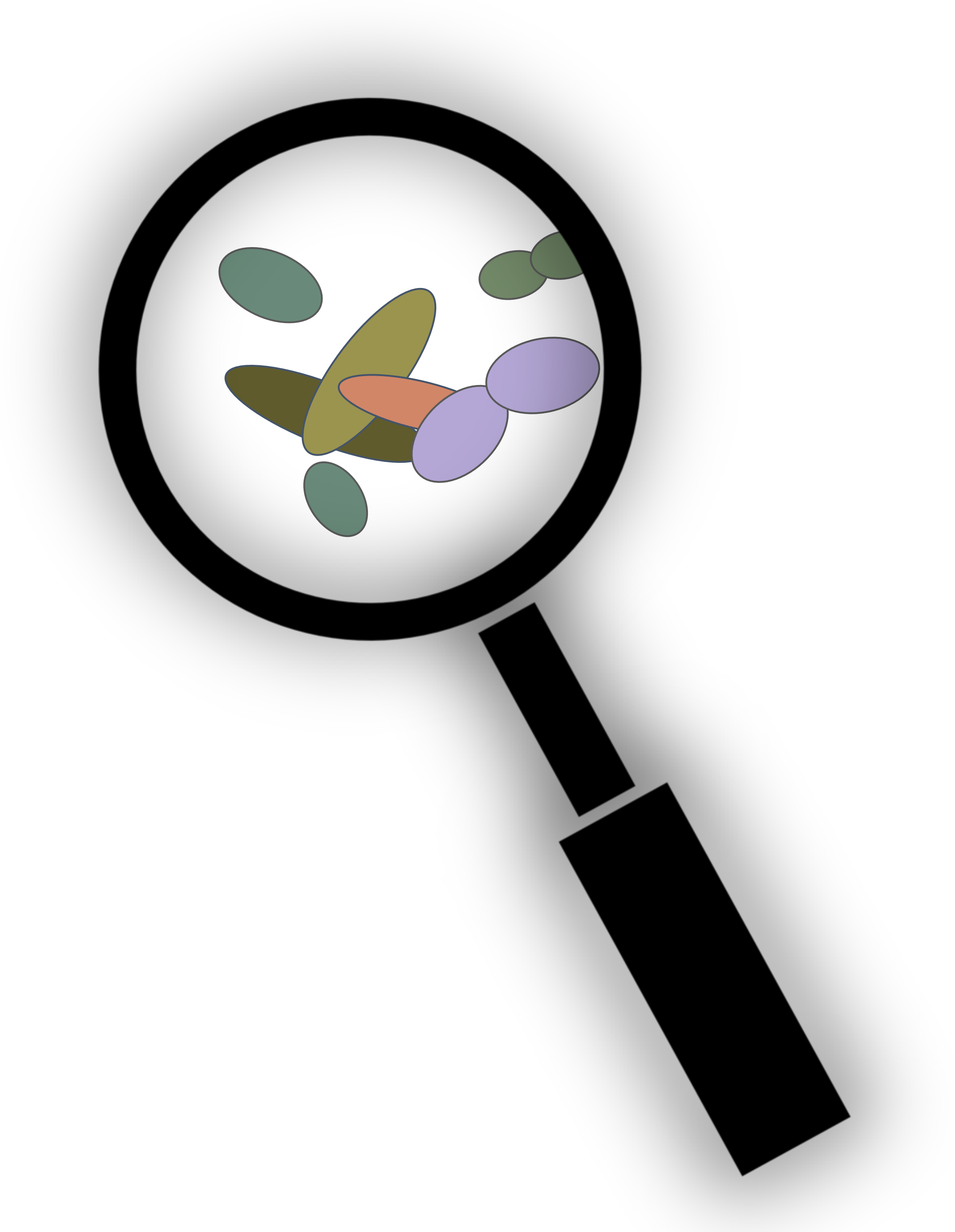
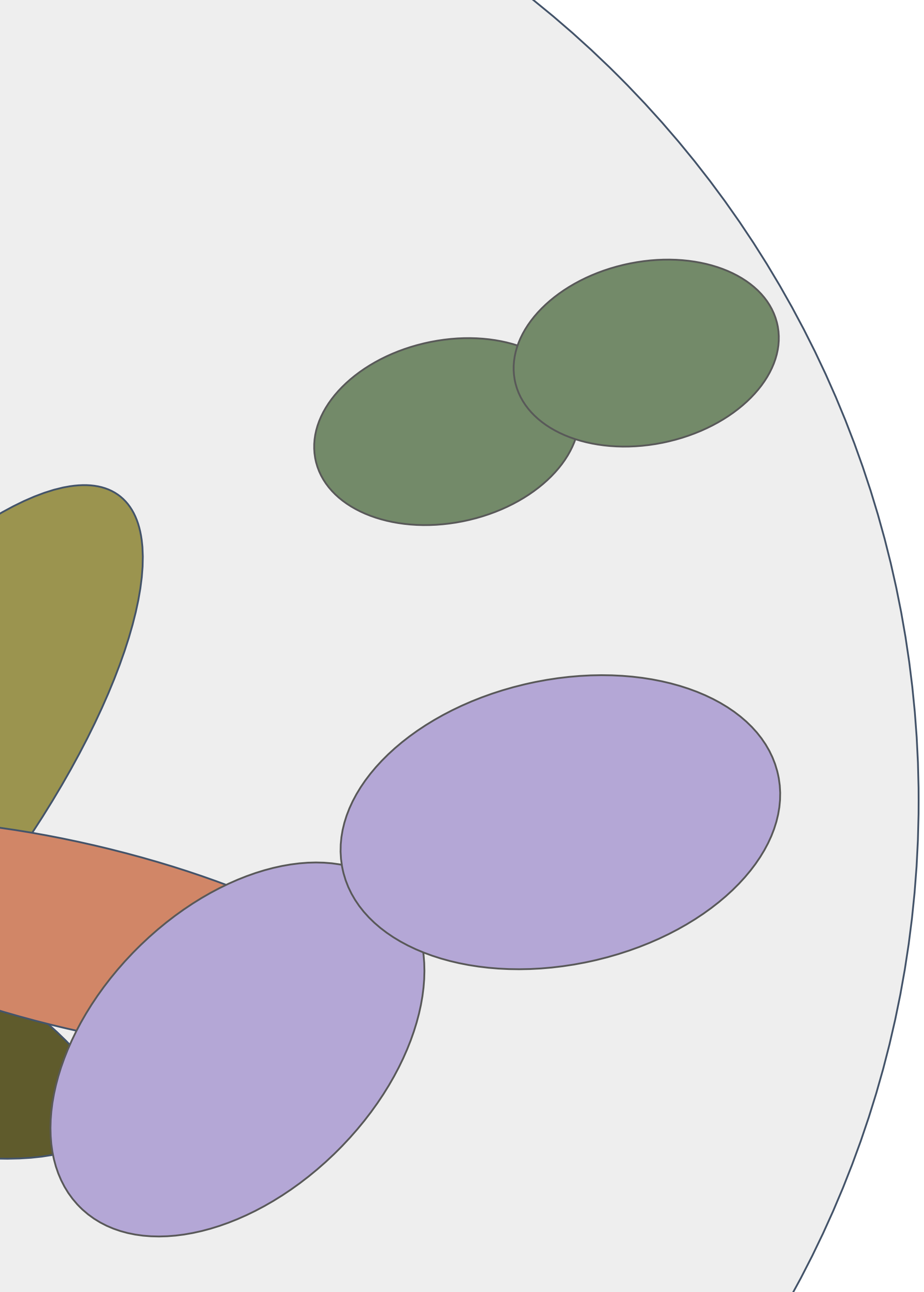


Bacterial genomes are key to understanding strain diversity



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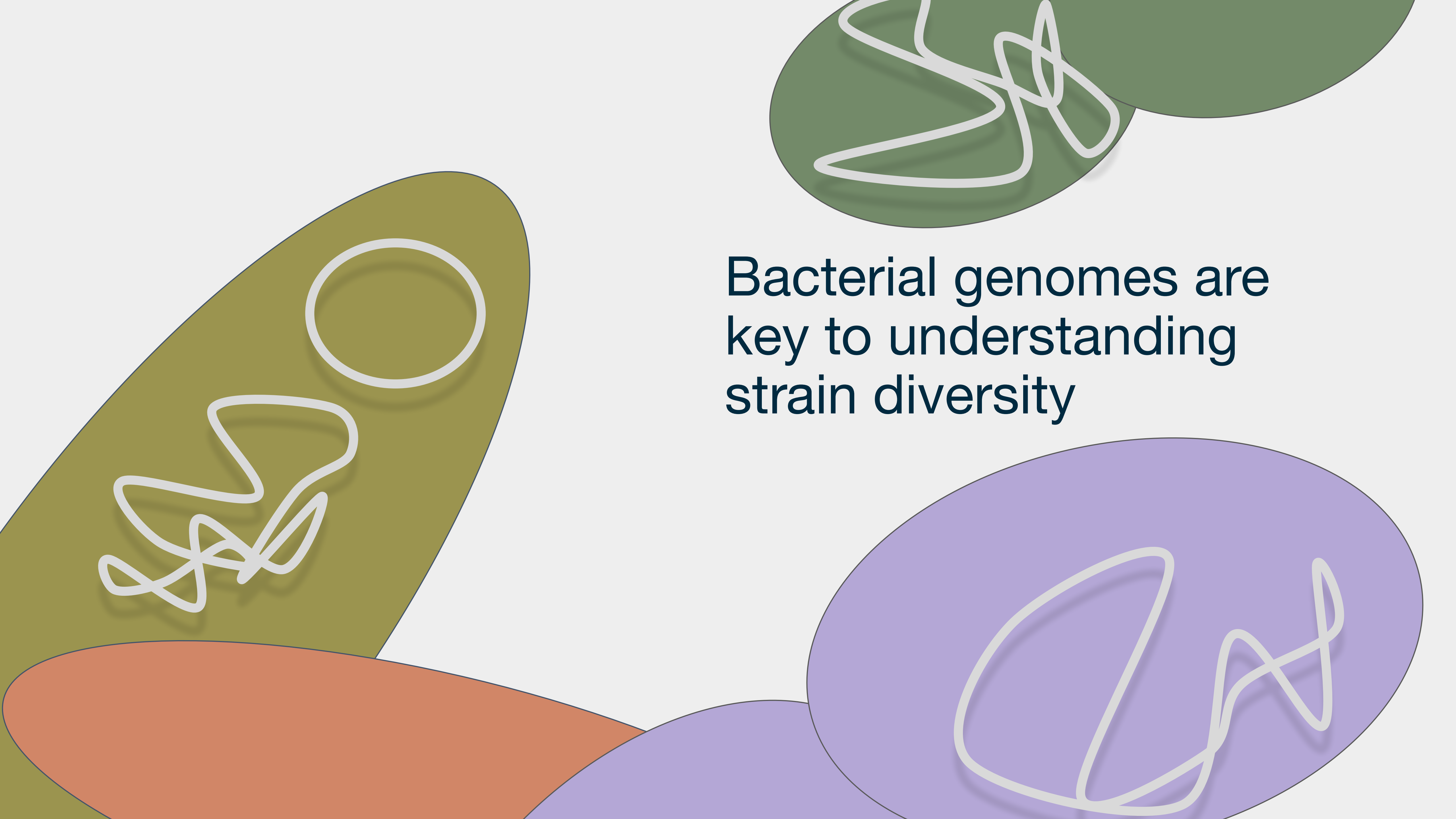

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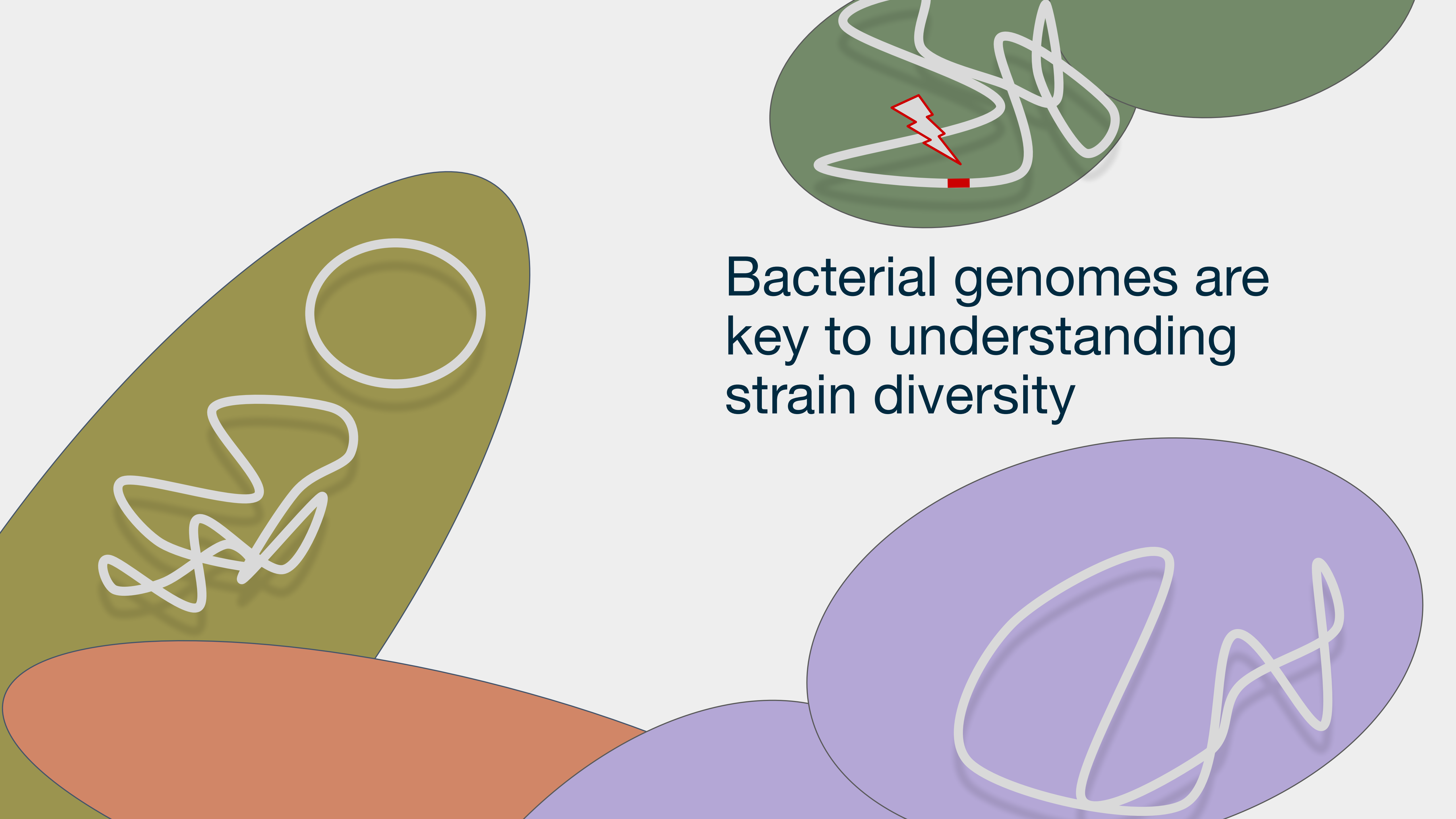

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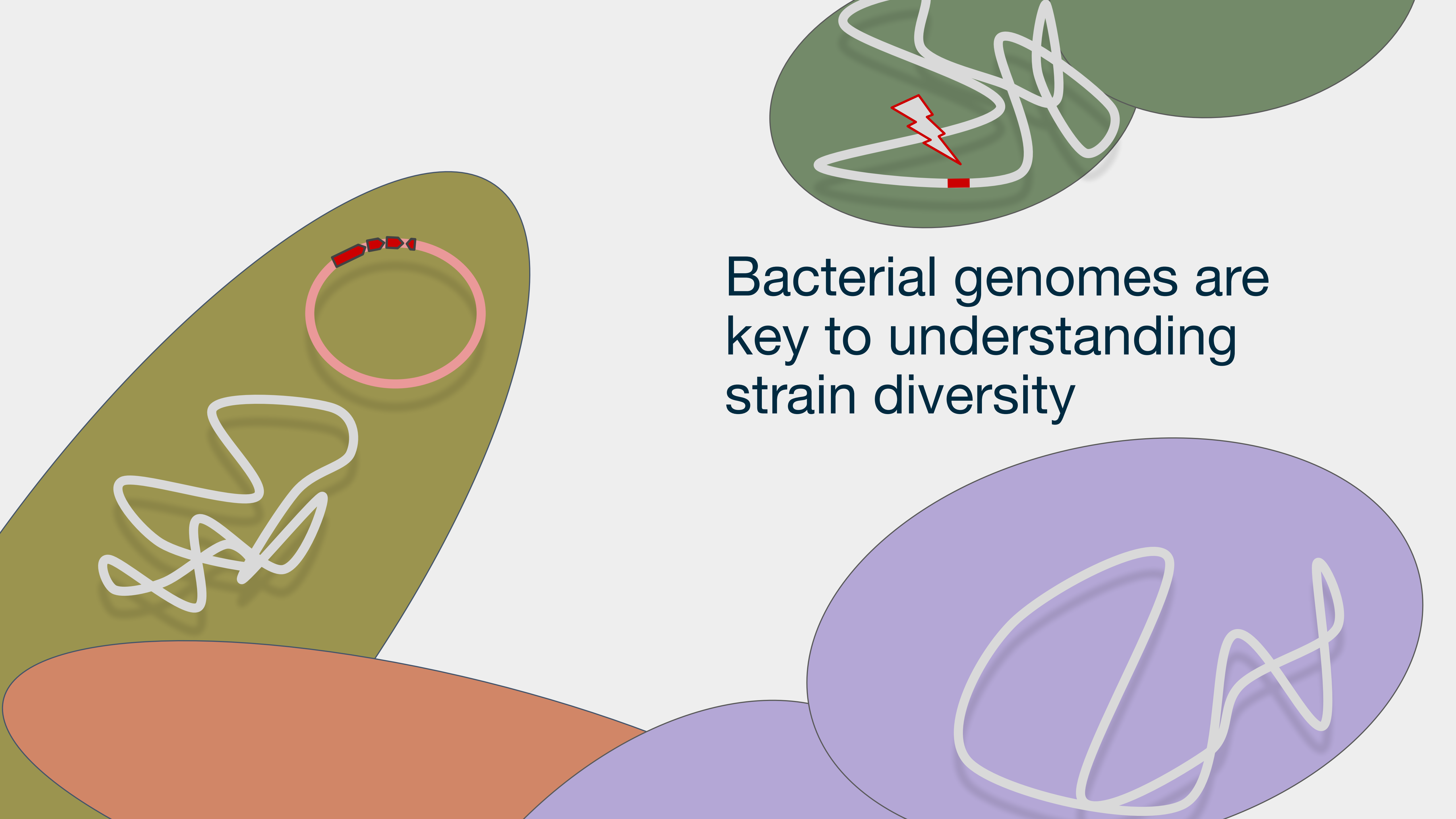

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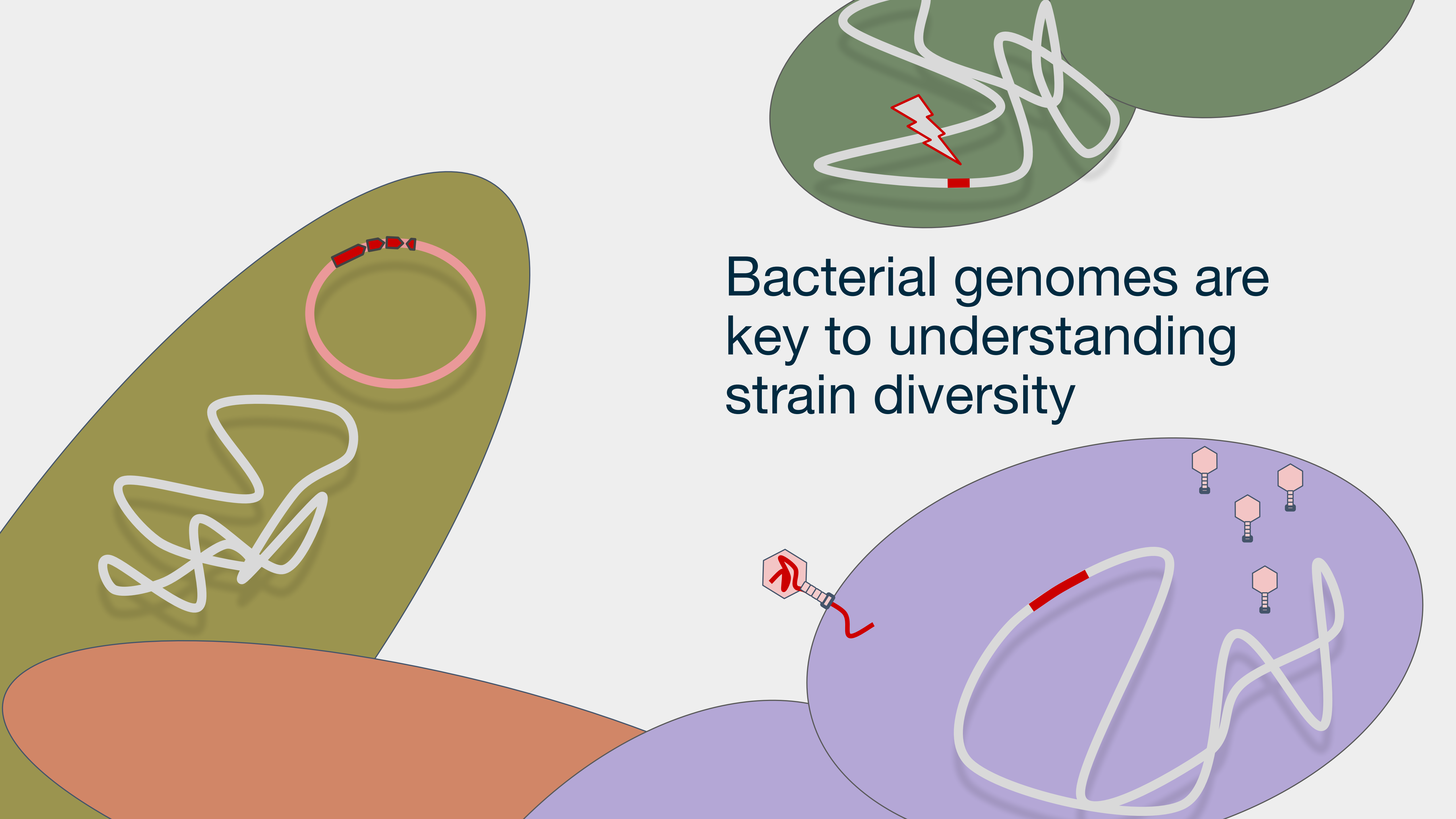
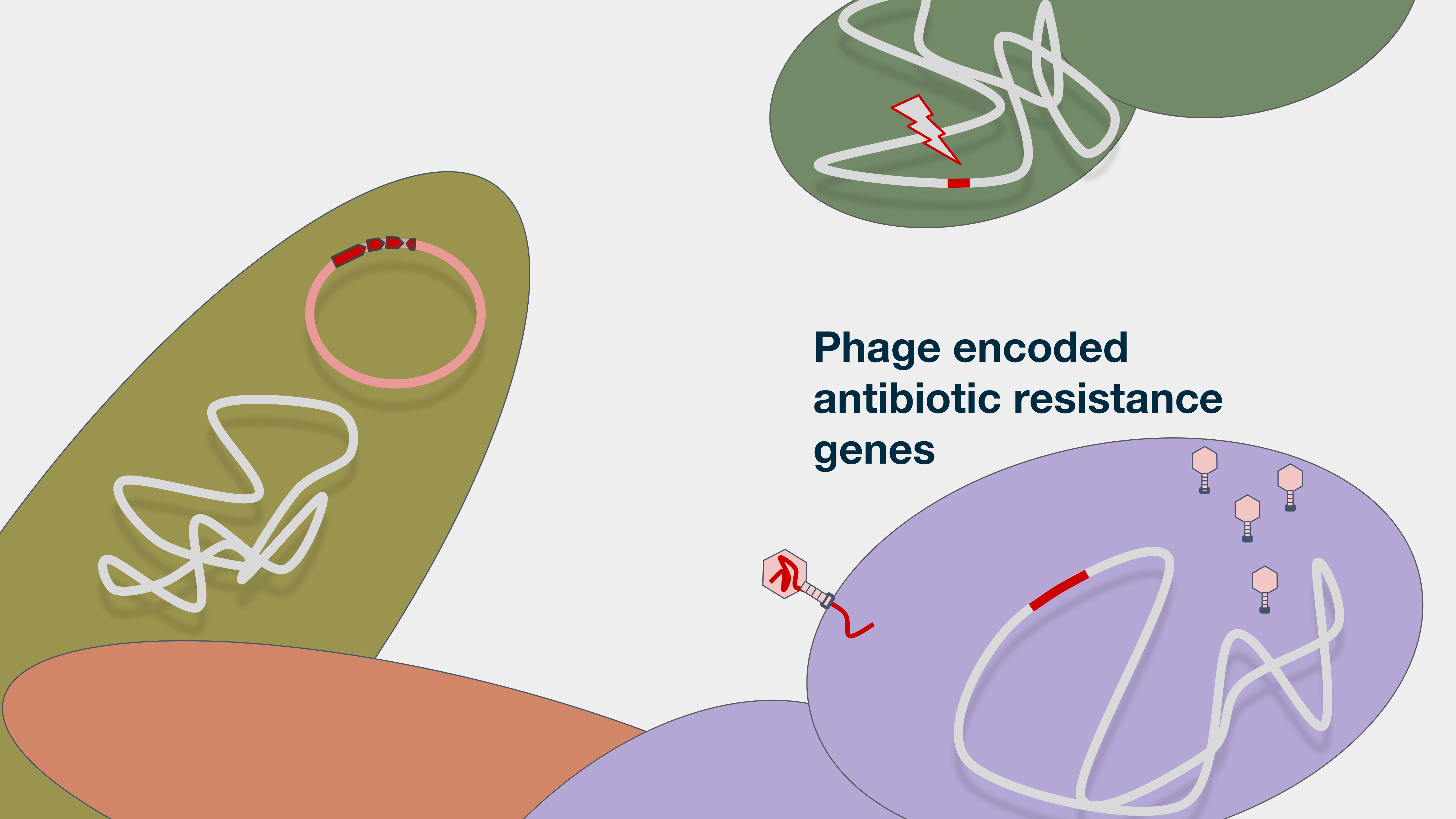
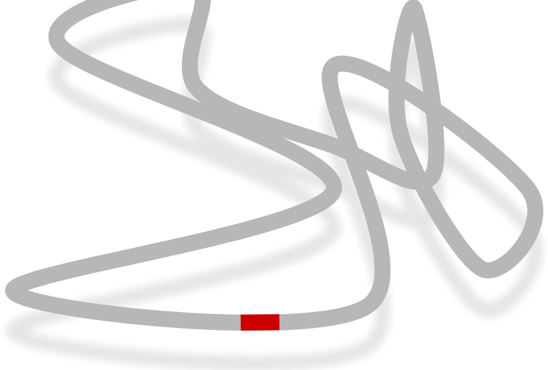


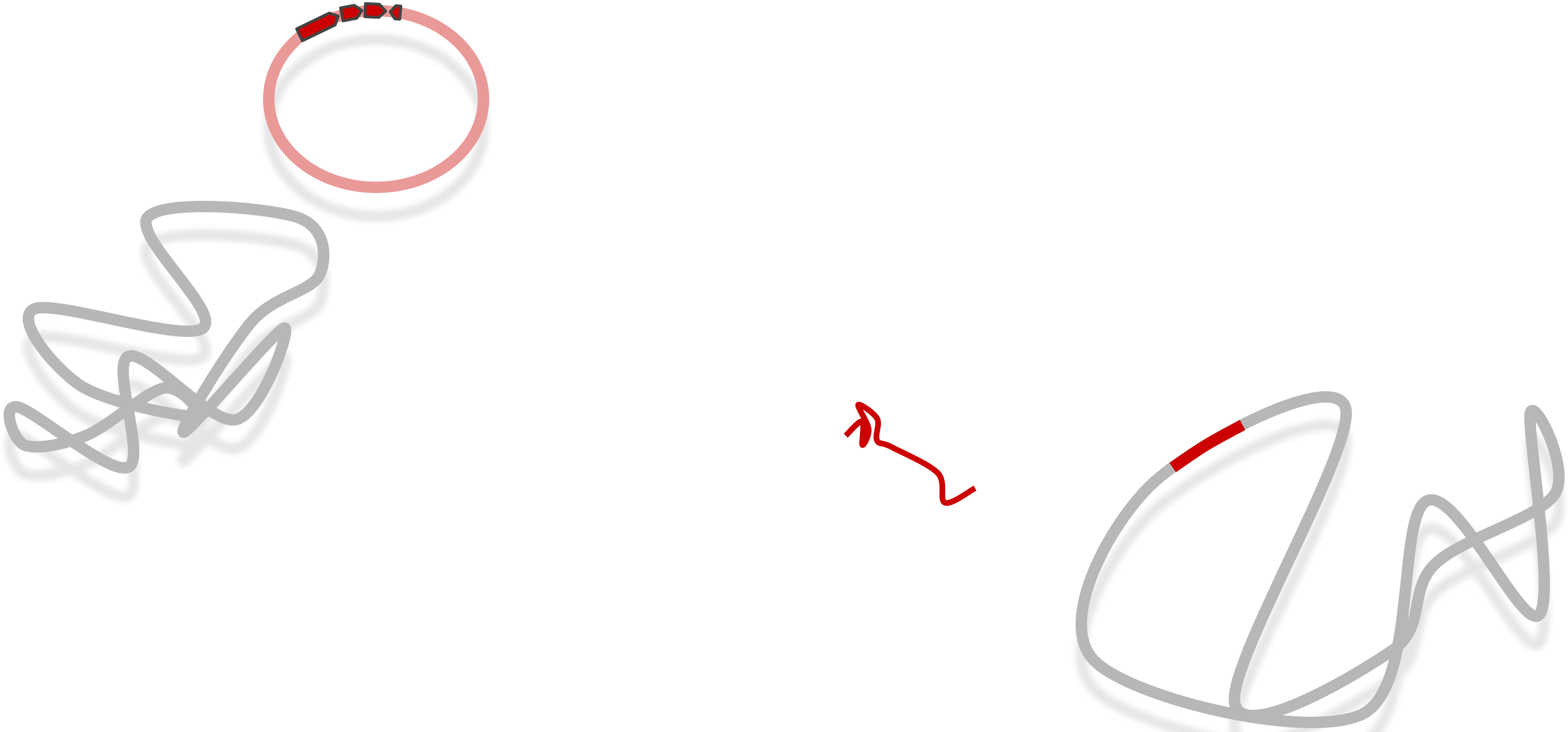
Bacterial genomes are key to understanding strain diversity

Phage encoded antibiotic resistance genes

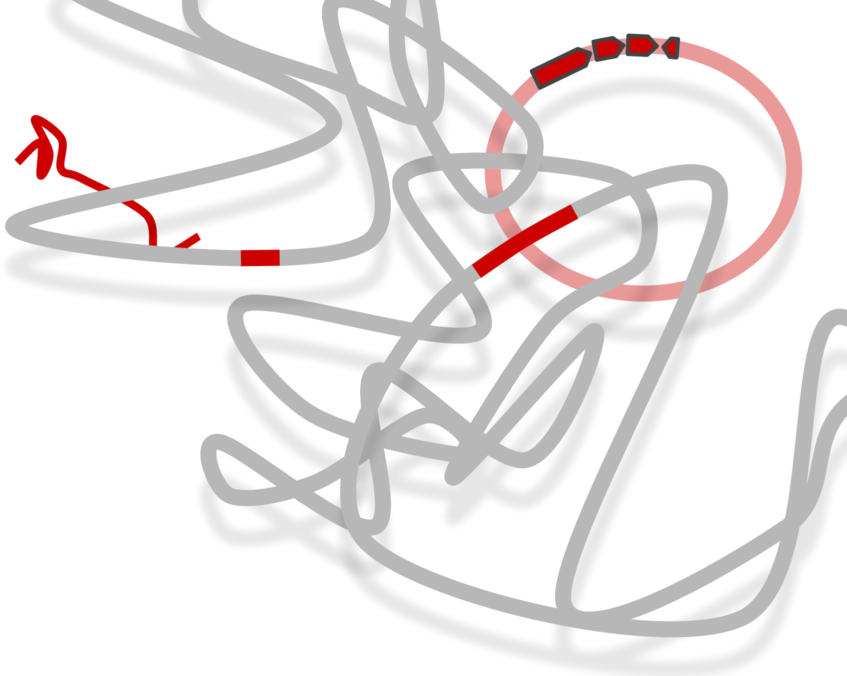


Metagenomic sequencing surveys all genomes





Short-read, shotgun metagenomes enable modern microbiome science



Requirements:

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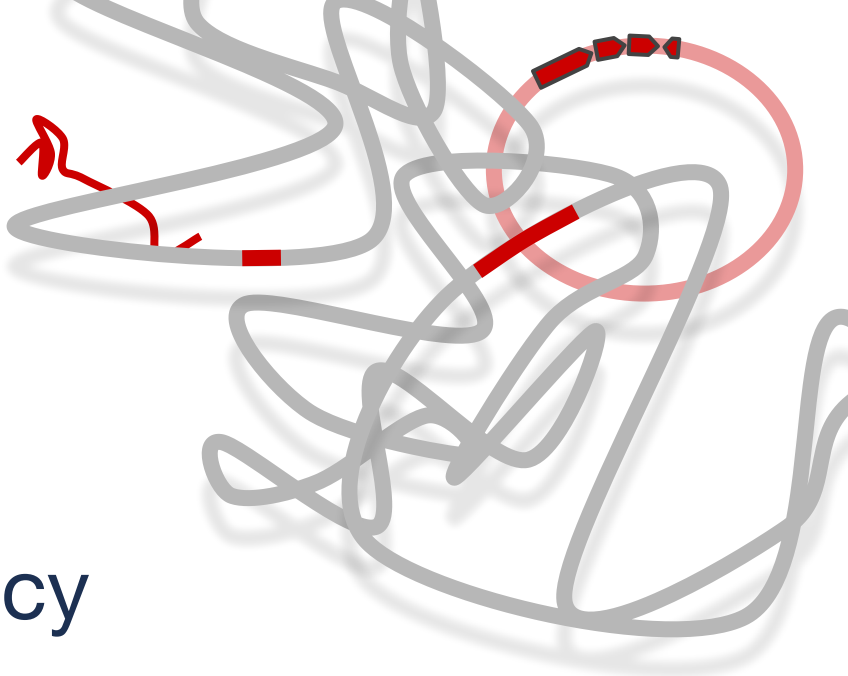

Requirements:

- strain-resolved genome sequences
  - ➢ high accuracy

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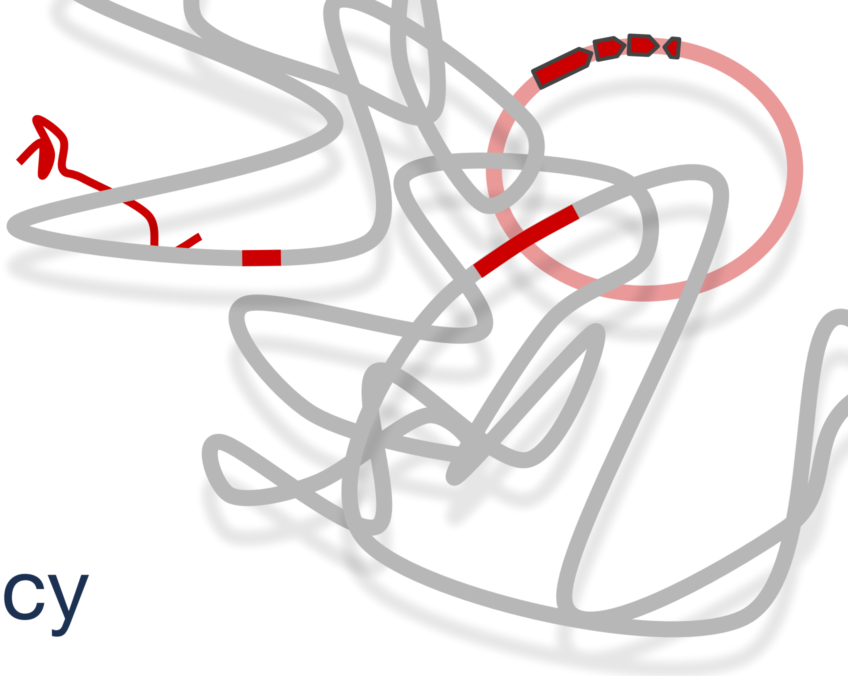

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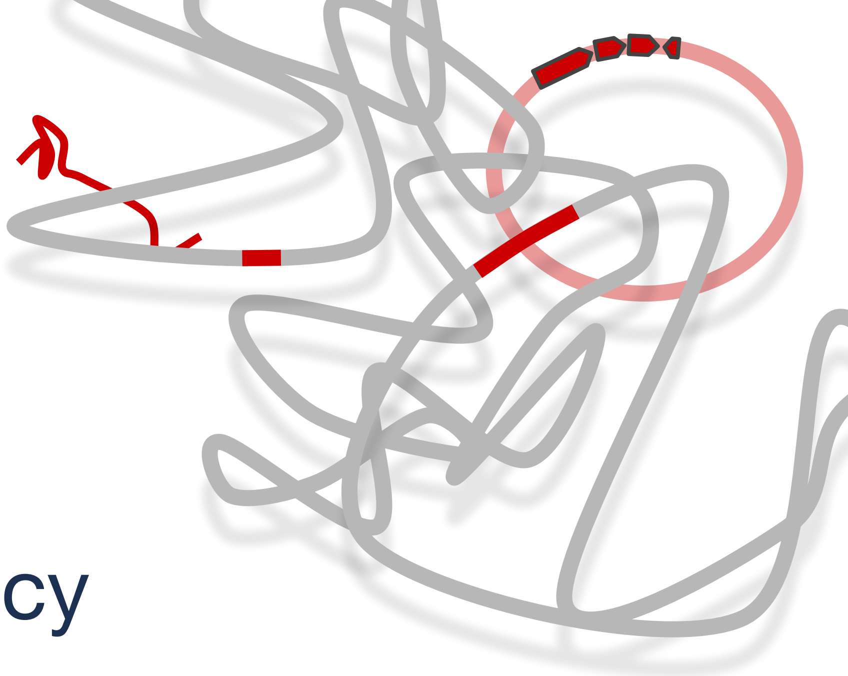

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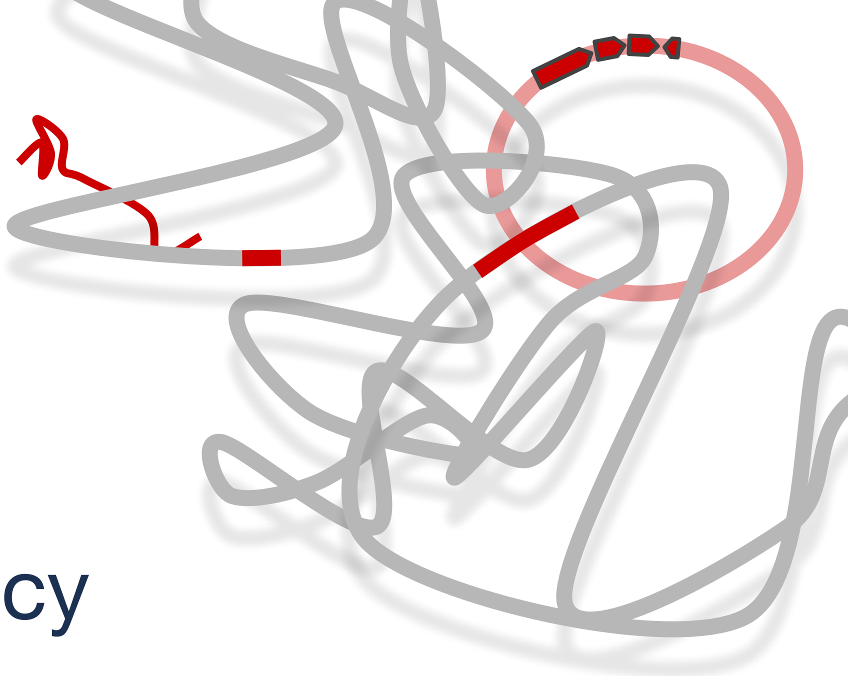

Requirements:

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● long sequences	➢ ...

Turning short reads into long sequences

ACGT

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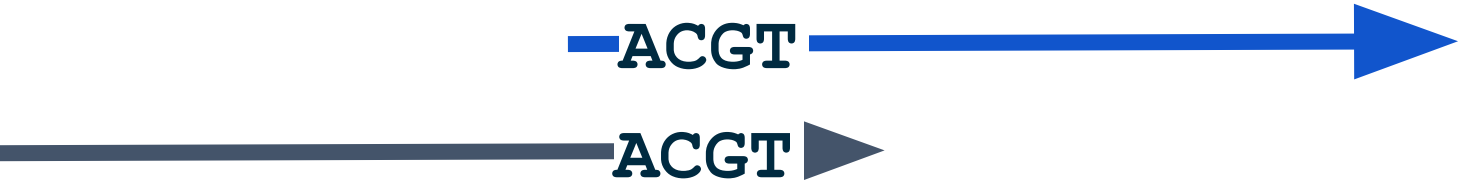

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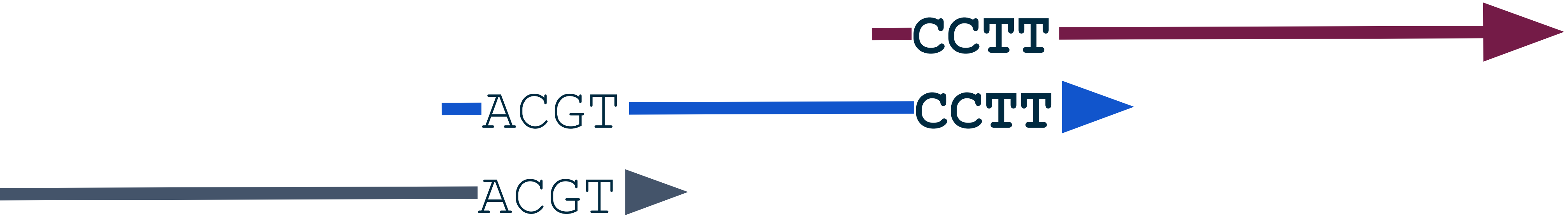

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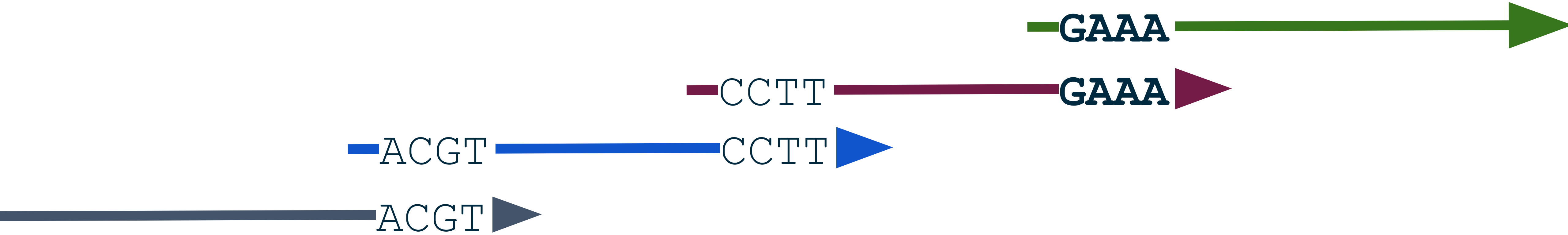

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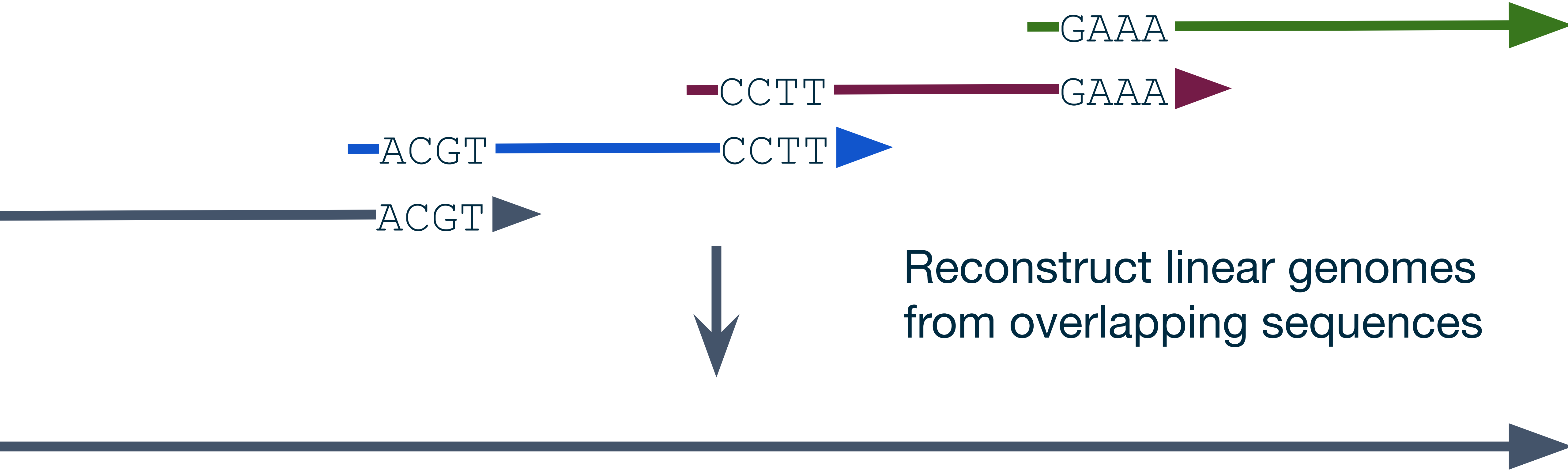
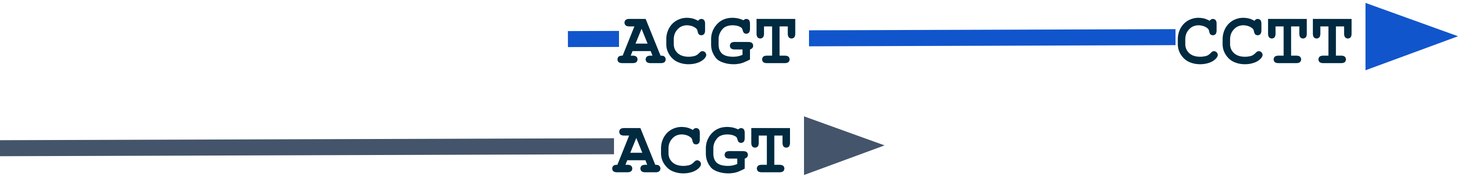


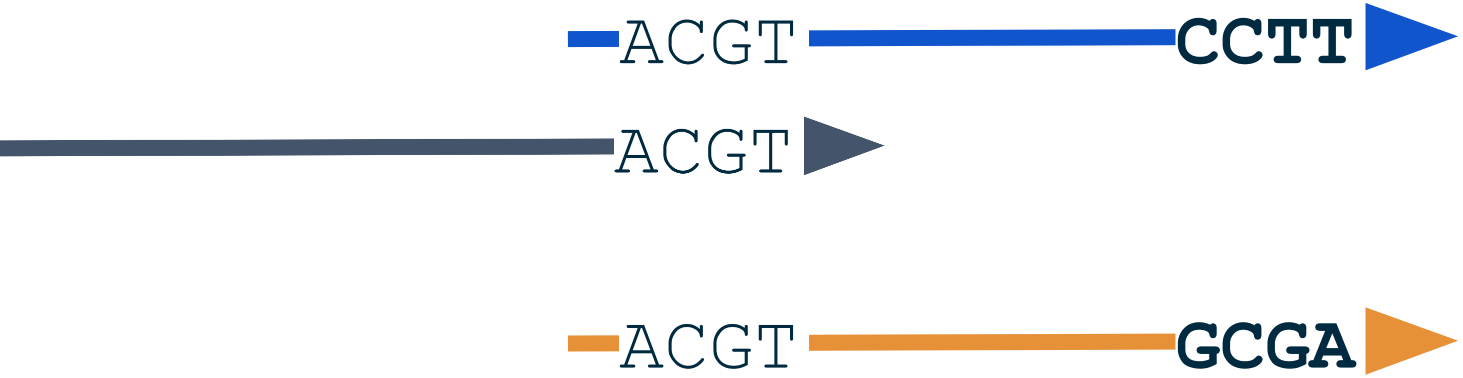
Reconstruct linear genomes from overlapping sequences

...GGTAGAGCGTGGACGTAGGGTTAACCTTAGAAAGCTAGAAAACCGCGCGCCCT...

Problem:



Problem: Closely related strains make read-chaining ambiguous



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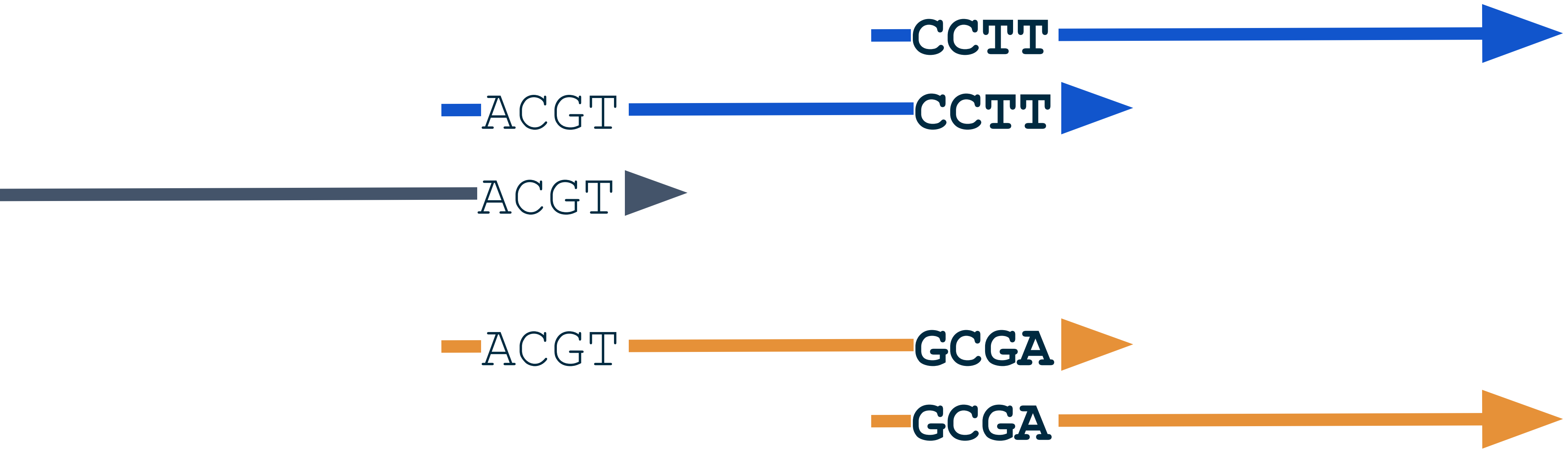

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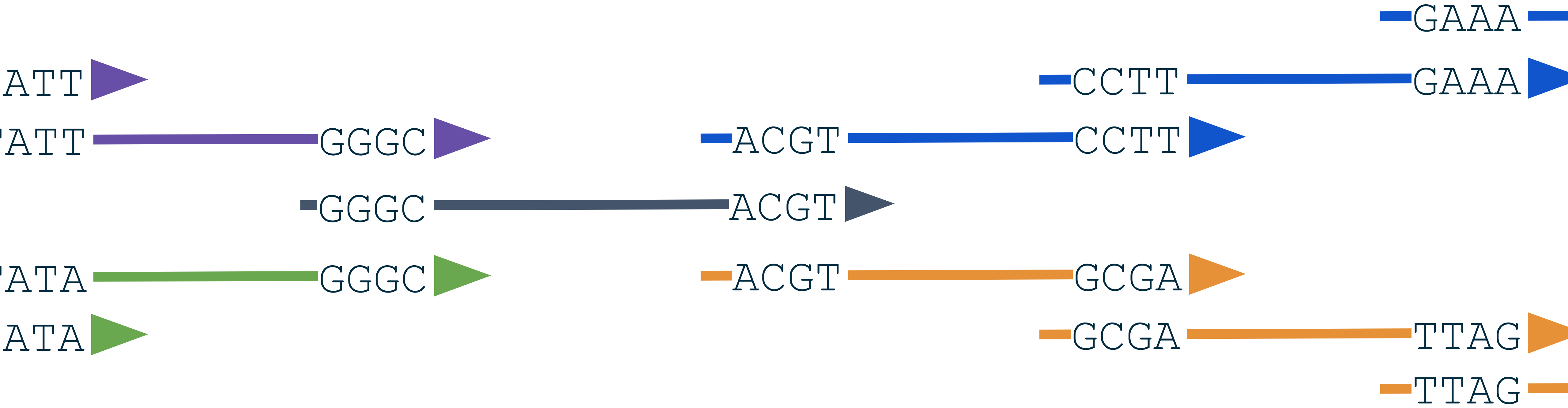
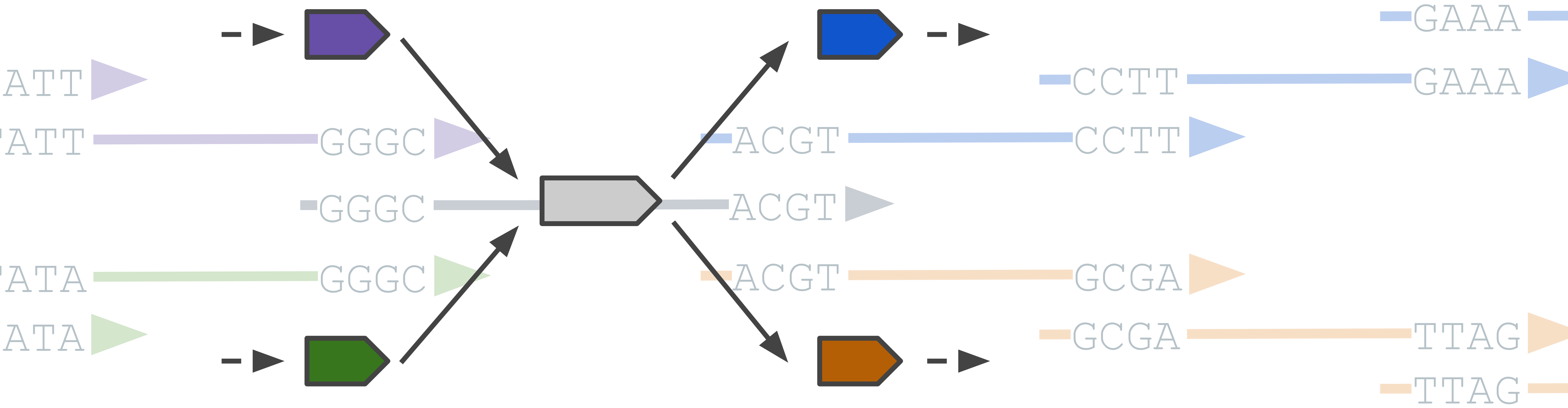


Can be represented as a graph of sequences linked by their overlaps



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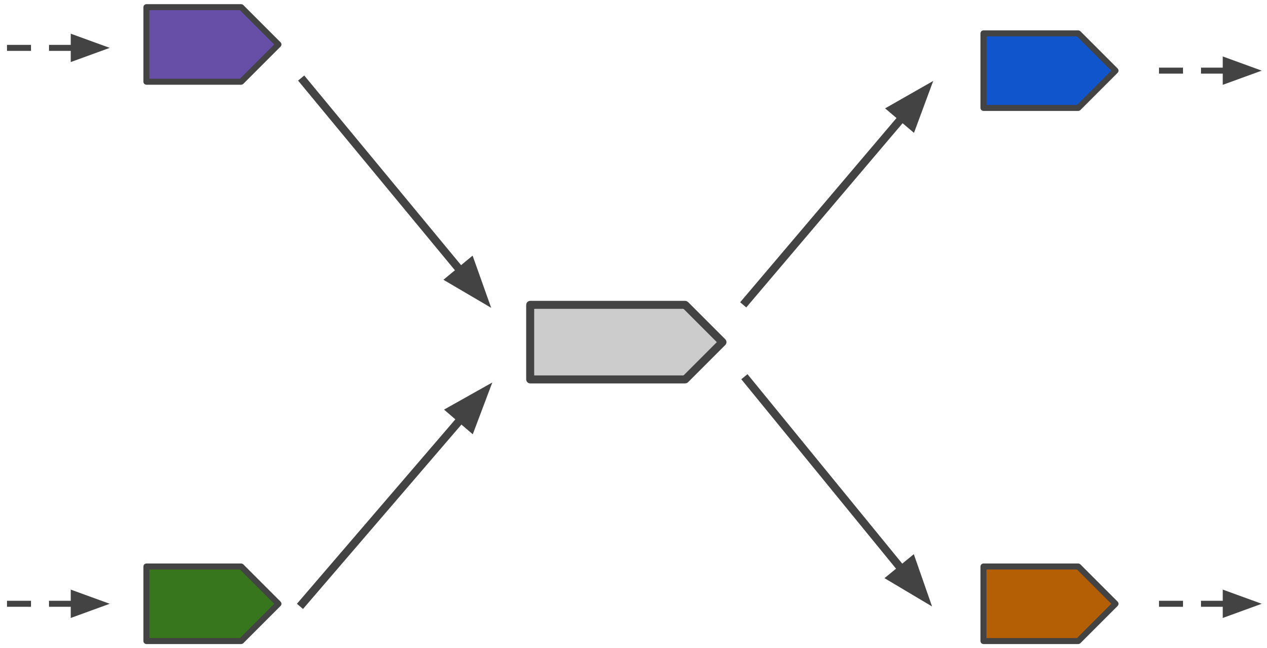

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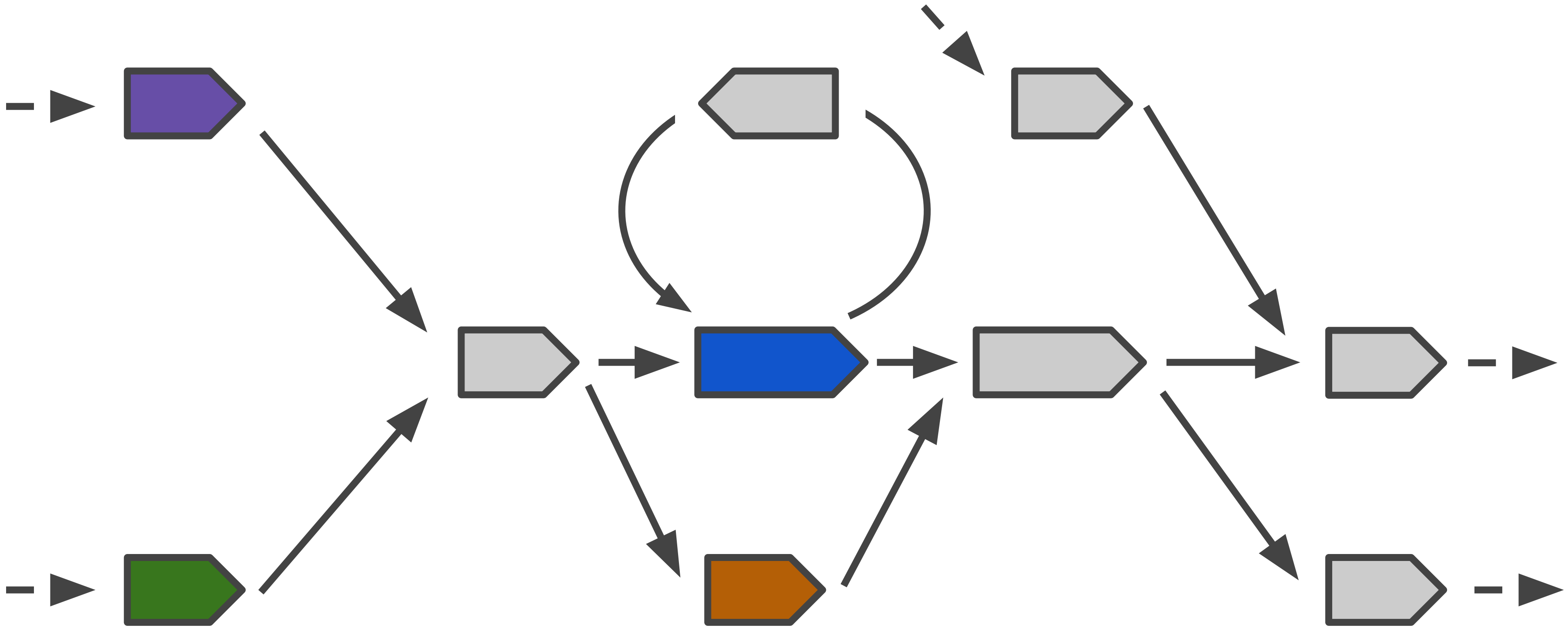

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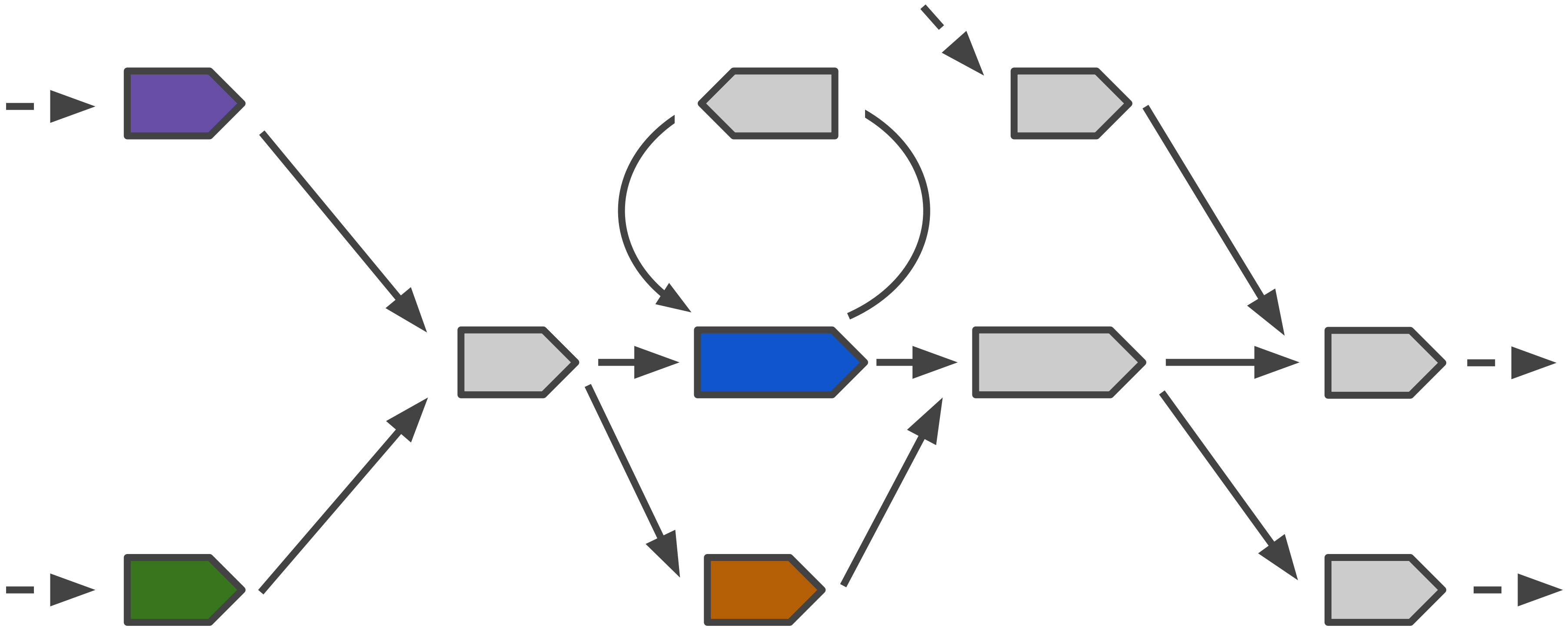
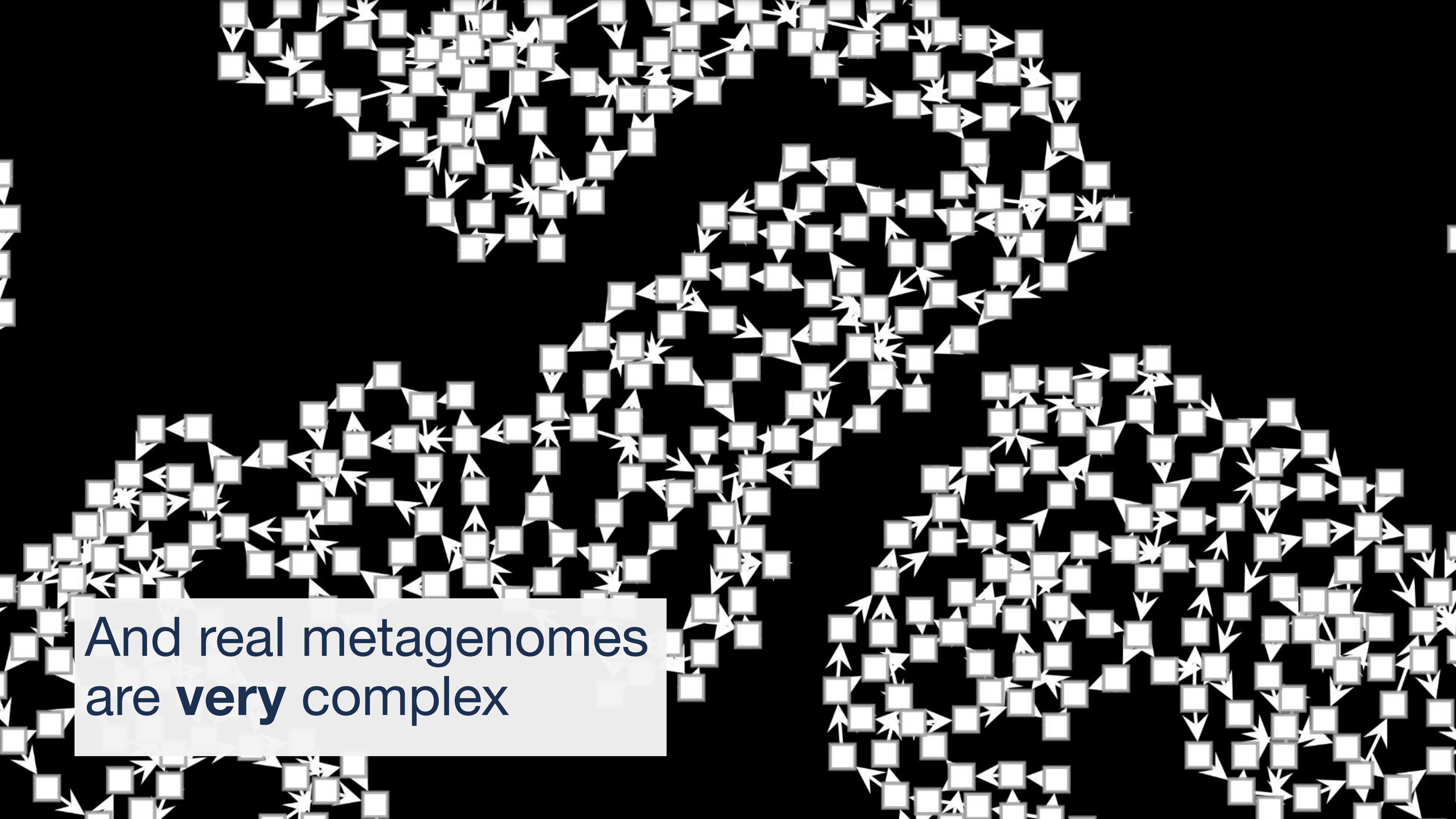
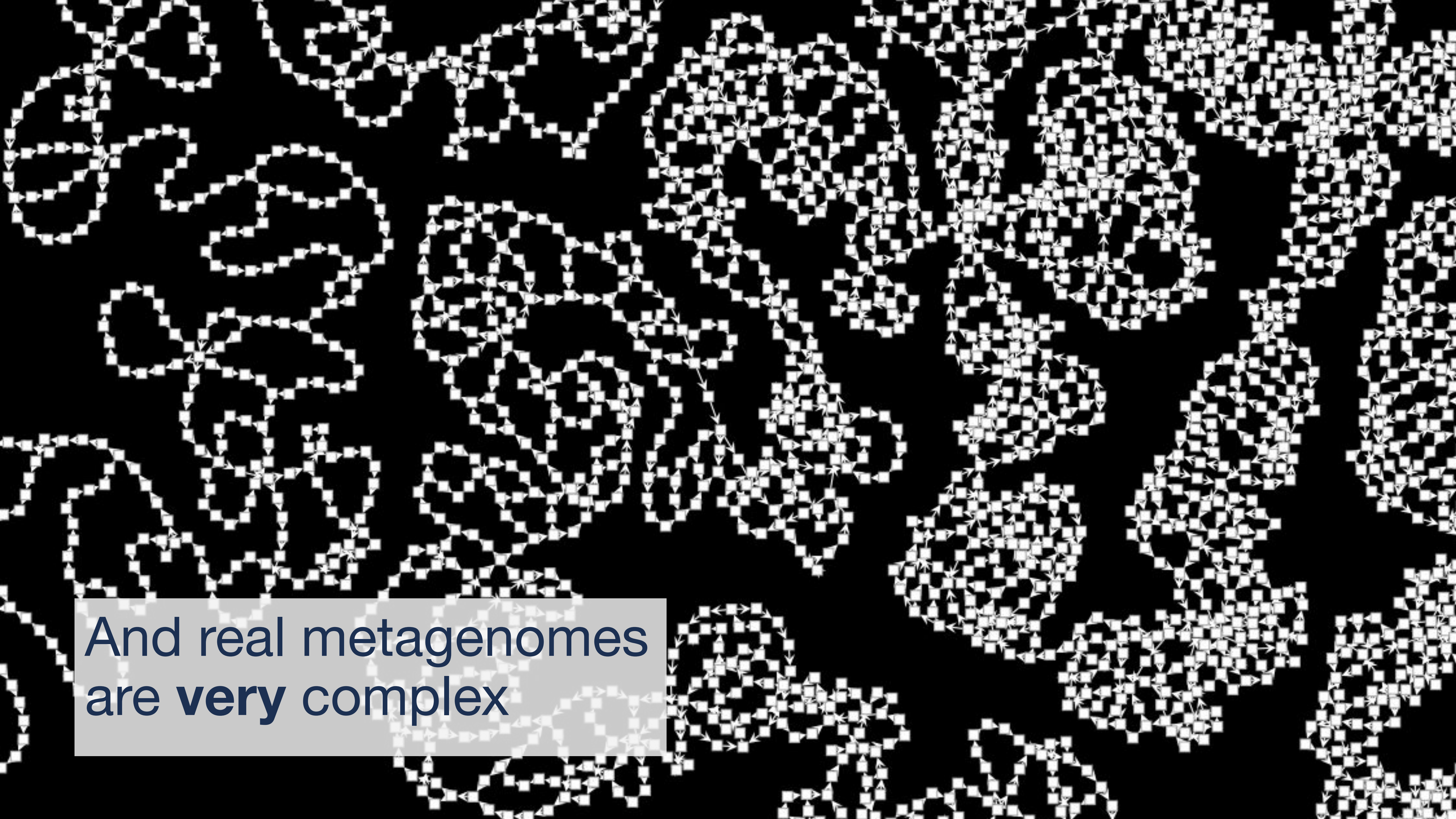


(This problem also comes up for mRNA alternative splicing)



And real metagenomes are **very** complex



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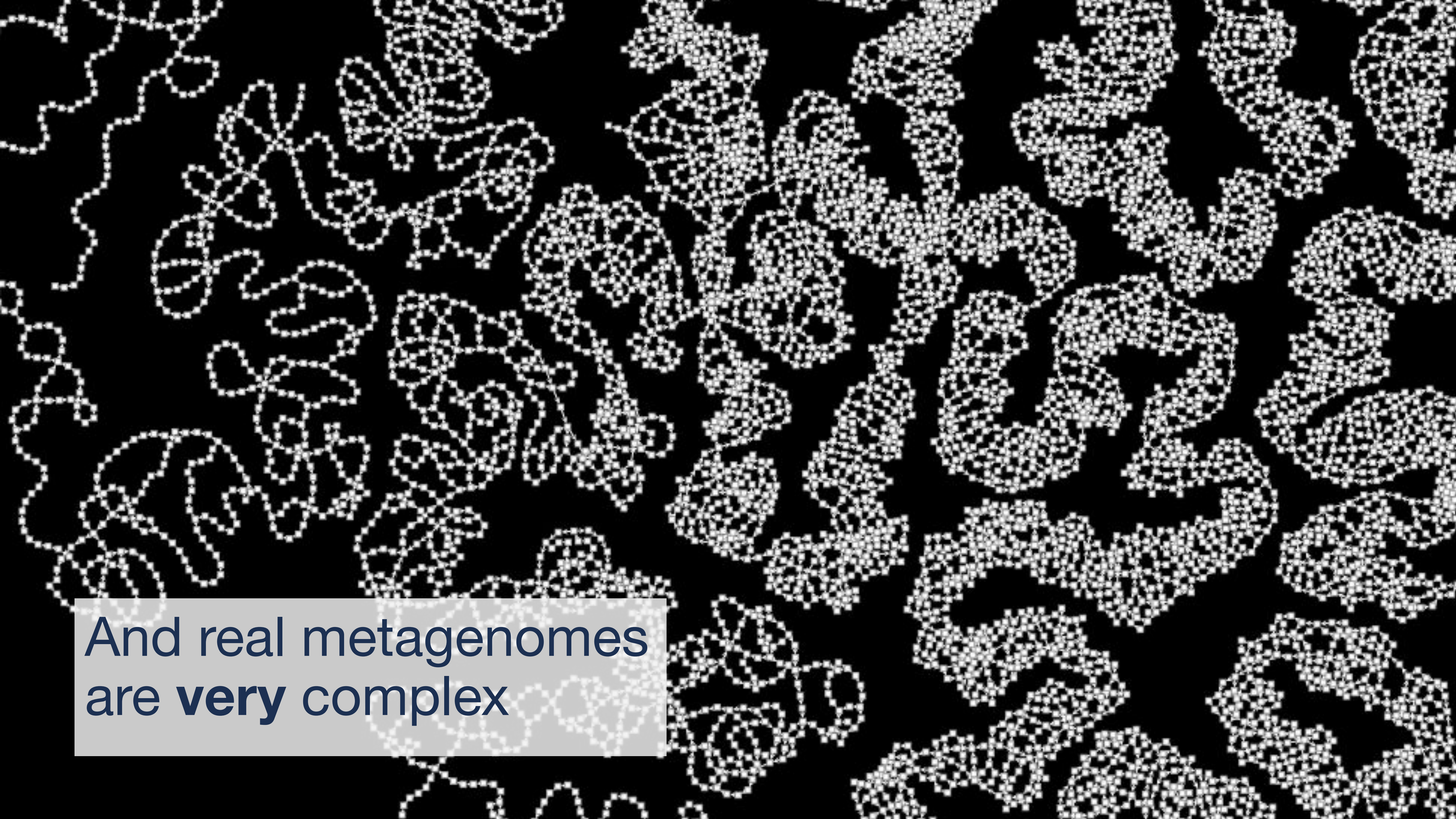

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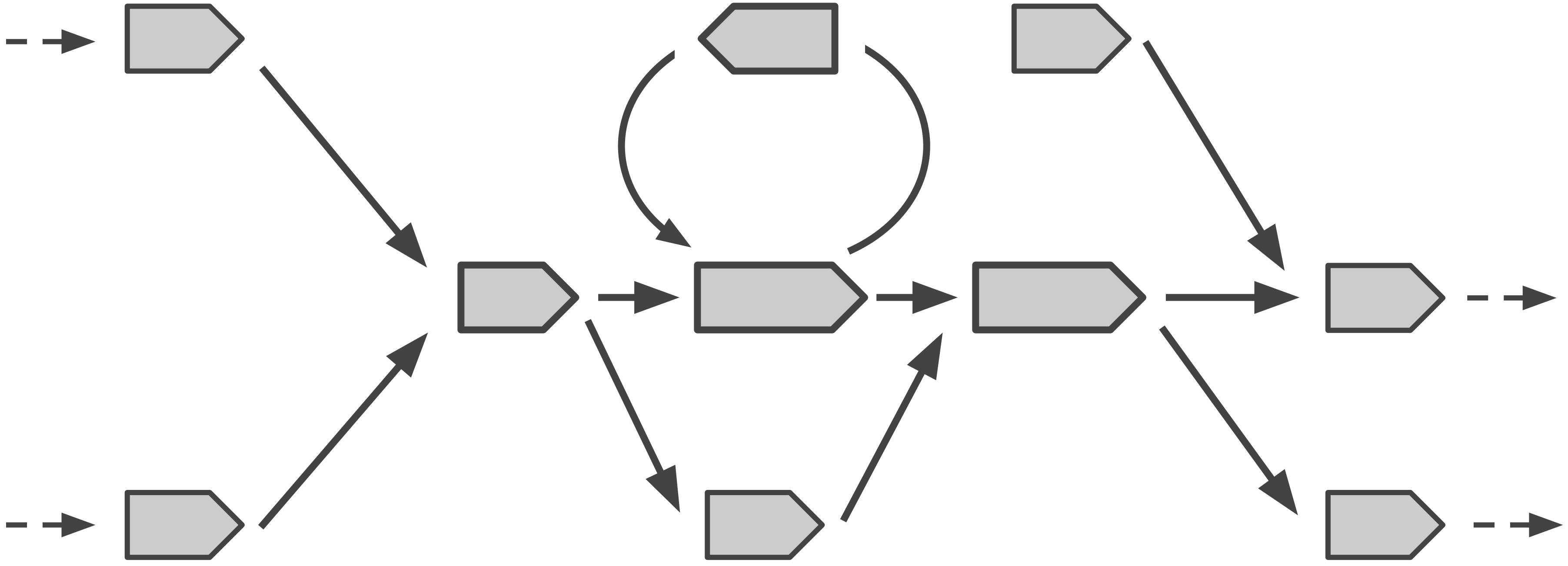
Real genomic sequences are paths on the graph



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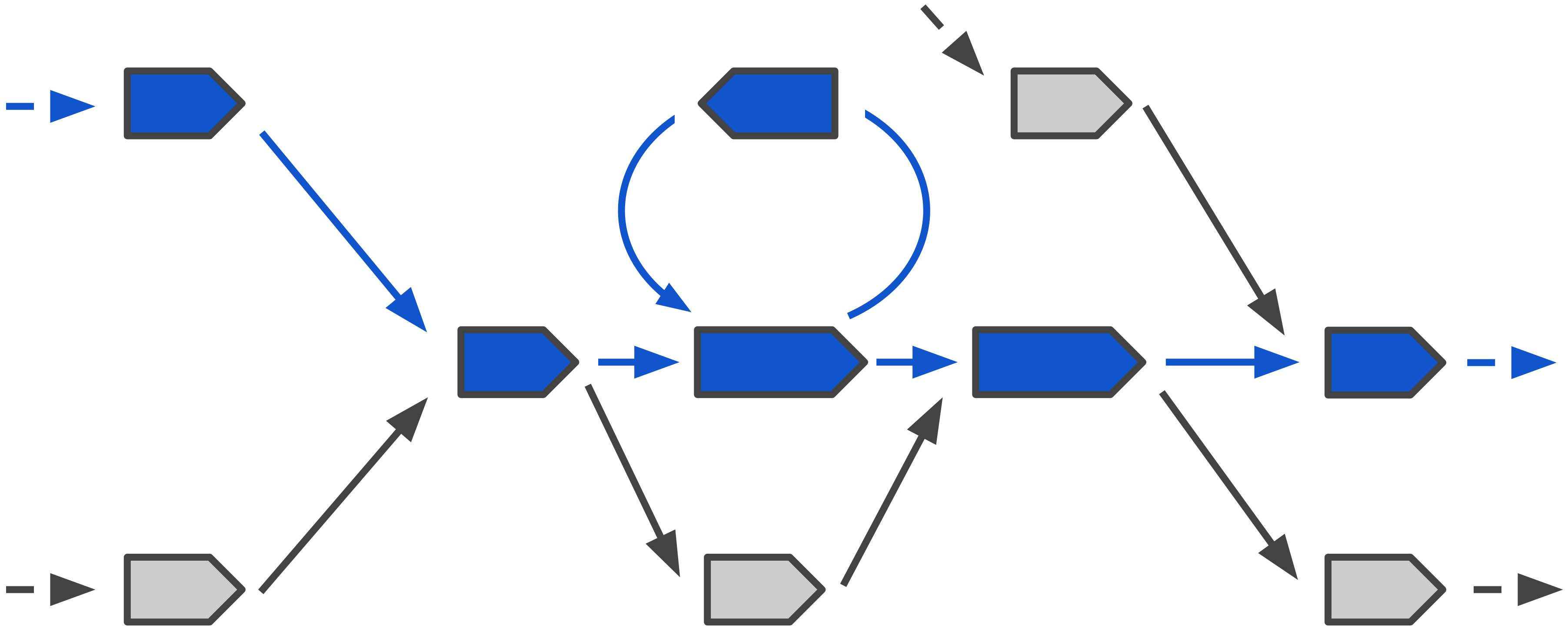

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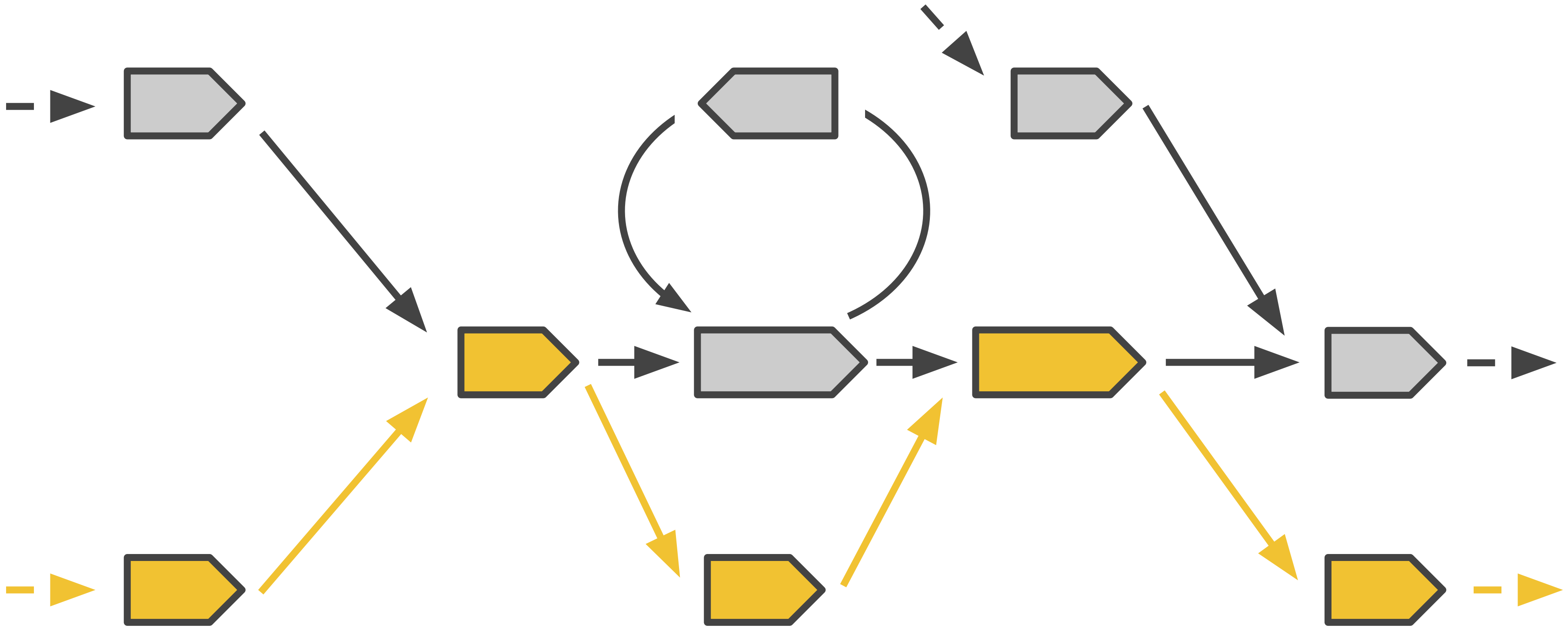

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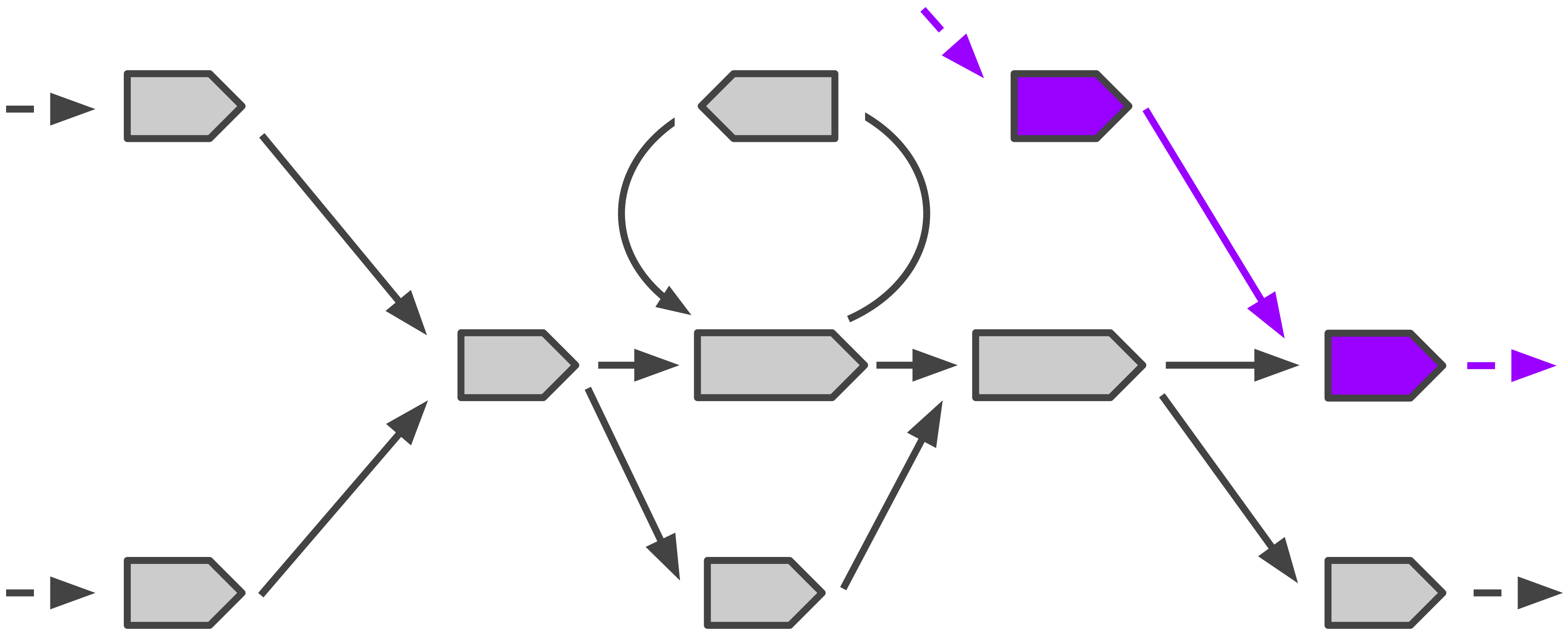
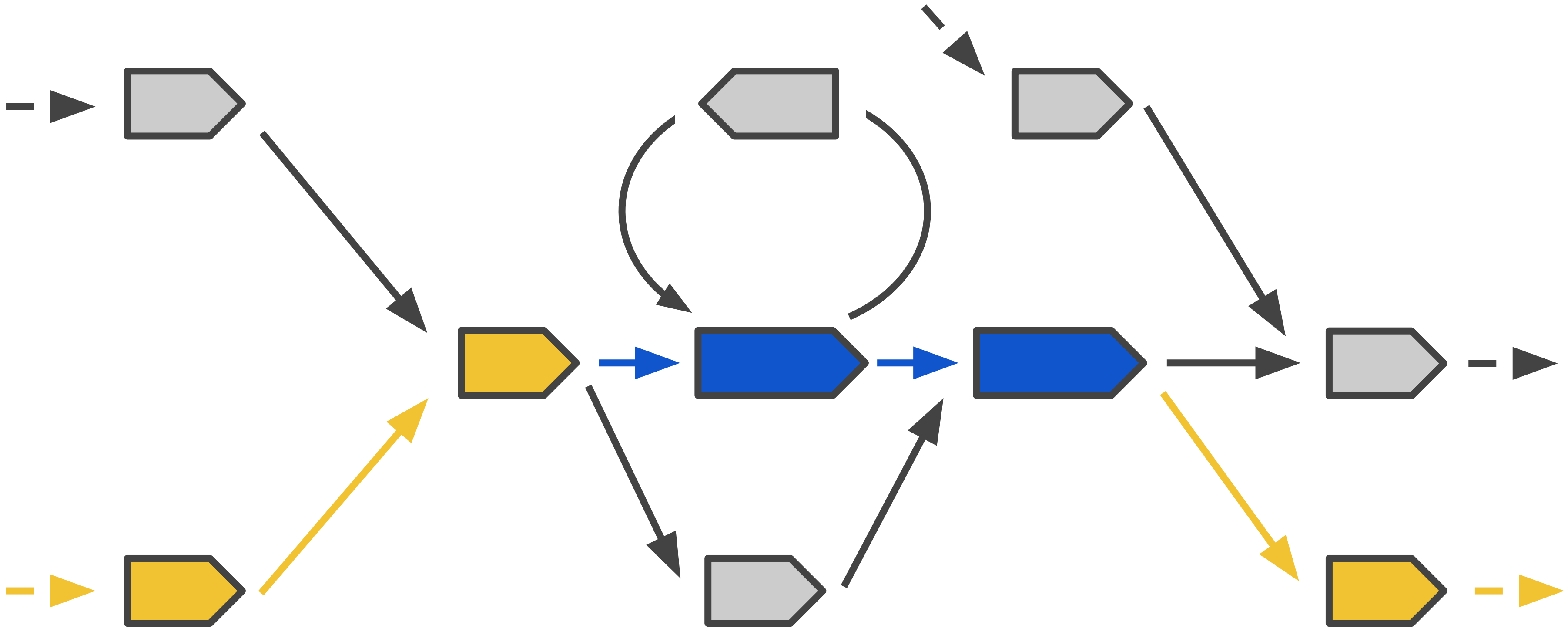


Lots of incorrect paths also exist…



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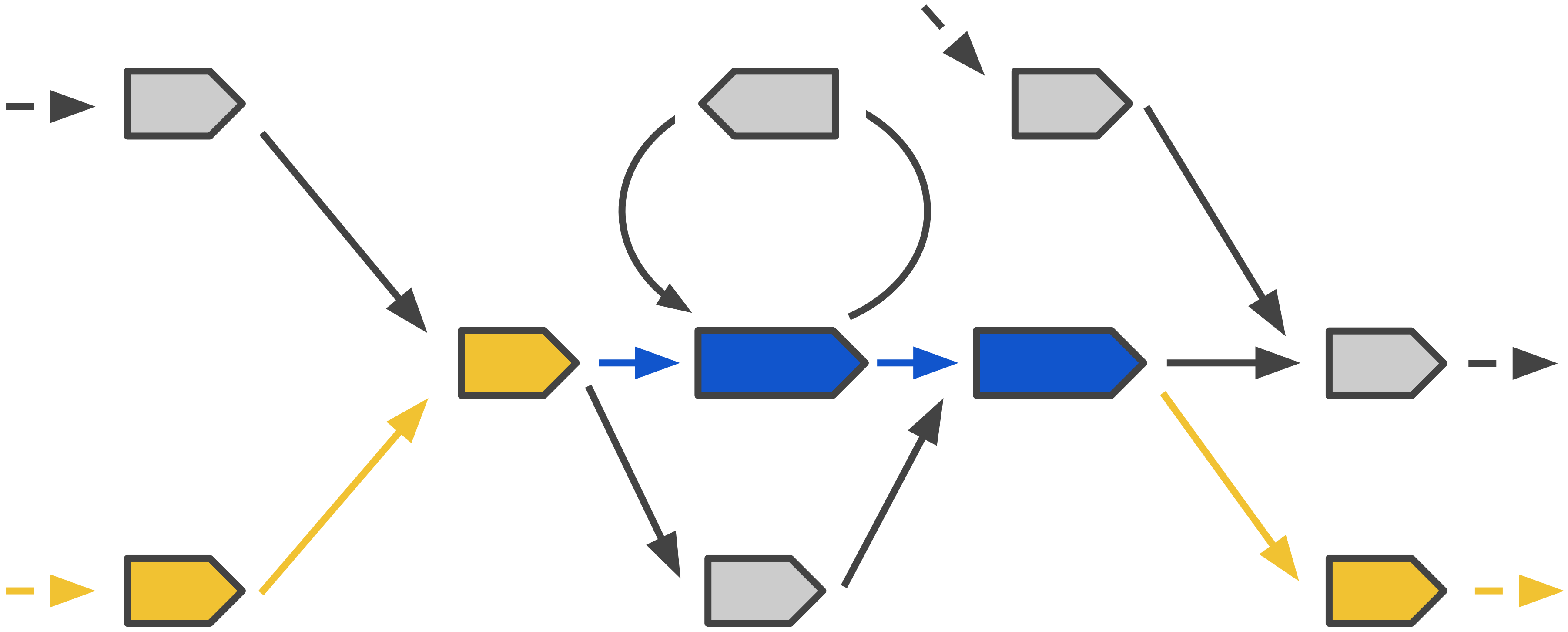

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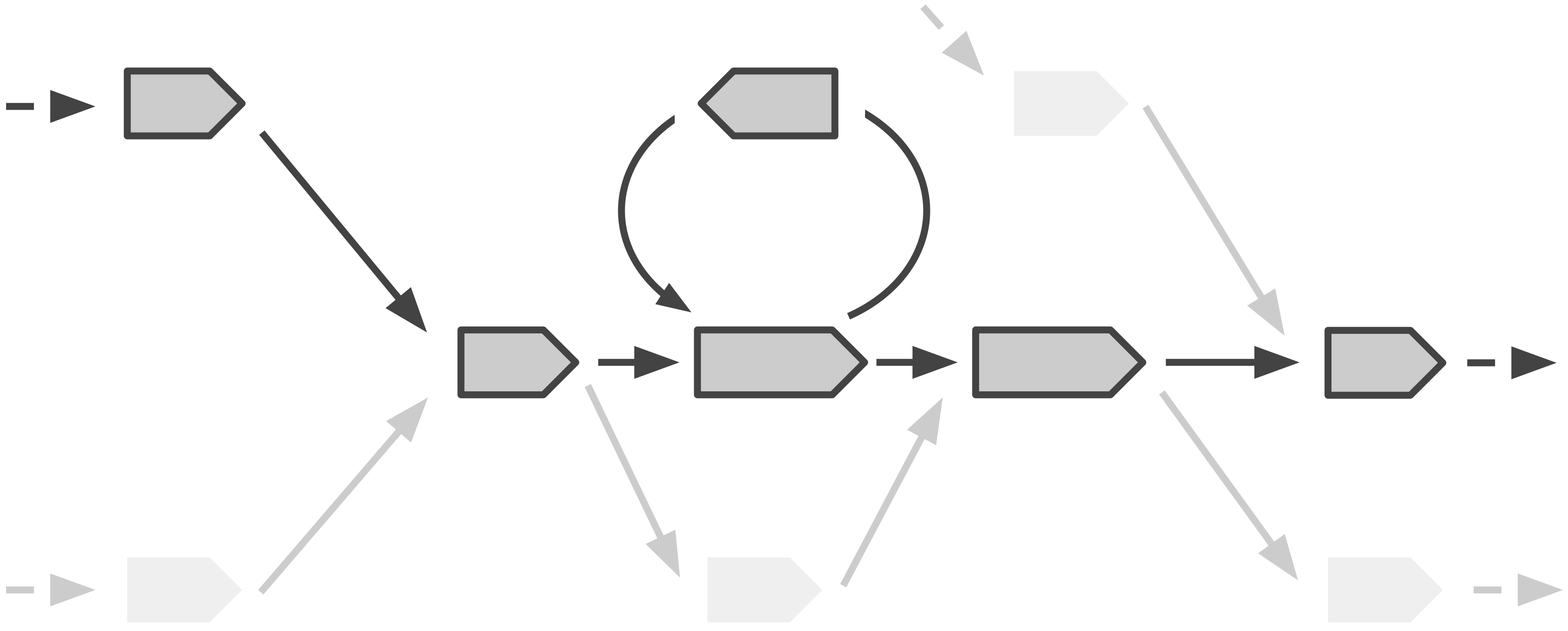

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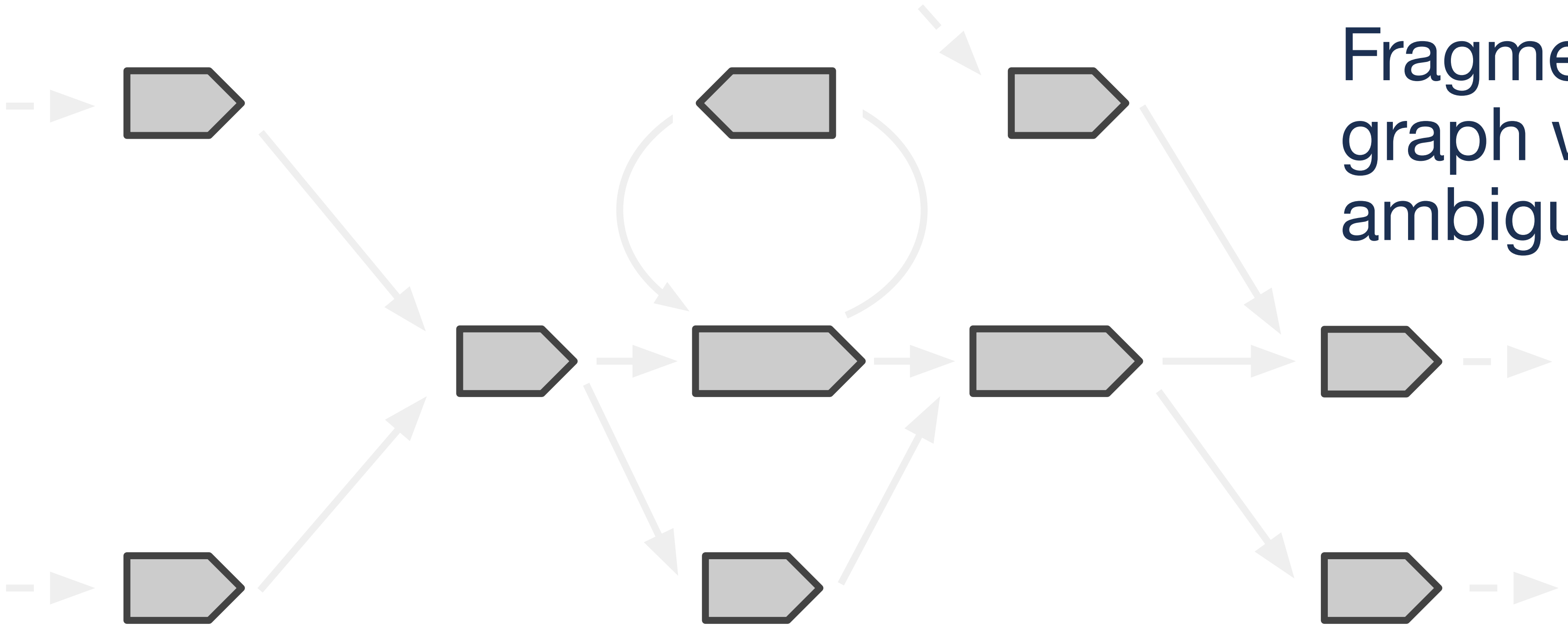

Standard Tools:

Filter out low-abundance sequences

Fragment the graph when it's ambiguous

Untangling the hairball

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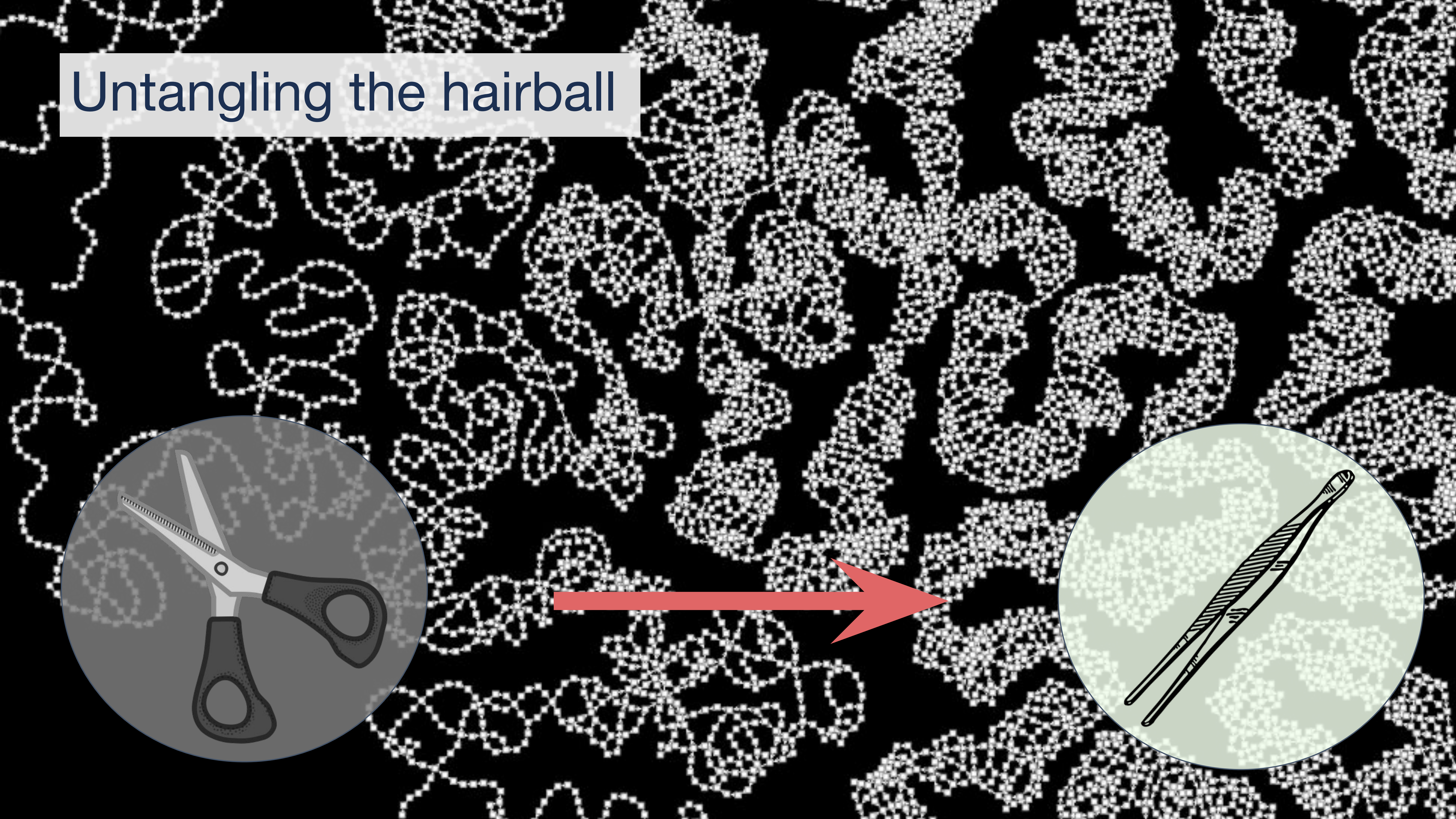

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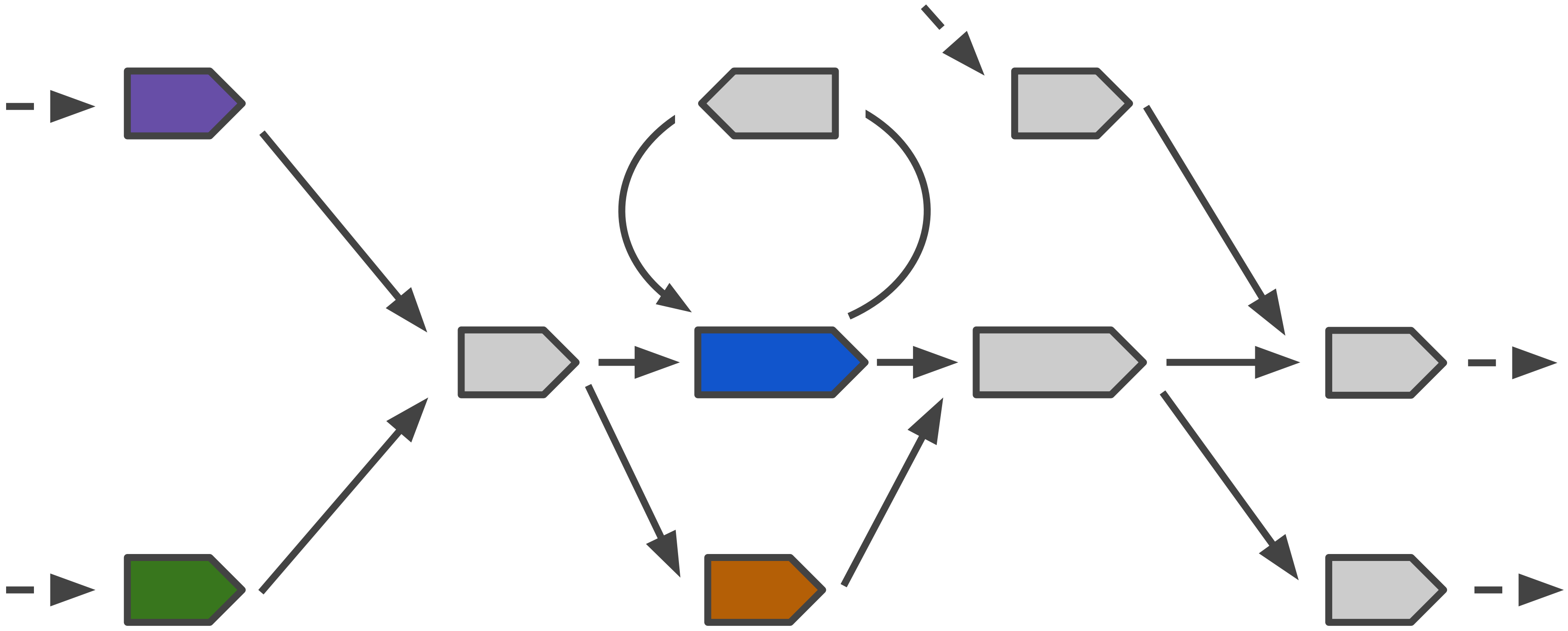
StrainZip:

Untangling the metagenome graph

How can we recover long, accurate genome sequences from short reads?



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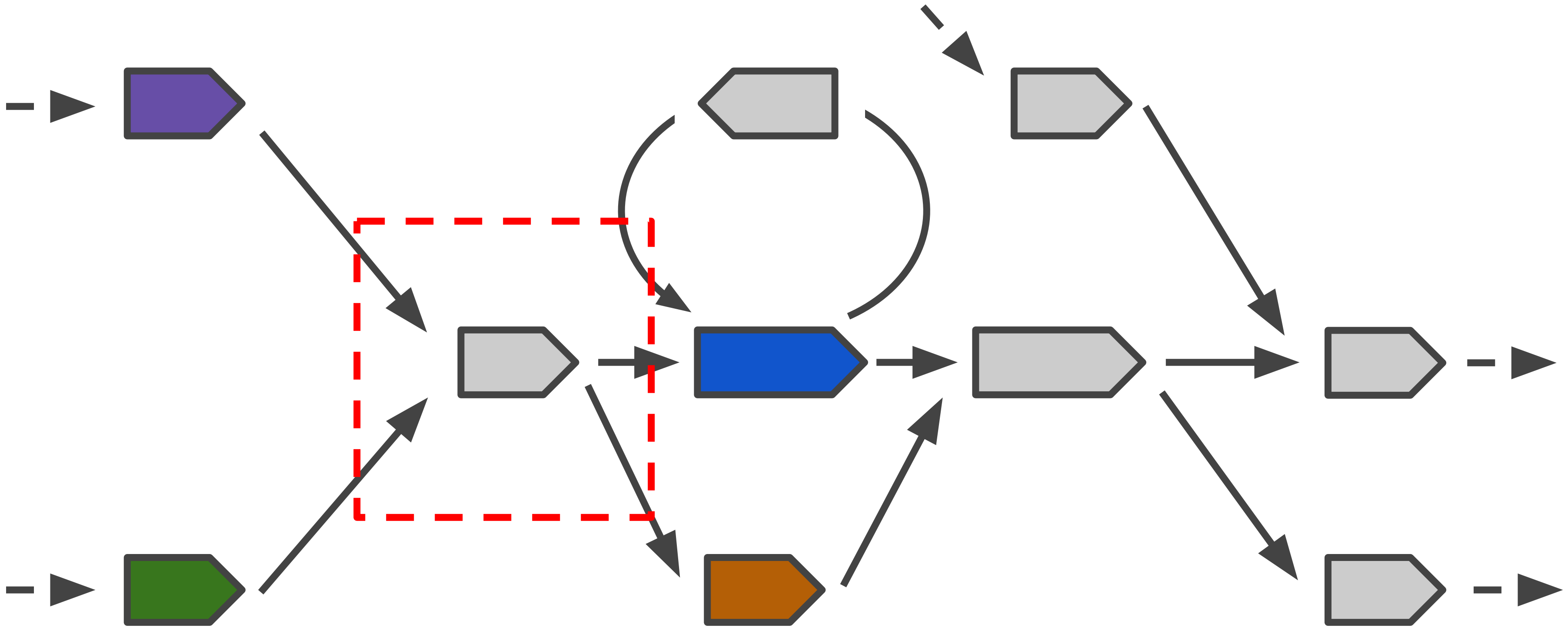
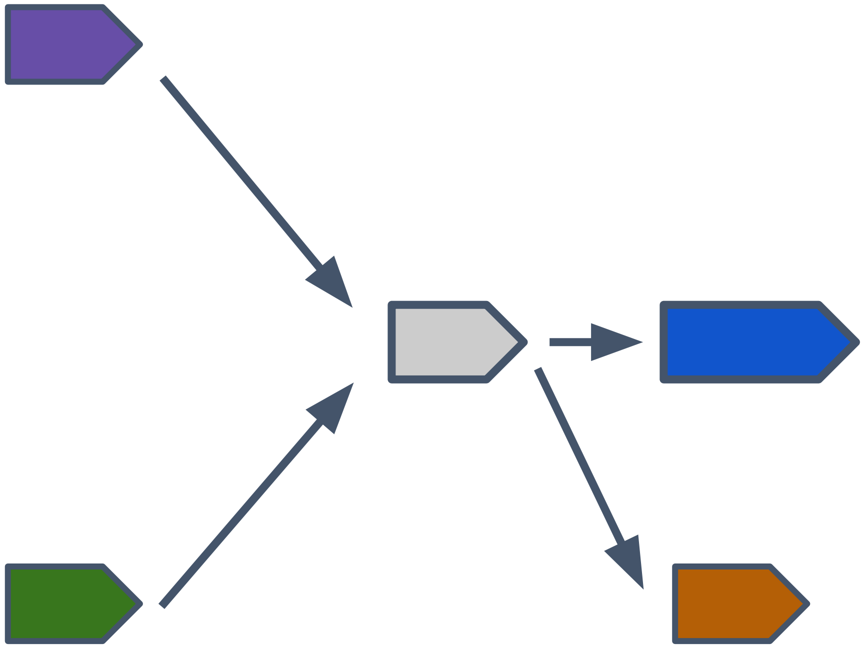


Focus on just one junction at a time



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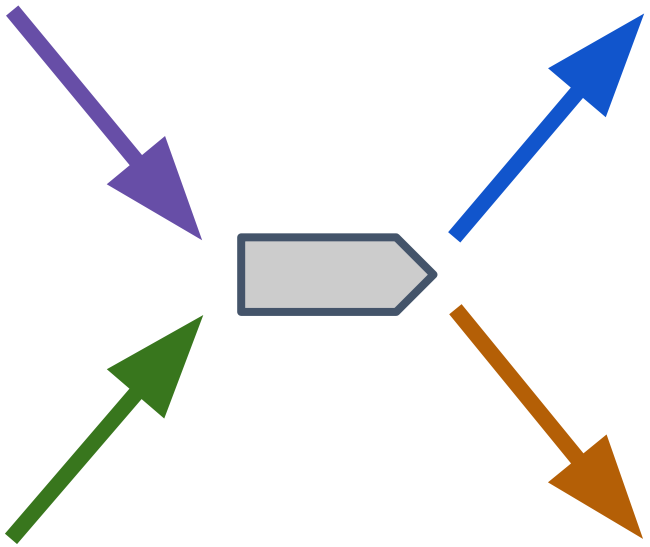

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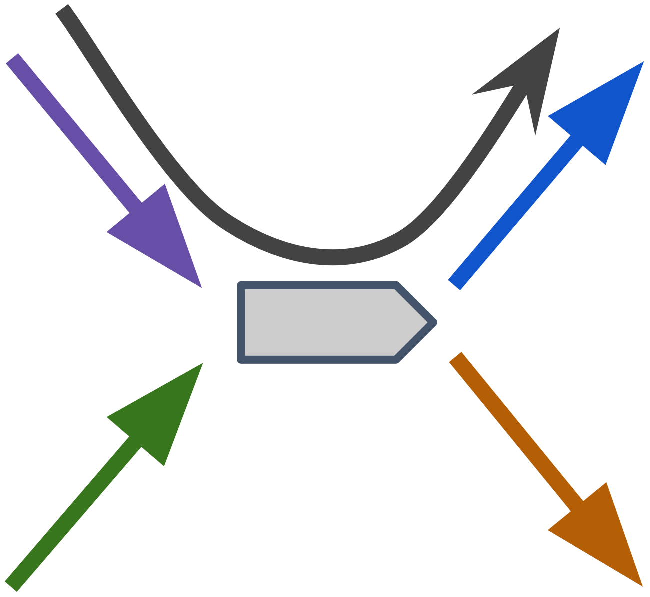

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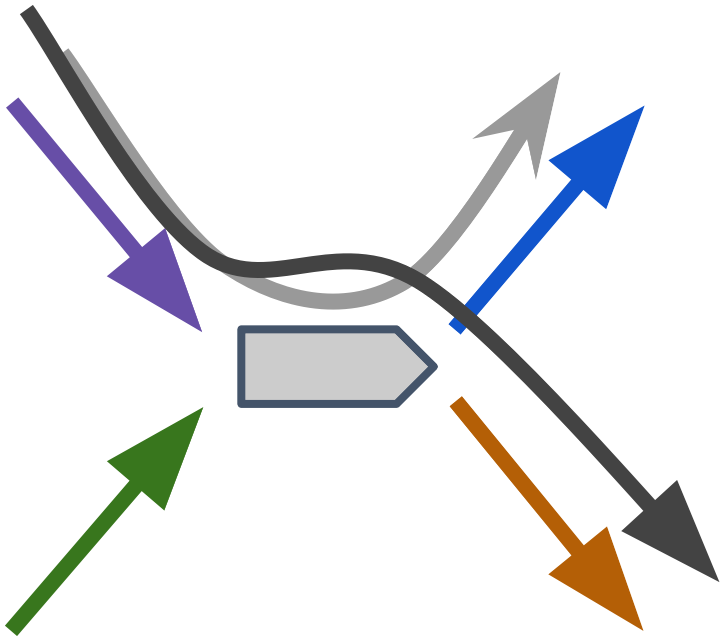

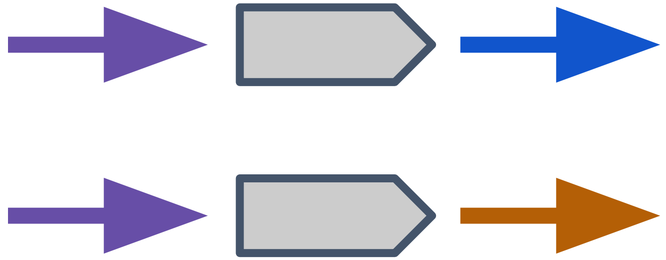

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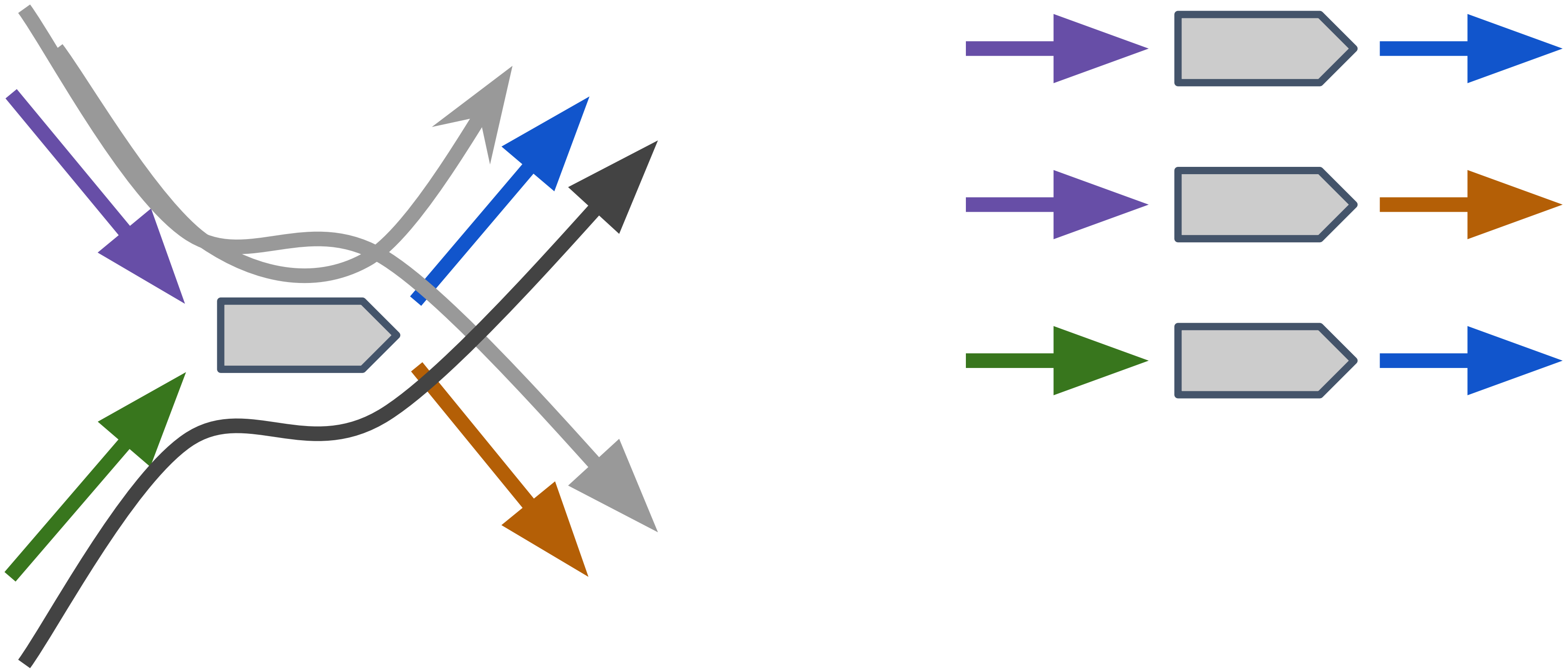

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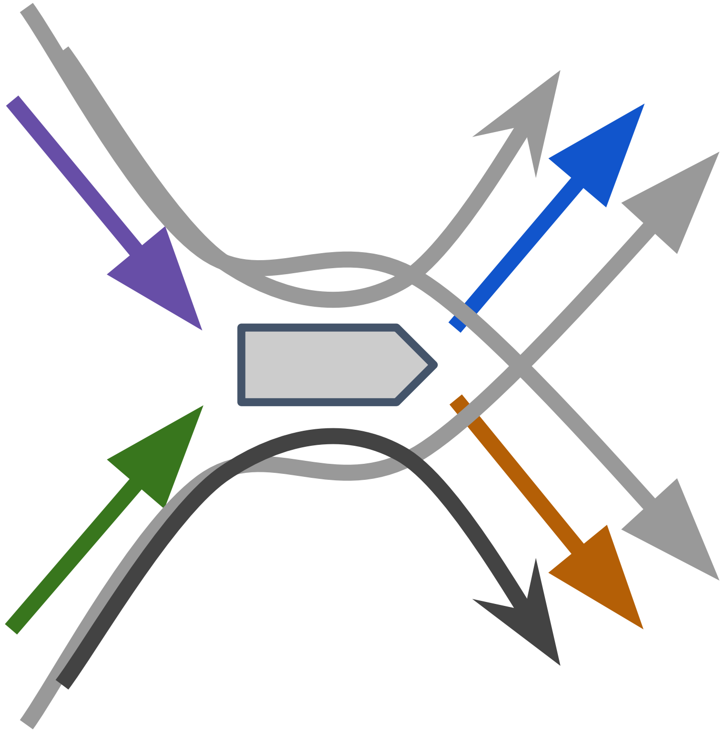
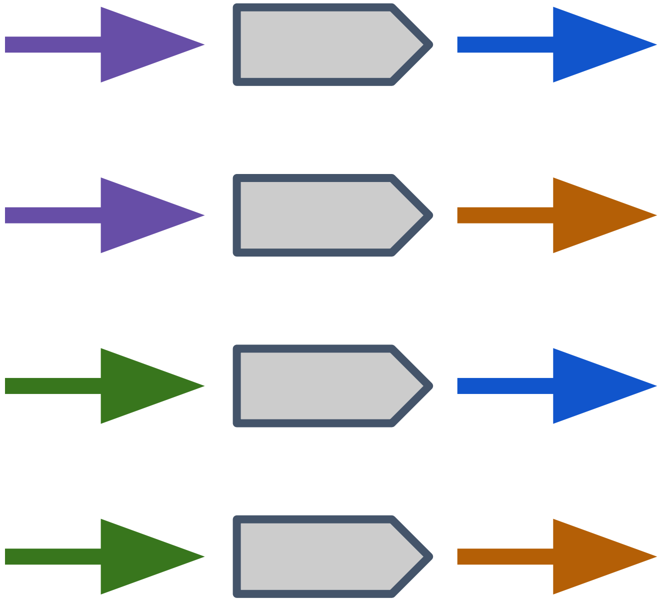
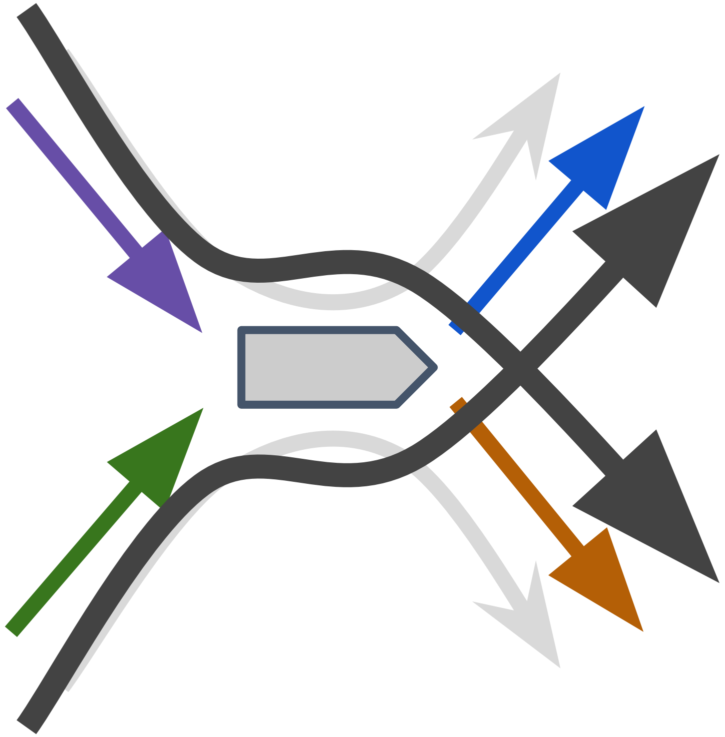




Focus on just one junction at a time
Select local paths



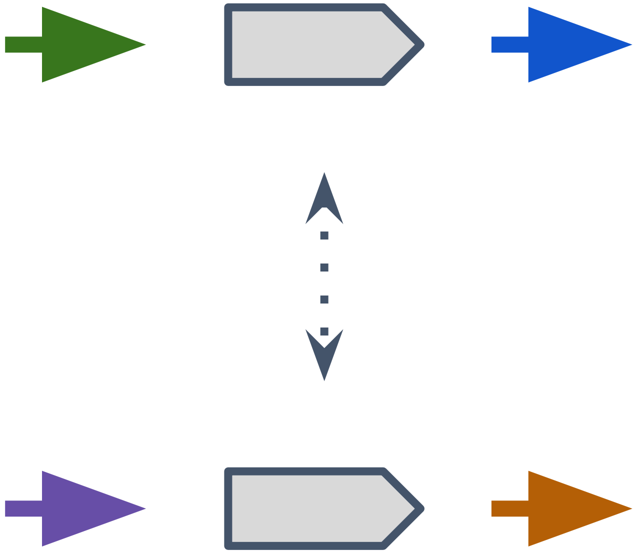
$$\underset{X}{\begin{array}{|c|c|c|c|}\hline \mathbf{1} & \mathbf{1} & 0 & 0 \\ \hline 0 & 0 & \mathbf{1} & \mathbf{1} \\ \hline \mathbf{1} & 0 & \mathbf{1} & 0 \\ \hline 0 & \mathbf{1} & 0 & \mathbf{1} \\ \hline \end{array}} \times \underset{\beta}{\begin{array}{|c|c|c|}\hline p_{1,1} & p_{1,2} & p_{1,3} \\ \hline p_{2,1} & p_{2,2} & p_{2,3} \\ \hline p_{3,1} & p_{3,2} & p_{3,3} \\ \hline p_{4,1} & p_{4,2} & p_{4,3} \\ \hline \end{array}} \approx \underset{Y}{\begin{array}{|c|c|c|}\hline e_{1,1} & e_{1,2} & e_{1,3} \\ \hline e_{2,1} & e_{2,2} & e_{2,3} \\ \hline e_{3,1} & e_{3,2} & e_{3,3} \\ \hline e_{4,1} & e_{4,2} & e_{4,3} \\ \hline \end{array}}$$

Sparse linear regression across multiple samples

Focus on just one junction at a time

Select local paths

Unzip

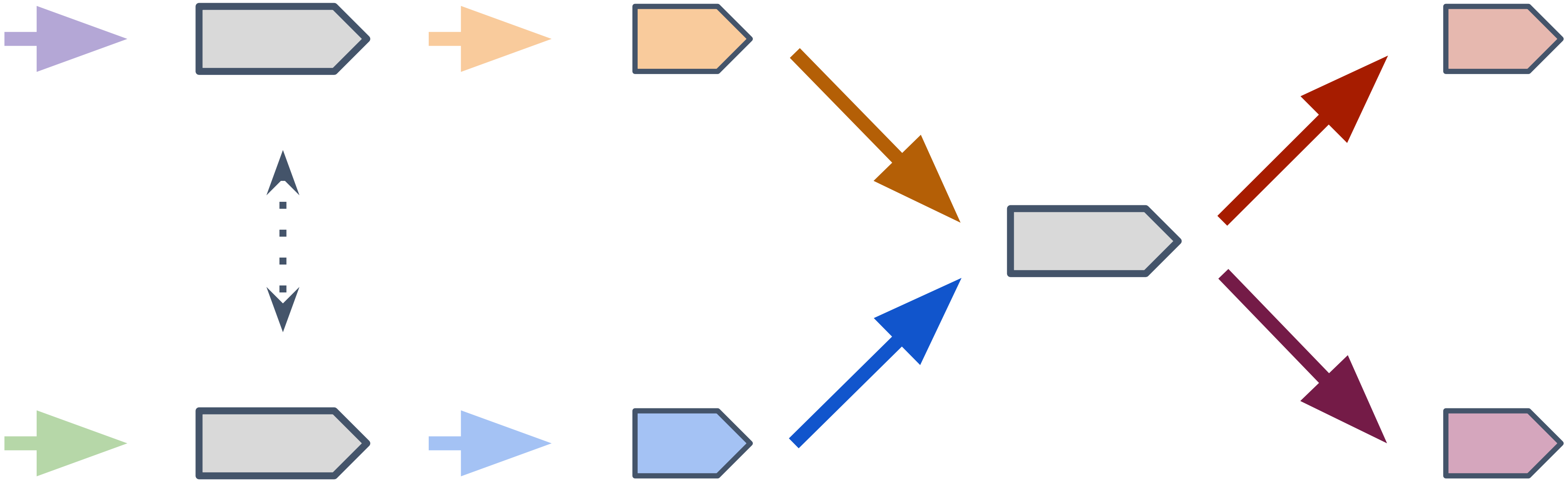


Focus on just one junction at a time

Select local paths

Unzip

Repeat



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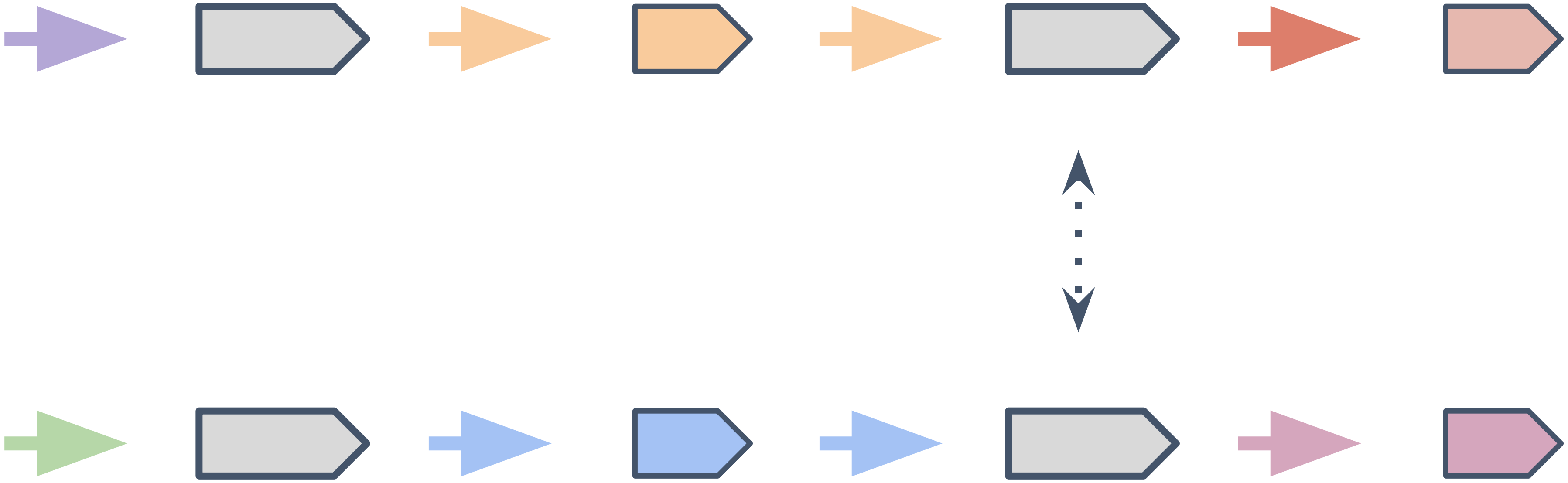

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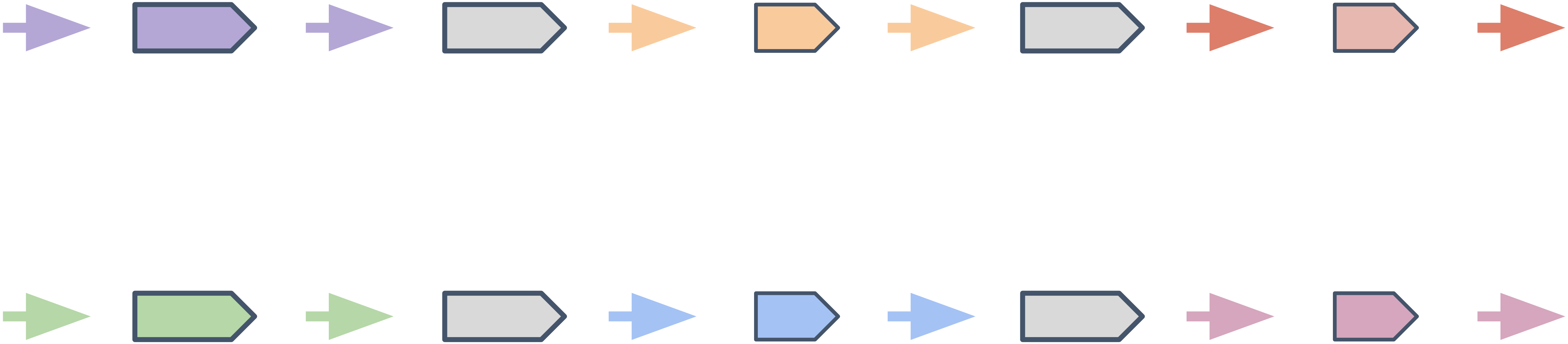

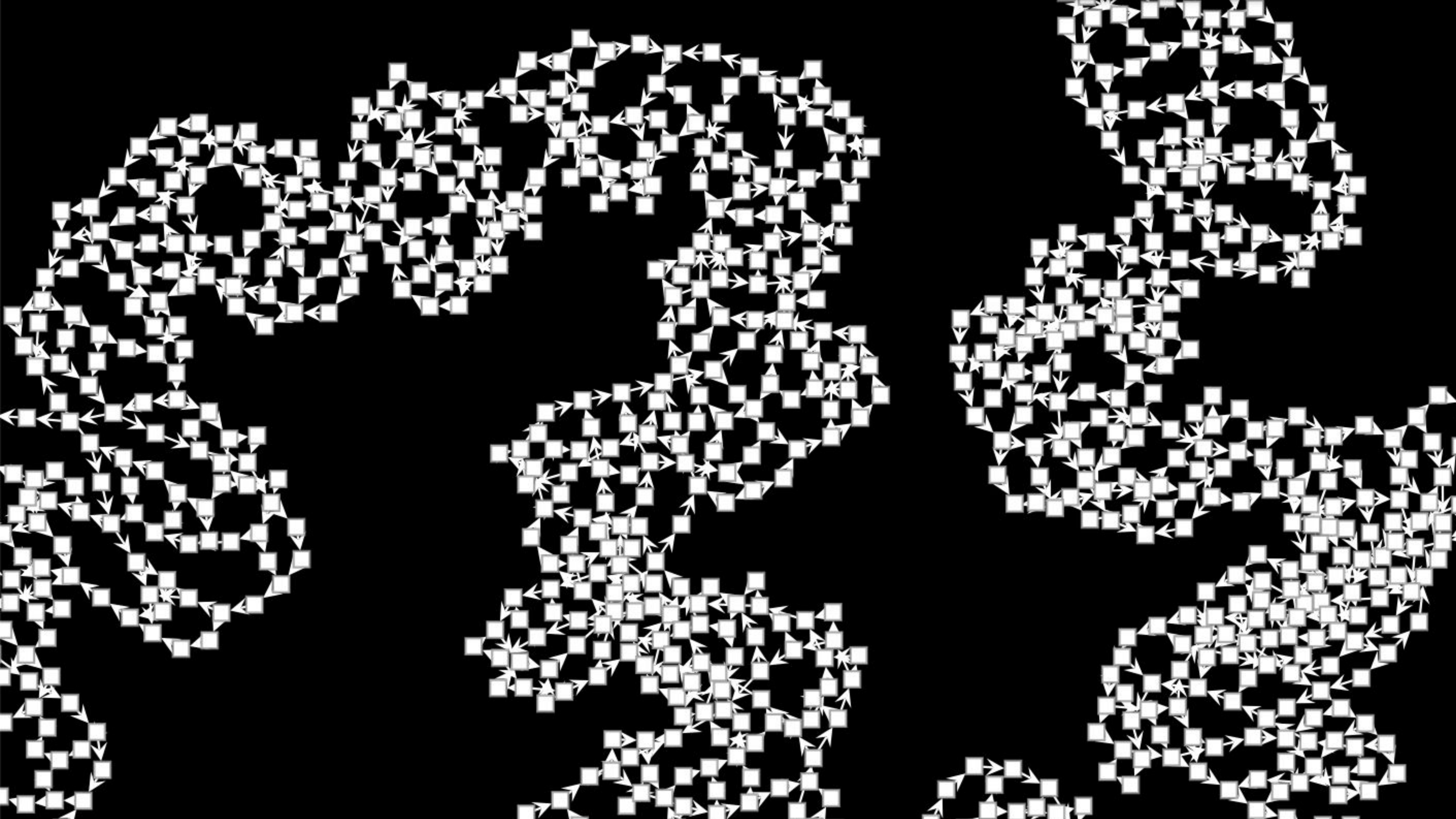

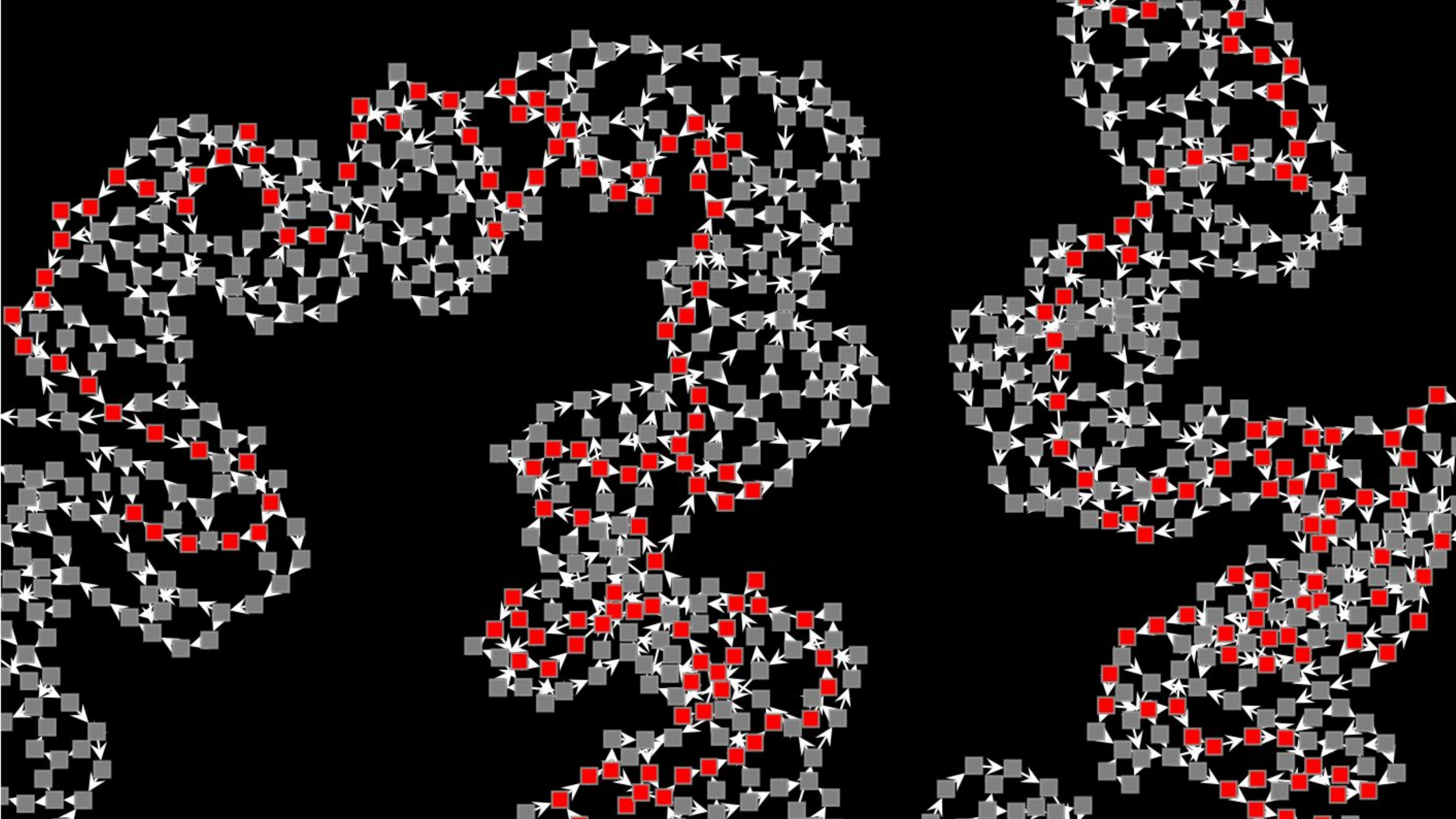

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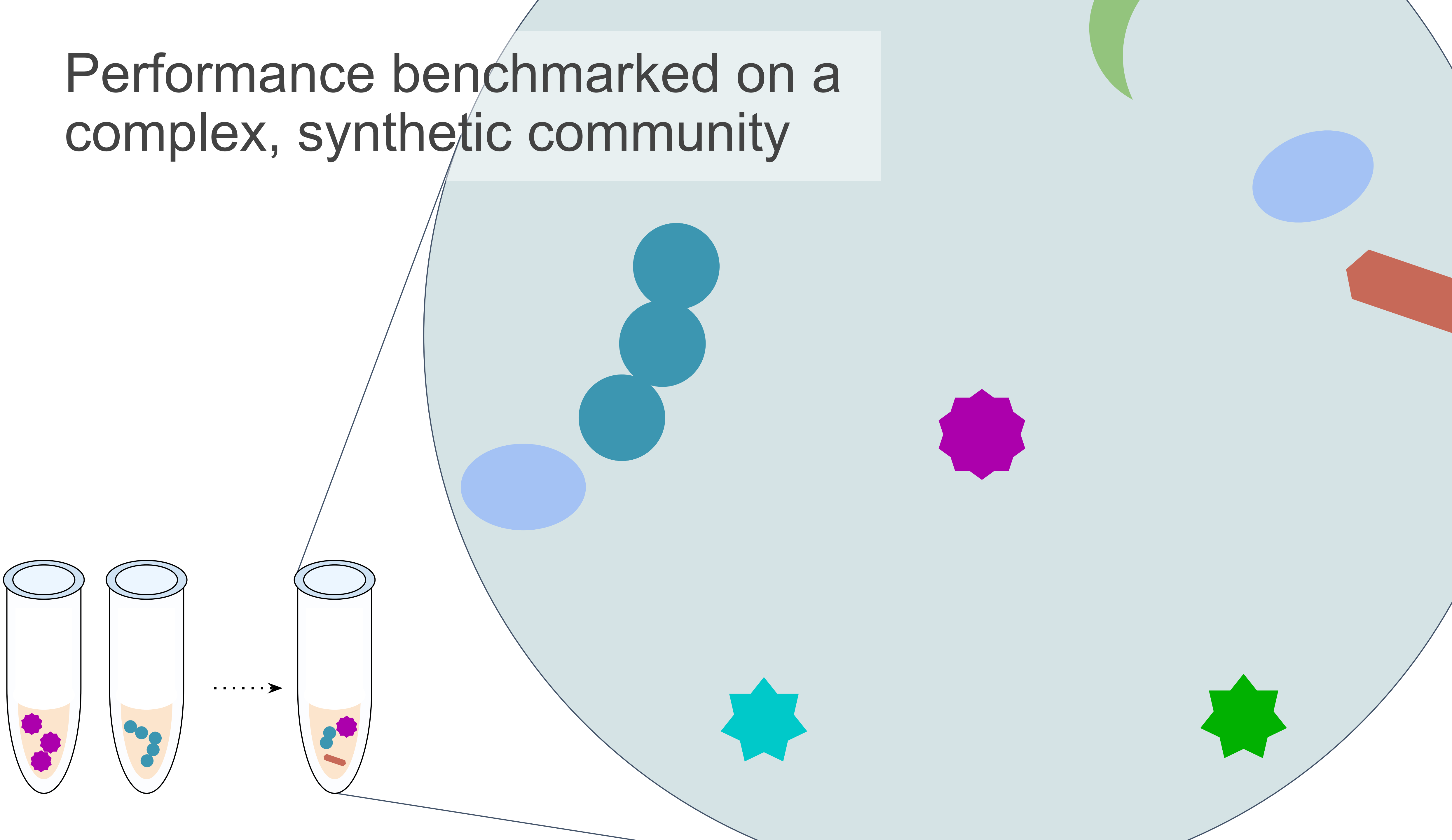
Strain-resolved discovery

Performance benchmarked on a complex, synthetic community



Antibiotic resistance genes are widespread in the gut microbiome

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- Detection can inform treatment

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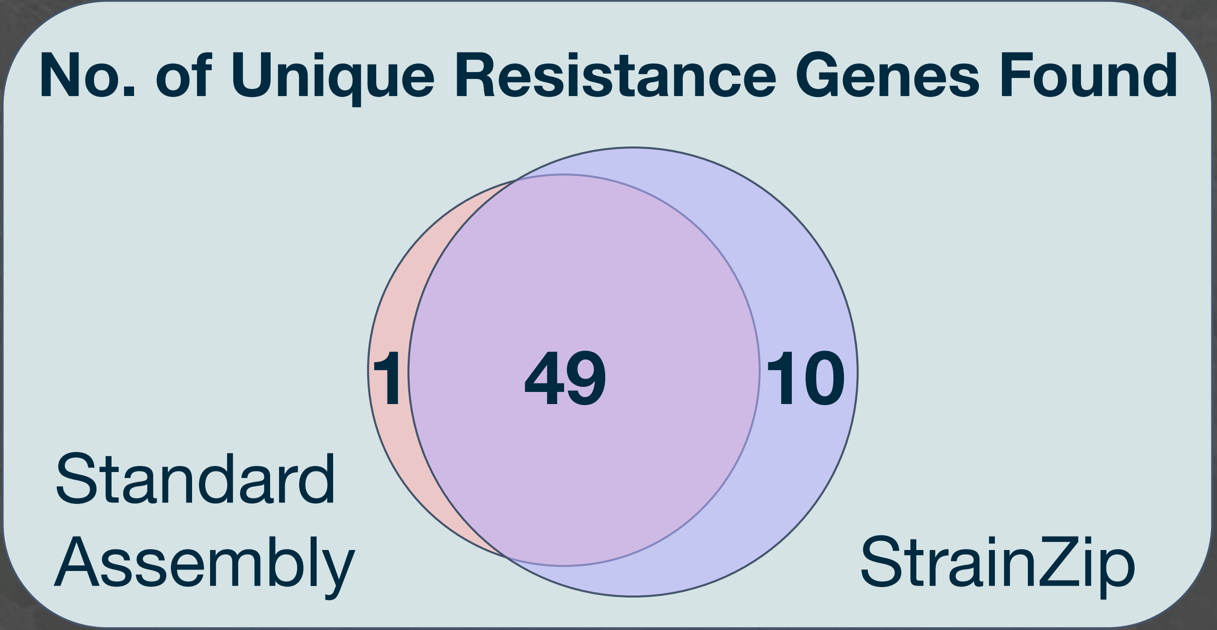
- Detection can inform treatment



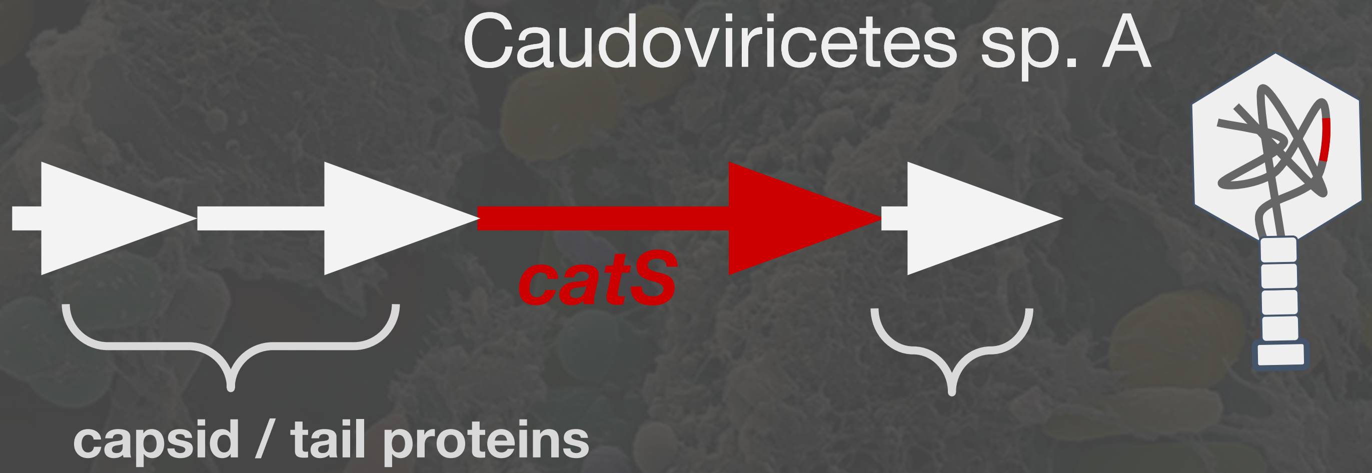
No. of Unique Resistance Genes Found

Antibiotic resistance genes are widespread in the gut microbiome

- Detection can inform treatment

Antibiotic resistance genes are widespread in the gut microbiome

- Detection can inform treatment
- Can be carried in phage genomes



Antibiotic resistance genes are widespread in the gut microbiome

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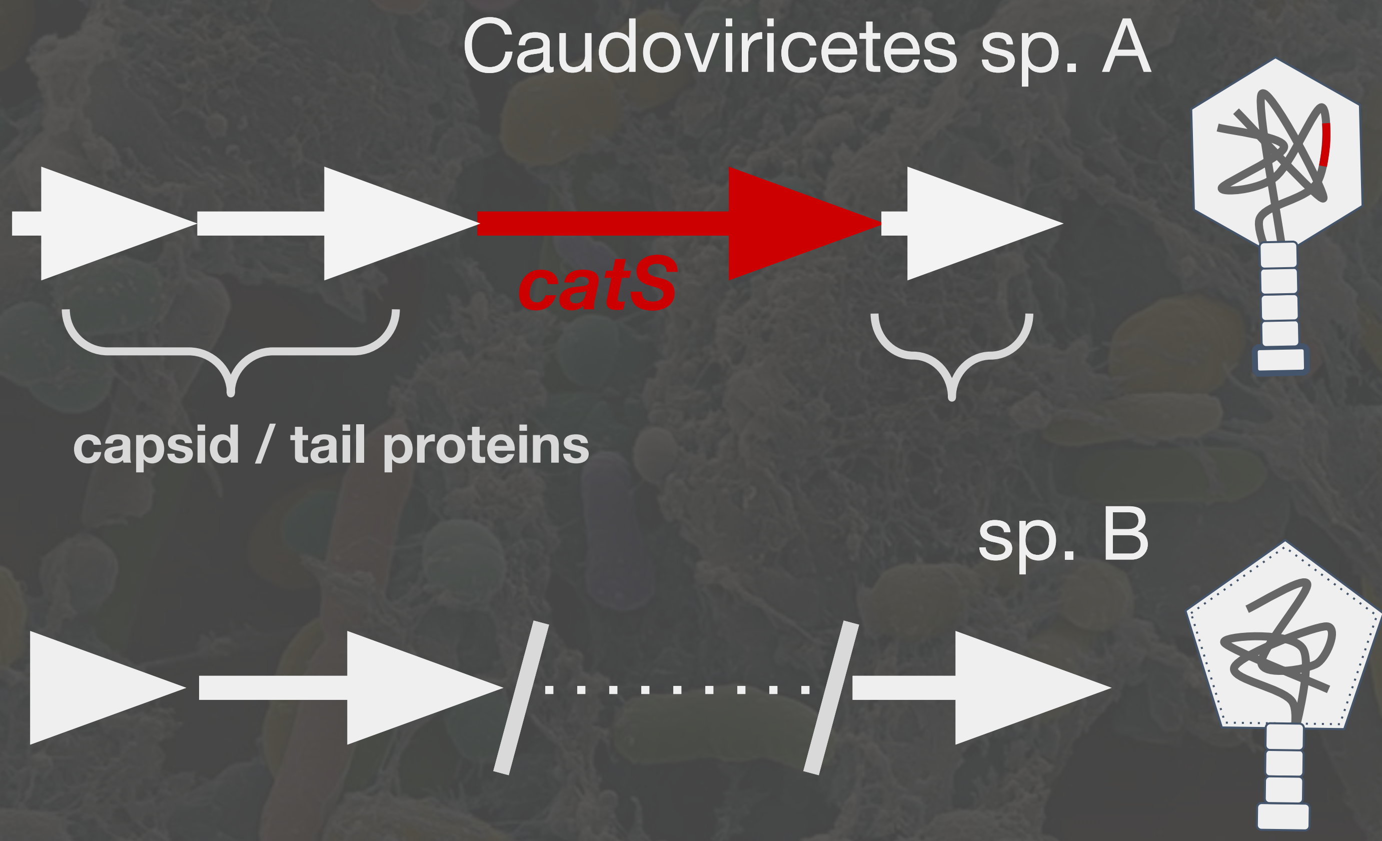
- Long sequence fragments provide useful information



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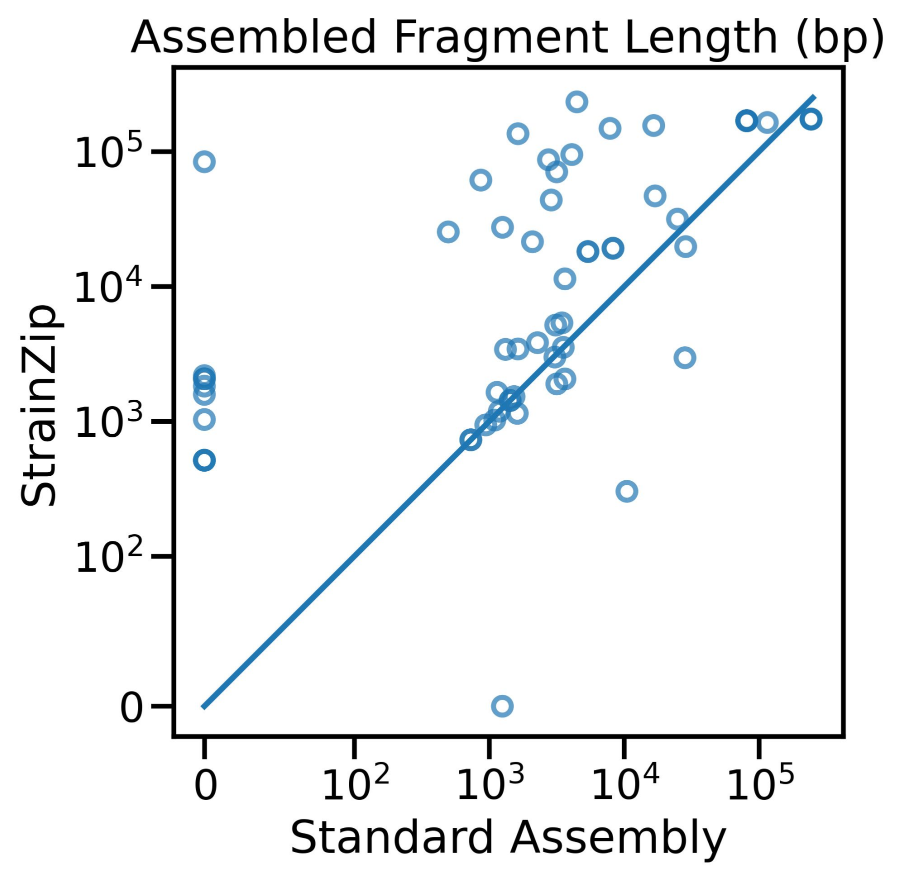
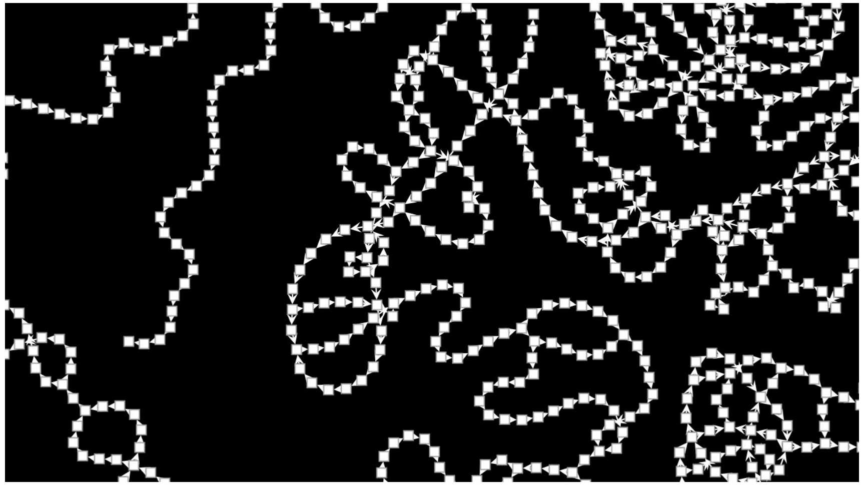
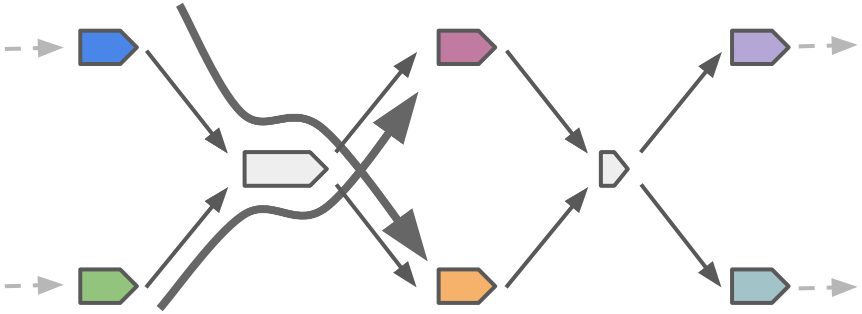


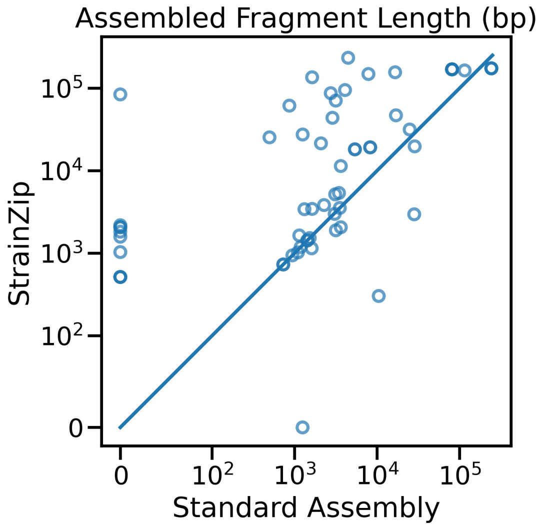
Complex Metagenome Graphs



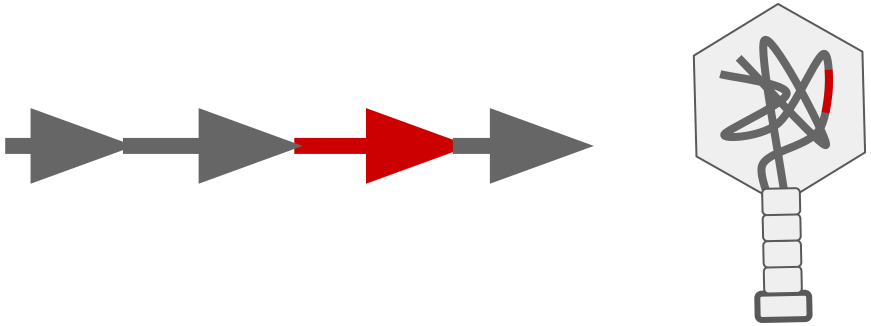
StrainZip Iteratively Unzips Junctions



Strain-Resolved Metagenomics



Antibiotic Resistance Potential of Phage



Gladstone Scientific Retreat 2024

Thank You!
Questions?