



RecyclesEU

recycleseu

Recycles EU

RECYCLES WORKSHOP

Metagenomics and metabarcoding approaches to describe ecological systems and infer their development

5th, 6th & 7th of July 2022

Metagenomics Analysis of the Resistome and Phageome in Wastewater Treatment Plants in Winnipeg Manitoba

Speaker/authors

Kadir Yanac

GA: 872053 — H2020 - MSCA - RISE-2019



European
Commission



Outline



1. A Brief Introduction: Why is metagenomic analysis of phages important?
2. Materials and Methods
3. Workflow for metagenomic analysis



1. Why is metagenomic analysis of phages important?



The most abundant organisms in the biosphere

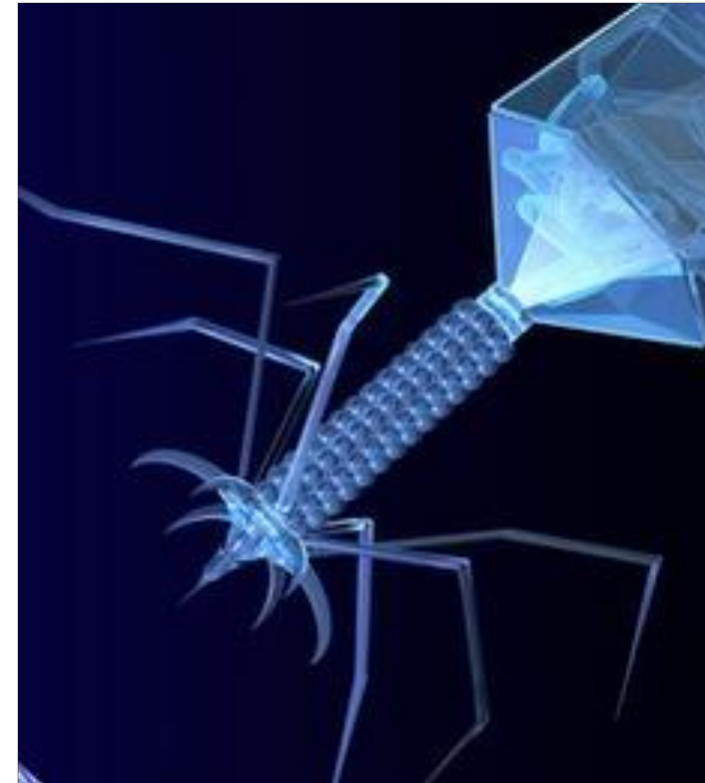
Vectors of horizontal gene transfer (HGT)
(Antibiotic resistance gene (ARG) transfer)

Drivers of bacterial evolution

Fecal pollution indicators

Can be used to control bacterial communities in wastewater

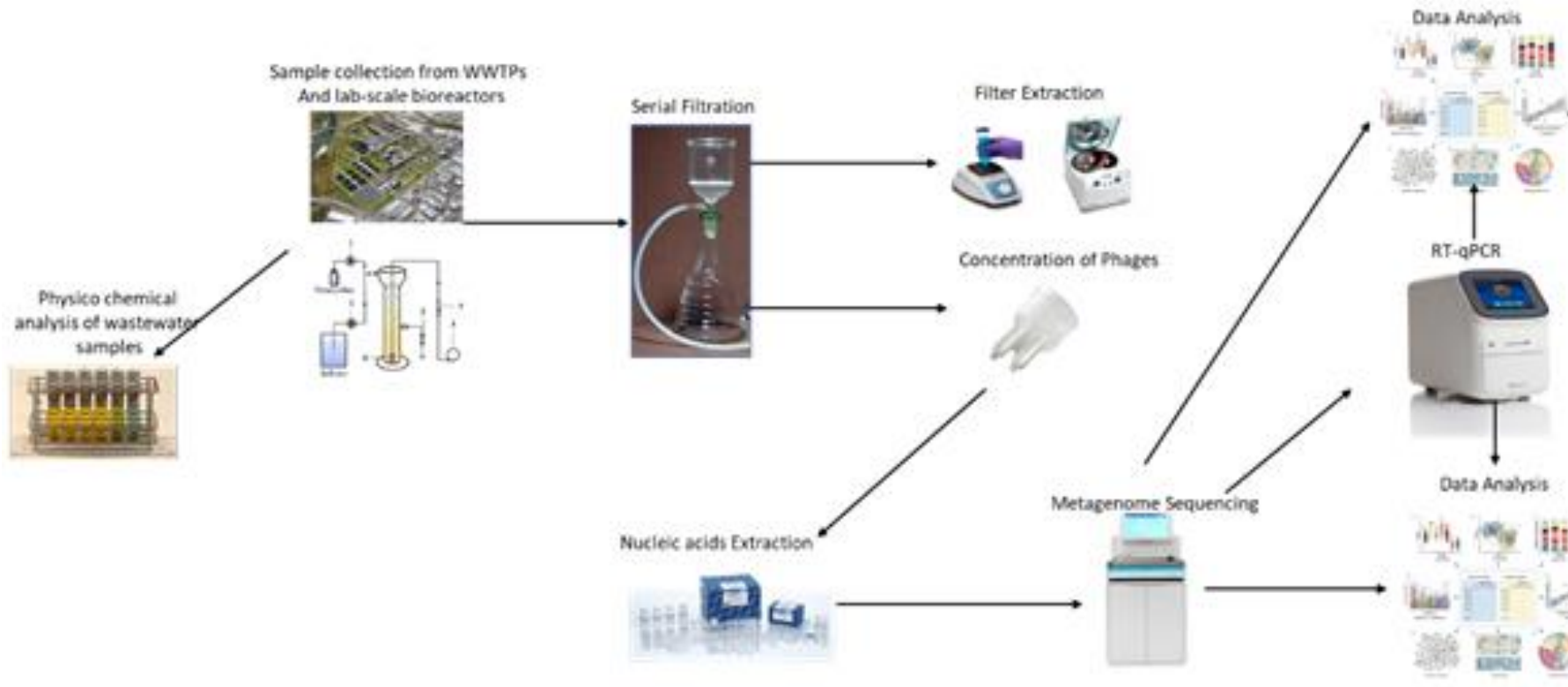
Can be used to cure bacterial infections



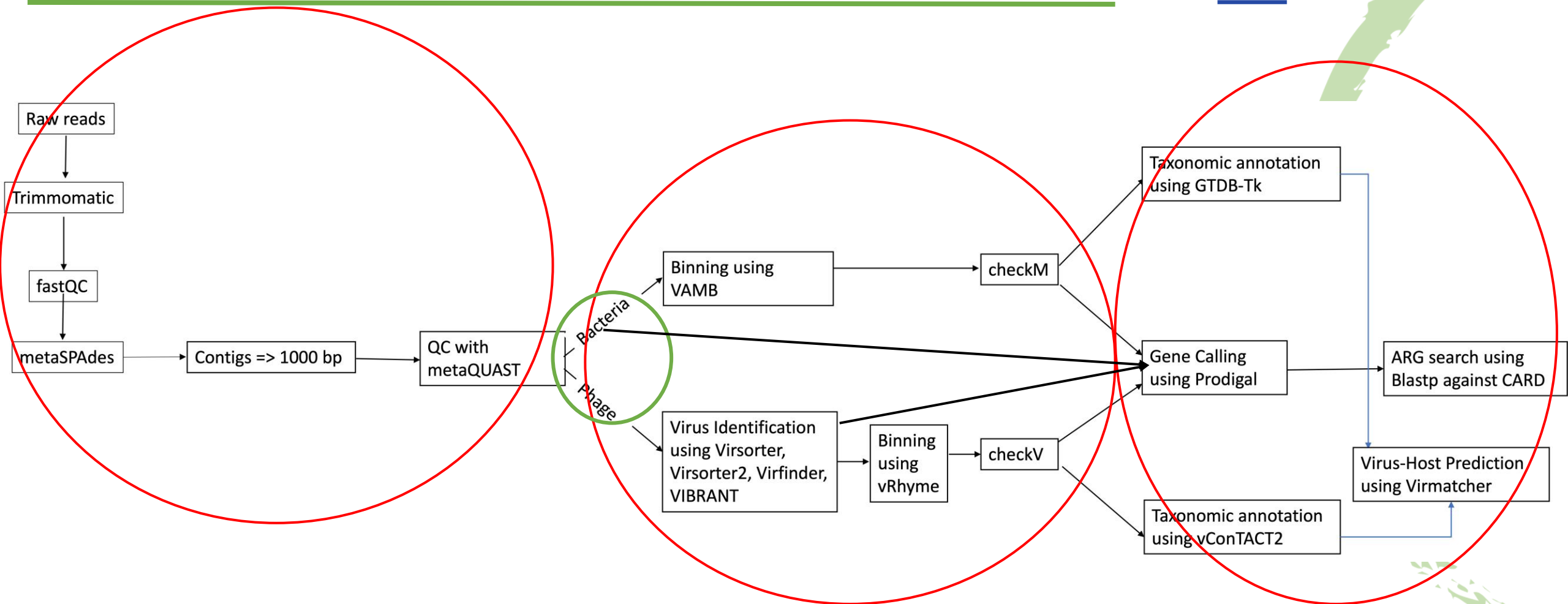
2. Sample Collection and Processing



- Our objective is to assess changes in phage and resistome profiles throughout WWTPs and the potential of phages as the carriers of ARGs



3. Workflow for Metagenomic Analysis



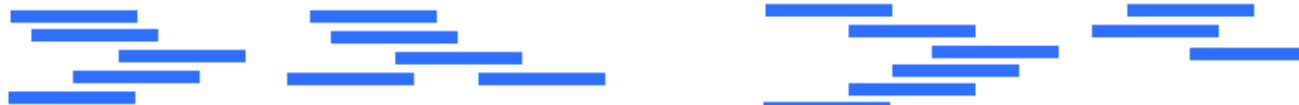
3. Workflow for Metagenomic Analysis



Genome



Reads



Contigs



Scaffolds



3. Workflow for Metagenomic Analysis



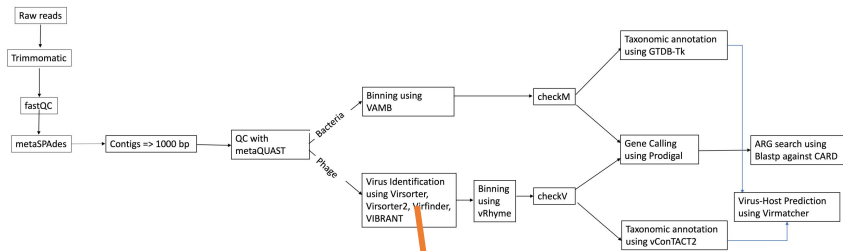
Bacterial DNA
contamination in
phage reads

- Bacterial contamination level in phage samples can be assessed using viromeQC, mapping reads against bacterial databases such as 16S SILVA Collection and/or targeting 16S rRNA using qPCR

3. Workflow for Metagenomic Analysis



Virus Identification



Virus Identification
using Virsorter,
Virsorter2, Virfinder,
VIBRANT

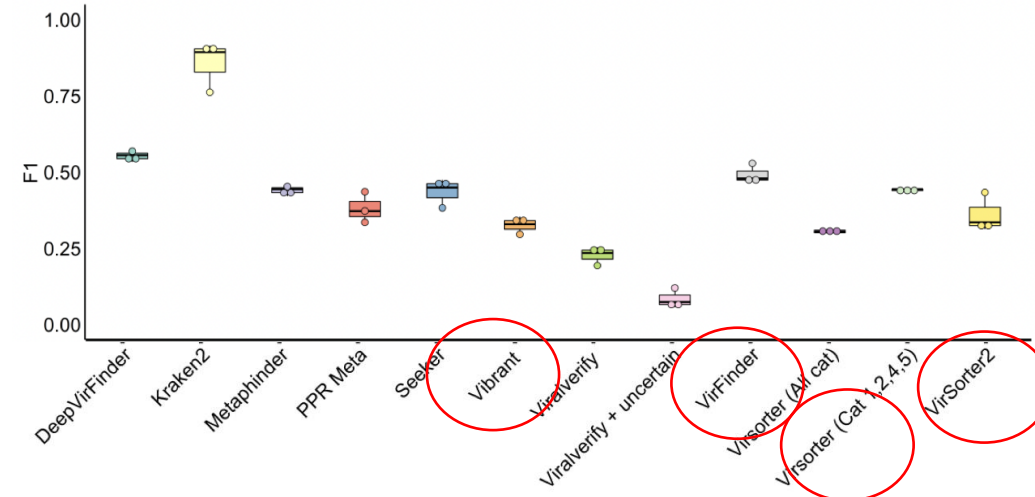


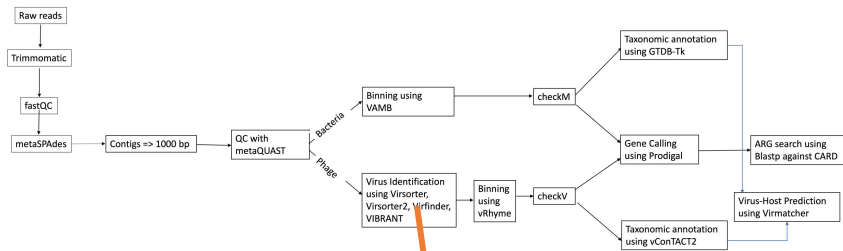
Figure 1. Comparison of viral identification tools on uneven mock community samples (1)

1. Ho, S. F. S., Wheeler, N., Millard, A. D., & van Schaik, W. (2022). Gauge your phage: Benchmarking of bacteriophage identification tools in metagenomic sequencing data. *bioRxiv*, 2021.2004.2012.438782. doi:10.1101/2021.04.12.438782

3. Workflow for Metagenomic Analysis



Virus Identification



Virus Identification
using Virsorter,
Virsorter2, Virfinder,
VIBRANT

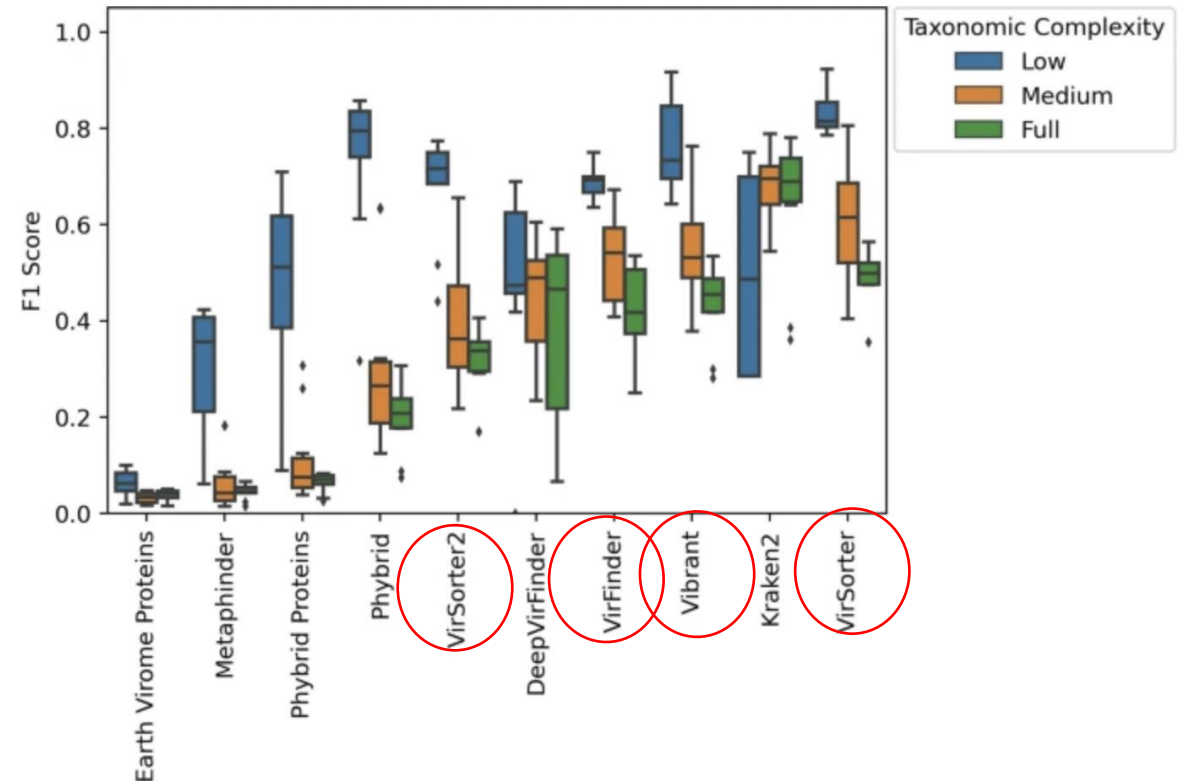


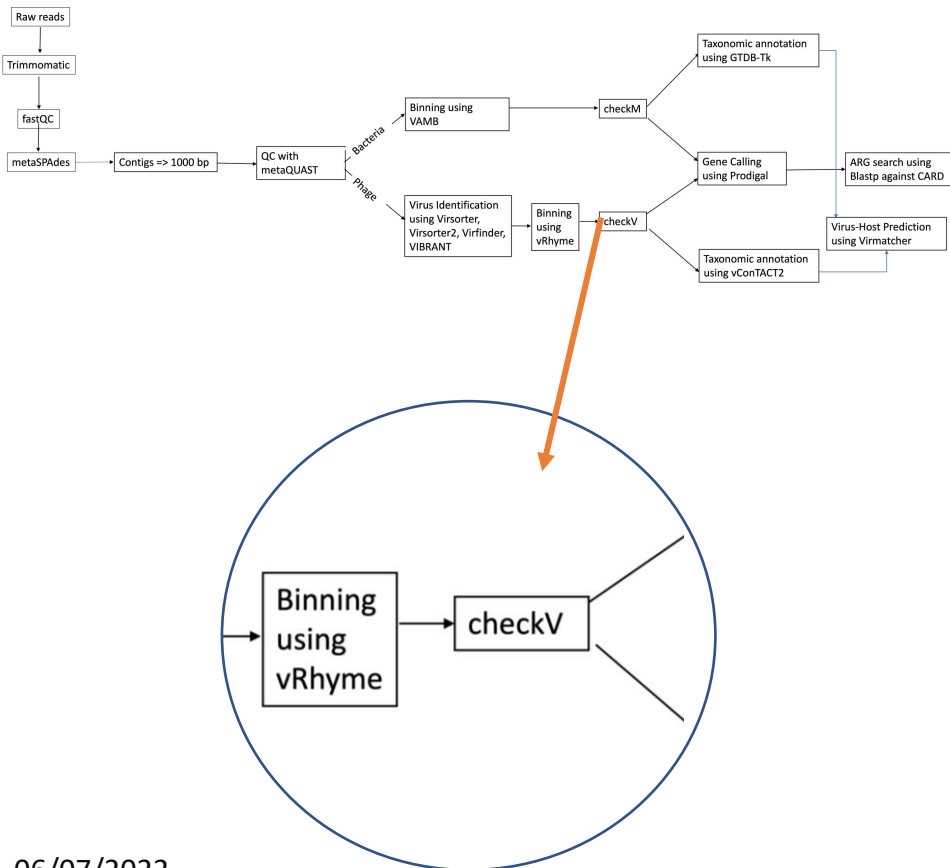
Figure 2. Comparison of viral identification tools on viral communities with different complexities (2)

2. Glickman, C., Hendrix, J. & Strong, M. Simulation study and comparative evaluation of viral contiguous sequence identification tools. *BMC Bioinformatics* **22**, 329 (2021). <https://doi.org/10.1186/s12859-021-04242-0>

3. Workflow for Metagenomic Analysis



Viral Metagenome Binning

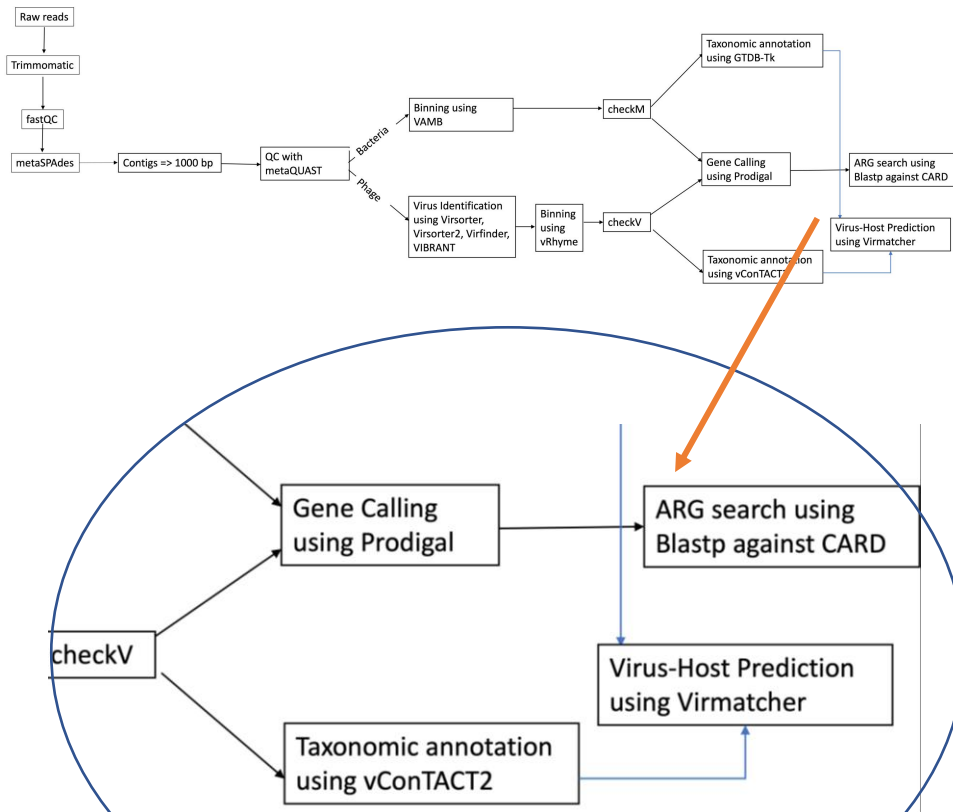


- A recently published tool, vRhyme, was used for binning phage genomes from metagenome (contigs).
- Metagenome assembled genomes (MAGs) predicted by binning tools can generate stronger inferences regarding viral metabolic potential, taxonomy, and community interactions.
- checkV can be used to assess the quality of MAGs, including estimation of completeness for genome fragments and identification of closed genomes.

3. Workflow for Metagenomic Analysis



Functional and Taxonomic Annotation



- Prodigal was used to annotate bacterial, archaeal and viral genomes
- Annotated genes (open reading frames (ORFs)) were used as queries to search for ARGs against the comprehensive antibiotic resistance database (CARD) with e value of $\leq 1 \times 10^{-5}$, query coverage $\geq 60\%$, and percent identity $\geq 70\%$
- vConTACT2 is used for viral taxonomic annotation and classification of unclassified and new phages/viruses

3. Workflow for Metagenomic Analysis



vConTACT2 in combination with Cytoscape

- vConTACT2 can be used to predict unknown phages.
- It can be used to help to distinguish between the classification of genomes as phage, plasmid or unknown.

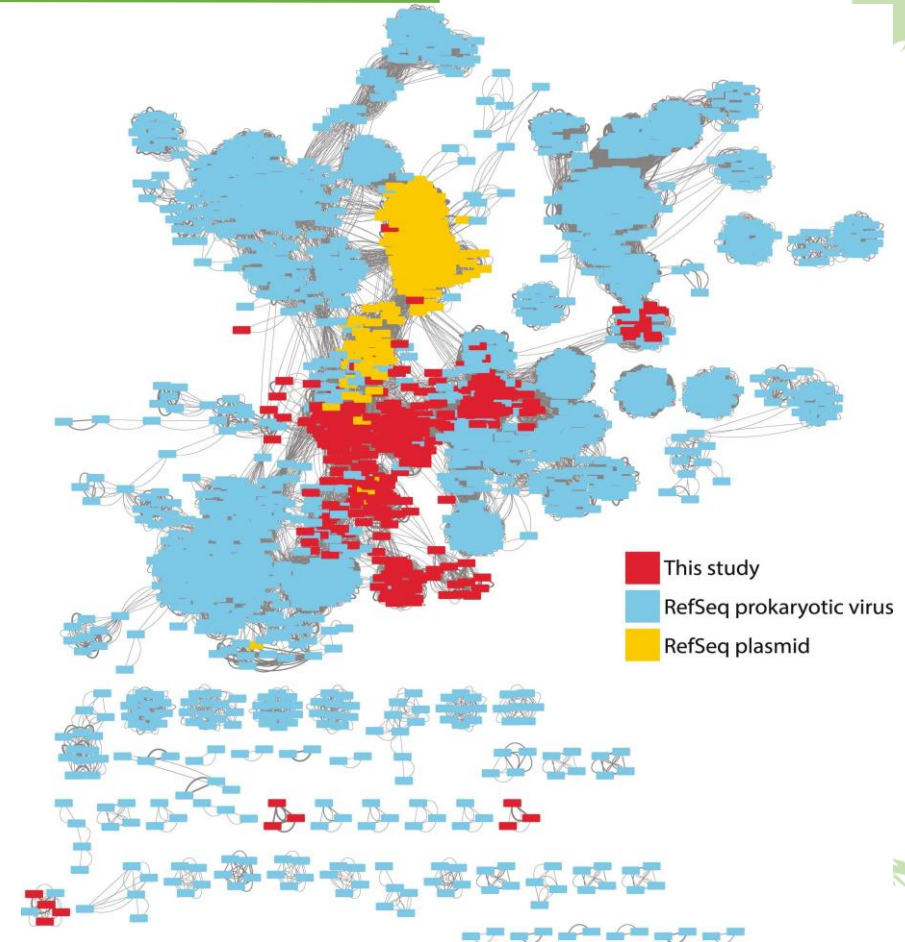
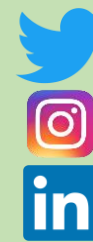


Figure. Protein-clustering network for phages and plasmids (1)

1. Al-Shayeb, B., Sachdeva, R., Chen, L.-X., Ward, F., Munk, P., Devoto, A., . . . Banfield, J. F. (2020). Clades of huge phages from across Earth's ecosystems. *Nature*, 578(7795), 425-431. doi:10.1038/s41586-020-2007-4



RecyclesEU

recycleseu

Recycles EU

RECYCLES WORKSHOP

Metagenomics and metabarcoding approaches to describe ecological systems and infer their development

5th, 6th & 7th of July 2022

Metagenomics Analysis of the Resistome and Phageome in Wastewater Treatment Plants in Winnipeg Manitoba

Speaker/authors

Kadir Yanac

GA: 872053 — H2020 - MSCA - RISE-2019



European
Commission

