

Research in Progress

Establishing an Understanding of the Gut Virome

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Schloss Lab Research Meeting
May 18, 2016

Objectives

- Introduction to some ongoing projects
- Identify gaps in data presentation
- Share preliminary data
- Get valuable feedback through discussion

Outline

- Phage - bacteria infectious interaction modeling
- Establishing gut virome protocol

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Why Do We Care?

- Host range is crucial for describing a virome, but currently limited by narrow annotation.
- Phages are important mediators of horizontal gene transfer (transduction) but we lack a “road map” of origins and destinations.
- Prediction of contig hosts will allow for identification/characterization independent of nucleotide similarity to known phages (important due to lack of references).

Potential Applications

- Found understanding of phage tropism nestedness across populations and within the gut.
- Map potential avenues of transduction between bacteria (e.g. antibiotic resistance genes).
- Understand mechanisms of population control by broader phage predation.
- Properly identify phages with acknowledgement of broader host tropism. Addressing problems with current taxonomic classifications.

Predicting Infectious Interactions

Existing Approaches

- CRISPR Targeting
- Nucleotide Similarity Between Phages & Bacteria
- Gene Similarity Between Phages & Bacteria

Predicting Infectious Interactions

Existing Approaches

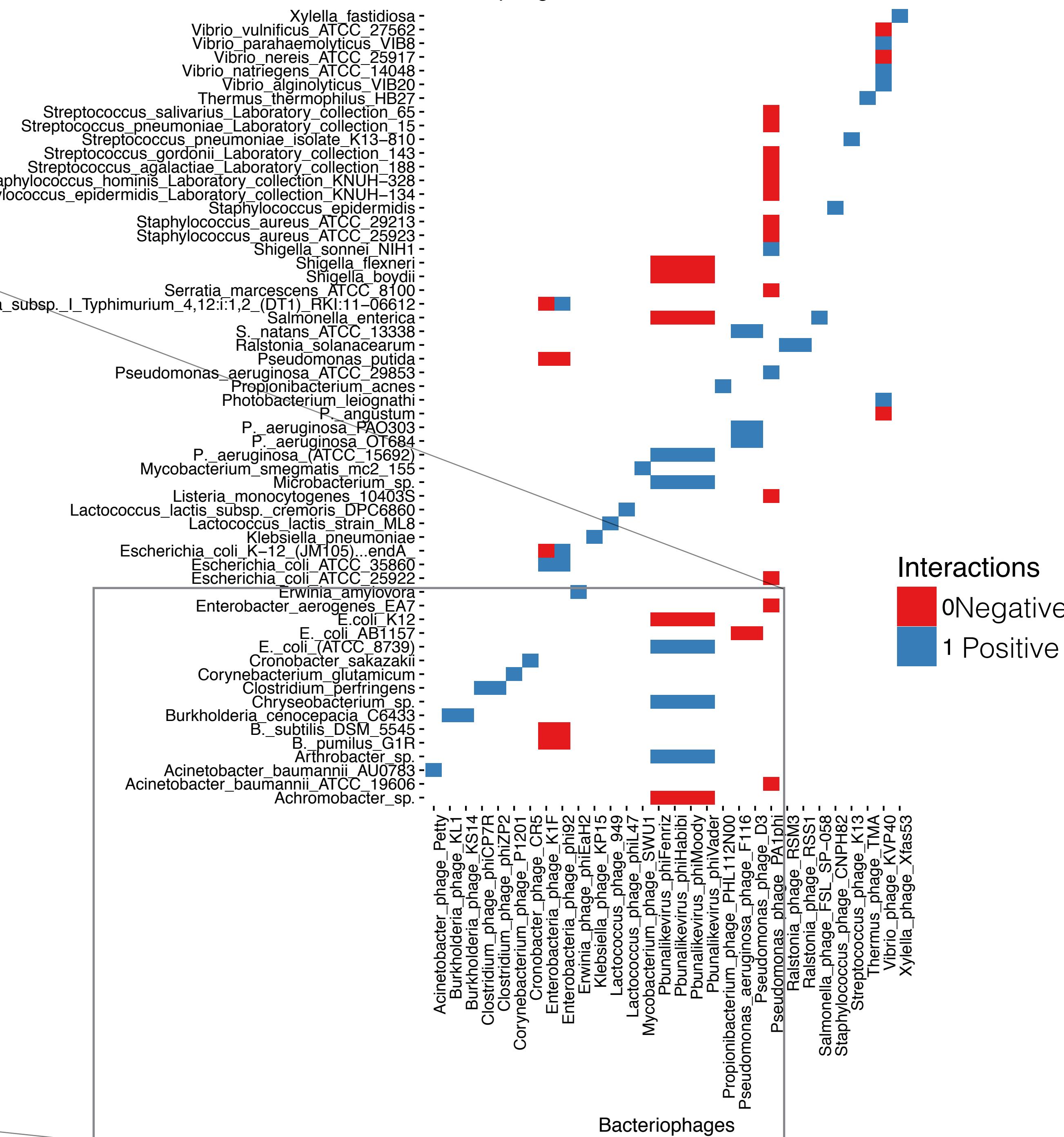
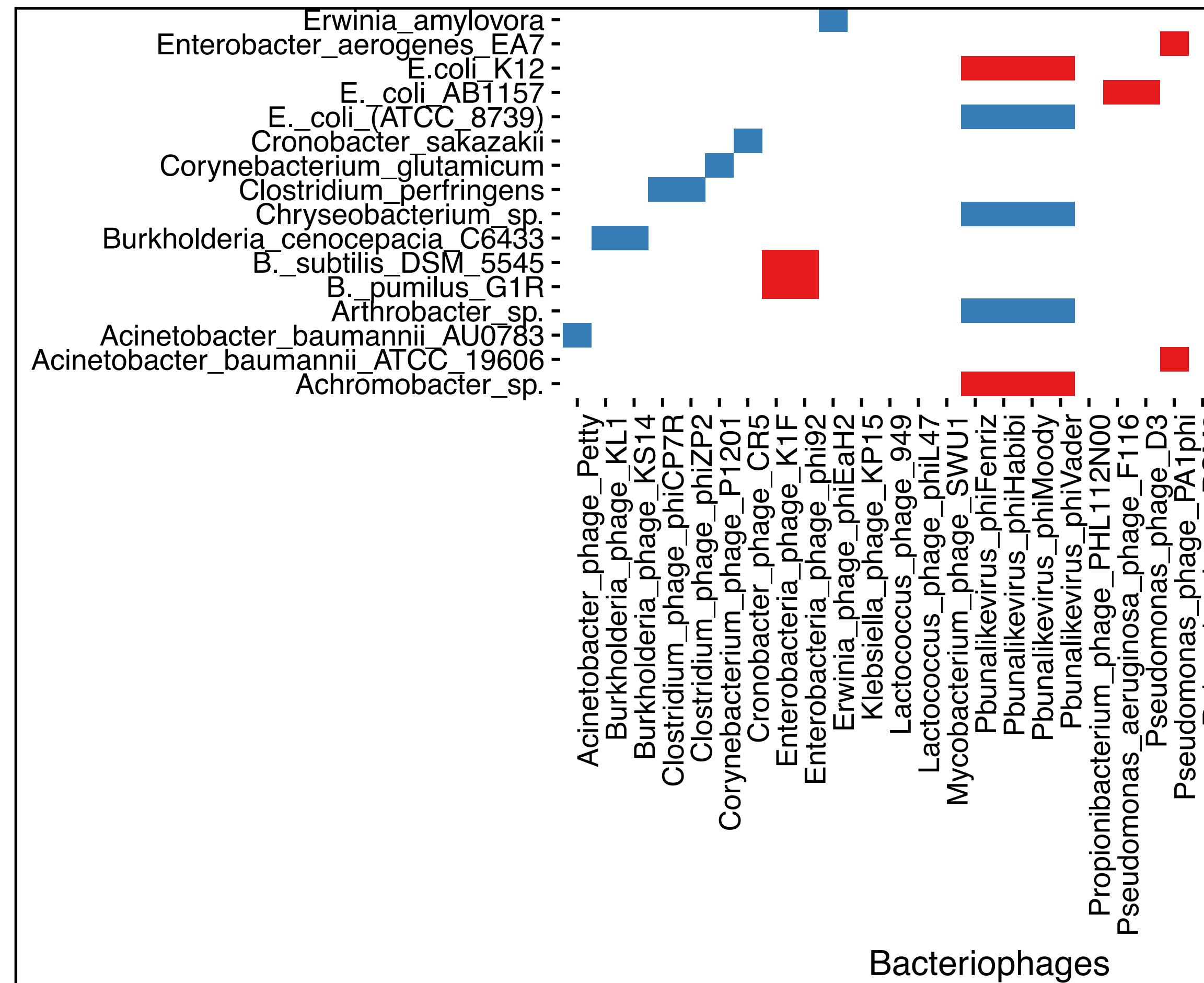
- CRISPR Targeting
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Our New Approaches

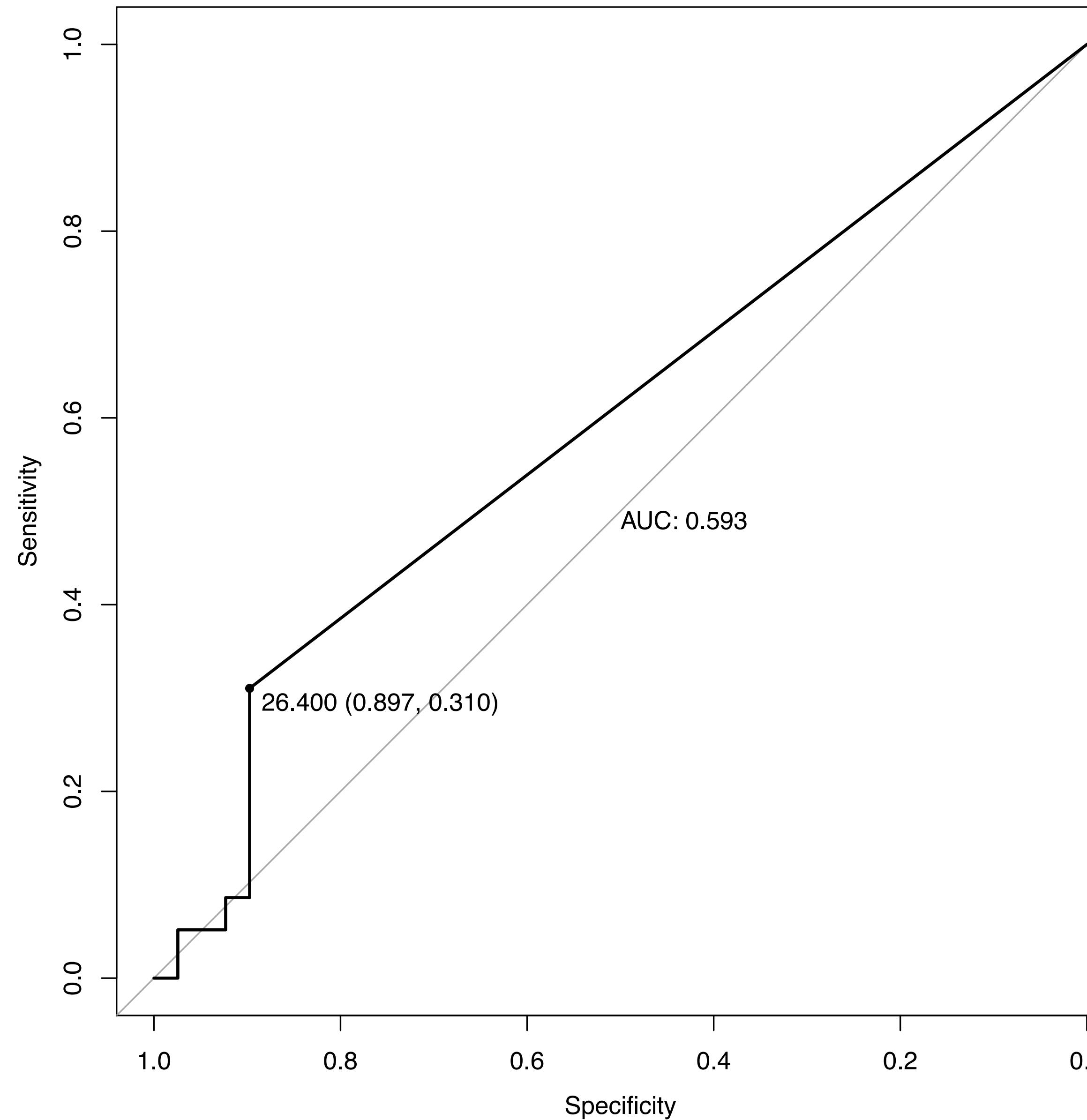
- Connecting Established PFAM Interactive Domains
- Connecting Established Uniprot Genes
- Establishing Gene Presence Correlations

Experimentally Validated Interaction Benchmarking.

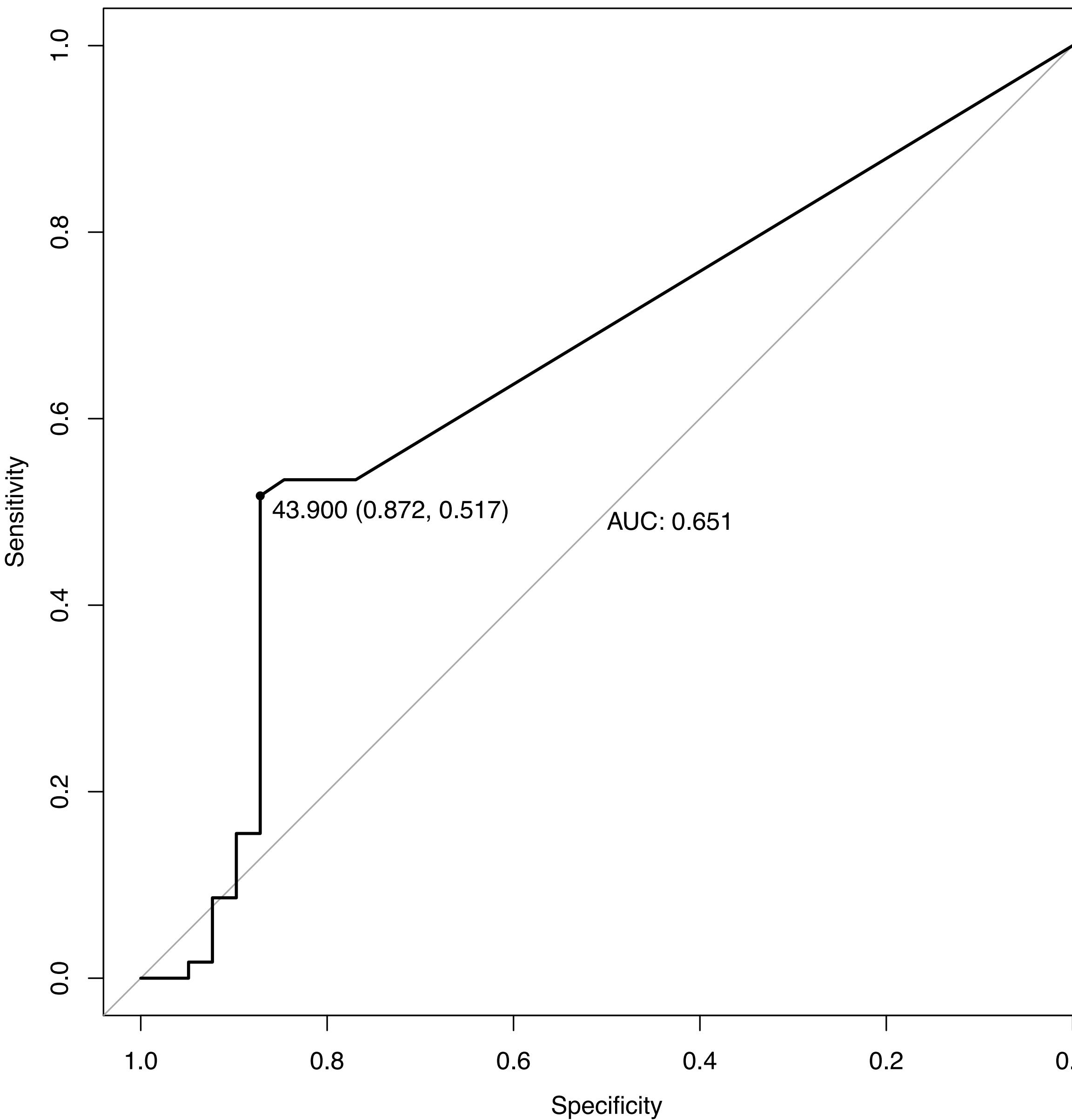
Bacteriophage – Bacteria Benchmark Interactions



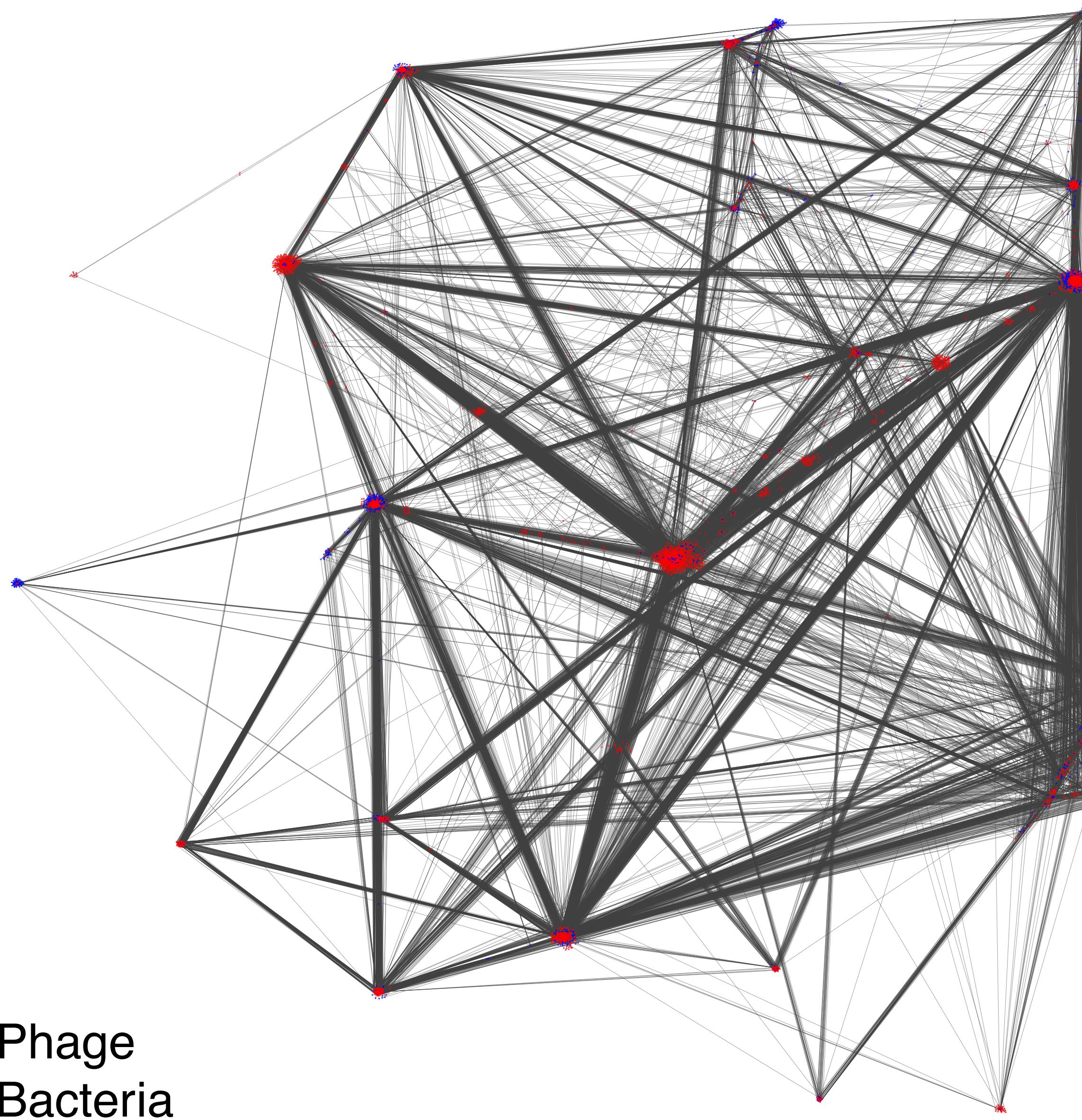
Predicting Known Interactions



Improved Prediction with Protein Blast Similarity

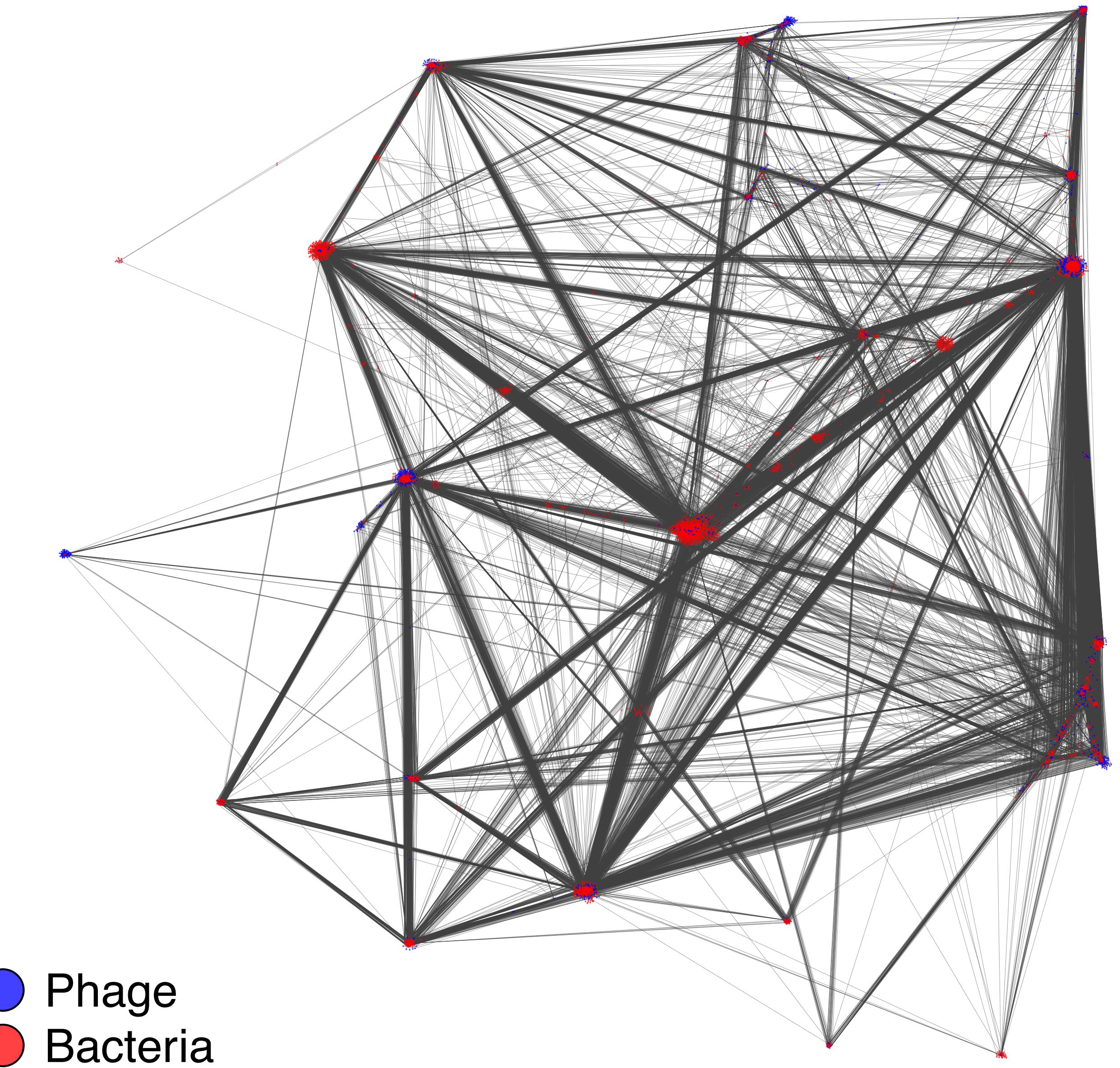


Using Predictions to Build a Network



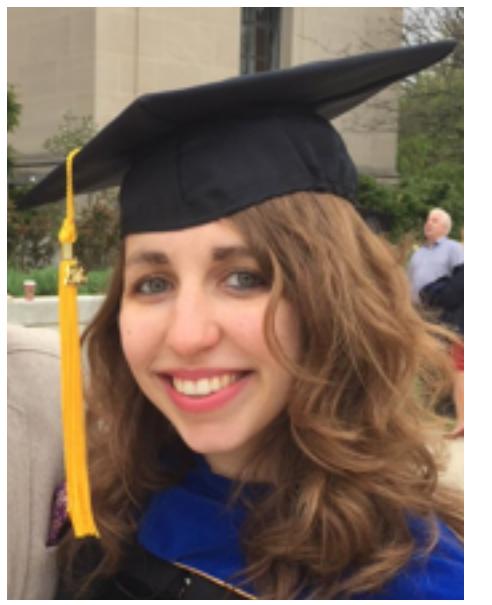
Using Networks to Understand Biology

- Clustering by bacterial taxa
- Crossover between clusters by broadly infectious phages
- More accurate definitions of phages by their hosts
- Powering answers to tropism patterns and transduction



Example: Connecting Bacteria by Shared Phages

Triadic Closures



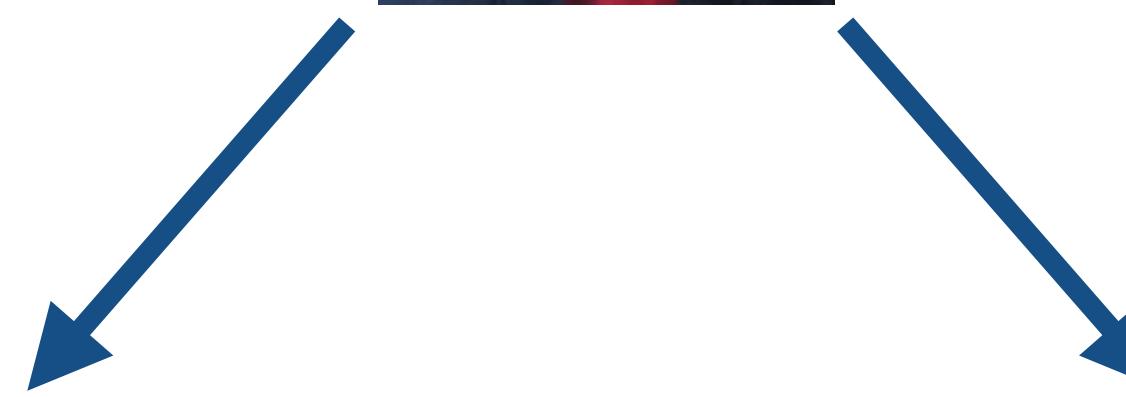
Example: Connecting Bacteria by Shared Phages

Triadic Closures



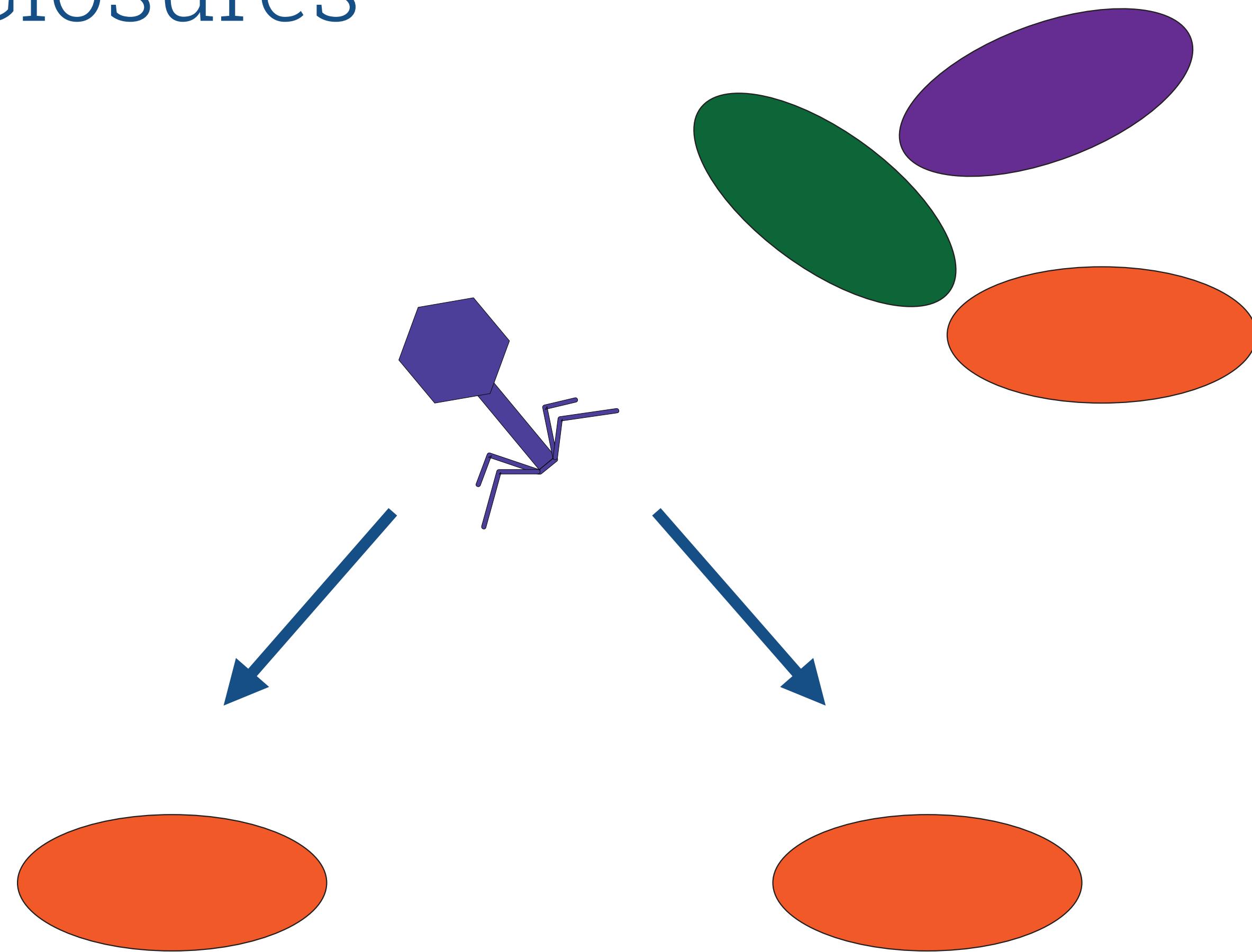
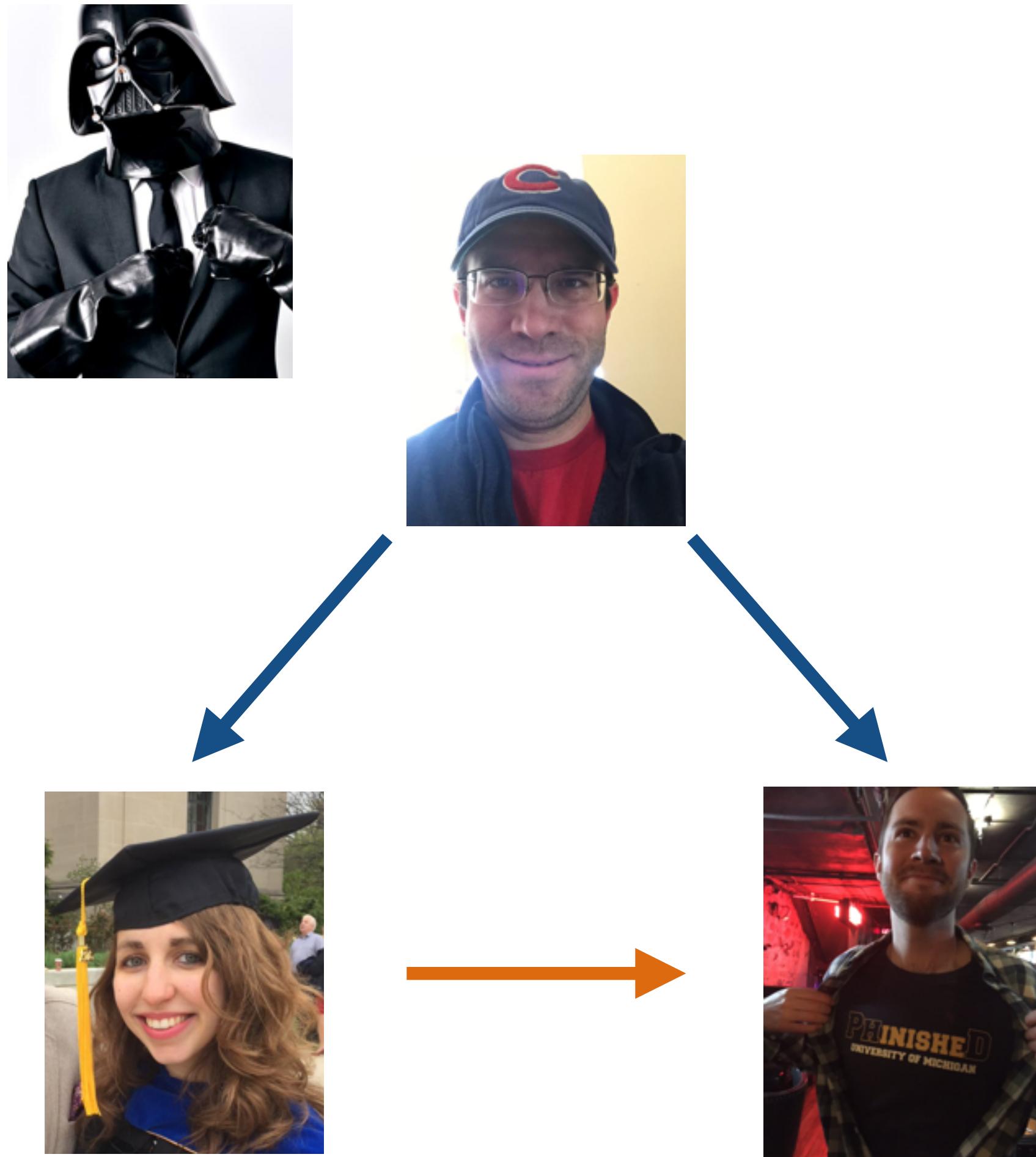
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Triadic Closures



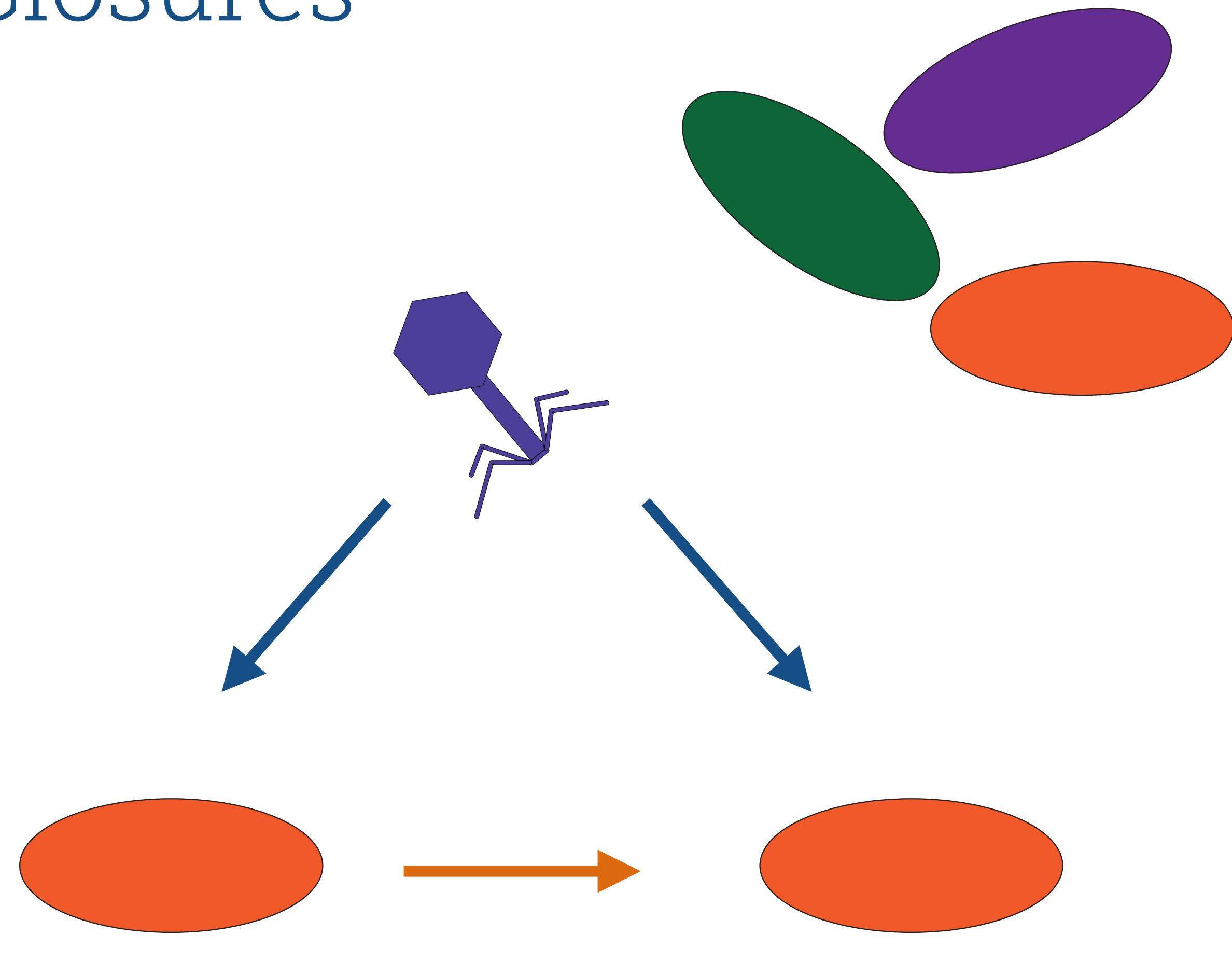
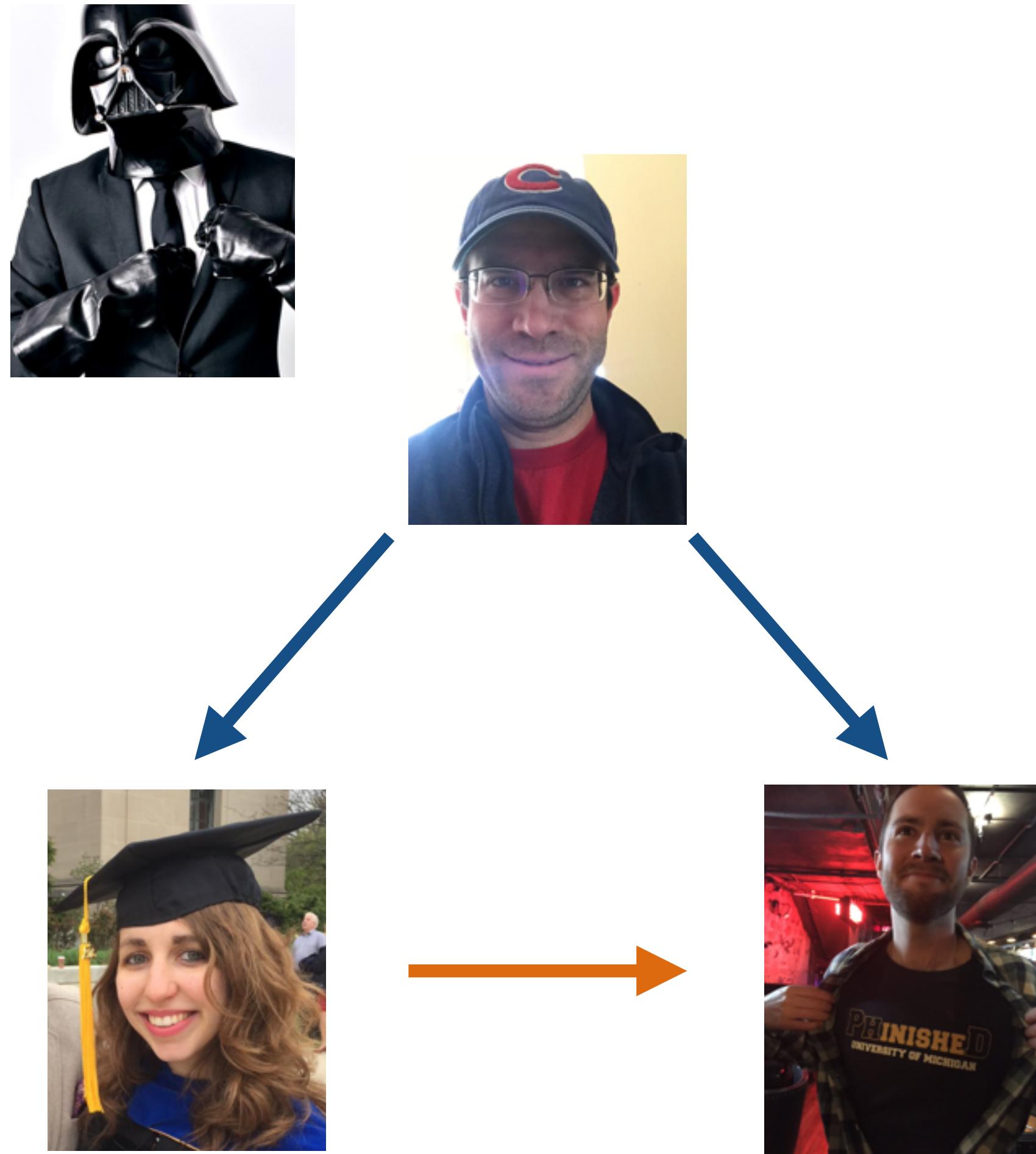
Example: Connecting Bacteria by Shared Phages

Triadic Closures

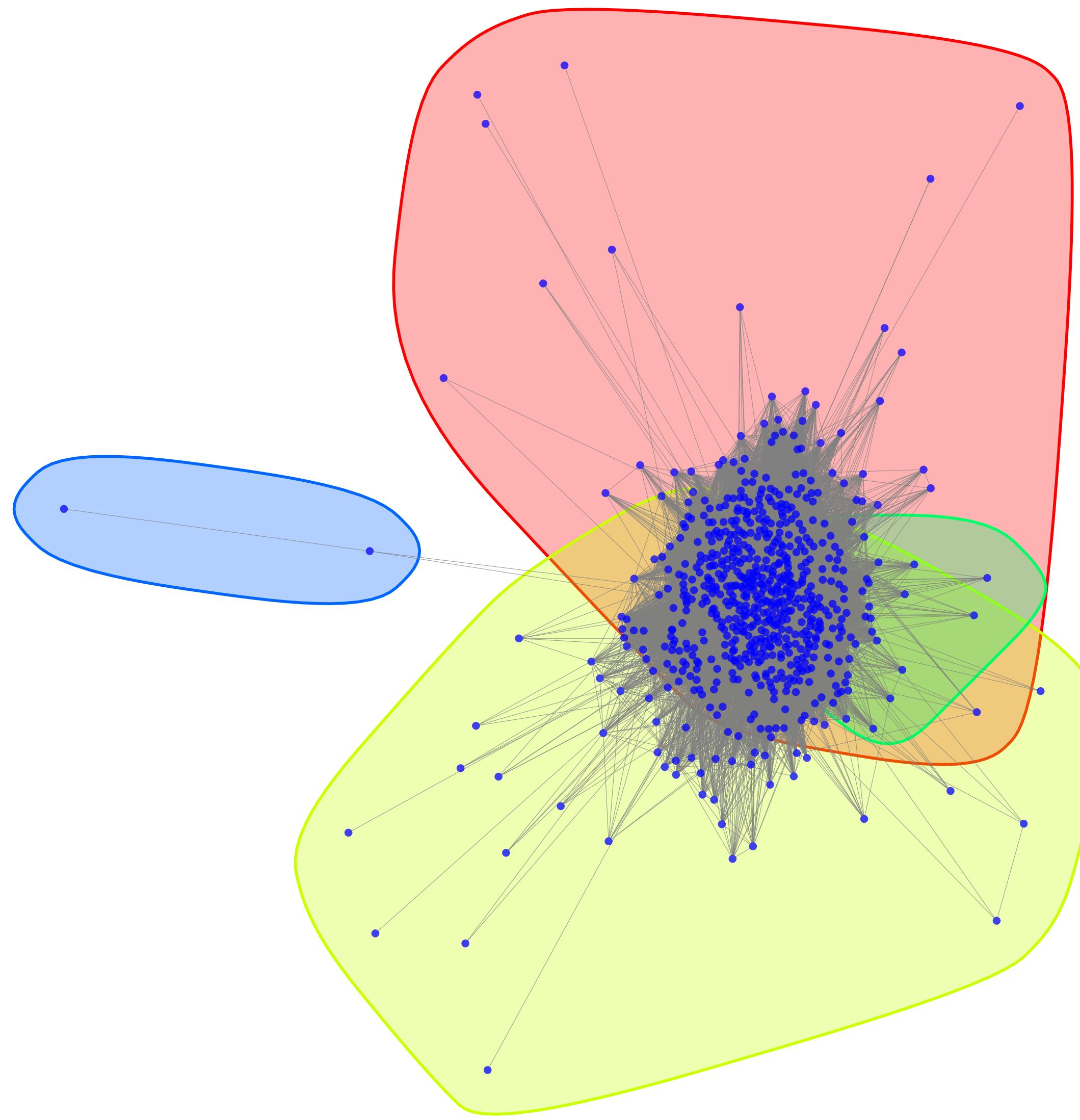


Example: Connecting Bacteria by Shared Phages

Triadic Closures



Bacteria Cluster By Shared Phages



Conclusions

- We can identify a portion of interactions (TP=51%) while avoiding 9 in 10 false positive interactions (TN=87%).
- Clustering reveals a potential nested trophic structure with predatory crosstalk.
- We can cluster bacteria by their shared predators (transducers).

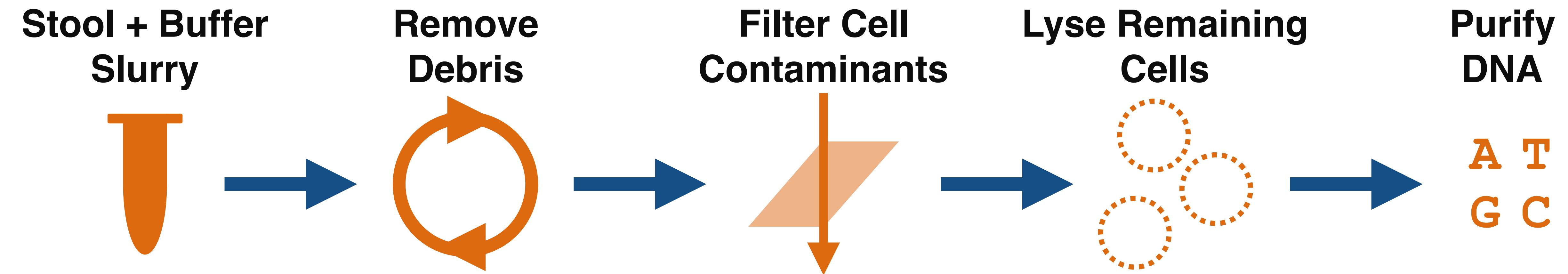
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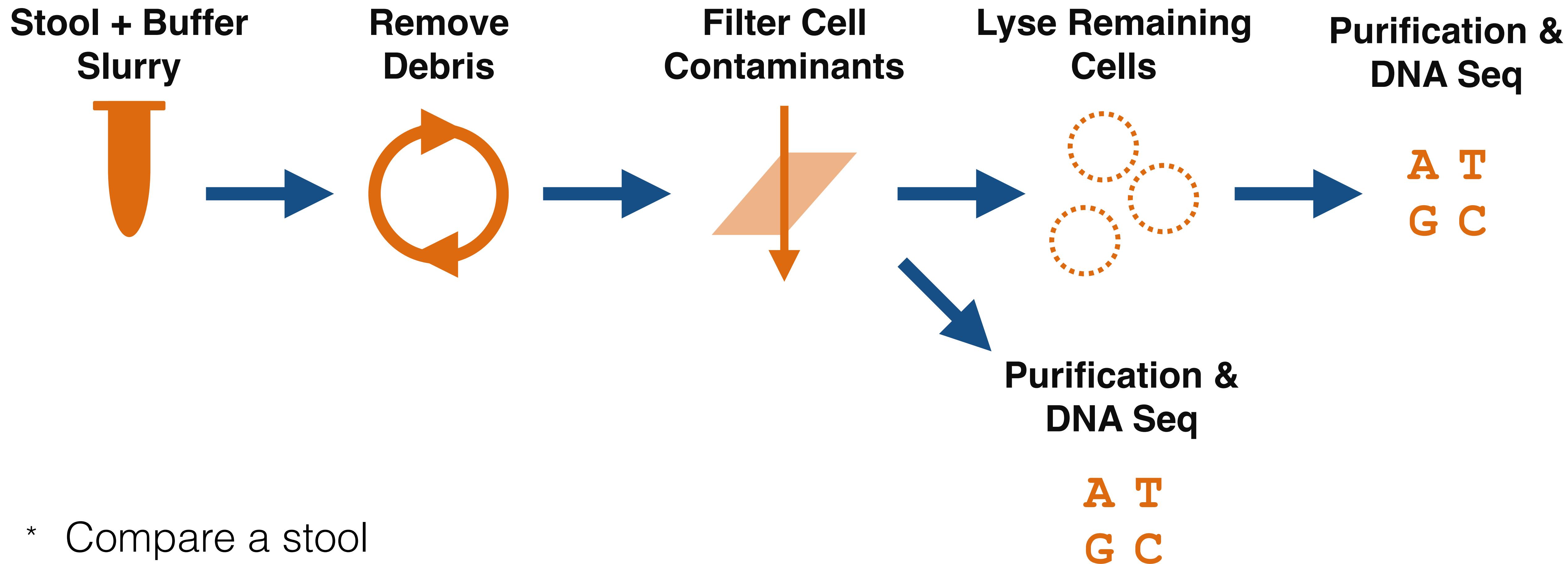
Why Do We Care?

- We cannot (properly) study the virome without a functional purification and sequencing protocol.
- Our data and analyses will benefit from a protocol optimized for purity (garbage in, garbage out).
- We can inform experimental design with an understanding of biomass limitations.

Virome Purification Protocol

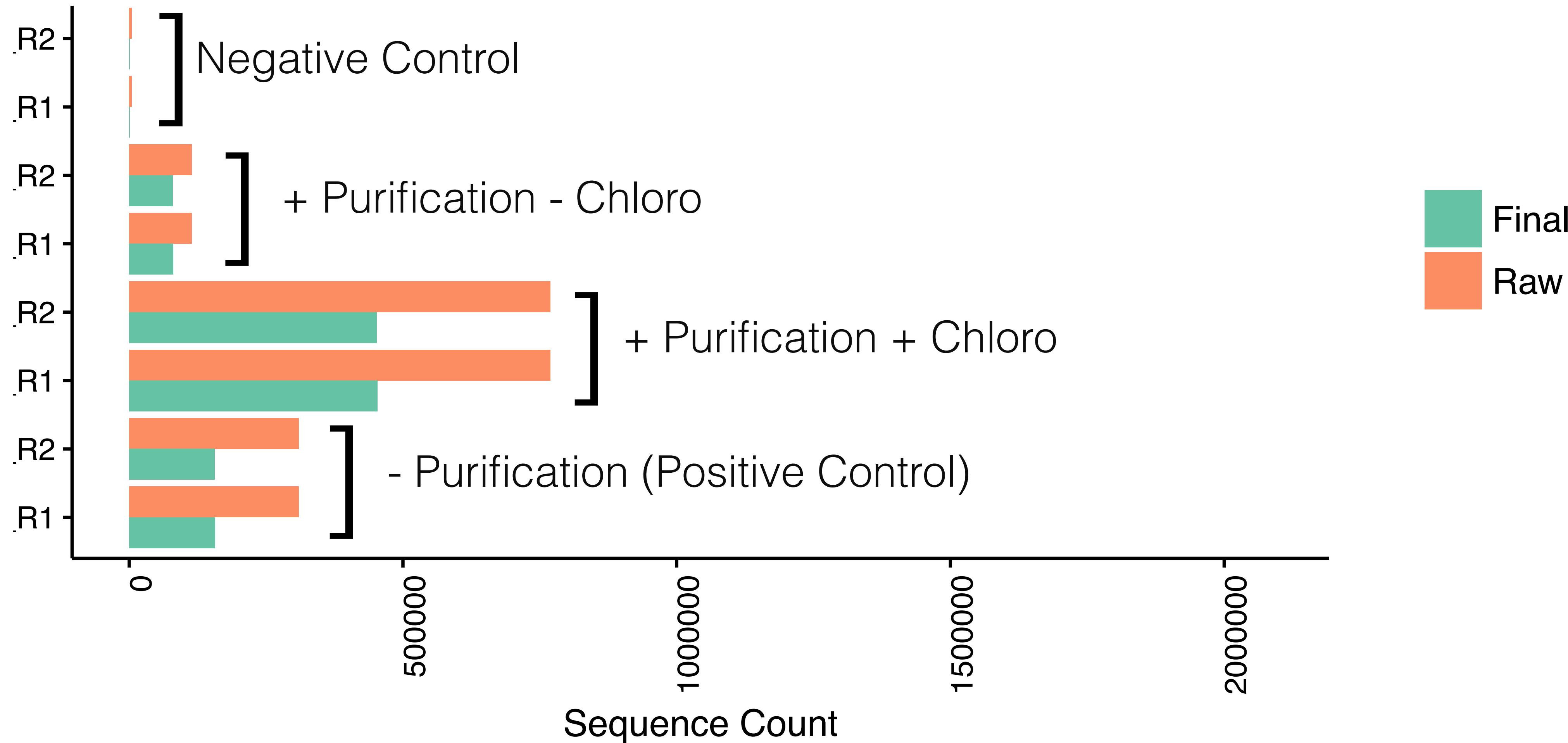


Impact of Chloroform Treatment

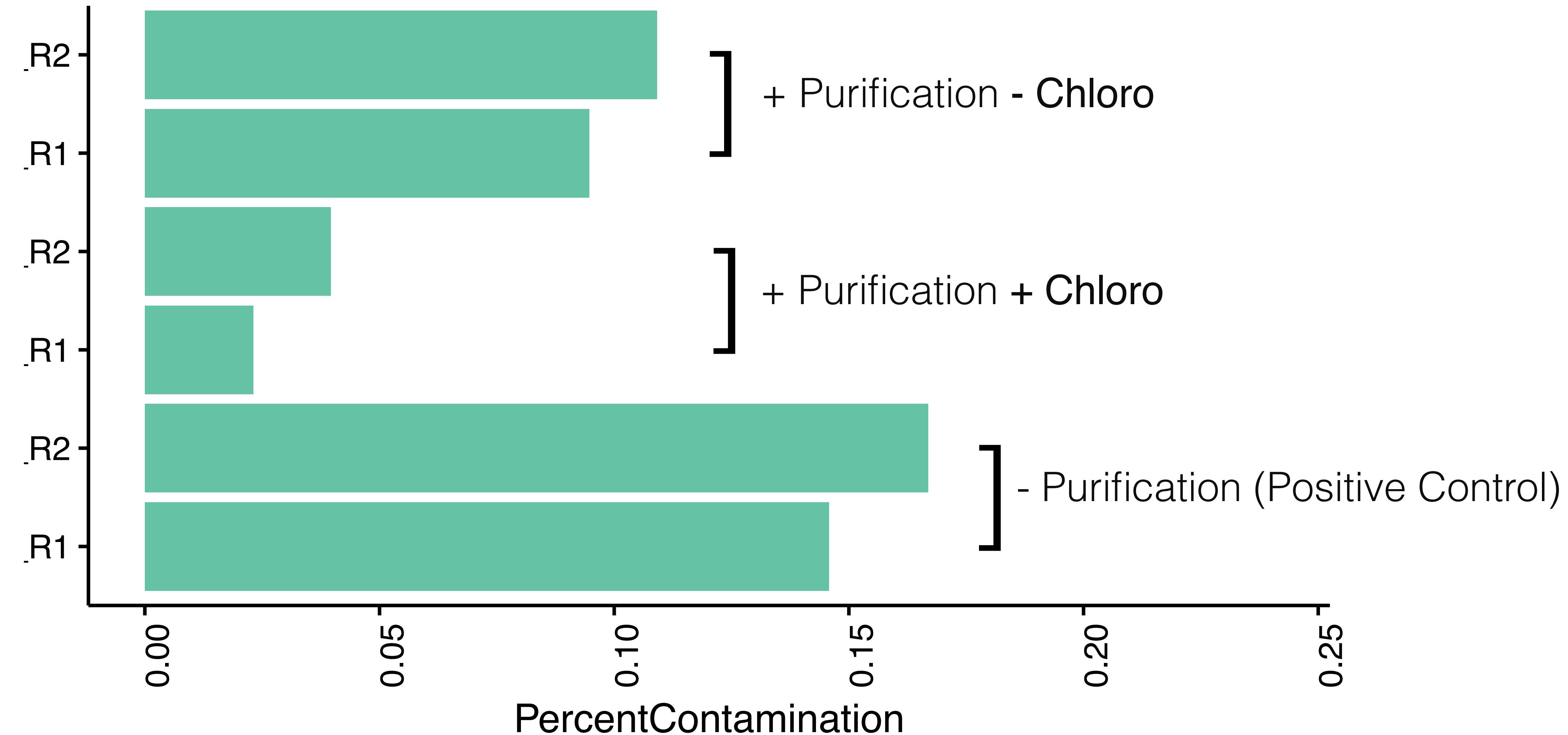


* Compare a stool slurry for continuity.

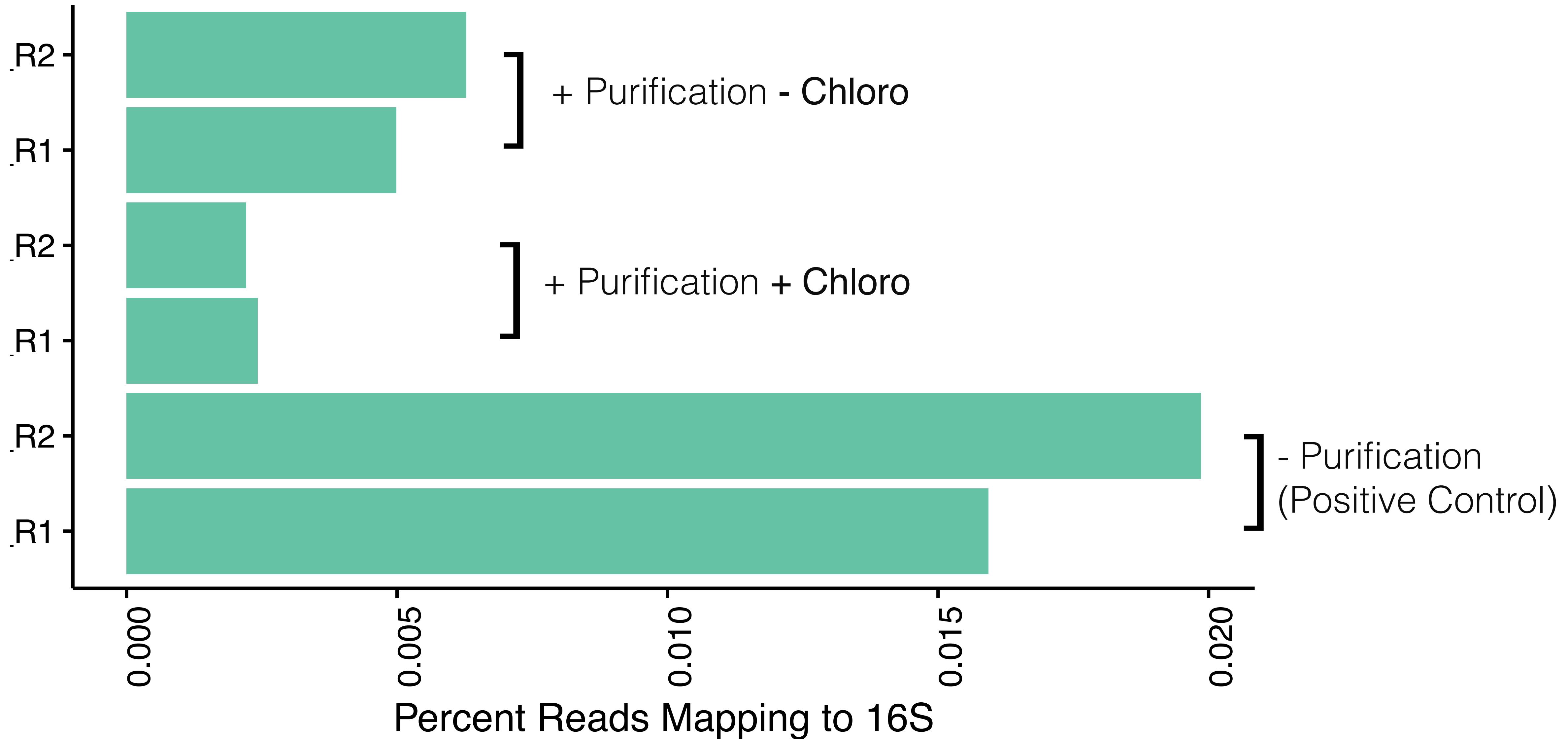
Verifying QC Sequence Count



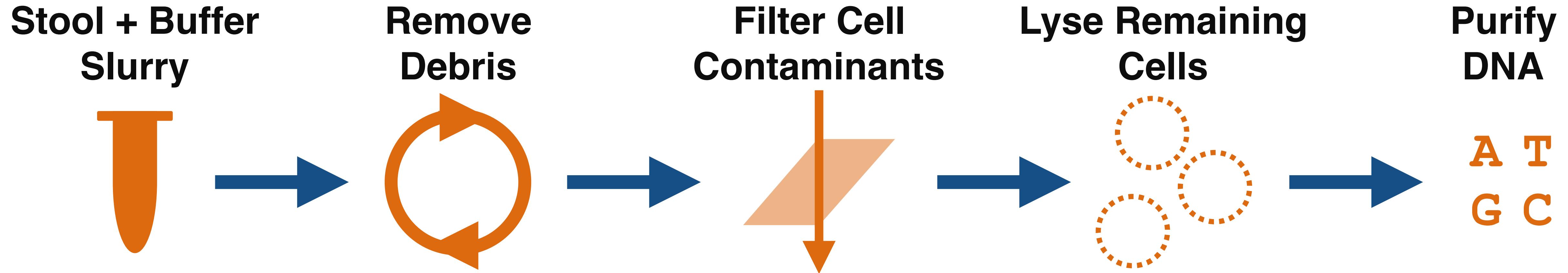
Chloro Reduces Mouse Contamination



Chloro Reduces Bacterial Contamination



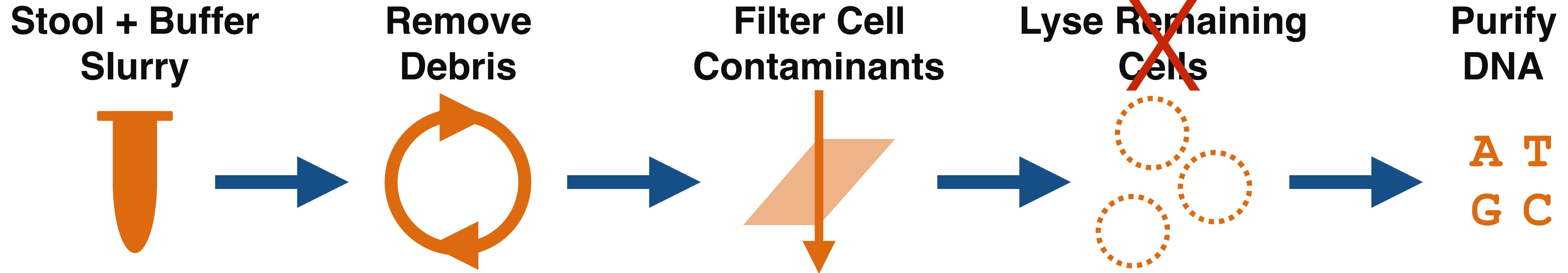
Defining the Sampling Threshold



Measuring Required Mouse Fecal Pellets



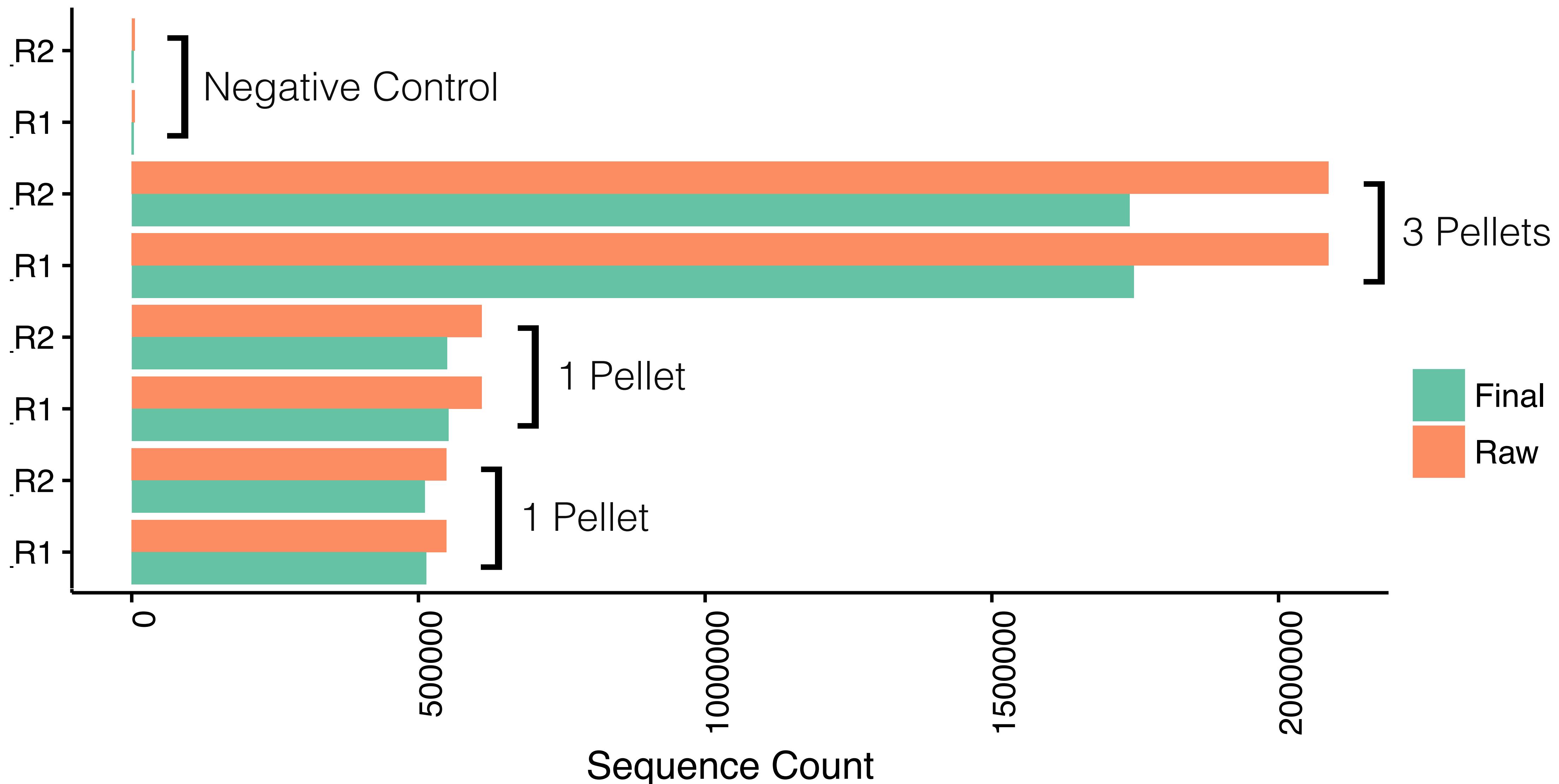
Defining the Sampling Threshold



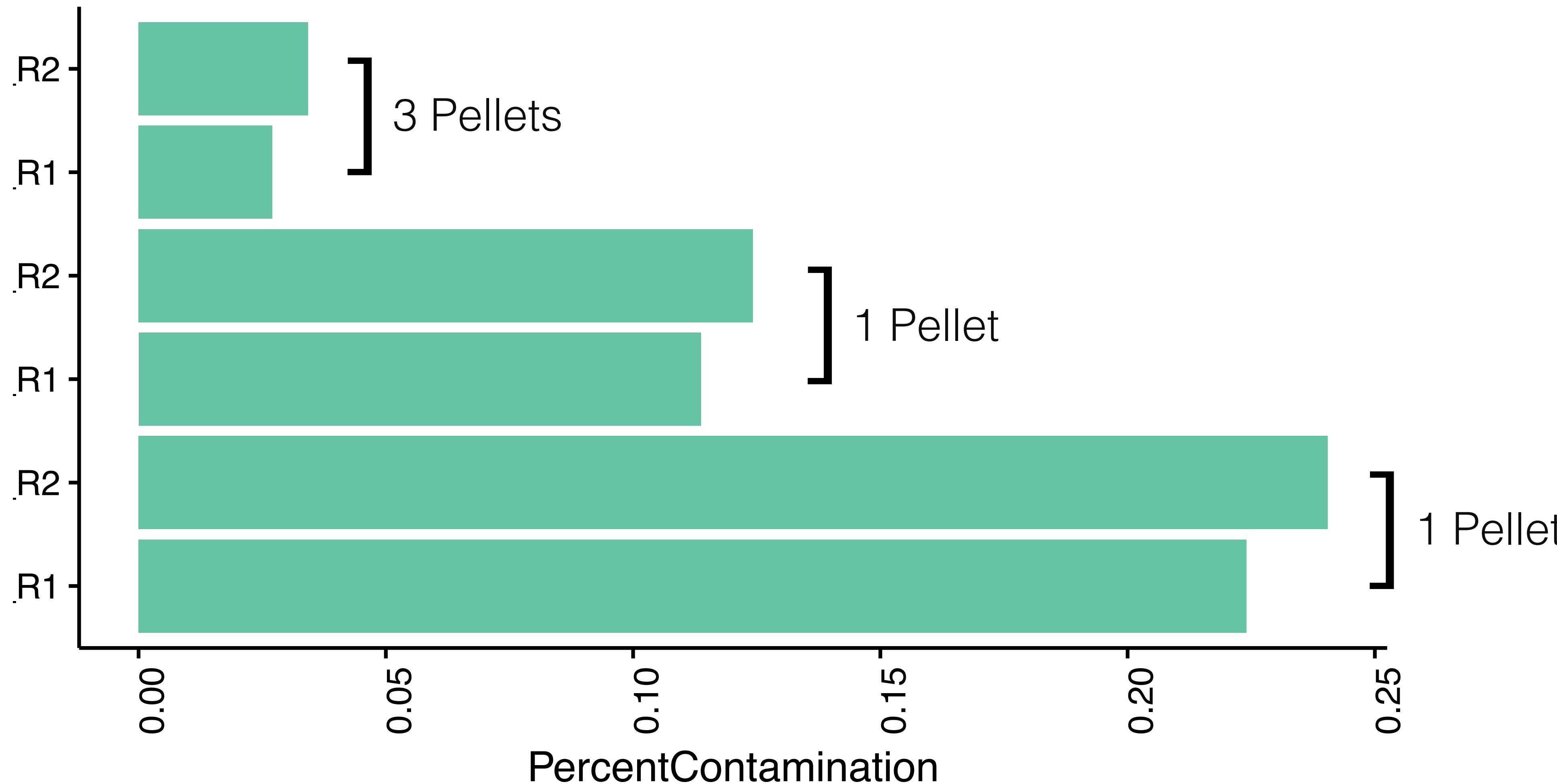
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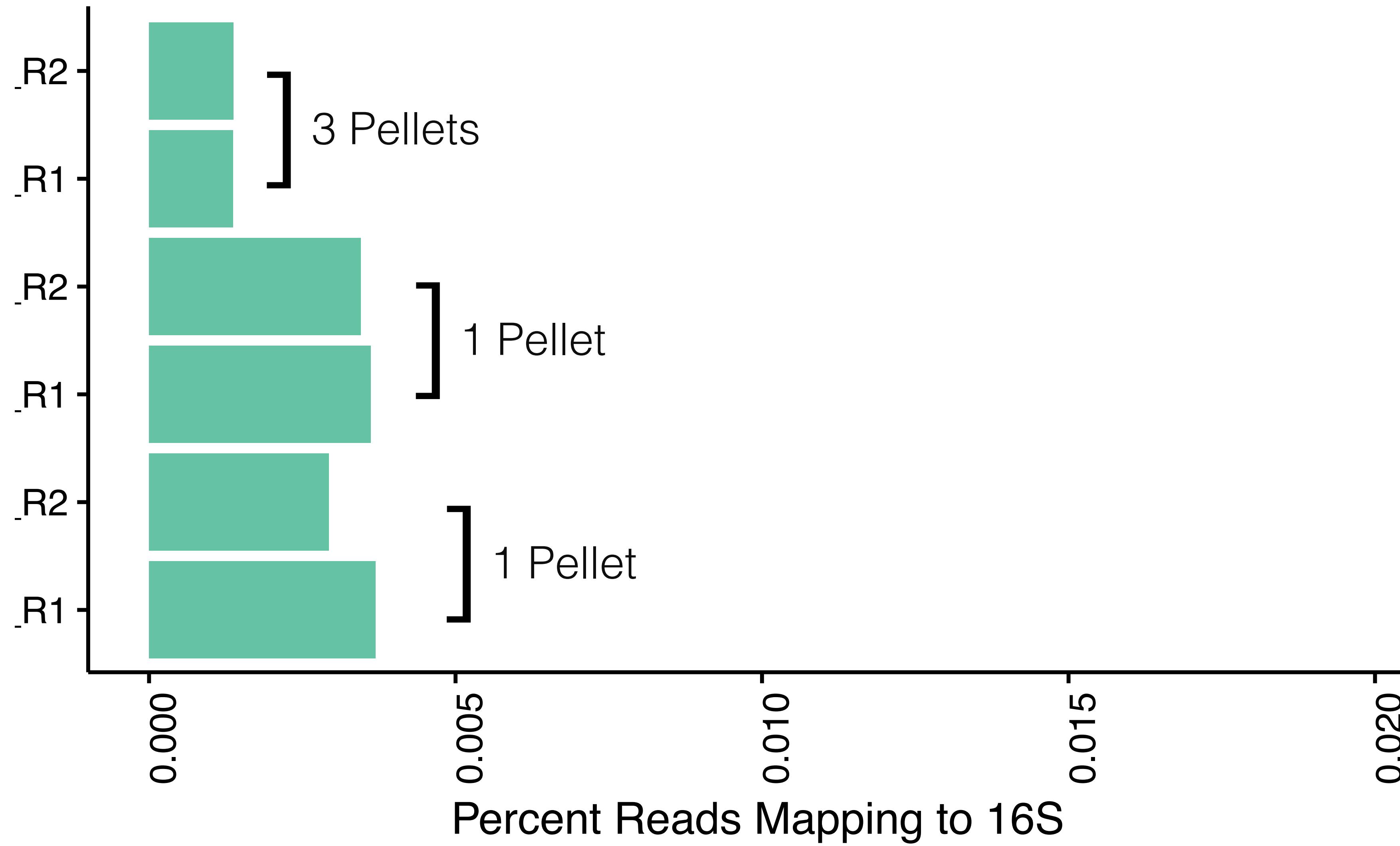
Single Pellets Result in Quality Sequences



3 Pellet Samples Had Least Mouse DNA



Comparable Bacterial DNA



Conclusions

- Caveat: pilot data.
- Chloroform treatment yields purer virome DNA (less contamination).
- Minimum of one mouse stool pellet (~0.05 g) is required for sequencing.

Future Directions: Building on our Foundation

- Network methods for virome analysis
- The colon cancer virome
- C diff virome
- Cystic fibrosis virome
- Phage therapy effects and utility as tools



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