Agriculture & Agri-Food Canada Phage Genomics Workshop

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Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada

UNIVERSITY of GUELPH

PART 2

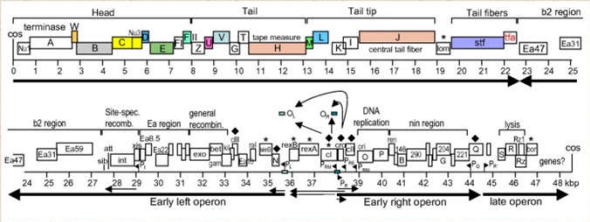
Genome annotation

APOLOGY 1: NO AUTOANNOTATION SOFTWARE PACKAGE IS PERFECT. YOU WILL NEED TO DO A LOT OF PROOFREADING TO ACHIEVE A HIGH-QUALITY ANNOTATED VIRAL GENOME
APOLOGY 2: THE DFAST SERVER MAY BE DOWN

Outline

- ❑ Online primary genome annotation with DFAST, PHAROKKA or RAST – **PART 2A**
- ❑ Massaging *.gbk file
- ❑ Identification of missing coding sequences using UGENE – **PART 2B**
- ❑ GenBank file types & their interconversion – **PART 2C**
- ❑ Examination of proteins for conserved motifs – **PART 2D**
- ❑ Updated GenBank flatfile (*.gbk)

Annotation outcome



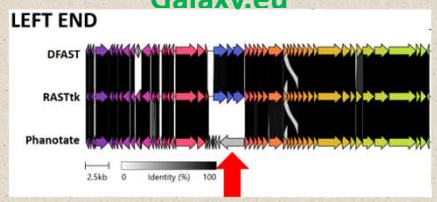
- We want to identify the location and function of all the genes on our phage genome:
- Two main classes encode proteins and tRNAs

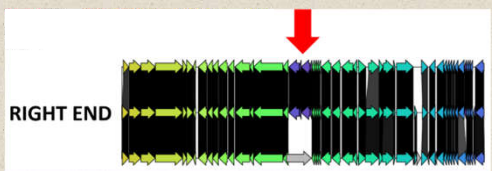
Automated Annotation – PART 2A

- Proksee - https://proksee.ca/ uses Prokka
- RAST - Rapid Annotation using Subsystem Technology (https://rast.nmpdr.org/)
- DFAST - DDBJ Fast Annotation and Submission Tool. (https://dfast.nig.ac.jp/)
- BV-BRC - Bacterial and Viral Bioinformatics Resource Center (https://www.bv-brc.org/app/Annotation)
- GenSAS v6.0 - https://www.gensas.org/
- MicroScope Microbial Genome Annotation & Analysis Platform - https://mage.genoscope.cns.fr/microscope/home/index.php
- Bakta Web - https://bakta.computational.bio/

All except DFAST & Bakta require free online registration

Use Pharokka with caution _ Galaxy.eu

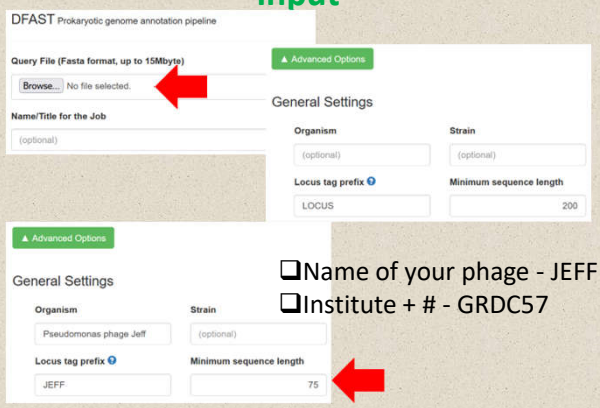




First pass annotation using DFAST

- DDBJ Fast Annotation and Submission Tool
- associated with DNA Data Bank of Japan
- Tanizawa Y et al. 2018. Bioinformatics. **34(6):** 1037-1039. doi: 10.1093/bioinformatics/btx713. PMID: 29106469
- https://dfast.ddbj.nig.ac.jp/
- click on "Start your project"

Input



- Name of your phage - JEFF
- Institute + # - GRDC57

AMR/Virulence Factors

Results

- 17 seconds latter:

Genome Statistics

Total Length (bp)	66,776
No. of Sequences	1
GC Content (%)	55.6%
N50	66,776
Gap Ratio (%)	0.0%
No. of CDSs	91
No. of rRNA	0
No. of tRNA	0
No. of CRISPRS	0
Coding Ratio (%)	91.6%

Download Files

Genbank Flat File :	annotation.gbk
GFF3-formatted File :	annotation.gff
Genome Fasta File :	genome.fna
Protein Fasta File :	protein.faa
CDS Fasta File :	cds.fna
RNA Fasta File :	rna.fna
Feature Table :	features.tsv
Pseudogene Summary :	pseudogene_summary.tsv
Genome Statistics :	statistics.txt
Zip Archive :	annotation.zip

What do they all mean?

*.faa Protein FASTA file of the translated CDS sequences.

*.ffn Nucleotide FASTA file of all the prediction transcripts (CDS, rRNA, tRNA, tmRNA, misc_RNA)

*.fna Nucleotide FASTA file.

*.gbk This is a standard GenBank flatfile

*.gff This is the master annotation in GFF3 format, containing both sequences and annotations.

*.tsv Tab-separated file of all features

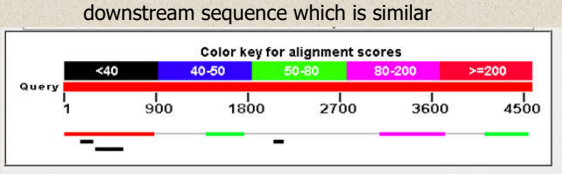
Problems with DFAST

- it is prokaryote annotation pipeline NOT a phage pipeline, therefore annotations are incomplete and skewed.
- while you can change the translation code from 11 (default) to 4 you cannot change it to 15 which is used by some gut phages.
- splicing not recognized:
  - in GenBank record this is reported as CDS join(99941..100807,101606..103957)
  - important with members of the *Herelleviridae*, such as *Staphylococcus* phages; and, *Campylobacter* phages

Introns and Inteins – rare but

- the gene encoding the aerobic ribonucleoside diphosphate reductase (large subunit) from *Campylobacter* phage vB_CcoM-IBB_35 is located on a 4.5 kb region which homologous to a 2.3 kb region from *Klebsiella* phage vB_KleM-RaK2. N.B. proteins have similar mass.

N.B. the thin grey line joining blocks indicates a downstream sequence which is similar

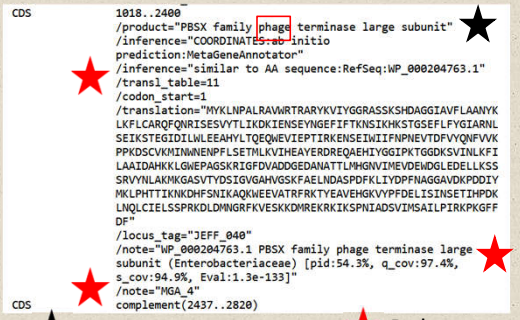


Editing primary annotation (*.gbk) files

- No matter what program you use you will have to edit the results
- Notepad++ is better than Notepad

Open in Notepad++

- Install from: https://notepad-plus-plus.org/

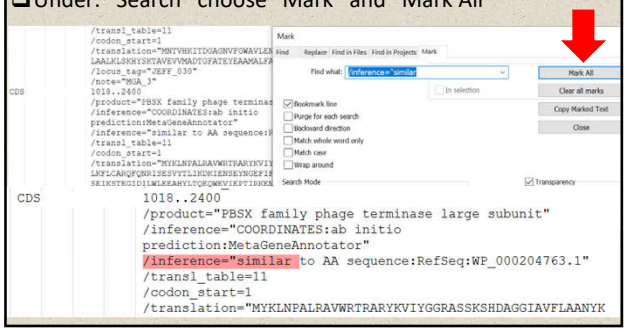


```
CDS             1018..2400
                /product="PBSX family phage terminase large subunit"
                /inference="COORDINATES:ab initio
                prediction:MetaGeneAnnotator"
                /inference="similar to AA sequence:RefSeq:WP_000204763.1"
                /transl_table=11
                /codon_start=1
                /translation="MYKLNPALRAVWRTRARYKVIYGGRASSKSHDAGGIAVFLAANYK
                LKFLCARQFQNRISESVYTLIKDKIENSEYNGEFIFTKNSIKHKSTGSEFLFYGIARNL
                SEIKSTEGIDILWLEEAHYLTQEQWEVIEPTIRKENSEIWIIFNPNEVTDFVYQNFVVK
                PPKDSCVKMINWNENPFLSETMLKVIHEAYERDREQAEHIYGGIPKTGGDKSVINLKFI
                LAAIDAHKKLGWEPAGSKRIGFDVADDGEDANATTLMHGNVIMEVDEWDGLEDELLKSS
                SRVYNLAKMKGASVTYDSIGVGAHVGSKFAELNDASPDFKLIYDPFNAGGAVDKPDDIY
                MKLPHTTIKNKDHFSNIKAQKWEEVATRFRKTYEAVEHGKVYPFDELISINSETIHPDK
                LNQLCIELSSPRKDLDMNGRFKVESKKDMREKRKIKSPNIADSVIMSAILPIRKPKGFF
                DF"
                /locus_tag="JEFF_040"
                /note="WP_000204763.1 PBSX family phage terminase large
                subunit (Enterobacteriaceae) [pid:54.3%, q_cov:97.4%,
                s_cov:94.9%, Eval:1.3e-133]"
                /note="MGA_4"
CDS             complement(2437..2820)
```

★ Comment ★ Delete

Open in Notepad++

- Install from: https://notepad-plus-plus.org/
- Notes: https://www.youtube.com/watch?v=wVfbFe57h2o
- Under: “Search” choose “Mark” and “Mark All”



Mark All/Delete

```
CDS             1018..2400
                /product="PBSX family phage terminase large subunit"
                /inference="COORDINATES:ab initio
                prediction:MetaGeneAnnotator"
                /inference="similar to AA sequence:RefSeq:WP_000204763.1"
                /transl_table=11
                /codon_start=1
                /translation="MYKLNPALRAVWRTRARYKVIYGGRASSKSHDAGGIAVFLAANYK
                LKFLCARQFQNRISESVYTLIKDKIENSEYNGEFIFTKNSIKHKSTGSEFLFYGIARNL
                SEIKSTEGIDILWLEEAHYLTQEQWEVIEPTIRKENSEIWIIFNPNEVTDFVYQNFVVK
                PPKDSCVKMINWNENPFLSETMLKVIHEAYERDREQAEHIYGGIPKTGGDKSVINLKFI
                LAAIDAHKKLGWEPAGSKRIGFDVADDGEDANATTLMHGNVIMEVDEWDGLEDELLKSS
                SRVYNLAKMKGASVTYDSIGVGAHVGSKFAELNDASPDFKLIYDPFNAGGAVDKPDDIY
                MKLPHTTIKNKDHFSNIKAQKWEEVATRFRKTYEAVEHGKVYPFDELISINSETIHPDK
                LNQLCIELSSPRKDLDMNGRFKVESKKDMREKRKIKSPNIADSVIMSAILPIRKPKGFF
                DF"
                /locus_tag="JEFF_040"
                /note="WP_000204763.1 PBSX family phage terminase large
                subunit (Enterobacteriaceae) [pid:54.3%, q_cov:97.4%,
                s_cov:94.9%, Eval:1.3e-133]"
                /note="MGA_4"
```

- Under “Search”, choose “Bookmark” and “Remove Bookmarked Lines”
- It will not remove multiple lines so you will have to manually accomplish this
- shorten “product” to terminase, large subunit

Edit top of *.gbk file

```
LOCUS       sequence1             66776 bp    DNA     linear   BCT 03-FEB-2025
DEFINITION  Pseudomonas phage Jeff unspecified. DNA, contig: sequence1.
ACCESSION   sequence1
VERSION     sequence1.1
KEYWORDS    .
SOURCE      Pseudomonas phage Jeff unspecified.
  ORGANISM  Pseudomonas phage Jeff unspecified.
            .
COMMENT     Annotated by DFAST (https://dfast.nig.ac.jp).
```

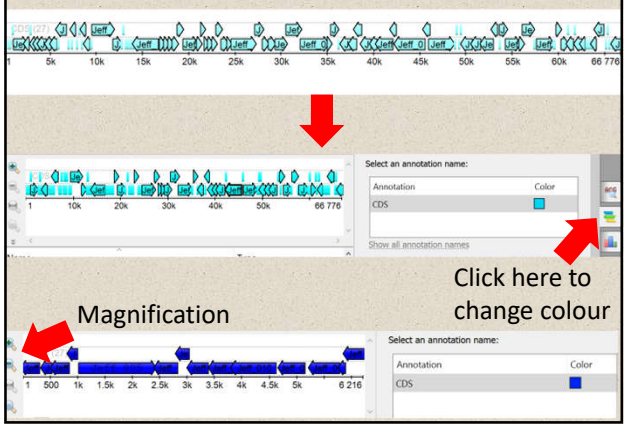
```
LOCUS       Jeff            66776 bp    DNA     linear   PHG 28-JAN-2025
DEFINITION  Pseudomonas phage Jeff, complete genome.
ACCESSION   Pseudomonas phage Jeff
VERSION     Pseudomonas phage Jeff
KEYWORDS    .
SOURCE      .
  ORGANISM  Pseudomonas phage Jeff
            Viruses; Duplodnaviria; Heunggongvirae; Uroviricota; Caudoviricetes;
            Pbunavirus; Pseudomonas phage Jeff
FEATURES             Location/Qualifiers
```

This will be discussed in this workshop BUT in many cases the taxonomy can be readily assessed from the BLASTN hits

Visualizing & editing GBK files using UGENE – PART 2B

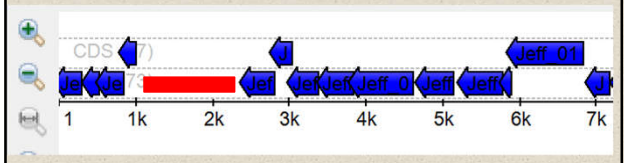
- Rose R et al. 2019. Bioinformatics **35(11):** 1963-1965 doi: 10.1093/bioinformatics/bty901. PMID: 30358807.
- Window, Linus & Mac versions
- http://ugene.net/
- podcasts: https://www.youtube.com/@UniproUGENE

UGENE

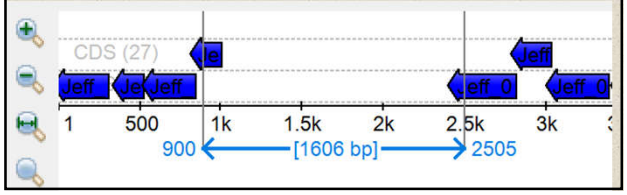


Screen for missing genes

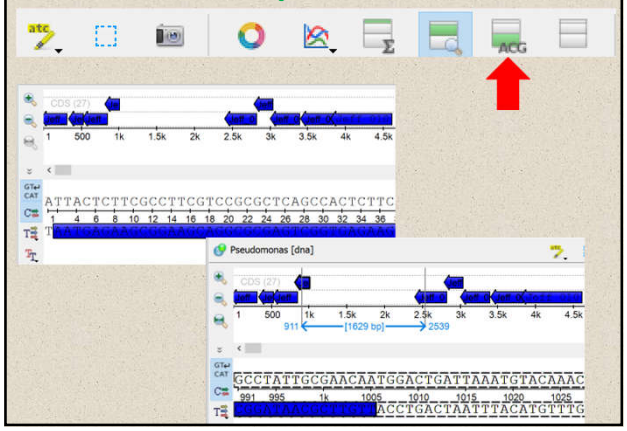
- appear as gaps



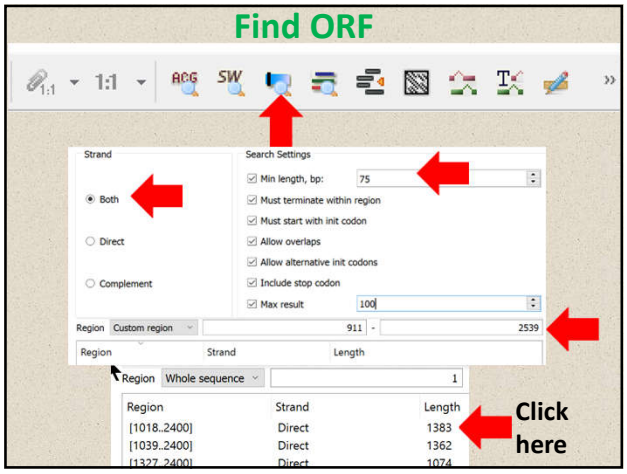
- Highlight "missing region"



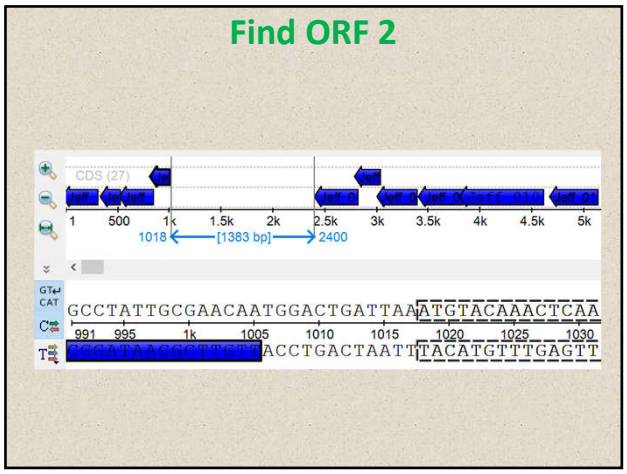
See sequence data



Find ORF

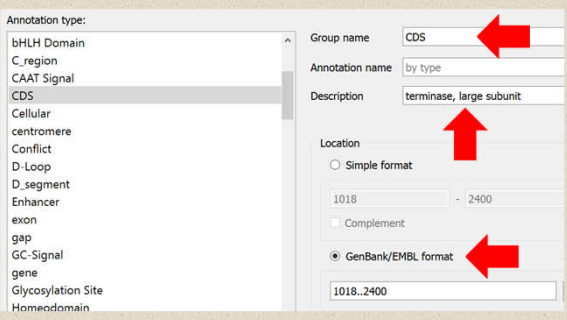


Find ORF 2



Add new annotation

- Under "Actions" in the top bar choose:
  - "Add" and "New annotation"



Alternatives

- Geneious Prime – commercial https://www.geneious.com/
- NCBI's Genome Workbench – Windows, Mac, Ubuntu; discontinued 2024 (https://ftp.ncbi.nlm.nih.gov/toolbox/gbench/ver-3.9.1/)
- DNA Master

https://phagesdb.org/DNAMaster/

ORF versus CDS

- Open Reading Frames vs Coding Sequences
- DFAST identified 91 CDSs in *Pseudomonas* phage Jeff
- NCBI's Open Reading Frame Finder "ORF finder" ORFs found: 795 Genetic code: 11 Start codon: 'ATG' and alternative codons

https://www.ncbi.nlm.nih.gov/orffinder/

- Basic difference is that CDSs are preceded by ribosome-binding sites

CDS, ORF and Genes - optional

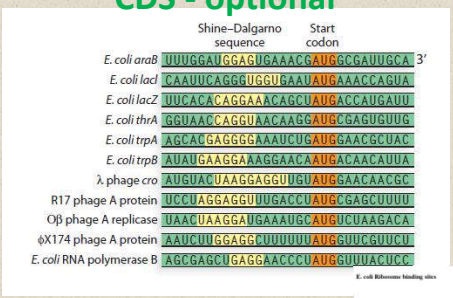
"Most protein sequences are derived from translations of **Co**D**ing** **S**equence (CDS) derived from gene predictions. A **Co**D**ing** **S**equence (CDS) is a region of DNA or RNA whose sequence determines the sequence of amino acids in a protein. It should not be mixed up with an **O**pen **R**eading **F**rame (ORF), which is a series of DNA codons that does not contain any STOP codons. All CDS are ORFs, but not all ORFs are CDS..."
(https://www.uniprot.org/help/cds_protein_definition)

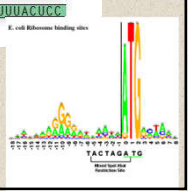
ORF vs CDS 2 - optional

- ❑ an ORF is a sequence that has a length divisible by three and is bounded by stop codons
- ❑ stop codons - TAA, TAG or TGA
- ❑ may not specify a protein

(Sieber P, Platzer M, Schuster S. 2018. The Definition of Open Reading Frame Revisited. Trends in Genetics, 34 (3): 167-170)

CDS - optional





N.B. No matter what the "start" codon it is always translated as M (Met, methionine)

File format interconversions - PART 2C

- Genome2D – conversion
- Baerends RJ et al. 2004. Genome Biol. **5(5):** R37. doi: 10.1186/gb-2004-5-5-r37. PMID: 15128451.
- http://genome2d.molgenrug.nl/g2d_tools_conversions.html
- **Warning**: is the conversion what you expected? Are there any spurious characters? Do all the proteins begin with M?

Solution

- use UGENE to examine/alter start of this gene
- manually change start in Notepad/Notepad++

BLASTp as an annotation tool

- run your proteins against Caudoviricetes (taxid:2731619)
- always compare size of your protein with the consensus of it NCBI "hits"
- don't concentrate on first "hit" since it may say "DNA polymerase" while all the rest say "hypothetical protein"
- Geneious has a very useful BLAST tool; more user friendly than NCBI BLAST

What do I call my gene product (i.e. protein)?

- "phage hypothetical protein" – redundant, all these proteins are "phage proteins"
  → hypothetical protein
- "gp87" (gp = gene product)
  - gp200 describes radically different proteins in *Listeria, Enterococcus, Mycobacterium, Rhodococcus, Sphingomonas, Pseudomonas, Bacillus* and *Synechococcus* phage genomes
  - Add /note="similar to gp43 of Escherichia phage T4"

Gene Product Nomenclature 2

- /product="UboA"; "Mcp"; "NrdA"; "hypothetical protein SA5_0153/152"; "ORF184" (as bad as gp184); "RNAP1"; "32 kDa protein"
  - Bad because they don`t mean anything to the casual (or informed) reader.
- Unless you are a bioinformatician or biostatistician be very conservative in recording "hits." Could you convince your grandmother?, if not, list as a "hypothetical protein" but do take a stand -"putative DNA polymerase" is cowardly
- "Grandmother rule"

Use Consistent Nomenclature

- All of these describe homologs of the products related to the coliphage T4 *rIIA* protein!

rIIA protector from prophage-induced early lysis
protector from prophage-induced early lysis
protector from prophage-induced early lysis rIIA
membrane-associated affects host membrane ATPase
rIIA membrane-associated affects host membrane ATPase
phage rIIA lysis inhibitor
rIIA protector
rIIA
rIIA protein
membrane integrity protector
hypothetical protein
unnamed protein product !!!!!!
protein of unknown function

Nomenclature Sins

- ❑hypothetical protein → DNA polymerase with no or poor quality evidence is far worse than:
  DNA polymerase → hypothetical protein
- ❑Miss-annotation creep → database poisoning

- ❑Be cautious about employing BLASTp hits in naming proteins – is there additional evidence to back the designation up?

Resources

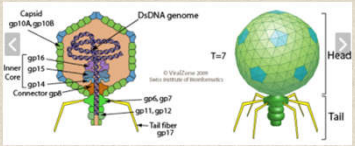
- ❑UniProt Knowledgebase (UniProtKB) is a catalog of information on proteins with is manually curated and reviewed (check **Proteomes**). (https://www.uniprot.org/). Includes a BLAST feature.

Entry	Entry name		Protein names	Gene names	Organism
P00806	ENLYS_BPT7		**Endolysin**	3.5	Enterobacteria phage T7 T7)
P00581	DPOL_BPT7		**DNA-directed DNA polymerase**	5	Enterobacteria phage T7 T7)
P03696	DNBI_BPT7		**Single-stranded DNA-binding protein...**	2.5	Enterobacteria phage T7 T7)
P03726	EXLYS_BPT7		**Peptidoglycan transglycosylase gp16**	16	Enterobacteria phage T7 T7)
P00638	EXRN_BPT7		**Exonuclease**	6	Enterobacteria phage T7 T7)
P00969	DNLI_BPT7		**DNA ligase**	1.3	Enterobacteria phage T7 T7)
P00641	ENDO_BPT7		**Endonuclease I**	3	Enterobacteria phage T7 T7)
P19726	CAPSA_BPT7		**Major capsid protein**	10	Enterobacteria phage T7

e.g. "capsid protein" versus head protein

Resources 2

- ❑ ViralZone (https://viralzone.expasy.org/) - a knowledge resource to understand virus diversity. Click on proteome for any viral genus.
- ❑ Linked to UniProt Knowledgebase (UniProtKB)



Extending the annotation

- using the phage_proteins.faa file
- general protein motif searches:
  - Batch Web CD-Search Tool - https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi
    Change "Expect value" to 0.00001
  - InterPro - https://www.ebi.ac.uk/interpro/
  - Open latter results (*.tsv) in Excel

InterPro

- Under "Advanced options" consider running separately:

Families, domains, sites & repeats

☑ NCBIfam ☑ SFLD ☑ PANTHER ☑ HAMAP ☑ PROSITE profiles ☑ PROSITE patterns ☑ SMART ☑ CDD ☑ PRINTS
☑ Pfam ☑ PIRSF

Other sequence features

☑ Phobius ☑ SignalP ☑ Coils ☑ MobiDBLite ☑ TMHMM ☑ SignalP_EUK ☑ SignalP_GRAM_POSITIVE
☑ SignalP_GRAM_NEGATIVE ☑ AntiFam ☑ FunFam ☑ PIRSR

Transmembrane domains

- using the protein.faa file
- a good resource:
  - DeepTMHMM - https://services.healthtech.dtu.dk/services/DeepTMHMM-1.0/
- in the case of Jeff six proteins, all identified as "hypothetical proteins," were identified to possess transmembrane domains (TMDs)
- in *.gbk file change "hypothetical protein" to "hypothetical membrane protein" and add: /note="three transmembrane domains identified using DeepTMHMM"

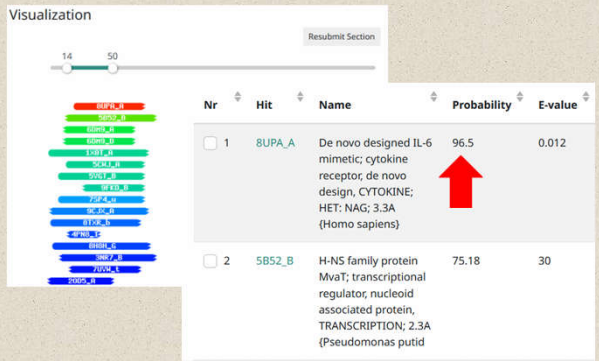
HHpred

- ❑ Identification based upon similar structure

https://toolkit.tuebingen.mpg.de/tools/hhpred

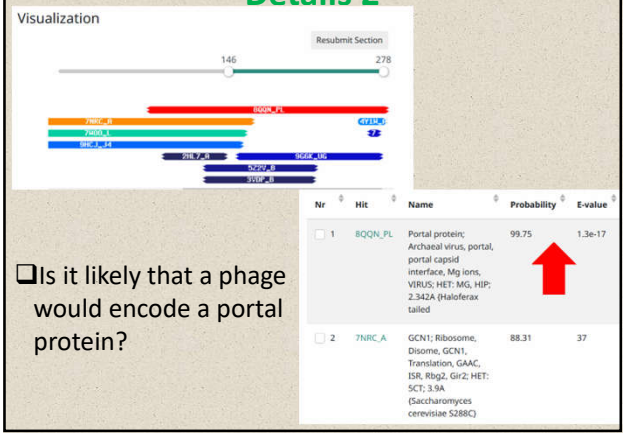
- ❑ Introduction - https://seaphages.org/video/87/
- ❑ HHpred is useful for remote protein homology detection and structure prediction
- ❑ cannot be run remotely in batch mode
- ❑ worth remote installation if you are seriously into phages

Details



❑Is it likely that a phage would encode a cytokine?

Details 2



❑Is it likely that a phage would encode a portal protein?

Phage therapy important questions

- ❑ Is my phage temperate or lytic?
- ❑ Does it carry virulence determinants?
- ❑ Does it carry antimicrobial resistance genes?

  - ➢ The latter two points are dealt with by DFAST
  - ➢ Alternative approaches listed next

Phage therapy important questions

- ❑ Is my phage temperate or lytic?
  - ➢ Phage Lifestyle Prediction Servers

Phage AI

  - ➢ https://www.phage.ai/
  - ➢ requires registration

PhaBOX

  - ➢ https://phage.ee.cityu.edu.hk/phabox
  - ➢ PhageGE – Lifestyle prediction
  - ➢ https://jason-zhao.shinyapps.io/PhageGE_Update/

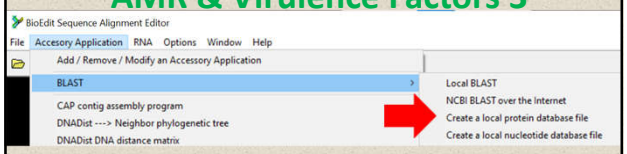
AMR & Virulence Factors

- ❑ Antimicrobial resistance – CARD (Comprehensive Antibiotic Resistance Database) - https://card.mcmaster.ca/
  - ➢ Use RGI Resistance Gene Identifier with DNA sequence at: https://card.mcmaster.ca/analyze/rgi

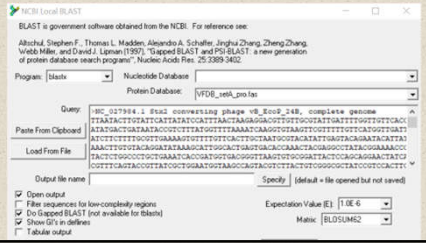
AMR & Virulence Factors 2

- Virulence factors can be found using VFanalyzer at VFDB (Virulence factors of Pathogenic Bacteria)
  - https://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi
  - SLOW
- OR: Local BLAST versus VFDB "Protein sequences of core dataset)" using BioEdit 7.7 (Windows)
  - https://bioedit.software.informer.com/
  - OR UGENE

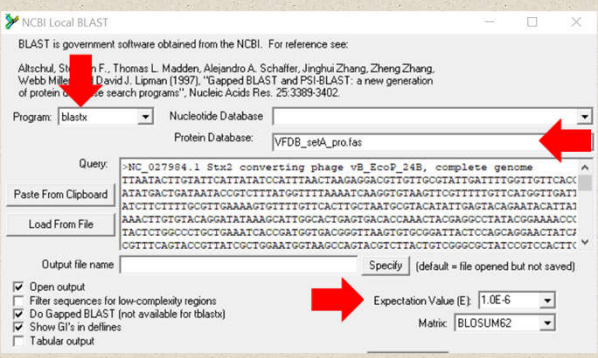
AMR & Virulence Factors 3



- Select "VFDB_setA_pro.fas"



AMR & Virulence Factors 4



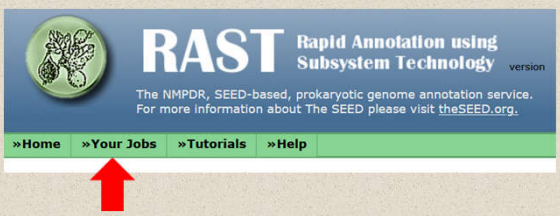
- "Do Search"
- Use caution in interpreting "hits"

End of Part 2

Questions?

Notes on RAST

Create new project in RAST



- ❑ Choose this
- ❑ Select “Upload New Job”

RAST Step 2

File Upload:

Sequences File Browse... Jeff.fasta

Use this data and go to step 2

Contig statistics

Statistic	As uploaded	After splitting into scaffolds
Sequence size	66776	66776
Number of contigs	1	1
GC content (%)	55.6	55.6
Shortest contig size	66776	66776
Median sequence size	66776	66776
Mean sequence size	66776.0	66776.0
Longest contig size	66776	66776
N50 value		
L50 value	1	1

RAST Step 3

Genome information:

Taxonomy ID: 2731619 Fill in form based on NCBI taxonomy-ID.

Use this number for Caudoviricetes

Taxonomy string: Viruses; Duplodnaviria; Heunggongvirae; Uroviricota; Caudoviricetes

Domain: Bacteria Archaea Viruses

Genus:

Insert Virus here

Species: Pseudomonas phage Jeff

Strain:

Genetic Code: 11 (Archaea, most Bacteria, most Virii, and some Mitochondria)
4 (Mycoplasmaea, Spiroplasmaea, Ureoplasmaea, and Fungal Mitochondria)

Use this data and go to step 3

RAST Step 4

Upload a Genome

Complete Upload

Please consider the following options for the RAST annotation pipeline

RAST Annotation Settings:

Choose RAST annotation scheme	RASTtk
Customize RASTtk pipeline	☐ Yes
Automatically fix errors?	☑ Yes
Fix frameshifts?	☐ Yes
Build metabolic model?	☐ Yes
Compute similaritiess?	☐ Yes
Turn on debug?	☐ Yes
Set verbose level	0
Disable replication	☐ Yes

Finish the upload

RAST – waiting for results

RAST Rapid Annotation using Subsystem Technology version

The NMPDR, SEED-based, prokaryotic genome annotation service.
For more information about The SEED please visit theSEED.org.

»Home »Your Jobs »Tutorials »Help

Your upload will be processed as job 1588956. View job status

Go back to the genome upload page to add another annotation job.

You can view the status of your project on the status page.

Job Details #1588956

» Available downloads for this job: RASTtk workflow Download Update download files

» Share this genome with selected users

» Back to the Jobs Overview

Genome Upload has been successfully completed.

Genome ID - Name:	2731619.784 - Virus Pseudomonas phage Jeff
Job:	#1588956
User:	kropinsk
Date:	Thu Feb 20 09:40:01 2025
Genetic code:	11
Annotation scheme:	RASTtk
Preserve gene calls:	no
Automatically fix errors:	yes
Fix frameshifts:	no
Backfill gaps:	yes

Check periodically since this page does not refresh

AK1

RAST Jobs Overview

Job	Owner	ID	Name	Num contigs	Size (bp)	Creation Date	Annotation Progress	Status
1588956	Kropinski, Andrew	2731619.784	Virus Pseudomonas phage Jeff	1	66776	2025-02-20 09:40:01	[view details]	complete

» Browse annotated genome in SEED Viewer

» Available downloads for this job: Genbank Download Update download files

» Share this genome with selected

» View Close Strains for this job

» Back to the Jobs Overview

Genome Upload has been suc

Genome ID - Name: 2731
Job: #158
User: kropi
Date: Thu F
Genetic code: 11
Annotation scheme: RAST
Preserve gene calls: no
Automatically fix errors: yes
Fix frameshifts: no
Backfill gaps: yes

Rapid Propagation has been s

Quality Check has been succ

For detailed explanations of the terms used in our quality report, please refer to our wiki.

- Amino-Acid FASTA file
- DNA Contigs
- EMBL
- EMBL (EC numbers stripped)
- GFF3
- GFF3 (EC numbers stripped)
- GTF
- GTF (EC numbers stripped)
- Genbank
- Genbank (EC numbers stripped)
- Genbank merged (for use in e.g. Artemis)
- Genome Typed Object (GTO)
- Genome directory
- Nucleic-Acid FASTA file
- RASTtk workflow
- Spreadsheet (Excel XLS format)
- Spreadsheet (tab-separated text format)

Cleaning up RAST output

No locus tags

```
CDS             9476..11773
                /db_xref="SEED:fig|2731619.784.peg.21"
                /translation="MFKLSWIFGRKKDSAACSESAPEKVAQIPQHDPLDPMIKLGRIR
                GWNVEPEKAPVIRSVKDFLEPGLSVAMDSAYGDGPTPAAKVAAGGQNPYVVPTMLQDW
                YNSQGFIGHQACAIISQHWLVDKACSMSGEDAARNGWELKSDGRKLSDEQSALIARRD
                MEFRVKDNLVELNRFKNVFGVRIALFVVESDDPDYYEKPFNPDGITPGSYKGISQVDP
                YWAMPQLTAASTADPSAEHFYEPDFWIISGKKYHRSHLVVVRGPQPPDILKPTYLFGG
                IPLTQRIYERVYAAERTANEAPLLAMSKRTSTIHVDVEKAIANEDAFNARLAFWIANR
                DNHGVKVLGIDEGMEQFDTNLADFDSIIMNQYQLVAAIAKTPATKLLGTSPKGFNATG
                EHETISYHEELESIQEHIFDPLLERHYLLLAKSEEIDVQLEIVWNFVDSTSSQQQAEL
                NNKKAATDEIYINSGVVSPDEVRERLRDDPRSGYNRLTDDQAETEPGMSPENLAEFEK
                AGAQSVKAKGEAERAEAQAGAVEGAGAGGPVPAAPRATKPLAKAAEEGASEAAEPPSRPD
                PKAELRNLLVDLLSKLQDLDDIKAPDGVDIEHNDAPGLKRTSKPGVSGMEPSVFSSNR
                IVGPRDHSELQRIKVNGITTLIENPRGSIRQGKDGSWRVQMKHHYGFIKGTKGADGDE
                VDCFVGPNLGSKRVFVVNQVNKEGQFDEHKCMLGFNNINDAKSGYLSCFRPGWDGLGS
                IHEVDLPAFRRWLANGDTTKPFGGE
                /product="Phage protein"
                /transl_table=11
CDS             11773..12609
                /db_xref="SEED:fig|2731619.784.peg.22"
                /translation="MAFKASKKRERRAPLPVGRGKPIIPSAGIEAWYRKQMKDMSKLM
                ISDYRNEIEKALSQPAAERFFARDESVNVLFKMTLRSLQQRWSRIFEGFAAKIAPEFV
                NRTEEAATAATLHSLSVAGVDQPRAAYNESVKNTLEAATTYNHTLITKIQEEVHEKIY
                TSVMLSLTSPNPEEQGTSGITNALRKVGKFSEDRIELIARDQTSKLYSSLSDERMAEN
                GVEEFEWLHSSAGKTPRHTHLEKDGKRFKLNDPRLWEGPKADQGPPGWAINCRCRKIP
                VI"
                /product="Phage minor capsid protein"
                /transl_table=11
```

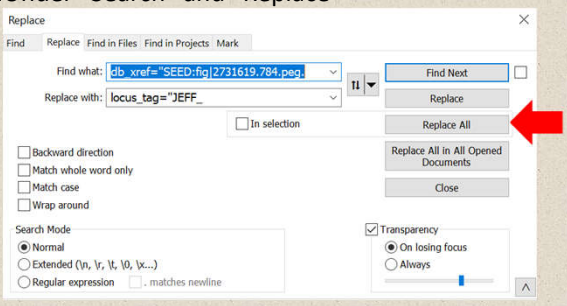
Phage is redundant

Slide 59

AK1 Andrew Kropinski, 2025-02-20

Clean-up using Notepad++

- Under “Search” and “Replace”



- Under “Search” and “Find” modify “Phage”

Final version

```
CDS             9476..11773
                /locus_tag="JEFF_21"
                /translation="MFKLSWIFGRKKDSAACSESAPEKVAQIPQHDPLDFMIKLGRIR
                GWNVEPEKAPVIRSVKDFLEPGLSVAMDSAYGDGPTPAAKVAAGGQNPYVVPTMLQDW
                YNSQGFIGHQACAIISQHWLVDKACSMSGEDAARNGWELKSDGRKLSDEQSALIARRD
                MEFRVKDNLVELNRFKNVFGVRIALFVVESDDPDYYEKPFNPDGITPGSYKGISQVDP
                YWAMPQLTAASTADPSAEHFYEPDFWIISGKKYHRSHLVVVRGPQPPDILKPTYLFGG
                IPLTQRIYERVYAAERTANEAPLLAMSKRTSTIHVDVEKAIANEDAFNARLAFWIANR
                DNHGVKVLGIDEGMEQFDTNLADFDSIIMNQYQLVAAIAKTPATKLLGTSPKGFNATG
                EHETISYHEELESIQEHIFDPLLERHYLLLAKSEEIDVQLEIVWNPVDSTSSQQQAEL
                NNKKAATDEIYINSGVVSPDEVRERLRDDPRSGYNRLTDDQAETEPGMSPENLAEFEK
                AGAQSVKAKGEAERAEAQAGAVEGAGGPVPAAPRATKPLAKAAEEGASEAAEPPSRPD
                PKAELRNLLVDLLSKLQDLDDIKAPDGVDIEHNDAPGLKRTSKPGVSGMEPSVFSSNR
                IVGPRDHSELQRIKVNGITTLIENPRGSIRQGKDGSWRVQMKHHYGFIKGTKGADGDE
                VDCFVGPNLGSKRVFVVNQVNKEGQFDEHKCMLGFNNINDAKSGYLSCFRPGWDGLGS
                IHEVDLPAFRRWLANGDTTKPFGGE"
                /product="hypothetical protein"
                /transl_table=11
CDS             11773..12609
                /locus_tag="JEFF_22"
                /translation="MAFKASKKRERRAPLPVGRGKPIIPSAGIEAWYRKQMKDMSKLM
                ISDYRNEIEKALSQFAAERFFARDESVNVLFKMTLRSLQQRWSRIFEGFAAKIAPEFV
                NRTEEAATAATLHSLSVAGVDQPRAAYNESVKNTLEAATTYNHTLITKIQEEVHEKIY
                TSVMLSLTSPNPEEQQTSGITNALRKVGKFSEDRIELIARDQTSKLYSSLSDERMAEN
                GVEEFEWLHSSAGKTPRHTHLEKDGKRFKLNDPRLWEGPKADQGPPGWAINCRCRKIP
                VI"
                /product="minor capsid protein"
                /transl_table=11
```

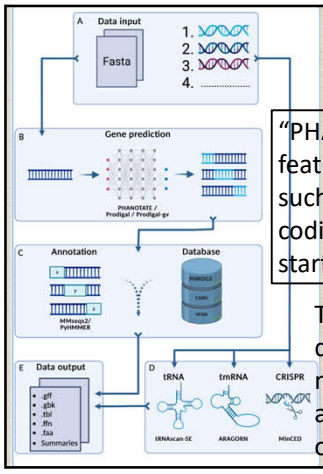
Notes on Pharokka

Pharokka – the only phage-specific pipeline

Galaxy EUROPE

Google Colab

Pharokka workflow



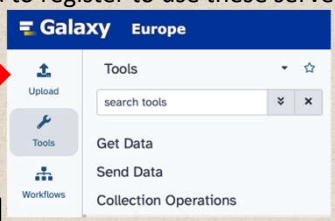
"PHANOTATE considers unique features of phage genomes such as small gene size, high coding density and alternative start codons"

The average protein-coding density of bacterial genomes is 87 % (McCutcheon and Moran 2012). Phage often >95%

Pharokka via Galaxy

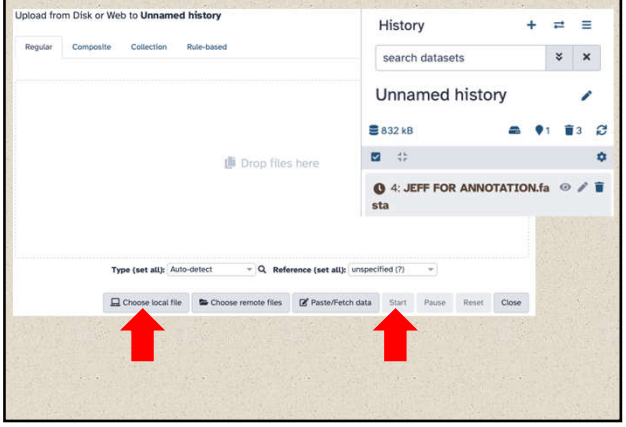
Galaxy EUROPE

- https://usegalaxy.eu/
- Also available at: https://usegalaxy.org.au/
- Need to register to use these servers

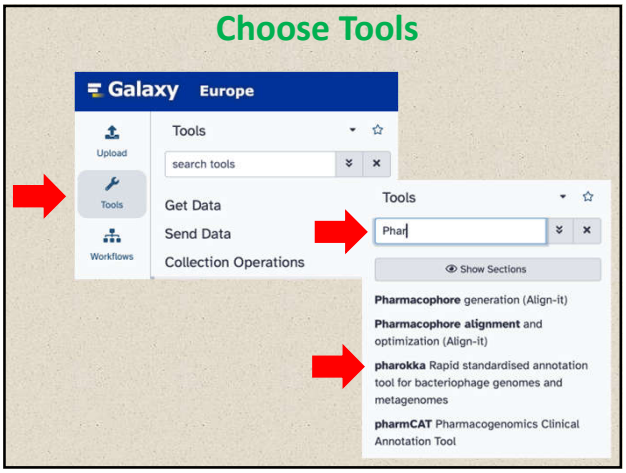


Start here

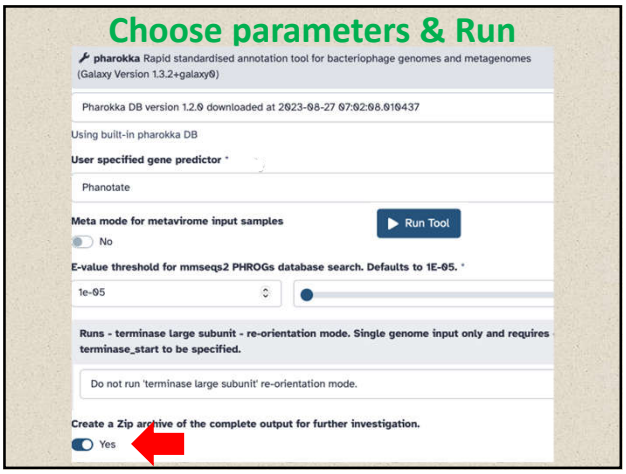
Uploading genome sequence



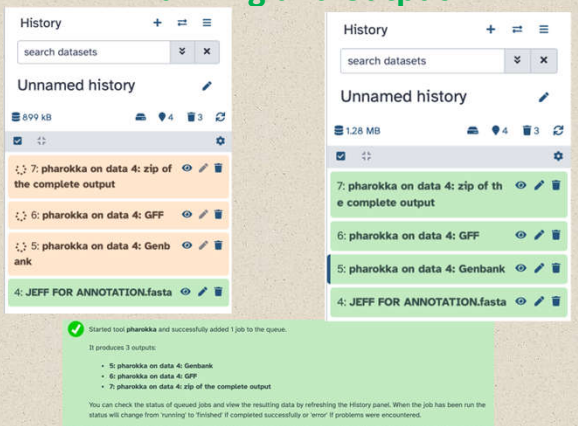
Choose Tools



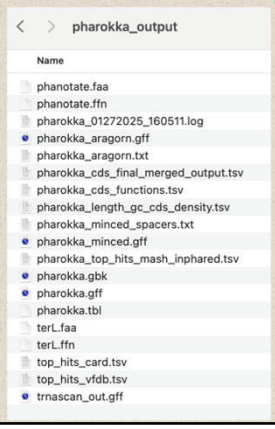
Choose parameters & Run



Running and output



Output



- ❑GenBank Flat File (*.gbk)
- ❑GFF3-formatted File (*.gff)
- ❑Genome Fasta File (*.fna)
- ❑Protein Fasta File (*.faa)
- ❑CDS Fasta File (*.fna)
- ❑RNA Fasta File (*.fna)
- ❑Feature Table (*.tsv)

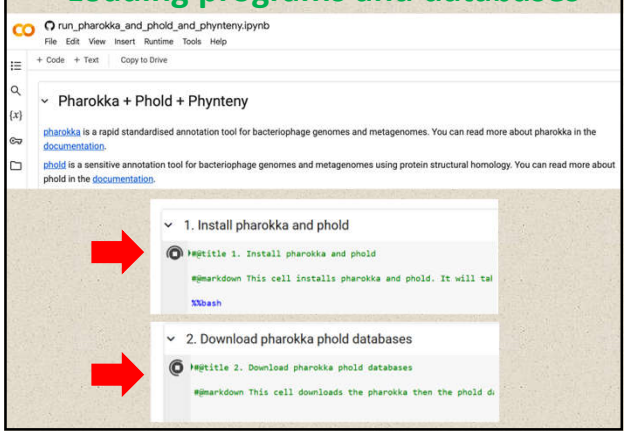
Pharokka via Google Colab

- ❑ https://colab.research.google.com/github/gbouras13/phold/blob/main/run_pharokka_and_phold_and_phynteny.ipynb
- ❑ Need to register to use this server
- ❑ Windows with Google Chrome **NOT** Mac M1/ Safari

Advantages of this version over Galaxy version

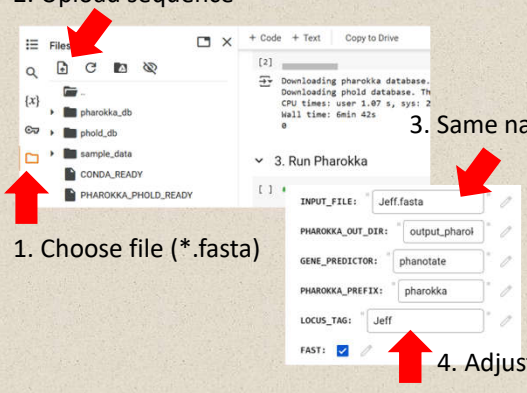
- ❑ Allows one to specify the Locus tag name – use name of phage
- ❑ Automatically generates zipped file

Loading programs and databases



Loading sequence

2. Upload sequence



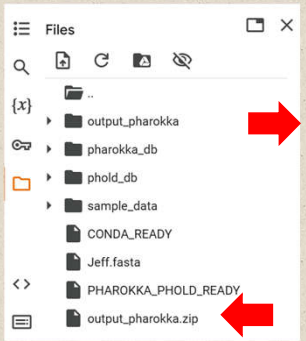
3. Same name

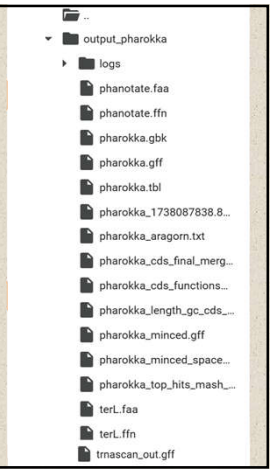
1. Choose file (*.fasta)

4. Adjust

Running Pharokka

Output





Problems with Pharokka

- ❑ locus_tags include the word CDS and are sequential from 0001 – N.B. phages don't have thousands of genes
- ❑ locus_tag numbers increase by one unit – N.B. if a CDS is missing you will have to use A, B, C after the number.
- ❑ translation code may not be included in *.gbk file.
- ❑ you cannot change the translation code (11). N.B. Some large, uncultivated phages of the gut microbiome - predominantly Lak phages and crAssphages - have recoded the TAG or TGA stop codon (genetic codes 15 and 4).

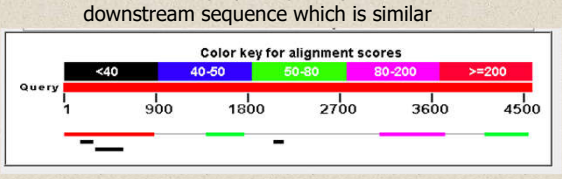
Problems with Pharokka 2

- annotation is **not perfect**
- splicing not recognized
- in GenBank record this is reported as CDS join(99941..100807,101606..103957)
- important with members of the *Herelleviridae*, such as *Staphylococcus* phages; and, *Campylobacter* phages

Introns and Inteins – rare but

- the gene encoding the aerobic ribonucleoside diphosphate reductase (large subunit) from *Campylobacter* phage vB_CcoM-IBB_35 is located on a 4.5 kb region which homologous to a 2.3 kb region from *Klebsiella* phage vB_KleM-RaK2. N.B. proteins have similar mass.

N.B. the thin grey line joining blocks indicates a downstream sequence which is similar

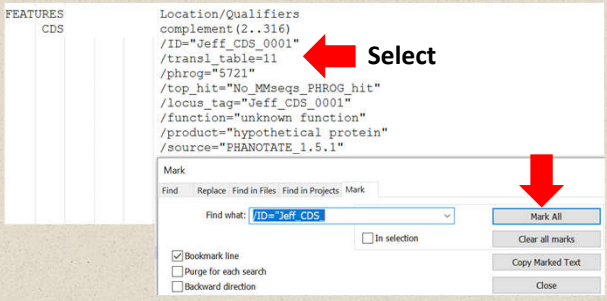


Editing primary annotation (*.gbk) files

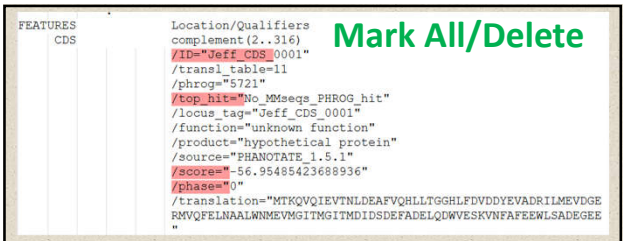
- No matter what program you use you will have to edit the results
- Notepad++ is better than Notepad

Open in Notepad++

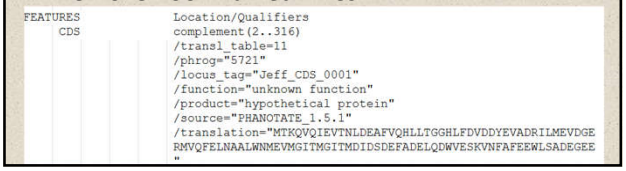
- ❑ Install from: https://notepad-plus-plus.org/
- ❑ Review: https://www.youtube.com/watch?v=wVfbFe57h2o
- ❑ Under: “Search” choose “Mark” and “Mark All”



Mark All/Delete

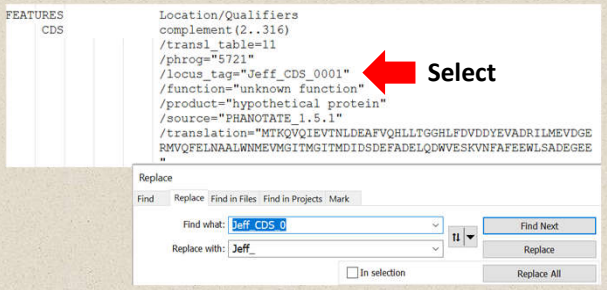


- ❑ Under “Search”, choose “Bookmark” and “Remove Bookmarked Lines”



Modify locus_tag

- ❑ Under “Search” choose “Replace” and “Replace All”



- ❑ Changes /locus_tag=“Jeff_CDS_0001“ to /locus_tag=“Jeff_001"

Progress

```
FEATURES             Location/Qualifiers
     CDS             complement(2..316)
                     /transl_table=11
                     /phrog="5721"
                     /locus_tag="Jeff_001"
                     /function="unknown function"
                     /product="hypothetical protein"
                     /source="PHANOTATE_1.5.1"
                     /translation="MTKQVQIEVTNLDEAFVQHLLTGGHLFDVDDYEVADRILMEVDGE
                     RMVQFELNAALWNMEVMGITMGITMDIDSDEFADELQDWVESKVNFAFEEWLSADEGEE
                     "

     CDS             complement(65814..66776)
                     /transl_table=11
                     /phrog="5834"
                     /locus_tag="Jeff_101"
                     /function="tail"
                     /product="tail length tape measure protein"
                     /source="PHANOTATE_1.5.1"
                     /translation="HQARPVGRAIHRRIVVSKLIPFNSIDGDVKFKLEVVDEAEIVLVD
                     VSVSTSQFRKFWPIADSLCWDEEKAEFVPRYKSSYLQGATEFSNRALLAFAAGQYYLRA
                     DQGRKDESEGVTSDESLTAMELDRENSRLDECLQNSIAREDKTAEVNRQLQLRIRDLEA
                     EVESKNSAIKGHQRRIDELLKHSQNLSAQNVEFSLELESKKTLLEALDKSLETLMADKD
                     RLEAAAKSAIDLENKAAETNSQLALRIRELEAELESQKALREESDRSLDGSIANANNQY
                     KLACEAREALSRAREDAEFYRIGMQRIAEIANRKPFEDC"
```

Edit top of *.gbk file

```
    Pseudomonas            66776 bp    DNA     linear   PHG 28-JA
ON  Pseudomonas phage Jeff, complete genome.
N   Pseudomonas
    Pseudomonas
    .
    .
SM  .
    .
            Location/Qualifiers
```

↓

```
    Jeff            66776 bp    DNA     linear   PHG 28-JAN-2025
ON  Pseudomonas phage Jeff, complete genome.
N   Pseudomonas phage Jeff
    Pseudomonas phage Jeff
    .
    .
SM  Pseudomonas phage Jeff
    Viruses; Duplodnaviria; Heunggongvirae; Uroviricota; Caudoviri
    Pbunavirus; Pseudomonas phage Jeff
            Location/Qualifiers
```

Editing DEFINITION, SOURCE and ORGANISM

- ❑ DEFINITION - Pseudomonas phage Jeff, complete genome
- ❑ SOURCE - Pseudomonas phage Jeff
- ❑ ORGANISM Pseudomonas phage Jeff

Viruses; Duplodnaviria; Heunggongvirae; Uroviricota; Caudoviricetes; Pbunavirus; Pseudomonas phage Jeff.

This will be discussed on the last day of this workshop BUT in many cases the taxonomy can be readily assessed from the BLASTN hits

Pharokka protein function summary

pharokka_cds_functions.tsv

Description	Count
CDS	101
connector	1
DNA, RNA and nucleotide metabolism	8
head and packaging	6
lysis	1
other	3
tail	13
unknown function	69
integration and excision	0
moron, auxiliary metabolic gene and host takeover	0
transcription regulation	0
tRNAs	0
CRISPRs	0
tmRNAs	0
VFDB_Virulence_Factors	0
CARD_AMR_Genes	0

Odd – Endolysin, holin, spannins

68%

Therapeutic potential

Pharokka/phanotate output

>Jeff_CDS_0101 tail length tape measure protein
HQARPVGRAIHRRIVVSKLIPFNSIDGDVKFKLEVVDEAEIVLVDVSV
STSQFRKFWPIADSLCWDEEKAEFVPRYKSSYLQGATEFSNRALLAF
AAGQYYLRADQGRKDESEGVTSDES.... **(total 320 amino acids)**

WEIRD

- ❑ BLASTp or PSI-BLAST versus Caudoviricetes (taxid:2731619)

tail length tape-measure protein [Pseudomonas phage E79]

Sequence ID: AXF41778.1 Length: **305** Number of Matches: **1**

Range 1: 1 to 305 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
603 bits(1555)	0.0	Compositional matrix adjust.	301/305(99%)	302/305(99%)	0/305(0%)

```
Query  16   VSKLIPFNSIDGDVKFKLEVVDEAEIVLVDVSVSTSQFRKFWPIADSLCWDEEKAEFVPR  75
            +SKLIPFNSIDGDVKFKLEVVDEAEIVLVDVSVS SQFRKFWP ADSLCWDEEKAEFVPR
Sbjct  1    MSKLIPFNSIDGDVKFKLEVVDEAEIVLVDVSVSASQFRKFWPTADSLCWDEEKAEFVPR  60
```