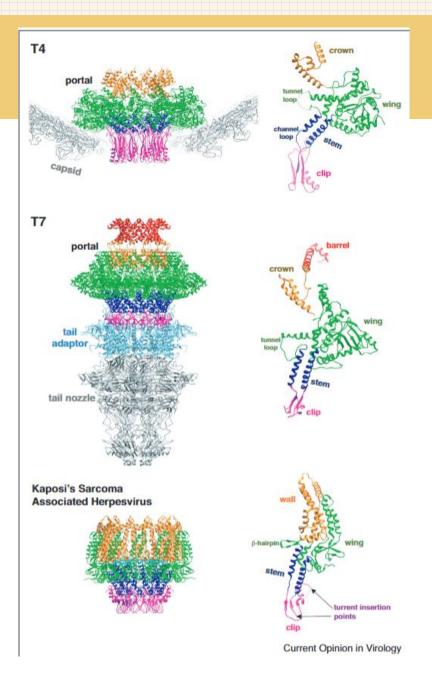
## MGV Database: Extracting Connector Protein

## **Importance of Bacteriophages**

- Affect our microbiome
- The microbiome has been shown to influence everything from physical health (ie obesity) to mental disorders (ie anxiety, depression)

## **Connector Protein**

- Helps package DNA into the capsid
- Assembly of the motor



## Goal

- 1. Find connector protein sequence
- 2. Simulate sequences

Improve understanding of the general connector protein structure

Eventual goal: We want to simulate the structure of an entire virus

## **Viral Protein Homology**

- Connector protein sequences aren't similar because the phages evolved the protein independently
- Common methods of annotating proteins:
  - Structural: modelling and comparing
  - Sequence: comparing sequence to database
- Homology = sequence related because of common ancestor
- Similarity = degree of likeness between sequences

# Phages have adapted the same protein fold to fulfill multiple functions in virion assembly

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Edited\* by Michael G. Rossmann, Purdue University, West Lafayette, IN, and approved June 21, 2010 (received for review April 28, 2010)

Evolutionary relationships may exist among very diverse groups of proteins even though they perform different functions and display little sequence similarity. The tailed bacteriophages present a uniquely amenable system for identifying such groups because of their huge diversity yet conserved genome structures. In this work, we used structural, functional, and genomic context comparisons to conclude that the head–tail connector protein and tail tube protein of bacteriophage  $\lambda$  diverged from a common ancestral protein. Further comparisons of tertiary and quaternary structures indicate that the baseplate hub and tail terminator proteins of bacteriophage may also be part of this same family. We propose that all of these proteins evolved from a single ancestral tail tube protein fold, and that gene duplication followed by differentiation

the connector and passes down the tail into the cell. The portion of the connector that is inserted into the head is composed of a dodecameric ring of the product of gene *B* (gpB), also known as the portal protein. The bottom surface of the connector (Fig. 1*A*), which interacts with the tail, is composed of gpFII (5). Another protein, gpW, is required for the stabilization of the DNA within the head and for the addition of gpFII (6, 7), suggesting that it may be positioned in the connector between gpB and gpFII. *Bacillus subtilis* phage SPP1 gp16, a protein with the same structure, function, and genomic position as gpFII (2) (Fig. 1 *A* and *C*), has been shown by cryoelectron microscopy (cryoEM) to form a 12-membered ring within the connector (8, 9). Although the number of molecules of gpFII in assembled phage particles has

## **Data**

- The paper analyzed bacteriophages found in the human gut microbiome
- 189,680 viral genomes
- Found many NEW species
- Clustered viral proteins using MMseqs2 and annotated using HMMER (pHMM that searches database for sequence homologs) against HMM databases (KEGG, TIGRFAM, Pfam, VOGDB)

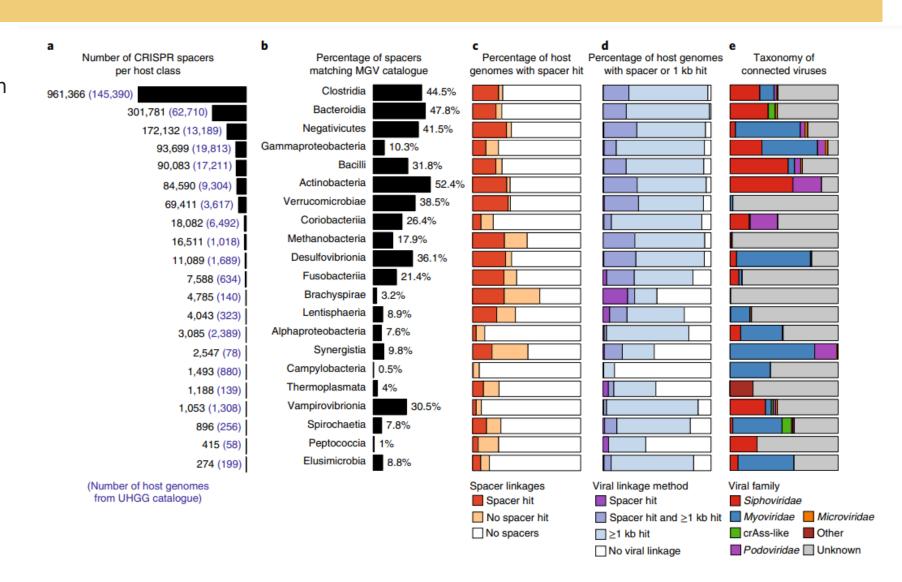
## Metagenomic compendium of 189,680 DNA viruses from the human gut microbiome

Stephen Nayfach 1,2 April David Páez-Espino 1,2, Lee Call 1,2, Soo Jen Low 3, Hila Sberro 4,5, Natalia N. Ivanova 1,2, Amy D. Proal 6, Michael A. Fischbach 1,8,9,10, Ami S. Bhatt 1,5, Philip Hugenholtz 1,3 and Nikos C. Kyrpides 1,2 April 1,2 Apri

Bacteriophages have important roles in the ecology of the human gut microbiome but are under-represented in reference databases. To address this problem, we assembled the Metagenomic Gut Virus catalogue that comprises 189,680 viral genomes from 11,810 publicly available human stool metagenomes. Over 75% of genomes represent double-stranded DNA phages that infect members of the Bacteroidia and Clostridia classes. Based on sequence clustering we identified 54,118 candidate viral species, 92% of which were not found in existing databases. The Metagenomic Gut Virus catalogue improves detection of viruses in stool metagenomes and accounts for nearly 40% of CRISPR spacers found in human gut Bacteria and Archaea. We also produced a catalogue of 459,375 viral protein clusters to explore the functional potential of the gut virome. This revealed tens of thousands of diversity-generating retroelements, which use error-prone reverse transcription to mutate target genes and may be involved in the molecular arms race between phages and their bacterial hosts.

## **Family**

- "Distribution of known viral families that are associated with each host class. Each host class is infected by a distinct repertoire of viral families"
- Interested in what families the proteins we find come from



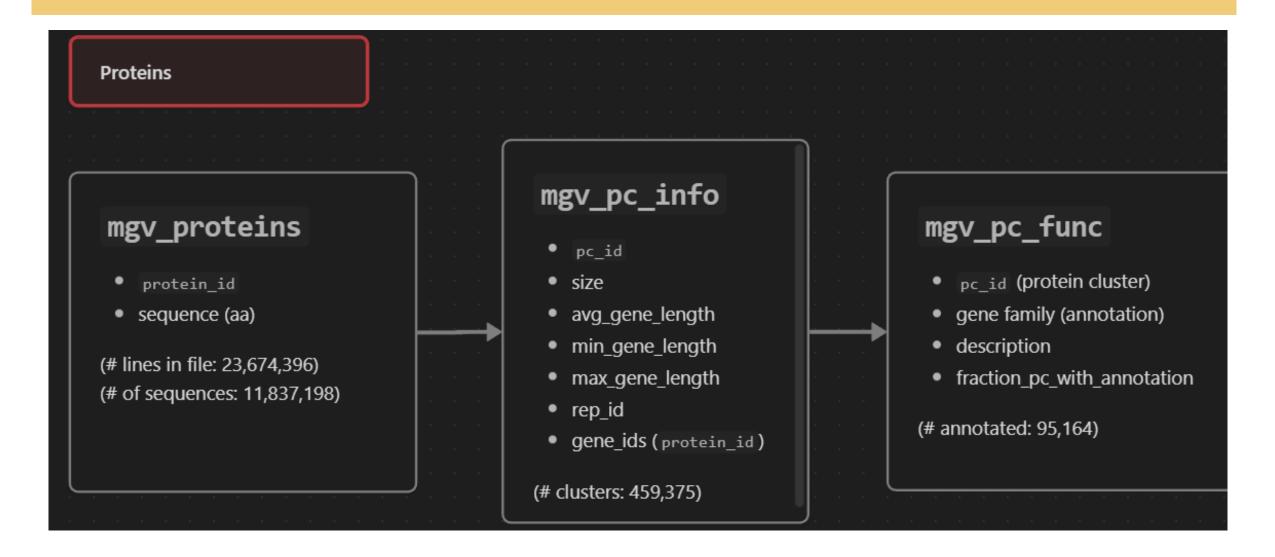
## Metadata

#### mgv\_contig\_info.tsv

- Metadata for the 189,680 viral genomes. Fields include:
- votu\_id: indicate the species-level viral OTU the genome belongs to
- checkv\_quality: medium quality (50-90% complete), high quality (>90% complete), complete (closed genome)
- prophage: whether or not the contig was flanked by DNA from the host (these regions were removed)
- temperate\_score: BACPHLIP output indicating the probability the virus lives a temperate lifestyle
- virulent\_score: BACPHLIP output indicating the probability the virus lives a virulent lifestyle
- completeness: CheckV estimated completeness
- gc: GC content
- stop\_codon\_readthrough: indicates whether the virus is predicted to read through a particular stop codon
- baltimore: baltimore classification
- ictv\_order, ictv\_family, ictv\_genus: annotations based on the ICTV taxonomy

contig_id	votu_id lengt	th checkv_quality	prophage	temperat	.e_score	virulen'	c_score	complete	eness	gc	stop_codor	n_readthrough	baltimore	ictv_ord	rder ictv_family
ctv_genus															
MGV-GENOME-0364	4295 OTU-61	1123 97376	Complete	No 6	0.0375	0.9625	98.26	31.6166	TAG	dsDNA	Caudoviral	les crAss-ph	nage NU'	4	
MGV-GENOME-0364	4296 OTU-61	1123 97376	Complete	No 6	0.0375	0.9625	98.26	31.6146	TAG	dsDNA	Caudoviral	les crAss-ph	nage NU'	4	
MGV-GENOME-0364	4303 OTU-05	5782 97388	Complete	No 6	0.035740	12	0.96426	98.28	27.9706	NULL	dsDNA Ca	audovirales	crAss-phage	e NULL	
MGV-GENOME-0364	4311 OTU-01	1114 97394	Complete	No	0.0375	0.9625	98.38	31.4485	TAG	dsDNA	Caudoviral	iles crAss-ph	nage NU'	<u> </u>	
MGV-GENOME-0364	4312 OTU-23	3935 97395	Complete	No 6	0.0138753	.3	0.98612	<i>A</i>	99.25	33.5777	TAG ds	lsDNA Caudovir	rales cr/	Ass-phage	NULL

## **Data**



## **Data**

- BaltimoreClassification:
- 7 classes based on nucleic acid (DNA / RNA), strandness (double / single), sense, method of replication

#### mgv\_sample\_info

- contig\_id
- assembly\_source
- assembly\_name
- study\_accession (# unique: 179,323)
- sample\_accession (# unique: 188,684)
- run\_accessions
- continent
- country\_code
- sex
- age
- health
- disease

#### mgv\_contig\_info.tsv.gz

- •Metadata for the 189,680 viral genomes. Fields include:
- contig\_id: indicate the species-level viral OTU the genome belongs to
- •checkv\_quality: medium quality (50-90% complete), high quality (>90% complete), complete (closed genome)
- •prophage: wheter or not the contig was flanked by DNA from the host (these regions were removed)
- •temperate\_score: BACPHLIP output indicating the probability the virus lives a temperate lifestyle
- •virulent\_score: BACPHLIP output indicating the probability the virus lives a virulent lifestyle
- •completeness: CheckV estimated completeness
- •gc: GC content
- •stop\_codon\_readthrough: indicates whether the virus is predicted to read through a particular stop codon
- •baltimore: baltimore classification
- •ictv\_order, ictv\_family, ictv\_genus: annotations based on the ICTV taxonomy

#### mgv\_votu\_representatives

- contig\_id
- vOTU

(# vOTUs: 54,118)

#### mgv\_contigs

- contig id
- sequence (DNA)

(# lines: 189,681)

(# sequences: 189,680)

## mgv\_host\_assignments .tsv.gz

- contig
- host: (# unique: 246)
- host\_phylum: (# unique: 102000)
- host\_class: (# unique: 102197)
- host\_order: (# unique: 127,548)
- host\_family: (# unique: 145047)
- host\_genus: (# unique: 141839)
- host\_species: (# unique: 112148)

(# lines: 170,093)

## **Annotations in Dataset**

#### Paper:

- Clustered data using MMseqs2
- Annotated 20% using HMMER:
  - HMMER: detects homology by comparing a profile-HMM (a Hidden Markov model constructed explicitly for a particular search) to either a single sequence or a database of sequences.

### • Pfam:

collection of protein families (MSA and HMMs)

#### Results

- 411 portal proteins
- 146 connector

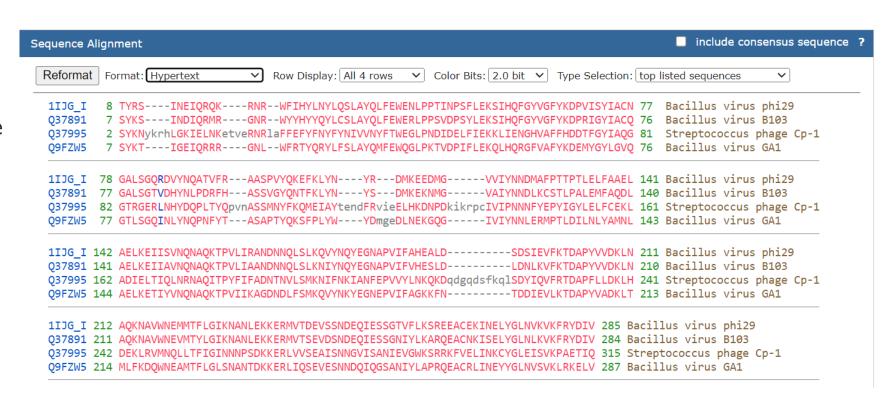
annotation	vpc_id	protein_id	protein_seq
abla	abla	abla	7
Phage gp6-like head-tail connector protein	VPC-8627	MGV-GENOME-0282701_34	MSLDDEKILEKIKFSCRIDDDI
Phage gp6-like head-tail connector protein	VPC-16699	MGV-GENOME-0270537_10	MLSMADFEDTVLINVKEDLA
Phage gp6-like head-tail connector protein	VPC-135993	MGV-GENOME-0232097_34	MSIKNLMGTVTDDDLQLTKT
Phage gp6-like head-tail connector protein	VPC-545	MGV-GENOME-0260596_65	MEYTTLEQVKIRLKQFHIDTV
Phage gp6-like head-tail connector protein	VPC-456140	MGV-GENOME-0209946_11	MSGEAAAFKPPNRTERTKER:

## Workflow

- 1. Find connector protein domain (in literature / NCBI)
- 2. BLAST
- 3. Filter alignment results
  - want very low e-values and high bit scores
- 4. Check if the proteins are annotated / clustered (mgv\_pc\_info.tsv.gz)
- 5. Model some proteins in list / cluster
  - to make sure the results are correct

## 1. Connector Domain

- Query: Phage connector domain (from NCBI, bacteriophage phi29) →
- Database: MGV database
- BLAST results: 540 sequences
- Filtered by e-value: 2e-60 as a threshold (was just the highest "significant" e-value)
- Found protein cluster (in database) that matched most of the BLAST outputs (5464 sequences)



## 2&3. BLAST results

```
201
                                                                    2e-60
MGV-GENOME-0089456 15 # 11494 # 12366 # 1 # ID=606 15;partial=00;...
                                                             200
                                                                    2e-60
MGV-GENOME-0098546_17  # 11992  # 12870  # 1  # ID=1255_17;partial=00...
                                                             200
                                                                    2e-60
MGV-GENOME-0102802 18 # 12383 # 13201 # 1 # ID=206 18;partial=00;...
                                                             199
                                                                    2e-60
MGV-GENOME-0102795 18 # 12383 # 13201 # 1 # ID=204 18;partial=00;...
                                                             199
                                                                    2e-60
MGV-GENOME-0102791 18 # 12383 # 13201 # 1 # ID=203 18;partial=00;...
                                                             199
                                                                    2e-60
```

## E-value

 number of expected hits of similar quality (score) that could be found just by chance

## Bit score

 Required size of a sequence database in which the current match could be found just by chance

### **2&3. BLAST**

```
Score
Sequences producing significant alignments:
                                                                    (Bits) Value
                                                                              2e-36
MGV-GENOME-0212193 24 # 19174 # 20226 # 1 # ID=267 24;partial=00;... 140
MGV-GENOME-0159433 29 # 20711 # 21538 # 1 # ID=491 29;partial=00;... 138
                                                                             2e-36
MGV-GENOME-0210500 19 # 15471 # 16532 # 1 # ID=861 19;partial=00;... 139
                                                                              3e-36
MGV-GENOME-0117354 6 # 5749 # 6810 # 1 # ID=496 6;partial=00;star... 139
                                                                              4e - 36
MGV-GENOME-0222640 14 # 9874 # 10935 # -1 # ID=1055 14:partial=00... 136
                                                                             4e-35
MGV-GENOME-0209211 21 # 16647 # 17711 # 1 # ID=1419 21;partial=00... 135
                                                                              1e-34
MGV-GENOME-0131812 19 # 12507 # 12902 # -1 # ID=367 19;partial=00... 76.6
                                                                             9e-15
MGV-GENOME-0191353 6 # 4015 # 4557 # -1 # ID=2682 6;partial=00;st... 75.1
                                                                             7e - 14
MGV-GENOME-0191353 5 # 3534 # 3938 # -1 # ID=2682 5;partial=00;st... 63.9
                                                                             3e - 10
MGV-GENOME-0105632 1 # 3 # 485 # -1 # ID=725 1;partial=10;start t... 58.9
                                                                              3e-08
MGV-GENOME-0214625 28 # 24444 # 25526 # 1 # ID=514 28;partial=00;... 55.5
                                                                              3e-06
MGV-GENOME-4395318_4 # 2697 # 3779 # 1 # ID=1794_4;partial=00;sta... 53.5
                                                                              1e-05
MGV-GENOME-0215696 35 # 28616 # 29707 # -1 # ID=1451 35;partial=0... 51.6
                                                                              5e-05
```

- BLAST results from using the entire connector protein as the query.
- After checking if the results correspond to any protein clusters, I found VPC\_8016, which has exactly the same number of proteins in the cluster as there are in the filtered BLAST results (269 proteins)

## 4. Check for annotation & family

#### **Annotaation**

```
VPC-34 pfam PF05352.12 Phage Connector (GP10) 1
```

#### **Families**

```
167 NULL
1 Papillomaviridae
5296 Podoviridae
```

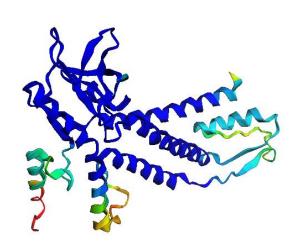
## 5. Modeling

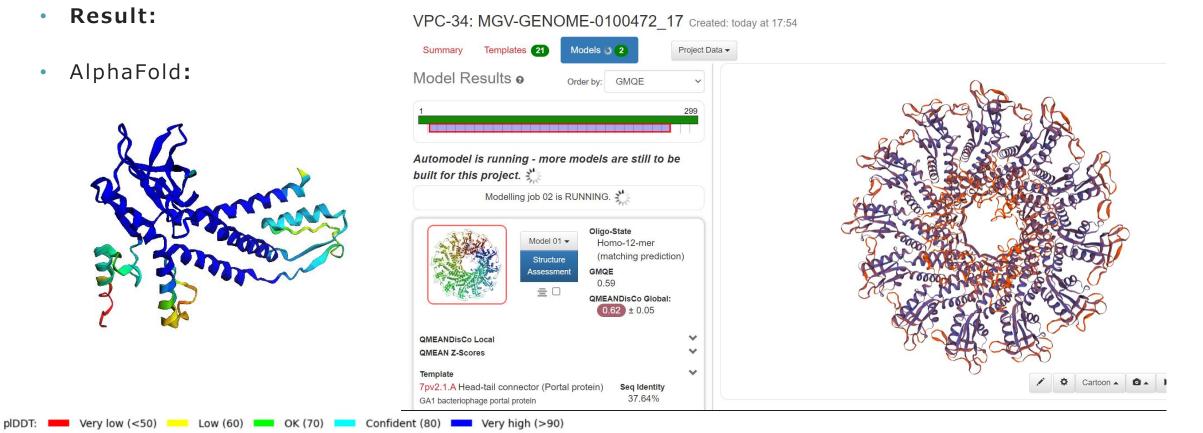
Modeled some of the proteins found in the protein cluster and in BLAST results (VPC-34)

Swiss-MODEL:

Result:

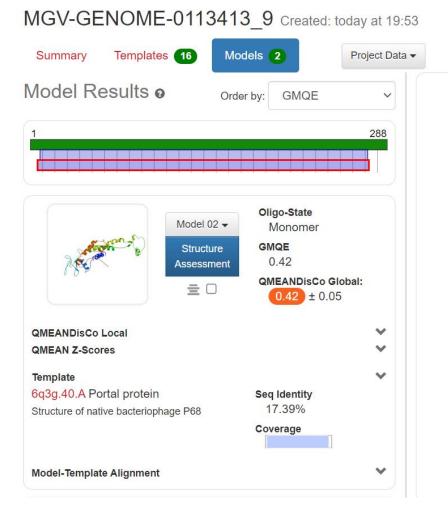
AlphaFold:

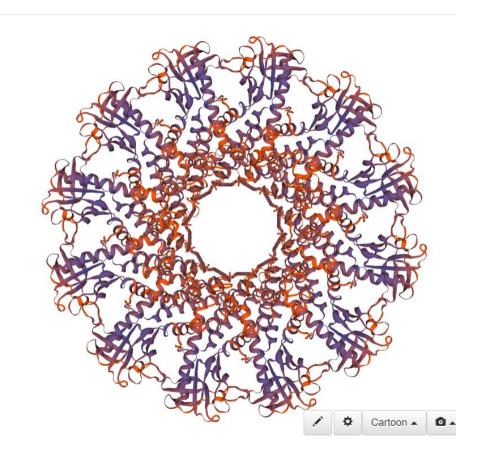




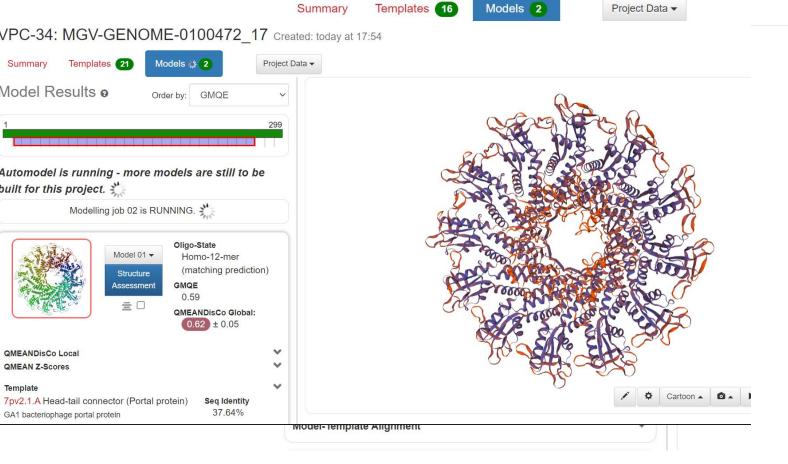
## 5. Modelling

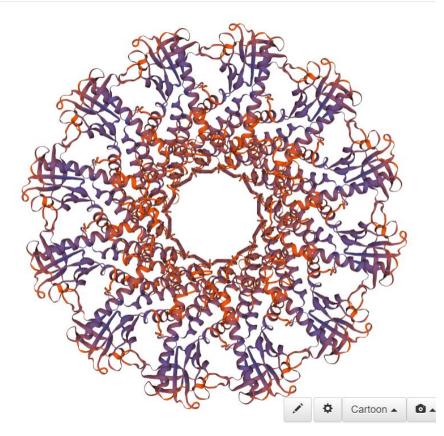
Modeledprotein found inVPC-34 but notin BLAST results





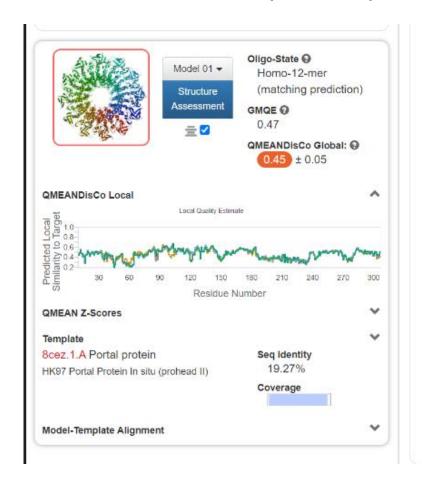
#### MGV-GENOME-0113413\_9 Created: today at 19:53

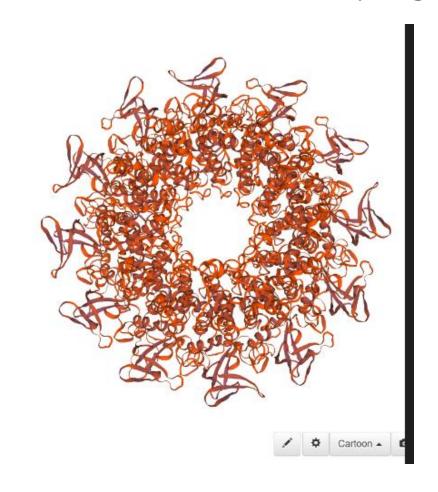




## **Query Sequence**

>YP\_010091615.1 portal protein [uncultured Caudovirales phage]





## **BLAST: Query Sequence**

```
(base) claireh@claire-virtualbox:/media/sf_shared_folder/Bacteriophage/MGV$ cat blast protein/filtered query.txt
MGV-GENOME-0192513 23 # 17497 # 18534 # 1 # ID=3086 23;partial=00... 172
                                                                             8e-49
MGV-GENOME-0212292 17 # 12116 # 13165 # 1 # ID=320 17;partial=00;... 170
                                                                             5e-48
MGV-GENOME-0172516 15 # 10958 # 12007 # -1 # ID=1136 15;partial=0...
                                                                             5e-48
                                                                     170
MGV-GENOME-0210072 17 # 11748 # 12797 # 1 # ID=55 17;partial=00;s...
                                                                             5e-48
                                                                     170
MGV-GENOME-0226859 40 # 31378 # 32427 # 1 # ID=795 40;partial=00;...
                                                                     170
                                                                             6e-48
MGV-GENOME-0187969 20 # 13343 # 14392 # -1 # ID=372 20;partial=00...
                                                                             1e-47
                                                                     169
MGV-GENOME-0183924 16 # 11035 # 12084 # -1 # ID=1677 16;partial=0...
                                                                     169
                                                                             2e-47
(base) claireh@claire-virtualbox:/media/sf_shared_folder/Bacteriophage/MGV$ cat blast protein/filtered connector dom
MGV-GENOME-0104393 9 # 6790 # 7770 # 1 # ID=513 9;partial=00;star... 254
                                                                             6e-81
MGV-GENOME-0122635 24 # 15385 # 16266 # 1 # ID=1448 24;partial=00...
                                                                     236
                                                                             1e-74
MGV-GENOME-4313378 4 # 3458 # 4342 # -1 # ID=783 4;partial=00;sta...
                                                                     233
                                                                             3e-73
MGV-GENOME-0094600 4 # 3582 # 4466 # -1 # ID=892 4;partial=00;sta...
                                                                     233
                                                                             3e-73
MGV-GENOME-0094502 14 # 10883 # 11767 # 1 # ID=877 14;partial=00;...
                                                                     233
                                                                             3e-73
MGV-GENOME-0095706 5 # 3584 # 4468 # -1 # ID=1053 5;partial=00;st...
                                                                     231
                                                                             2e-72
MGV-GENOME-0099638 13 # 9158 # 10123 # 1 # ID=1418 13;partial=00;...
                                                                     231
                                                                              2e-72
```

- BLAST results using query sequence aren't quite as good as using connector domain but they're still good

## Query Seq. (cont)

#### **Annotation**

```
MGV-GENOME-0192513_23 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region MGV-GENOME-0212292_17 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region MGV-GENOME-0172516_15 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region MGV-GENOME-0210072_17 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region MGV-GENOME-0226859_40 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region MGV-GENOME-0187969_20 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region MGV-GENOME-0165836_19 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region MGV-GENOME-0142829_6 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region MGV-GENOME-0142829_6 VPC-8016 Cytidine and deoxycytidylate deaminase zinc-binding region
```

## Query Seq. (cont)

MGV-GENOME-0192513\_23, labelled as Cytidine and deoxycytidylate deaminase zinc-binding region

