# Enrichment of Bacterial Virulence Factors in Bacteriophages

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# Outline

# Bacterial Virulence Factors

## Number of Cases

The number of NTM cases is estimated over 100K

## Increasing Case

The rate of cases is estimated to grow at 8% every year

## Populations at risk of developing NTM

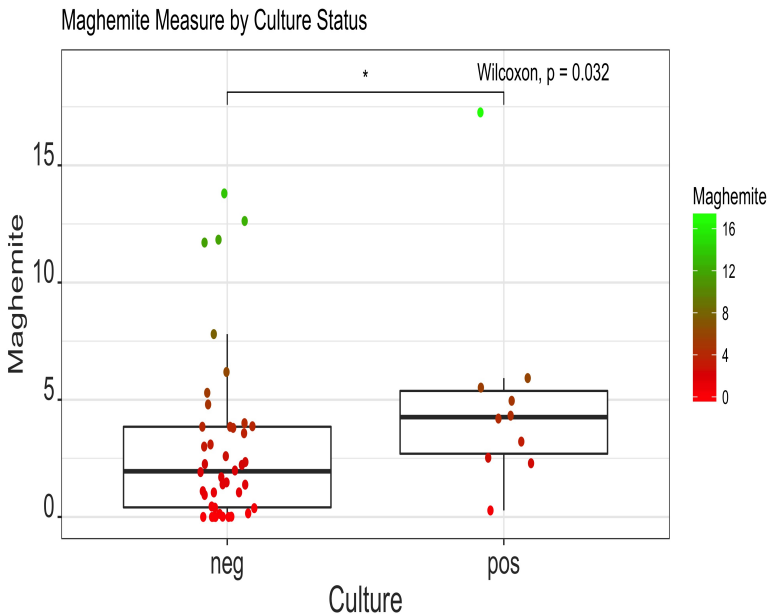
- Immunocompromised individuals
- Patients with lung damage or malfunction
- Residents of warm costal areas especially Hawaii

Strollo SE, et al. Ann Am Thorac Soc. 2015
Adjemian J, et al. Am J Respir Crit Care Med. 2012

# Importance of Phages as Vectors of Virulence Factors

## Hawaiian Soil Project

Identifying important soil characteristics for NTM soil culture




## Pulmonary NTM

- 90% of NTM cultures are from respiratory samples
- The lung has the lowest abundance of bacteriophages among human niches

O'Brien, R., et al. American Review of Respiratory Disease 1987
Aziz R., et al. Frontiers in microbiology 2015

# Of "Viral" Importance

## Bacteriophages (Phages)

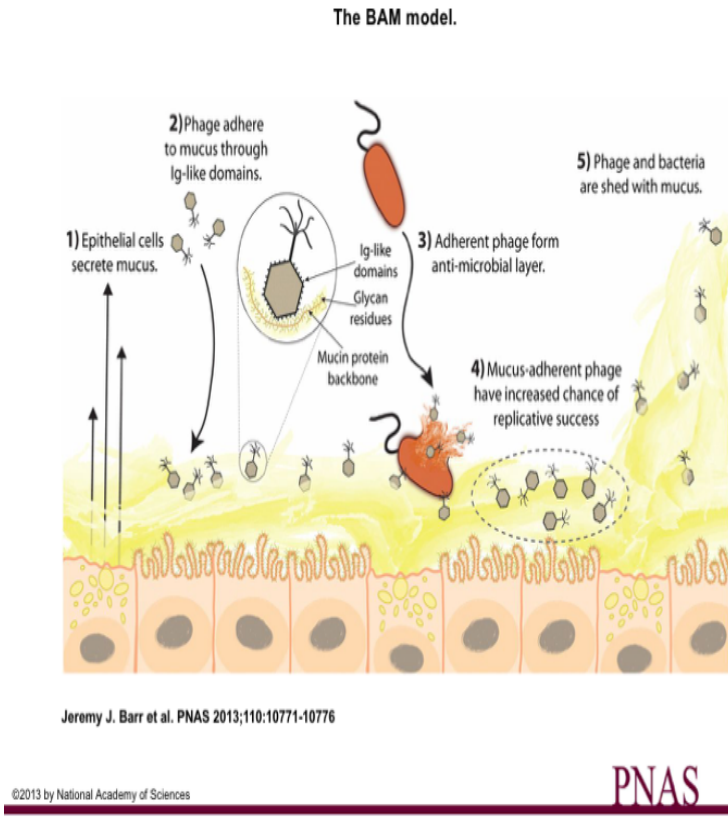
Phages are DNA viruses that infect prokaryotes

## Bacteriophage Adherence to Mucus (BAM)

- Phages act as an innate immune system in mucosal tissues
- Prior studies identified Ig-like motifs in induced phages from Pseudomonas cultures

## Phages in the Lungs

Does phage abundance in the lungs affect bacterial biofilm formation?




Barr, Jeremy, et al., PNAS 2013
Tariq, Mohammad, et al., Frontiers in Microbiology 2015

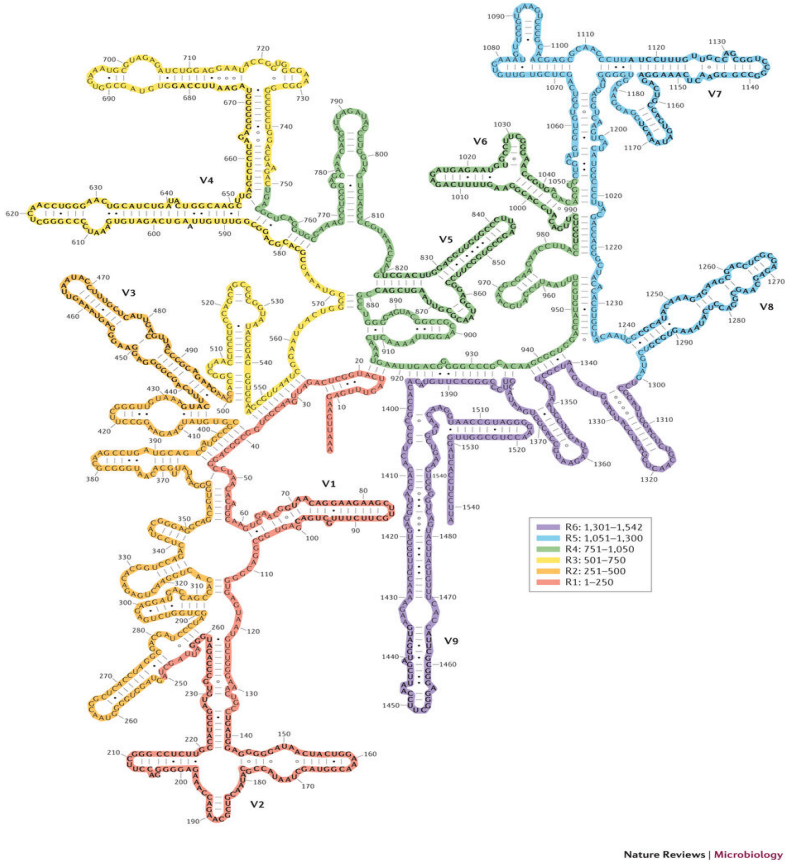
# Molecular Methods to Study Phages

## Difficulties of phage study

- Lack of universal marker gene
- Sequence heterogeneity
- Misclassification in databases

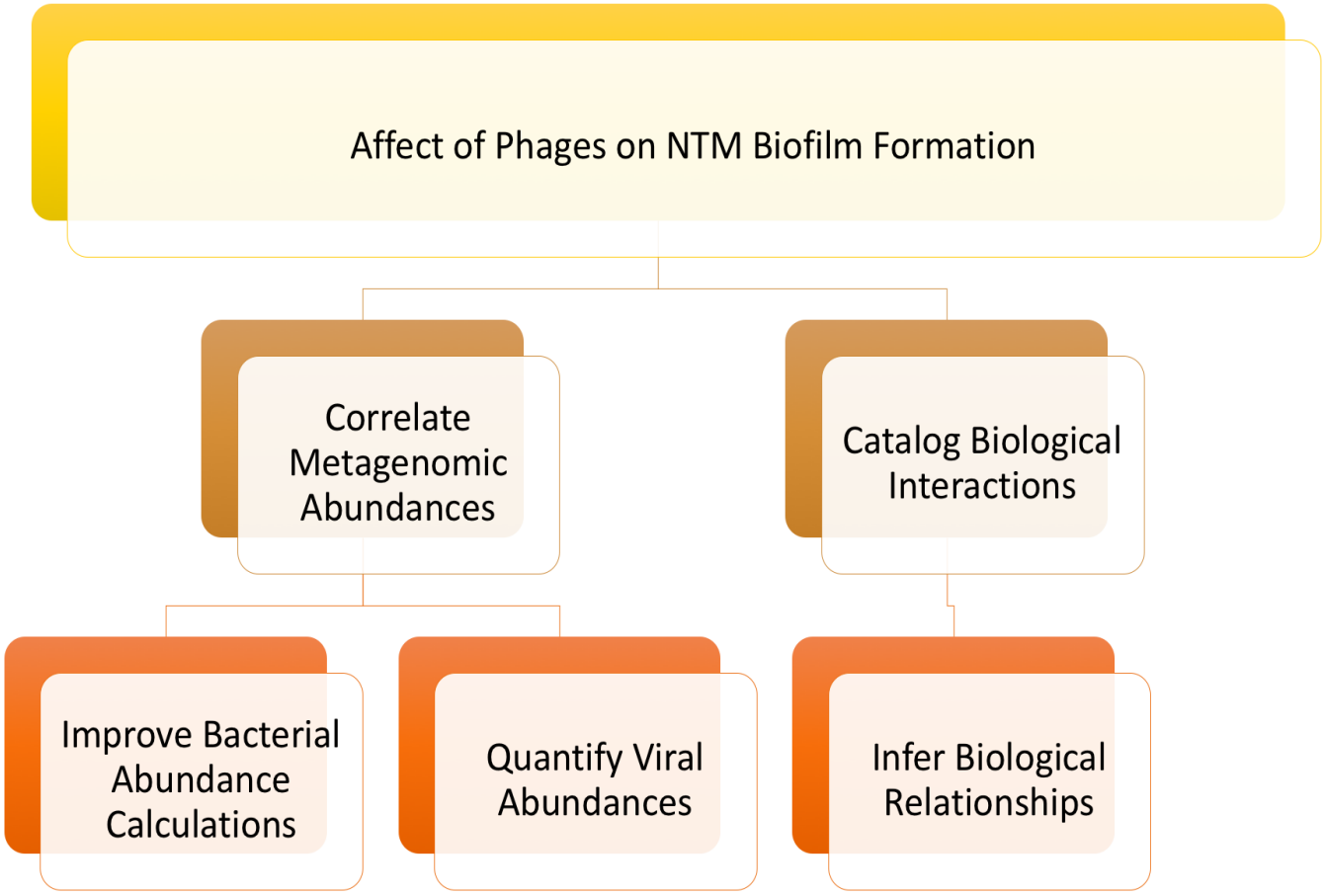
## Phage Isolation Methods

- Biological filtration
- In silico methods




Yarza, P., et al. Nature Reviews Microbiology 2014

# Objective


Affect of Phages on NTM Biofilm Formation
Correlate Metagenomic Abundances
Catalog Biological Interactions
Improve Bacterial Abundance Calculations
Quantify Viral Abundances
Infer Biological Relationships

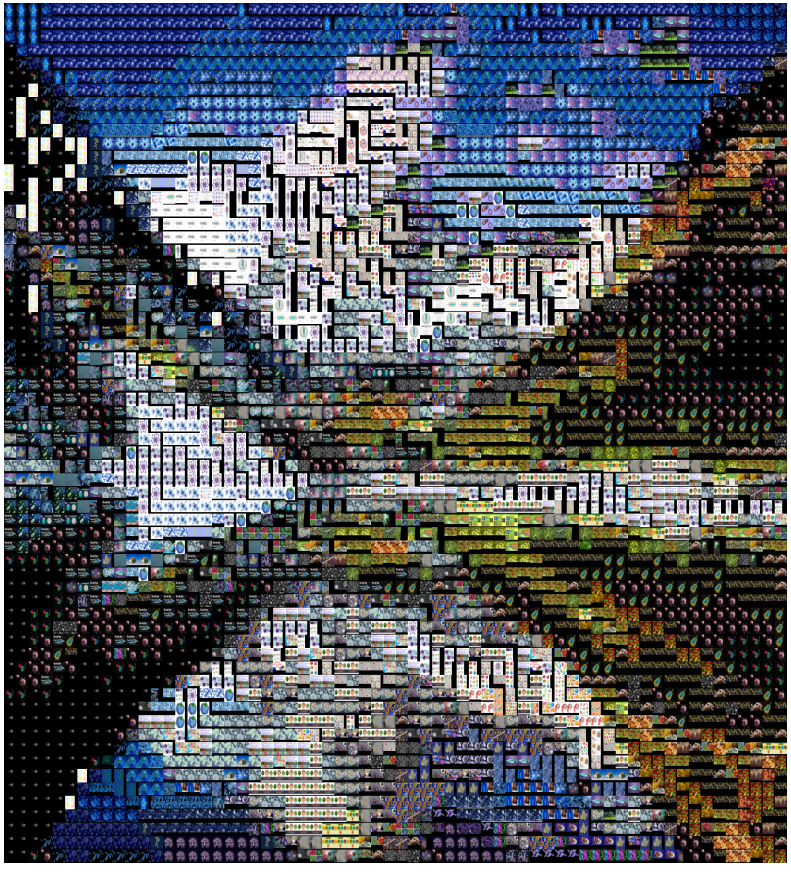
# Metagenomics

## What is Metagenomics?

Unbiased study of all genetic material in a sample

## Importance of Metagenomics

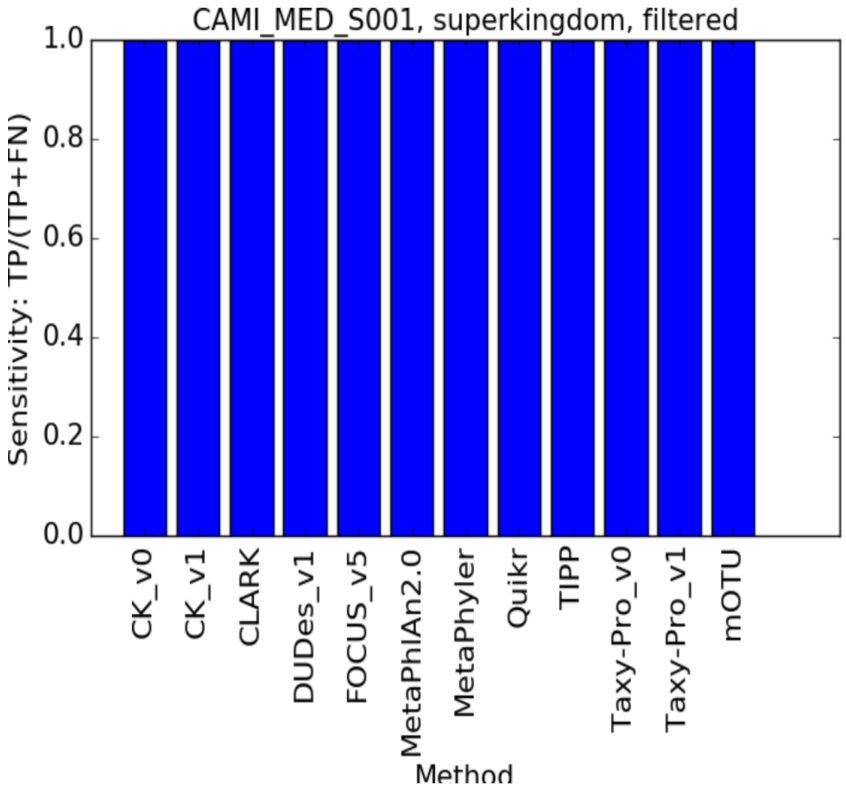
- Functional capabilities of a sample
- Species level distinctions
- Due to lack of a universal gene marker, phages are studied by metagenomics

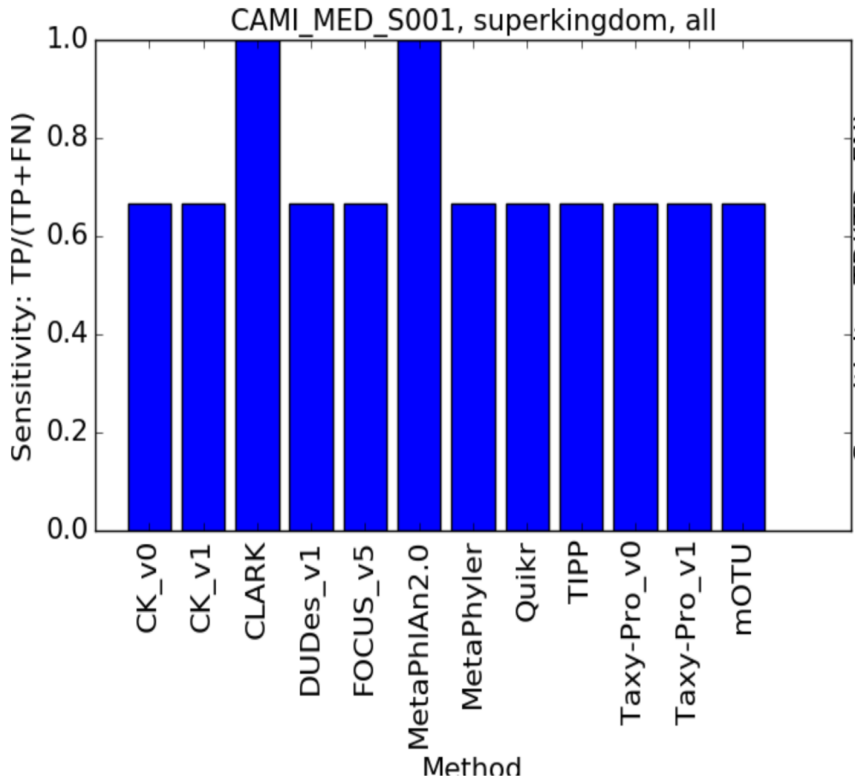


# Metagenomics Gold Standard

## Critical Assessment of Metagenome Interpretation (CAMI)

- Comprehensive simulation study of tools from all levels of analysis (binners, assemblers, taxonomic profilers)
- Viral and plasmids affected the performance of taxonomic profilers and abundance calculations




Sczyrba, A., et al. Nature Methods 2017

# Viral Filtration Simulation Study

## Study Design

30 simulated mixed metagenomes are used to compare the viral contiguous sequence (contigs) identification performance of multiple tools

## Sequencing Depth of Experiment

Each metagenome is comprised of 10 million reads

## Complexity of Metagenomes

8 bacteria and 8 phages comprise the low complexity samples in each metagenome

# Genomes in Simulation

## Virus - 0.12 Mb

- Bacillus phage Pony
- Caulobacter phage CcrColossus
- Mycobacterium phage Bxb1
- Mycobacterium phage Che9d
- Mycobacterium phage TM4
- Pseudomonas phage vB-PaeM-C2-10-Ab1
- Staphylococcus phage CNPH82
- uncultured phage crAssphage

## Bacteria - 4.72 Mb

- Bacillus subtilis subs. subtilis 168
- Clostridium acetobutylicum ATCC 824
- Clostridium perfringes str. 13
- Lactococcus lactis subsp. lactis Il1403
- Pseudomonas aeruginosa LESB58
- Staphylococcus aureus subsp. aureus N315
- Streptococcus pyogenes M1 476
- Xylella fastidiosa 9a5c

# Tools Used in Study

## Assembler

MEGAHIT - Effective at assembling viromes
Roux, S, et al. PeerJ 2017

## Viral Contig Identification Methods

VirFinder - Viral contig K-mer identification model
Ren, Jie, et al. Microbiome 2017

Blastx - Filtering against a viral protein database
Camacho C., et al. BMC Bioinformatics 2008

VirSorter - Hybrid HMM and gene marker database
Roux, S., et al. PeerJ 2015

vHMM - Iterative HMM trained on viral genes
Paez-Espino, D., et al. Nature 2016

# Performance Measurements

## True Viral Contigs

True viral contigs are defined by BLAST hits (E-value 10^05) against a custom database of the reference phages and bacterial prophage elements.

## Term Definitions

TP = True Positive FP = False Positive FN = False Negative

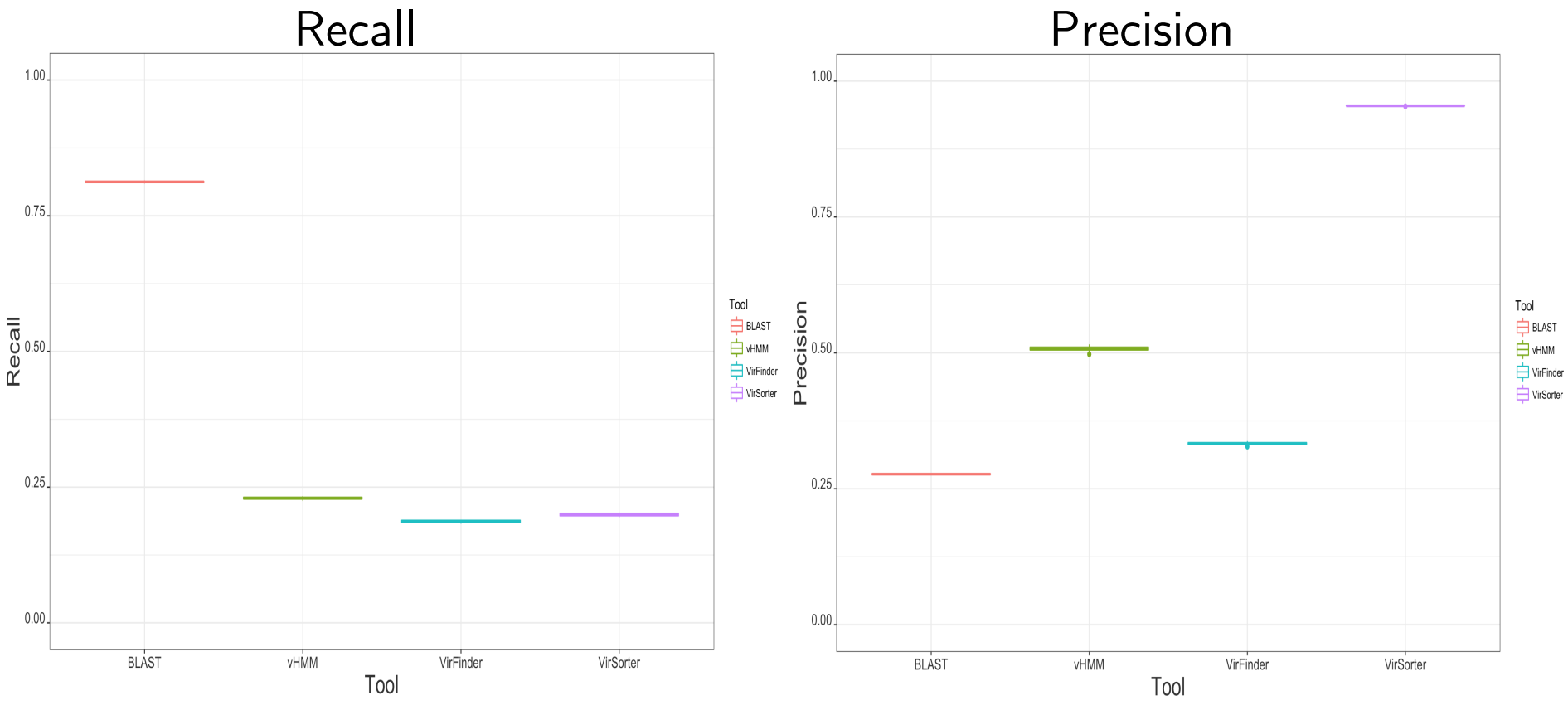
## Performance Metrics

- Recall = TP / (TP + FN)
- Precision = TP / (TP + FP)

# Recall and Precision

## Order of Tools
from left to right

BLAST, vHMM, VirFinder, VirSorter



# Conclusions

## Performance

The variance of tool performance suggests that no single tool is optimal for viral filtration

## Tool Parameter Optimization

Used default tool parameters for all tools

- Lenient BLASTX filtering resulting in false positives
- vHMM model is the second iteration

# Future Directions

## Expansion of Tools

Inclusion of binners MetaBat and MetaWatt 3.5

## Expanded Dataset

Filter viral elements from CAMI data

## Combinations of Tools

Explore how the tools perform in combinations

# Creating Custom BLAST Databases

## Demand for Custom Databases

The Strong Lab uses custom databases to identify mycobacterium elements in metagenomics

## Current Tools

- makeblastdb from Command-line BLAST allows users to create custom BLAST databases
- makeblastdb requires local sequences to create usable database

# Batch Sequence Retrieval

## Current Methods

- NCBI Webserver
- ESearch / Efetch function
- biomartr R package

## Test Case

Retrieve protein sequences of three Mycobacterium subspecies

- Mycobacterium avium paratuberculosis
- Mycobacterium abscessus massiliense
- Mycobacterium abscessus bolletii

# NCBI Webserver

## Batch Download Process

- Query Assembly: "Mycobacterium abscessus subsp. massiliense"[Organism] OR "Mycobacterium abscessus subsp. bolletii"[Organism] OR "Mycobacterium avium subsp. paratuberculosis"[Organism]
- Select Complete Genome Tab
- Click "Download Assemblies" button and select Protein FASTA from File Type drop down

## Difficulties

- Query string must to be specific
- Length of query string can become unwieldy
- Sparse information on download procedure and examples

# Efetch and biomartr

## Efetch Retrieval Process

Efetch can be utilized via Python or via command-line

```
handle = Entrez.efetch(db="nuccore", id= UID_List ,
rettype = "fasta_cds_aa")
```

## biomartr Sequence Retrieval

```
library(biomartr)
meta.retrieval(kingdom = "bacteria", group =
"Actinobacteria", db = "genbank", type = "proteome")
```

## Difficulties

- Entrez.efetch function requires *a priori* knowledge of Entrez UID
- biomartr only able to bulk download phylum level
- Programming skills important [not required for Efetch]

# Genomic Retrieval and Blast Database Creation (GRAB)

## A Batch Retrieval System for Biologists

Well documented command line tool and web interface (in development)

## GRABs Sequences by Taxonomy

- GRAB requires the name of organisms and the taxonomic level
- GRAB retrieves genomic, coding sequences, or protein sequences

## GRAB Sequence Retrieval

```
python GRAB.py -m protein -q
paratuberculosis,massiliense,bolletii -l subspeices
```

# Finding Prophages

## Prophage Discovery Problem

Same difficulties as gene prediction: finding signal in data

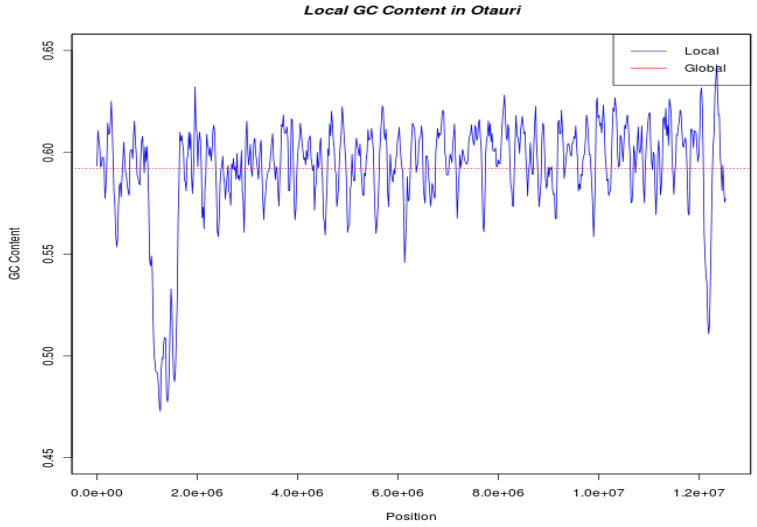
# Prophage Discovery Tools

## Number of Tools

10 tools listed at Omic Tools for prophage discovery

## Methods Used

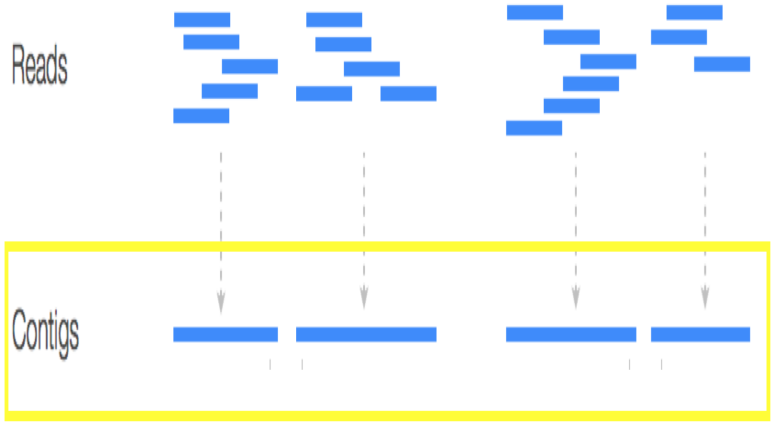
- Sequence similarity
- Hidden Markov models
- Transcription direction
- Protein length
- Sliding window GC content
- Phage specific kmer



# Prophage Discovery Methods

## Top Down Methods

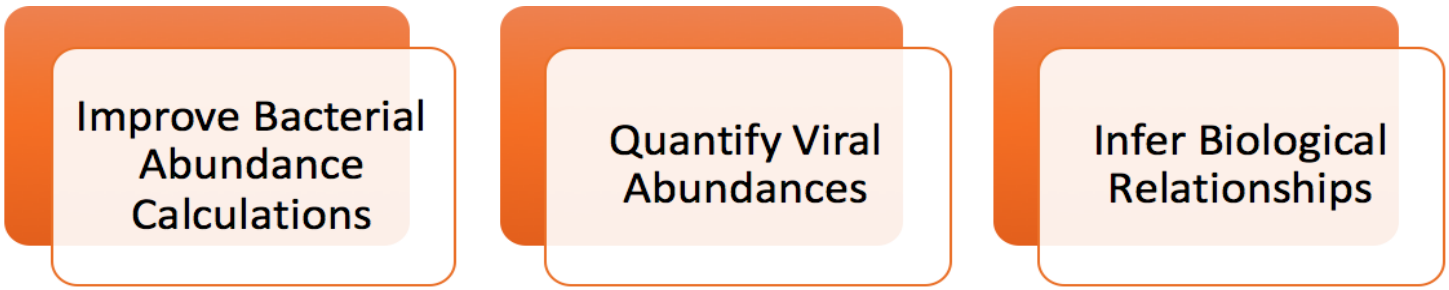
All prophage discovery methods find prophages within contiguous sequences or genomes




## Potential Prophage Tool Pitfalls

- Metagenomics produces short contigs that are discarded
- In metagenomics, prophage hosts may not be identified

# Concluding Remarks




## Metagenomic Simulation Study

Effectively identifying viral elements improves bacterial abundance calculation

## GRAB

Viral GRAB will contribute to a focus on phages specific to lung infections

## Building Up Domains

Allows for the identification of prophages elements in metagenomics

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# Questions?

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