ViWrap: A modular pipeline to identify, bin, classify, and predict viral-host relationships for viruses from metagenomes

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https://github.com/AnantharamanLab/ViWrap

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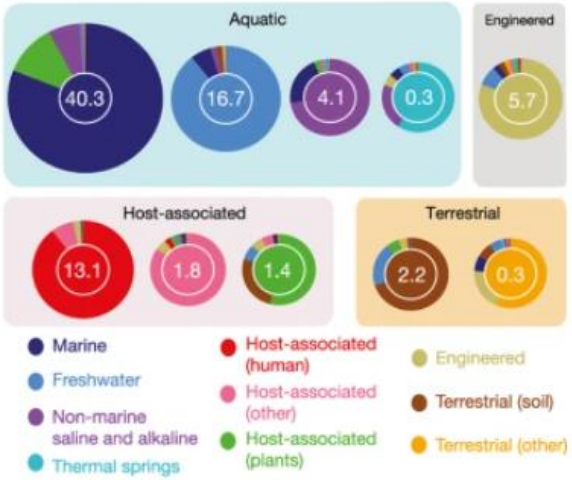
Introduction

Virome and viral genomes reconstructed from metagenomes

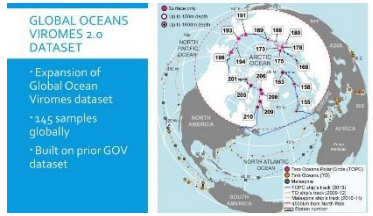
Uncovering Earth's virome

David Paez-Espino & Nikos C. Kyrpides
Nature 2016 (DOE-JGI)

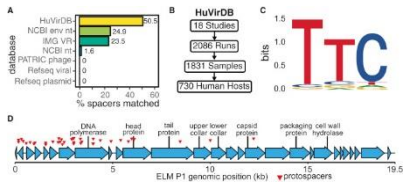
Virome:
metagenomes specifically targeting the viral fraction of environmental samples



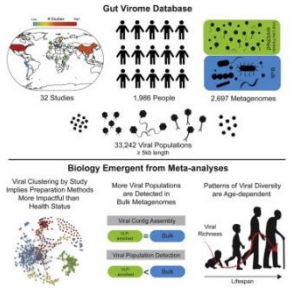
Viral genomes reconstructed from metagenome:
viral genomes reconstructed from bulk metagenomes



2019
Global Oceans Viromes (GOV) v2.0



2019
Human virome database (HuVirDB)



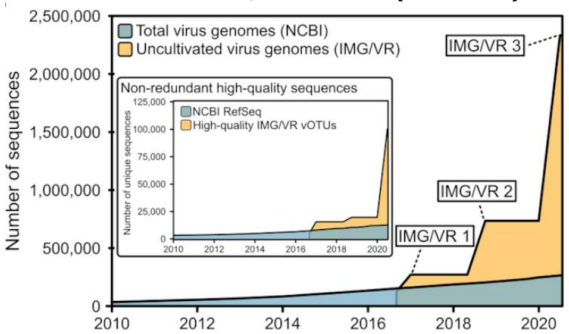
2020
Gut Virome Database (GVD)

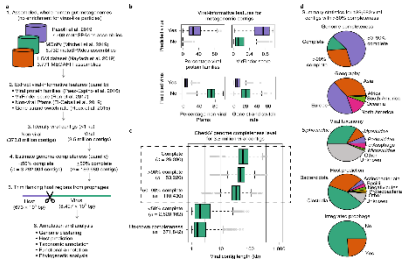
(Uses both methods)

2021
IMG V/R database (v3)

- contain viruses from all environments
- keep updating constantly

IMG V/R v3, NAR 2021 (DOE-JGI)





2021
Metagenomic Gut Virus (MGV)

Background

VIBRANT

Virus Identification By iteRative ANnoTation

Virus Identification by a hybrid machine learning and protein similarity approach
Anantharaman Lab, UW–Madison, 2020

jiarong/**VirSorter2**

customizable pipeline to identify viral sequences from (meta)genomic data

A multi-classifier, expert-guided identifier
Sullivan & Roux Lab, OSU and DOE JGI, 2021

jessieren/**DeepVirFinder**

Identifying viruses from metagenomic data by deep learning

A kmer based identifier by convolutional neural networks algorithm
Sun Lab, USC, 2020

Tools for virus identification

NCBI RefSeq Virus genomes
(NCBI)

VOG HMMs
(http://vogdb.org)

IMG/VR V3 high-quality vOTUs with taxonomy assigned
(DOE IMG)

Databases for virus taxonomy classification

vRhyme

Virus Binning Using Machine Learning

Virus binning by scaffold coverage effect size and nucleotide feature
Anantharaman Lab, UW–Madison, 2022

Virus binning

vConTACT2

Making whole genome gene-sharing networks for distance-based hierarchical clustering and taxonomy prediction
Sullivan Lab, OSU, 2019

MrOlm/**drep**

Rapid comparison and dereplication of genomes

Clustering and dereplicating microbial/viral genomes based on sequence identity
Banfield Lab, UC Berkeley, 2017

Virus clustering

CheckV

Checking the quality and completeness of viral genomes
Banfield Lab, UC Berkeley, 2017

Quality Check

iPHOP

An integrated machine-learning framework for host prediction
Roux Lab, DOE JGI, 2022

Host prediction

Lack of an integrated pipeline/wrapper

Workflow

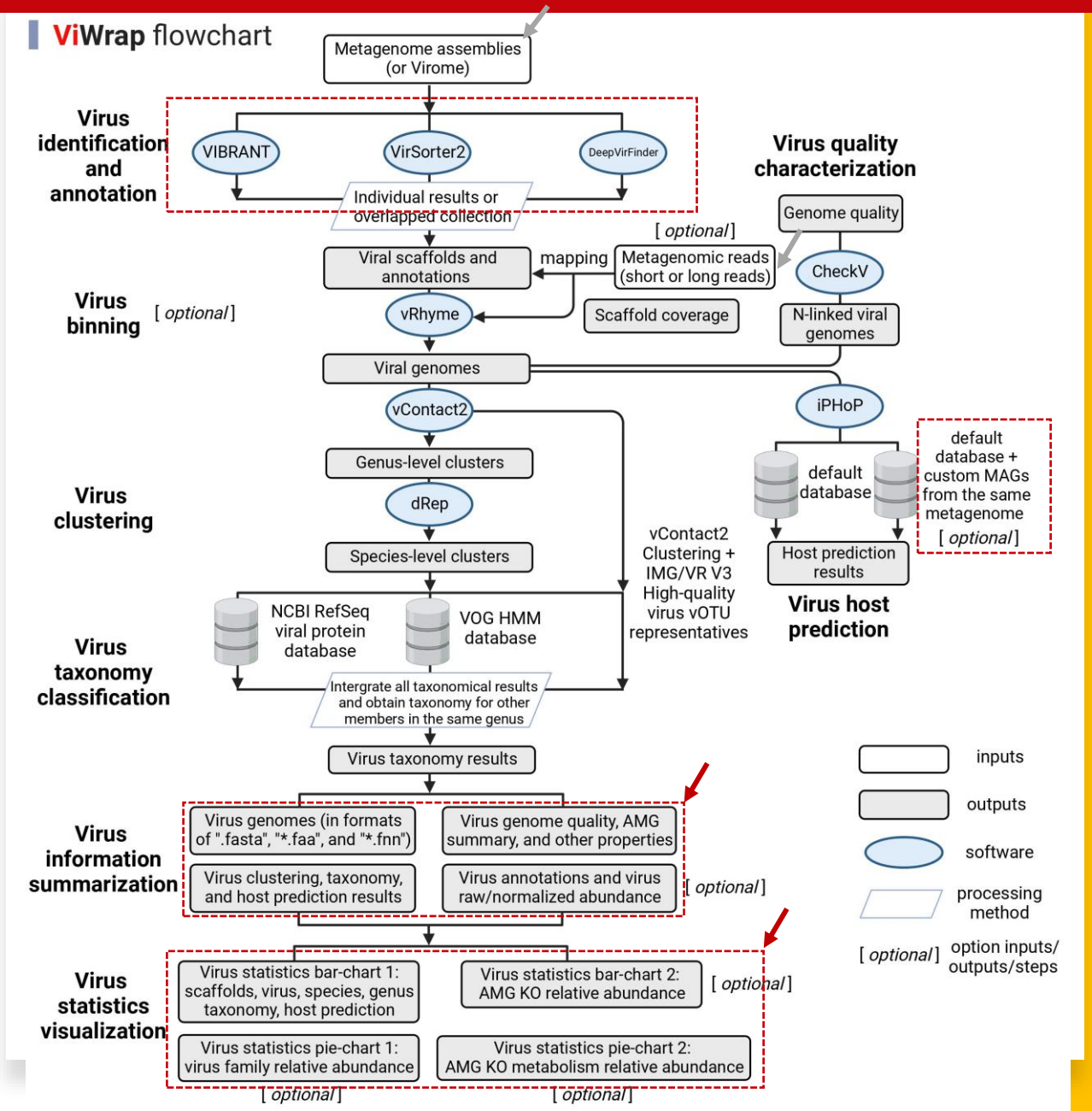
Step 1 Three virus identifiers

Step 2 Metagenomic reads mapping, virus binning, and quality check

Step 3 Cluster viruses into genus and species and assign taxonomy

Step 4 Use iPHoP to predict hosts for viruses

Step 5 Summarize to obtain results and visualize virus statistics



Result layout

All result folders

Organized intermediate folders

- `00_VIBRANT_input_metageome_stem_name` : the virus identification result (would be "00_VirSorter_input_metageome_stem_name", "00_DeepVirFinder_input_metageome_stem_name", "00_VIBRANT_VirSorter_input_metageome_stem_name", or "00_VIBRANT_VirSorter_DeepVirFinder_input_metageome_stem_name")
- `01_Mapping_result_outdir` : the reads mapping result
- `02_vRhyme_outdir` : vRhyme binning result
- `03_vConTACT2_outdir` : vConTACT2 classifying result
- `04_Nlinked_viral_gn_dir` : N-linked viral genome as CheckV inputs
- `05_CheckV_outdir` : CheckV result
- `06_dRep_outdir` : dRep clustering result
- `07_iPHoP_outdir` : iPHoP result for host prediction
- `08_ViWrap_summary_outdir` : Summarized results
- `09_Virus_statistics_visualization` : Visualized statistics of viruses
- `ViWrap_run.log` : running log file containing the issued command and time log

Results In virus statistics visualization folder

```
> 09_Virus_statistics_visualization
    > Result_visualization_inputs
        - virus_statistics.txt
        - virus_family_relative_abundance.txt
        - KO_ID_relative_abundance.txt
        - KO_metabolism_relative_abundance.txt
    > Result_visualization_outputs
        - virus_statistics.png # the 1st bar-chart
        - virus_family_relative_abundance.png # the 1st pie-chart
        - KO_ID_relative_abundance.png # the 2nd bar-chart
        - KO_metabolism_relative_abundance.png # the 2nd pie-chart
        - virus_statistics.pdf
        - virus_family_relative_abundance.pdf
        - KO_ID_relative_abundance.pdf
        - KO_metabolism_relative_abundance.pdf
```

Results in ViWrap summary folder

```
> 08_ViWrap_summary_outdir
    - Genus_cluster_info.txt # Virus genus clusters
    - Species_cluster_info.txt # Virus species clusters
    - Host_prediction_to_genome_m90.csv # Host prediction result at genome level
    - Host_prediction_to_genus_m90.csv # Host prediction result at genus level
    - Sample2read_info.txt # Reads counts and bases
    - Tax_classification_result.txt # Virus taxonomy result
    - Virus_annotation_results.txt # Virus annotation result
    > Virus_genomes_files # Contains all fasta, faa, ffn files for virus genomes
        - vRhyme*.fasta
        - vRhyme*.faa
        - vRhyme*.ffn
    > Virus_normalized_abundance.txt # Normalized virus genome abundance (normalized by 100M reads/sample)
    > Virus_raw_abundance.txt # Raw virus genome abundance
    > Virus_summary_info.txt # Summarized property for all virus genomes
```

Summary and take home message

- ViWrap integrates currently available tools/databases for both comprehensive and stringent virus screening.
- It is flexible for options of identifying methods, metagenomic reads, and custom microbial genomes for various application scenarios.
- It has a one-stop, user-friendly workflow and generates easy-to-read/parse results.

  - It can be used for various environmental settings, including natural, man-made, and human microbiome-related environments
  - ViWrap is publicly available via GitHub (https://github.com/AnantharamanLab/ViWrap). A detailed description of software usage and result interpretation can be found on the website.

Demonstration

Create the ViWrap conda environment

```
zhichao@sulfur:~cd /storage1/data11/ViWrap
zhichao@sulfur:/storage1/data11/ViWrap$ conda create -c bioconda -p /slowdata/yml_environments/ViWrap python=3.8 biopython mamba numpy pandas pyfastx
```

Get into the conda environment

```
zhichao@sulfur:/storage1/data11/ViWrap$ conda activate /slowdata/yml_environments/ViWrap
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap$
```

Git clone ViWrap package and make it executable

```
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11$ git clone https://github.com/AnantharamanLab/ViWrap
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11$ cd ViWrap
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap$ chmod +x ViWrap scripts/*.py
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap$ PATH=`pwd`:$PATH
```

Demonstration

Set up the conda environments

```
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap$ ViWrap set_up_env --conda_env_dir /slowdata/yml_environments/
```

```
### Set up conda env ###

[2022-10-30 19:36:16] | Looks like the input parameter is correct
[2022-10-30 19:37:38] | ViWrap-VIBRANT conda env has been installed
[2022-10-30 19:38:18] | ViWrap-vRhyme conda env has been installed
[2022-10-30 19:39:15] | ViWrap-vContact2 conda env has been installed
[2022-10-30 19:39:47] | ViWrap-CheckV conda env has been installed
[2022-10-30 19:40:38] | ViWrap-dRep conda env has been installed
[2022-10-30 19:40:55] | ViWrap-Tax conda env has been installed
[2022-10-30 19:43:50] | ViWrap-iPHoP conda env has been installed
[2022-10-30 19:44:07] | ViWrap-GTDBTk conda env has been installed
[2022-10-30 19:44:34] | ViWrap-vs2 conda env has been installed
[2022-10-30 19:44:56] | ViWrap-Mapping conda env has been installed
[2022-10-30 19:45:59] | ViWrap-DVF conda env has been installed
ViWrap-VIBRANT conda env path has been checked
ViWrap-vRhyme conda env path has been checked
ViWrap-vContact2 conda env path has been checked
ViWrap-CheckV conda env path has been checked
ViWrap-dRep conda env path has been checked
ViWrap-Tax conda env path has been checked
ViWrap-iPHoP conda env path has been checked
ViWrap-GTDBTk conda env path has been checked
ViWrap-vs2 conda env path has been checked
ViWrap-DVF conda env path has been checked
ViWrap-Mapping conda env path has been checked
The total running time is 0:09:48 (in "hr:min:sec" format)
```

Demonstration

Set up ViWrap database

```
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap$ ViWrap download --db_dir ./ViWrap_db  --conda_env_dir /slowdata/yml_environments
```

```
### Welcome to ViWrap ###

[2022-10-30 19:55:08] | Looks like the input conda software is correct
```

…

```
Set VIBRANT_DATA_PATH to /storage1/data11/ViWrap/ViWrap_db2/VIBRANT_db
Downloading VIBRANT databases to /storage1/data11/ViWrap/ViWrap_db2/VIBRANT_db...

This script will download, extract subsets and press HMM profiles for VIBRANT.
This process will require ~20GB of temporary free storage space, but the final size requirement is ~11GB in the form of pressed HMM databases.
Please be patient. This only needs to be run once and will take a few minutes.
Logger started. Check log file for messages and errors.

VIBRANT v1.2.1 is good to go!
See example_data/ for quick test files.

VIBRANT databases are downloaded successfully. Please see log file for any error messages.

[2022-10-30 20:04:07] | VIBRANT db has been set up
```

…

```
2022-10-30 22:31:04 (11.3 MB/s) - './ViWrap_db2/gtdbtk_r202_data.tar.gz' saved [50840267340/50840267340]

[2022-10-30 22:39:17] | GTDB-Tk db has been set up
[2022-10-30 22:44:05] | VirSorter2 db has been set up
Cloning into './ViWrap_db2/DVF_db_tmp'...
Updating files:  93% (28/30)^MUpdating files:  96% (29/30)^MUpdating files: 100% (30/30)^MUpdating files: 100% (30/30), done.
[2022-10-30 22:44:09] | DVF db has been set up
The total running time is 2:49:01 (in "hr:min:sec" format)
```

It takes several hours to complete, depending on the internet speed. While, it only needs to do once

Demonstration

Test ViWrap

```
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap$ ViWrap -h
ViWrap v1.2.0: Analyzing wrapper for virus from metagenome

Usage: ViWrap <task> [options]

Task:
run          Run the full wrapper for identifying, classifying, and characterizing virus genomes from metagenomes
run_wo_reads Run the full wrapper for identifying, classifying, and characterizing virus genomes from metagenomes without metagenomic reads
download     Download and setup the ViWrap database
set_up_env   Set up the conda environments for all scripts
clean        Clean redundant information in each result directory


options:
  -h, --help  show this help message and exit
```

Test ViWrap run

```
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap$ ./ViWrap run -h
        Run the full wrapper for identifying, classifying, and characterizing virus genomes from metagenomes

        Usage: ViWrap run --input_metagenome <input metagenome assemblies> --input_reads <input metagenomic reads> --out_dir <output directory> [options]

        Example 1: ViWrap run --input_metageome /path/to/Lake_01_assemblies.fasta \
                              --input_reads /path/to/Lake_01_T1_1.fastq,/path/to/Lake_01_T1_2.fastq,/path/to/Lake_01_T2_1.fastq,/path/to/Lake_01_T2_2.fastq \
                              --out_dir ./ViWrap_Lake_01_outdir \
                              --identify_method vb-vs \
                              --conda_env_dir /path/to/ViWrap_conda_environments

        Example 2: ViWrap run --input_metageome /path/to/Lake_01_assemblies.fasta \
                              --input_reads /path/to/Lake_01_T1_1.fastq,/path/to/Lake_01_T1_2.fastq,/path/to/Lake_01_T2_1.fastq,/path/to/Lake_01_T2_2.fastq \
                              --out_dir ./ViWrap_Lake_01_outdir \
                              --db_dir /path/to/ViWrap_db \
                              --identify_method vb-vs \
                              --conda_env_dir /path/to/ViWrap_conda_environments \
                              --threads 30 \
                              --virome \
                              --input_length_limit 2000 \
                              --custom_MAGs_dir /path/to/custom_MAGs_dir
```

Demonstration

Run ViWrap

```
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap$ vi run_ViWrap2.sh
```

```
python ViWrap run --input_metagenome /storage1/data11/ViWrap/Guaymas_scaffolds_min1000.subset.fasta \
                  --input_reads /storage1/Reads/HydroPlume/Guaymas/Guaymas_final_reads.subset10perc_1.fastq,/storage1/Reads/HydroPlume/Guaymas/Guaymas_final_reads.subset10perc_2.fastq,/storag
e1/Reads/HydroPlume/Guaymas/Guaymas_final_reads.subset15perc_1.fastq,/storage1/Reads/HydroPlume/Guaymas/Guaymas_final_reads.subset15perc_2.fastq \
                  --out_dir ./ViWrap_outdir_vb_vs \
                  --conda_env_dir /slowdata/yml_environments \
                  --threads 20 \
                  --input_length_limit 5000 \
                  --db_dir /storage1/data11/ViWrap/ViWrap_db \
                  --identify_method vb-vs \
                  --custom_MAGs_dir /storage1/data11/ViWrap/Guaymas_bins
```

```
### Welcome to ViWrap ###

The issued command is:
/storage1/data11/ViWrap/ViWrap run --input_metagenome /storage1/data11/ViWrap/Guaymas_scaffolds_min1000.subset.fasta --input_reads /storage1/Reads/HydroPlume/Guaymas/Guaymas_final_reads.subse
t10perc_1.fastq,/storage1/Reads/HydroPlume/Guaymas/Guaymas_final_reads.subset10perc_2.fastq,/storage1/Reads/HydroPlume/Guaymas/Guaymas_final_reads.subset15perc_1.fastq,/storage1/Reads/HydroPl
ume/Guaymas/Guaymas_final_reads.subset15perc_2.fastq --out_dir ./ViWrap_outdir_vb_vs --db_dir /storage1/data11/ViWrap/ViWrap_db --identify_method vb-vs --conda_env_dir /slowdata/yml_environme
nts --threads 20 --input_length_limit 5000 --custom_MAGs_dir /storage1/data11/ViWrap/Guaymas_bins

[2022-10-29 11:06:16] | Pre-check inputings. In processing...
[2022-10-29 11:06:16] | Looks like the input metagenome and reads, database, and custom MAGs dir (if option used) are now set up well, start up to run ViWrap pipeline
[2022-10-29 11:06:16] | Run VIBRANT-VirSorter2 method. Run VIBRANT to identify and annotate virus from input metagenome. In processing...
[2022-10-29 11:21:16] | Run VIBRANT-VirSorter2 method. Run VIBRANT to identify and annotate viruses from input metagenome. Finished
[2022-10-29 11:21:16] | Run VIBRANT-VirSorter2 method. Run VirSorter2 to identify viruses from input metagenome. Also plus CheckV to QC and trim, and KEGG, Pfam, and VOG HMMs to annotate viru
ses. In processing...
[2022-10-29 15:04:36] | Run VIBRANT-VirSorter2 method. Run VirSorter2 the 1st time to identify viruses from input metagenome. Finished
[2022-10-29 15:05:48] | Run VIBRANT-VirSorter2 method. Run CheckV the 1st time to QC and trim viruses identified from VirSorter2 1st run. Finished
[2022-10-29 15:50:07] | Run VIBRANT-VirSorter2 method. Run VirSorter2 the 2nd time for CheckV-trimmed sequences. Finished
[2022-10-29 15:51:39] | Run VIBRANT-VirSorter2 method. Run CheckV the 2nd time to get viral and host gene counts. Finished
[2022-10-29 15:53:28] | Run VIBRANT-VirSorter2 method. Run VIBRANT to check "keep2" and "manual_check" groups and get the final VirSorter2 virus sequences. Finished
[2022-10-29 15:53:28] | Map reads to metagenome. In processing...
[2022-10-29 16:34:11] | Map reads to metagenome. Finished
[2022-10-29 16:34:11] | Run vRhyme to bin viral scaffolds. In processing...
[2022-10-29 16:35:45] | Run vRhyme to bin viral scaffolds. Finished
[2022-10-29 16:35:45] | Run vContact2 to cluster viral genomes. In processing...
[2022-10-29 17:32:22] | Run vContact2 to cluster viral genomes. Finished
[2022-10-29 17:32:22] | Run CheckV to evaluate virus genome quality. In processing...
[2022-10-29 17:36:40] | Run CheckV to evaluate virus genome quality. Finished
[2022-10-29 17:36:40] | Run dRep to cluster virus species. In processing...
[2022-10-29 17:36:47] | Run dRep to cluster virus species. Finished
[2022-10-29 17:36:47] | Conduct taxonomic charaterization. In processing...
[2022-10-29 17:42:43] | Conduct taxonomic charaterization. Finished
[2022-10-29 17:42:43] | Conduct Host prediction by iPHoP. In processing...
[2022-10-29 18:19:49] | Conduct Host prediction by iPHoP. Finished
[2022-10-29 18:19:49] | Conduct Host prediction by iPHoP using custom MAGs. In processing...
[2022-10-30 09:22:15] | Conduct Host prediction by iPHoP using custom MAGs. Finished
[2022-10-30 09:22:17] | Get virus genome abundance. Finished
[2022-10-30 09:22:17] | Get virus sequence information. Finished
[2022-10-30 09:22:17] | Visualize the result. Finished
The total running time is 22:16:01 (in "hr:min:sec" format)
```

Demonstration

Result

```
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap/ViWrap_outdir_vb_vs$ ls
00_VIBRANT_VirSorter_Guaymas_scaffolds_min1000.subset  02_vRhyme_outdir     04_Nlinked_viral_gn_dir  06_dRep_outdir   08_ViWrap_summary_outdir           ViWrap_run.log
01_Mapping_result_outdir                               03_vConTACT2_outdir  05_CheckV_outdir         07_iPHoP_outdir  09_Virus_statistics_visualization
```

```
(/slowdata/yml_environments/ViWrap) zhichao@sulfur:/storage1/data11/ViWrap/ViWrap_outdir_vb_vs/08_ViWrap_summary_outdir$ ls
Genus_cluster_info.txt             Host_prediction_to_genus_m90.csv  Species_cluster_info.txt       Virus_genomes_files             Virus_raw_abundance.txt
Host_prediction_to_genome_m90.csv  Sample2read_info.txt              Tax_classification_result.txt  Virus_normalized_abundance.txt  Virus_summary_info.txt
```