Metagenomic Exploration the Sequel: Development of tools for viral and bacterial sequence analysis

Cody Glickman

CPBS Update Talk

Nov 12th, 2018

Research Update Outline

Virulence Factors in Bacteriophages

Glickman C., Hendrix J., Strong M. Computational identification and analysis of bacterial virulence factors embedded into bacteriophage genomes. Poster session accepted at: Rocky 18: 2018 Dec 8-10; Snowmass, CO

Building Up Domains: Lysogenic Host Discovery

Incorporated into NCBI's Virus Discovery Project

Hybrid Viral Contig Prediction

Glickman C., Strong M. Hybrid Viral Identification in Metagenomics. In preperation: Early 2019

Progress of Other Projects

Asthma Environmental Microbiome

Koon P., Glickman C., Epperson L.E., Strong M., Clemente J.C., Vicencio A., Diette G., Bose S. Household determinants of the indoor environmental microbiome in an urban asthmatic population. Poster submitted: ATS 2019: 2019 May 17-22; Dallas, TX

Clinical NTM Gene Databases

Glickman C., Epperson L.E., Hasan N., Strong M. Clinical NTM Gene Database. In preperation: Late 2018

Duobiome: 18S/16S Parallel Analysis

Glickman C., Russell P., Epperson L.E., Strong M. DuoBiome: A workflow for mixed metagenomes. In preperation: Early 2019

Genomic Retrieval and Blast Database Creation

Glickman C., Strong M. Batch retrieval and BLAST database creation tool. Poster session accepted at: Modelling microbial communities and functions. ISME 17: 2018 Aug 12-17; Leipzig, Germany

Hawaiian Soil Chemistry and Culture

Glickman C., Virdi R., Epperson L.E., Strong M., Nelson S., Honda J. Relationship Between Soil Mineral Characteristics and Non-Tuberculosis Mycobacterium Growth. In preperation: Early 2019

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

Viral Focus

Bacteriophages (Phages)

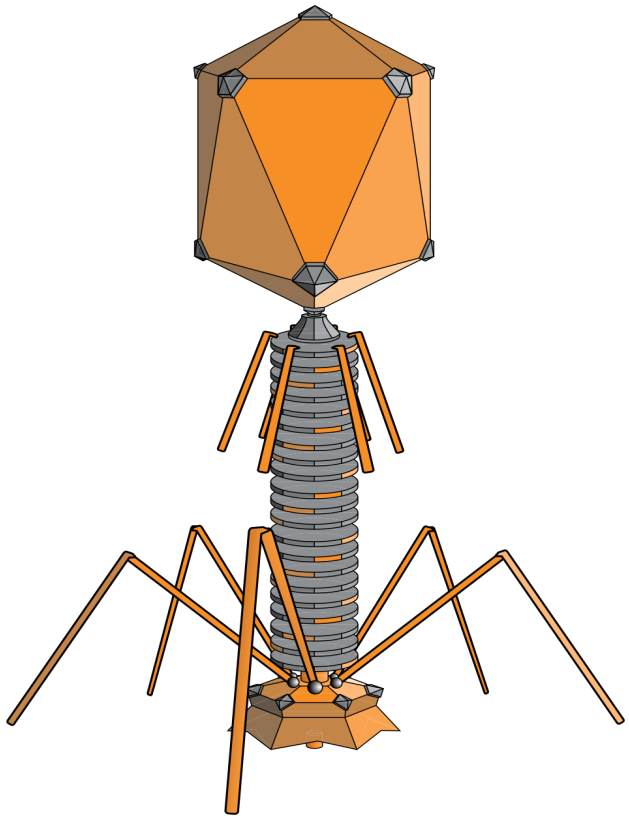
Phages are DNA viruses that infect prokaryotes

Phage Diversity

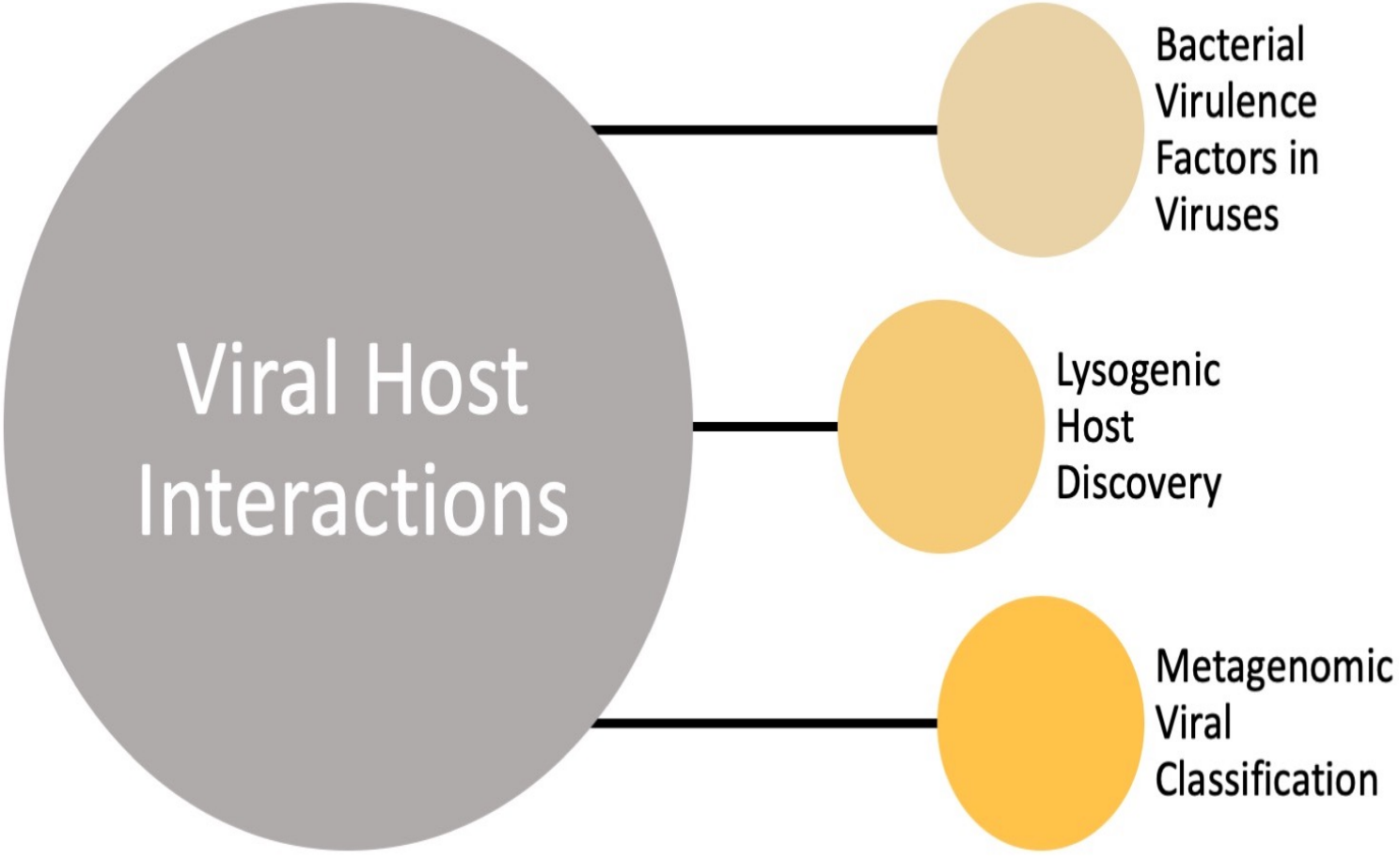
How phage abundance and diversity effects susceptibility to NTM lung infection

Phages as Vectors

How phages carry bacterial genes within clinical NTM infections



Objective


Viral Host Interactions
Bacterial Virulence Factors in Viruses
Lysogenic Host Discovery
Metagenomic Viral Classification

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Virulence

Virulence Defined

The capacity of a microorganism to proliferate despite host defenses

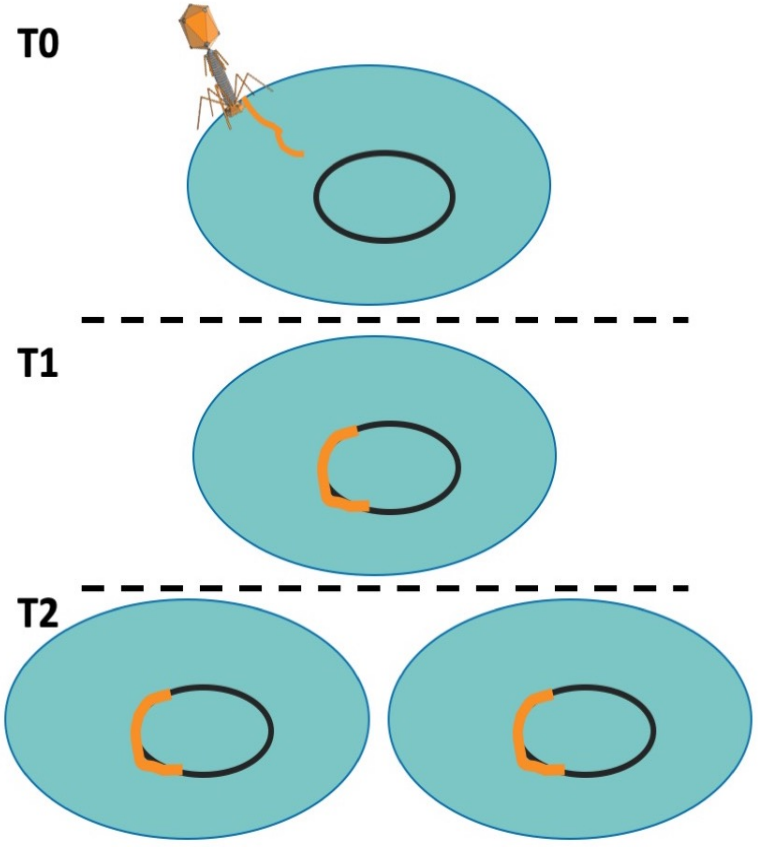
Influences on Virulence

- Number of microorganisms
- Composition of the mobile genetic reservoir
- Location of niche
- Host immune capabilities

Phages as a Genetic Reservoir of Virulence Factors Genes

Phages and Pathology

Virulence Factors that cause cholera, dysentery, botulism, and food poisoning are carried on phage elements.



Objective

Characterize the abundance of bacterial virulence factors within phages

Data

Virulence Protein Databases

- VFDB
  Chen, Lihong, et al. Nucleic Acids Research (2005)
- PatricVF
  Wattam, AR, et al. Nucleic Acids Research (2017)

Virulence HMMs

- pFam
  Bateman, Alex, et al. Nucleic Acids Research (2004)
- pVOG
  Grazziotin, AL, et al. Nucleic Acids Research (2016)

Phage Protein Database

Methods

Sequence Annotation Methods

Hidden Markov Models due to variation from rapid mutation rate

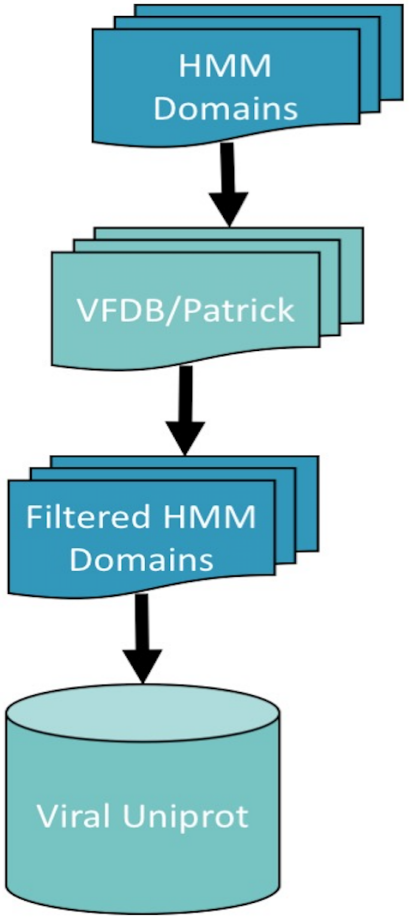
Normalizing By Gene Count

Hit Percentage $= P$
Hit Count $= HC$
Gene Count $= GC$

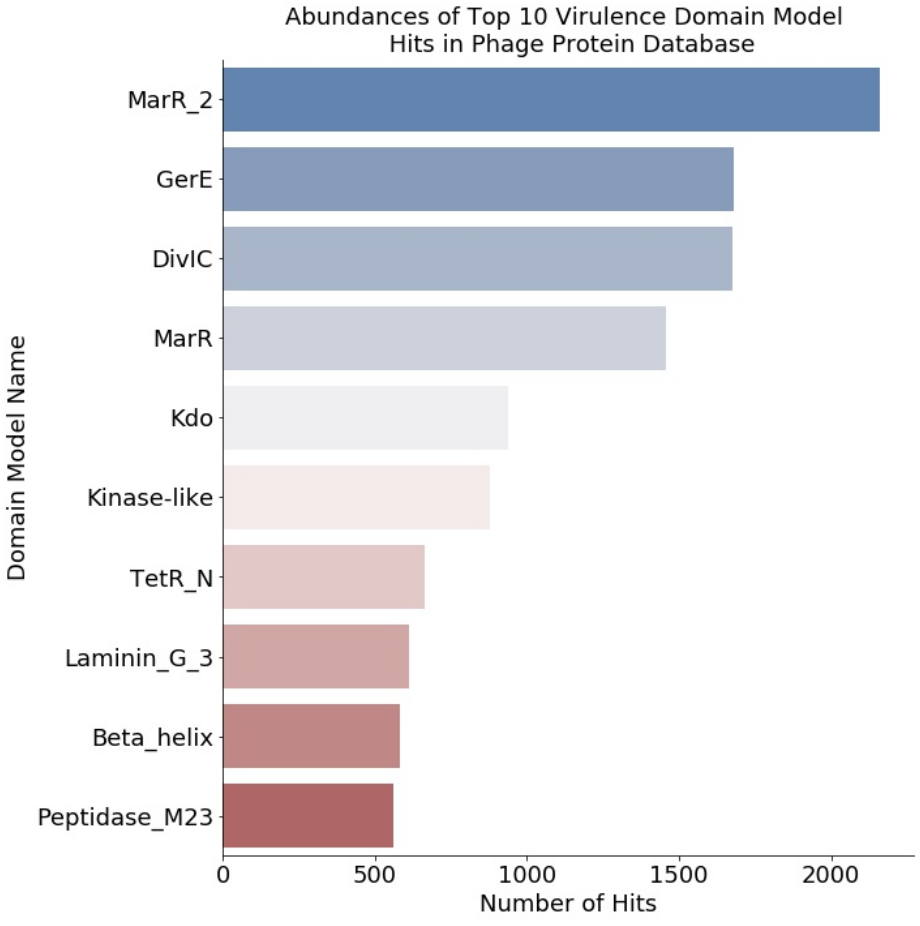
$$P = HC/GC$$

Filtering By Phage Abundance

Streptococcus phage:
Genera abundance greater than 30



HMM Hit Distribution



MarR_2/MarR

Domain involved in antibiotic resistance

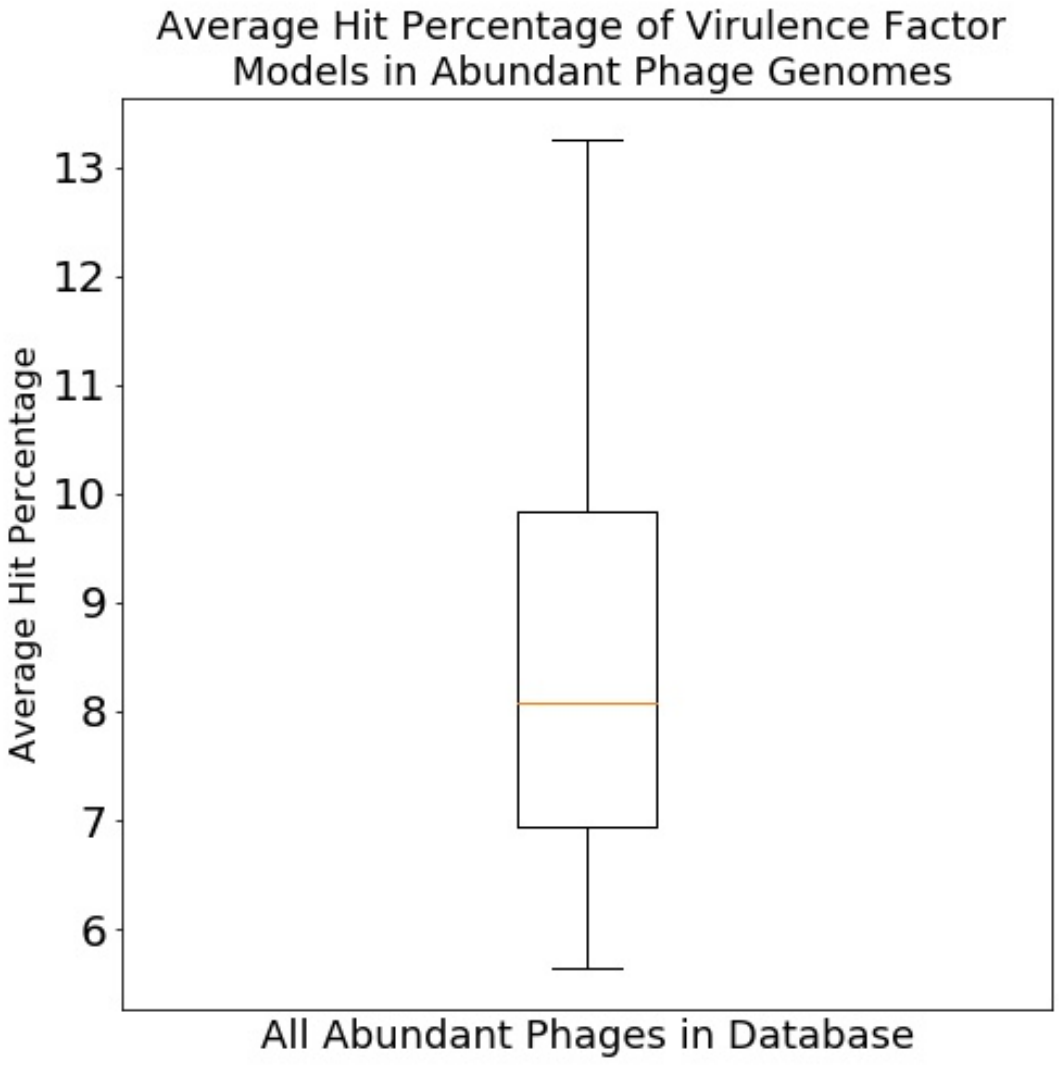
DivIC

Part of sporulation process

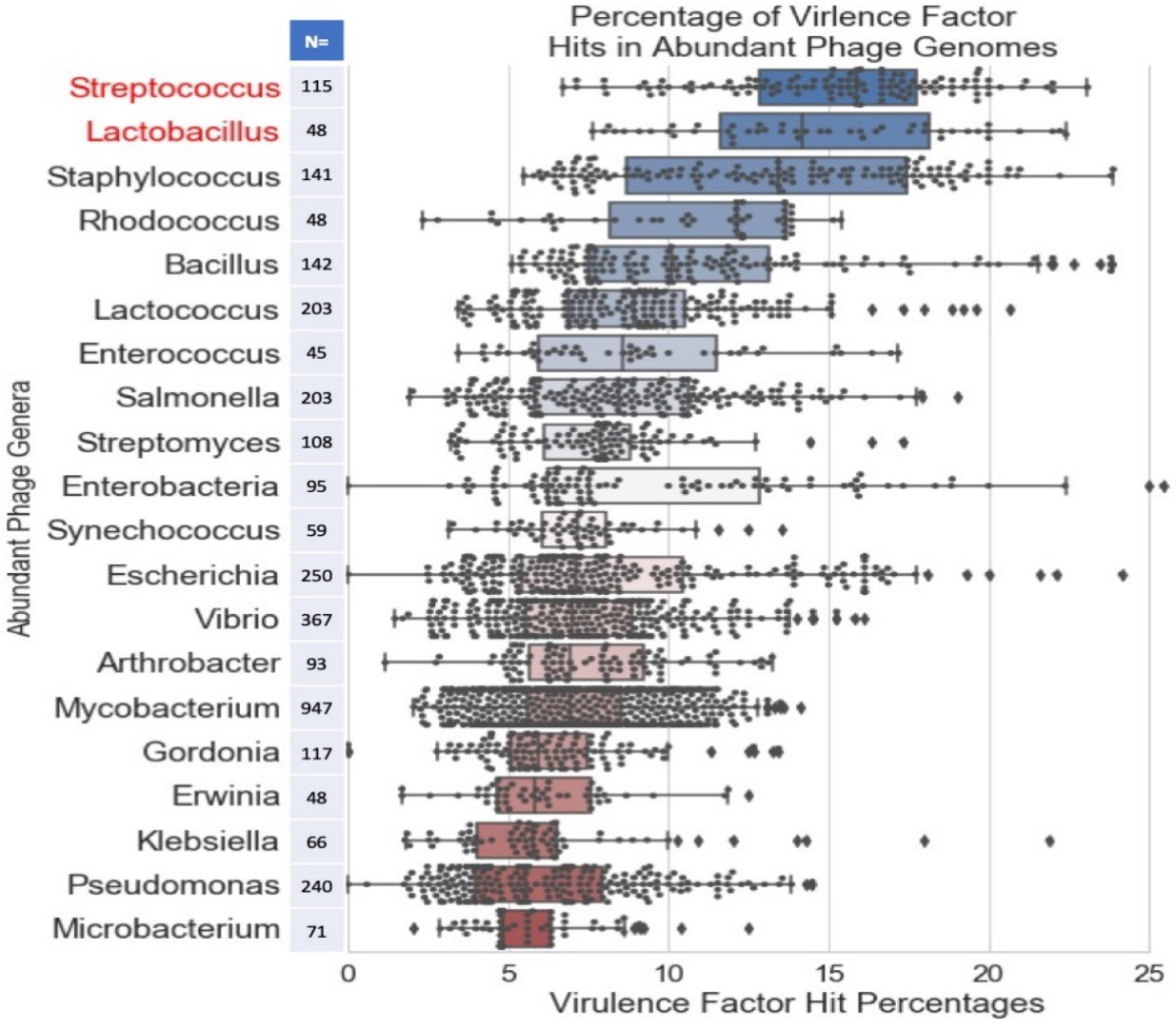
GerE

DNA binding domain found in virulence factors

Distribution of Hit Percentage in All Phages



Abundant Phage Distributions by Genera Name



Random Set of PFAMs

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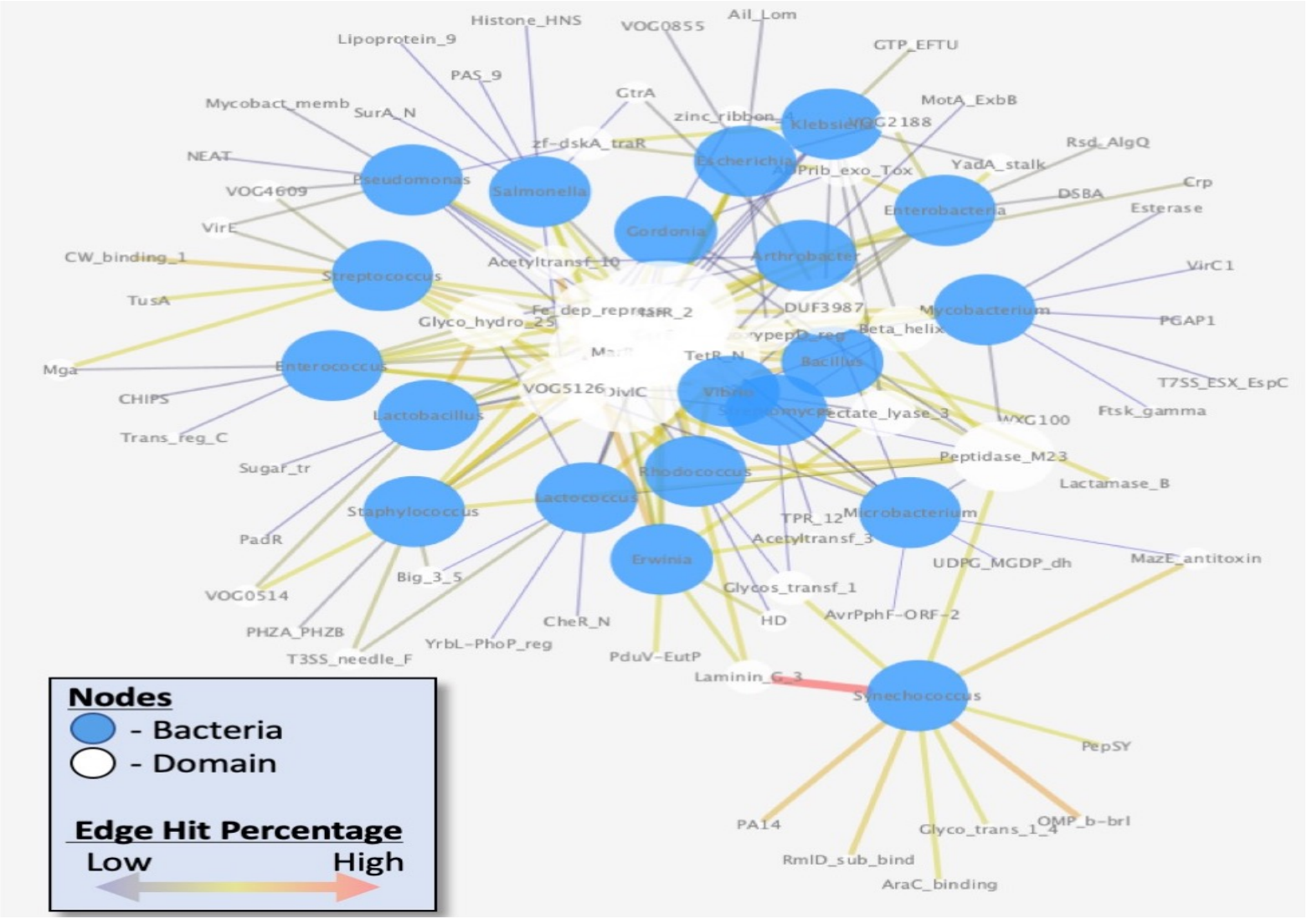
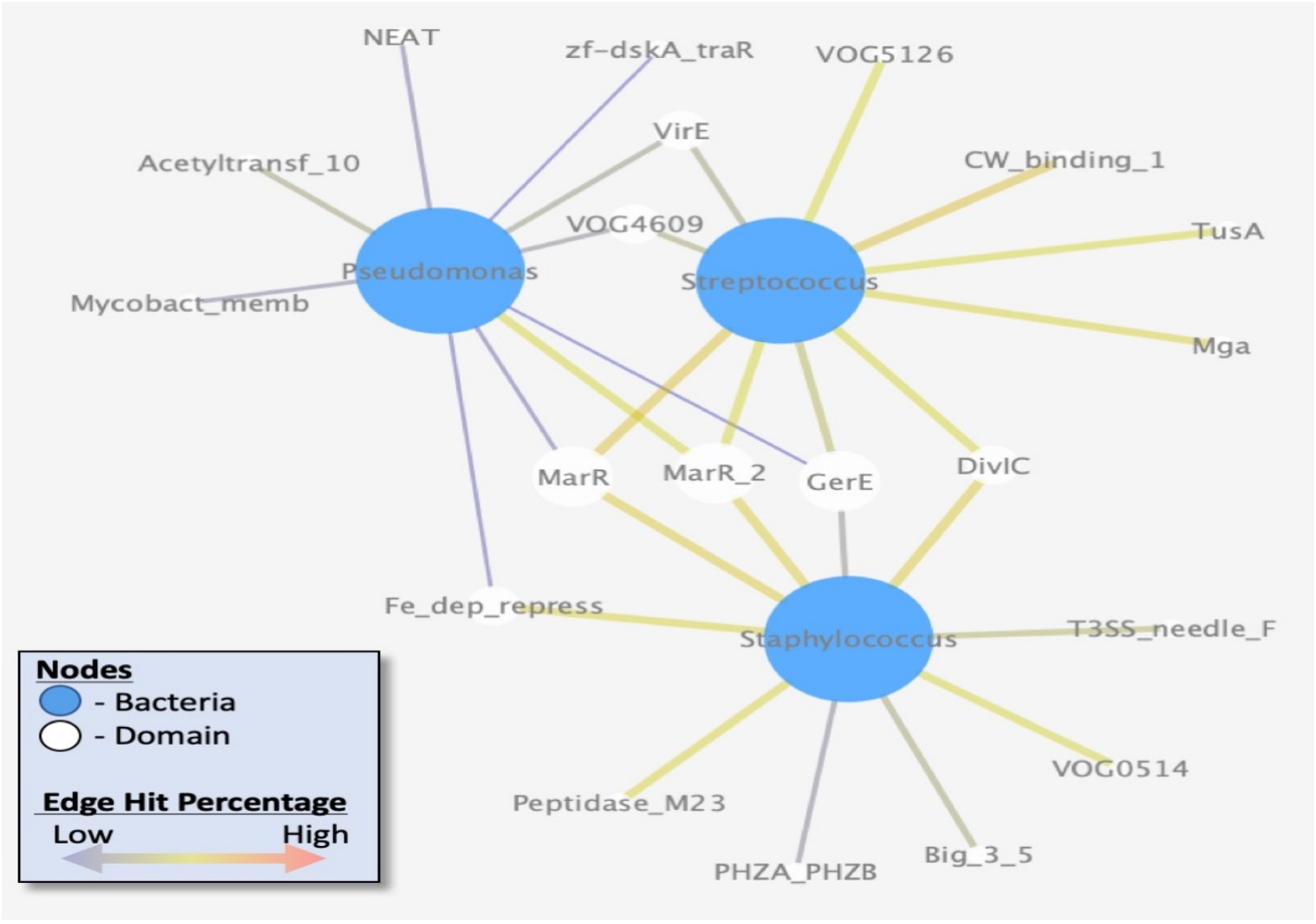
Full Top Domain Network



Pathogen Subset Domain Network



Comparison to Integrated Phages from Clinical NTM

Describe

Conclusions and Future Directions

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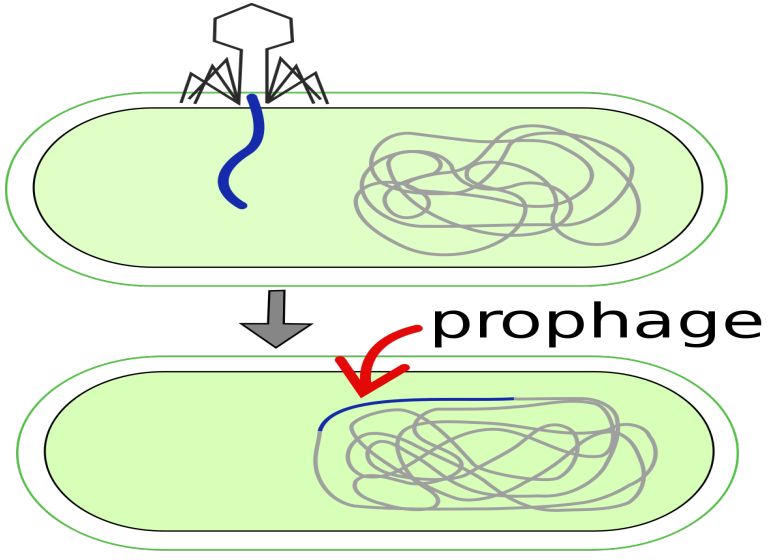
Endogenous Viral Elements (Prophages)

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

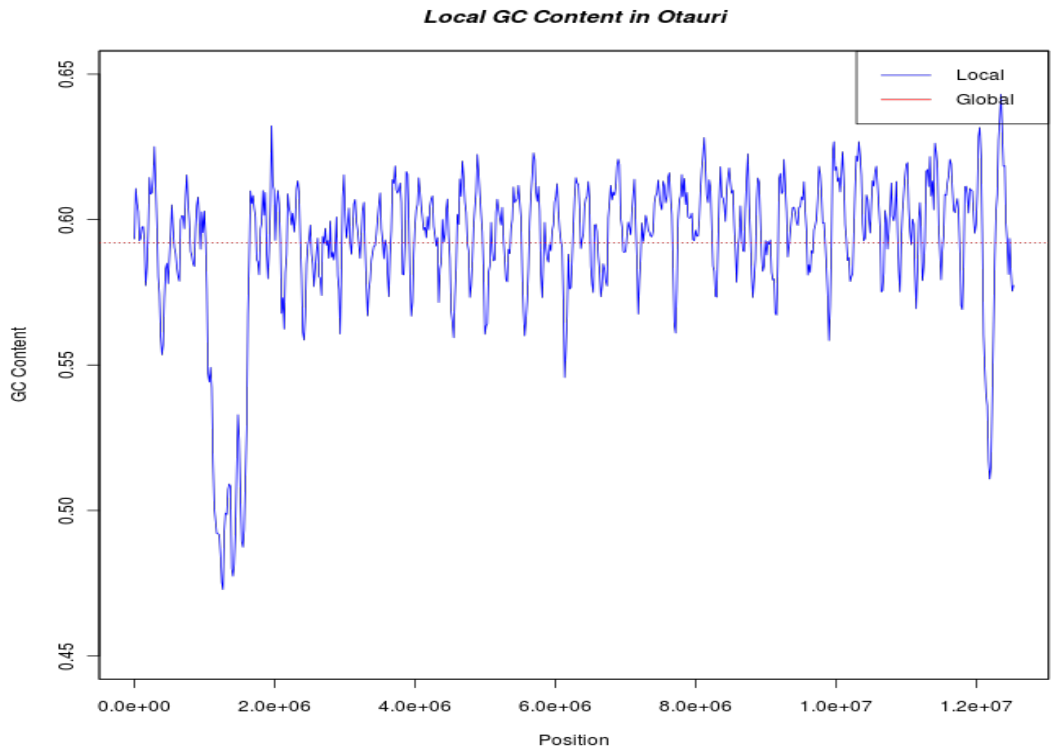
Prophage Discovery Problem

Same difficulties as gene prediction: finding signal in data

Prophage Discovery Tools

Current Methods Use

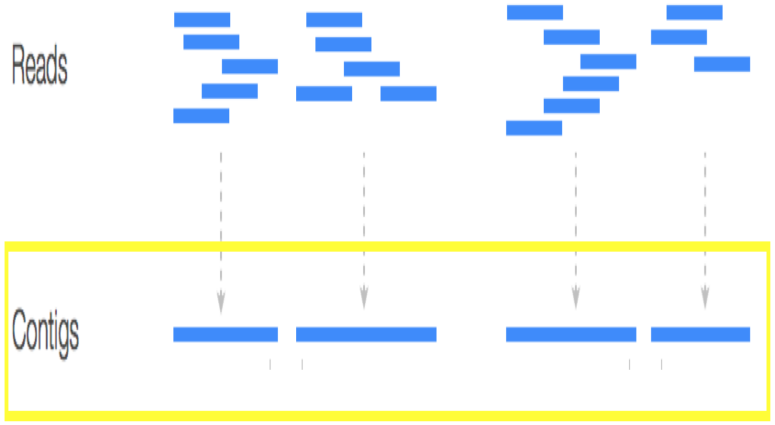
- Sequence similarity
- Hidden Markov models
- Transcription direction
- Protein length
- Sliding window GC content
- Phage specific kmer



Prophage Discovery Methods

Top Down Methods

All prophage discovery methods find prophages within contiguous sequences or genomes



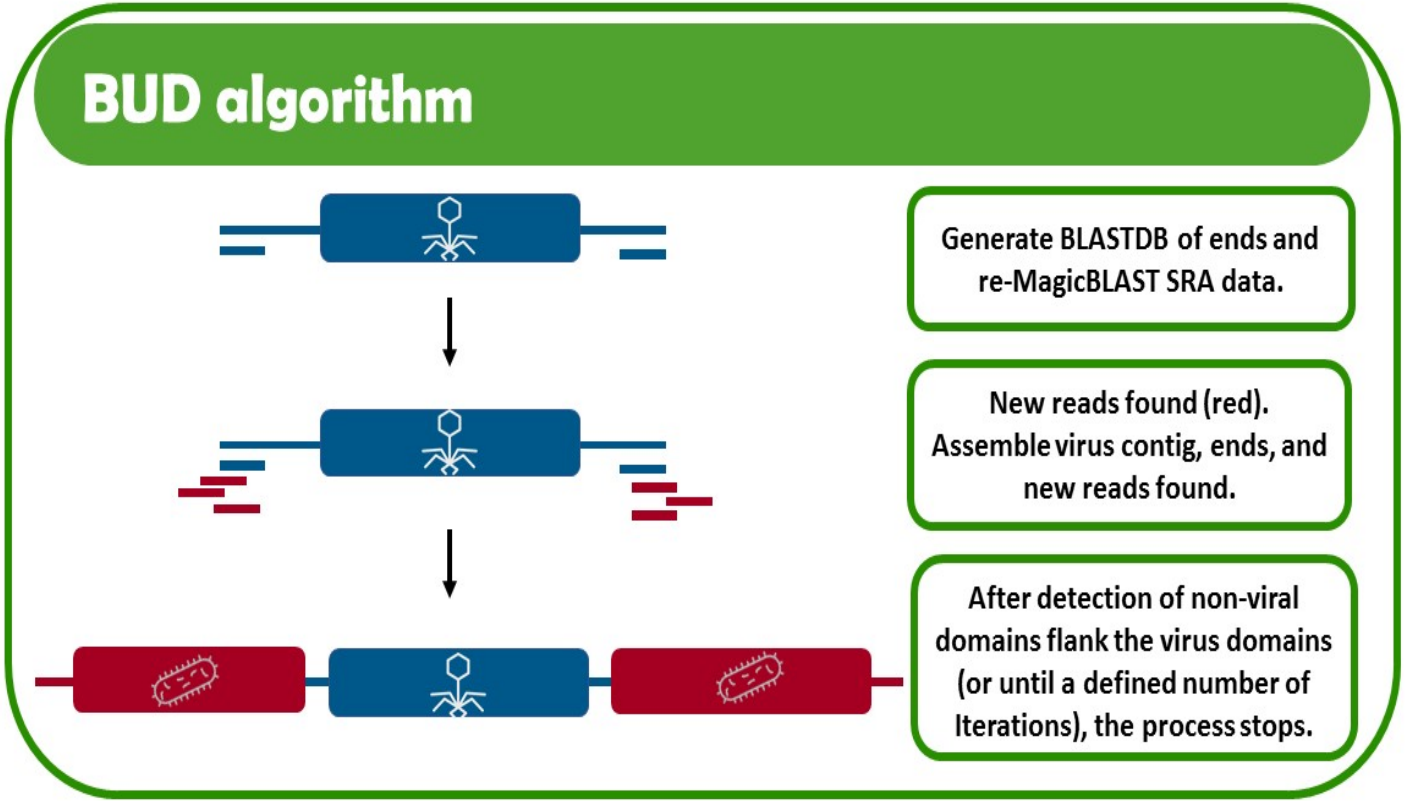
Potential Prophage Tool Pitfalls

Metagenomic sequencing produces short contigs that are discarded in current discovery methods

Building Up Domains (BUD) Algorithm

Initialization

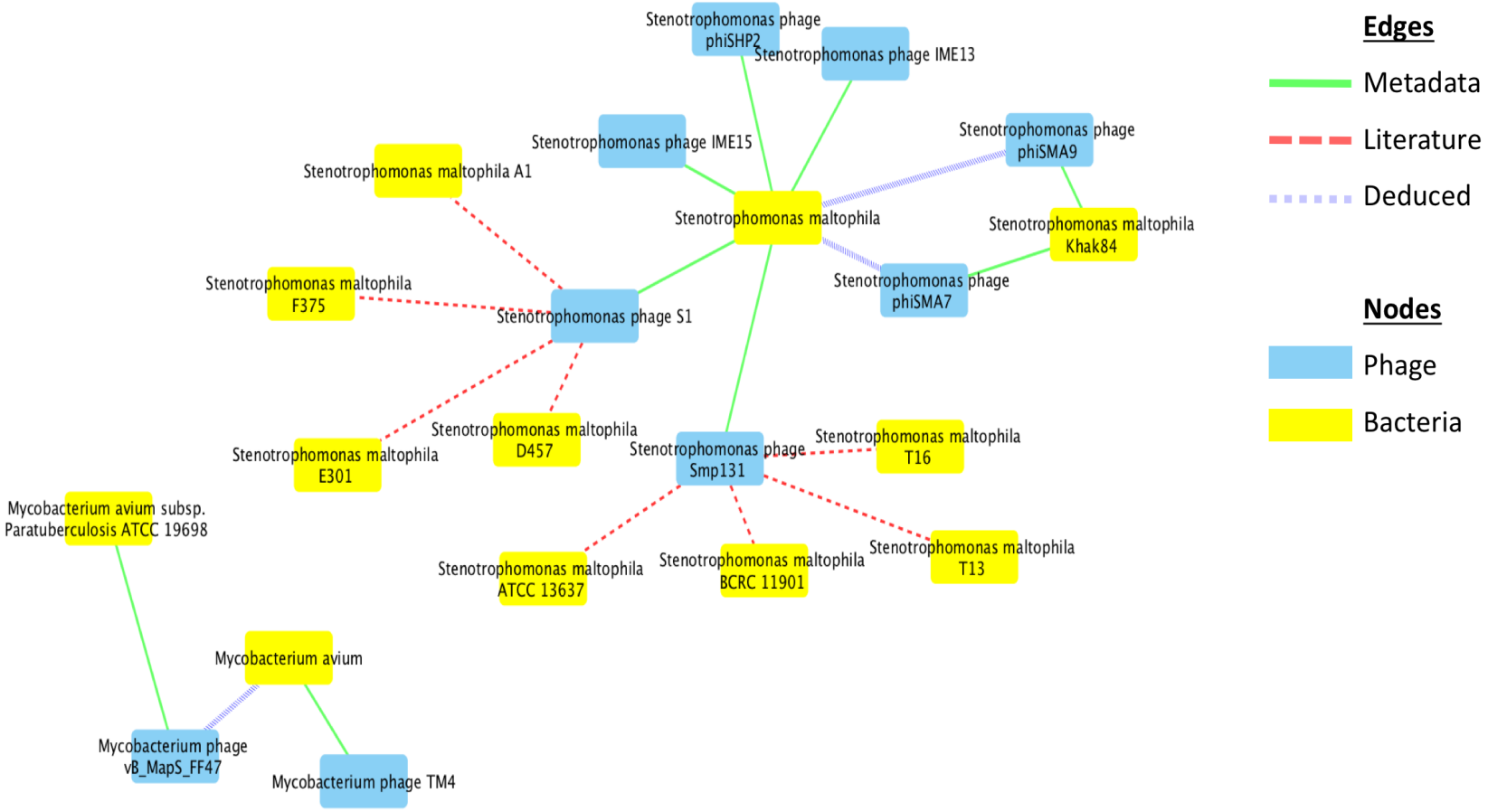
- Metagenomic reads are filtered by BLAST against Viral RefSeq
- BLAST hits are assembled into contigs



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

Overlap Consensus BUD

Create a version of BUD for local metagenomic sequences

Integration into Viral Discovery Project

Describe Project::

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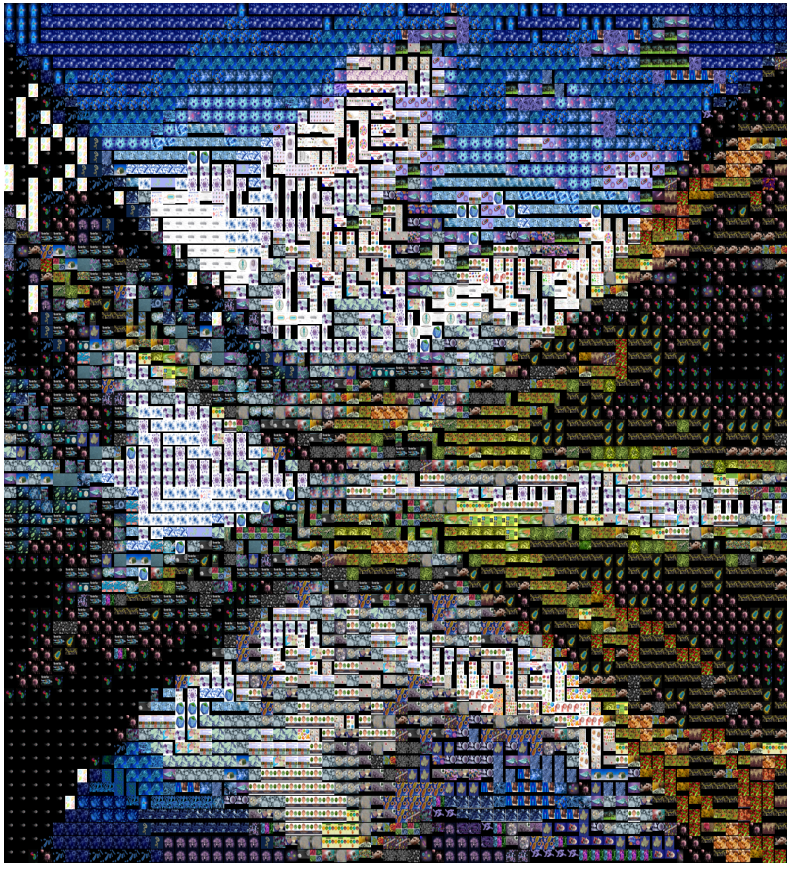
Metagenomics

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



Methods to Isolate Phages in Metagenomics

Biological Isolations

Filtrations and density gradiants to collect small particles

Lim, Y.W., et al. JoVE 2014

Sequence Similarity

Mapping to genomes, BLAST, and Hidden Markov Models

Roux, S., et al. PeerJ 2015

Machine Learning Methods

Linear discriminant analysis classifier on sequence k-mer profiles

Ren, Jie, et al. Microbiome 2017

Current Tool Limitations

Sequence Similarity

Acessibility to top performing tool is limited to integrated environment

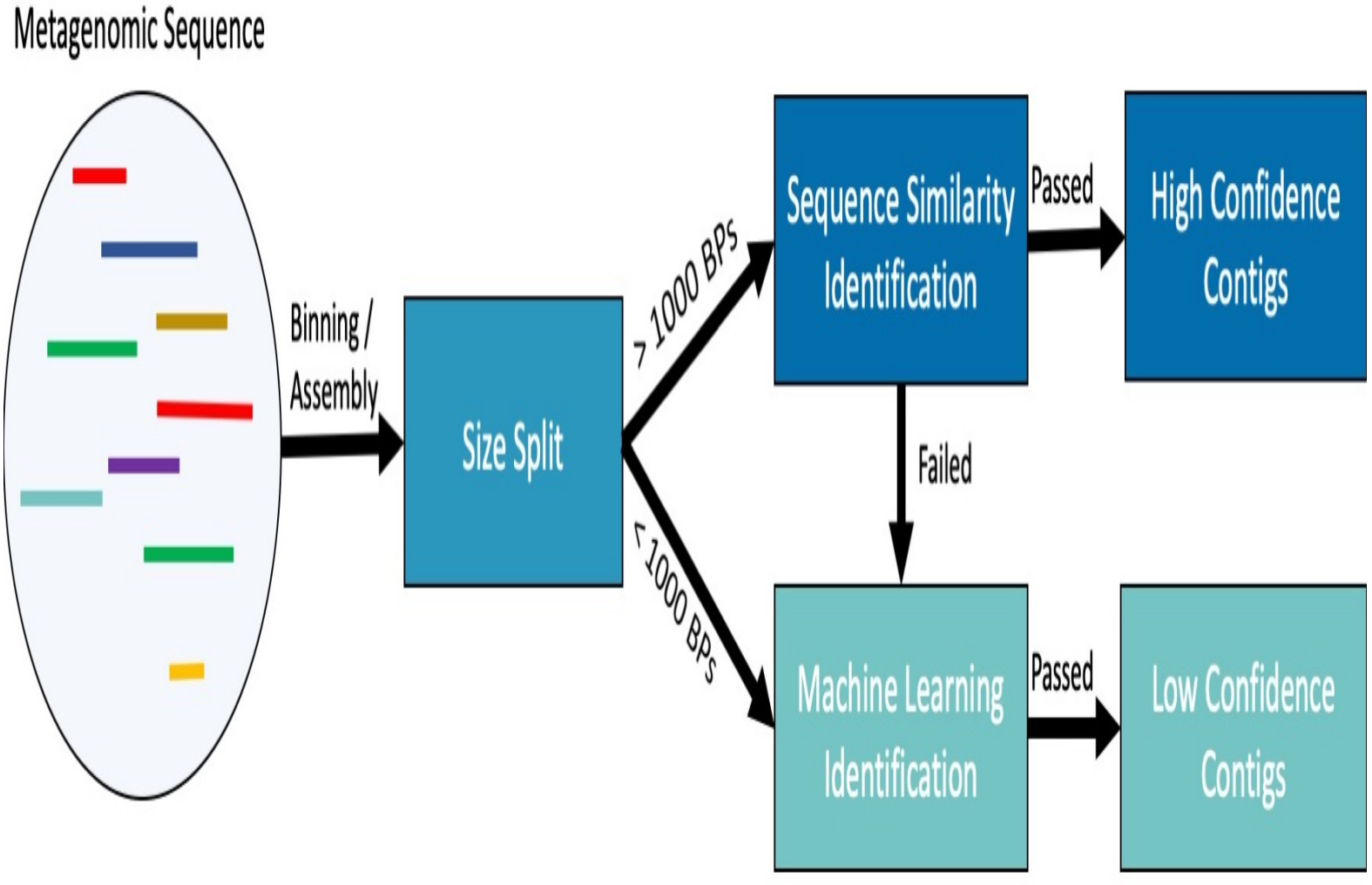
Roux, S., et al. PeerJ 2015

Machine Learning Methods

Limited to R workflow

Ren, Jie, et al. Microbiome 2017

Two-Step Hybrid Model



Methods

HMMs from Earth Virome
Python developed model with standalone operability

Comparison

Performance Comparison Using Critical Assessment of Metagenome Interpretation (CAMI) Data

Sczyrba, A., et al. Nature Methods 2017

Future Directions

Concluding Remarks

Virulence Factors in Bacteriophages

Optimized methods to simultaneously explore eukaryotic and prokaryotic communties

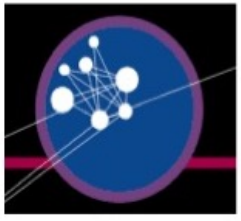
Building Up Domains: Lysogenic Host Discovery

A hybrid model to identify phage elements in metagenomics and connect them with bacteria

Hybrid Viral Contig Prediction

First quantification of bacterial virulence factors within phage genomes

Acknowledgements



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Questions?

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