Metagenomic Exploration: Evaluation and development of novel tools for viral and bacterial sequence analysis

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CPBS Update Talk

Jan 29th, 2018

Research Update

Asthma Environmental Microbiome

Building Up Domains

Clinical Features of Cystic Fibrosis and the Microbiome

Genomic Retrieval and Blast Database Creation

Hawaiian Soil Chemistry and Culture

Microbiome OTU Grouping Workflow

Metagenomic Simulation Study

Virome Statistics Pipeline

Nontuberculous Mycobacterial (NTM) Infections

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

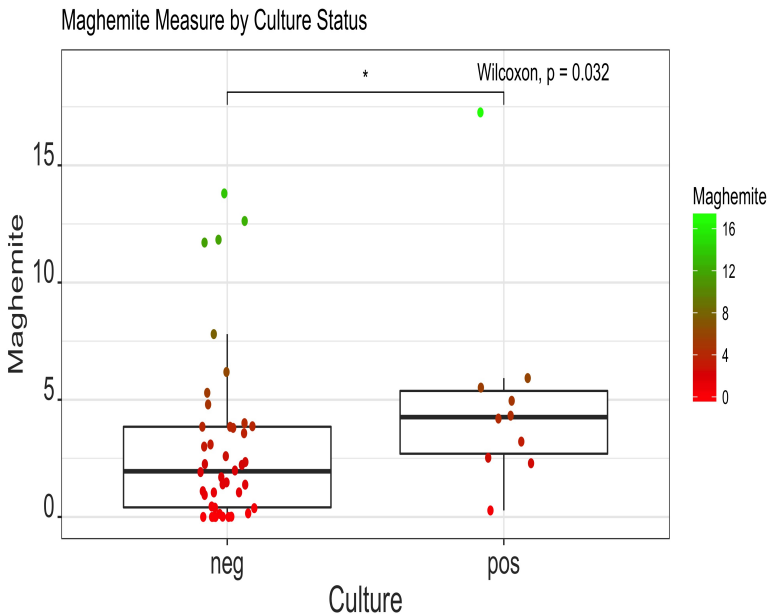
- Immunocompromised individuals
- Patients with lung damage or malfunction
- Residents of warm costal areas especially Hawaii

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

Understanding Why NTM Develops

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)

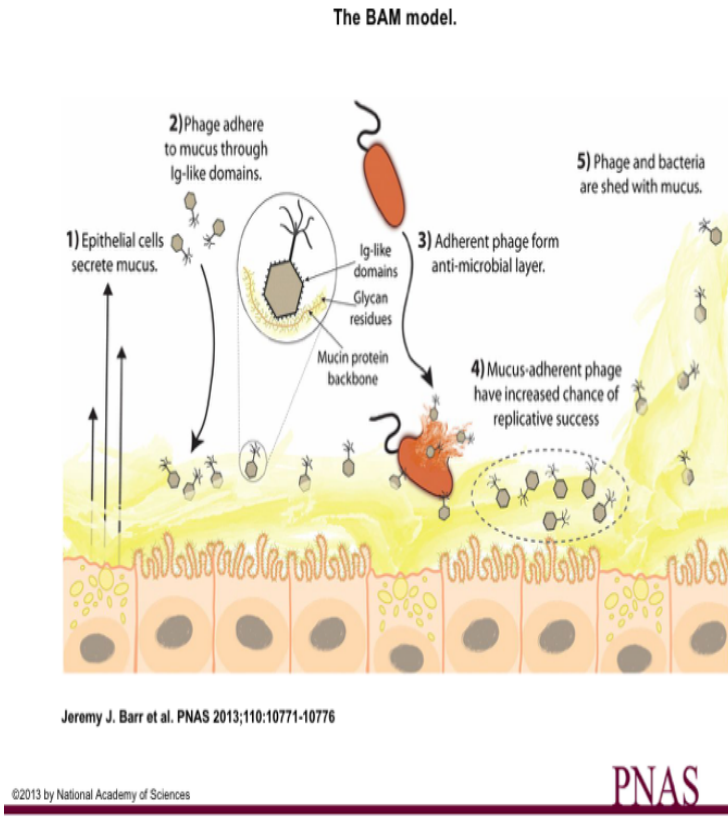
Phages are DNA viruses that infect prokaryotes

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

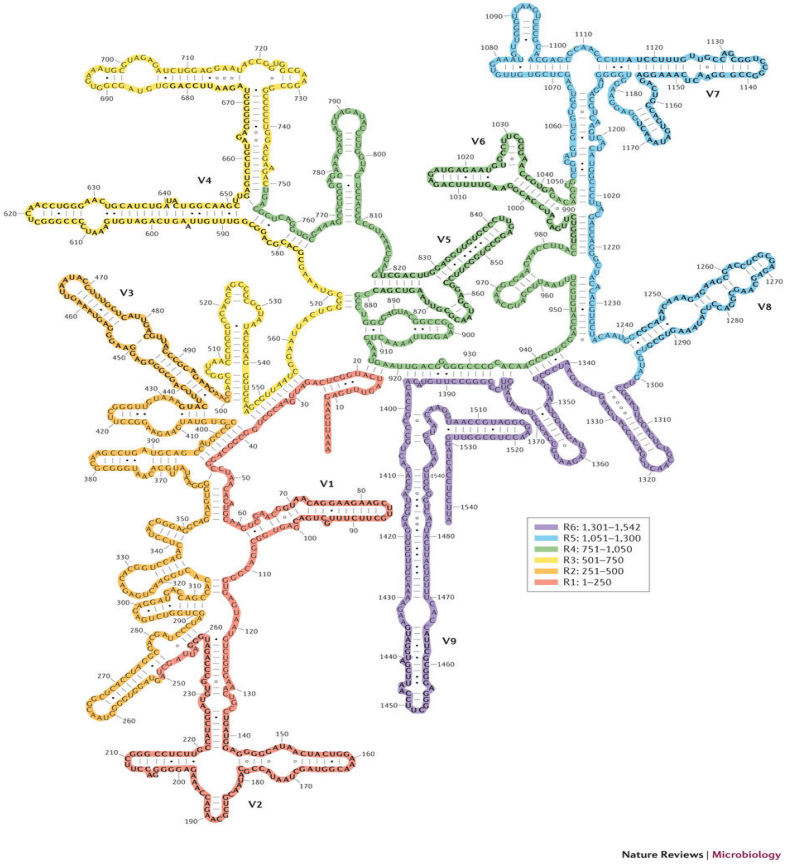
Molecular Methods to Study Phages

Difficulties of phage study

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

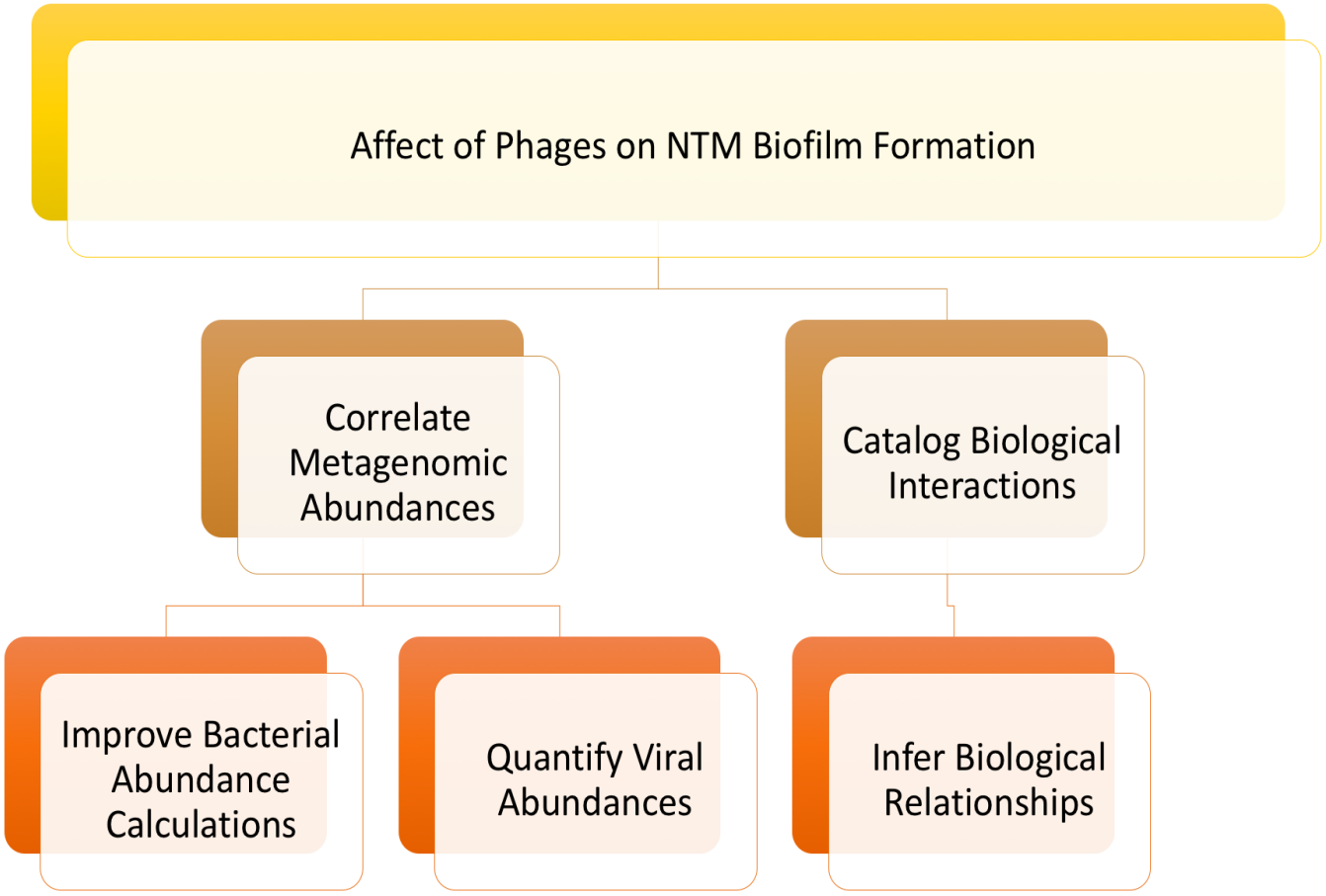
Phage Isolation Methods

- 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

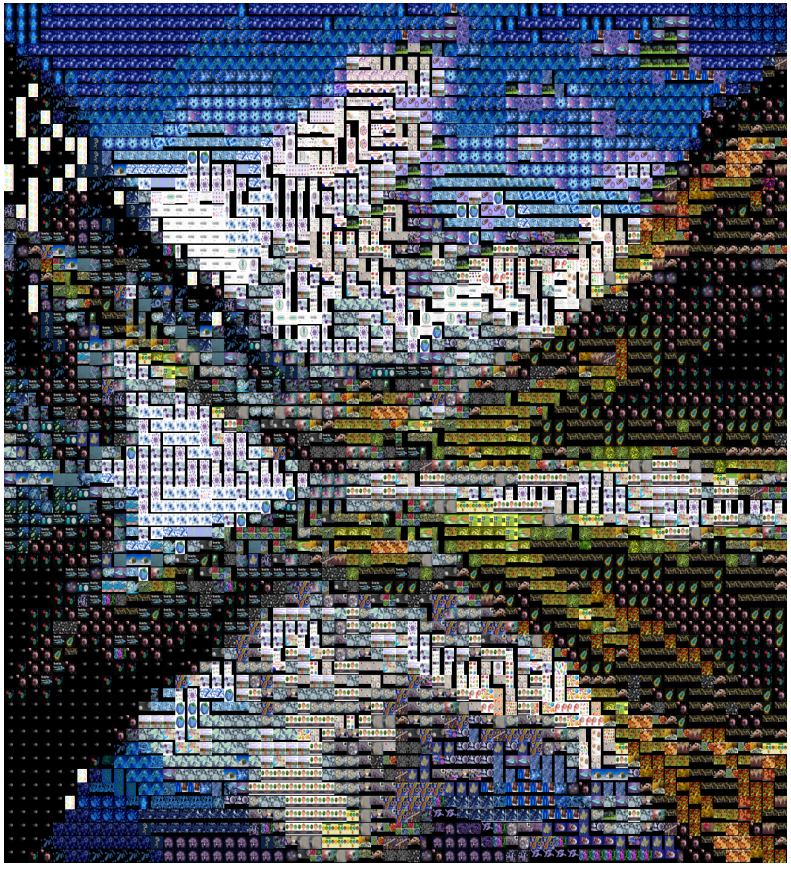
Metagenomics

What is Metagenomics?

Unbiased study of all genetic material in a sample

Importance of Metagenomics

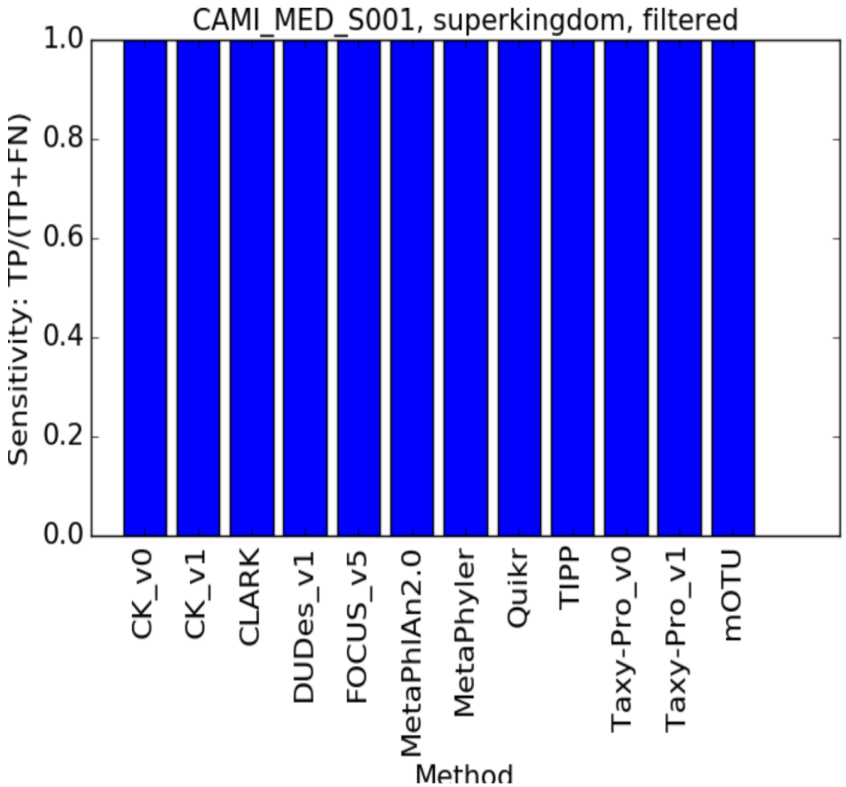
- Functional capabilities of a sample
- Species level distinctions
- Due to lack of a universal gene marker, phages are studied by metagenomics

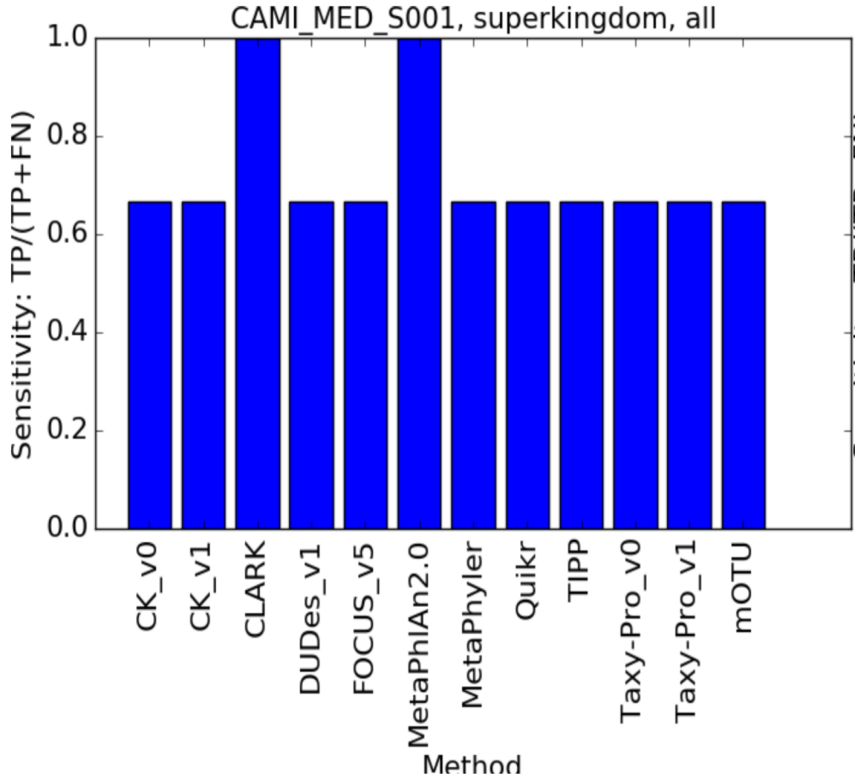


Metagenomics Gold Standard

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

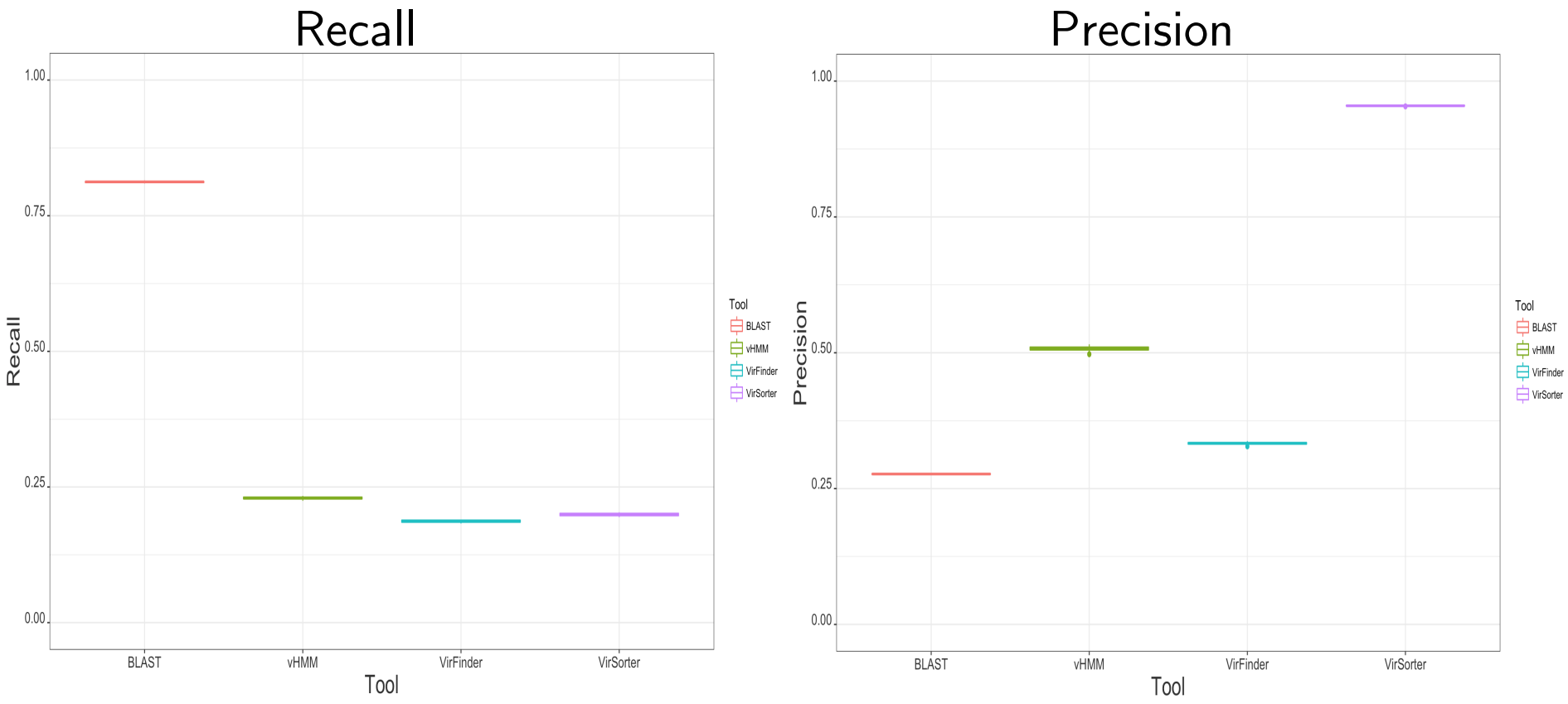
Performance Metrics

- 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

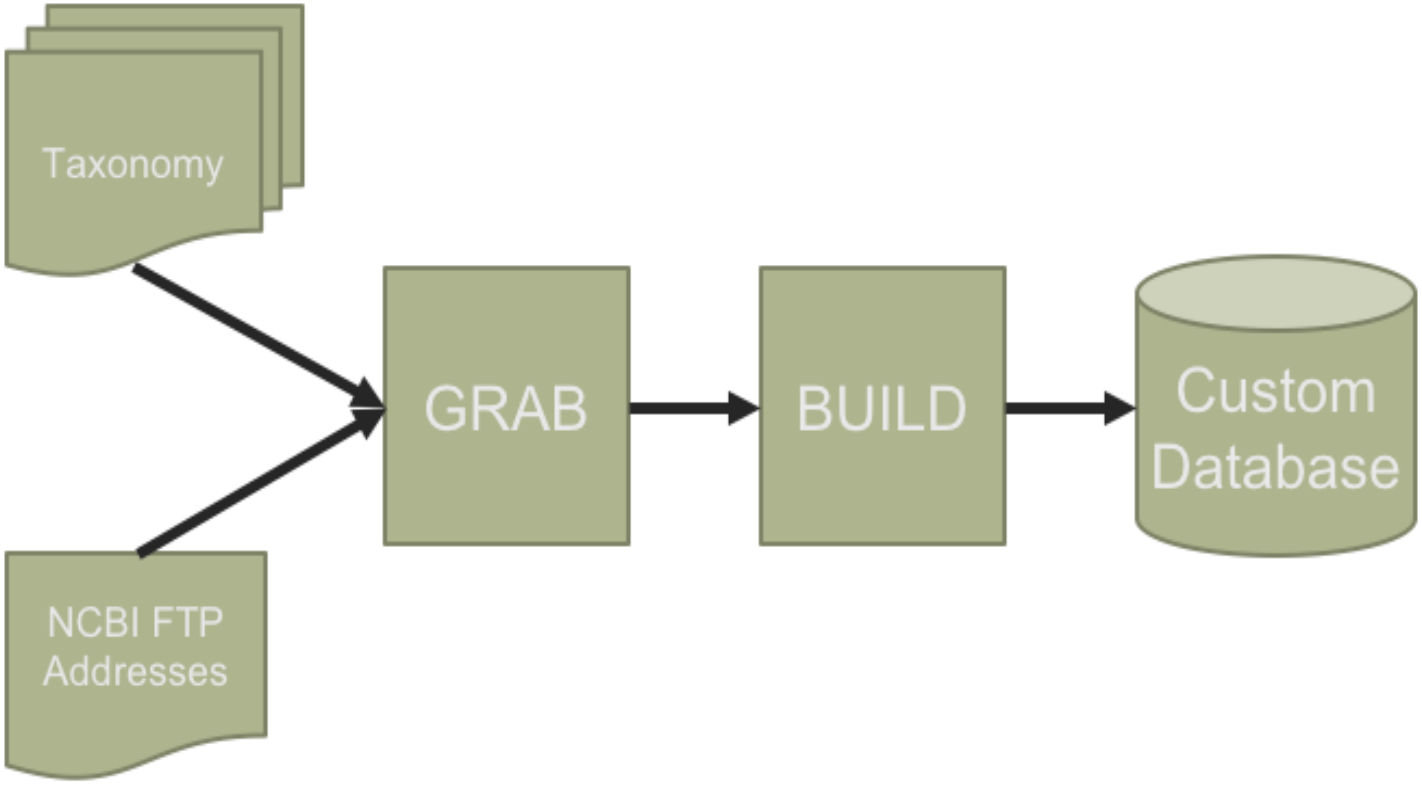
- GRAB requires the name of organisms and the taxonomic level
- GRAB retrieves genomic, coding sequences, or protein sequences

GRAB Sequence Retrieval

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

Genomic Retrieval and Blast Database Creation

GRAB Pipeline



Demand for GRAB

Nine questions related to bulk download of protein or genomic sequences (Over 58K views)

Four question related to bulk download of protein or genomic sequences (Over 3.5K views)

Future Directions

Webserver

Shiny web application in development

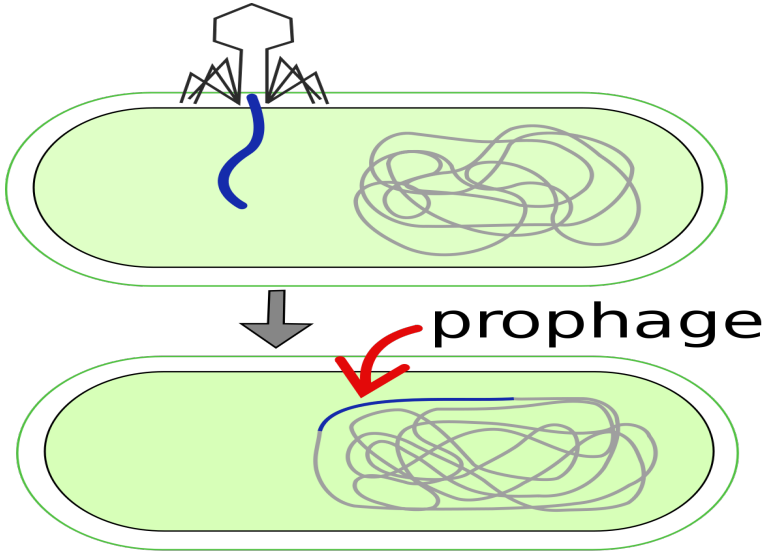
Viral GRAB

- Expansion of GRAB to viral elements
- Features include ability to filter viruses by genetic material type

Endogenous Viral Elements (Prophages)

Lysogenic Life Cycle

Viruses can integrate into host for an extended period of time



Importance of Prophages

Prophages can confer advantages to host improving survival

Prophages are important to the emergence of pathogenic bacteria

Canchaya C., et al. Curr Opin Microbiol 2003
Wagner PL. & Waldor MK. Infect Immune 2002

Finding Prophages

Prophage Discovery Problem

Same difficulties as gene prediction: finding signal in data

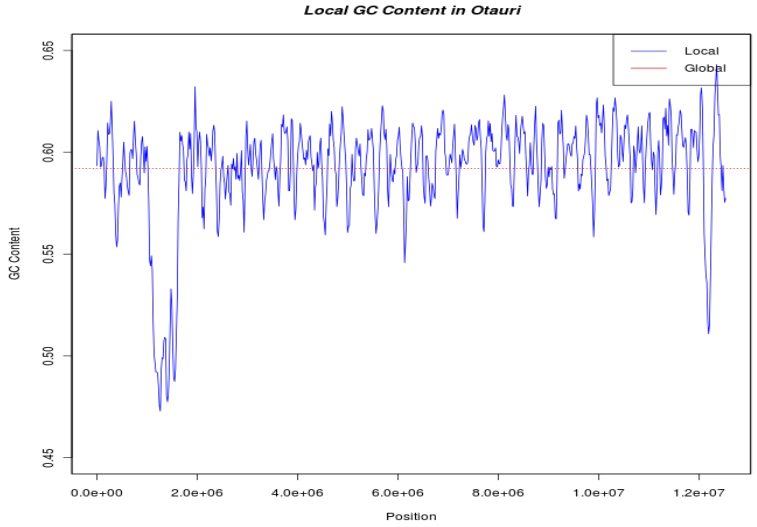
Prophage Discovery Tools

Number of Tools

10 tools listed at Omic Tools for prophage discovery

Methods Used

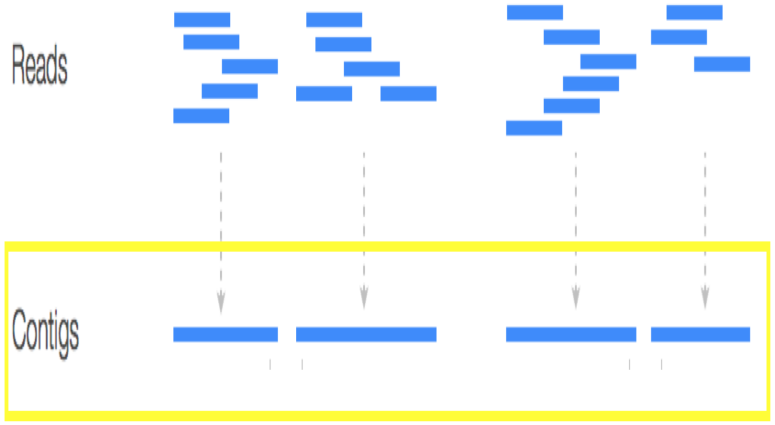
- Sequence similarity
- Hidden Markov models
- Transcription direction
- Protein length
- Sliding window GC content
- Phage specific kmer



Prophage Discovery Methods

Top Down Methods

All prophage discovery methods find prophages within contiguous sequences or genomes



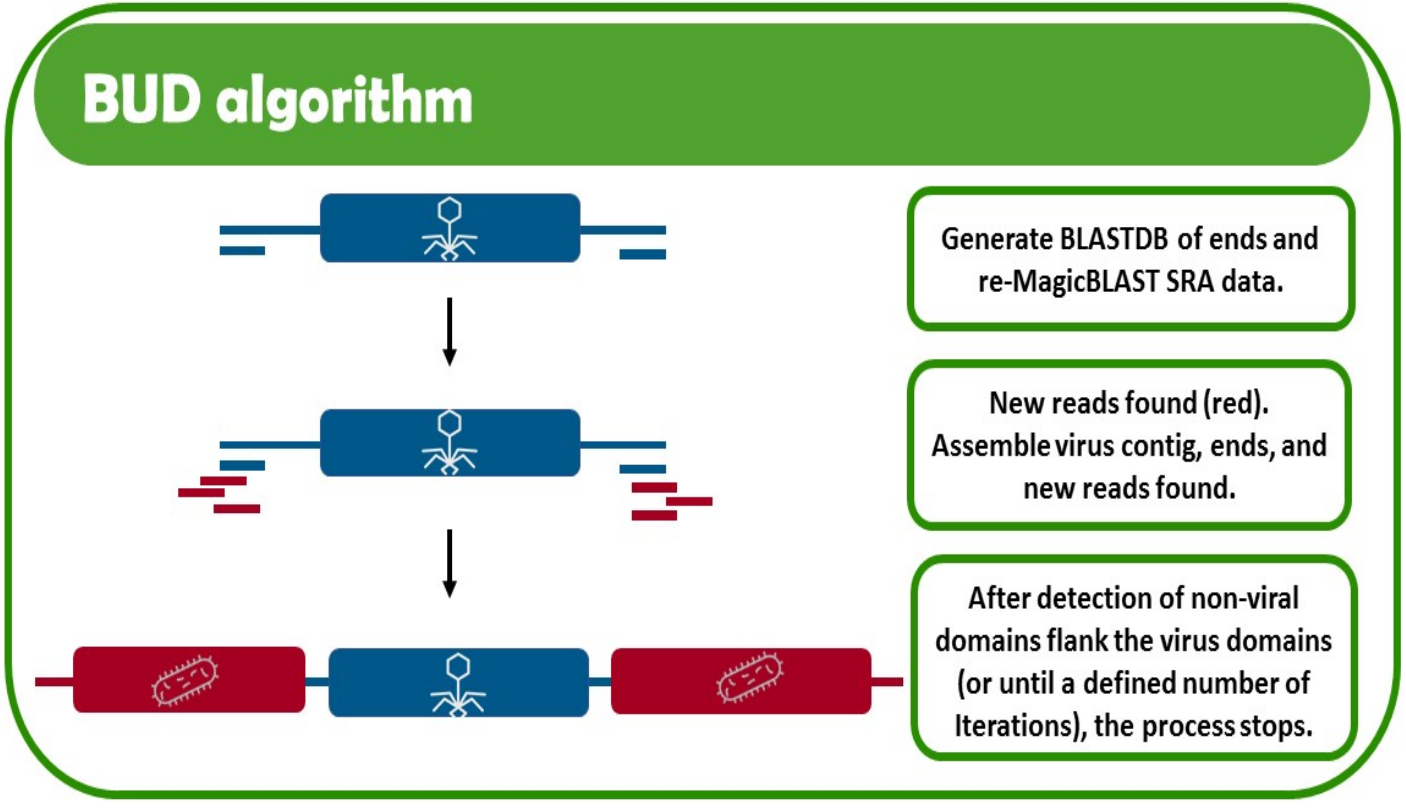
Potential Prophage Tool Pitfalls

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

Building Up Domains (BUD) Algorithm

Initialization

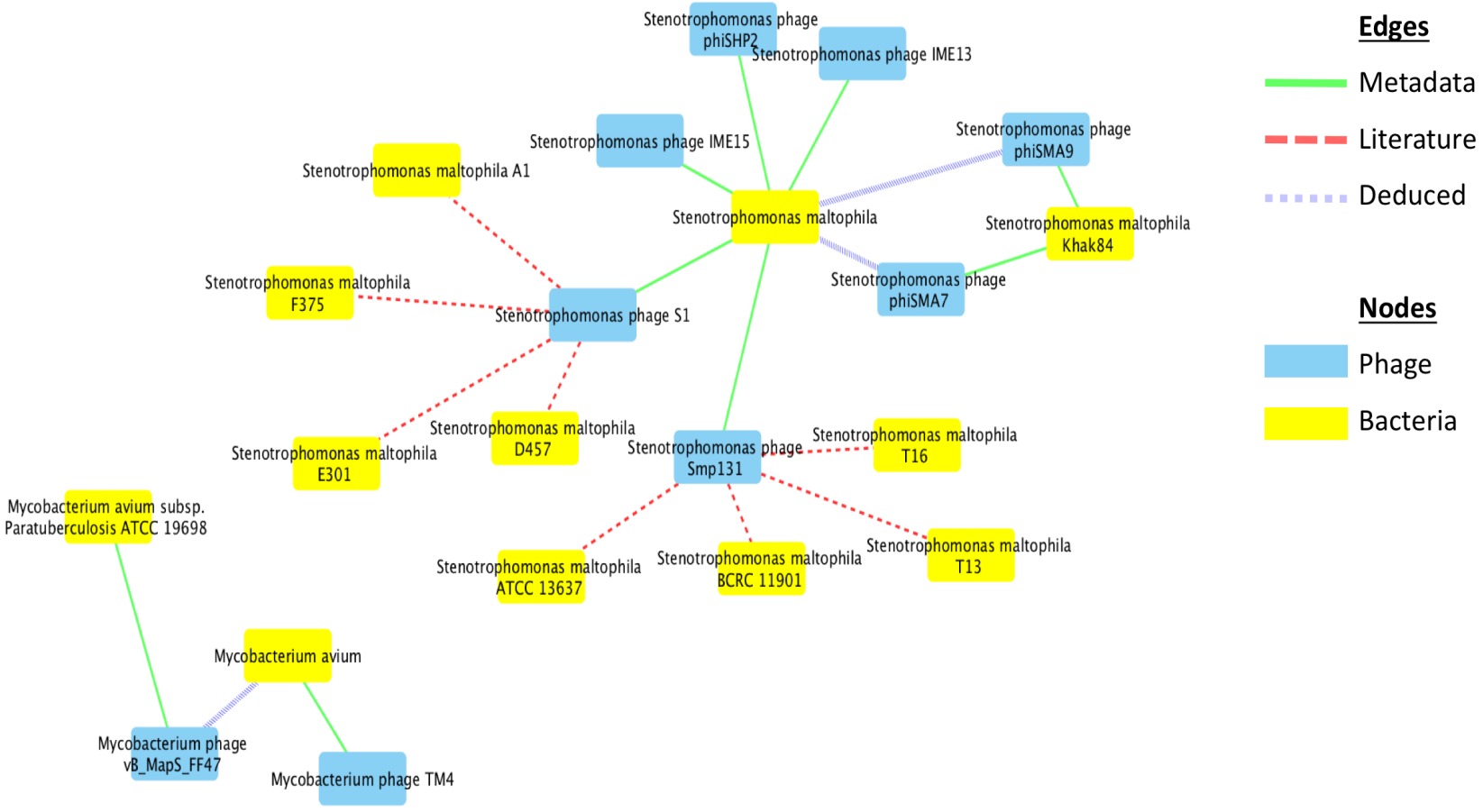
- Metagenomic reads are filtered by BLAST against Viral RefSeq
- Remaining reads are assembled into contigs



Potential Uses of BUD

Expanding Known Phage Host Range

BUD has the potential to identify novel hosts for prophages



Current Implementations of BUD

ViruSpy

- Originally written for NCBI Hackathon
- BUD Algorithm written in Perl and BASH
- Utilized Magic-BLAST for streaming of reads

EndoVir

NCBI Collaborators Jan Buchman and Ben Busby

- Written in Python
- Implementation of BUD with Magic-BLAST

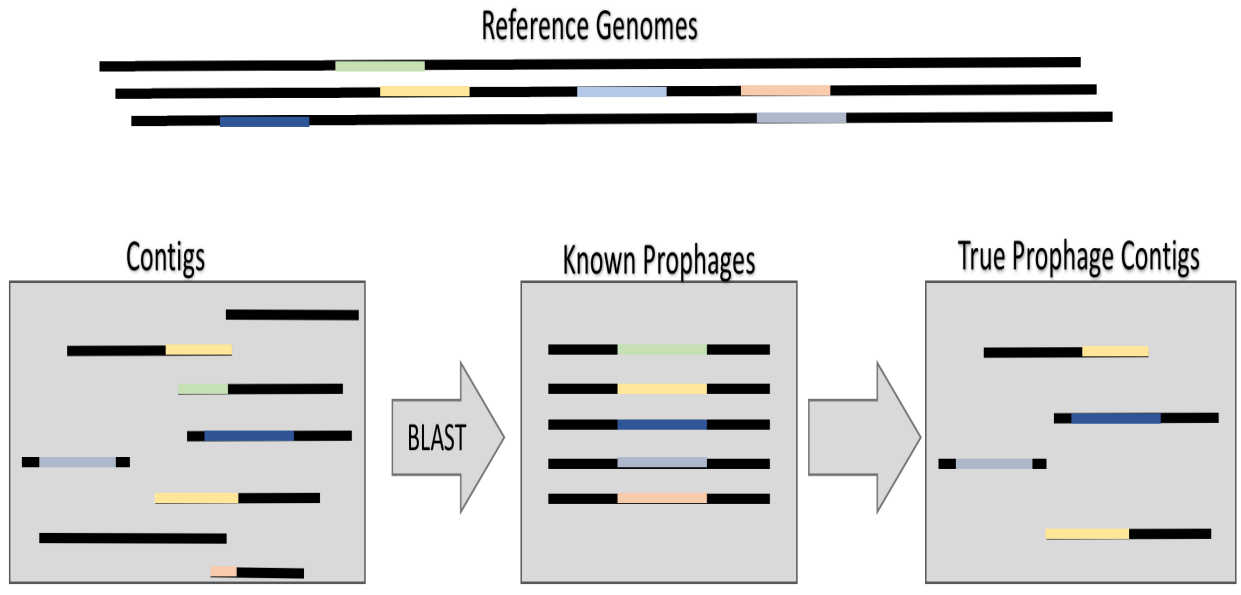
Future Directions

Magic-BLAST Streaming

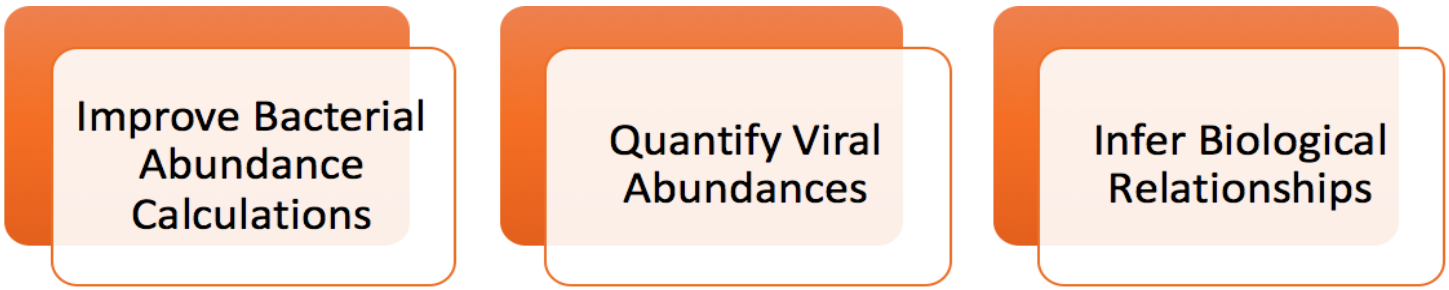
Create a version of BUD for local metagenomic sequences

Testing Performance of BUD

Using the simulated dataset from previous study to compare the performance of identifying prophages by current tools against BUD



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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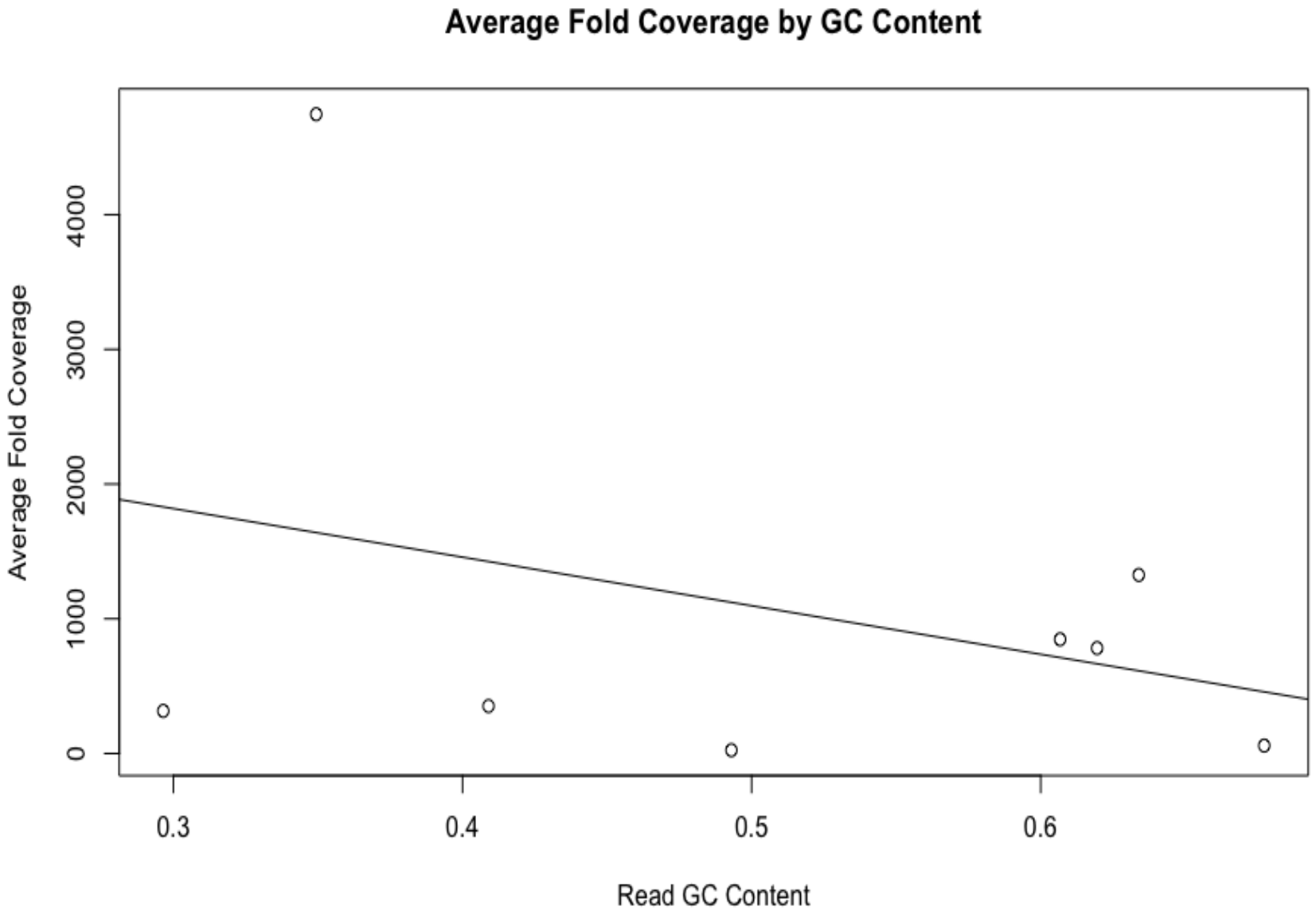
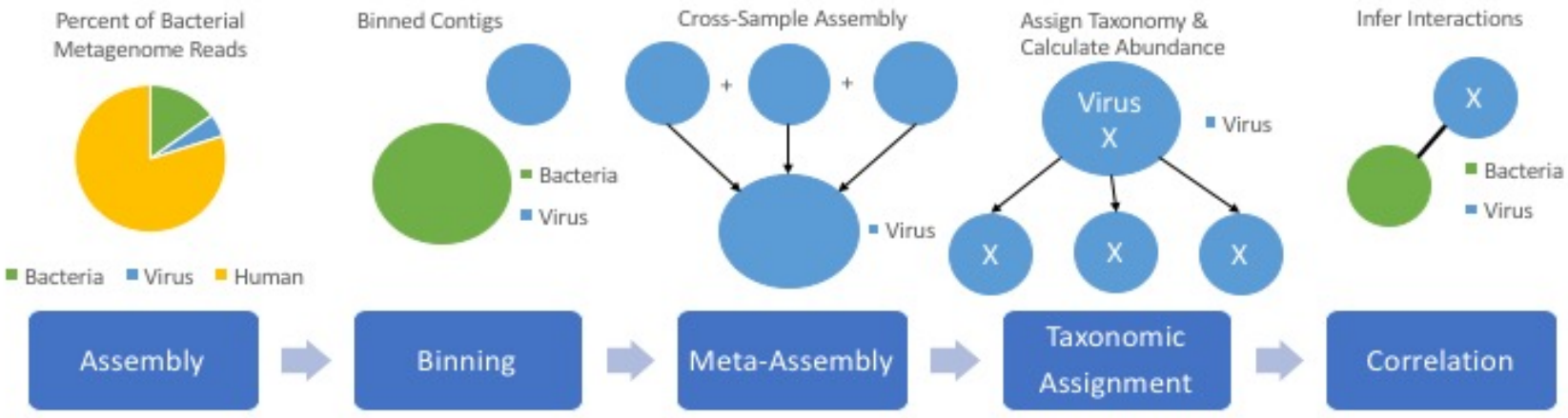
Bias in Average Fold Coverage by GC



My Pipeline


Percent of Bacterial Metagenome Reads
Bacteria
Virus
Human
Binned Contigs
Bacteria
Virus
Cross-Sample Assembly
Virus
Assign Taxonomy & Calculate Abundance
Virus X
Virus
X
X
X
Infer Interactions
X
Bacteria
Virus
Assembly
Binning
Meta-Assembly
Taxonomic Assignment
Correlation

Tools Used in Study Continued

Simulation Tools

BBMAP - a suite of tools designed for sequencing data
Bushnell, B., JGI 2016

Taxonomic Identification

Kraken - A reference-free K-mer taxonomic identifier
Wood, Derrick E., and Steven L. Salzberg Genome 2014

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

Prophage Identification

Phaster - A popular prophage discovery web tool
Arndt, David, et al., Nucleic Acids Research 2016