Biological sequence analysis via deep learning

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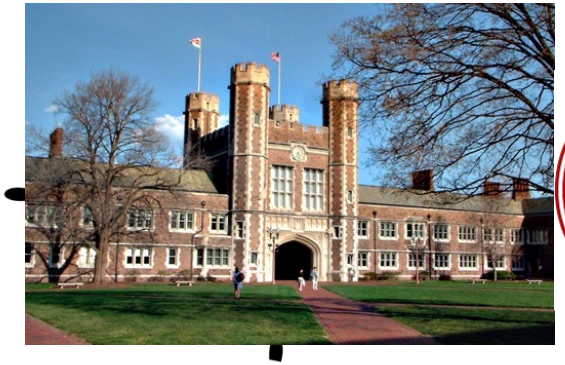
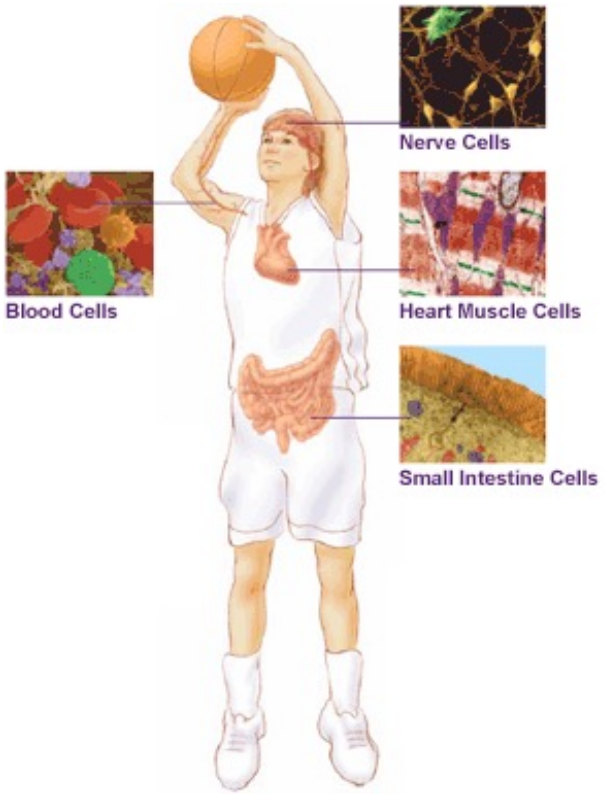
How to give an interdisciplinary talk: assume zero knowledge but infinite intelligence of the audience. - From Gary Stormo's talk

Yanni Sun, PhD in Computer Science & Engineering, research area: bioinformatics, sequence analysis, application of deep learning for genomic data analysis

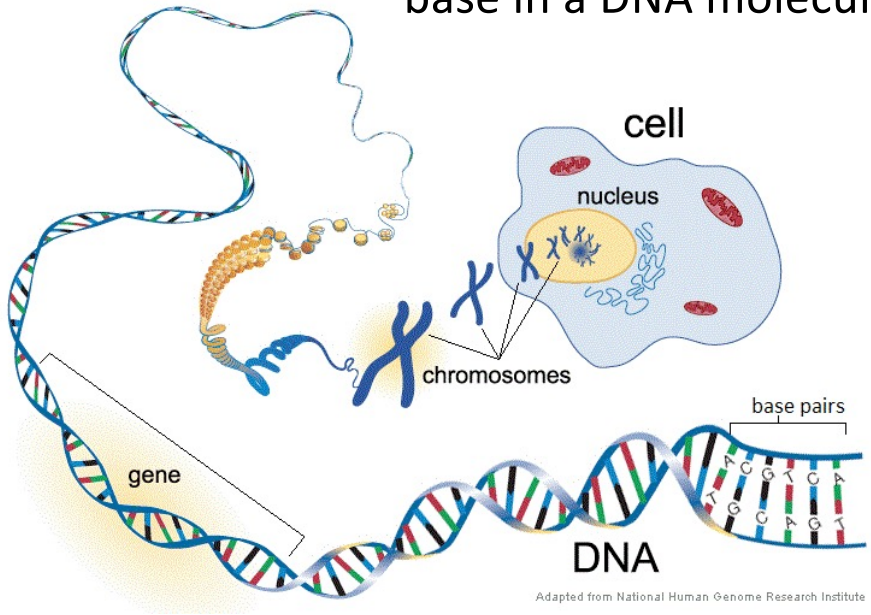


Genomic data: genomes

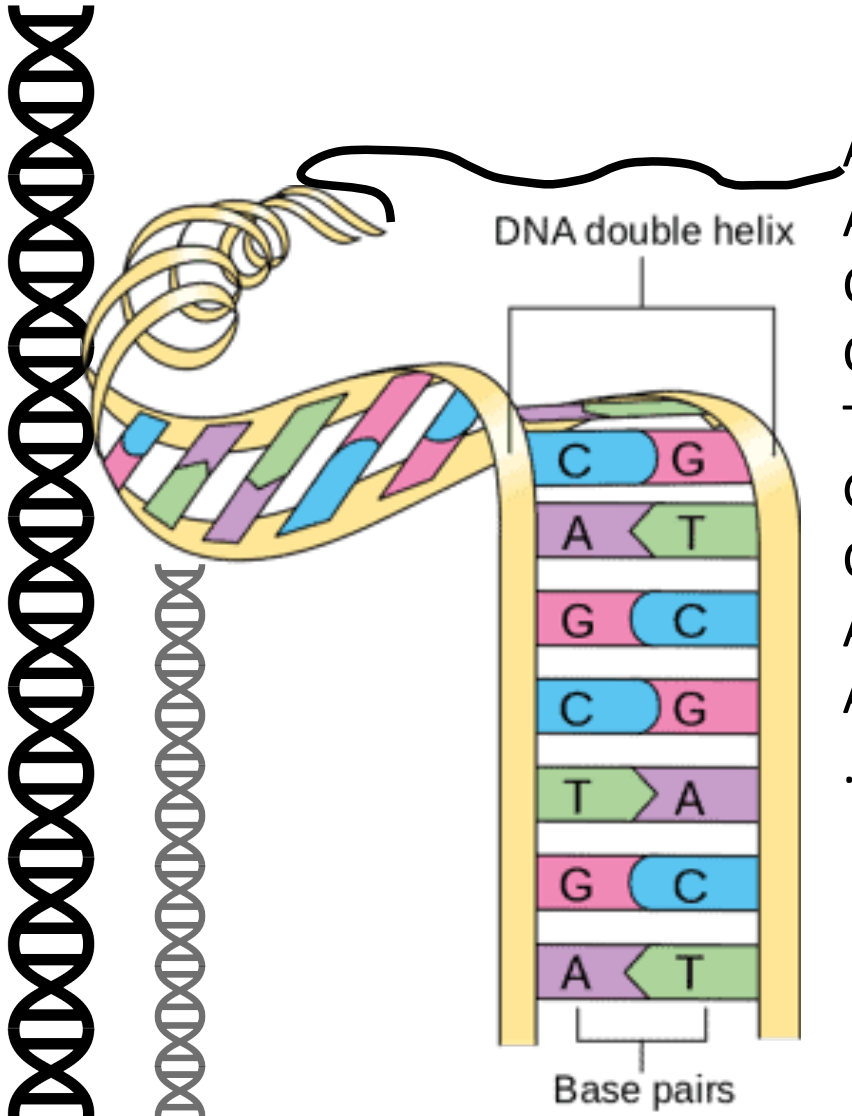


The primary structure of DNA sequence: a string defined on four bases: A, C, G, and T:

Sequencing : determine each base in a DNA molecule



The genome is in every cell except red blood cell and sex cells.



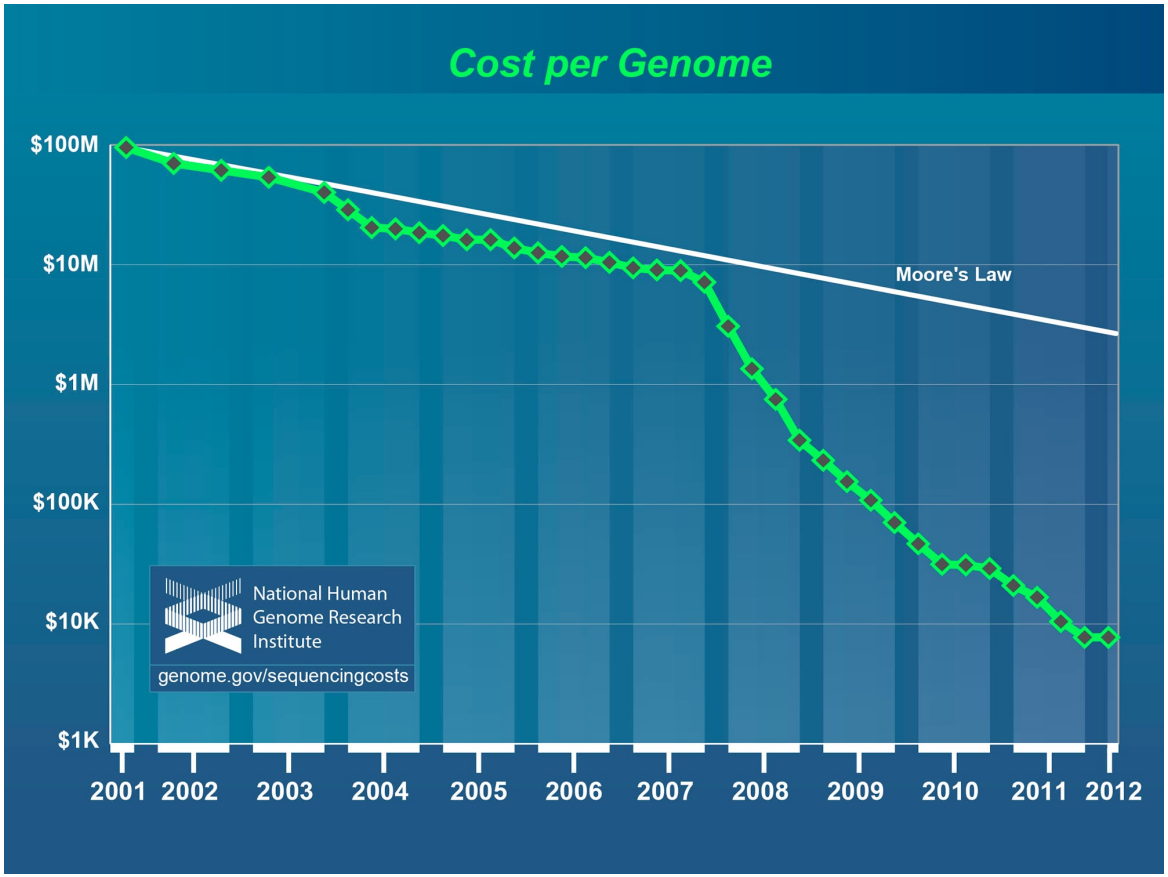
ATGGGCAAGTCAGAAAGTCAGATGG
ATATAACTGATATCAACACTCCAAAG
CCAAAGAAGAAACAGCGATGGACT
CCACTGGAGATCAGCCTCTCGGTCCT
TGTCCTGCTCCTCACCATCATAGCTGT
GACAATGATCGACTCTATGCAACCTA
CGATGATGGTATTTGCAAGTCATCAG
ACTGCATAAAATCAGCTGCTCGACTG
ATCCAAAACATGGATGCCACCACTG
… …

Language of Life

- Contains all functions that make each of us unique

https://nebula.org/blog/dna-structure-model/

BIG genomic data

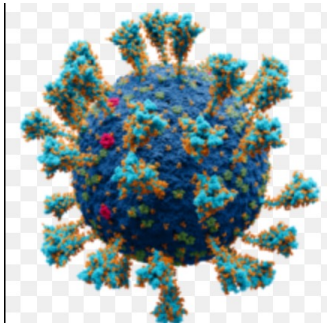


Low cost → fast accumulation of sequencing data

Sequence Read Archive at NIH: 11,141,607,428,443,304 bases (~11,141 terabases)

Fast accumulation of sequenced genomes

Species	*T2 phage*	*Escherichia coli*	*Drosophila melanogaster*	*Homo sapiens*	*Paris japonica*
Genome Size	170,000 bp	4.6 million bp	130 million bp	3.2 billion bp	150 billion bp
Common Name	 Virus	 Bacteria	 Fruit fly	 Human	 Canopy Plant

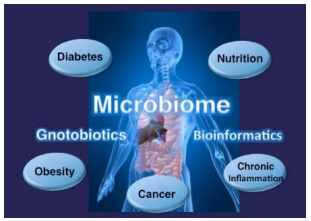


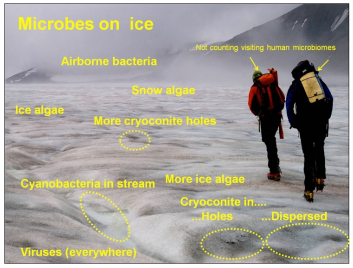
Length: ~30,000 bp

Pictures downloaded from Wiki

Analyze microbial communities using next-generation sequencing data

- Microbial communities: groups of microbes (such as bacteria, viruses) that share a common living space





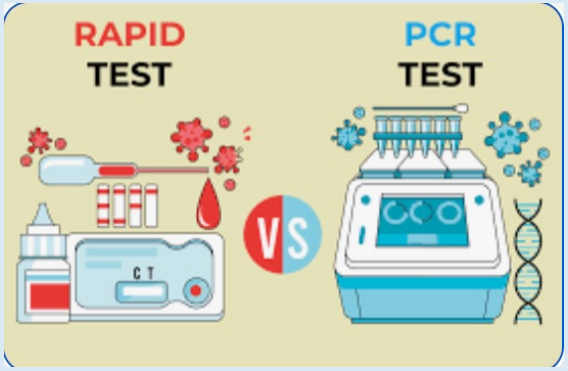
Powerful method to study microbiome: metagenomic sequencing

Sequencing machines (credit: illumina.com)

Algorithm/model input:

Bioinformatics algorithms, machine learning models

Sample **output**:
Bacterium 1: 50%
Bacterium 2: 45%
Virus 1: 4%
Virus 2: 0.5%
…



Picture credit: CommunityLab

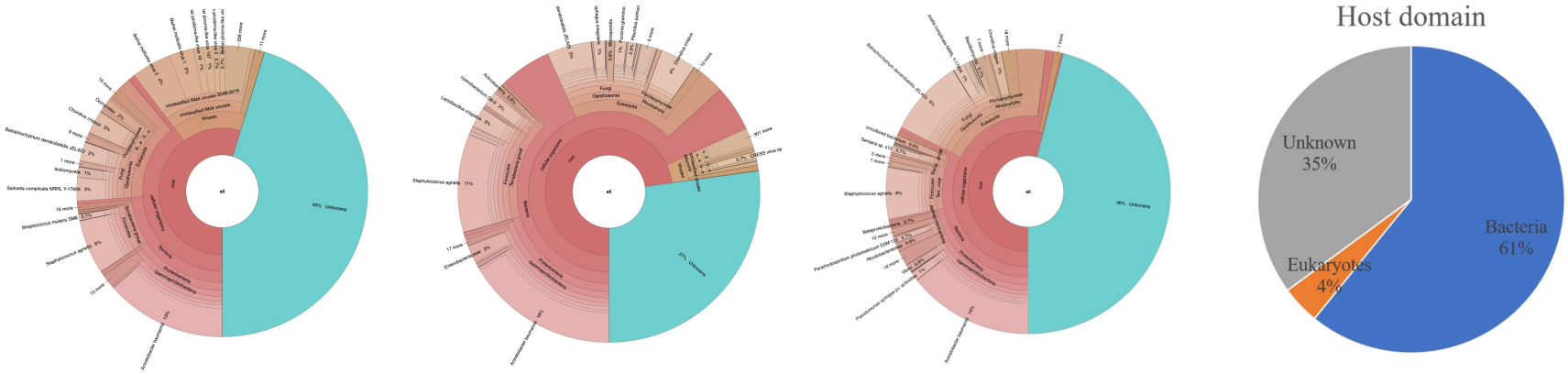
Targeted testing methods (RAT, PCR, etc.)

vs

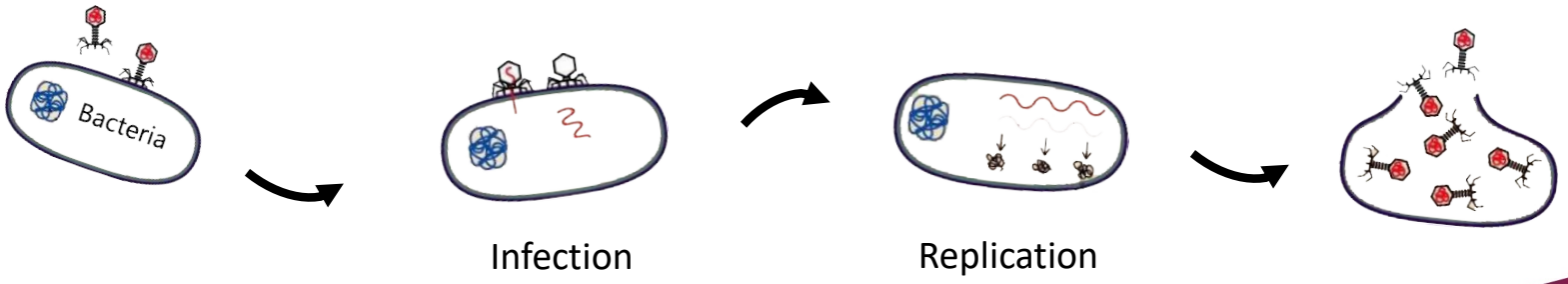
Metagenomic sequencing: broad spectrum

Dark matter in microbial communities: prokaryotic viruses

The most common and diverse entities



Life Cycle (lytic Cycle)

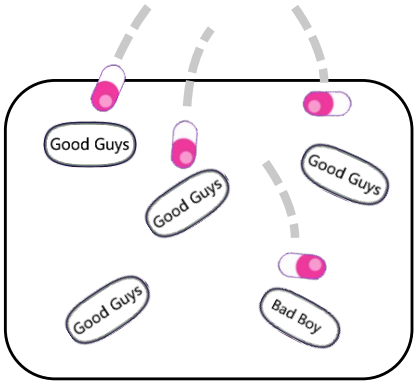


Material source: xconomy.com

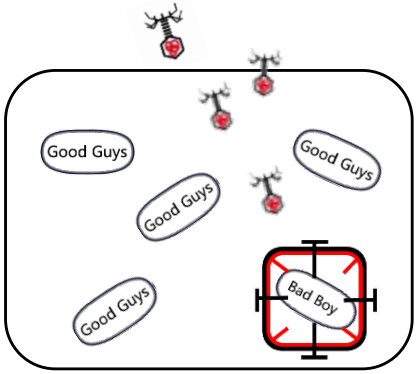
Phage therapy

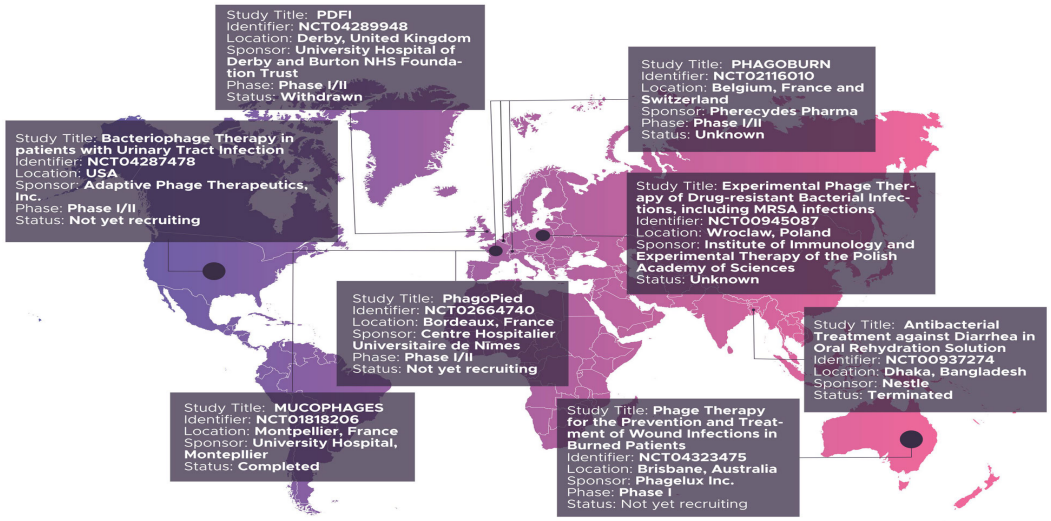
Used as antibiotics

Antibiotic (Area of effect)



Phages (targeted)





Adesanya et al. An exegesis of bacteriophage therapy: An emerging player in the fight against anti-microbial resistance. 2020

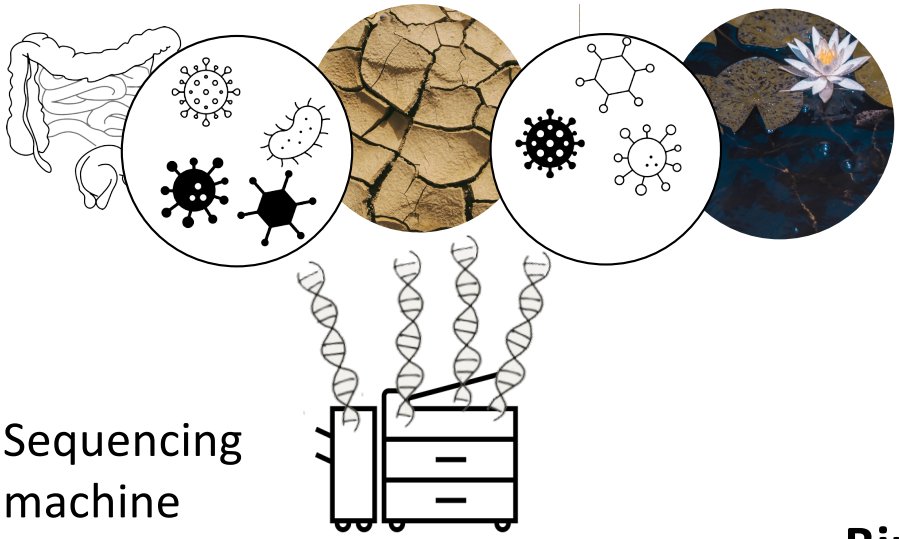
Two research problems

1. Identify phages from metagenomic data

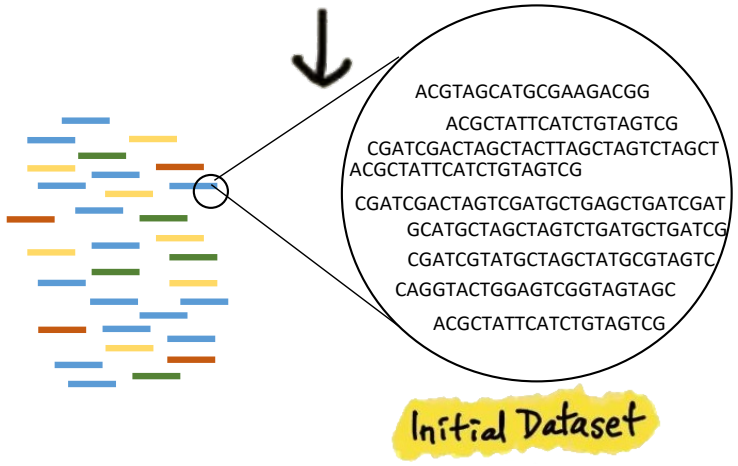
2. Find the phage-bacteria interactions

Jiayu Shang, Xubo Tang, Ruocheng Guo, **Yanni Sun***, Accurate identification of bacteriophages from metagenomic data using Transformer, Briefings in Bioinformatics, Volume 23, Issue 4, July 2022, bbac258,
Jiayu Shang, Jinzhe Jiang, and **Yanni Sun***, "Bacteriophage classification for assembled contigs using Graph Convolutional Network", the 29th Annual International Conference on Intelligent Systems for Molecular Biology and the 20th European Conference on Computational Biology (*ISMB/ECCB 2021)* July 25, 2021 **acceptance rate: 18%**
Jiayu Shang and **Yanni Sun***, "Detecting the hosts of bacteriophages using GCN-based semi-supervised learning", *BMC Biology,* 2021

Phage identification: problem formulation

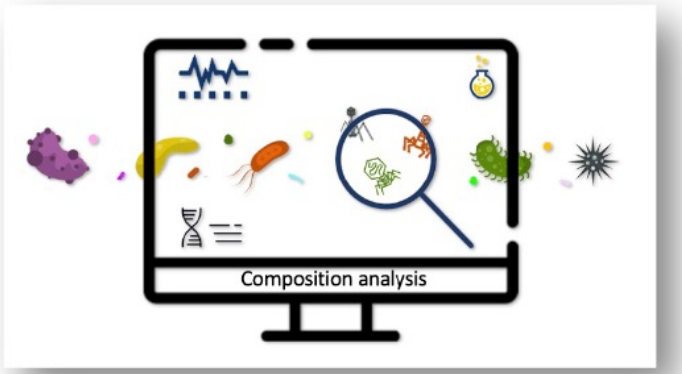


Sequencing machine



Initial Dataset

Binary classification



Phage identification

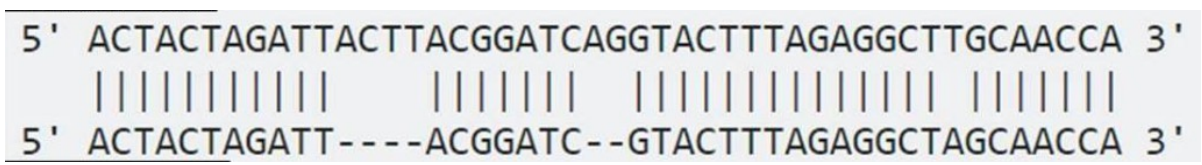
DNA sequences (**contigs**) obtained using sequencing data analysis

Material source: viralzone

Existing methods for phage identification

Comparison based:
compare a new DNA sequence with known phages

- Main feature: sequence similarity



Limitations of existing tools

-Limited reference genomes (viral dark matter)
-Diverged viral genomes (marginal sequence similarity)
-Massive amount of data (alignment-based tools can be slow)

Promising alternative: deep learning

- Automatic feature learning
- Mining degenerate patterns
- GPU for speedup

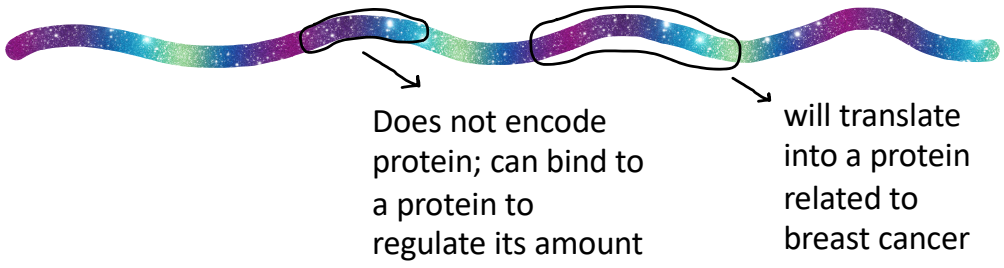
Methodology: towards more sensitive and accurate phage identification using

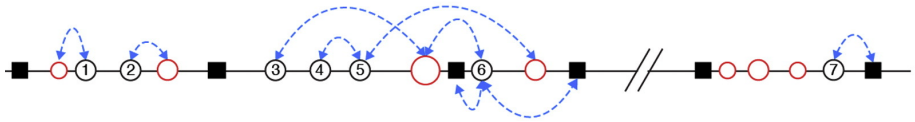
Transformer

Natural language vs. language of life

- **Alphabet**
  - English: {A,B,C,D, …, Z}
  - DNA: {A,C,G,T}

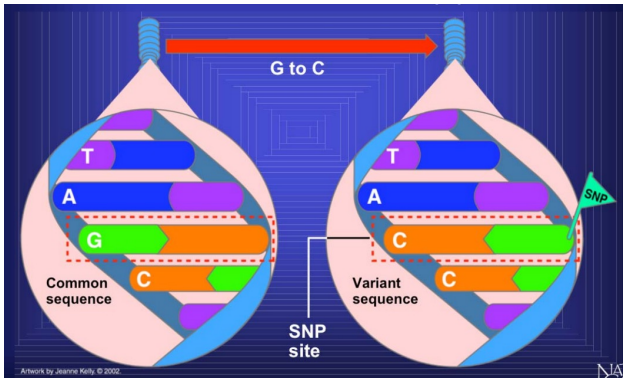
- **Words**
  - {happy, sad, student…}: delimited by spaces
  - What are the words for DNA sequences?

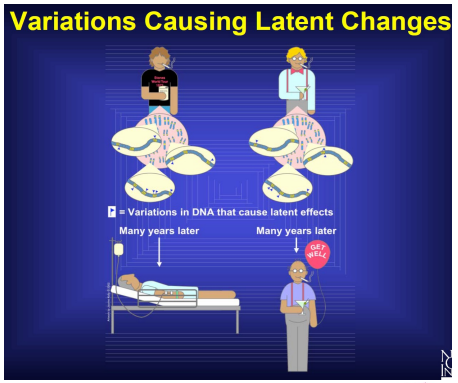




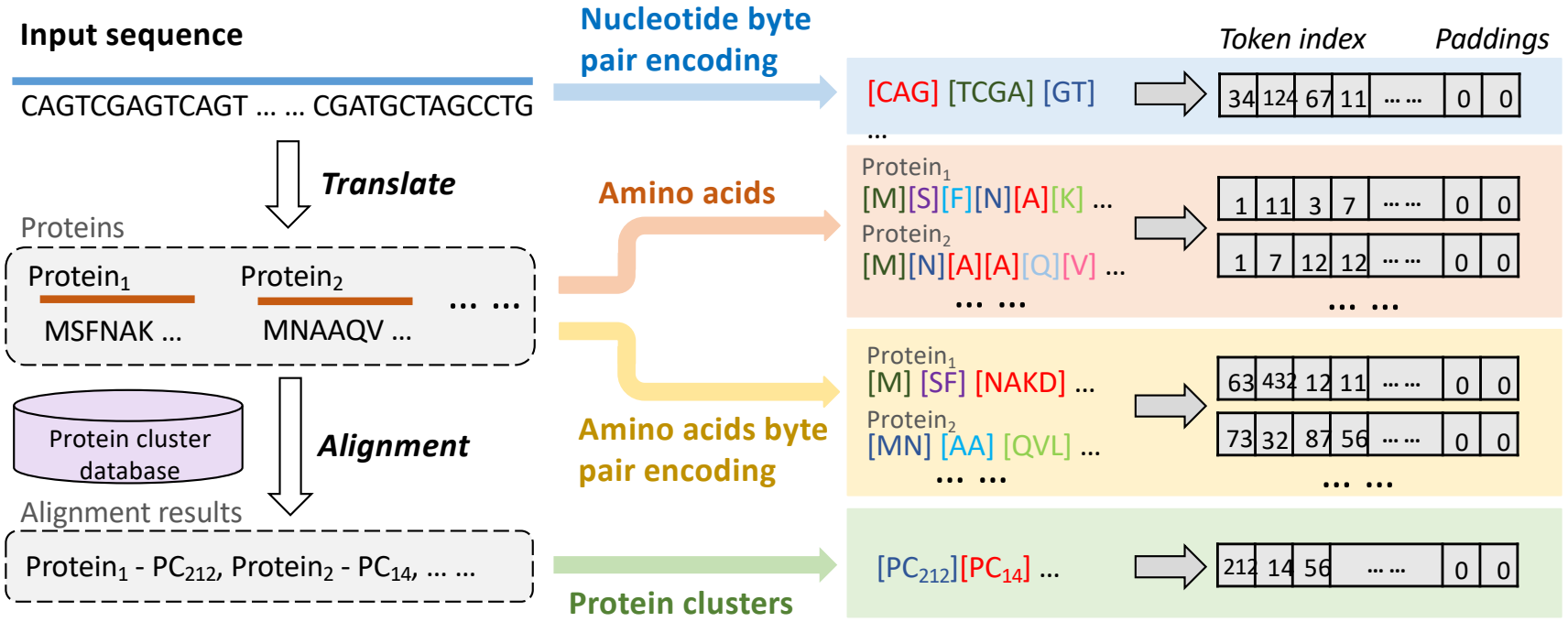
- **Interactions/associations**
  - She quickly corrected her mistakes. She corrected her mistakes quickly.

- **Errors in spelling**
  - I like my bok.
  - Variations in DNA → diseases





Token (word) construction



Byte pair encoding (BPE): count the most frequent nucleotide/ amino acid combinations in the corpus.

Nucleotide byte pair encoding tokenizer

Input sequence

CAGTCGAGT … … CGATGCG

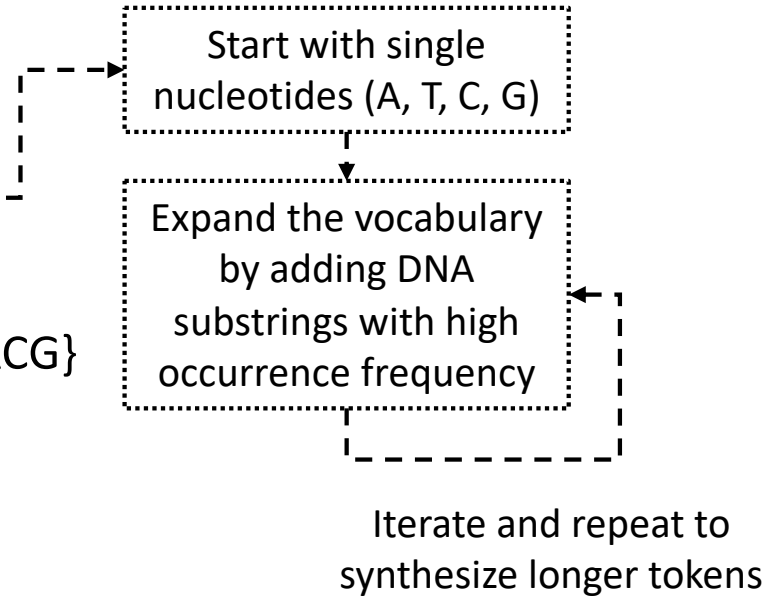
[CAG] [TCGA] [GT] …

Token index *Paddings*

34	124	67	11	… …	0	0

Build the BPE vocabulary

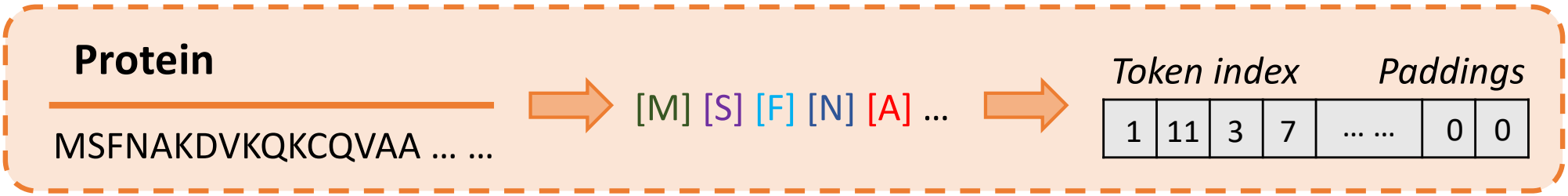
Iteration	Sequence	BPE Vocabulary
0	A C A C G A C G T	{A, C, G, T}
1	AC AC G AC G T	{A, C, G, T, AC}
2	AC ACG ACG T	{A, C, G, T, AC, ACG}
⋮	⋮	⋮



Count the most frequent nucleotide combinations.

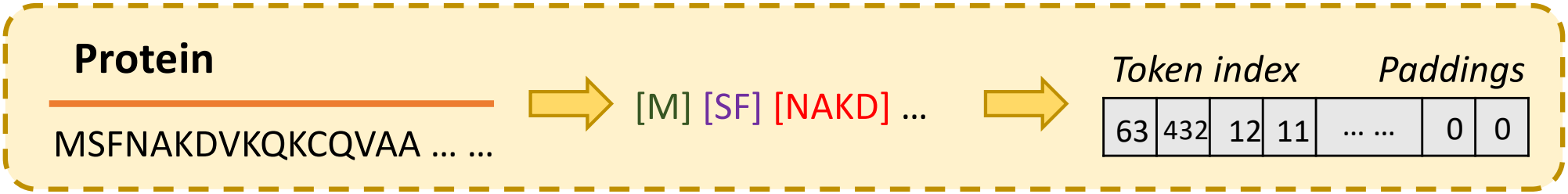
Easy to implement, but the encoded vector of the input sequence is usually too long.

Amino acids tokenizer



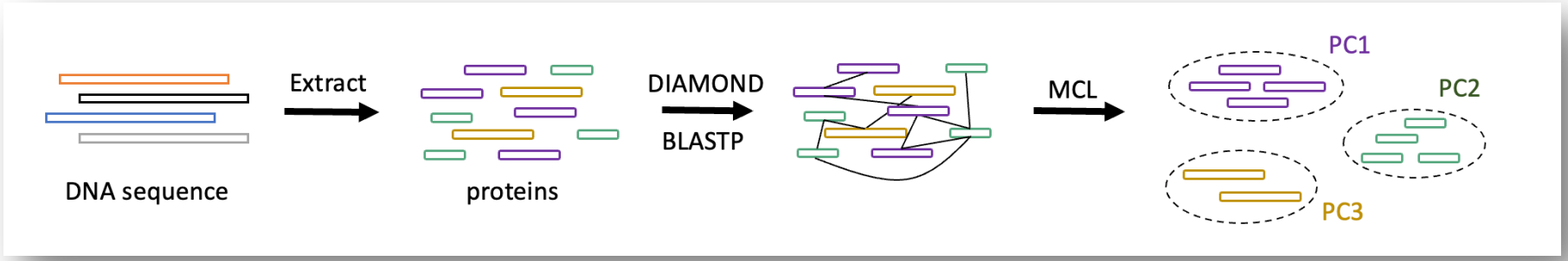
20 standard amino acids (*A, R, N, D, …*), undefined amino acid (*X*), and other amino acids (*OTHER*).

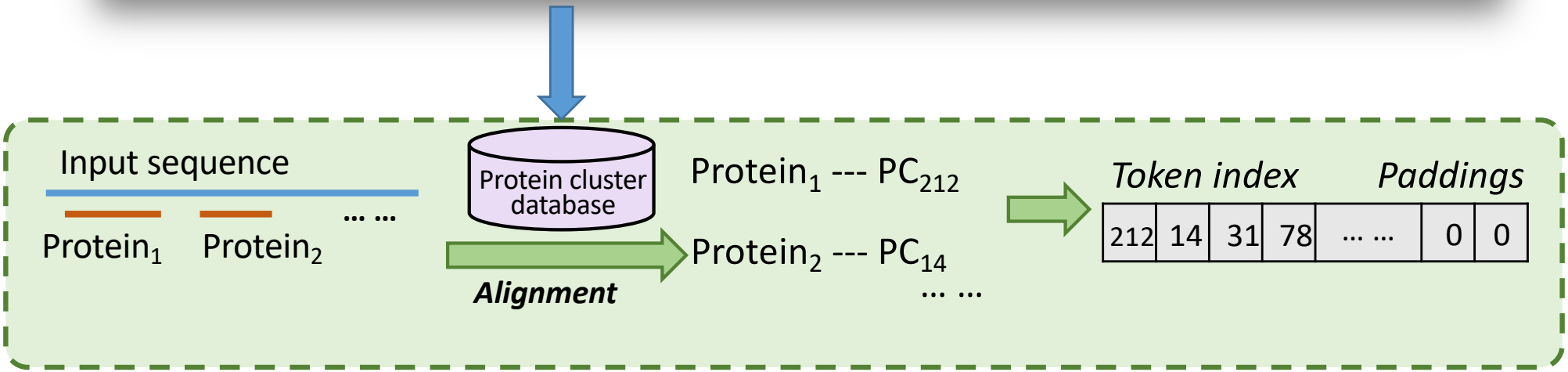
Amino acids byte pair encoding tokenizer



Amino acids BPE vocabulary: *count the most frequent amino acid combinations.*

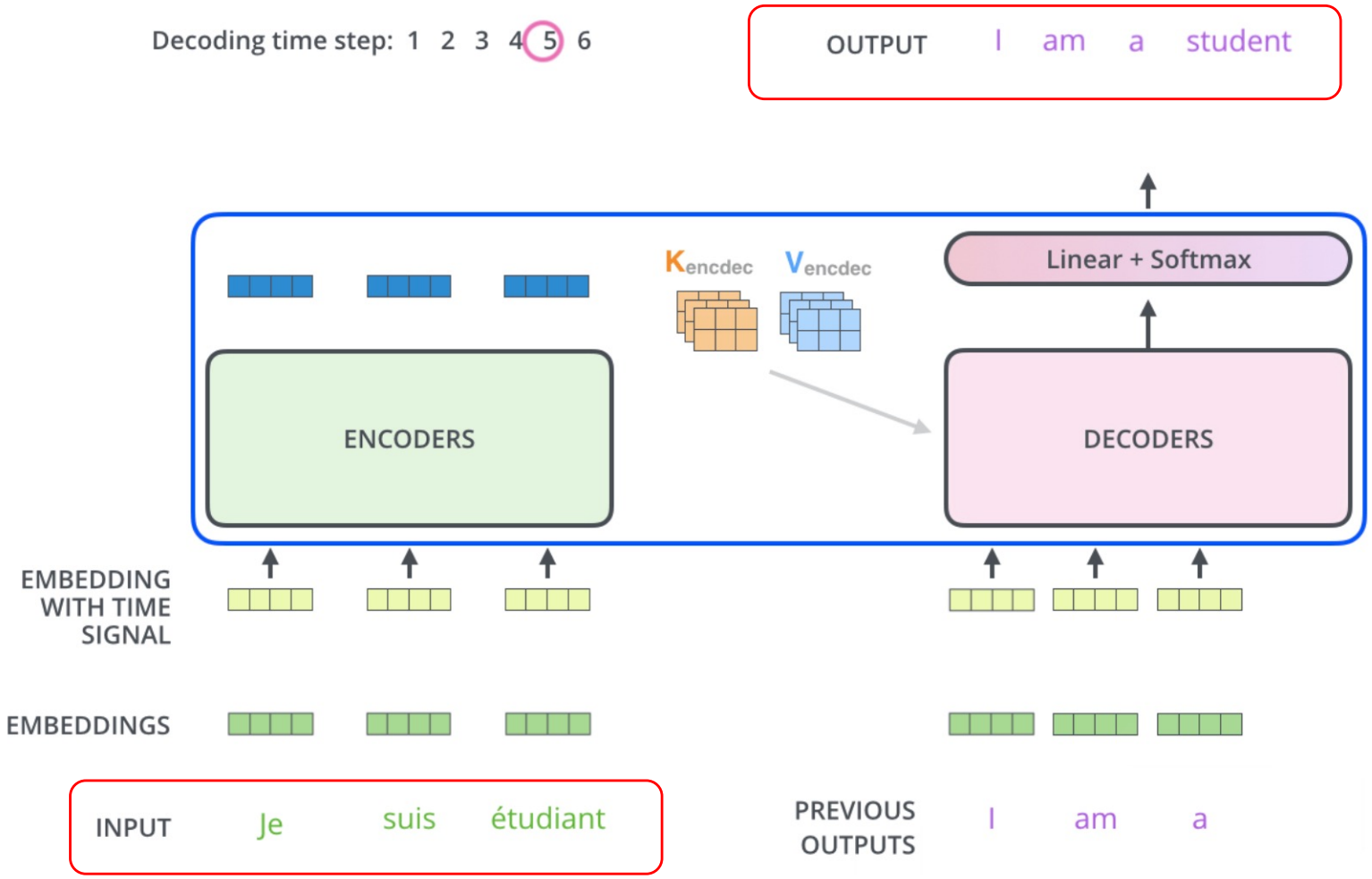
Protein cluster (PC) tokenizer





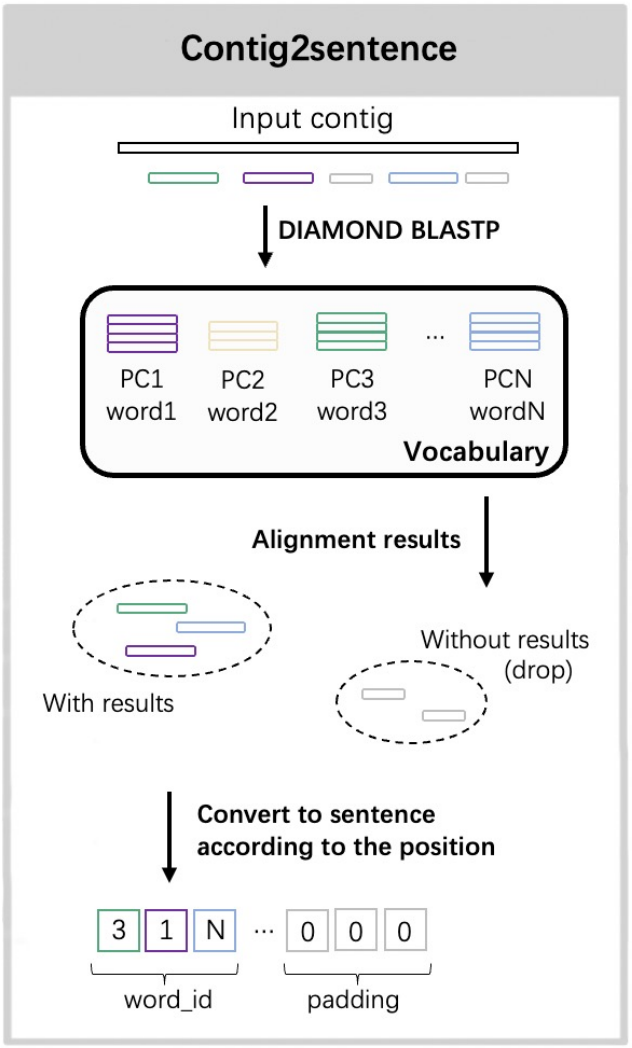
- Learn the importance of proteins
- Capture the correlation between different proteins

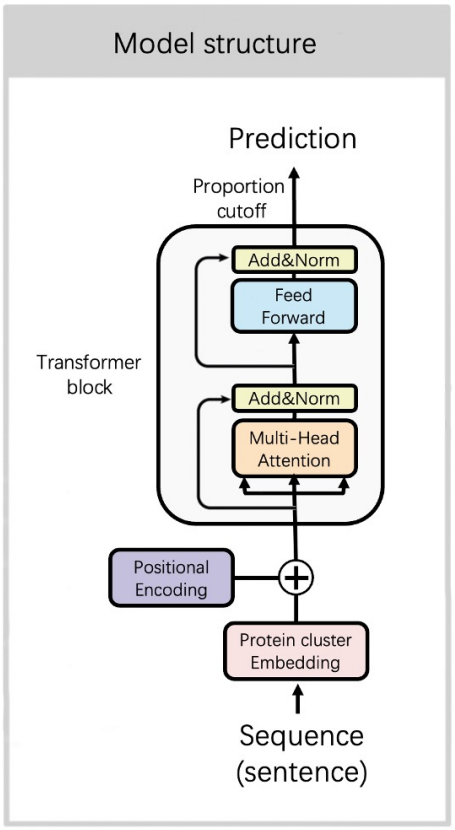
Transformer for translation



https://jalammar.github.io/illustrated-transformer/

Phage Identification with Transformer



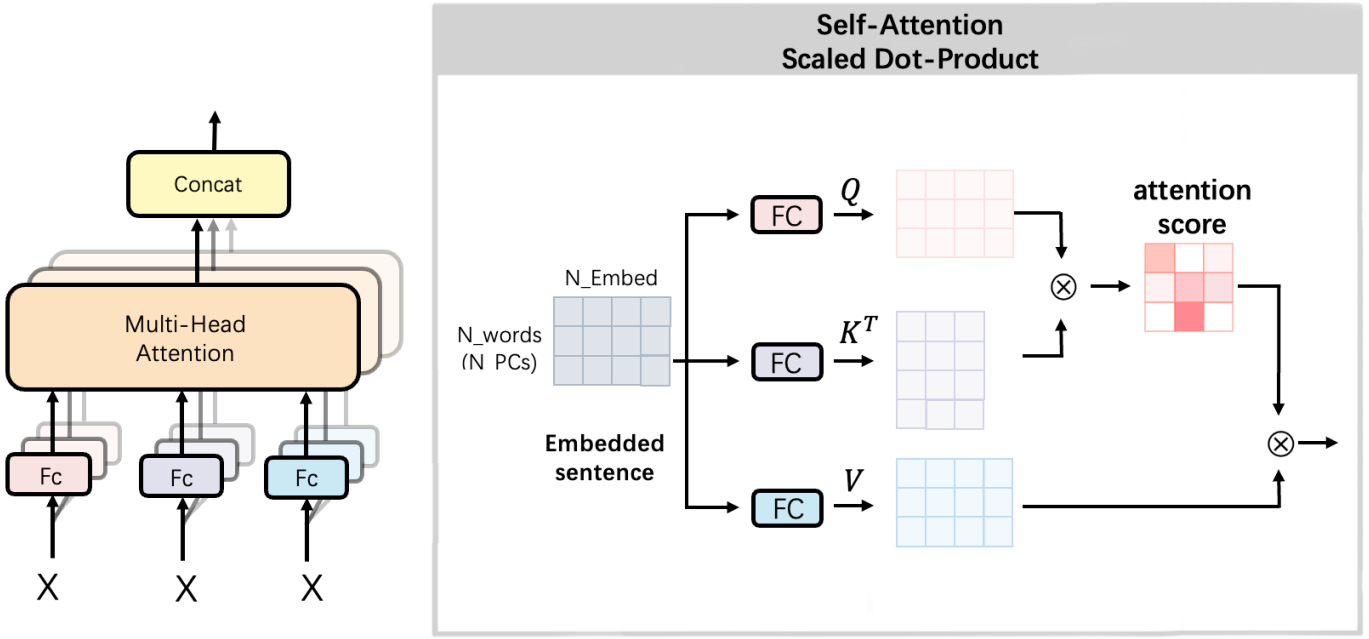


- Learn the importance of the PCs
- Learn the association between proteins

Multi-head attention:

- Learn the meaning of the word
- Learn the correlation between word

Phage Identification with Transformer



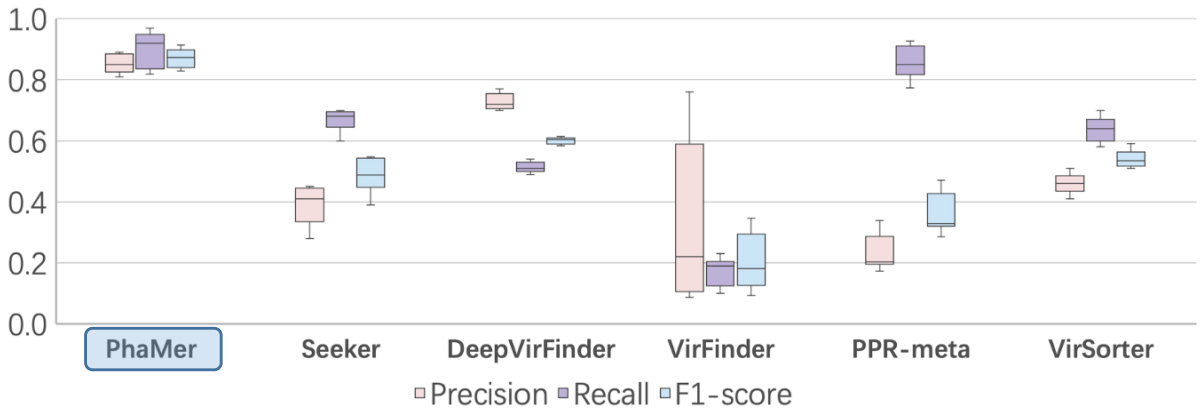
- The embedding of the protein sentence will be fed into multi-head attention module
- Value in the attention score matrix represents the strength of associations between two proteins

Phage Identification – Experimental results

Dataset

- Using phages as positive sample and their host as negative samples
  - May share common regions
- Testing on several independent datasets:
  - Low-similarity data
  - Mock dataset
  - IMG/VR database

Results

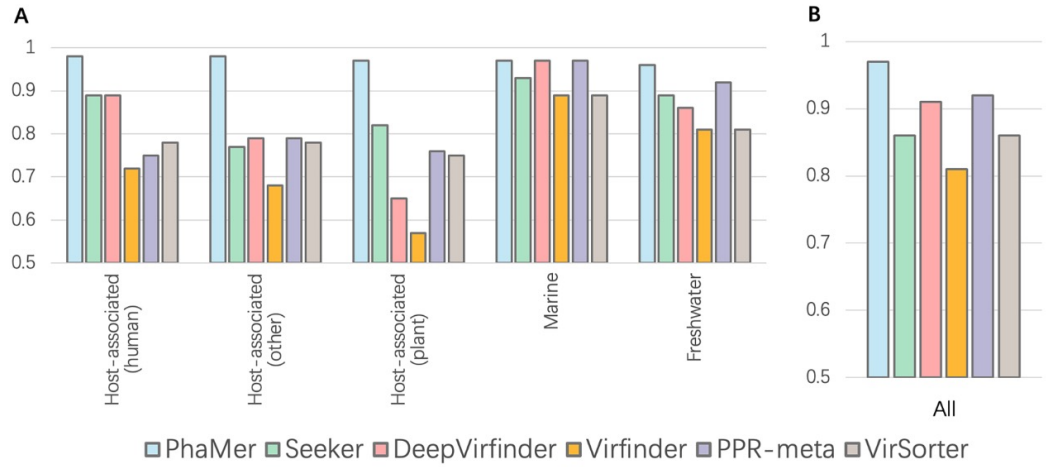


Mock dataset (European Nucleotide Archive PRJEB19901, ~30 species/strains)

Phage Identification – Experimental results

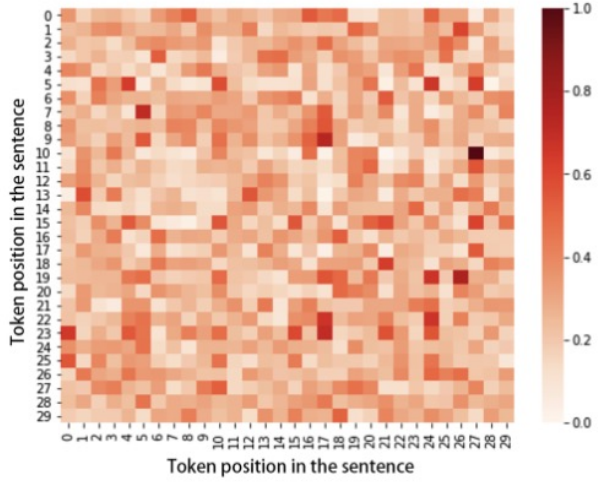
Results

Y-axis: recall



IMG/VR database

Visualization



Attention score matrix in Transformer

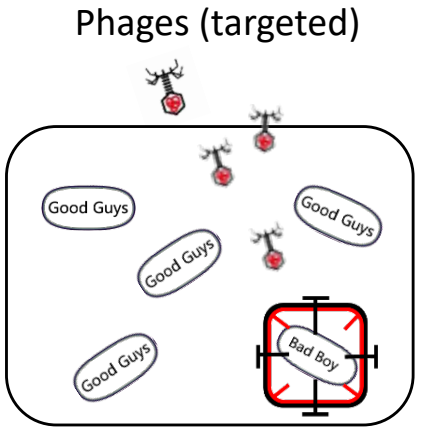
- Attention score matrix in Transformer

  The high score are the PCs contains structural protein (tail fiber/baseplate/…)

Host Prediction using GCN

Problem formulation

- Given a phage sequence, identify its bacterial hosts
  - Hosts' strains, species, genus, family etc.



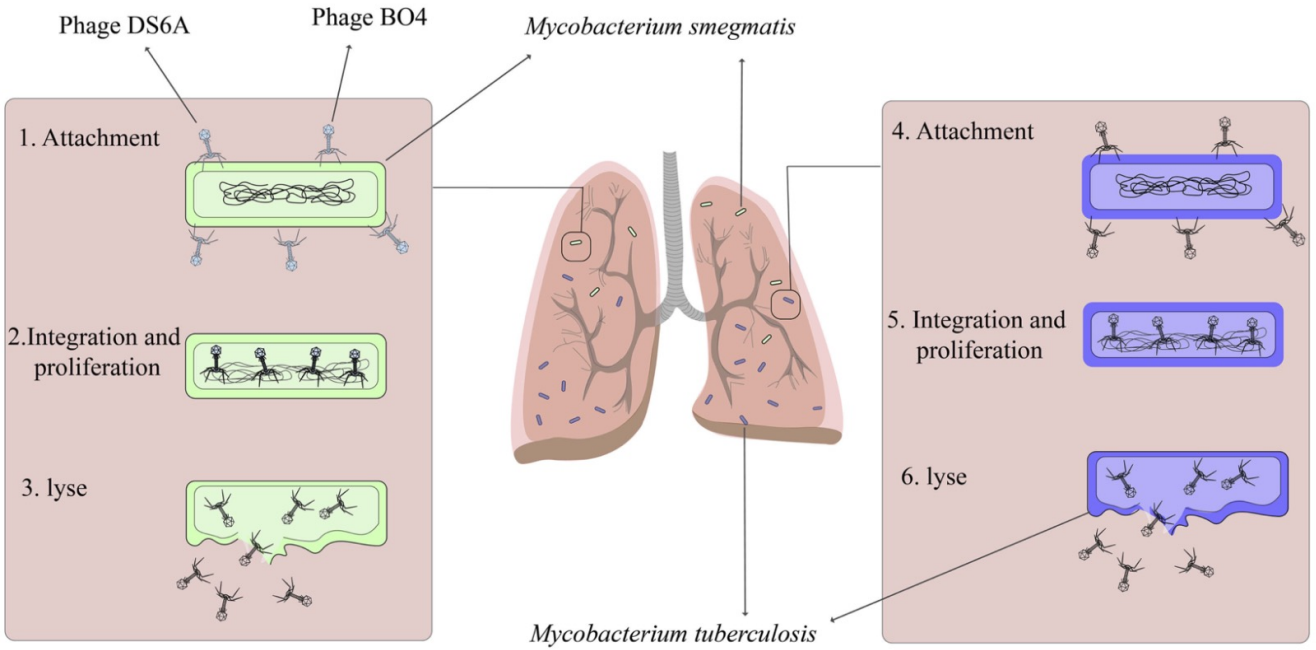
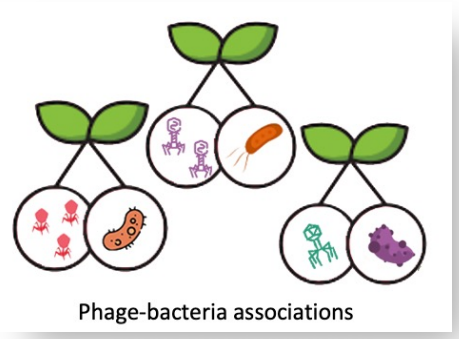


Figure 1 Steps involved in phage mediated *Mycobacterium tuberculosis* lysis using *Mycobacterium smegmatis*.

Azimi, T., Mosadegh, M., Nasiri, M. J., Sabour, S., Karimaei, S., & Nasser, A. (2019). Phage therapy as a renewed therapeutic approach to mycobacterial infections: a comprehensive review. *Infection and drug resistance*, *12*, 2943.

Host prediction: challenges

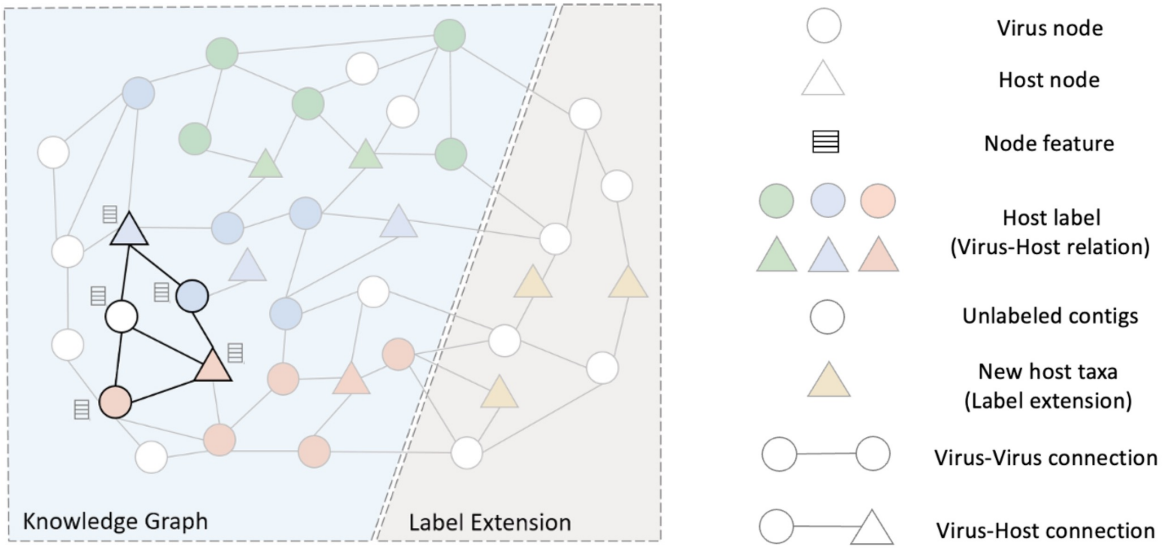


- Lack of known virus-host interactions
  - The number of known interactions dated up to 2020 only accounted for ~40% (1,940) of the phages at the NCBI RefSeq
  - Among the 60,105 prokaryotic genomes at the NCBI RefSeq, only 223 kinds of species have annotated interactions
- Not all phages share common regions with their host genomes
  - ~24% phages do not have significant alignments (e-value < 1e-5) with their hosts

Semi-supervised learning

- Limited known phages and large sequencing data → semi-supervised learning
  - Training on both labeled (known phages) and unlabeled (test) sequences

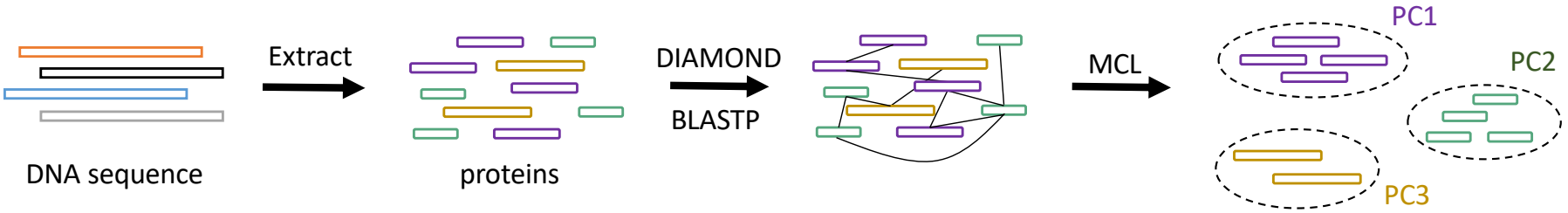
- Graph convolutional network (**GCN**)
  - Modeling the topological relationship between samples

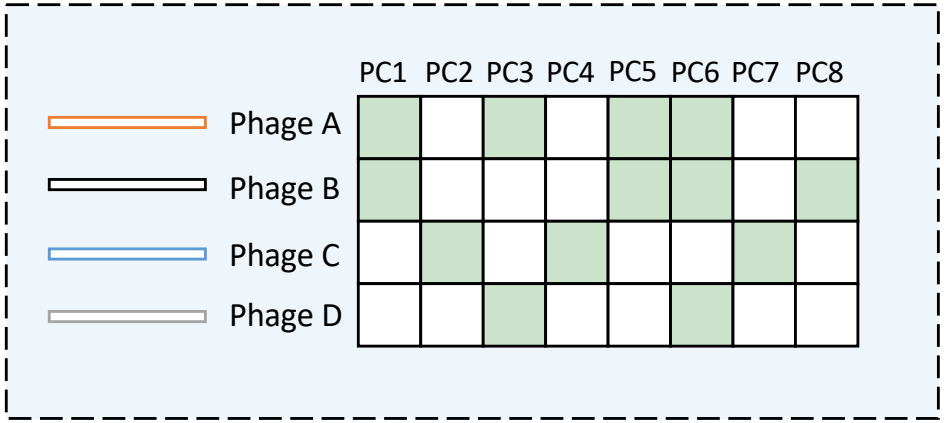


Knowledge graph

Edge construction I: virus-vs-virus

- **Similar protein organizations** -> might infect the same host
- **Protein cluster** construction using Markov Chain clustering (MCL)





Example (A and B):

$c = 3, n = 8, a = b = 4$

Share at least c protein clusters

$$P(X \geq c) = \sum_{i=c}^{\min(a,b)} \frac{C_a^i C_{n-a}^{b-i}}{C_n^b}.$$

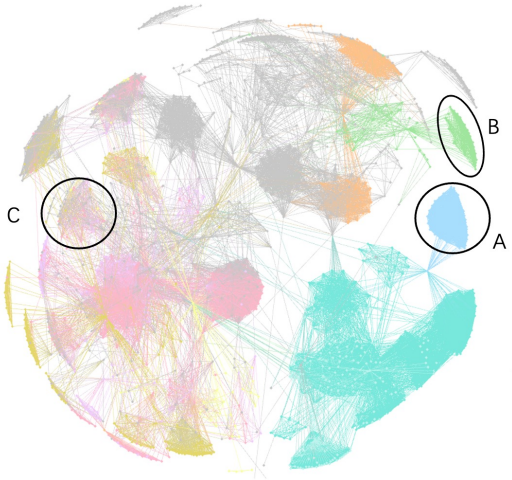
[1] Bolduc, Benjamin, et al. "vConTACT: an iVirus tool to classify double-stranded DNA viruses that infect Archaea and Bacteria." PeerJ 5 (2017)

Edge construction II

- Virus vs bacteria

$$virus\text{-}prokaryote = \begin{cases} 1 & \text{if } \exists \text{ CRISPR alignment} \\ & \text{or BLASTN } E_{value} < \tau_2 \\ & \text{or } \exists \text{ interaction in dataset} \\ 0 & \text{otherwise} \end{cases}$$

- Overview of the knowledge graph

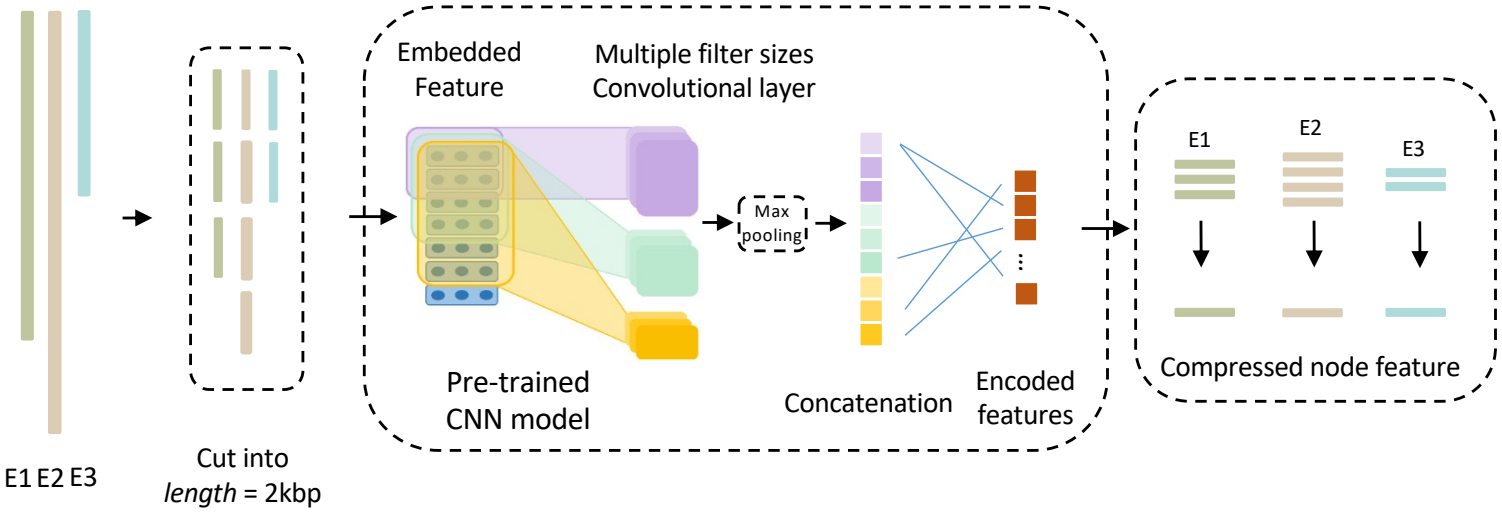


Host species	Proportion
Mycolicibacterium smegmatis	*21.23%*
Escherichia coli	*12.48%*
Pseudomonas aeruginosa	*7.33%*
Salmonella enterica	*5.50%*
Staphylococcus aureus	*4.48%*
Lactococcus lactis	*4.21%*
Cutibacterium acnes	*4.21%*
Vibrio cholerae	*2.65%*

Node feature encoding

Capture **motif-related** patterns from the DNA sequences

- Different **filter sizes** -> different **length** of motifs



Multiple filter sizes CNN structure

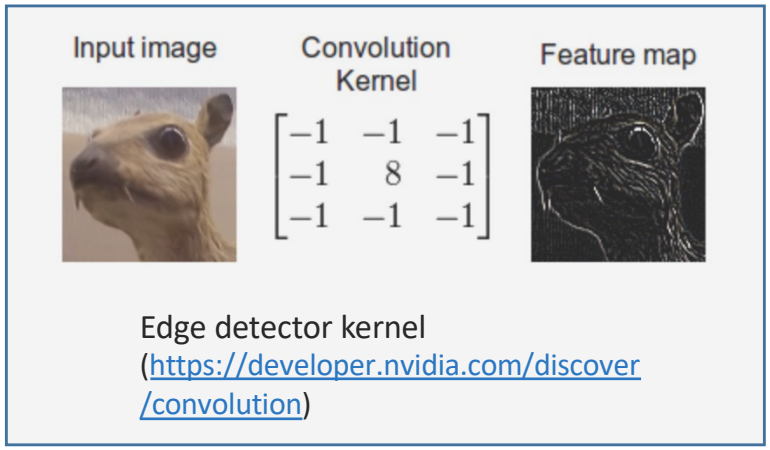
Motifs and convolution filters in CNN

- Conserved sequence patterns:
  - important features for genomic sequence classification
  - Can be represented by convolution filters

One hot encoding:

AACG →

A	1	1	0	0
C	0	0	1	0
G	0	0	0	1
T	0	0	0	0



Edge detector kernel (https://developer.nvidia.com/discover/convolution)

Zia, Amin & Moses, Alan. (2012).

Graph Convolutional Neural Network

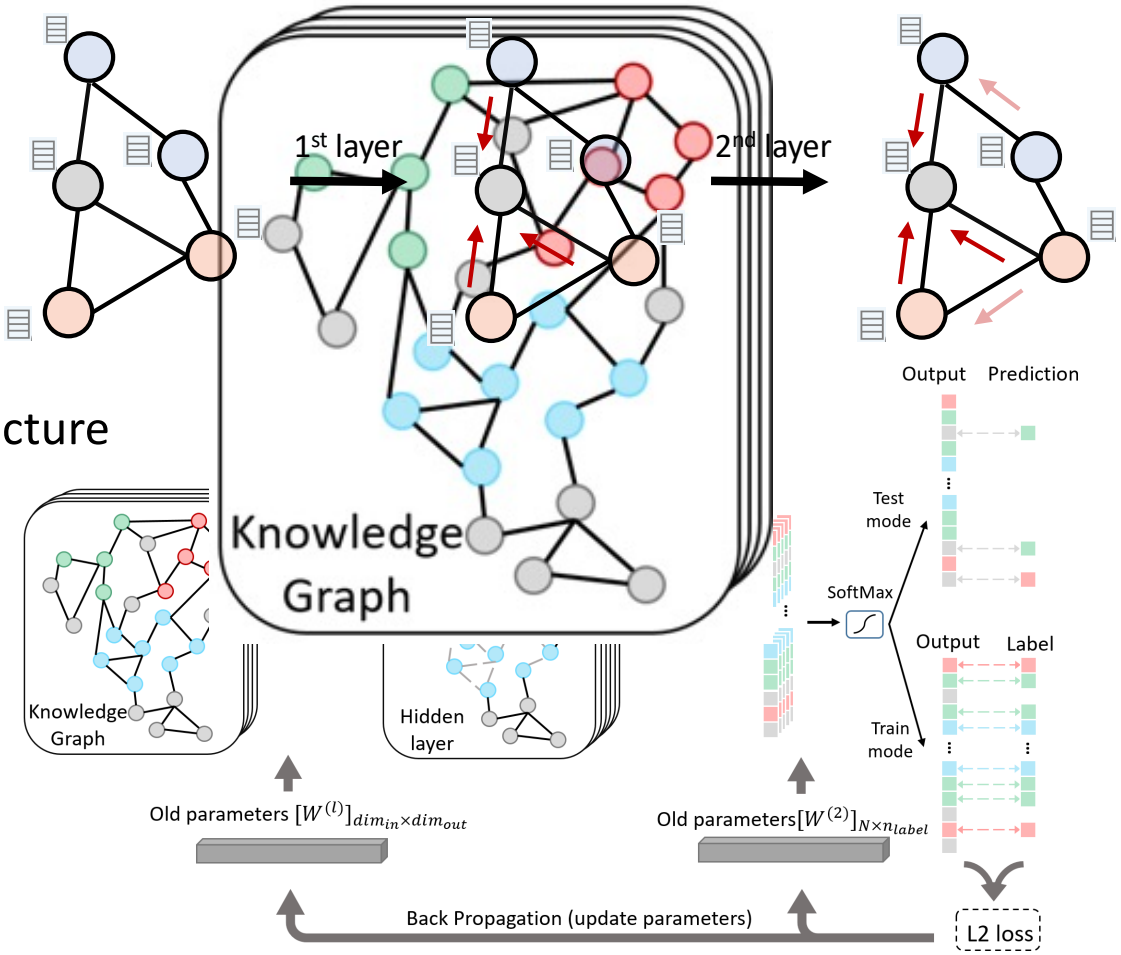
Key insight

$$H^{(l+1)} = ReLU(\tilde{D}^{-\frac{1}{2}}\tilde{G}\tilde{D}^{\frac{1}{2}}H^{(l)}W^{(l)})$$

$$Out = SoftMax(H^{(2)}W^{(2)})$$

- Using features from neighborhood

Structure

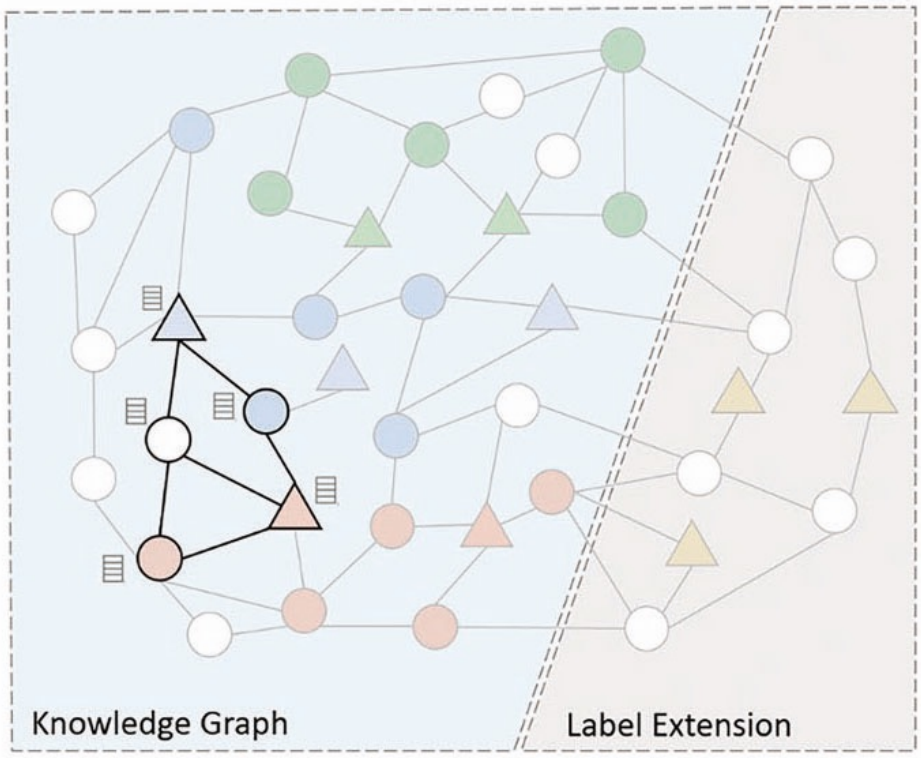


Graph convolutional neural network structure

Adapted Improvement 1

Graph extension

- Connecting the new bacteria by adding nodes and edges
- Training with the bacteria nodes (new labels)

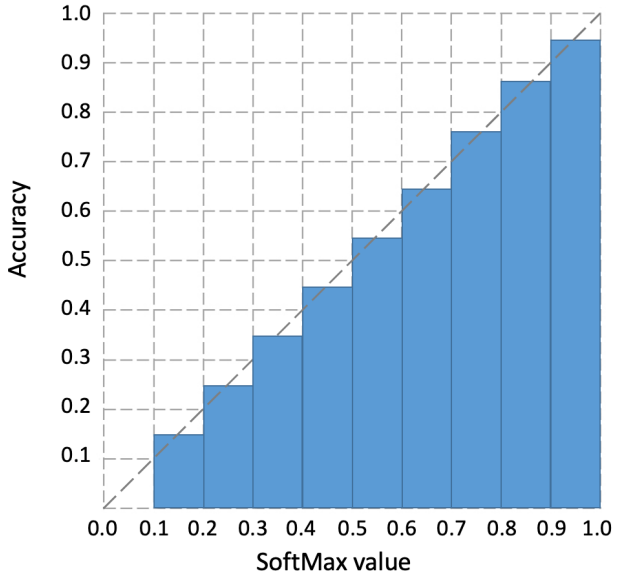


Label extension

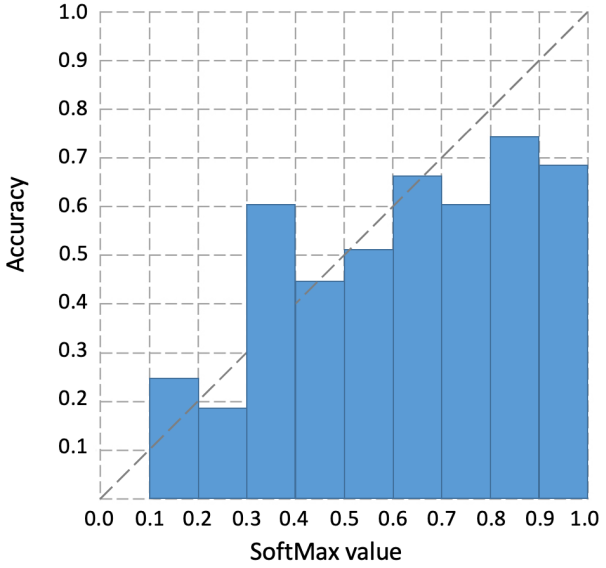
Adapted Improvement 2

How to represent confidence of the predictions

What we want



The real case



High SoftMax value ≠ High confidence

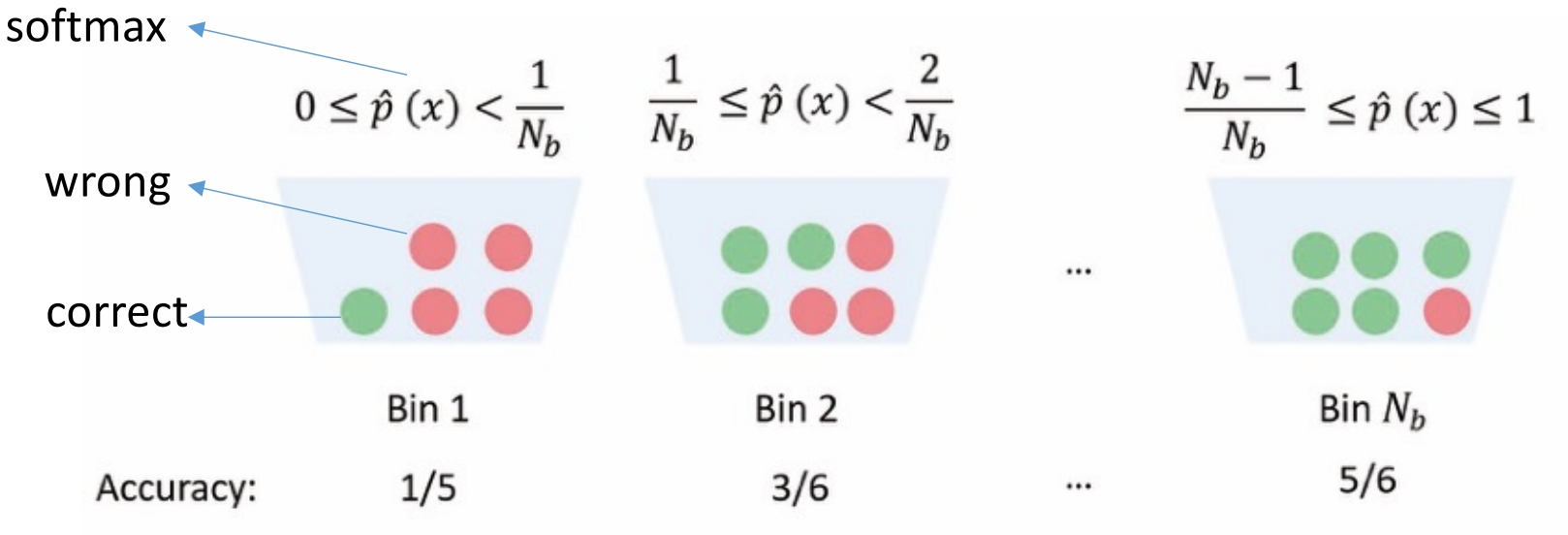
Adapted Improvement 2

- Expected calibrated error (ECE)
  - Divided the confidence score into several bins
  - Minimize $\|accuracy - confidence\|$ in each bin

$$\mathcal{L} = ECE + L2$$

$$ECE = \sum_i^{N_b} \frac{T_i}{T} |Acc_i - conf_i|$$

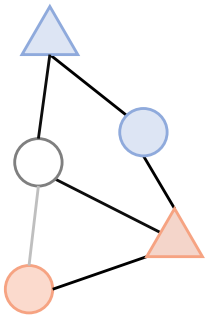
$$conf_i = \frac{\sum_j^{T_i} \hat{p}(x_{ij})}{S_i}$$



Theory of expected calibrated error (ECE)

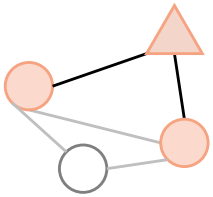
Hard cases for alignment-based methods

A) Has alignment results with bacteria in different taxa



269/656 phages have alignments with bacteria in different taxa at order level; 566/656 at family level; 656/656 at genus level.

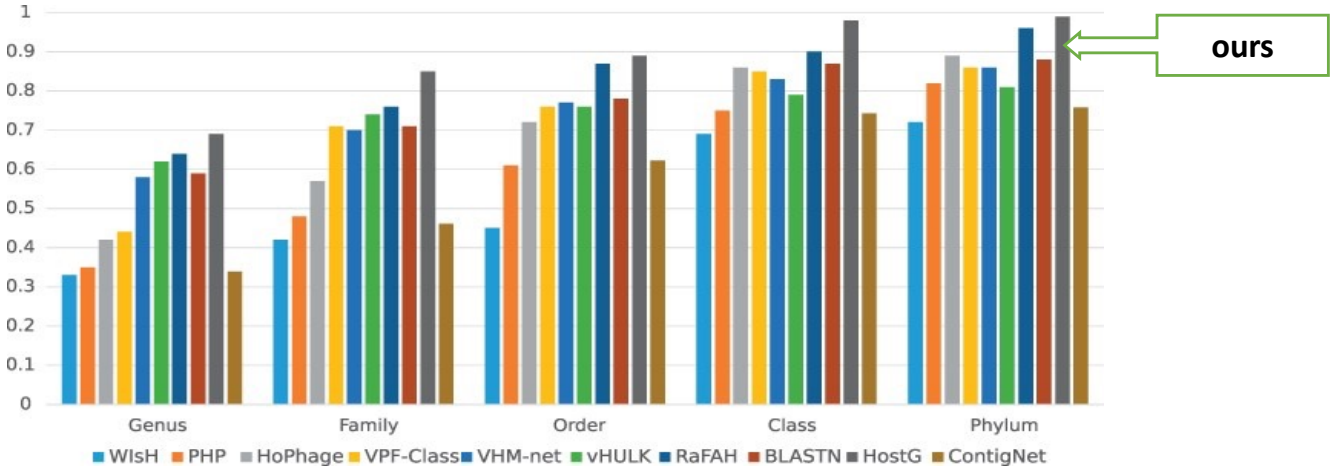
B) Has no alignment result with bacteria



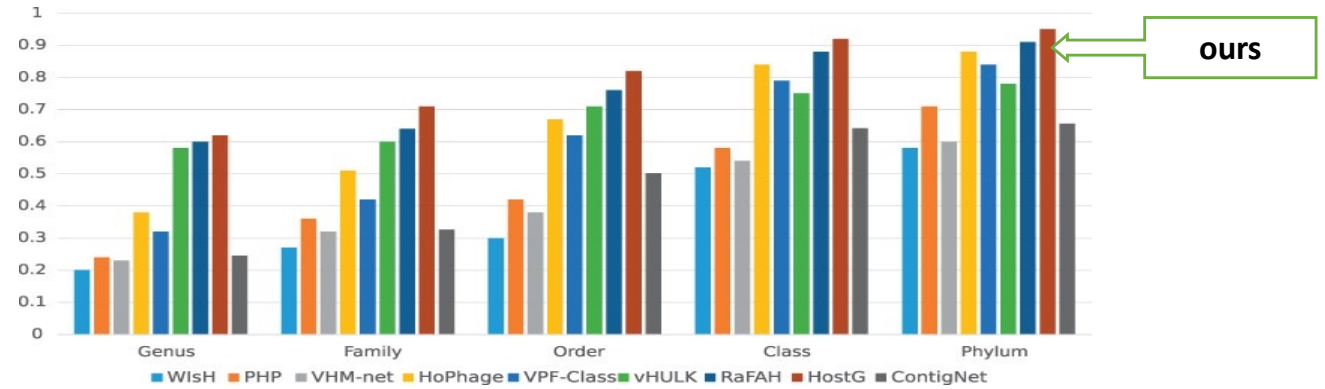
770/1426 phages have no alignment

Third-party evaluation

Host prediction accuracy for whole genomes from genus to phylum



Host prediction accuracy for whole genomes without alignment results from genus to phylum

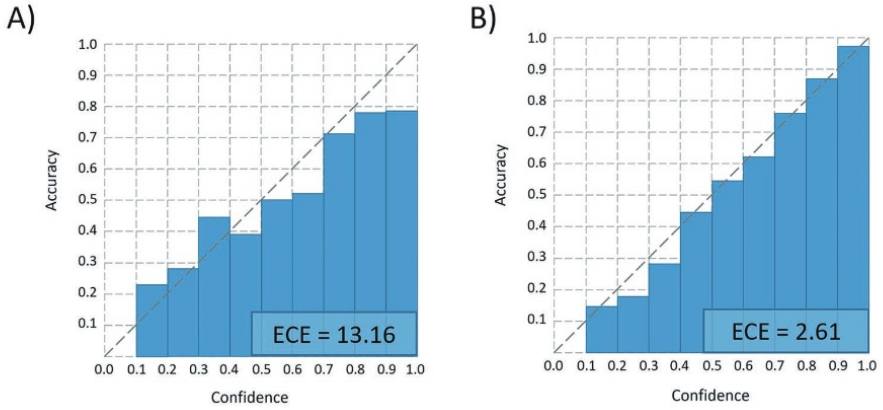


Bioinformatics, Volume 38, Issue Supplement_1, July 2022, Pages i45–i52, https://doi.org/10.1093/bioinformatics/btac239

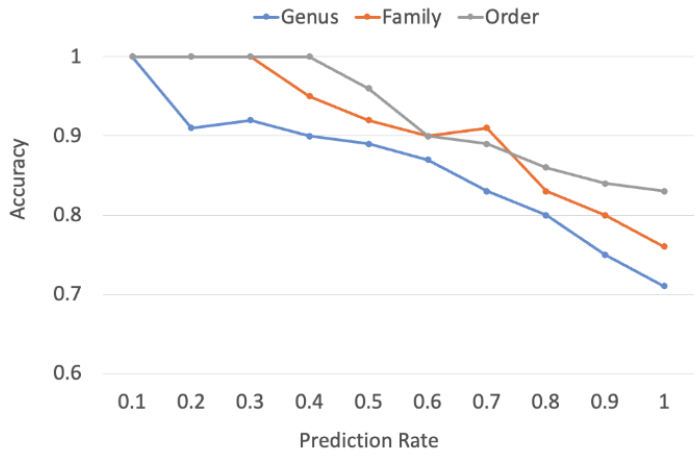
Experimental results

Improvement with ECE

- Accuracy vs. confidence (SoftMax value)



- Prediction with confidence

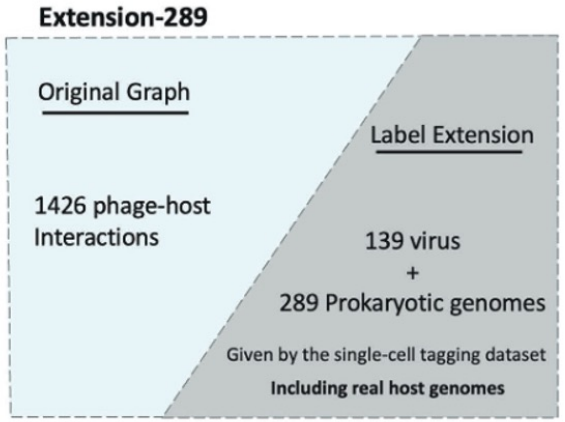


Experimental results

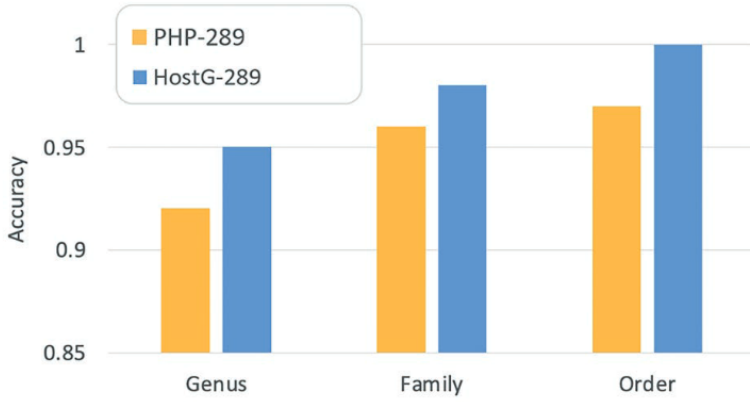
—— Single-cell viral tagging using a human stool sample

Improvement with label extension

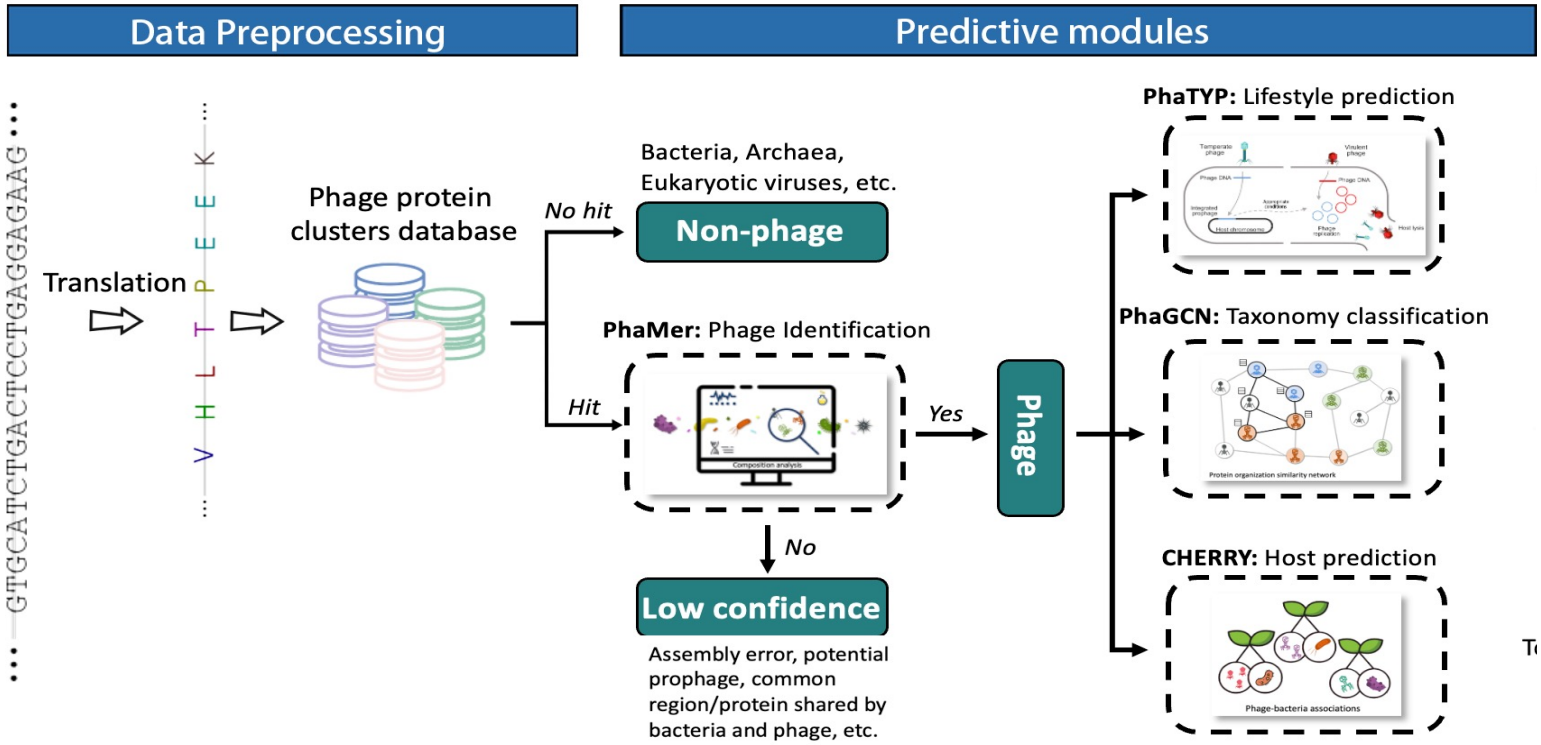
- Extension of the knowledge graph



- Performance of the label extension



PhaBOX

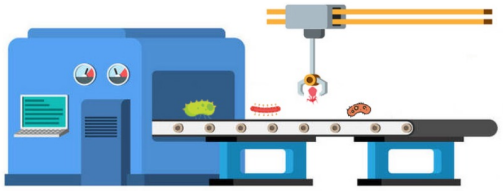


https://phage.ee.cityu.edu.hk/

Our web server

Our tools for phage sequence analysis

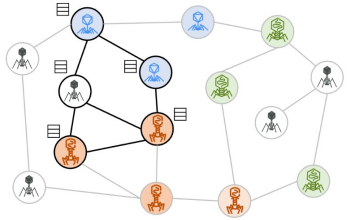
PhaMer



PhaMer is a python library for identifying bacteriophages from metagenomic data. PhaMer is based on a Transorfer model and rely on protein-based vocabulary to convert DNA sequences into sentences.

https://github.com/KennthShang/PhaMer

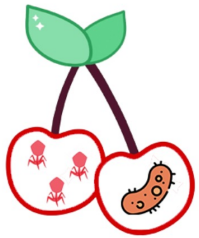
PhaGCN



PhaGCN is a GCN based model, which can learn the species masking feature via deep learning classifier, for new Phage taxonomy classification. To use PhaGCN, you only need to input your contigs to the program.

https://github.com/KennthShang/PhaGCN

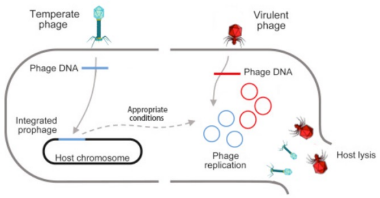
CHERRY



CHERRY is a python library for predicting the interactions between viral and prokaryotic genomes. CHERRY is based on a deep learning model, which consists of a graph convolutional encoder and a link prediction decoder.

https://github.com/KennthShang/CHERRY

Phage TYPE



PhaTYP is a python library for bacteriophages' lifestyle prediction. PhaTYP is a BERT-based model and rely on protein-based vocabulary to convert DNA sequences into sentences for prediction.

https://github.com/KennthShang/PhaTYP

CityU

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Acknowledgement

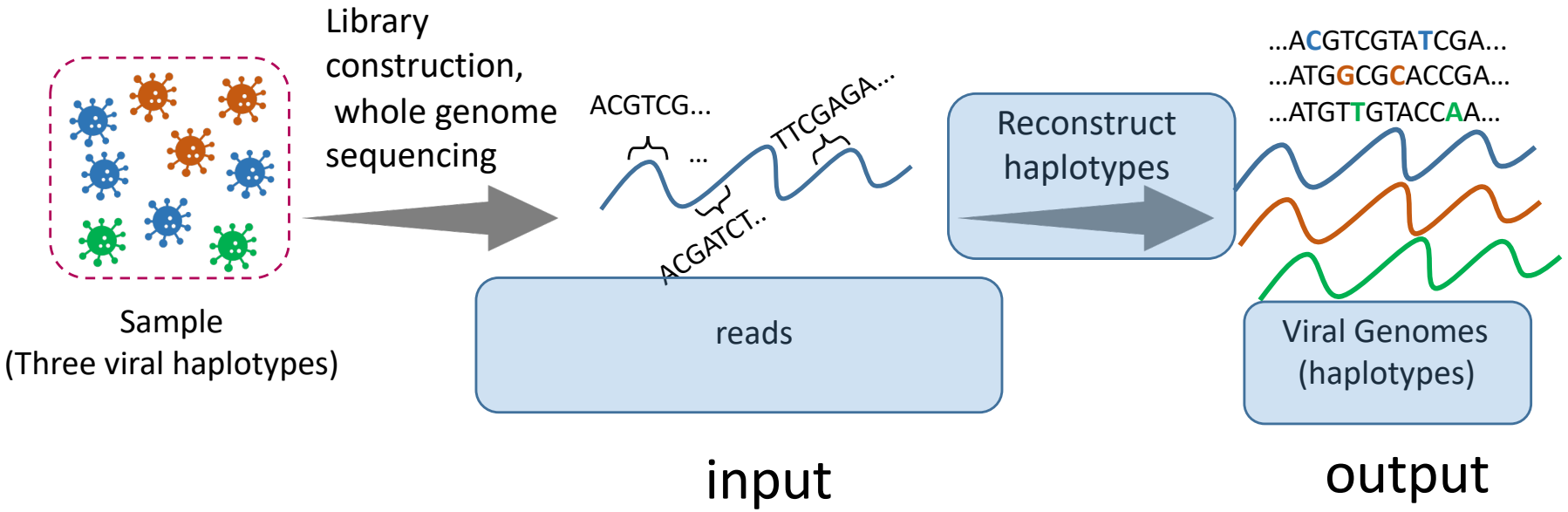
Funding: HKIDS, GRF, ITF, and City University of Hong Kong

Hong Kong Science Museum

Questions?

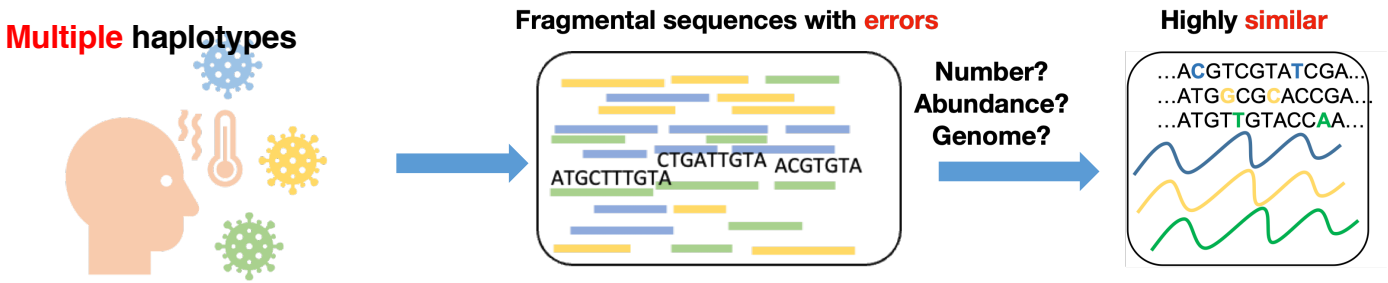
For more information, please visit the lab website:
https://yannisun.github.io/

Viral haplotype characterization using TGS

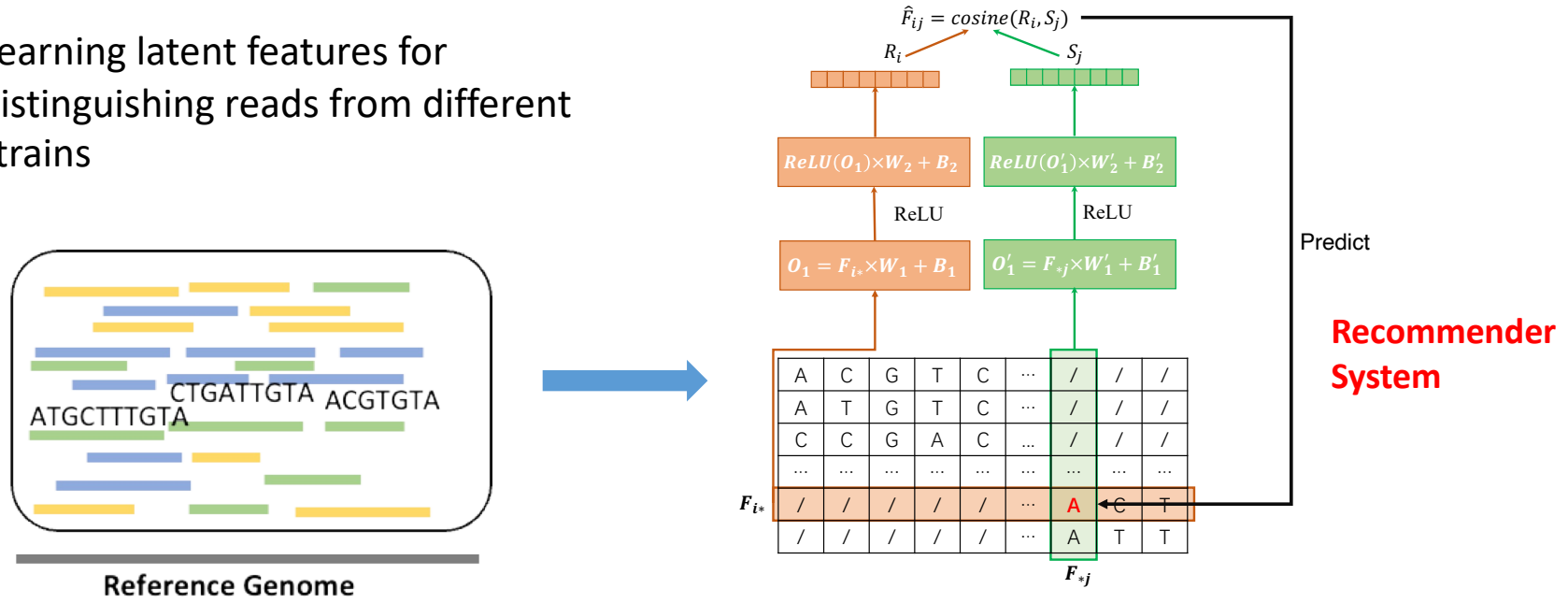


Generic assembly tools cannot produce all haplotypes

Viral haplotype reconstruction from TGS sequencing data



Learning latent features for distinguishing reads from different strains



Recommender System

Dehan Cai, Jiayu Shang, Sun Yanni. HaploDMF: viral Haplotype reconstruction from long reads via Deep Matrix Factorization. *Bioinformatics*. 2022; 29:btac708.

Dehan Cai & Yanni Sun. Reconstructing viral haplotypes using long reads. *Bioinformatics*. 2022; btac089.