CREATES Analysis Template

Free Response

Figure or Table Number: | 1 |

"Official" title for this figure or table (from the caption):

Taxonomic distribution of viral metagenome reads collected from six study sites on the Han River in South Korea.

My (simplified, decoded, in regular language) title for this figure or table:

Diversity of organisms represented in metagenome obtained from Han River samples

Analysis of the figure or table:

For each of the six samples, there is a pie chart indicating what percentage of the metagenome reads were virus, bacteria, archaea, or eukarya. There is also then a bar-chart further breaking down the bacteria and virus groups, showing what percentage of each taxonomic family of viruses is present and what percentage of each taxonomic class of bacteria is present.

In all 6 samples, viruses make up about 13% of the metagenome, while bacteria make up around 84%.

When we analyze the figure or table, we conclude:

The majority of the genetic material found in the metagenome was that of bacteria. However, there was still enough viral data to study.

Was the hypothesis supported? Why or why not?

They hypothesis that metagenomics could be used to study viral DNA present in the Han River was supported, as this figure shows that 13% of the metagenome was viral DNA data that could be further analyzed.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

This figure is quite simple, so I am not confused about it. However, I am not sure this figure is even necessary, as it does not show anything that cannot be explained in writing. Furthermore, I don't see any reason for family and class distributions to be included, as these are never referenced in the manuscript.

Figure or Table Number: 2

"Official" title for this figure or table (from the caption):

Sequence maps of contigs H4-C441, H1-C74, H4-C244, and H4-C367-bearing β-lactamase genes HRV-1 or HRVM-1. Red, HRV-1 or HRVM-1;

My (simplified, decoded, in regular language) title for this figure or table:

Placement of antibiotic resistance genes in predicted viral genomes

Analysis of the figure or table:

This figure shows two viral contigs and outlines where the antibiotic resistance genes are amongst all of the other phage genes that are included. The figure is color-coded, and the antibiotic resistance genes are red. DNA modification genes are green, structural genes are blue, and metabolic genes are yellow.

Overall, this figure provides a good understanding of the viral contig.

When we analyze the figure or table, we conclude:

The viral contigs assembled from the metagenome do include the antibiotic resistance genes.

Was the hypothesis supported? Why or why not?

They hypothesis that viral contigs would contain antibiotic resistance genes (ARGs) if viruses are serving as a reservoir was supported, as this figures shows the ARGs amongst other viral genes.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

Once again, I don't think this figure is necessary. The main information gleaned from this figure is the placement of the AGRs amongst the other viral genes, which is never referenced or expanded upon in the manuscript.

Figure or Table Number: 3

"Official" title for this figure or table (from the caption):

Maximum-likelihood phylogenetic tree of HRV-1 with representative enzymes of classes A, C, and D β-lactamases.

My (simplified, decoded, in regular language) title for this figure or table:

Phylogenetic tree showing what class of Beta-lactamases the first identified antibiotic resistance genes belongs to

Analysis of the figure or table:

This figure uses known sequences of class A, B, and C beta-lactamases to show where the first ARG falls. It falls into Class A.

Bacterial ARGs from the Han River metagenome also fall into Class A, signaling that the phages may be actively infecting bacterial hosts.

When we analyze the figure or table, we conclude:

The first ARG found in the viral metagenome is a Class A beta-lactamase.

Was the hypothesis supported? Why or why not?

This supports the hypothesis that viruses in the Han River may be carrying ARG and actively infecting bacterial hosts.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

I am a bit confused on the other sequences used for this tree. I know that the ones with purple circles are from the bacteria metagenome. Are all the other sequences also bacterial?

Figure or Table Number: 4

"Official" title for this figure or table (from the caption):

Maximum-likelihood phylogenetic tree of HRVM-1 with representative enzymes of subclasses B1, B2, and B3.

My (simplified, decoded, in regular language) title for this figure or table:

Phylogenetic tree showing what class of Beta-lactamases the second identified antibiotic resistance genes belongs to

Analysis of the figure or table:

Using the sequences of Subclass B1, B2, and B3 beta-lactamases, this tree shows where the second identified ARG falls. It falls into subclass B3, although it is quite distinct.

Some bacterial ARGs from the bacterial metagenome of the Han River also fall into Subclass B3, indicating that phages are infecting hosts.

When we analyze the figure or table, we conclude:

The second ARG found in the viral metagenome is a Subclass B3 beta-lactamase.

Was the hypothesis supported? Why or why not?

This supports the hypothesis that viruses in the Han River may be carrying ARGs and actively infecting bacterial hosts that live in the river.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

Just as in the prior figure, I am not sure if the other sequences included in the tree are all bacterial, or a mix of bacterial and viral genomes.

Figure or Table Number: 5

"Official" title for this figure or table (from the caption):

Minimum inhibitory concentrations (MICs) of β-lactams for *Escherichia coli* BL21 (DE3) transformants producing HRV-1 [*E. coli* BL21 (DE3)-pET-30a(+)-HRV-1] or HRVM-1 (*E. coli* BL21 (DE3)-pET-28a(+)-HRVM-1) or harboring the expression vectors pET-28a(+) or pET-30a(+) [*E. coli* BL21 (DE3)-pET-28a(+)/*E. coli* BL21 (DE3)-pET-30a(+)].

My (simplified, decoded, in regular language) title for this figure or table:

E. coli transformed with the antibiotic resistance genes found in the viral metagenome show increased resistance to a diverse set of antibiotic

Analysis of the figure or table:

This figure is a bar chart showing the minimum inhibitory concentration of 15 different drugs for E. coli that have been transformed with either the Class A beta-lactamase or the Subclass B3 beta-lactamase.

It shows that for 12 of the drugs, both antibiotic resistance genes cause their bacterial hosts to be more resistant to the drugs than the control bacteria. The ARG is exceptionally effective against penicillin and its variants.

When we analyze the figure or table, we conclude:

The ARGs found in the viral genomes are functional.

Was the hypothesis supported? Why or why not?

This supports the hypothesis that if the ARGs hosted in viruses are functional, then bacteria transformed with them will show more antibiotic resistant.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

I am curious about two things: 1) Why is the Class A drug more effective than the Subclass B3 ones in some cases. 2) Why do the ARGs work better against some of the drugs than others. I know the answers to both these questions have to do with the mechanism of the drug and the resistance genes, so I plan to research this further when I get a chance.

Figure or Table Number: 6

"Official" title for this figure or table (from the caption):

Genomic maps of the Han River bacterial metagenome contigs that harbor homologous ORFs to HRV-1 or HRVM-1.

My (simplified, decoded, in regular language) title for this figure or table:

Placement of antibiotic resistance genes in bacterial genomes

Analysis of the figure or table:

This figure shows six bacterial genomes found in the Han River and where the antibiotic resistance genes are amongst all of the other genes. The figure is color-coded the same way as Figure 2.

Overall, it shows that the same antibiotic resistance genes found in viral contigs are found in these bacterial hosts.

When we analyze the figure or table, we conclude:

The phages carrying the antibiotic resistance genes are actively infecting bacteria.

Was the hypothesis supported? Why or why not?

This supports the hypothesis that phages may transfer ARGs amongst bacterial populations.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

I am concerned that this might not be unambiguous evidence showing that phages are transferring the genes. It is always possible the bacteria are transferring the genes via conjugation, and the phage just happened to pick it up at some point.