

Research in Progress

Establishing an Understanding of the Gut Virome

Geoffrey Hannigan
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

Outline

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

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

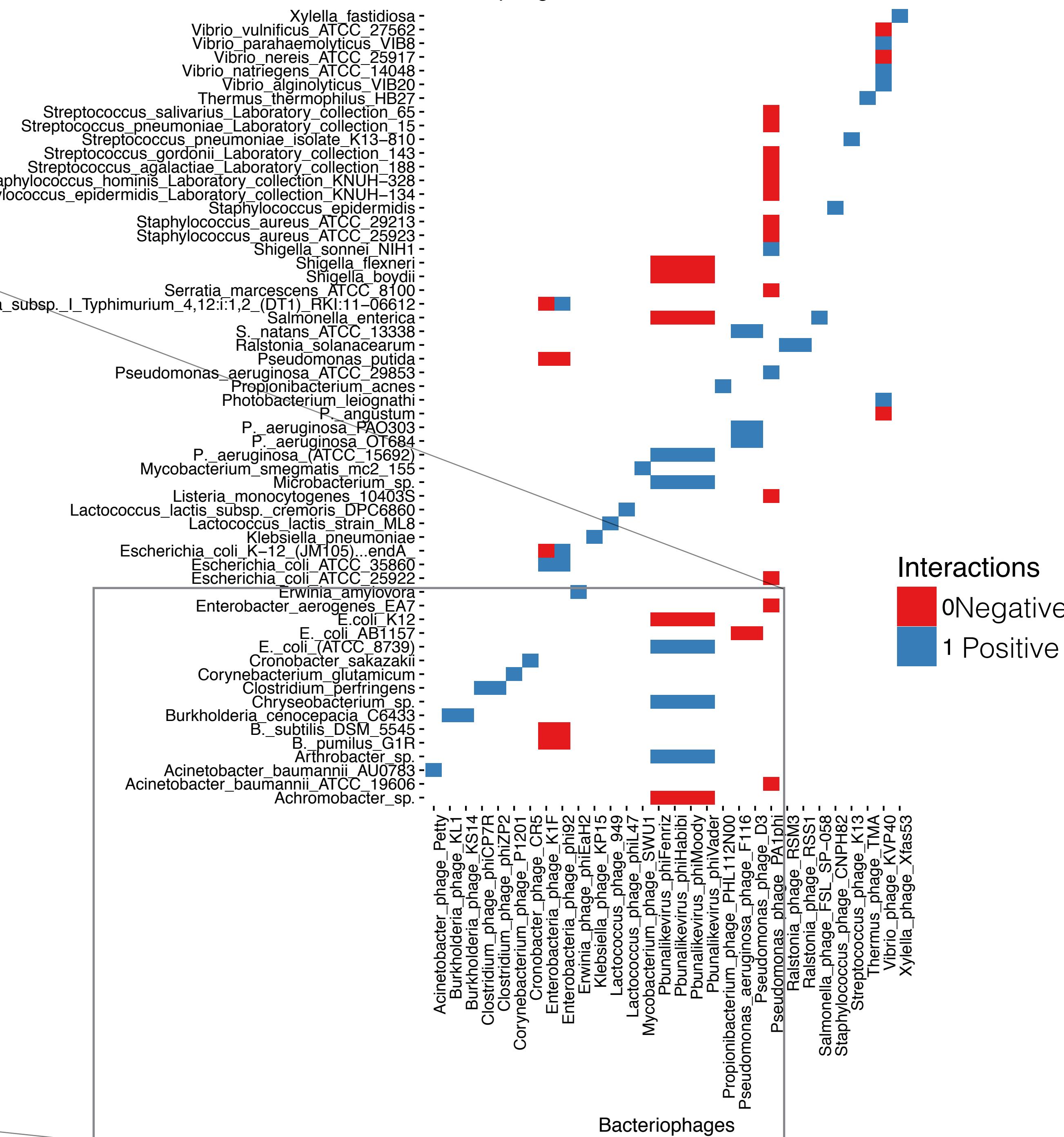
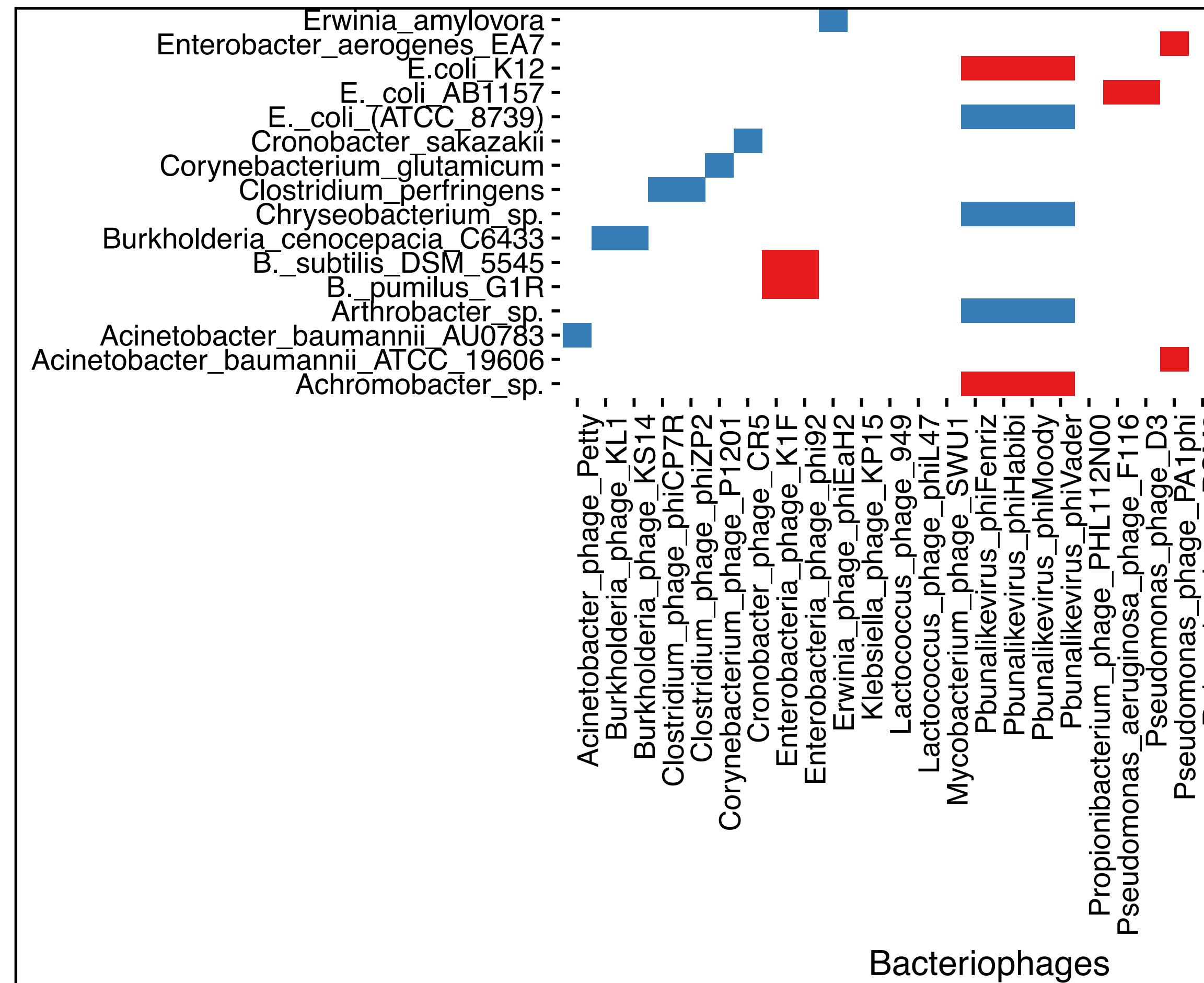
- CRISPR Targeting
- Nucleotide Similarity Between Phages & Bacteria
- Gene Similarity Between Phages & Bacteria

Our New Approaches

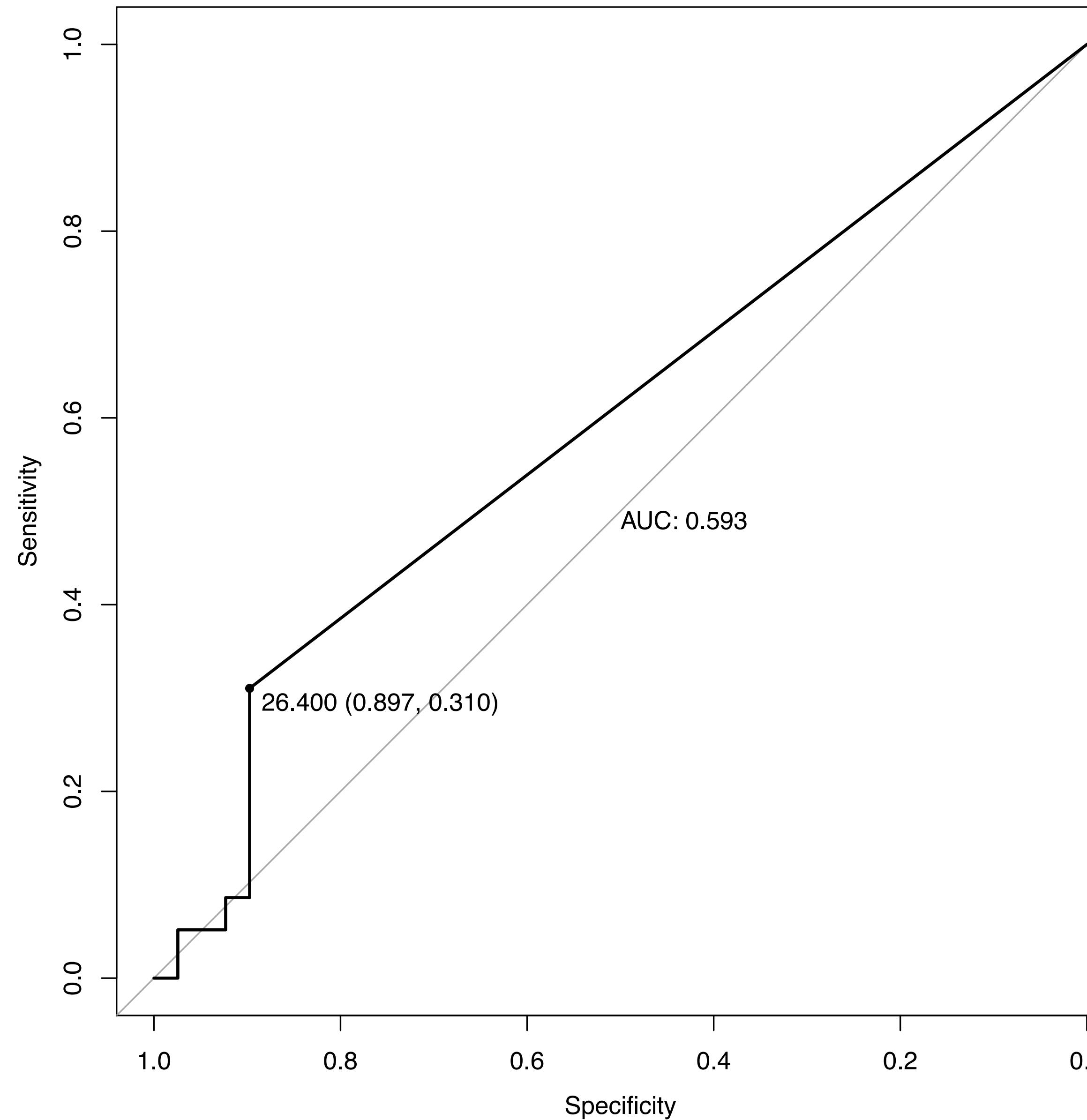
- Connecting Established PFAM Interactive Domains
- Connecting Established Uniprot Genes
- Establishing Gene Presence Correlations

Experimentally Validated Interaction Benchmarking.

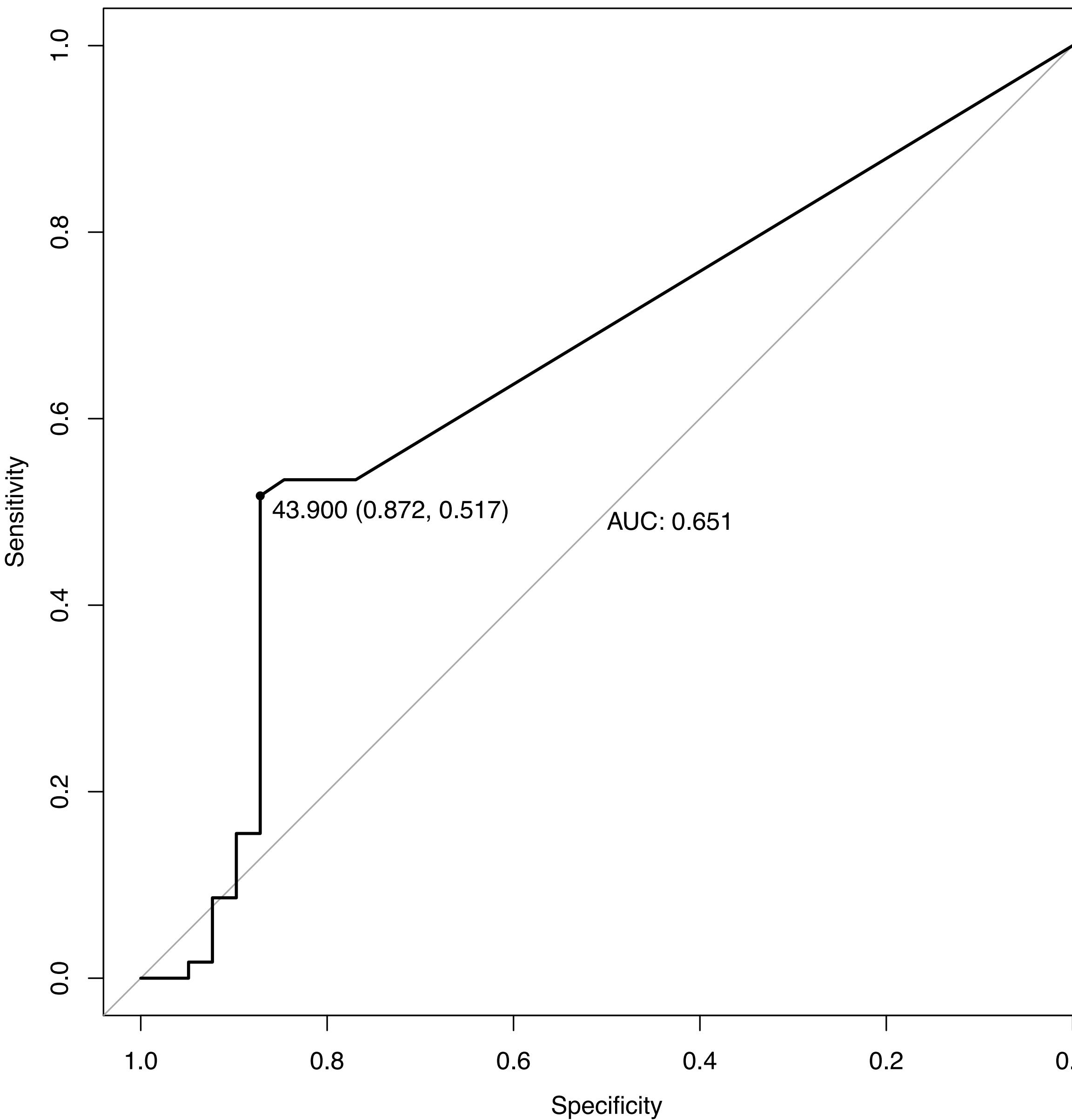
Bacteriophage – Bacteria Benchmark Interactions



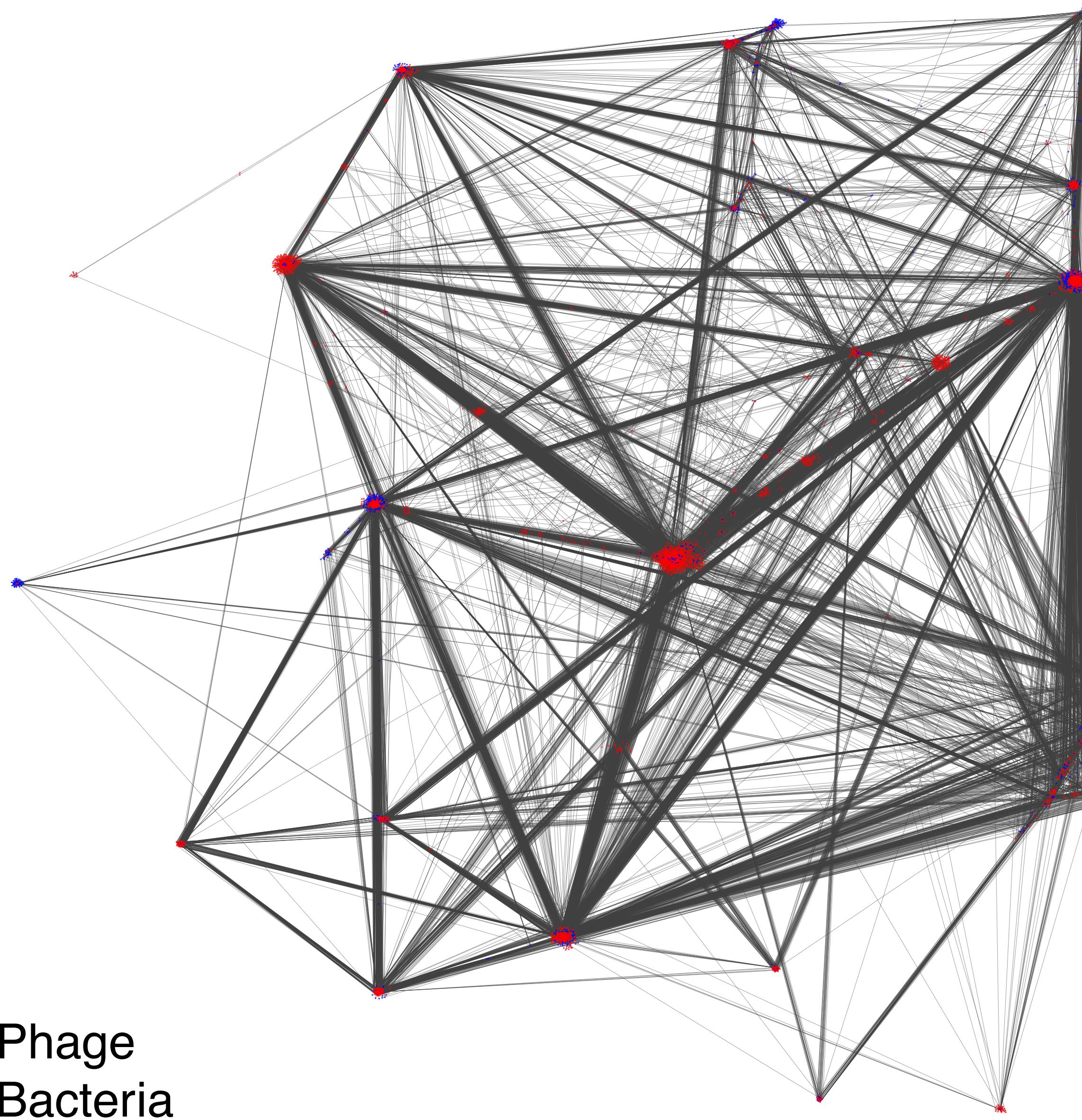
Predicting Known Interactions



Improved Prediction with Protein Blast Similarity

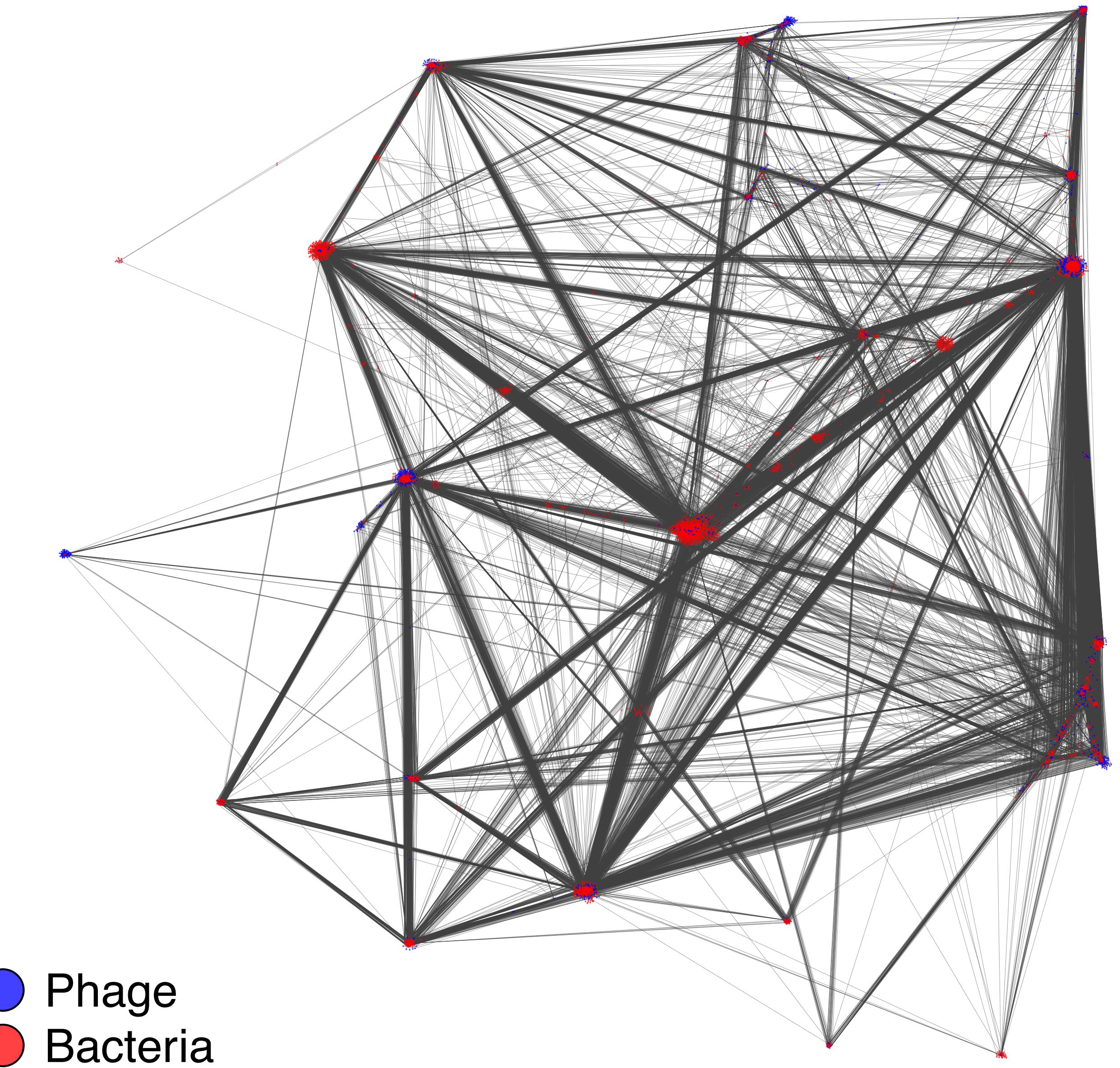


Using Predictions to Build a Network



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



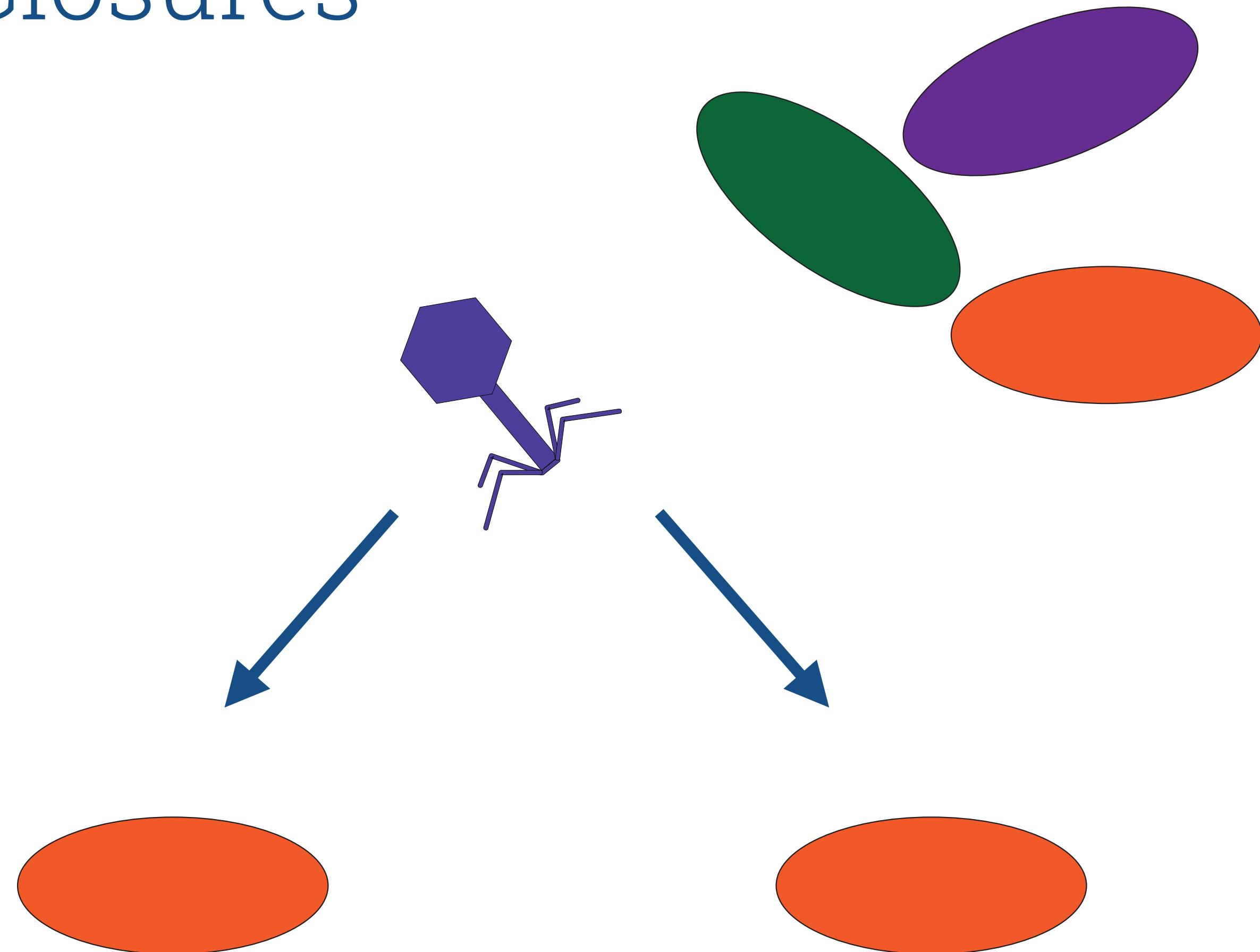
Example: Connecting Bacteria by Shared Phages

Triadic Closures



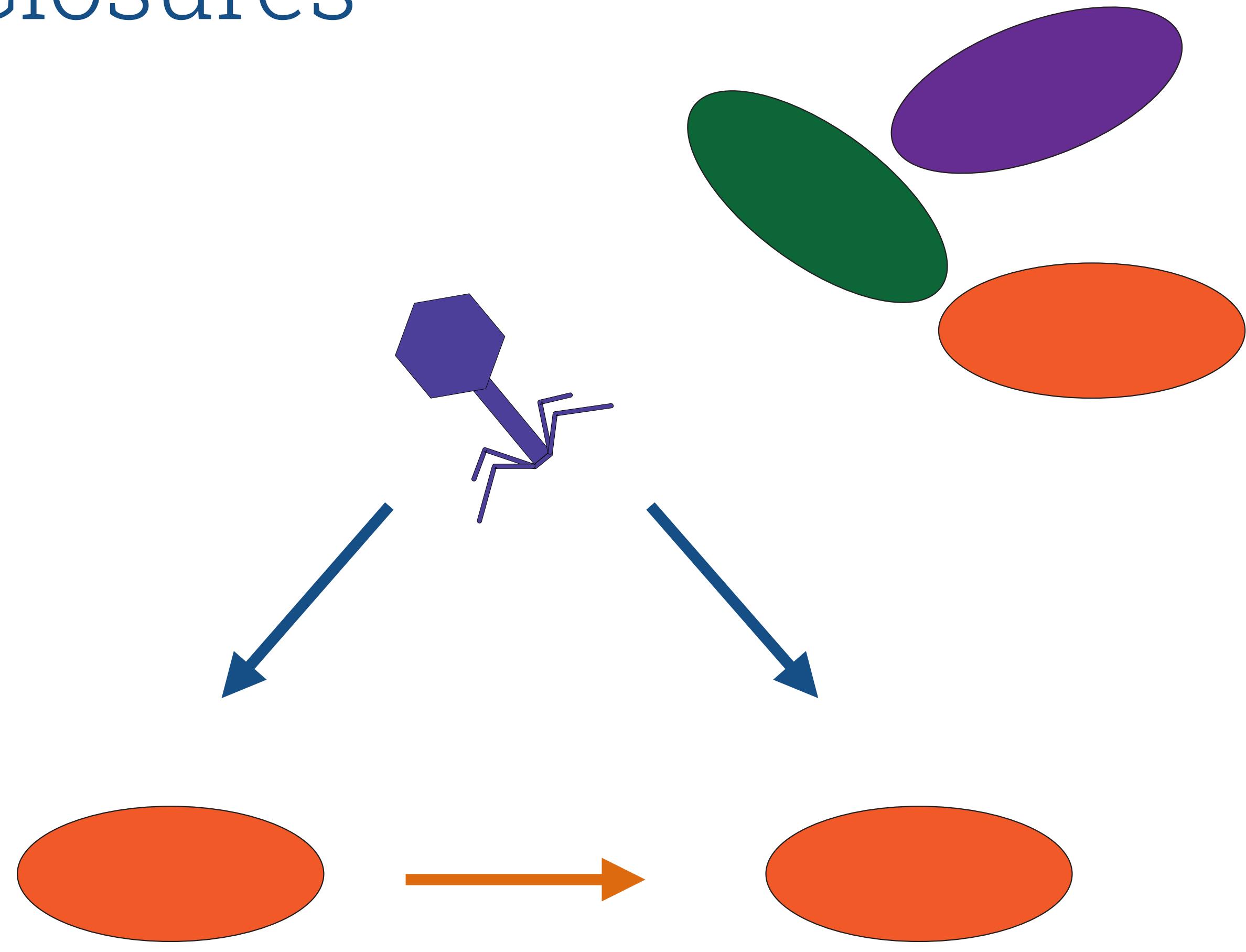
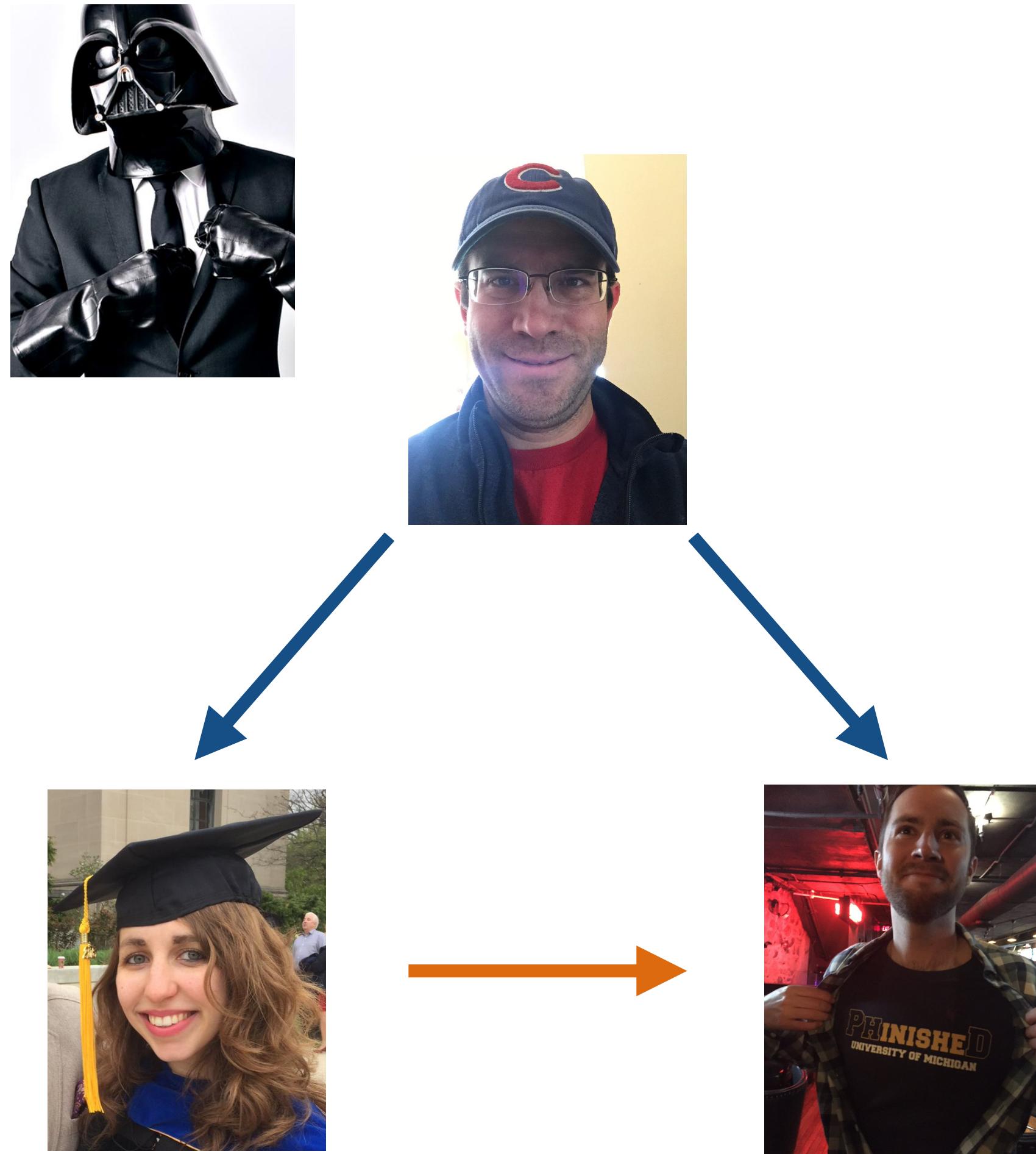
Example: Connecting Bacteria by Shared Phages

Triadic Closures

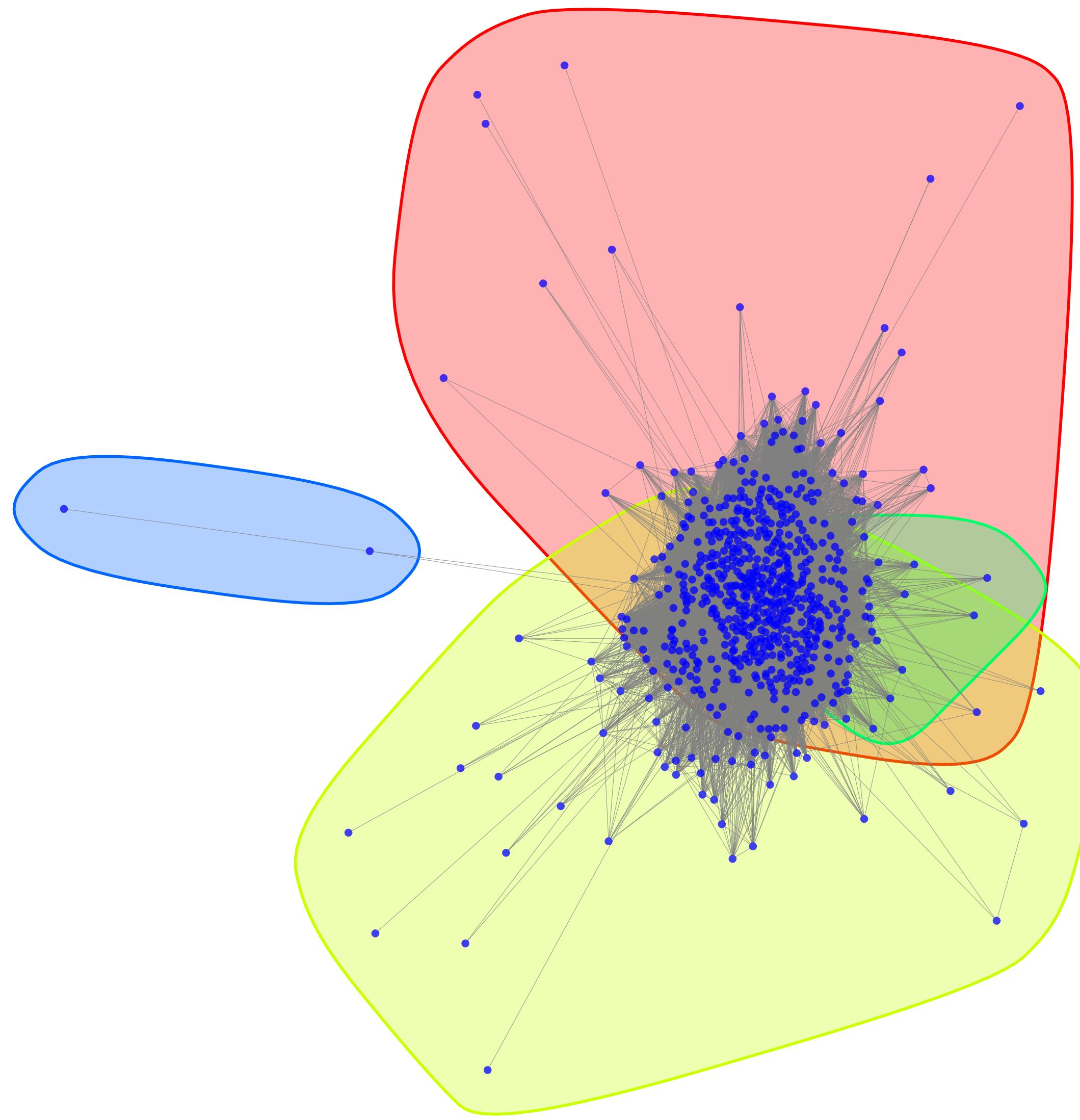


Example: Connecting Bacteria by Shared Phages

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).

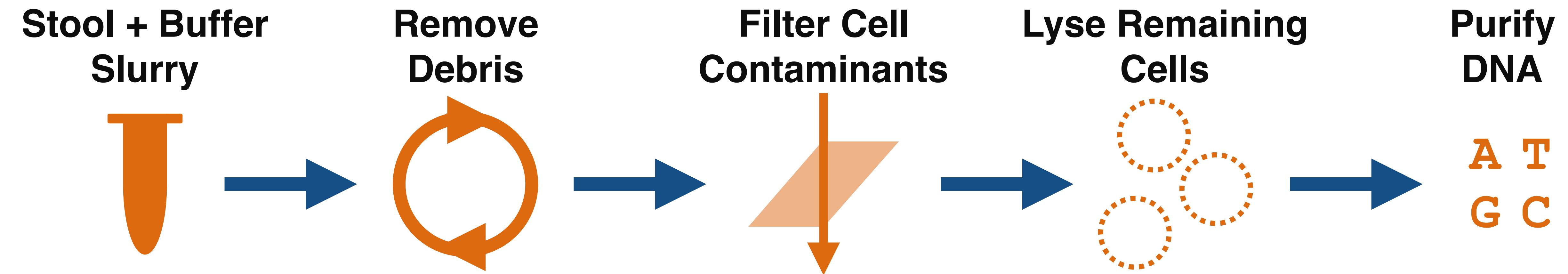
Outline

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

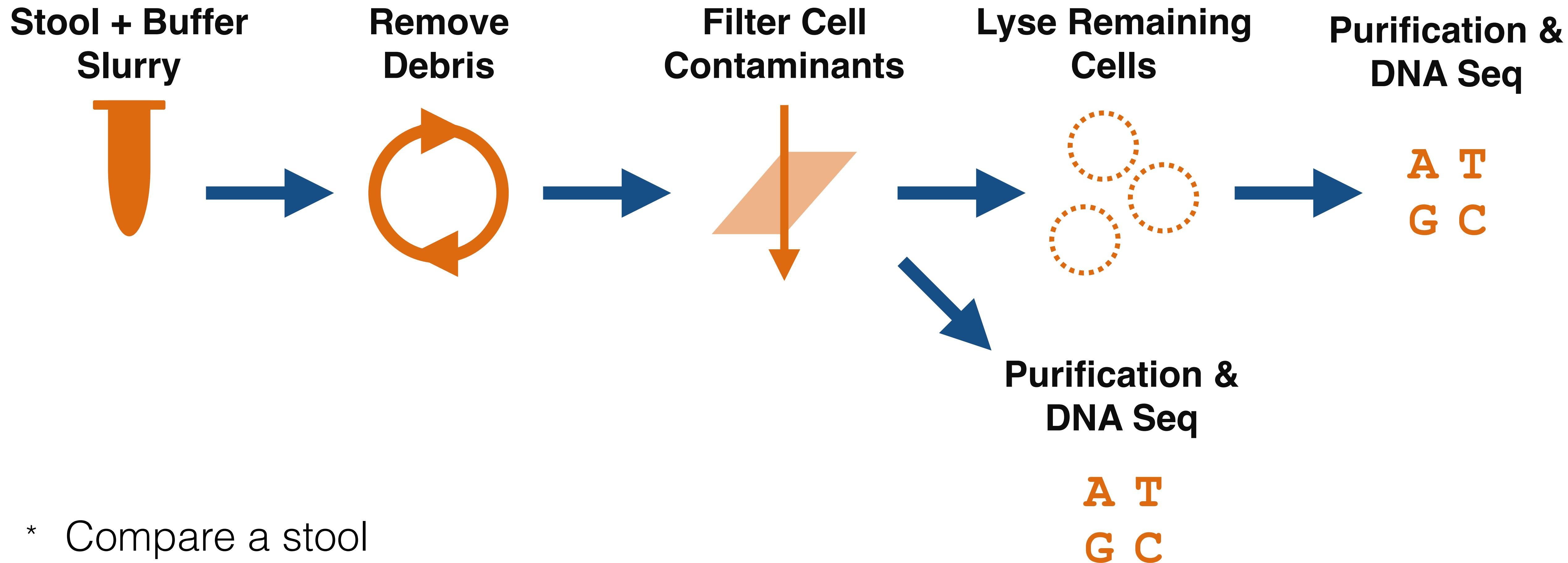
Why Do We Care?

- 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

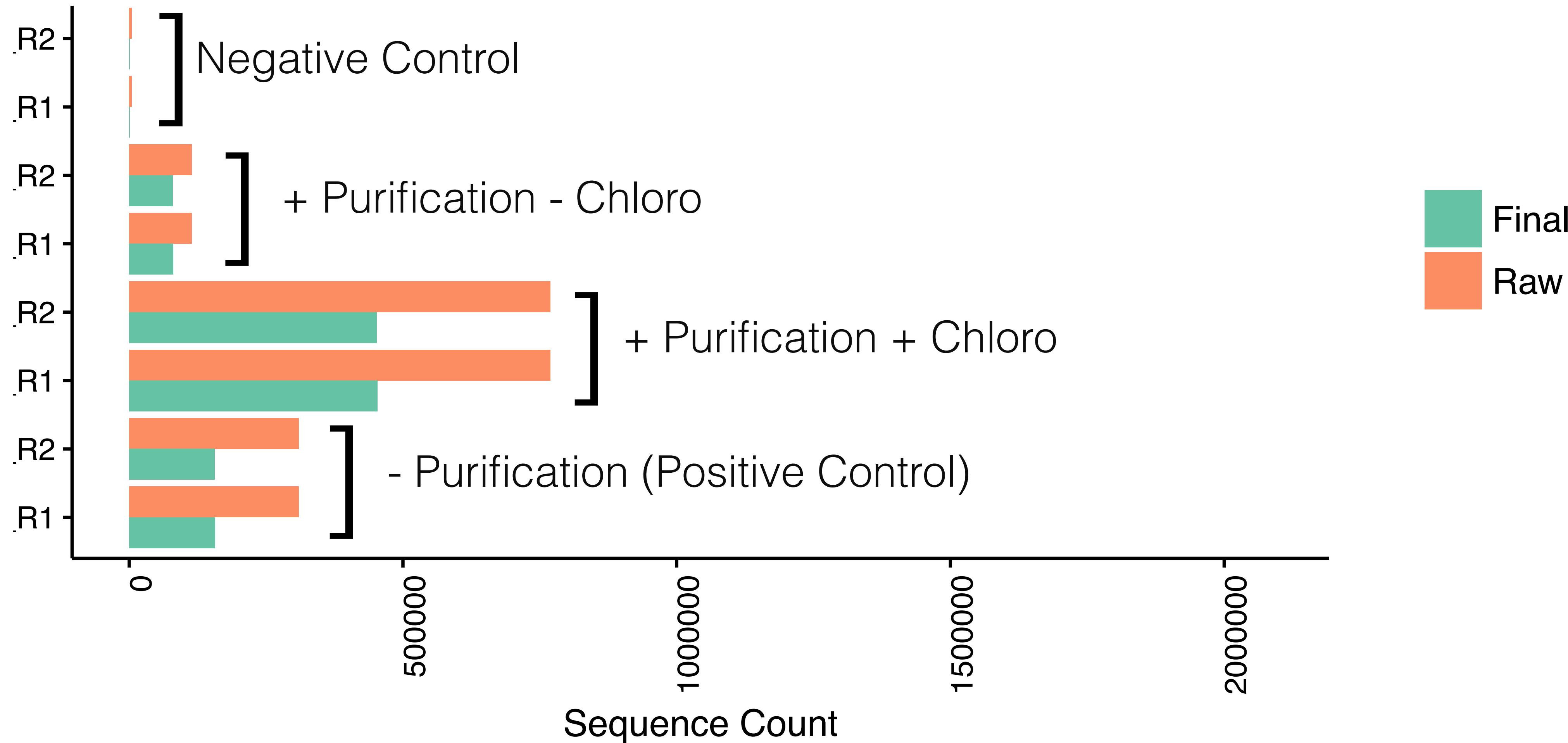


Impact of Chloroform Treatment

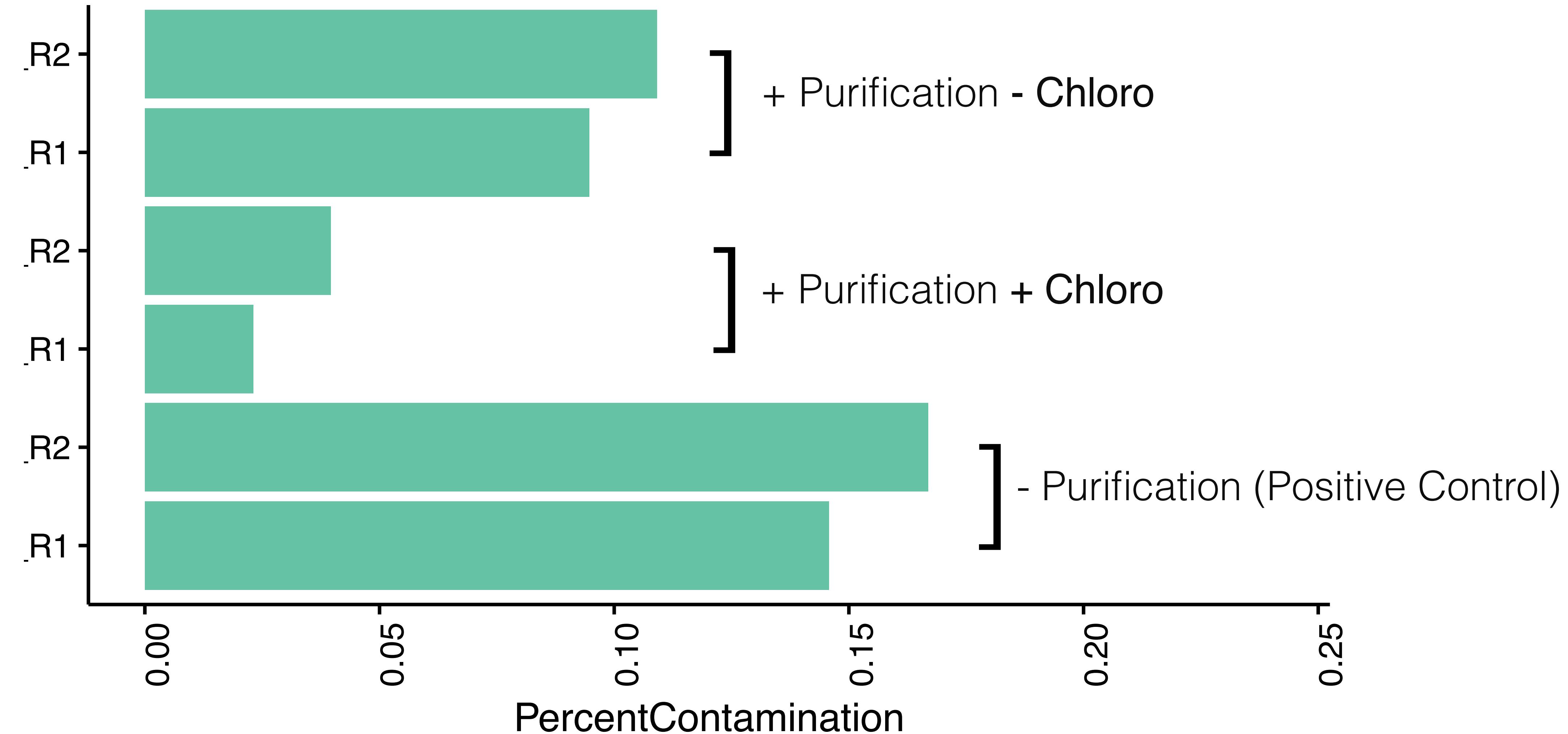


* Compare a stool slurry for continuity.

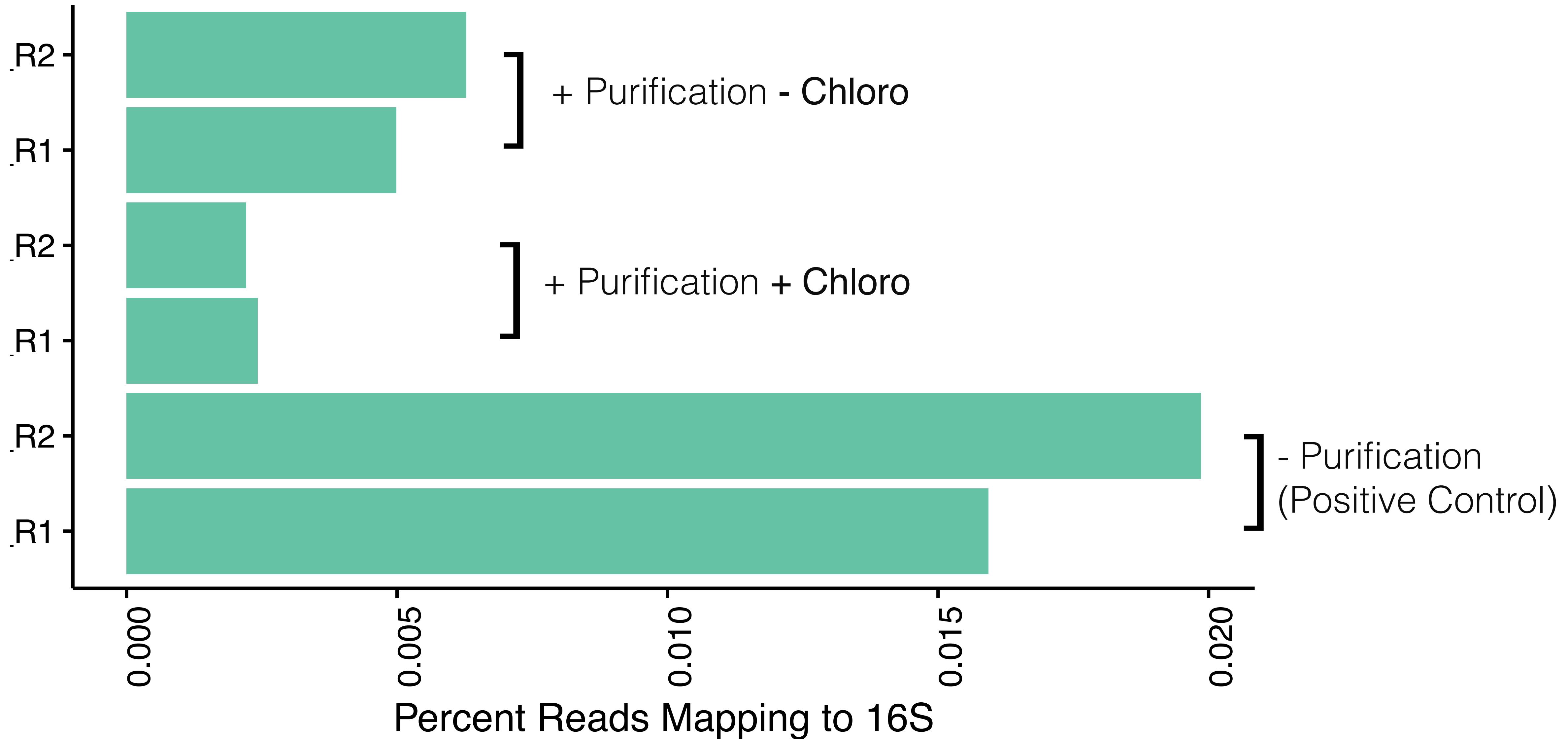
Verifying QC Sequence Count



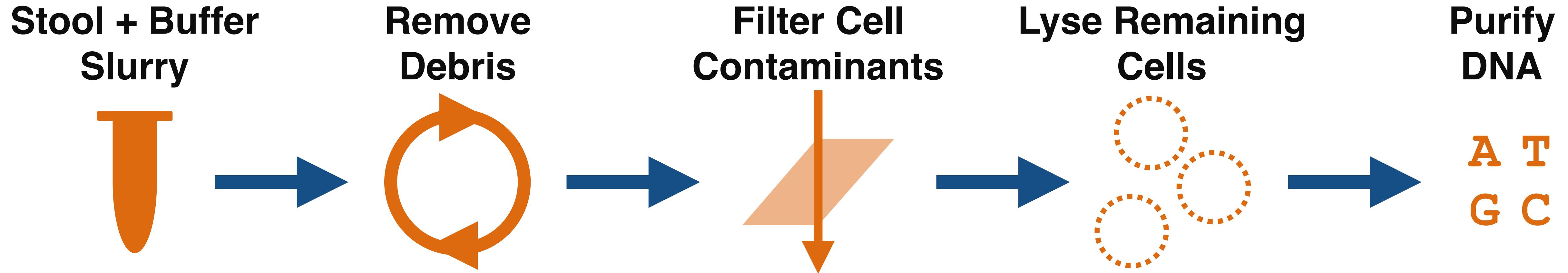
Chloro Reduces Mouse Contamination



Chloro Reduces Bacterial Contamination



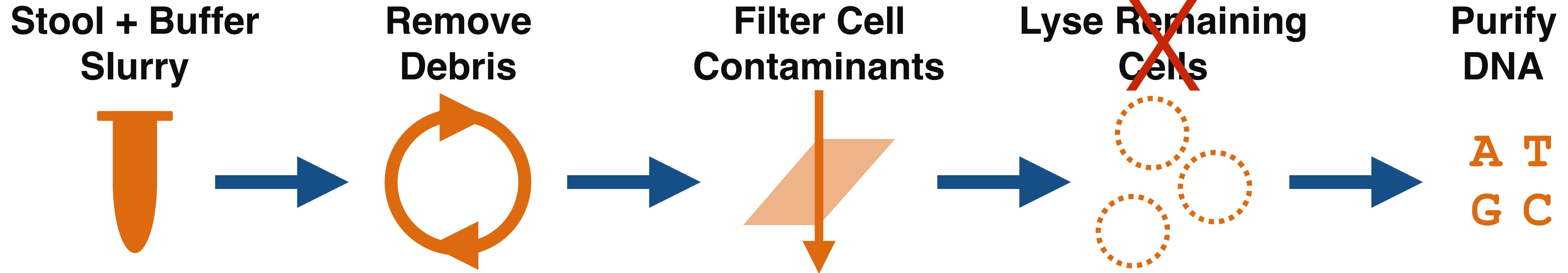
Defining the Sampling Threshold



Measuring Required Mouse Fecal Pellets



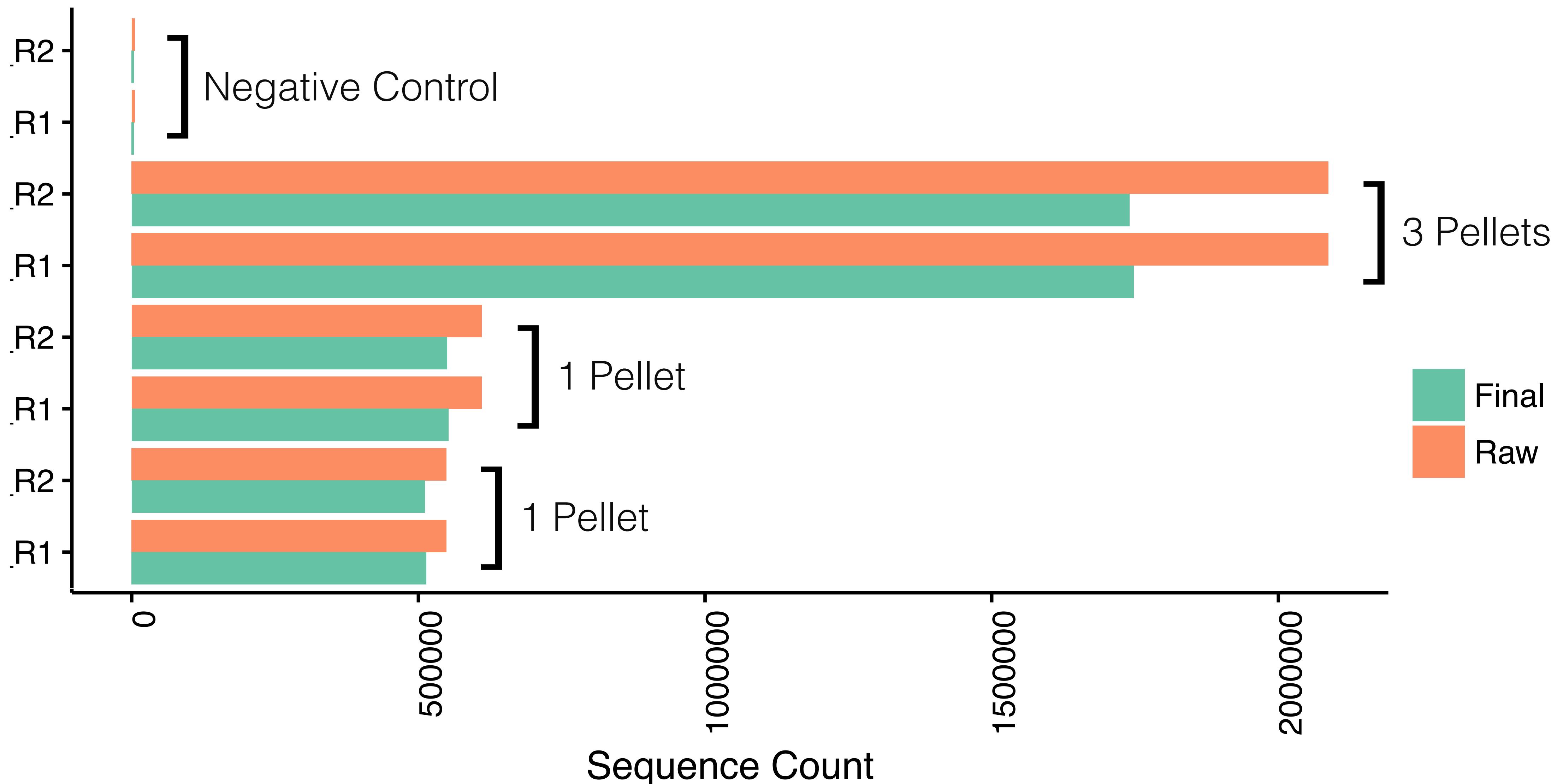
Defining the Sampling Threshold



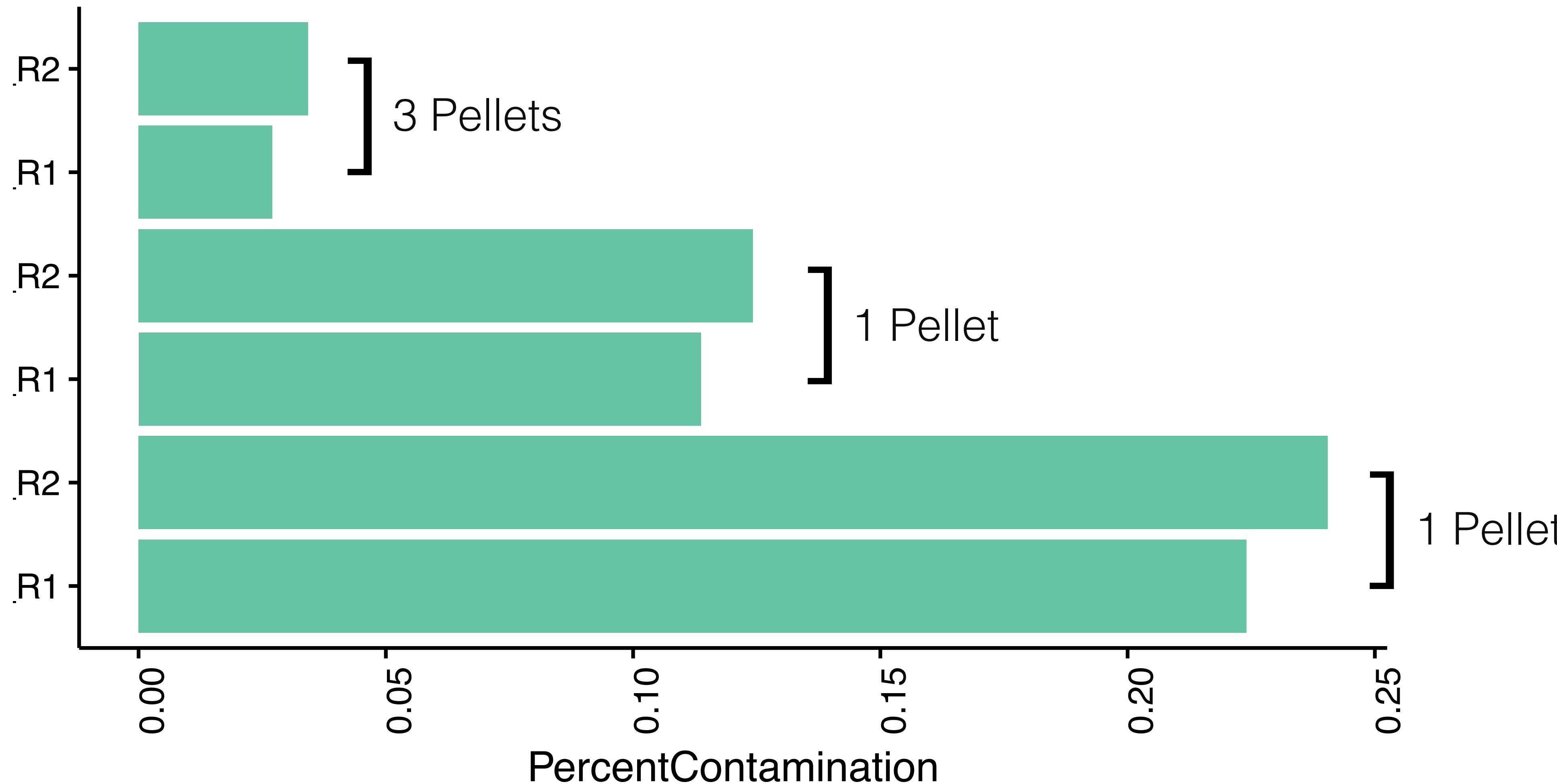
Measuring Required Mouse Fecal Pellets



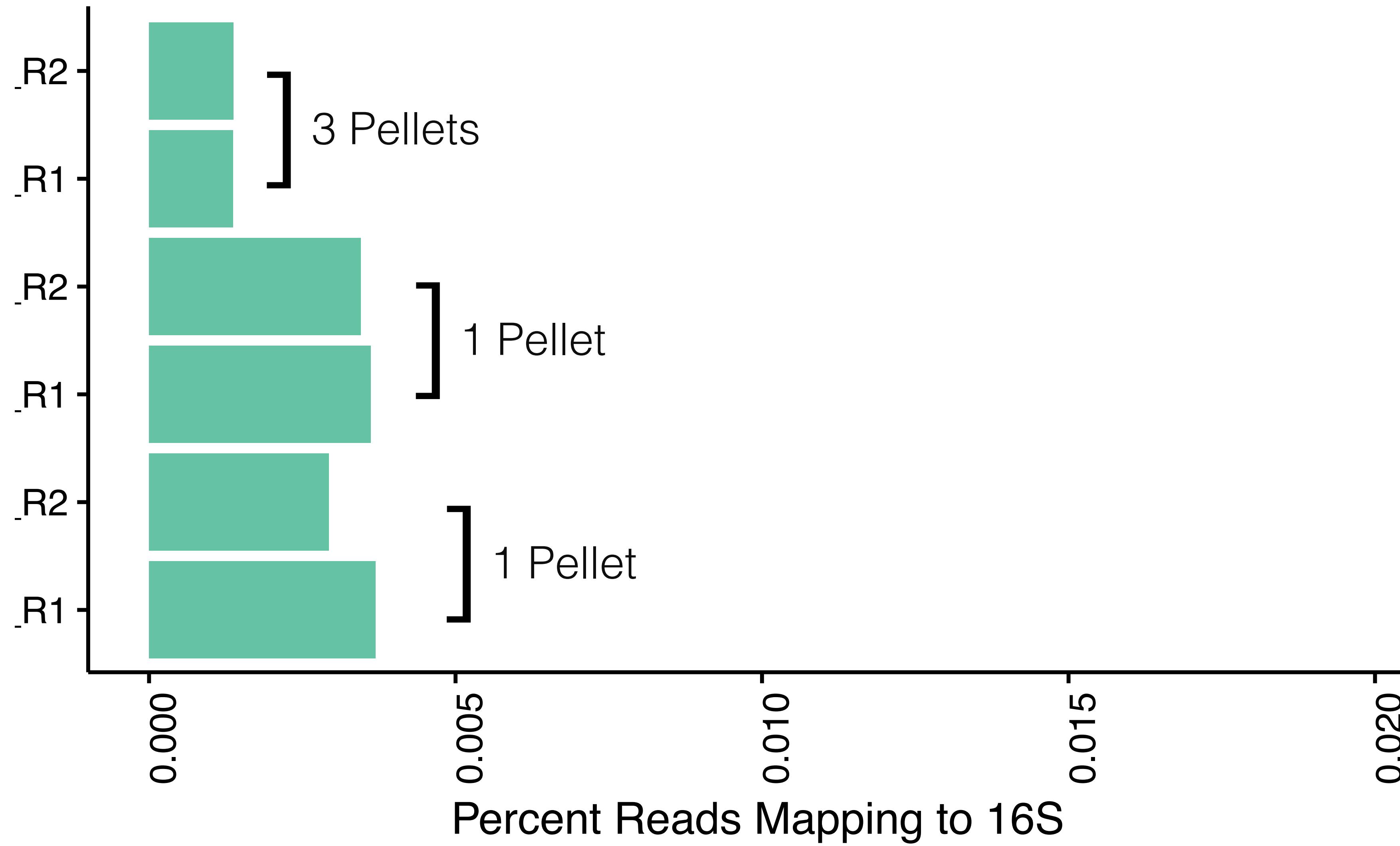
Single Pellets Result in Quality Sequences



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



Research in Progress

Establishing an Understanding of the Gut Virome

Geoffrey Hannigan
Schloss Lab Research Meeting
May 18, 2016