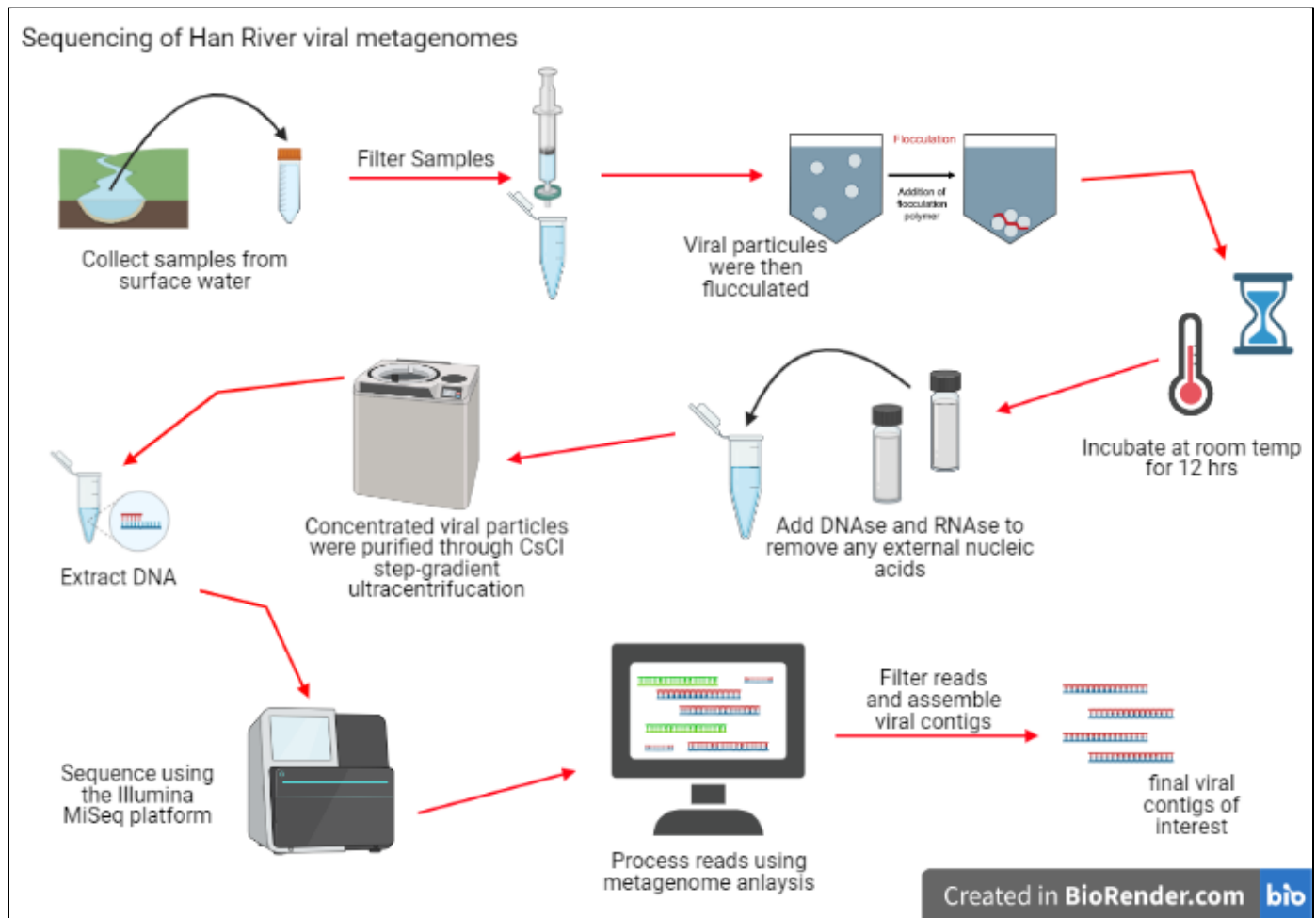
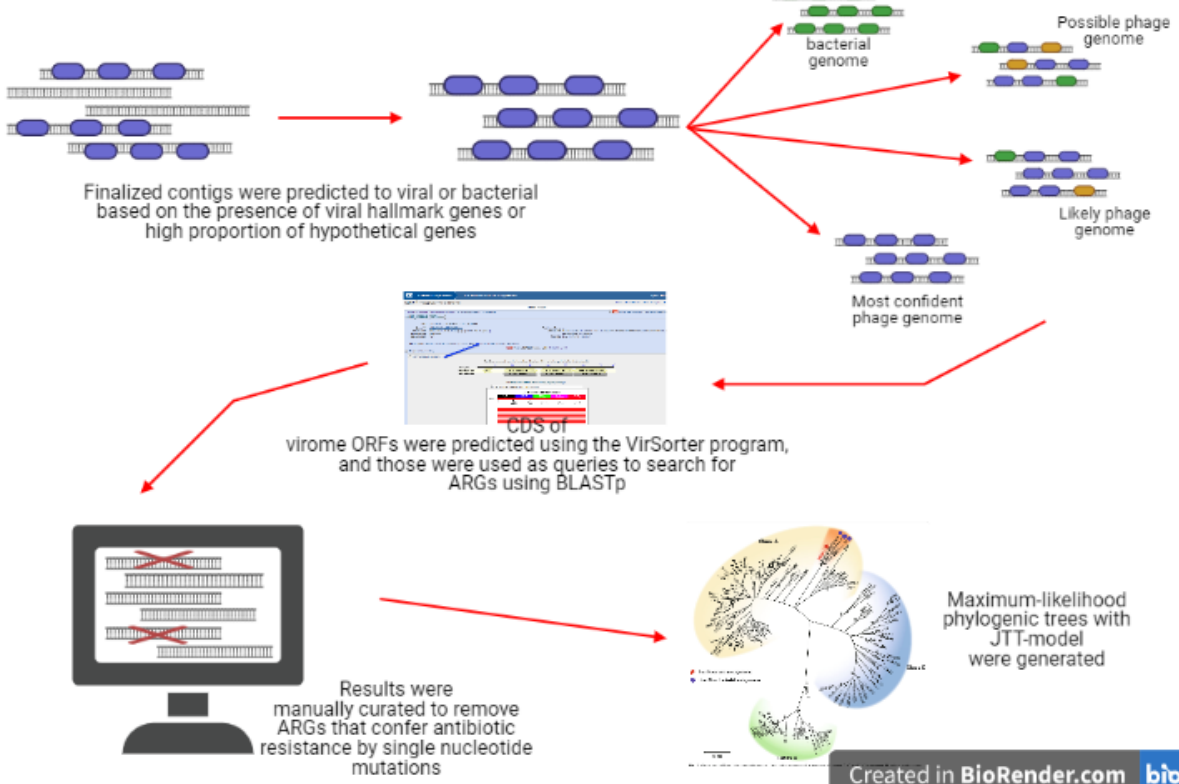


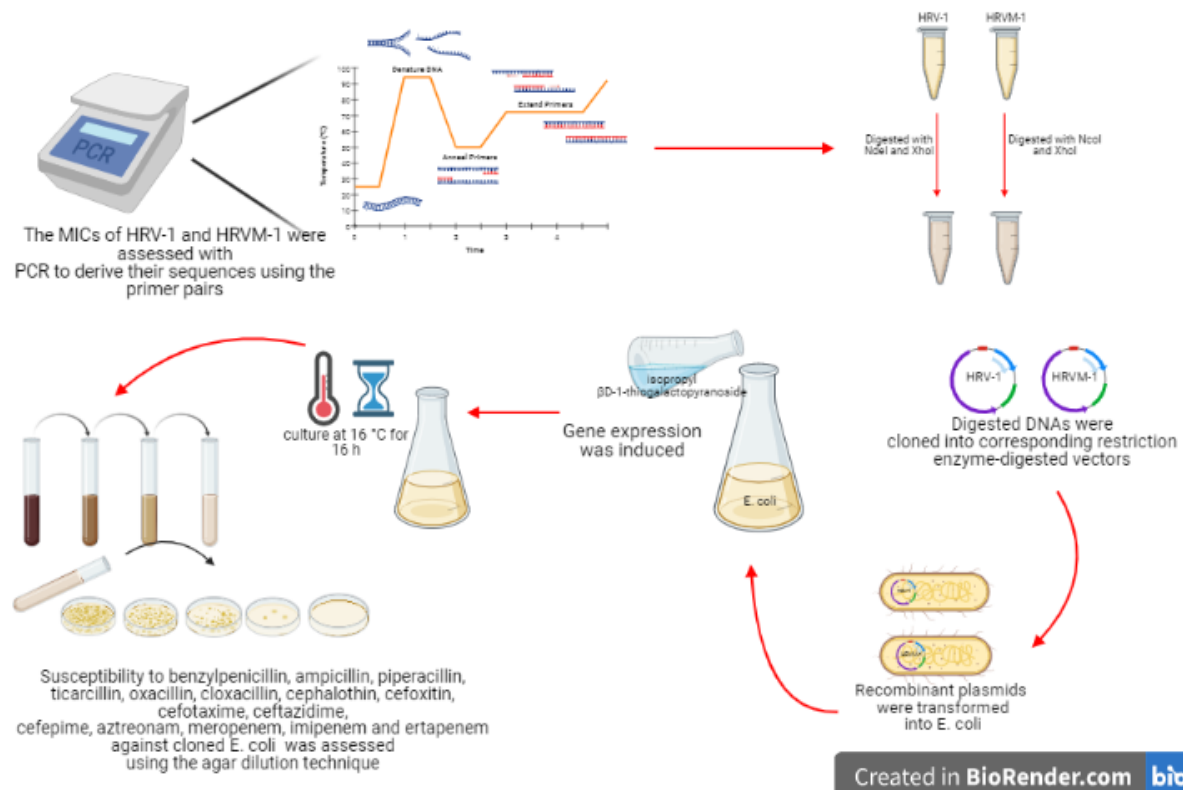
## Read and Annotate the Methods and Results:



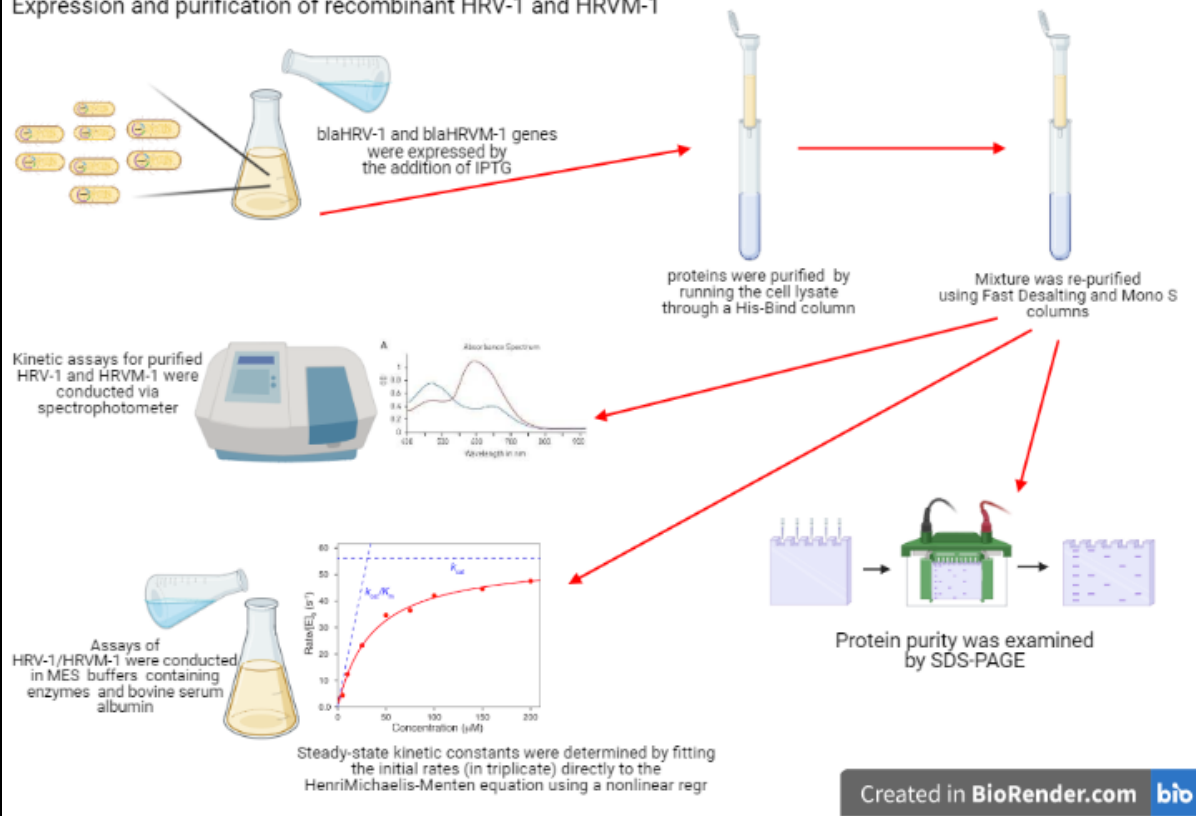
### Antibiotic resistance gene search and sequence analysis



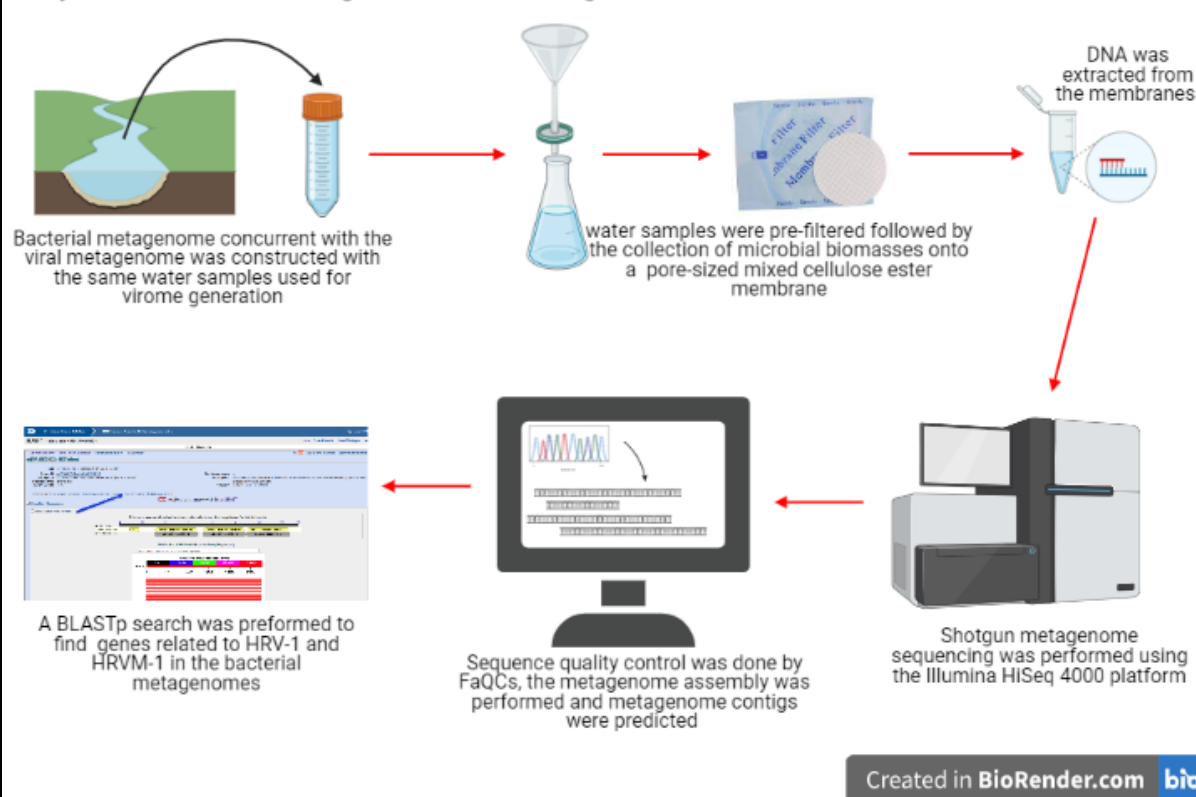
### Cloning and antimicrobial susceptibility tests of HRV-1 and HRVM-1 genes



## Expression and purification of recombinant HRV-1 and HRVM-1



## Analysis of HRV-1 and HRVM-1 genes in bacterial metagenomes



## **Renaming the Figures/Summary of Each**

Figure 1: Graphical display of the taxonomic distribution of viral metagenome reads collected from six study sites on the Han River in South Korea

Figure 2: Sequence maps of the four viral contigs, named H4-C441, H1-C74, H4-C244, and H4-C367, all of which include  $\beta$ -lactamase genes HRV-1 or HRVM-1

Figure 3: Phylogenetic tree of HRV-1 gene with representative enzymes of classes A, C, and D  $\beta$ -lactamases which groups HRV-1 into class A

Figure 4: Phylogenetic tree of HRVM-1 gene with representative enzymes of subclasses B1, B2, and B3 and which groups HRVM-1 into subclass B3

Figure 5: Minimum inhibitory concentrations (MICs) of  $\beta$ -lactams for *Escherichia coli* transformants expressing HRV-1 or HRVM-1 and in which the two strains show reduced susceptibility, ranging from 2- to 16-fold reductions, to the tested antibiotics

Figure 6: Genomic maps of the Han River bacterial metagenomic contigs that harbor homologous ORFs to the ARGs of interest HRV-1 or HRVM-1 with simplified sequence similarity shown

Annotated Figures can be found in a separate pdf

# Figure 1

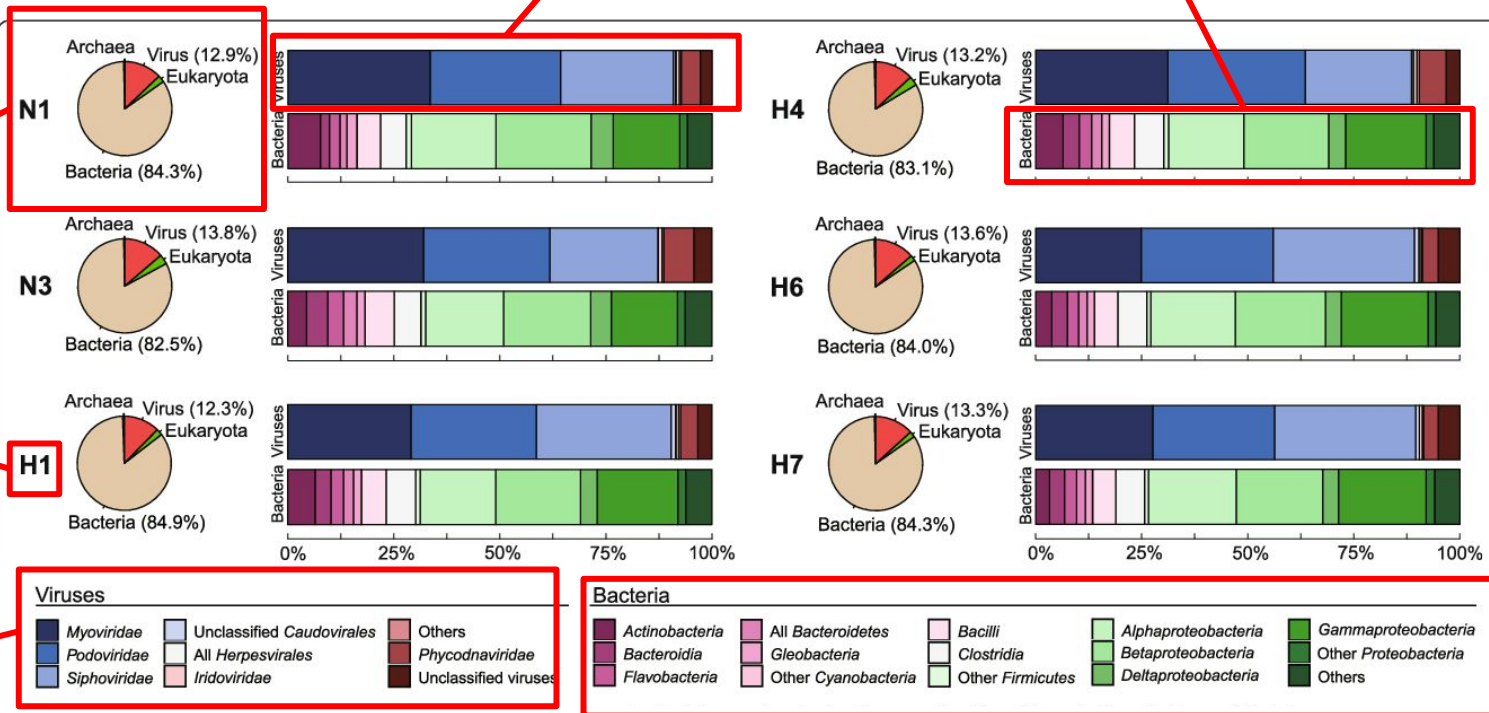
Each of the six samples are broken down into the percentage that belong to each domain and virus

The Han river flows from more pristine area (N1 and N3) to an urbanized area (H1, H4, H6, and H7)

Different virus families found in samples

Viral taxonomy distribution is described at the family level

bacterial taxonomy distribution is described at the class level



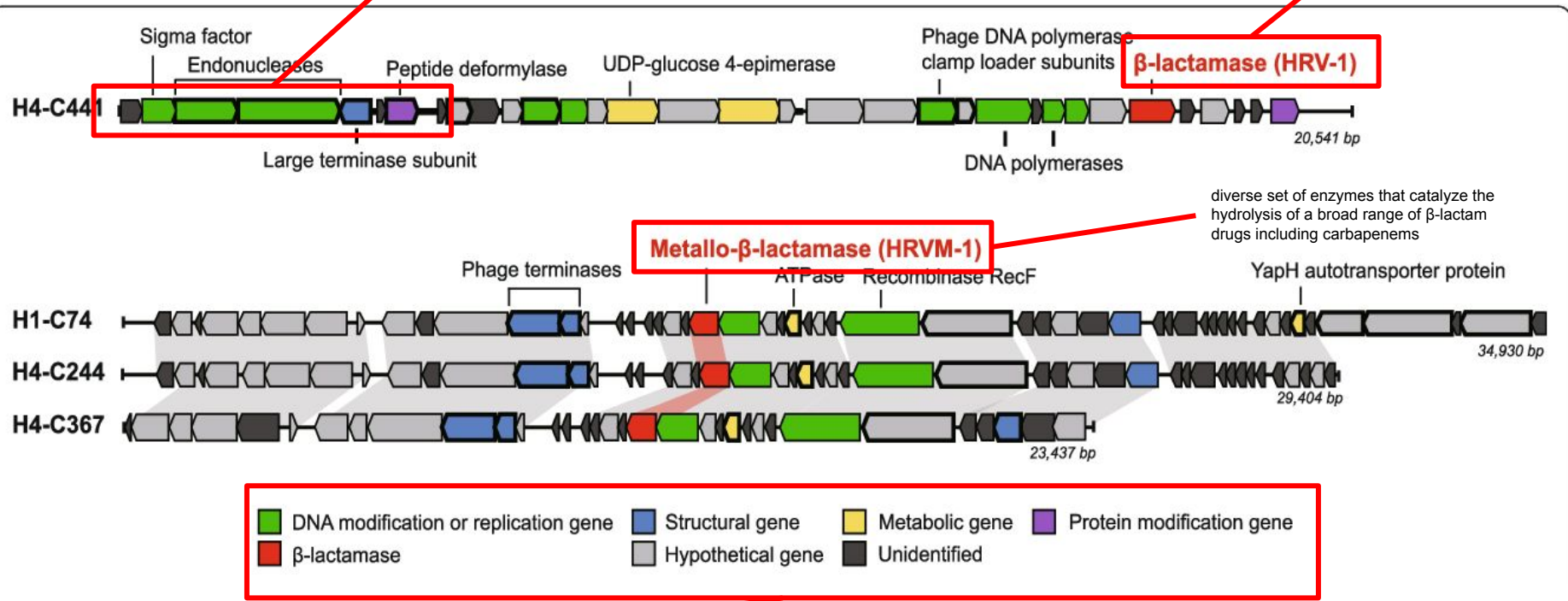
Different bacterial taxonomies found in samples

\*\*\* $\beta$ -lactamases, one of the widely found ARGs in diverse environments, were found in the 4 viral contigs shown in this figure

Class A (penicillinase-type) is the most common. The genes for class A beta-lactamases are widely distributed in bacteria, frequently located on transmissible plasmids in Gram-negative organisms, although an equivalent chromosomal gene has been found in a few species

# Figure 2

Conserved active sites common to class A



Labels used to annotate different parts of the viral contigs

# Figure 3

H4-c441-ORF28



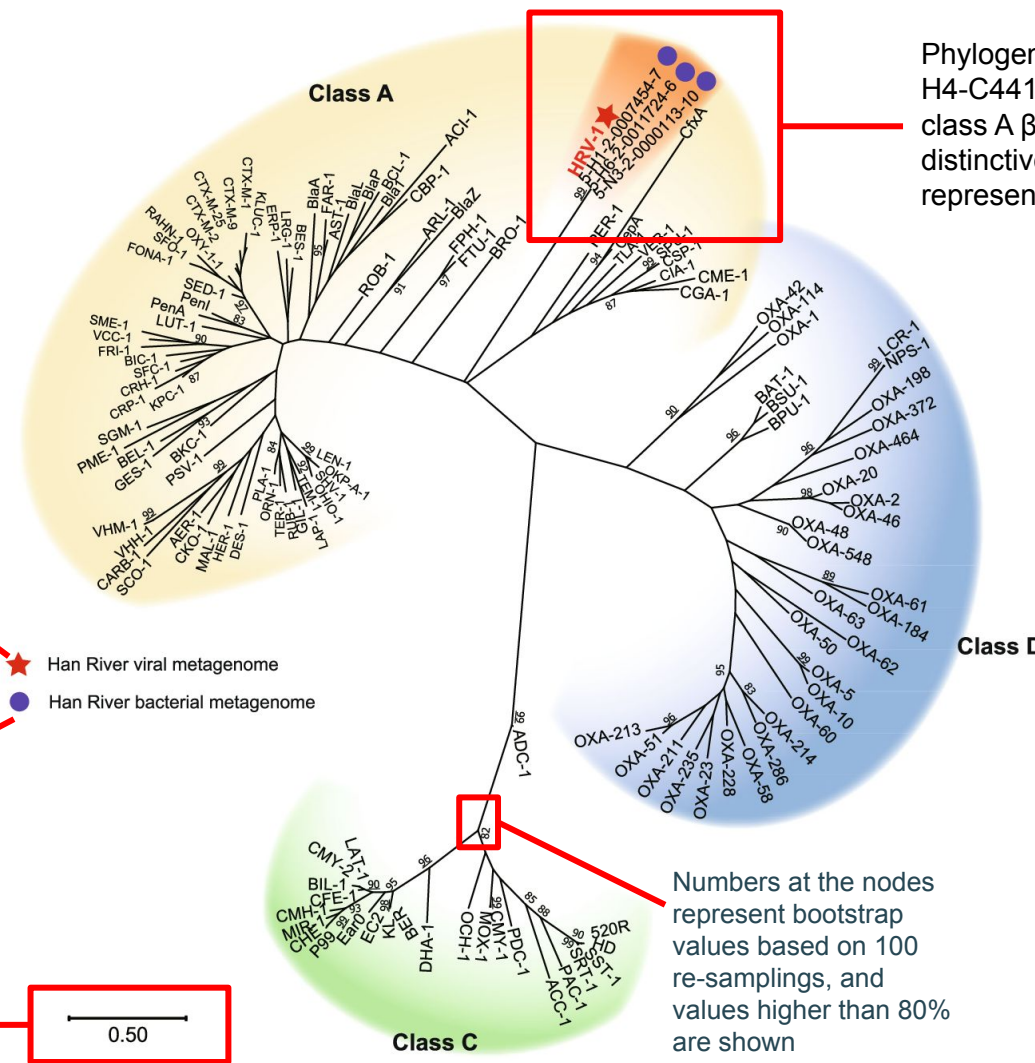
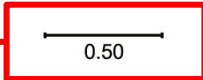
Han River viral metagenome



Han River bacterial metagenome

Metagenome sequences used for tree construction marked with blue

shows the length of branch that represents an amount genetic change of 0.50



Phylogenetic analyses showed that H4-C441-ORF28 was affiliated with class A  $\beta$ -lactamases but formed a distinctive clade apart from representative class A  $\beta$ -lactamases

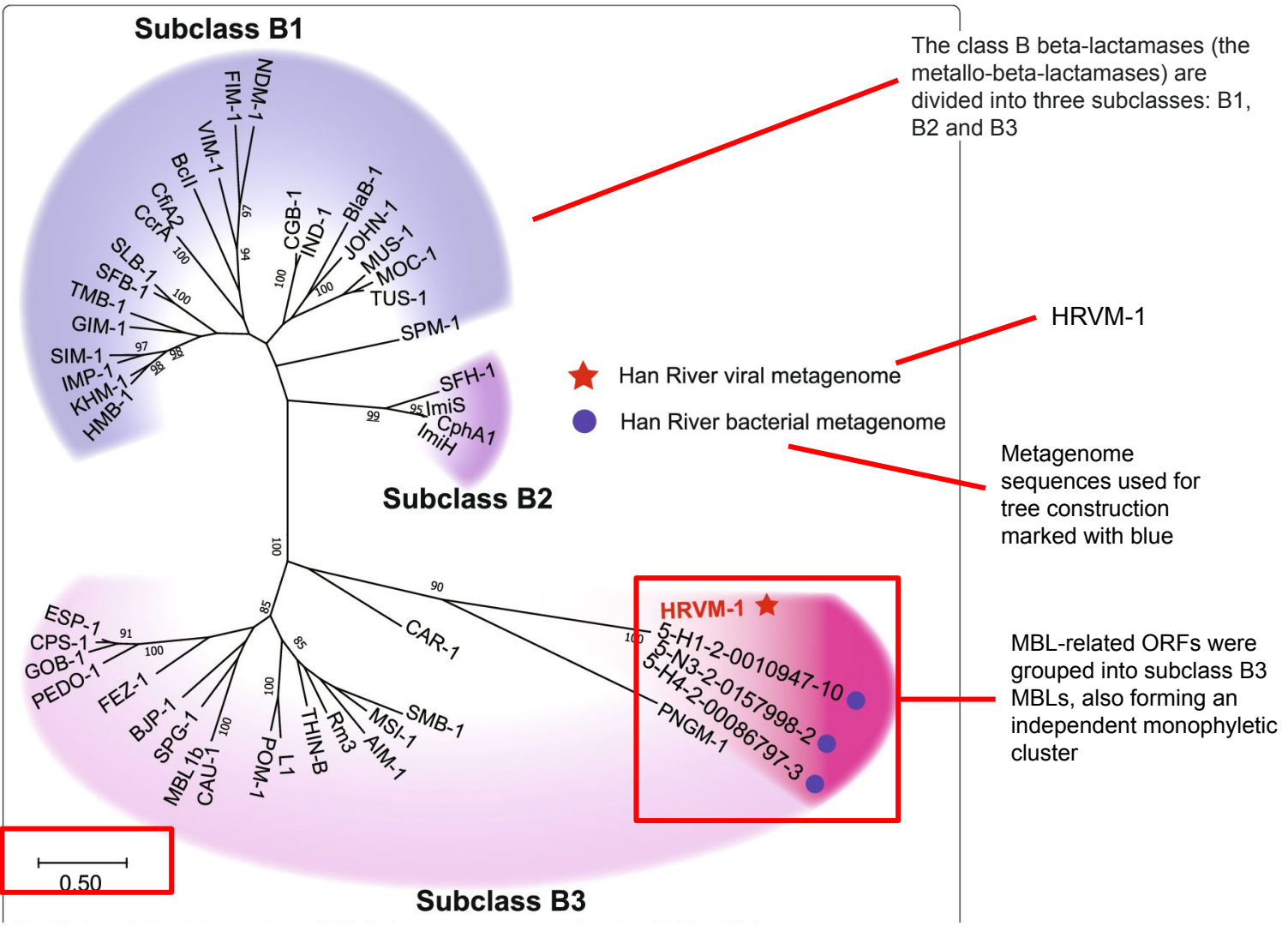
Numbers at the nodes represent bootstrap values based on 100 re-samplings, and values higher than 80% are shown

\*\*unique class A  $\beta$ -lactamase gene was named blaHRV-1, and its product was named HRV1 (Han River Virome  $\beta$ -lactamase-1)



# Figure 4

\*\*\*Novel gene and gene product were named as blaHRVM-1 and HRVM-1 (Han River Virome Metallo-β-lactamase-1)

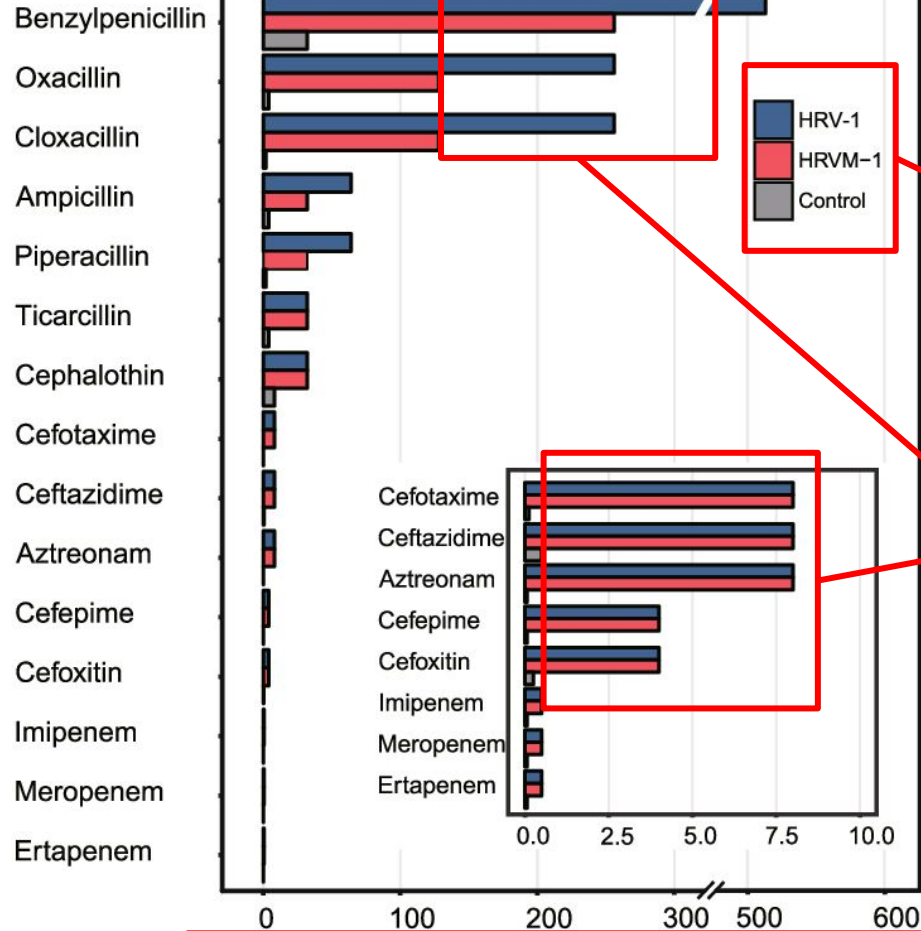




# Figure 5

Independent variable being tested/changed in experiment

**Antibiotics**



\*\*MIC: the lowest concentration of a chemical, usually a drug, which prevents visible growth of a bacterium or bacteria

Two viral contig samples + control

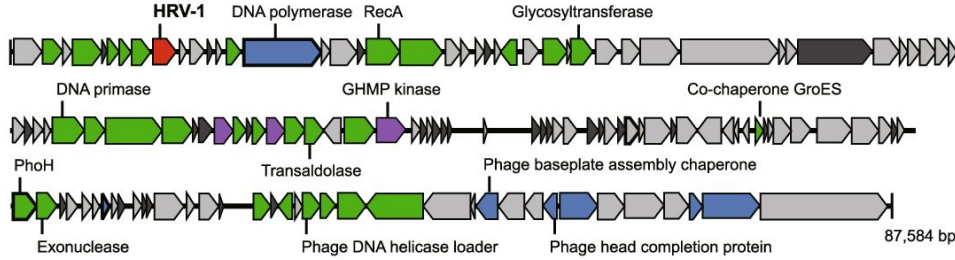
The two strains showed reduced susceptibility, ranging from 2- to 16-fold reductions

**Minimum inhibitory concentrations (MIC,  $\mu\text{g/ml}$ )**

Dependent variable being measured in experiment

# Figure 6

5-N3-2-0000113

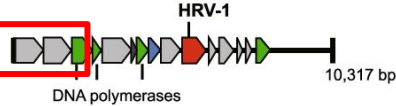


\*\*\*Despite these six contigs being discovered within bacterial metagenome sequences, they were predicted to be viral genomes and the metagenome contigs with these ORFs showed high synteny to the viral contigs containing blaHRV-1 and blaHRVM-1

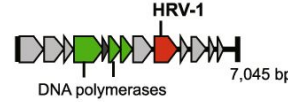
- suggests the presence of infectious phages or prophages carrying blaHRV-1 and blaHRVM-1 in the Han River bacterial communities

Direction of arrow indicates direction of transcription

5-H1-2-0007454

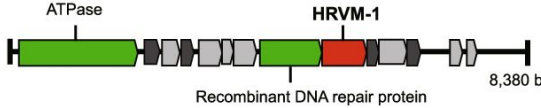


5-H6-2-0011724



simplified depiction of the sequence synteny between viral and bacterial metagenome contigs that carry HRV-1 or HRVM-1

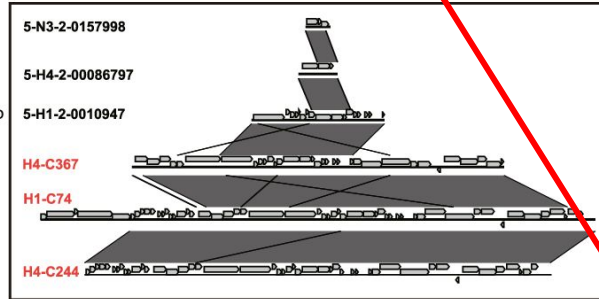
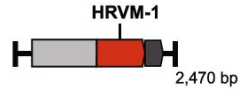
5-H1-2-0010947



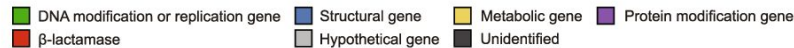
5-N3-2-0157998



5-H4-2-00086797



Red text represents viral contig and black is bacterial contig



Labels used to annotate different parts of the viral contigs