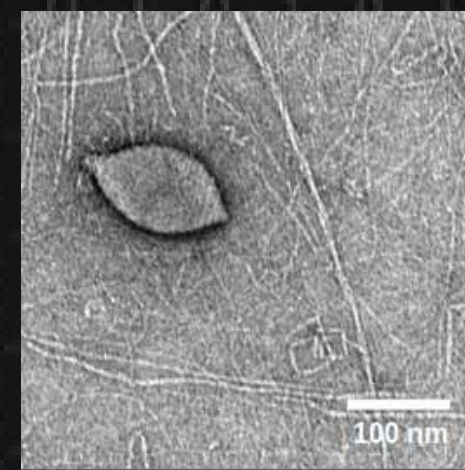
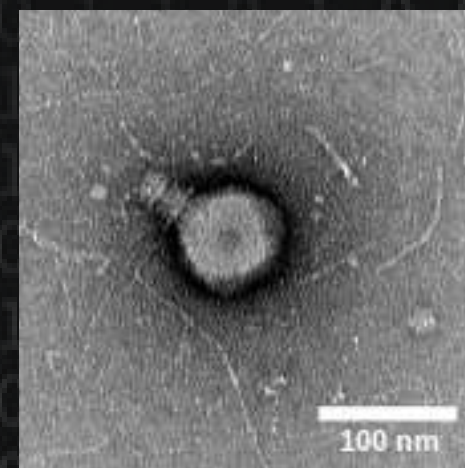
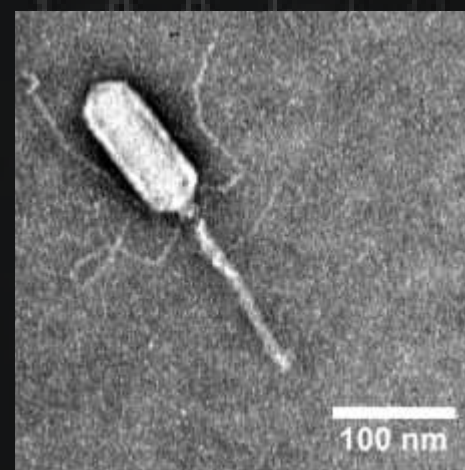
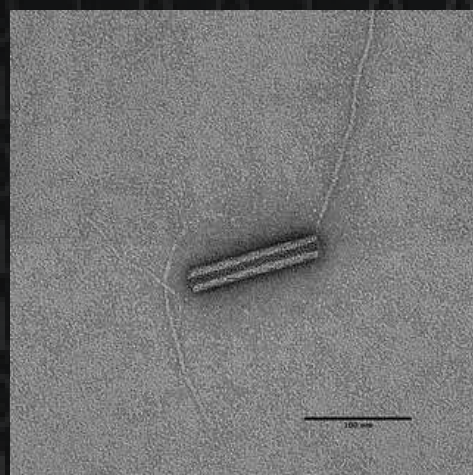


VIROMICS Sergio Sánchez Carrillo



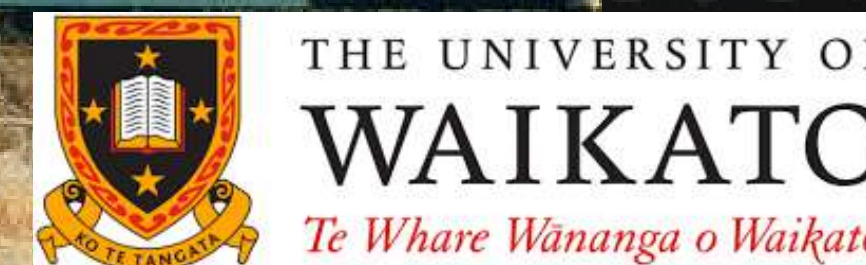
24/10/2024 Curso Aplicación de Herramientas -ómicas en Acuicultura

Gabinete de Formación CSIC

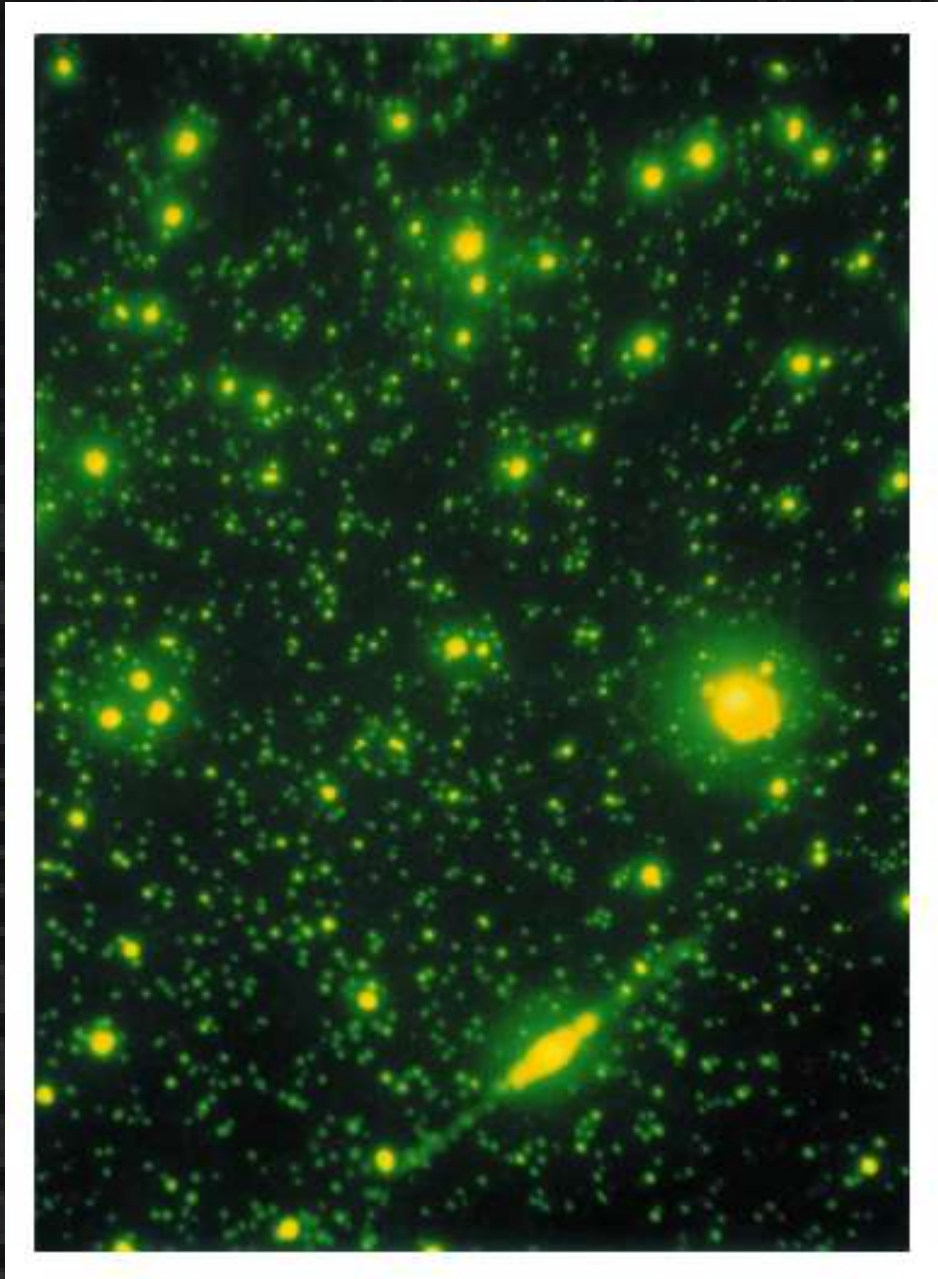
Outline

1. Alpine and polar viral metagenomics
2. Some theory about Viromics
3. Wetlab workflow
4. Drylab workflow
5. “Hands on” sea cucumbers dataset

1. Alpine and polar viral metagenomics



Environmental Viruses: Why?

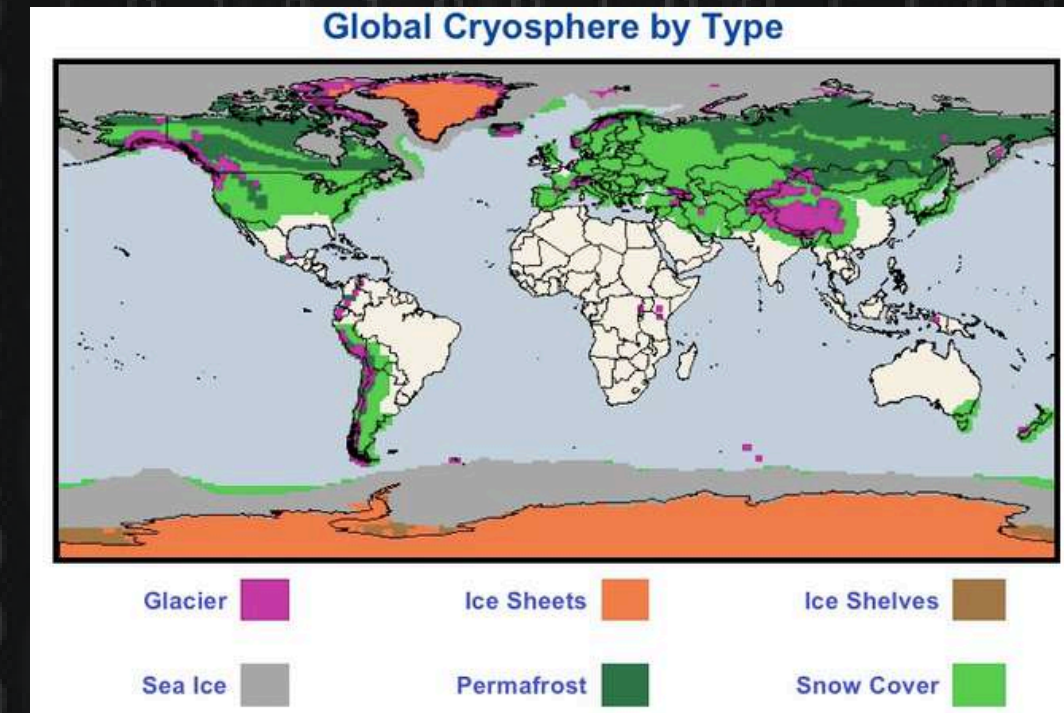


(Fuhrman, 1999)

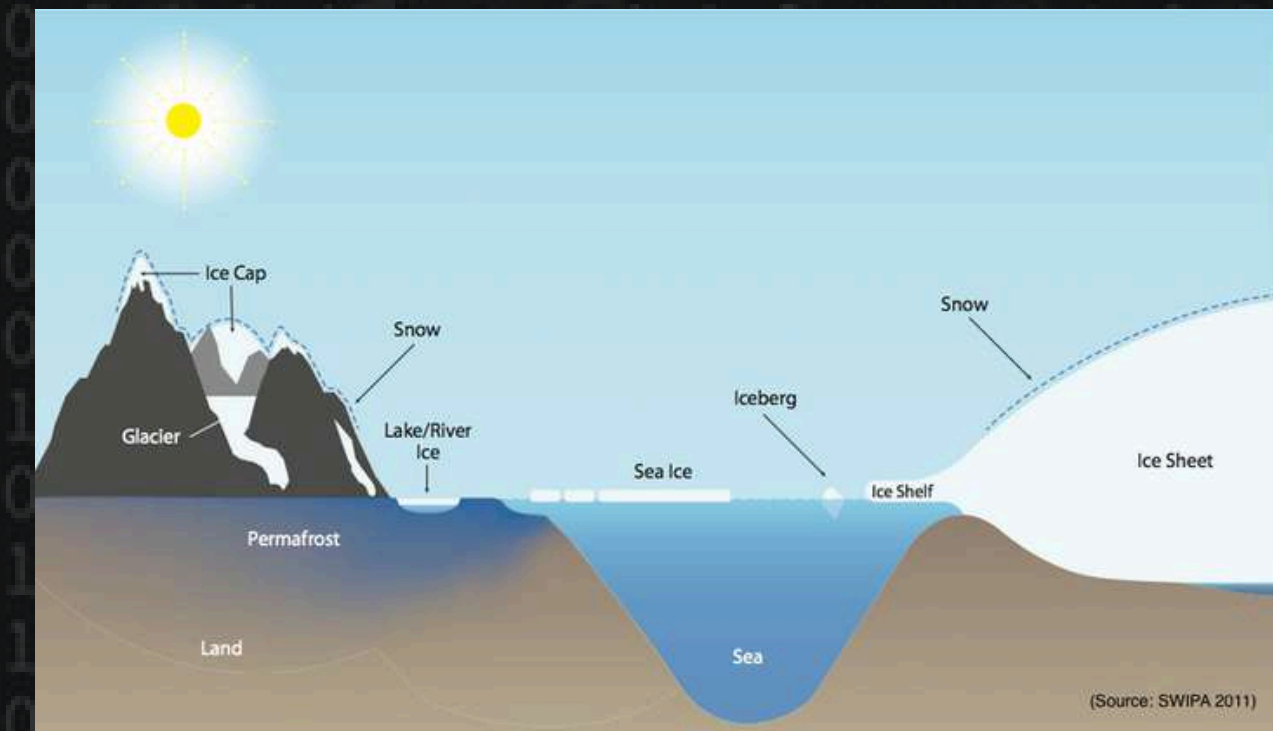
- Most abundant and genetically diverse bio-entities on Earth
- Reservoir of unknown genetic diversity
- Paramount role in microbial ecology and evolution (population limiters, metabolic reprogramming, evolutionary drivers)
- Key in biogeochemical cycles

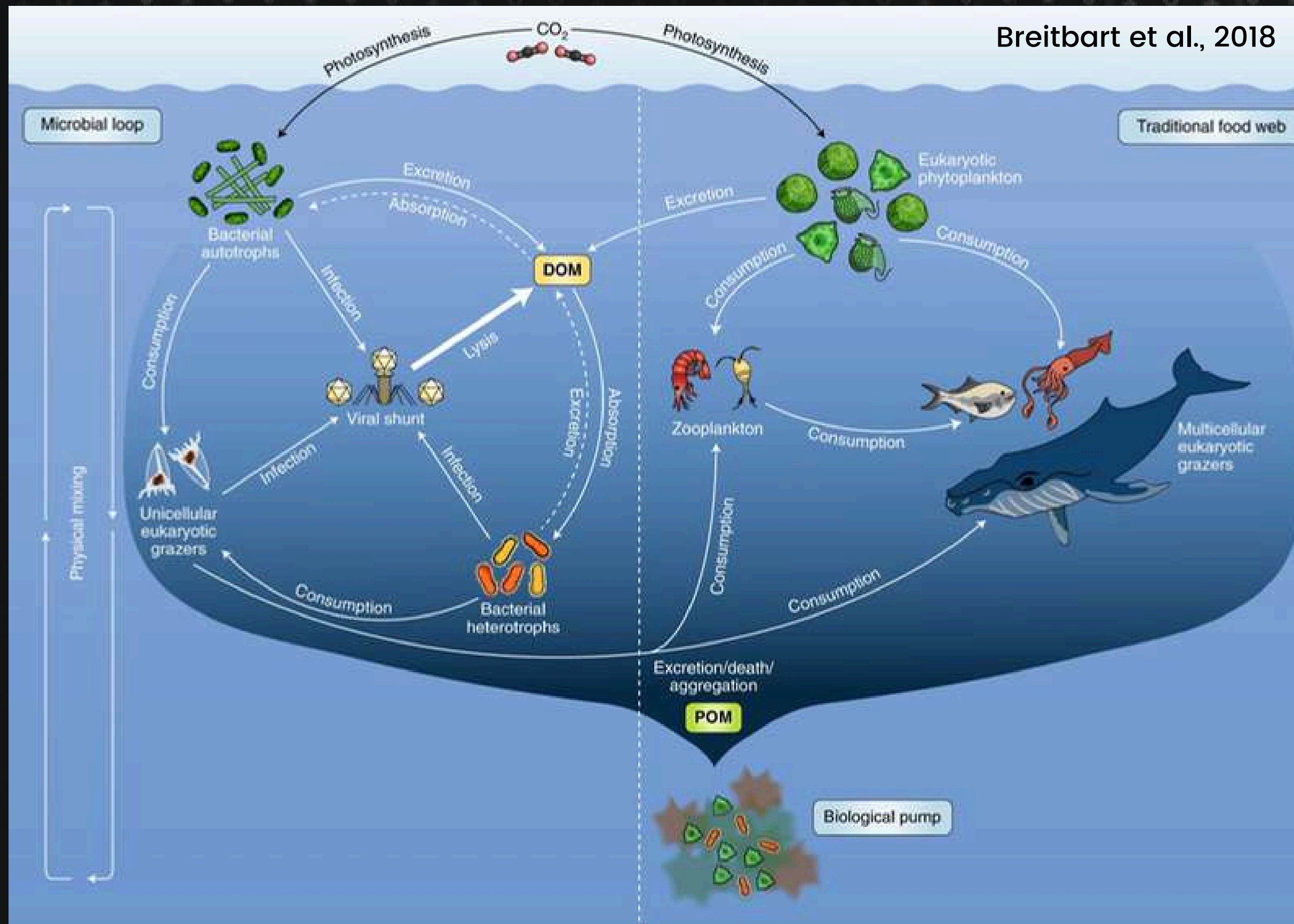
Cryosphere: Why?

- Widely distributed across Earth
- Key regulator of global climate (albedo, Global Ocean Circulation)
- Sustains highly diverse ecosystems
- Paramount role in global biochemical cycles
- Water supply for billions of people
- Highly susceptible to global change
- Relatively little known, especially in the case of viruses
- Exoplanet system analogues



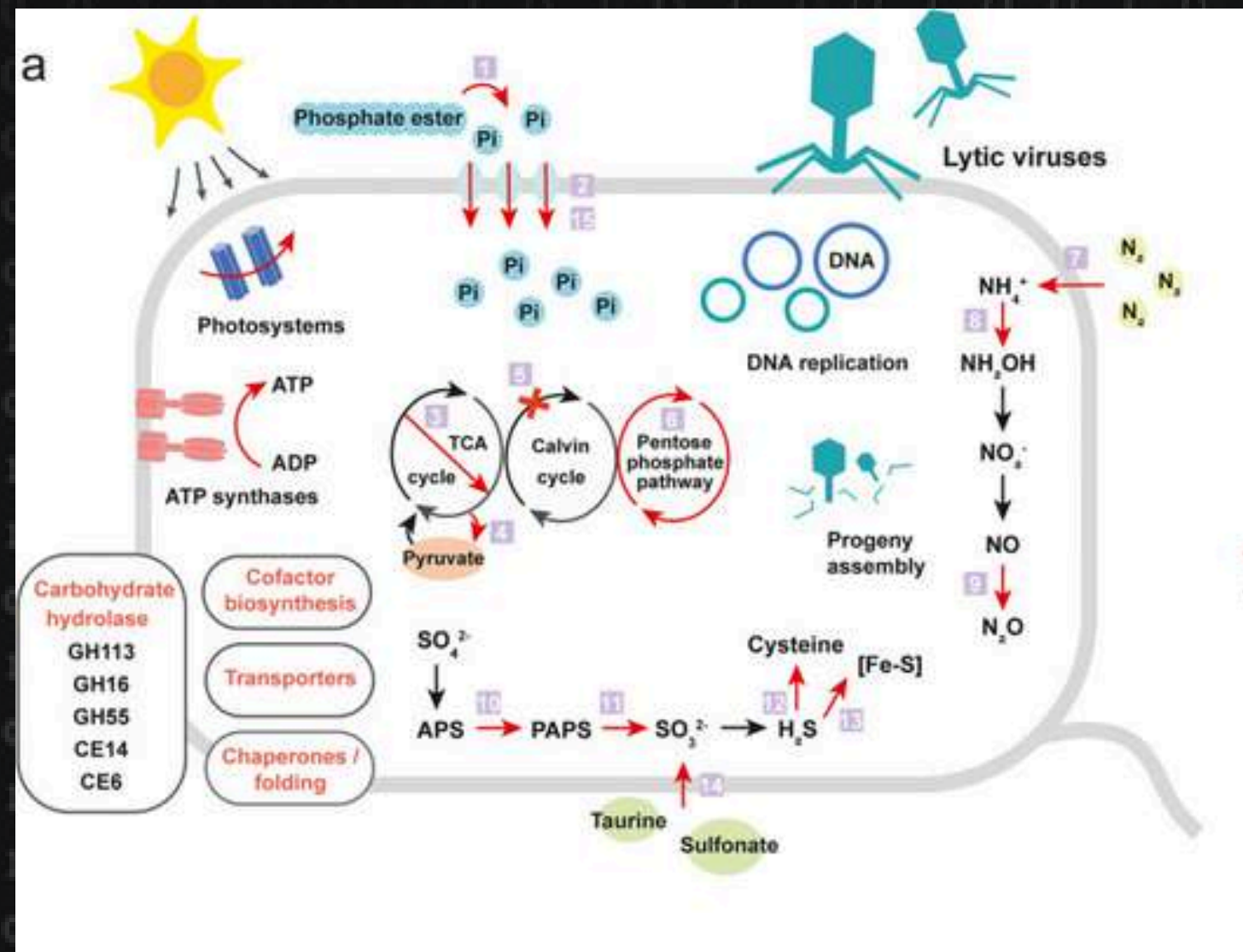
(<https://globalcryospherewatch.org>)





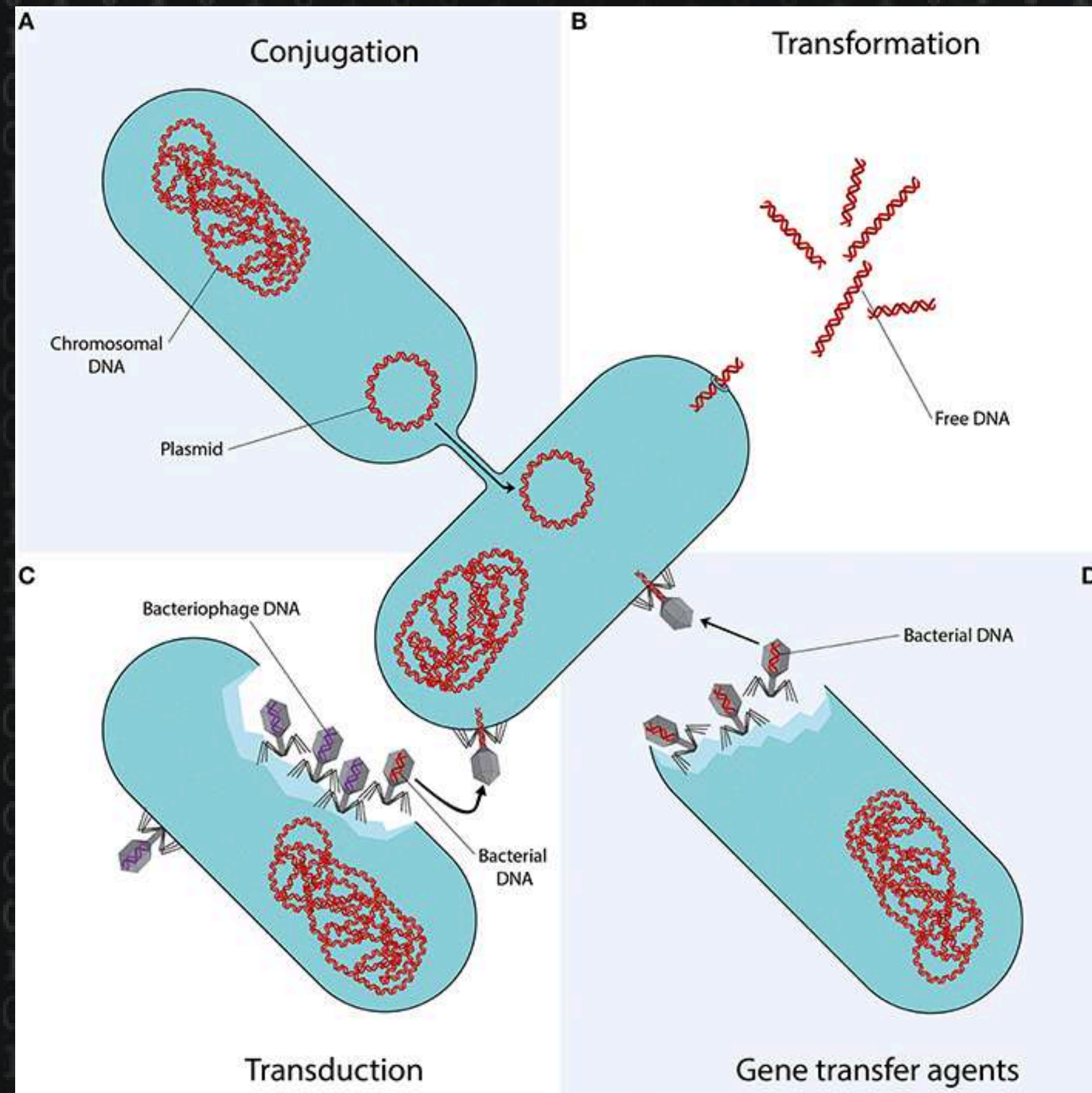
Microbial populations control \longrightarrow biogeochemical cycles

AMGs \longrightarrow Host metabolic reprogramming



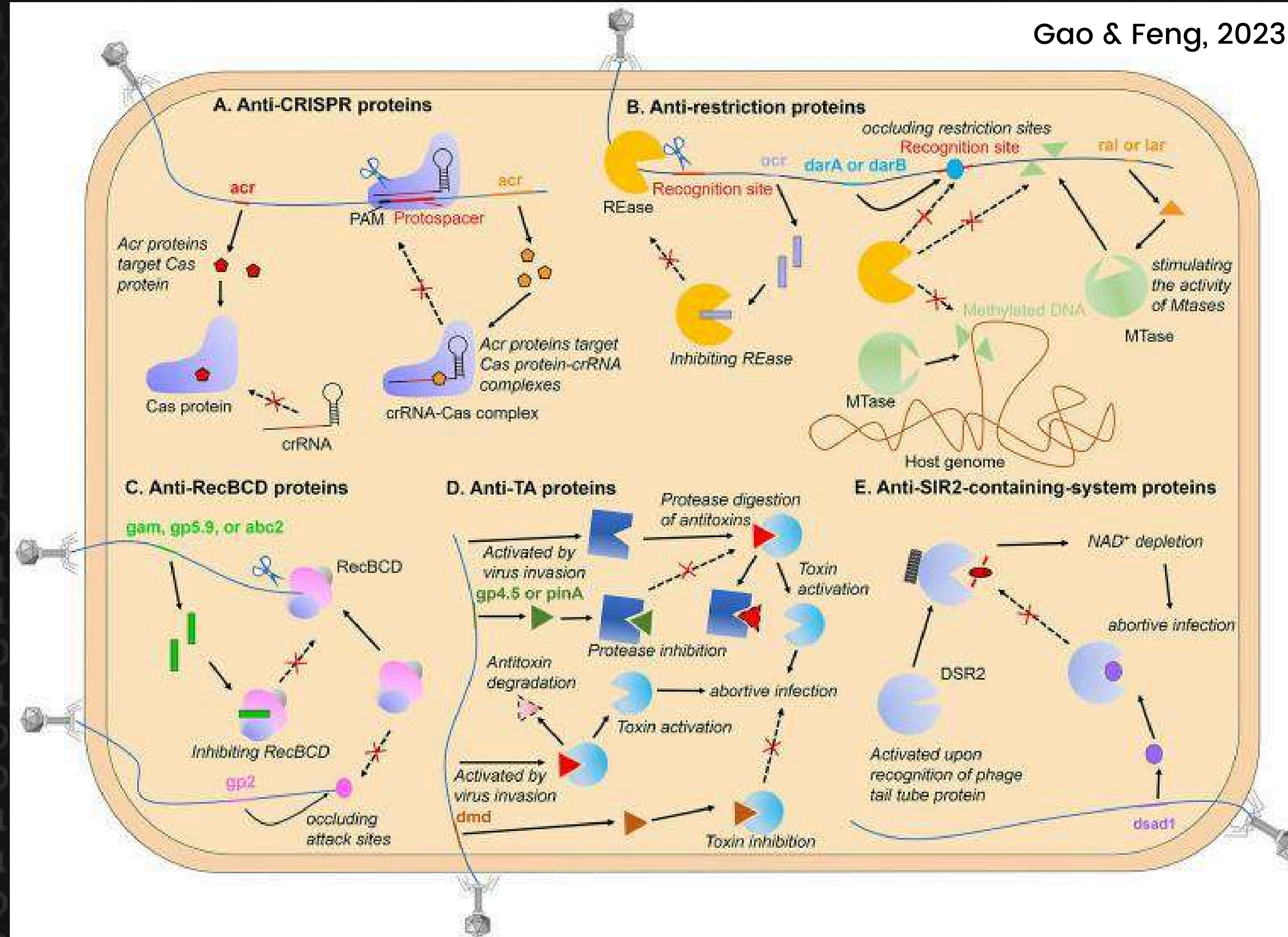
Wang et al., 2022

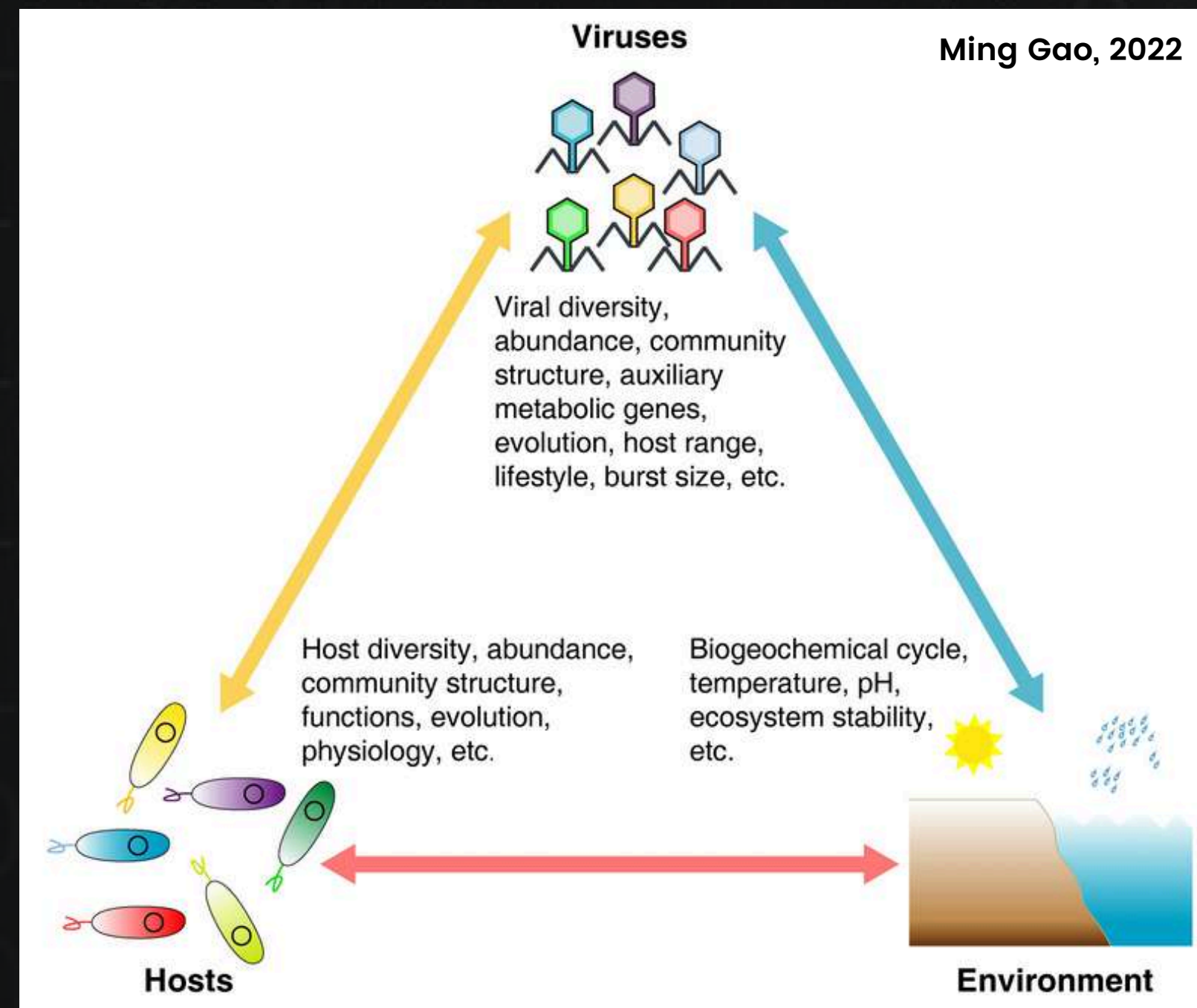
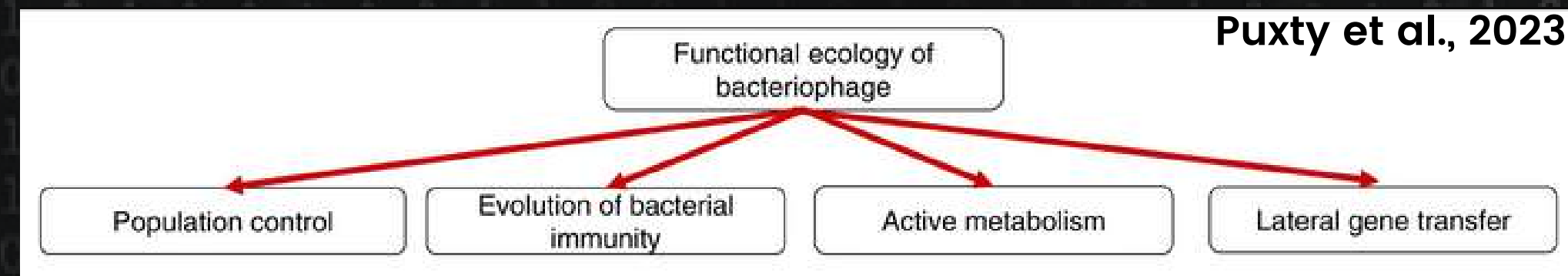
HGT → Host and virus evolution



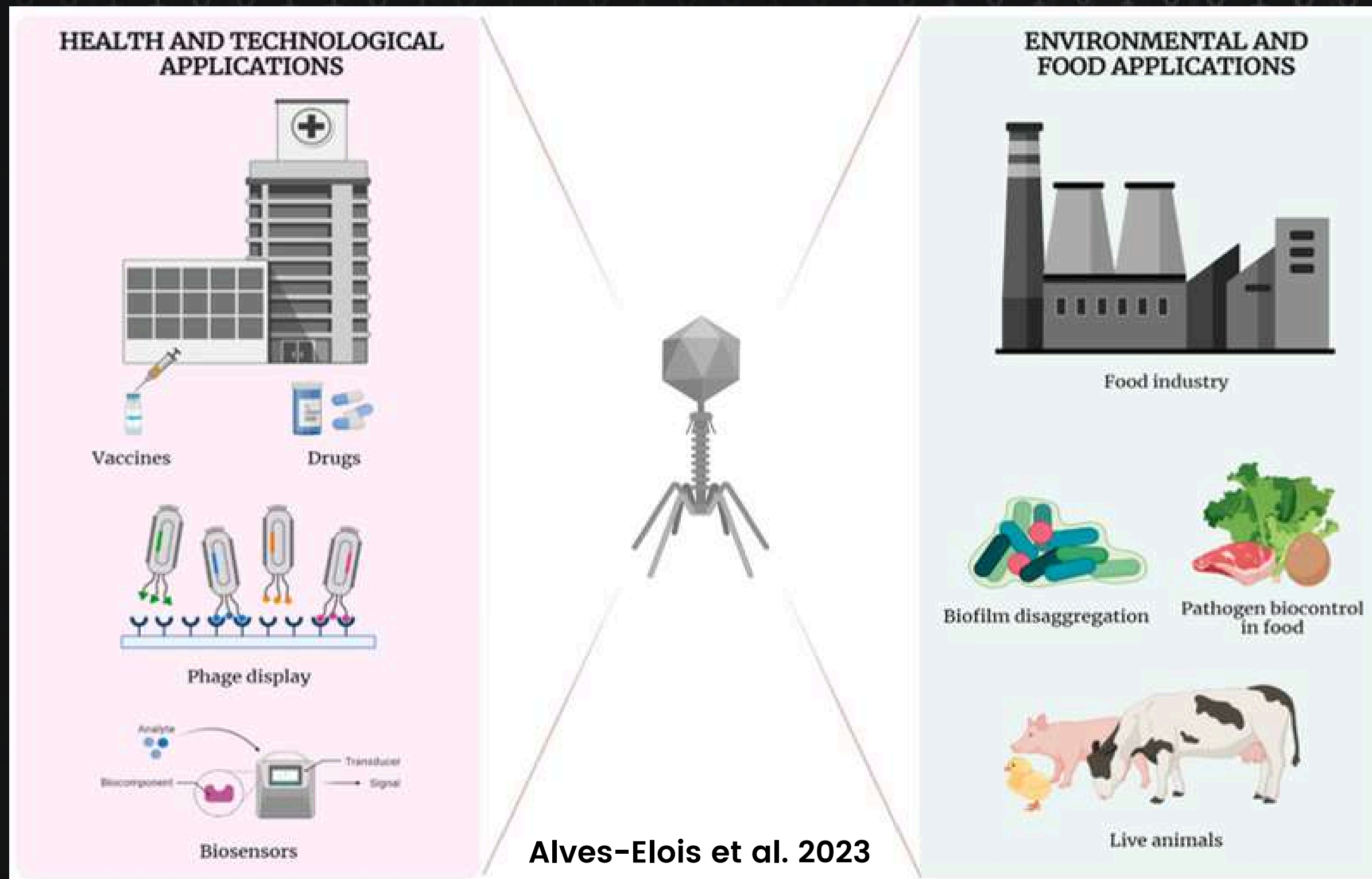
Wintersdorff et al, 2016

Overcoming bacterial antiviral response systems → Arms race





And even more...





MICROORDESA: Biodiversidad microbiana y viral en el P.N. de Ordesa y Monte Perdido

BBVA



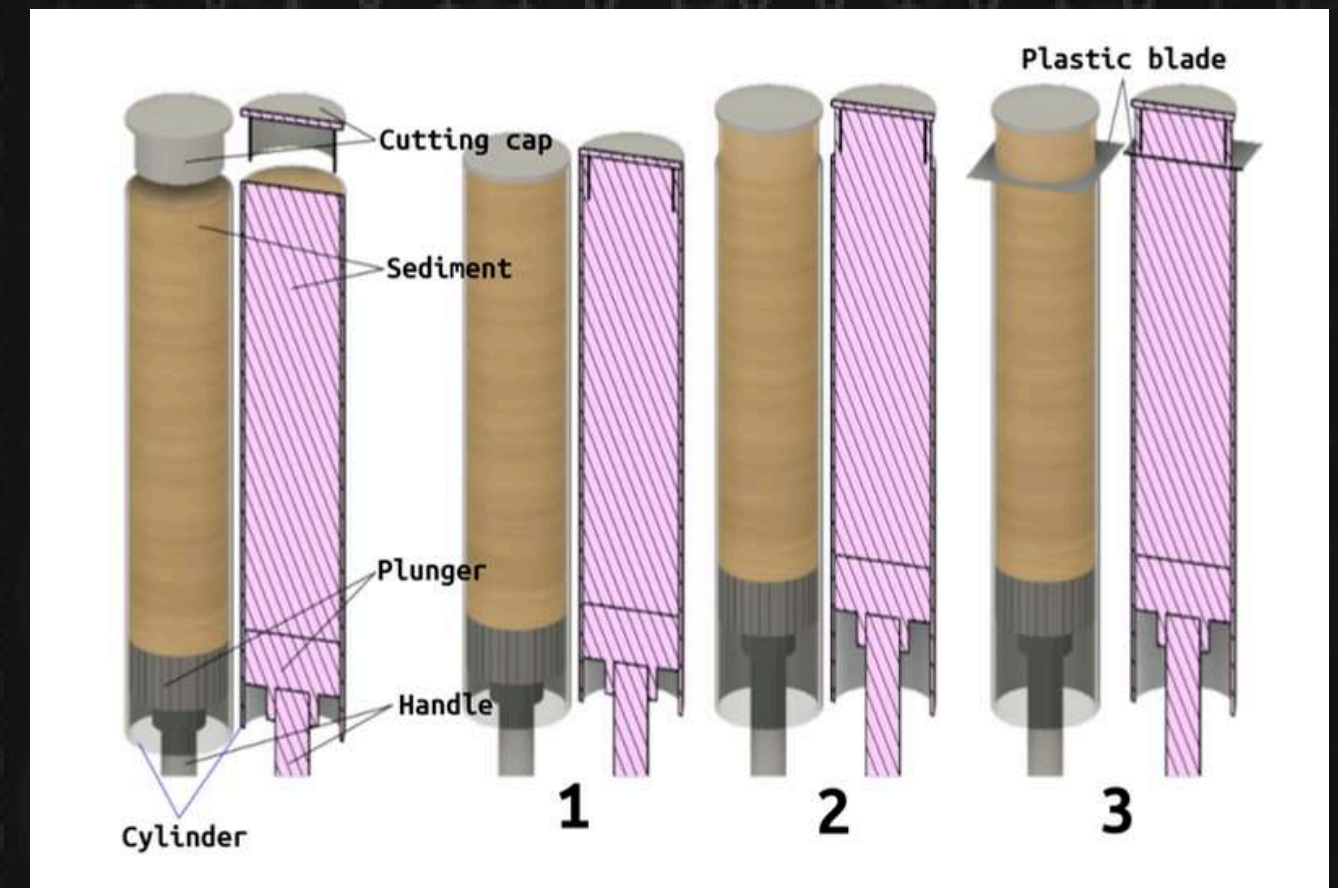
Miguel Bartolomé
Blas Valero-Garcés
Ana Moreno
J. Ignacio López-Moreno



DNA viruses of polar and alpine lake sediments



Name	Latitude	Longitude	Elevation (m asl)	Catchment area (Km2)	Lake area (ha)	Catchment/lake area ratio	Max Depth (m)	Thermal stratification	Trophic status
Escondido	62°37' S	61°0.4' W	92	8	1.6	5.0	5.3	Cold monomictic	Ultra-oligotrophic
Limnopolar	62°37' S	61°0.6' W	65	58	2.2	26.4	5.5	Cold monomictic	Ultra-oligotrophic
Marboré	42°41' N	0°2' E	2612	137	14.3	9.6	30	Dimictic	Ultra-oligotrophic
Tenndammen	78°06' N	15°02' E	5	210	15	14.0	2.5	Cold monomictic	Ultra-oligotrophic



Alejandra Vicente de Vera
&
Blas Blas Valero-Garcés



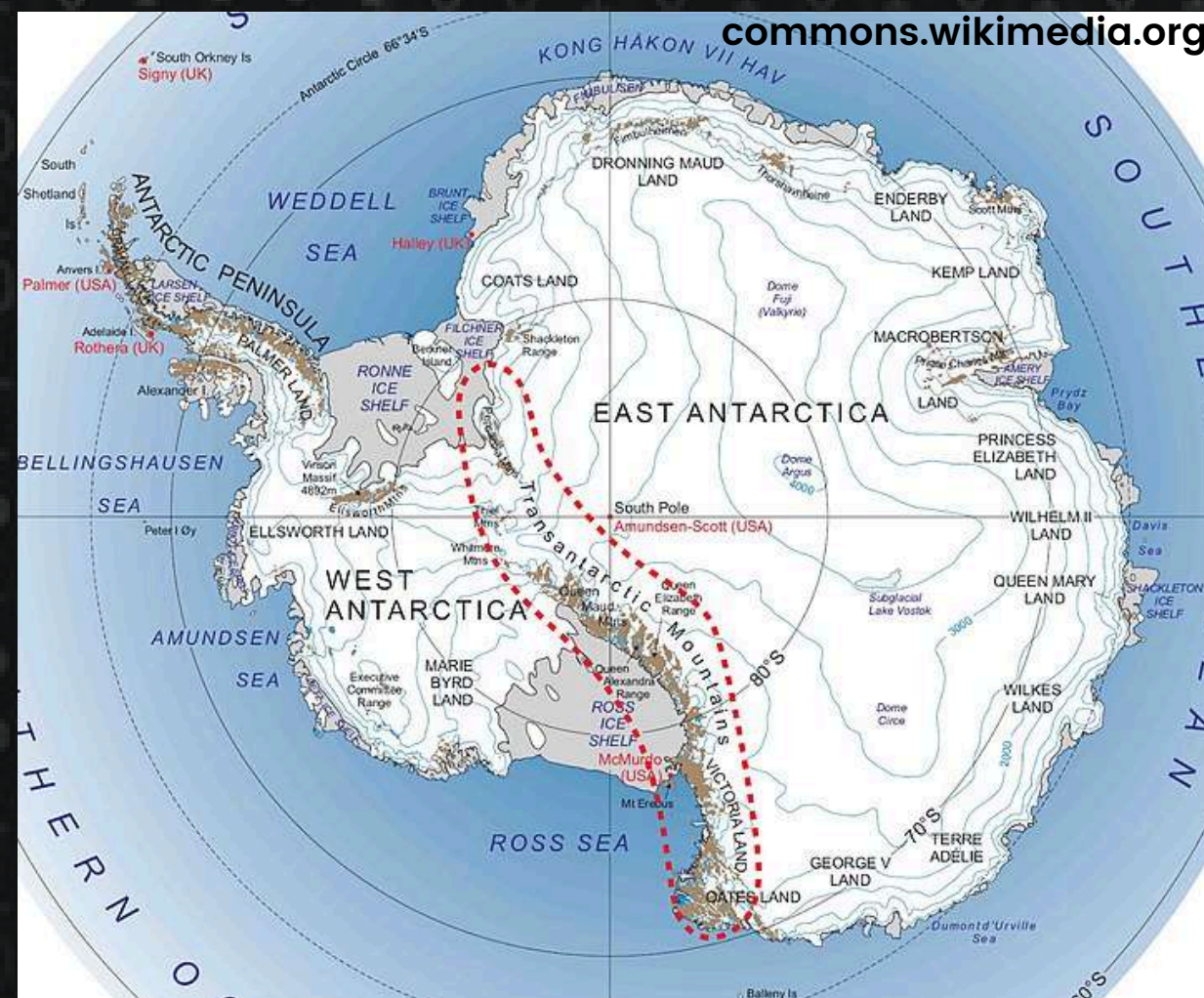


Diversity of DNA and RNA soil viruses in Antarctica

Ian R. McDonald & S. Craig Cary



Viral soil communities in ice-free soils in Antarctica



2. Some theory about Viromics

Viromics

Study of viral genomes and their interactions within a specific environment using high-throughput sequencing and bioinformatics techniques, focusing on understanding the diversity, structure, and function of viruses in the ecosystems. Viromics enables scientists to explore:

- Viral diversity
- Virus–host interactions
- Viral evolution
- Ecological roles

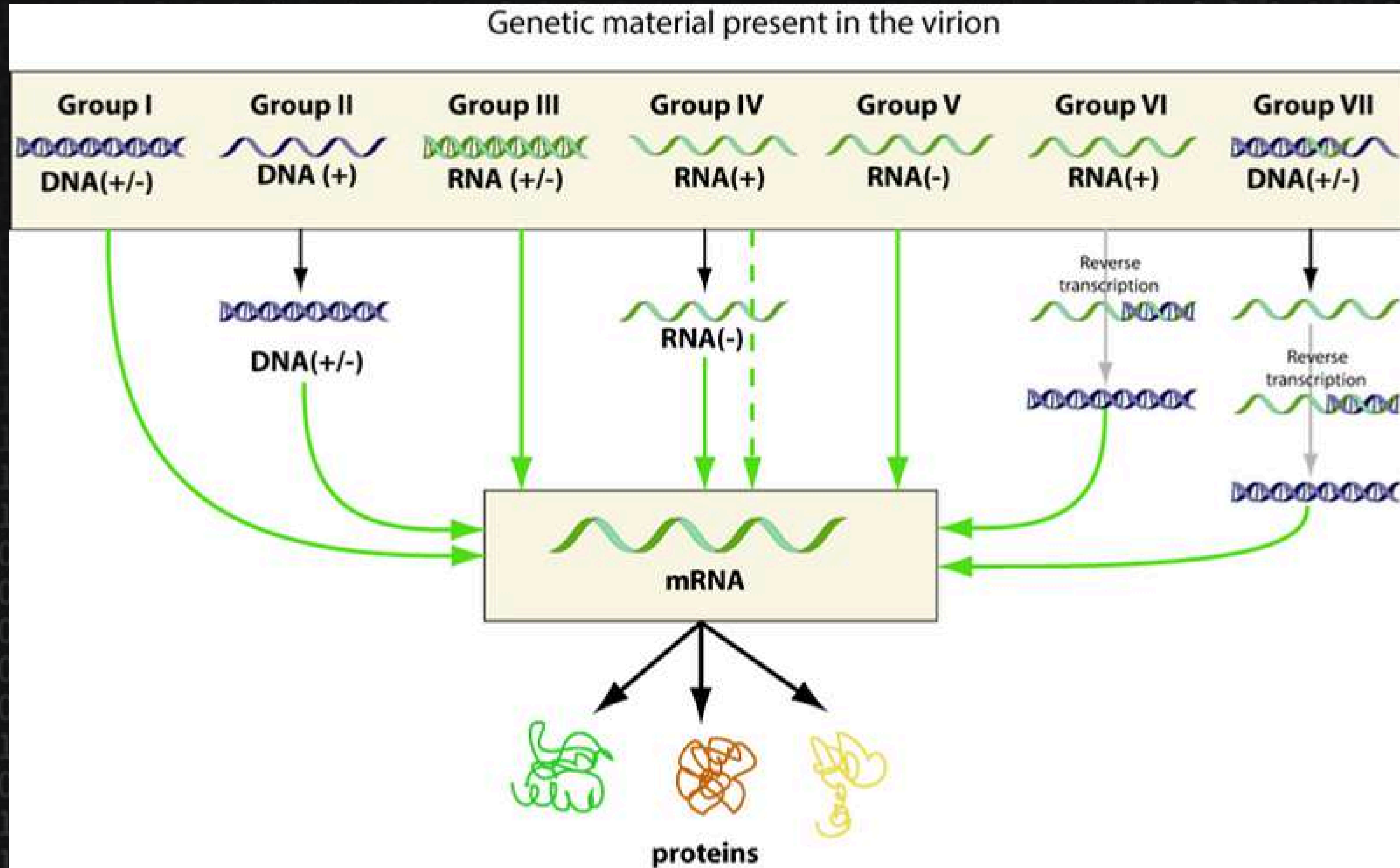
chatGPT

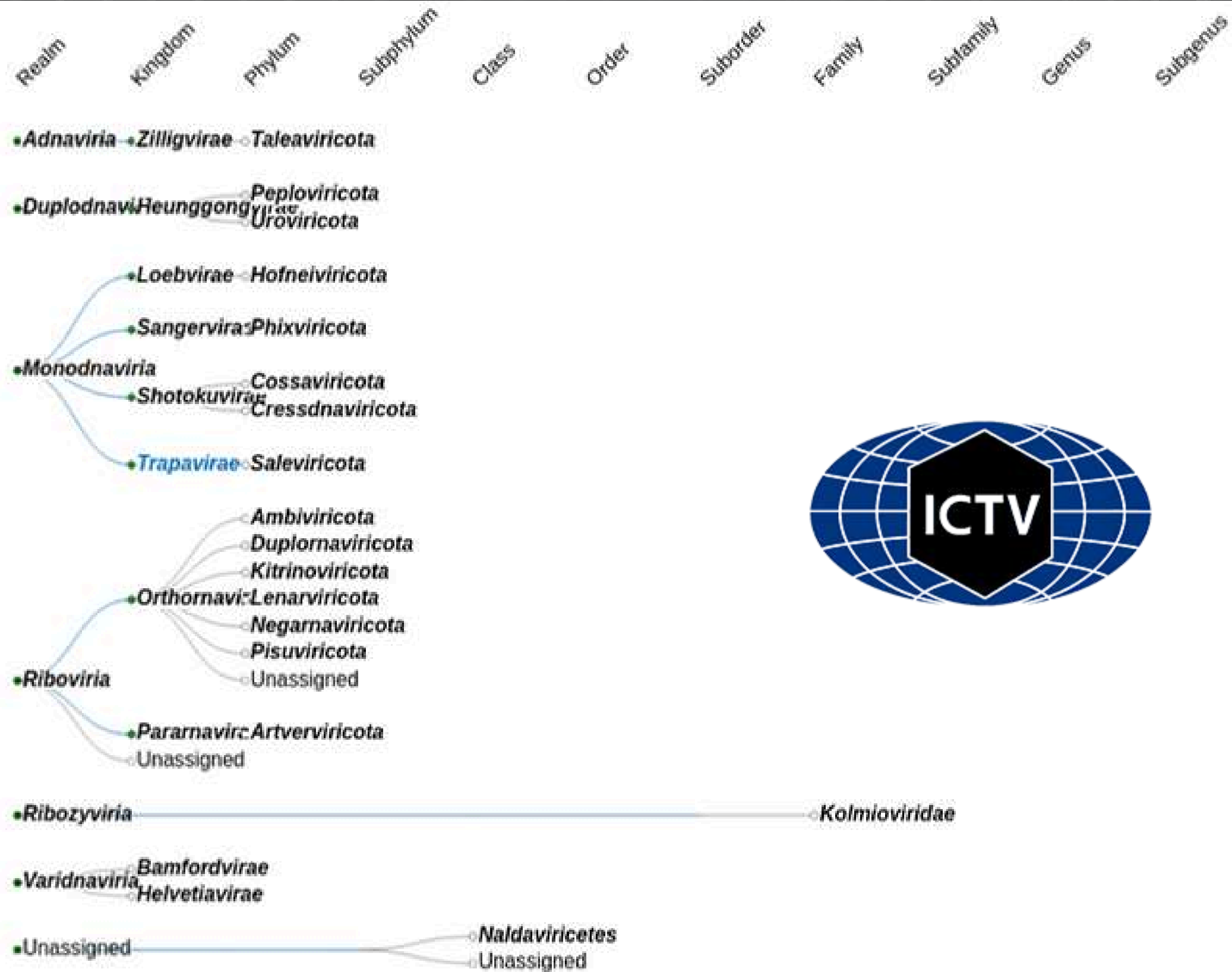
VLP enrichment
before sequencing?

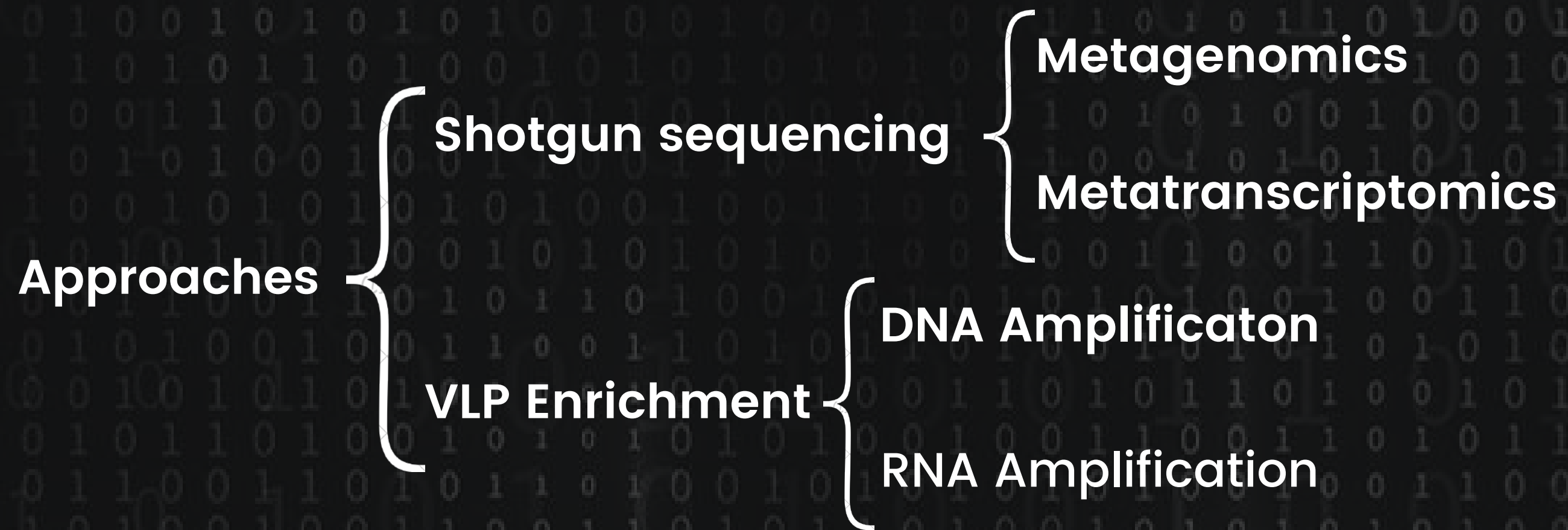


shotgun
sequencing?

Baltimore Classification







Is there any viral gene analogous to the universal gene 16S in Bacteria which we can amplify to study viruses?

Is viral metabarcoding possible?



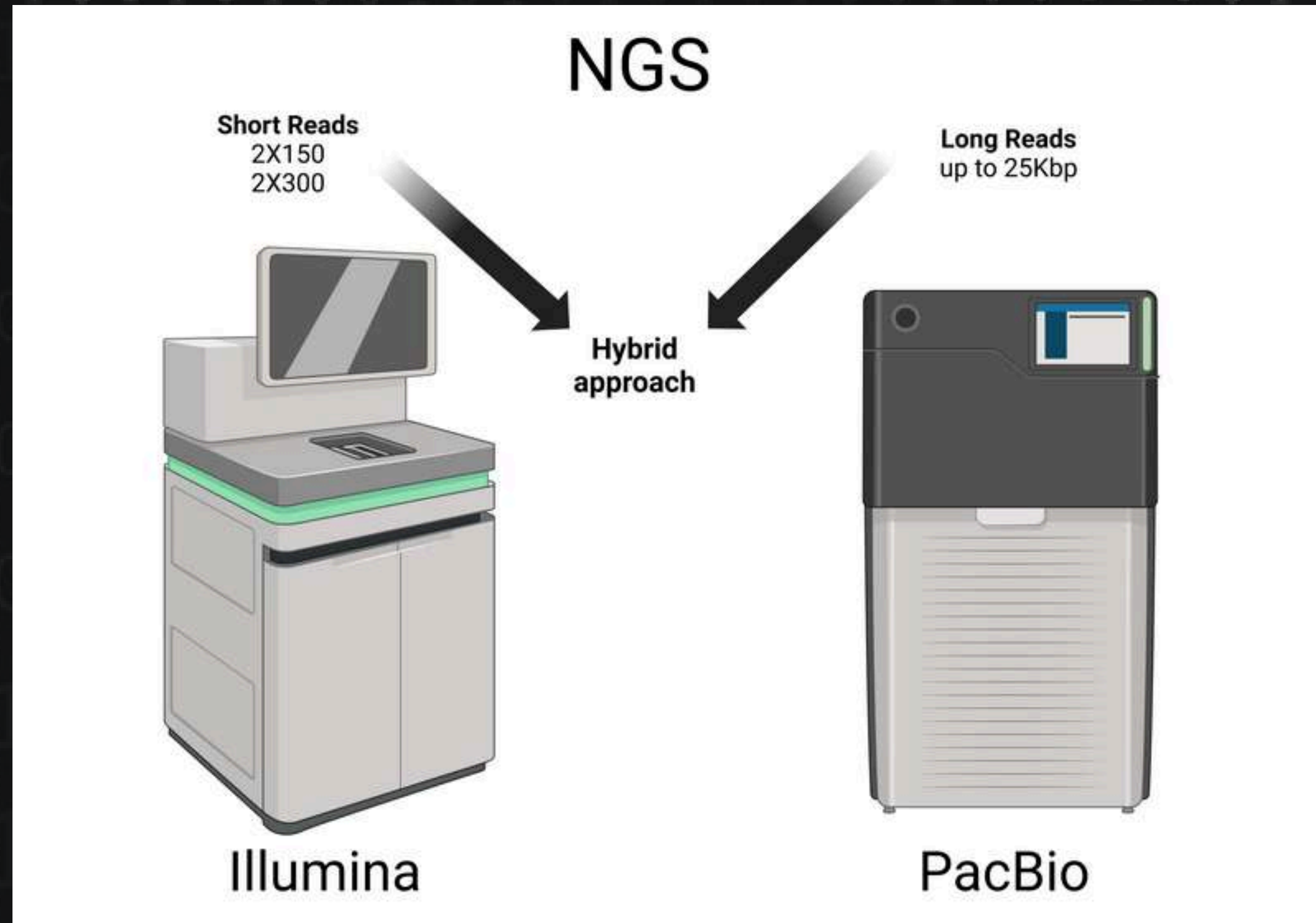
RdRP in RNA viruses?

Pros:

1. High Accuracy
2. High Throughput
3. Cost-Effective
4. Established Bioinformatics Tools

Cons:

1. Fragmentation of Genomes
2. Difficulty in Resolving Repeats and Complex Regions
3. Recombination and Diversity Detection



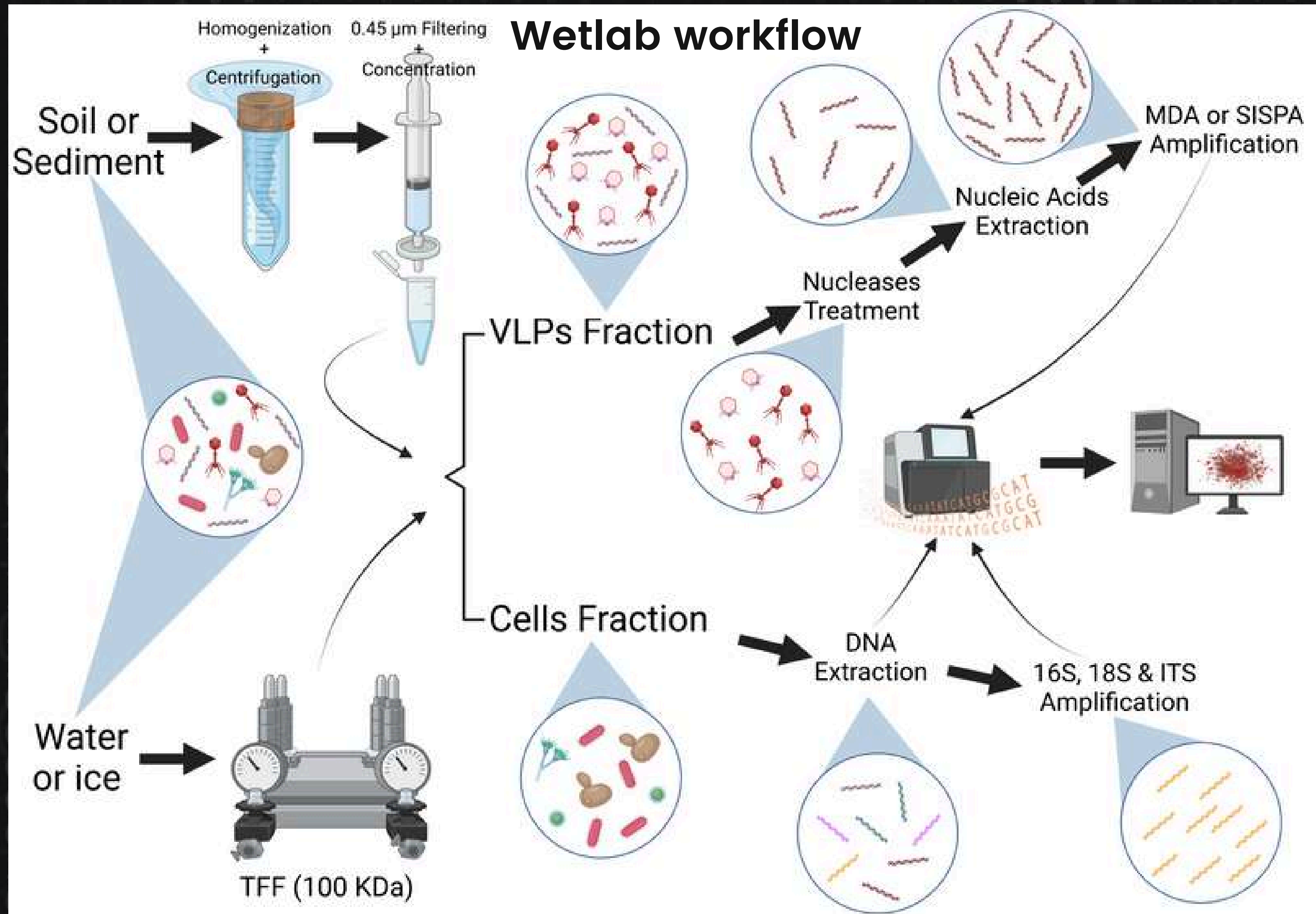
Pros:

1. Full Genome Assembly
2. Detection of Structural Variations.
3. Resolving Repetitive and Conserved Regions
4. Real-Time Sequencing

Cons:

1. Higher Error Rates
2. Lower Throughput
3. Cost Per Read
4. Bioinformatics Complexity

3. Wetlab workflow



Issues

The filtering pore diameter matters

0.45 μm VS 0.22 μm

0.45 μm : Prokaryotes contamination
0.22 μm : giant viruses lost



16S PCR check

PROs:

- Normally provides higher resolution of viral diversity

CONS:

- No Prokaryotes information
- Bias in frequencies

Amplification = Bias

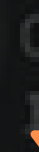
SISPA (RNA):

- low abundance viruses
- Small RNA viruses
- SSRNA Viruses
- Highly secondary structured
- High GC content
- Segmented viruses

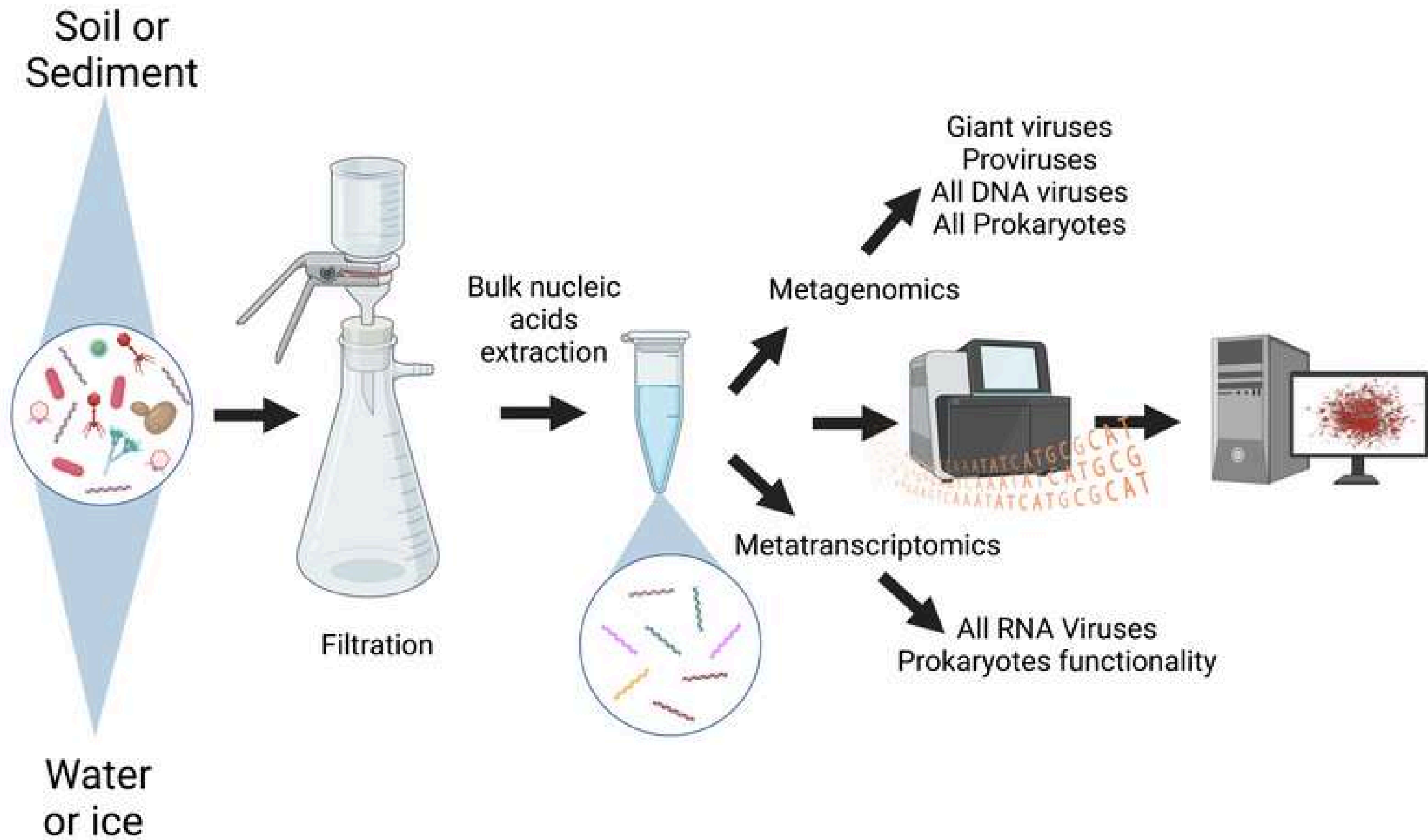


MDA (DNA):

- ssDNA
- cDNA
- Extreme GC content
- Secondary structured
- Repetitive regions
- low abundance viruses



Wetlab workflow



Issues

PROs:

- Normally provides higher resolution in terms of viral frequencies distribution
- Abundant information about Prokaryotes

CONS:

- Low proportion of viral sequences
- Lost of viral diversity
- Requires more bioinformatics (“needle in a haystack”)

The Big Issue



Avoid contamination!!!

- Sterile conditions (lab, tools, etc)
- Sample cleaning
- Controls

4. Drylab workflow

Common workflow:

1. Reads quality inspection
2. Quality filtering and trimming
3. Reads quality re-inspection
4. Assembling
5. Virus discovery
6. Binning
7. Quantification
8. Checking contigs quality
9. Functional annotation
10. Taxonomic annotation
11. Host prediction
12. Many others...

5. “Hands on” sea cucumbers dataset

sersancar/ **Viromics_Vigo**



Viromics day within the course "APLICACIÓN DE HERRAMIENTAS -ÓMICAS EN ACUICULTURA"

1

Contributor

0

Issues

0

Stars

0

Forks



sersancar/Viromics_Vigo: Viromics day within the course "APLICACIÓN DE HERRAMIENTAS -ÓMICAS EN...

Viromics day within the course "APLICACIÓN DE HERRAMIENTAS -ÓMICAS EN ACUICULTURA" - sersancar/Viromics_Vigo

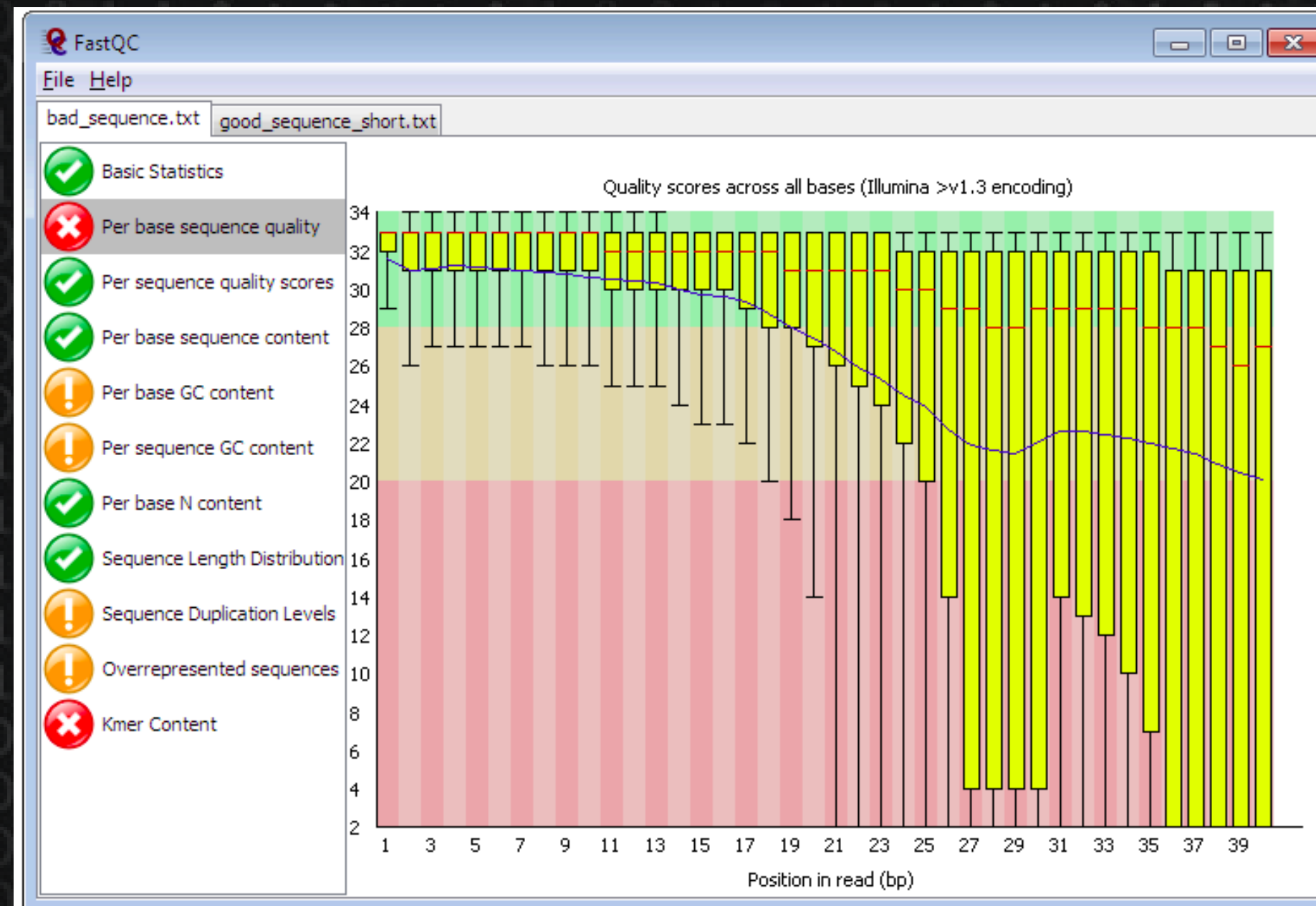


https://github.com/sersancar/Viromics_Vigo

Reads quality inspection

FastQC

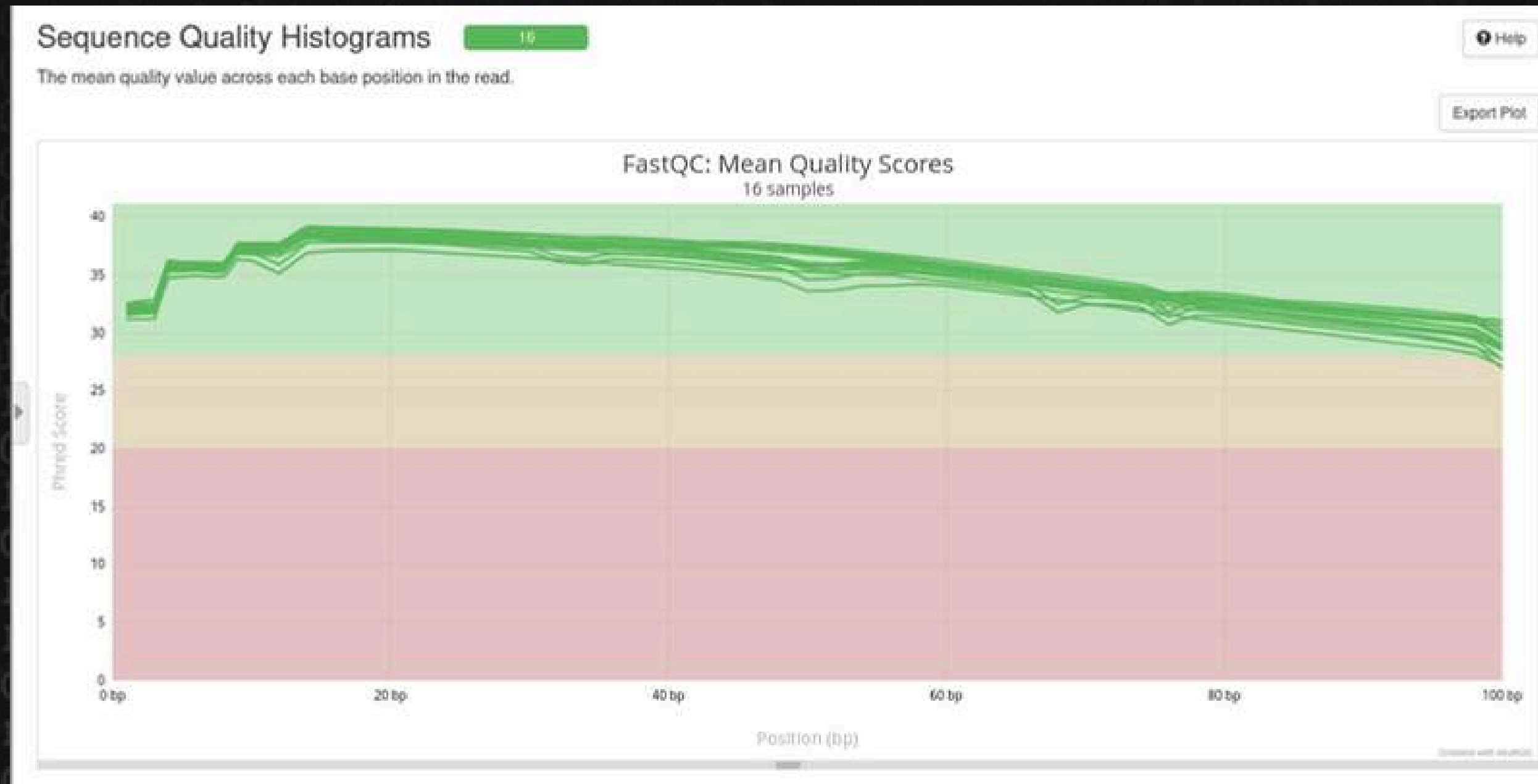
<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>



Reads quality inspection

MultiQC

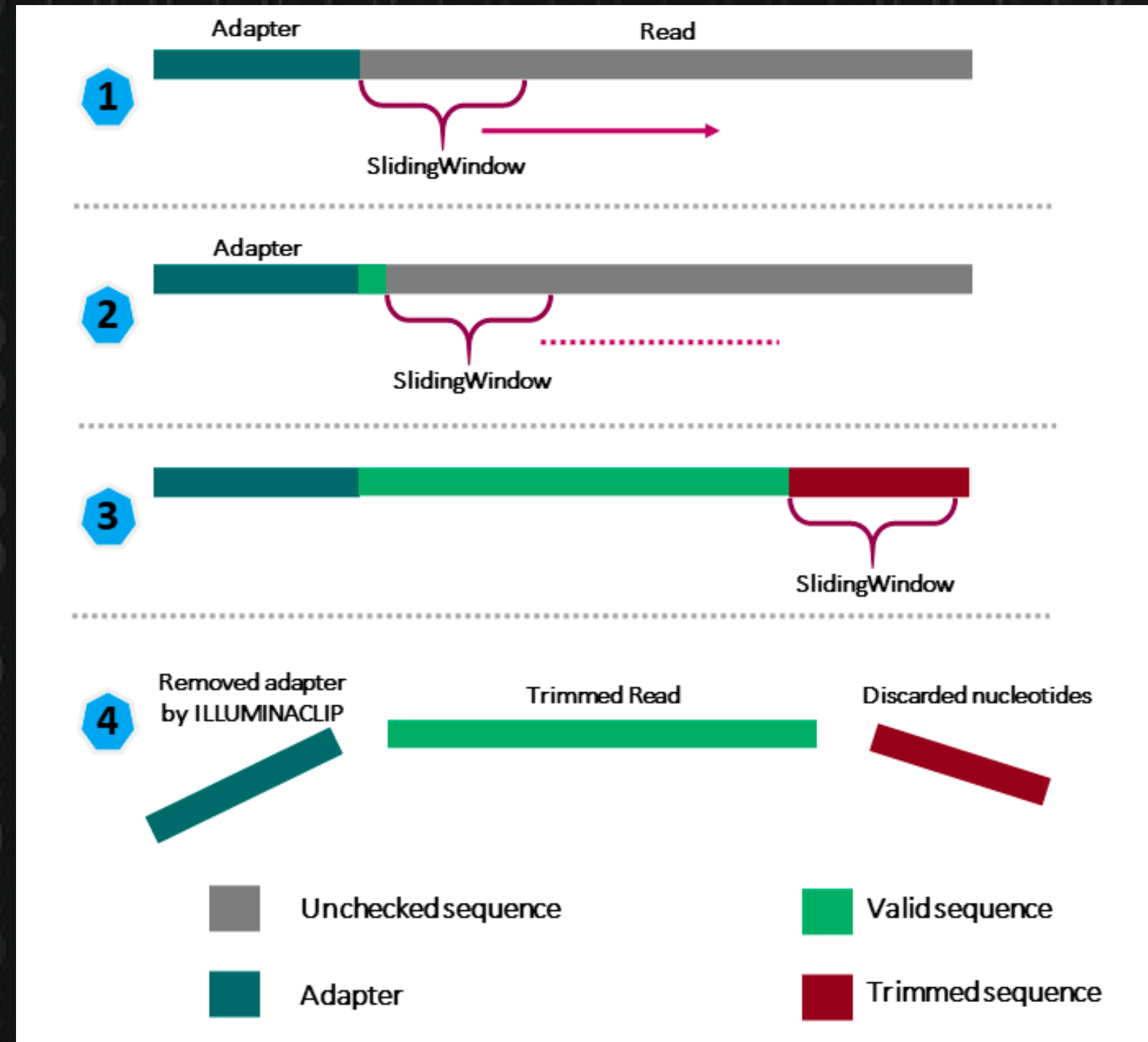
Ewels et al 2016



Quality filtering and trimming

Trimmomatic

Bolger, Lohse & Usadel 2014



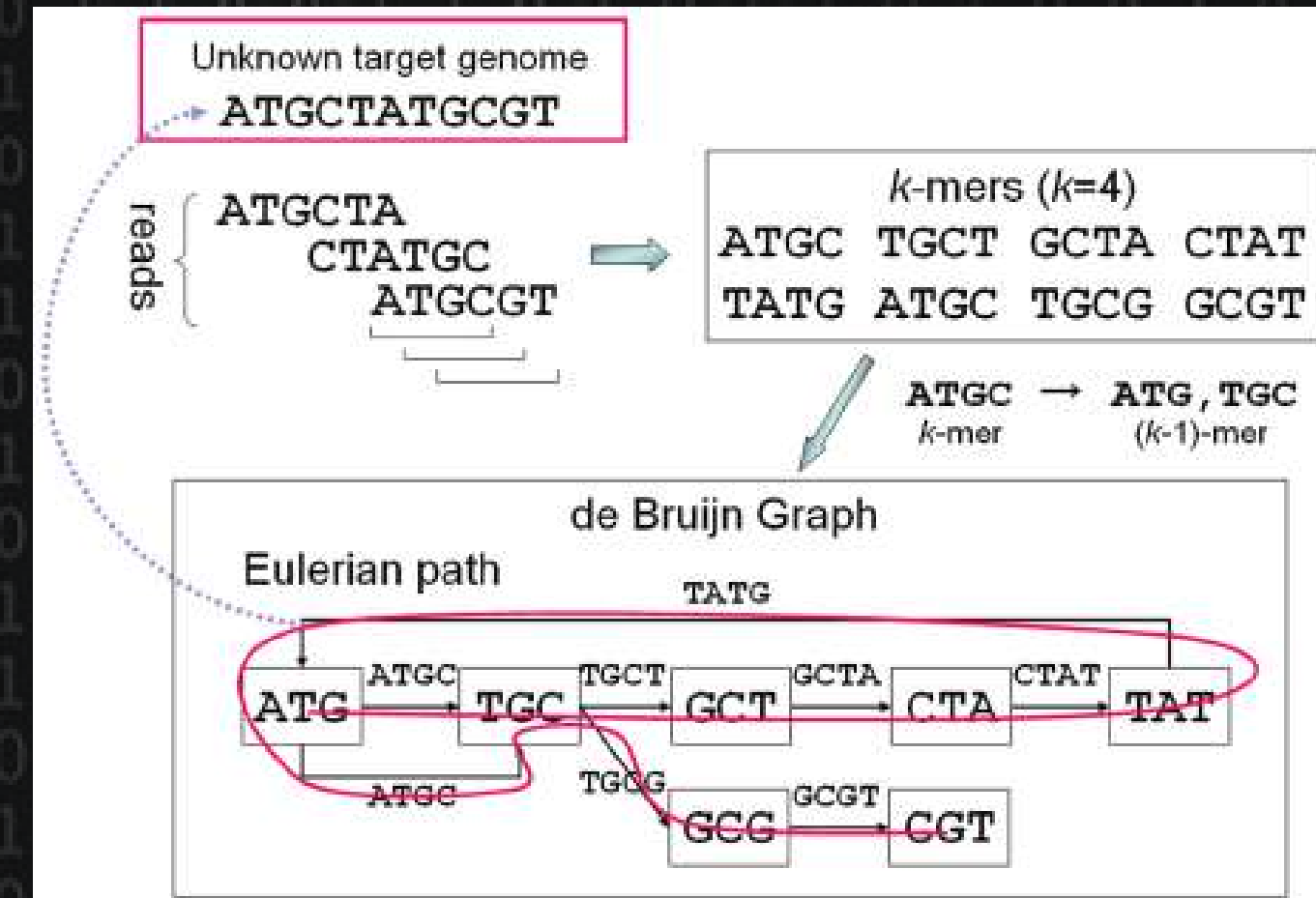
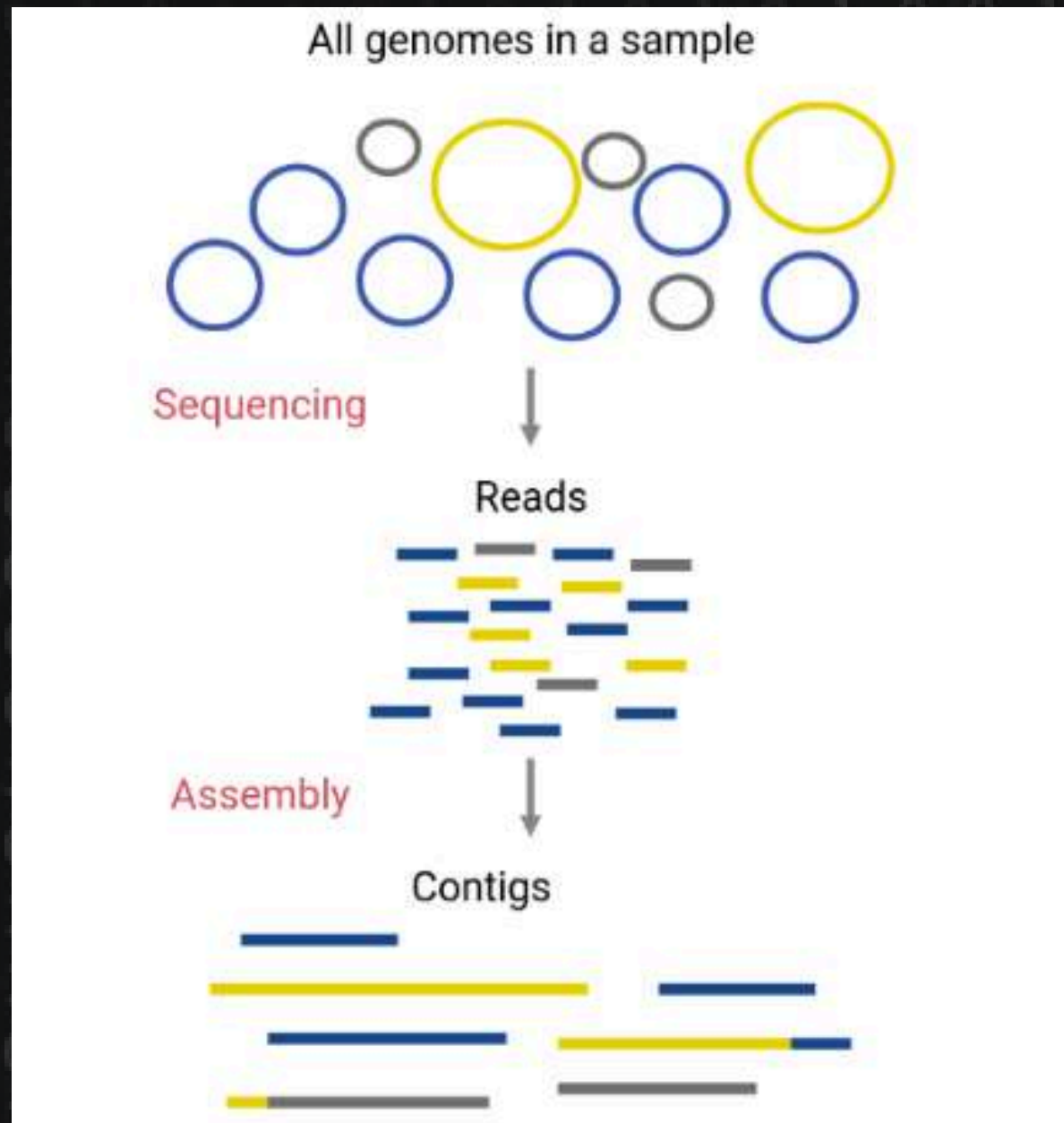
Assembling

SPAdes

Prjibelski et al. 2020

MEGAHIT

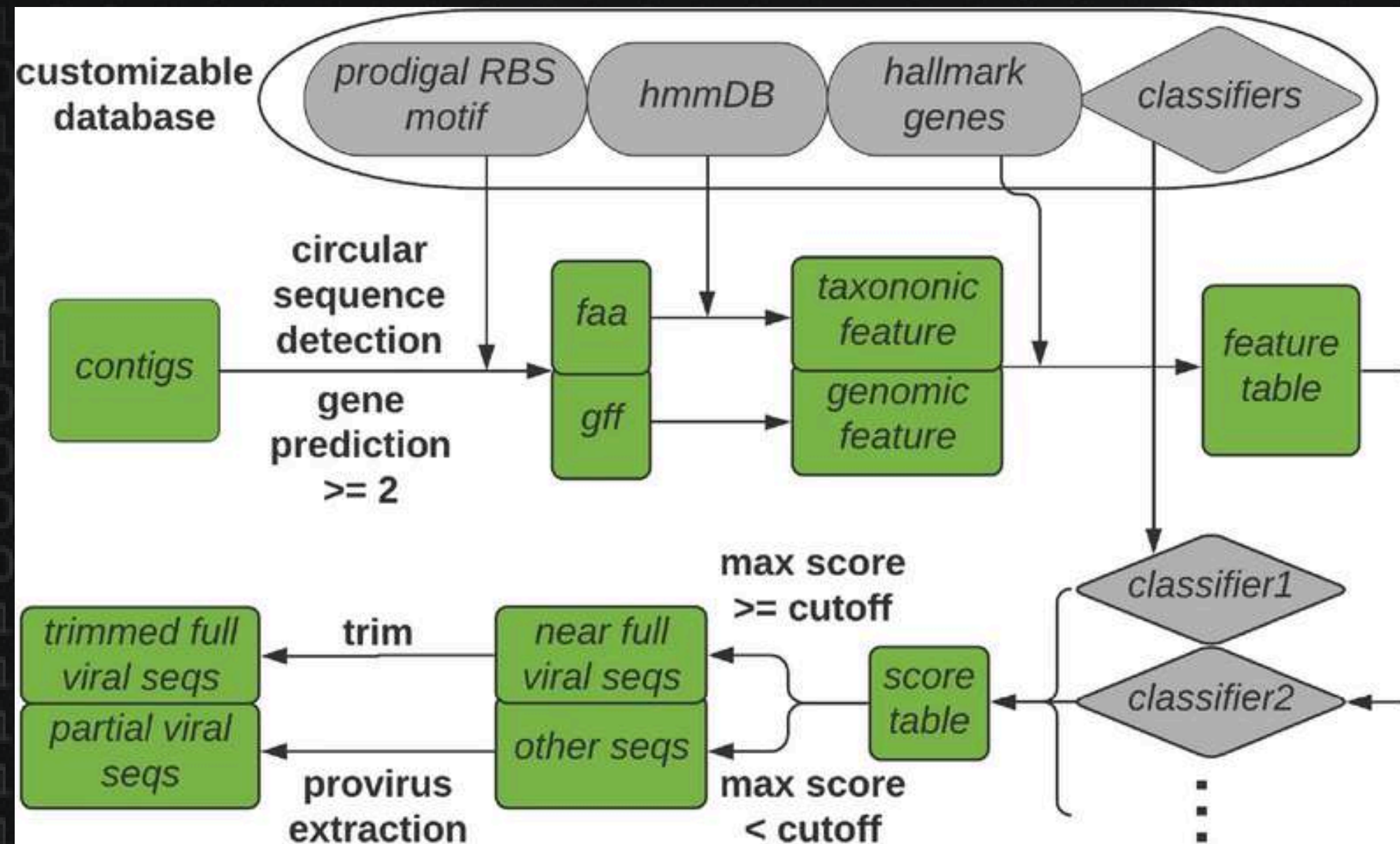
Li et al. 2015



Virus discovery

VirSorter2

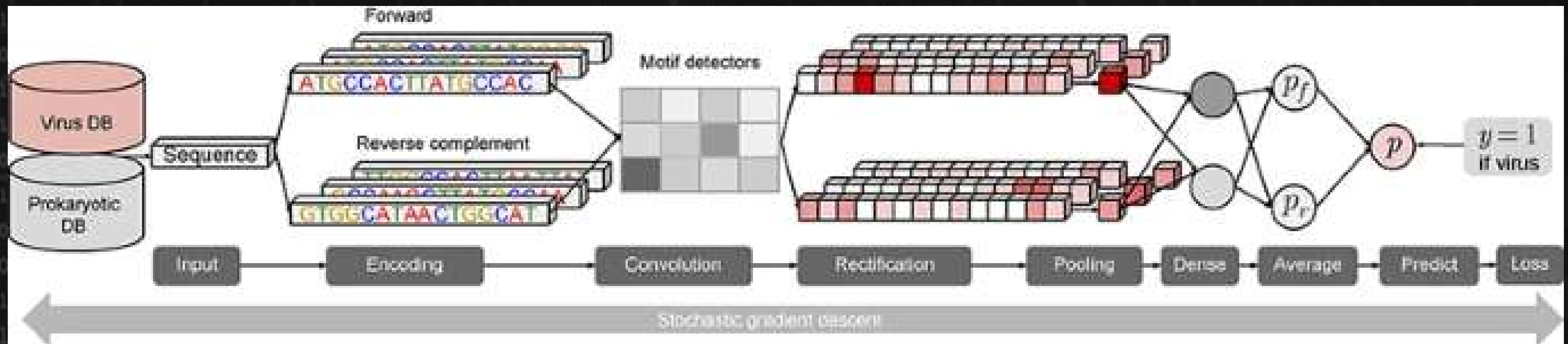
Guo et al. 2021



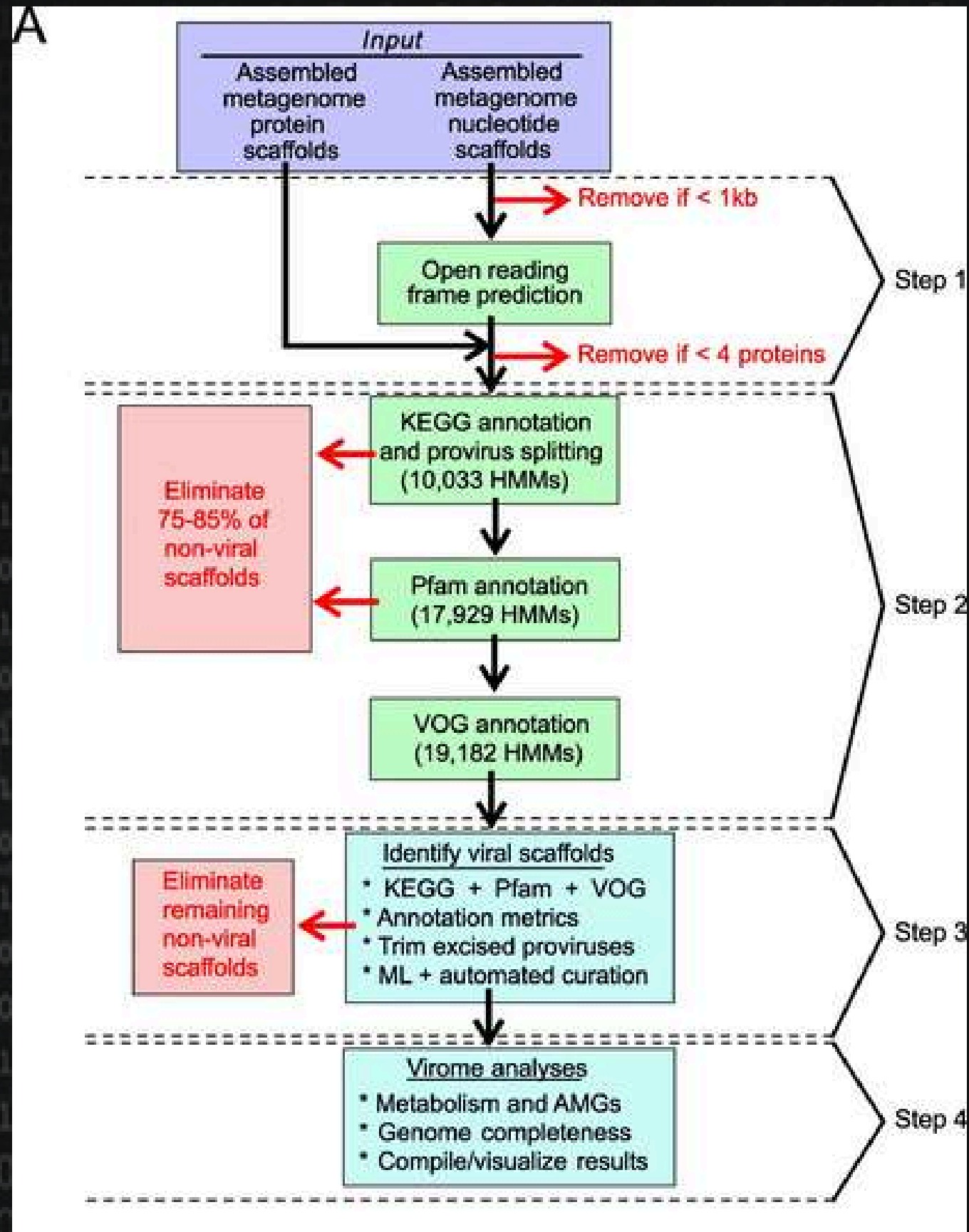
Virus discovery

DeepVirFinder

Ren et al. 2020

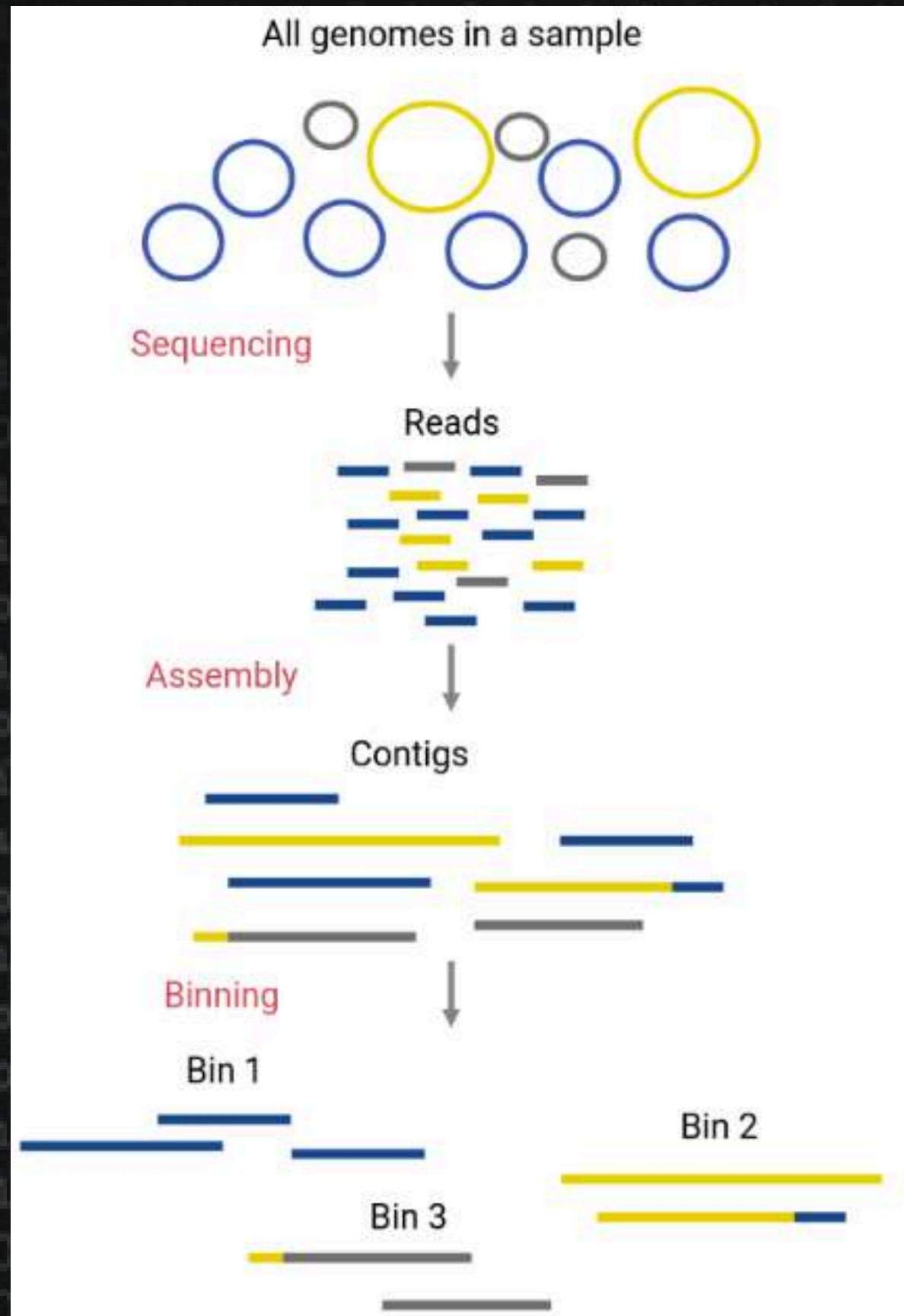


Virus discovery

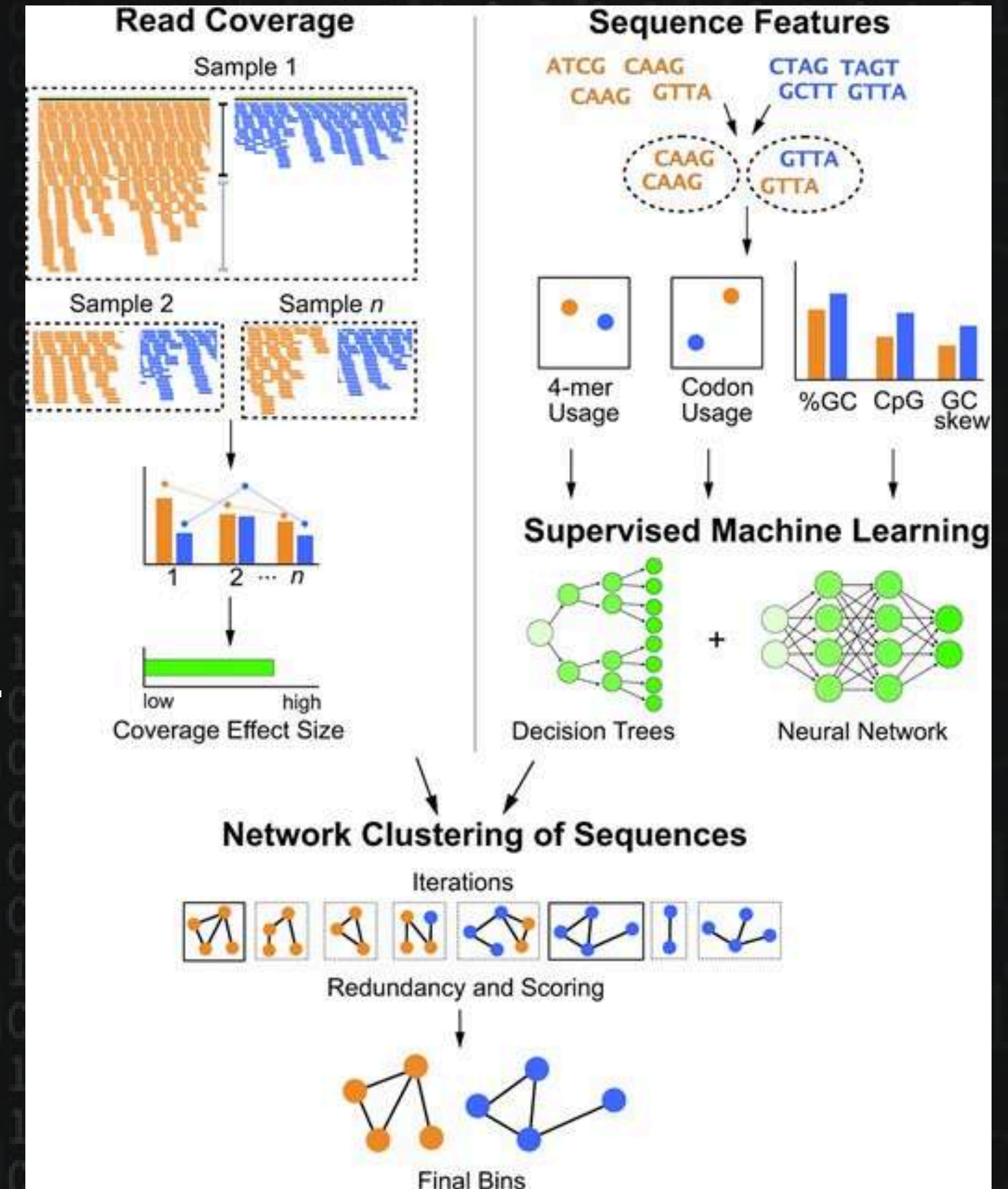


VIBRANT
Kieft, Zhou & Anantharaman 2022

Binning



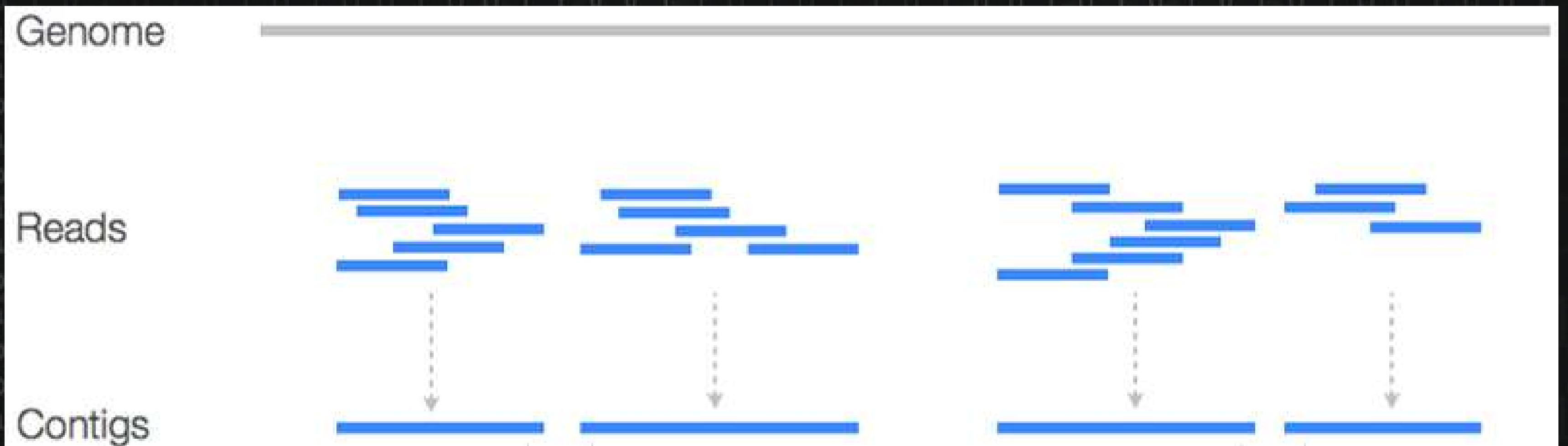
VRhyme
Kieft et al. 2022



Quantification

CoverM

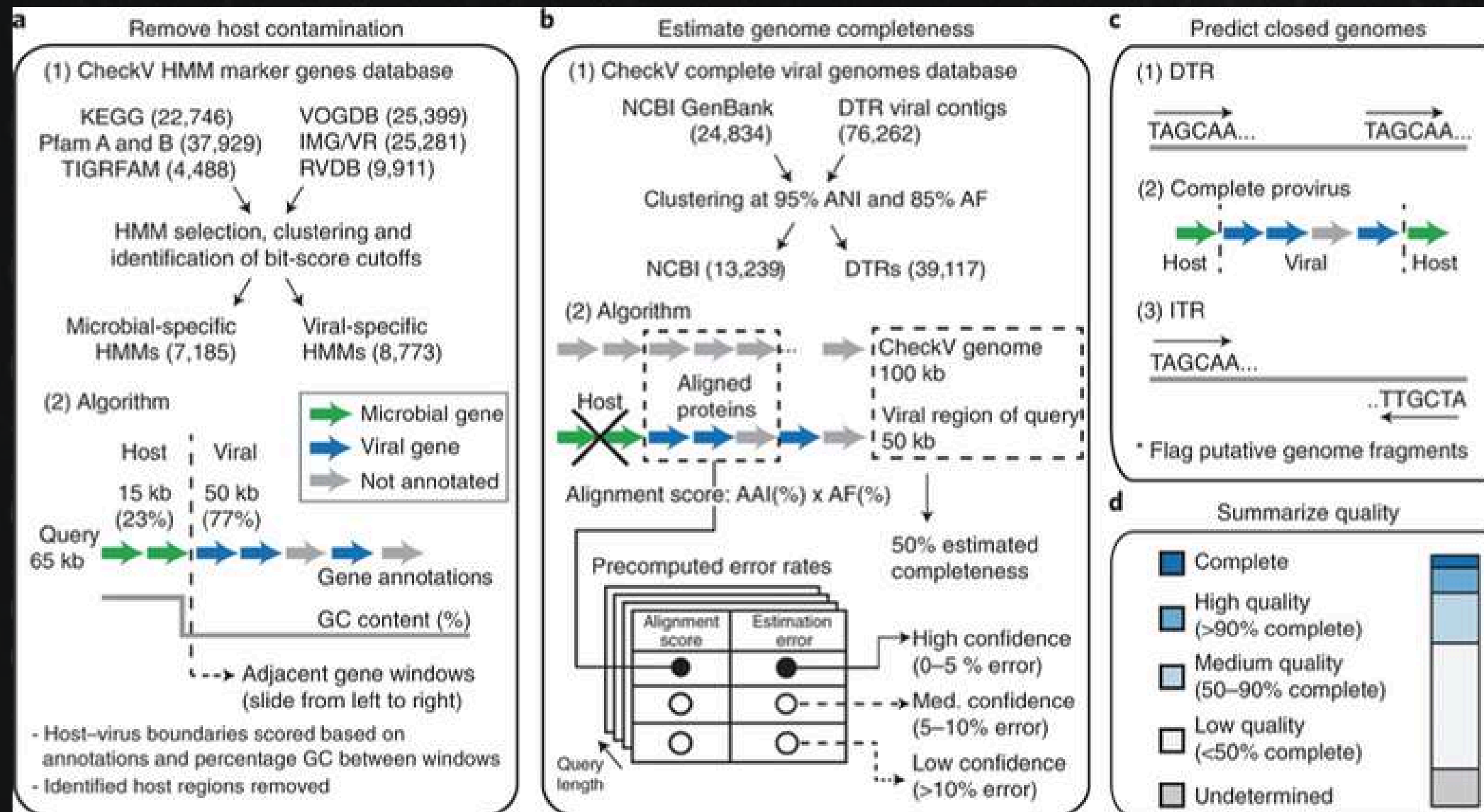
<https://github.com/wwood/CoverM>

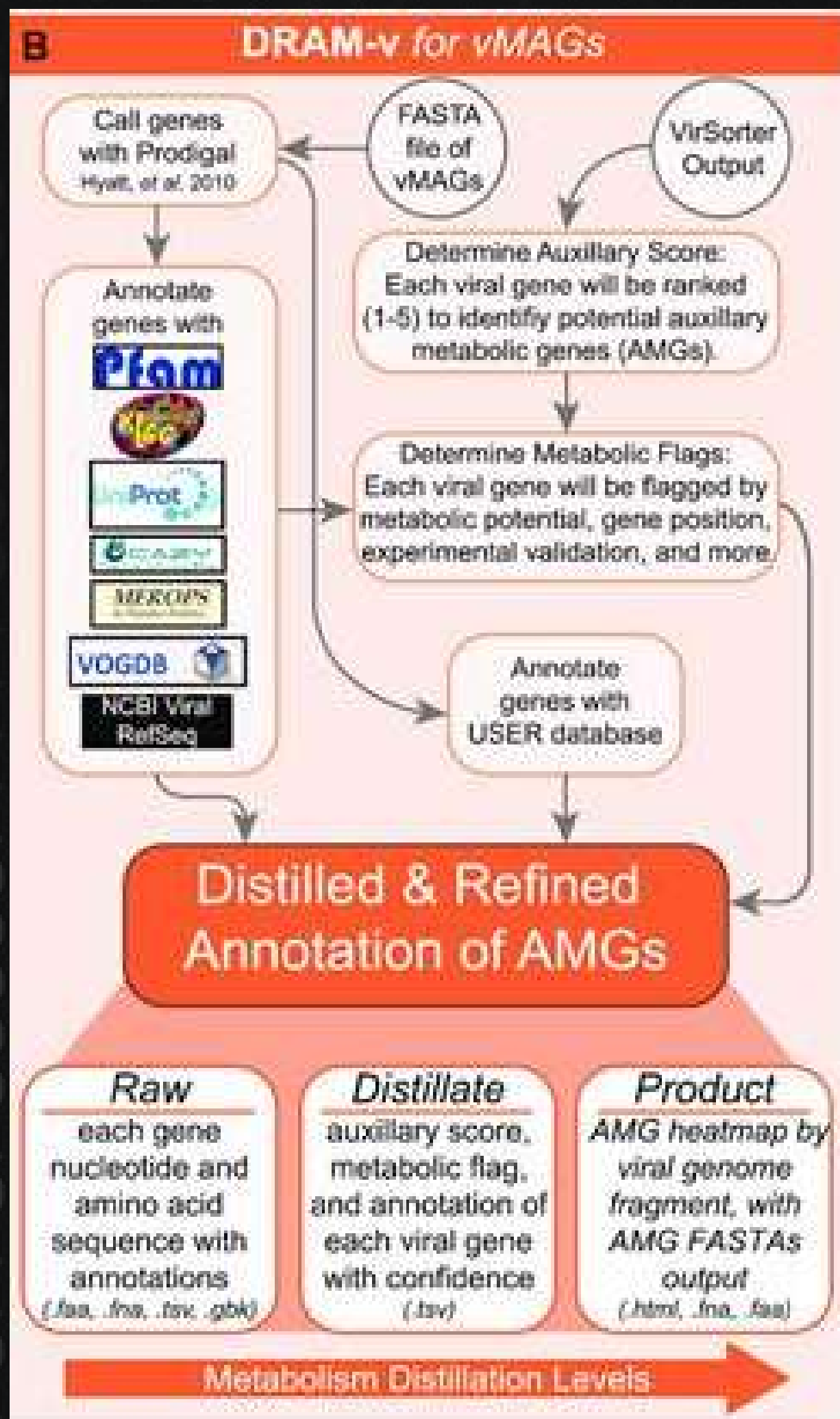


Checking contigs quality

CheckV

<https://www.nature.com/articles/s41587-020-00774-7>





Functional annotation

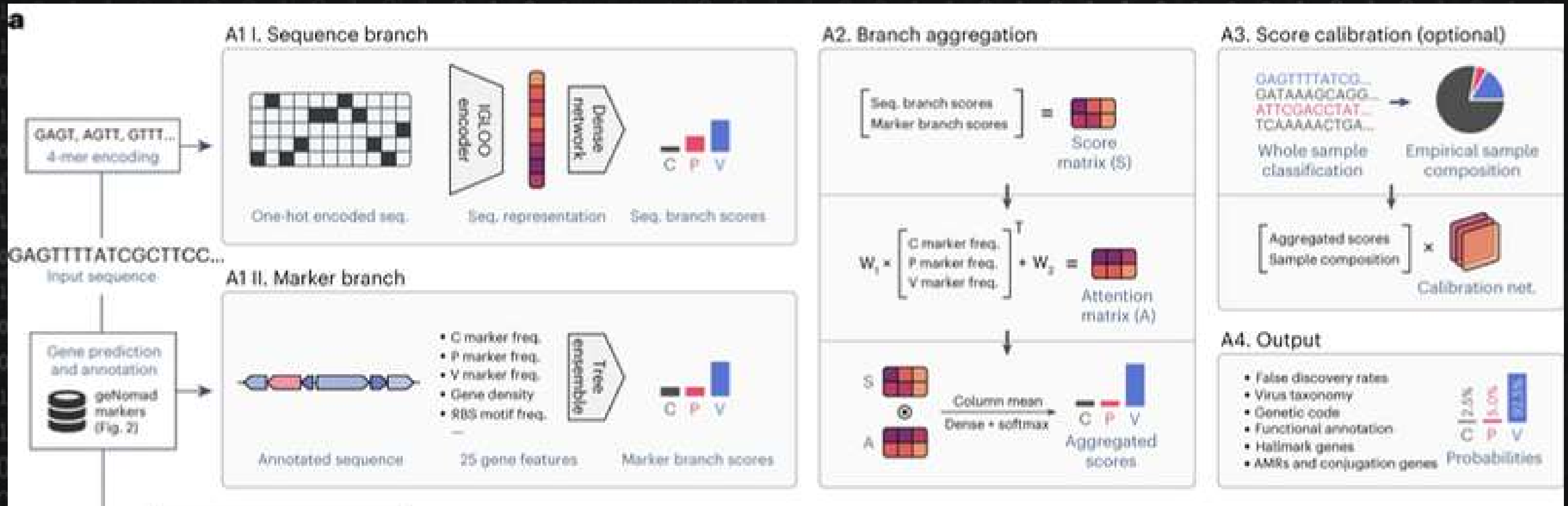
DRAM-v

Shaffer et al. 2020

Taxonomic annotation and many more....

geNomad

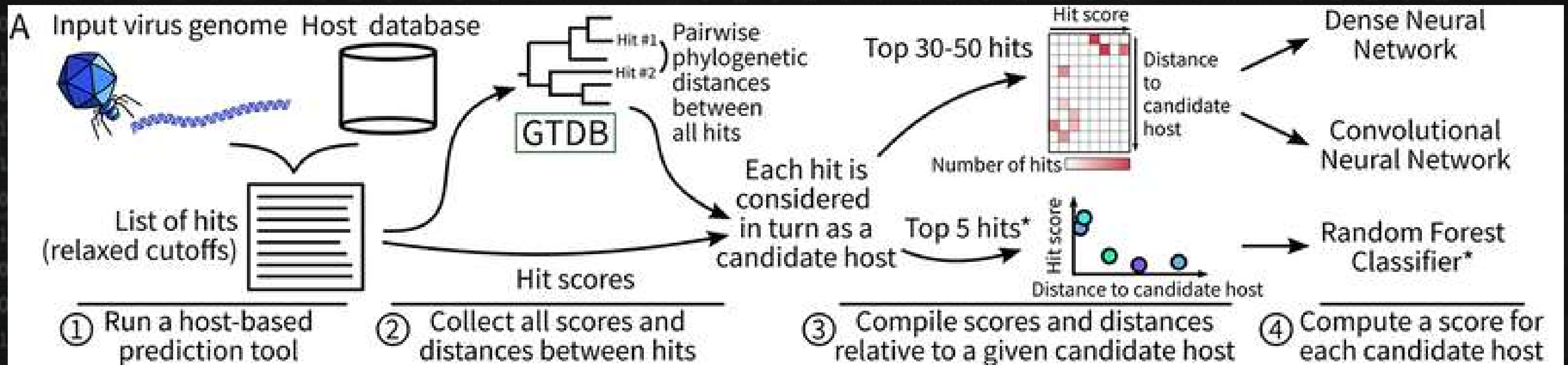
Camargo et al. 2024

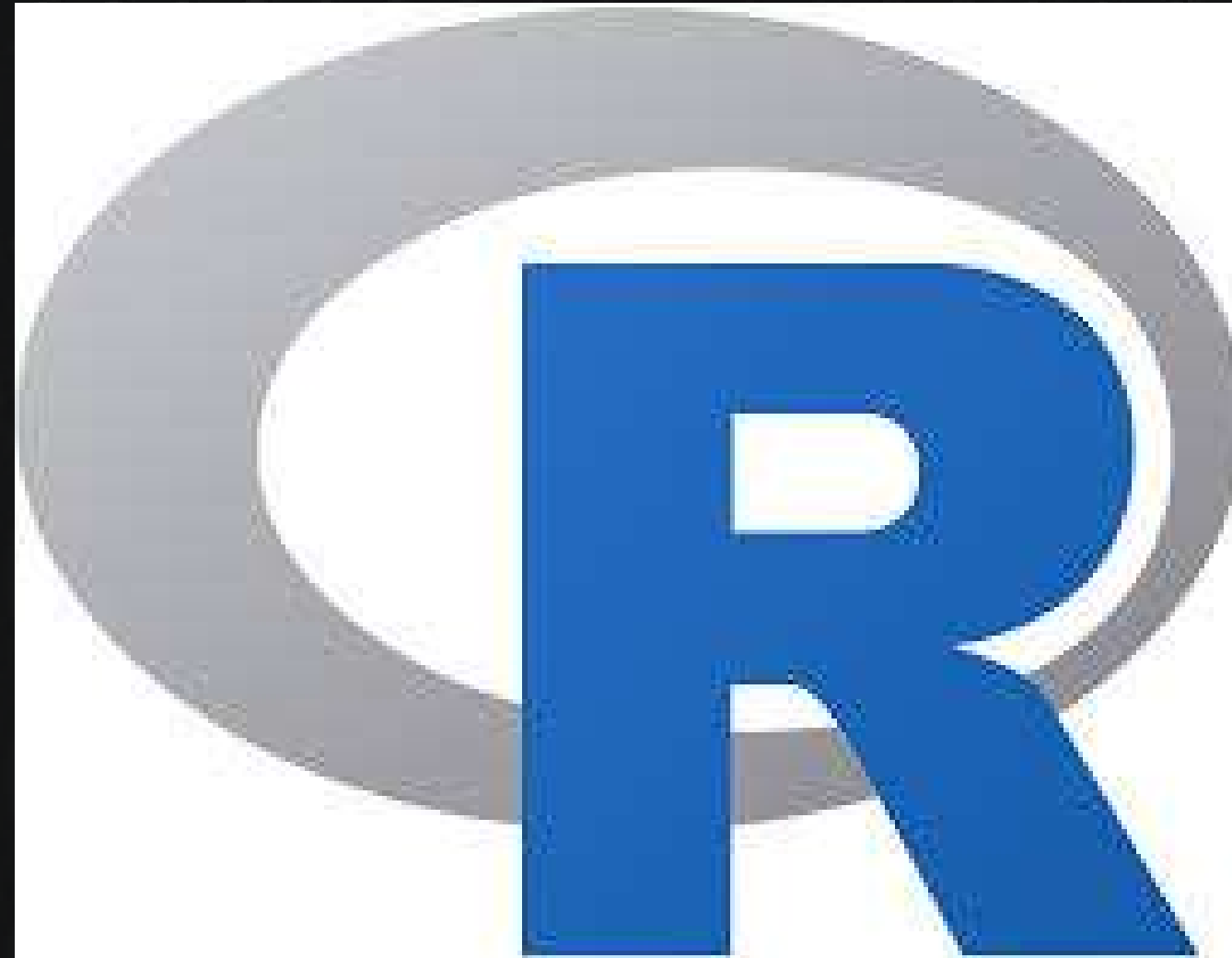


Host prediction

iPHoP

Roux et al. 2023





Data analysis

<https://www.r-project.org>

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<https://orcid.org/0000-0002-9271-7484>