Agriculture & Agri-Food Canada Phage Genomics Workshop

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Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada

UNIVERSITY of GUELPH

Acknowledgement

We thank Brian Anderson from DNASTAR Inc. for access to the latest version of Lasergene Suite software

DNASTAR

Biography

- Been working on phages since mid-1960s
- Held academic and government research positions
- Currently advise students & faculty at the University of Guelph – Adjunct Professor
- Past Chair, Bacterial and Archaeal Viruses Subcommittee of ICTV
- Genome Advisor to NCBI
- Sequenced: >150 phages
- Default-setting bioanalyst - Online Analysis Tools (http://molbiol-tools.ca)

If you're not a programmer...You're not a Bioinformatician! John H.E. Nash (PHAC)

- Apologies: I am a Windows and Mac person not a Unix/Linux user

Workshop Outline

- ❑ Phage genome assembly – emphasis on Illumina paired-end data
- ❑ Autoannotation coupled with manual proofreading
- ❑ Phage taxonomy

Objectives

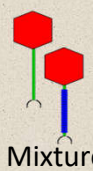
By the end of this workshop participants will

- ❑ have a deeper understanding of the steps involved in sequencing, assembling, and annotating phage genomes
- ❑ understand how phages are classified
- ❑ have an authoritative list of Internet resources and recommended software (commercial and free) for genome analysis.

PART 1

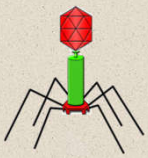
Genome Assembly

What We Want

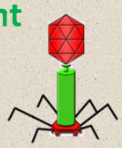


Mixture

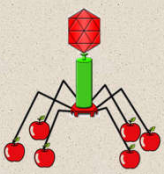
Incomplete



The goal:
- complete
- accurate



Frameshift



Faulty assembly
- chimeric phages

Outline

Paired-end Illumina sequence data

↓ Assemble
DNASTAR SeqMan NGen18

Primary assembly - contigs

↓ Trim
DNASTAR SeqMan Ultra
Reassemble

Reassembly

↓ Manipulation – RC, Cut/Paste, Splinting
SeqBuilder Pro

Genome for Annotation

Why emphasis on DNASTAR?

- ❑ Developed by geneticist Fred Blattner and computing science student John Schroeder (1984)
- ❑ I have been using their software since 1995
- ❑ Company responds readily to enquiries
- ❑ Software packages are **really** updated annually
- ❑ Available for Mac and PCs
- ❑ Intuitive software
- ❑ Excellent tutorials/videos

Non-commercial Alternatives

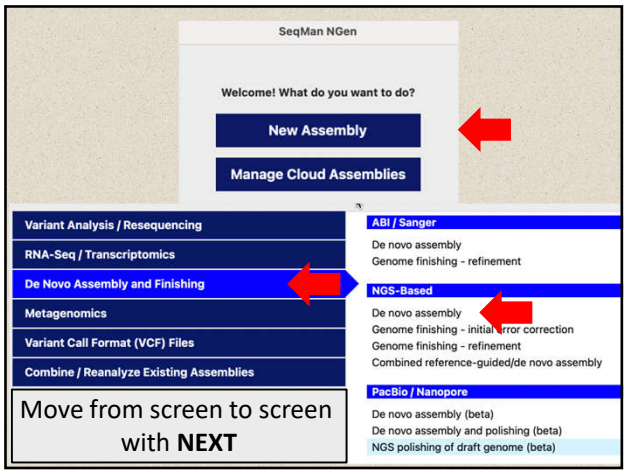
- SPAdes Genome Assembler
  - works with Ion Torrent, PacBio, Oxford Nanopore, and Illumina paired-end
  - Linux, macOS
  - URL: https://github.com/ablab/spades
  - Reference: Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. Using SPAdes De Novo Assembler. Curr Protoc Bioinformatics. 2020 Jun;70(1): e102. doi: 10.1002/cpbi.102. PMID: 32559359.
- **N.B.** I do not recommend Nanopore for phage sequencing

Setup for sequence assembly & analysis

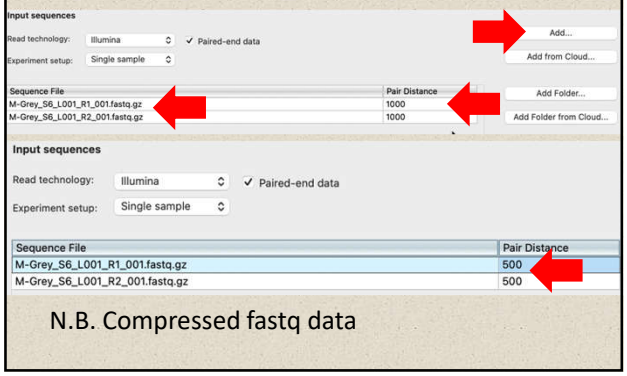
- Create a directory with the name of the phage under analysis
- Create four subdirectories:
  - Data
  - Assembly
  - Reassembly
  - Annotation

Genome assembly using SeqMan NGen18

- Step-by-Step

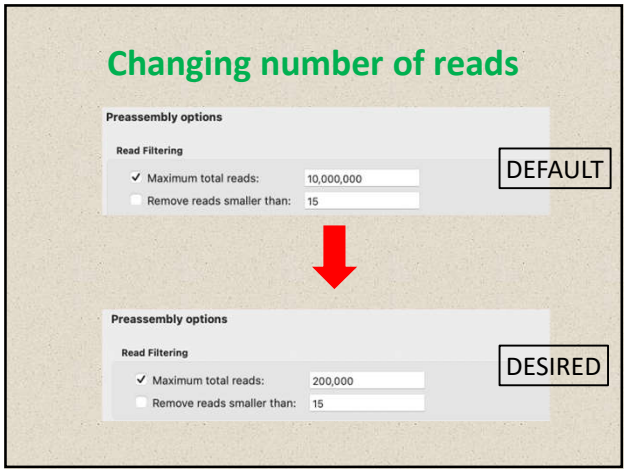


Adding Illumina data

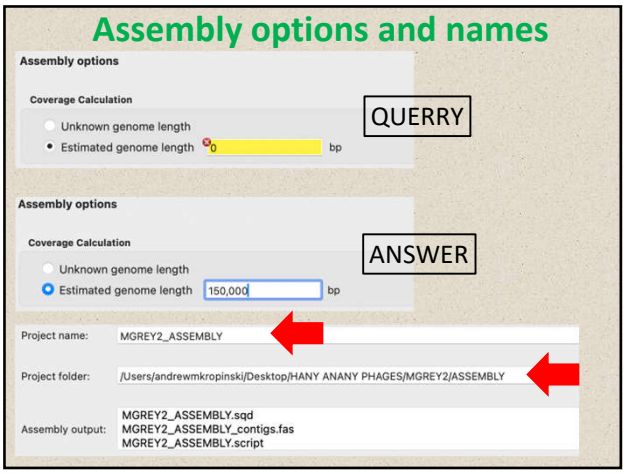


N.B. Compressed fastq data

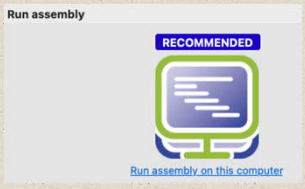
Changing number of reads



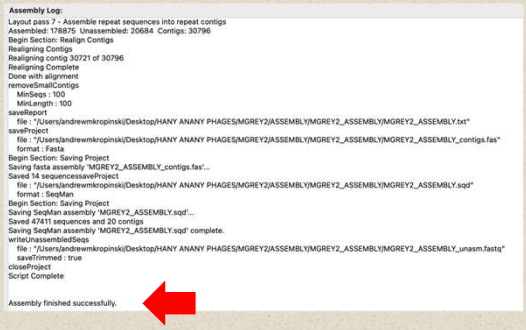
Assembly options and names



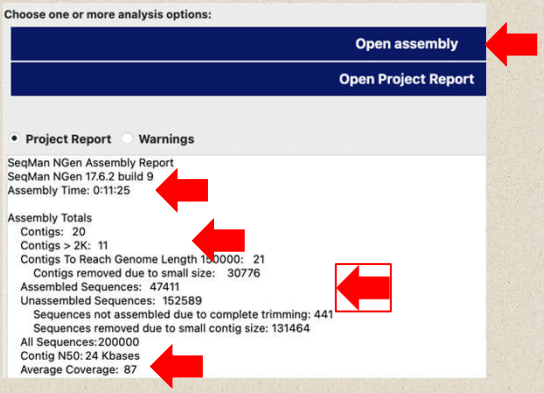
Run



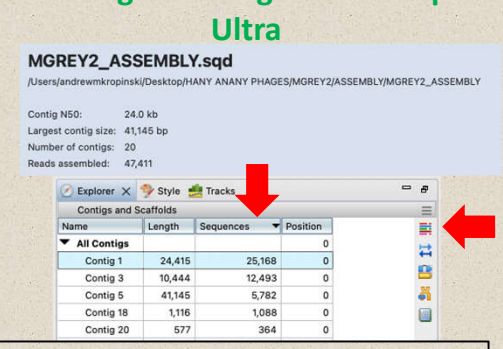
Finished



Useful data



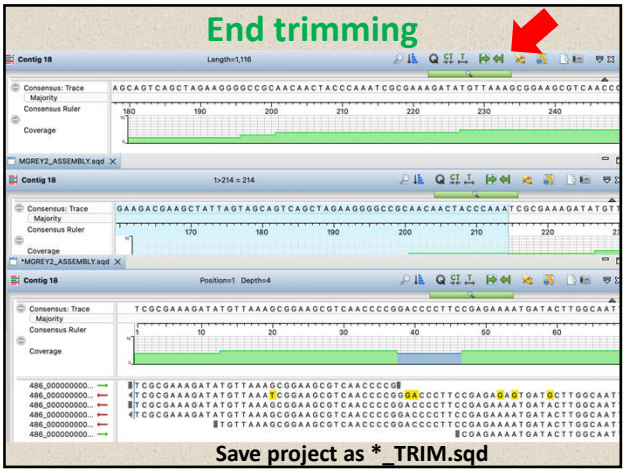
Examining & editing data in SeqMan Ultra



Click here to order by number of sequences

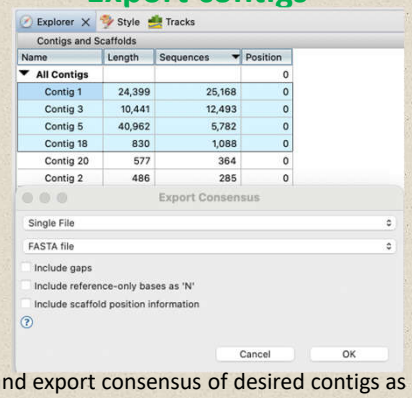
Click here to see a specific contig

End trimming



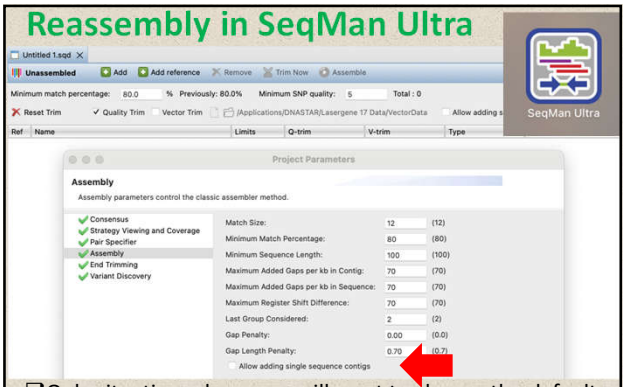
Save project as *_TRIM.sqd

Export contigs



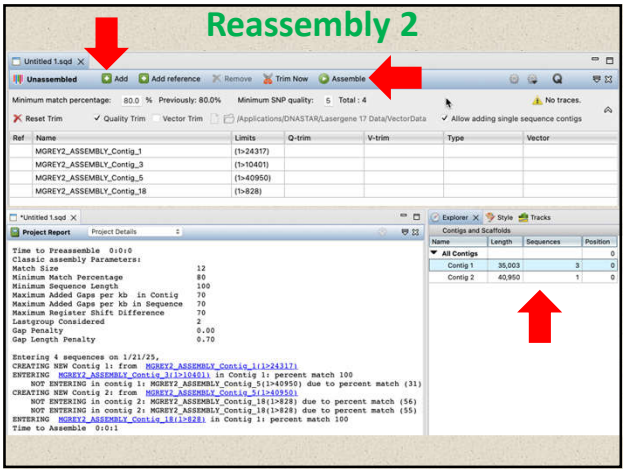
- Select and export consensus of desired contigs as a single file in fasta format (*.fas)

Reassembly in SeqMan Ultra

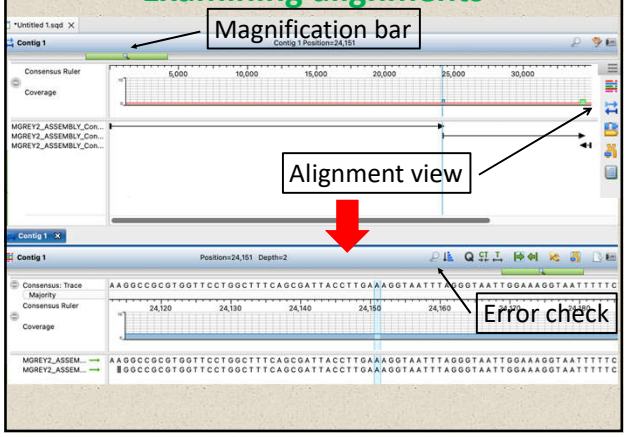


- Only situation where you will want to change the default assembly parameters
- Click to select

Reassembly 2

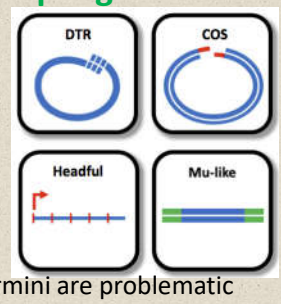


Examining alignments



Genome orientation - termini

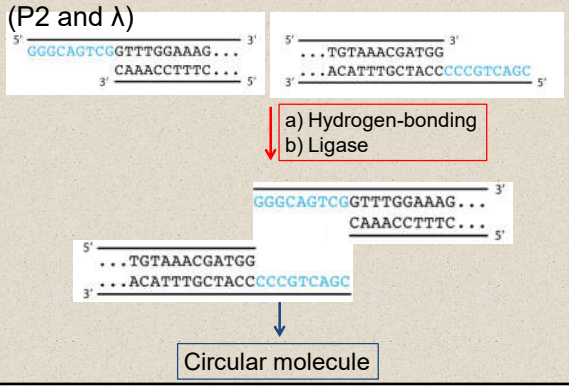
Bacteriophage Genome Termini



- ❑ Phage termini are problematic
- ❑ Bottom line – if your phage is related to an existing virus its genome should be colinear
- ❑ If a choice exists use RefSeq/ICTV homologs
- ❑ **Next six slides are for information only**

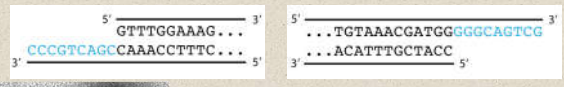
Termini of *Caudoviricetes*

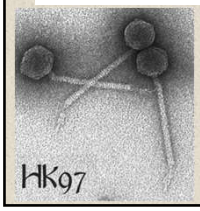
A. Cohesive (sticky) ends – 5'-extensions (P2 and λ)



Termini of Caudoviricetes 2

B. Cohesive (sticky) ends – 3'-extensions (HK97, D3, many mycobacteriophages)



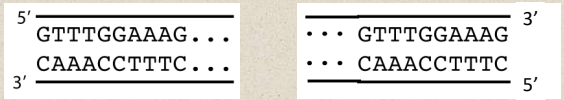


Site-specific packaging

(http://www.pitt.edu/~duda/HK97.html)

Termini of Caudoviricetes 3

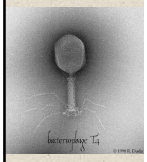
C. Terminal redundancy (TR; direct repeats (DR)) – *Autographivirinae* (T7, SP6, ϕKMV), T5 phage, *Listeria* phage A511

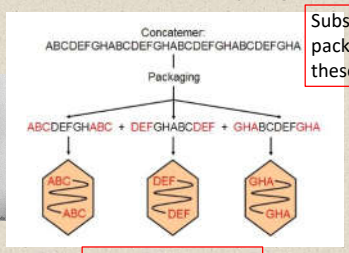


- *Escherichia* phage T7 – 160 bp
- *Listeria* phage A511 – 3,125 bp
- *Escherichia* phage T5 – 10,219 bp
- *Bacillus* phage SPO1 – 13,185 bp

Termini of Caudoviricetes 4

D. Terminal redundancy (TR) with Circular Permutation (CP) – *Escherichia* phages T4 and P1





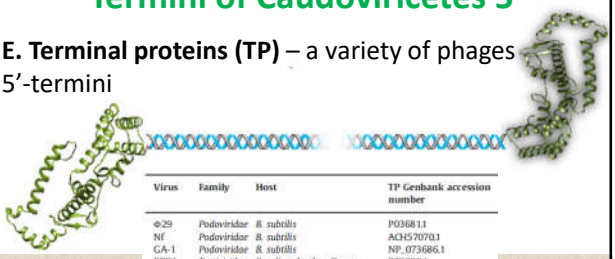
Substrate for packaging of all these phages

Headful packaging

N.B. No member of the class Caudoviricetes possess a circular genome

Termini of Caudoviricetes 5

E. Terminal proteins (TP) – a variety of phages 5'-termini

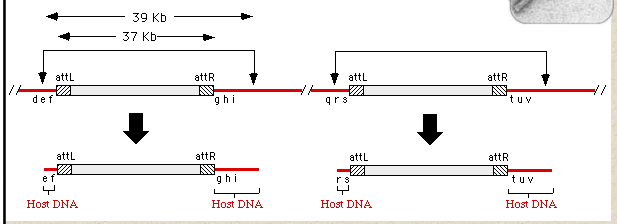


Virus	Family	Host	TP Genbank accession number
Φ29	*Podoviridae*	*B. subtilis*	P03681.1
Nf	*Podoviridae*	*B. subtilis*	ACH57070.1
GA-1	*Podoviridae*	*B. subtilis*	NP_073686.1
PRD1	*Tectiviridae*	*E. coli* and other Gram-negative	P09009.1
Bam35	*Tectiviridae*	*B. thuringiensis*	NP_943750.1*
Cp-1	*Podoviridae*	*S. pneumoniae*	NP_044816.1
Av-1	*Podoviridae*	*Actinomyces sp*	YP_001333658*
ΦCP24R	*Podoviridae*	*Clostridium perfringens*	AEW47836.1*
Asco-28	*Podoviridae*	*Lactococcus lactis*	ACA21480.1*
ΦYS61	Myoviridae	*Thermus thermophilus*	YP_006560295.1*

(http://www.sciencedirect.com/science/article/pii/S0042682214003742)

Termini of Caudoviricetes 6

F. Host DNA – coliphages Mu & D108



(http://www.sci.sdsu.edu/~smaloy/MicrobialGenetics/topics/transposons/Mu.html
http://2012.igem.org/Team:Marburg_SYNMIKRO/Project)

Termini of Caudoviricetes

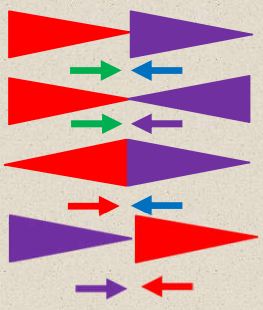
- PhageTerm - Garneau JR et al. 2017. Sci Rep. **7(1):**8292. doi: 10.1038/s41598-017-07910-5. PMID: 28811656.
- accessible via Galaxy Pasteur (https://galaxy.pasteur.fr/)
- video "How to run PhageTerm tool in Galaxy" https://www.youtube.com/watch?v=9y2gfUSLkgg
- for terminal repeats you can use the magnification bar in DNASTAR

What to do about gaps

- Occasionally assembly result in two or more contigs which will not collapse into one.
- Gap closure techniques:
  - Primer walking and Sanger sequencing – requires specialize sequencing abilities
  - PCR and Sanger sequencing
  - Splinting and reference-guided assembly

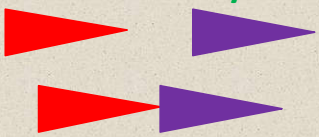
PCR and Sanger sequencing

- Two fragments
- Possibilities:



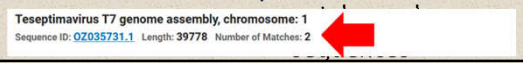
- Sequence amplicon

Splinting and reference-guided assembly

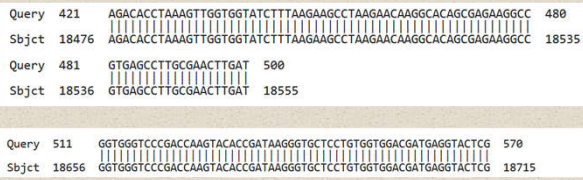


sequenceNNNNNsequence
(pseudogenome)

BLASTn versus Caudoviricetes (taxid: 2731619); exclude - Uncultured/environm

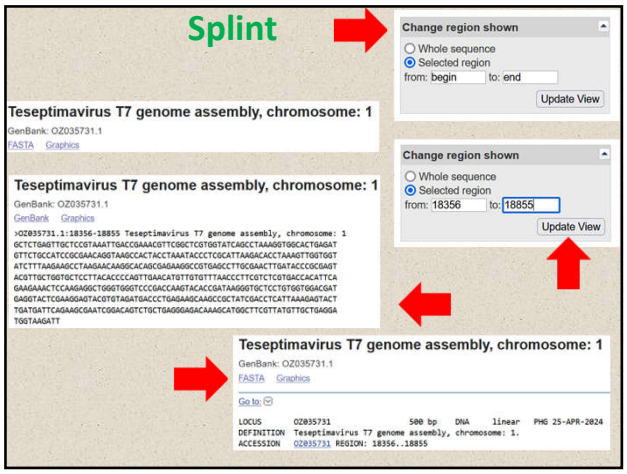


BLASTn results & splint



- ❑ gap in homologous genome of 99 bp (18556-18655)
- ❑ splint – homologous regions (200 bp) plus missing sequence
- ❑ 18356 - 18855

Splint



What next

- ❑ use splint to generate a single contig
- ❑ use this contig as the template in a reference-guided assembly with your Illumina sequence data.
- ❑ you will be able to export the consensus for further manipulations and analyses.

Nonaligned contigs

- ❑ what are they and should I worry?
  - ➢Unaligned fragments of your phage
  - ➢Host DNA
  - ➢Prophage DNA
  - ➢Second phage

Some general rules on genome termini

- ❑ T4-like phages begin with rIIA gene on complementary strand
- ❑ Many other phages begin with TerS/L

Pseudomonas phage vB_Pae_LESphi2, complete genome

GenBank: OQ594955.1

FASTA Graphics

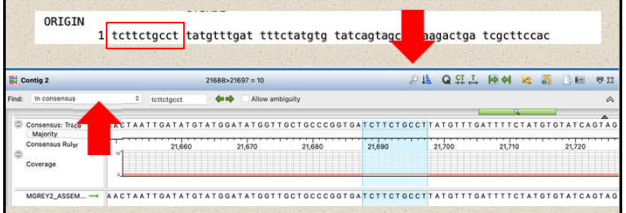
Go to:

```
LOCUS       OQ594955               42123 bp    DNA     linear   PHG 08-MAR-2024
DEFINITION  Pseudomonas phage vB_Pae_LESphi2, complete genome.
ACCESSION   OQ594955
VERSION     OQ594955.1
KEYWORDS    .
SOURCE      Pseudomonas phage vB_Pae_LESphi2
  ORGANISM  Pseudomonas phage vB_Pae_LESphi2
            Viruses; Duplodnaviria; Heunggongvirae; Uroviricota;
            Caudoviricetes.
```

Suppose the only homologous phage to your isolate is this one – shown by BLASTn analysis

Realigning your genome

- ❑ May require RC
- ❑ May require cutting and reassembly using SeqMan Ultra
- ❑ Requires SeqBuilder Pro



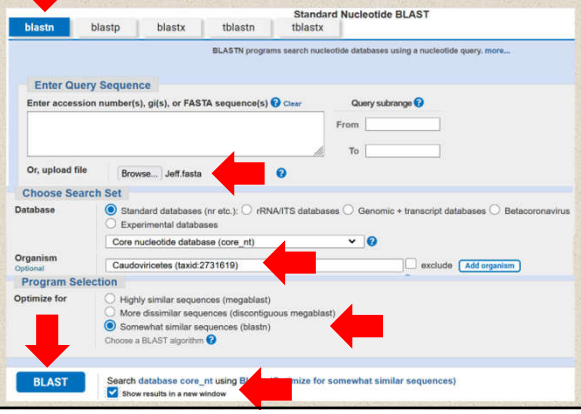
- ❑ Results in your phage genome being colinear with this phage

Is phage ready for annotation?

- ❑ Questions:
1. Is it full length?
2. Is it error free?

- ❑ Quick and dirty approaches:
1. BLASTn
2. BLASTx

BLASTn



BLASTn – complete example?

```
LOCUS       PQ039555              39435 bp    DNA     linear   PHG 18-SEP-2024
DEFINITION  Enterobacter phage vB_Ecl_MII_004, complete genome.
ACCESSION   PQ039555
VERSION     PQ039555.1
KEYWORDS    .
SOURCE      Enterobacter phage vB_Ecl_MII_004
  ORGANISM  Enterobacter phage vB_Ecl_MII_004
            Viruses.
REFERENCE   1  (bases 1 to 39435)
```

- ❑ BLASTn reveals it is complete and related to *Escherichia* phage Peacock (MK903279; 39233 bp)
- ❑ Caudoviricetes; *Autographiviridae; Studiervirinae; Kayfunavirus*

BLASTn – incomplete?

```
LOCUS       PP935704              15914 bp    DNA     linear   PHG 03-AUG-2024
DEFINITION  Salmonella phage vB_SE126_2P, complete genome.
ACCESSION   PP935704
VERSION     PP935704.1
KEYWORDS    .
SOURCE      Salmonella phage vB_SE126_2P
  ORGANISM  Salmonella phage vB_SE126_2P
            Viruses.
REFERENCE   1  (bases 1 to 15914)
```

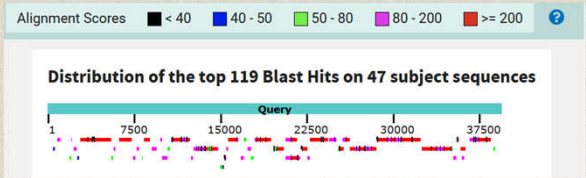
- ❑ BLASTn reveals it is incomplete

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Salmonella phage f18SE, complete genome	Salmonella phage f18SE	21603	22575	100%	0.0	91.90%	41868	NC_028698.
Salmonella phage f2SE, complete genome	Salmonella phage f2SE	21599	22572	100%	0.0	91.89%	41865	KU951146.1
Salmonella phage f3SE, complete genome	Salmonella phage f3SE	21594	22568	100%	0.0	91.89%	41867	KU951147.1
Salmonella phage fSE4S, complete genome	Salmonella phage fSE4S	21581	22554	100%	0.0	91.87%	41768	KT881477.1
Salmonella phage fSE1C, complete genome	Salmonella phage fSE1C	21547	22520	100%	0.0	91.81%	41720	KT962832.1
Salmonella phage vB_SenS_PVP-SE2, complete genome	Salmonella phage vB_SenS_PVP-SE2	18266	22585	100%	0.0	91.92%	42425	NC_073186.

BLASTx - frameshifts

```
LOCUS       PQ039555              39435 bp    DNA     linear   PHG 18-SEP-2024
DEFINITION  Enterobacter phage vB_Ecl_MII_004, complete genome.
ACCESSION   PQ039555
VERSION     PQ039555.1
```

- ❑ BLASTn reveals it is complete and related to *Escherichia* phage Peacock (MK903279; 39233 bp)
- ❑ BLASTx versus Escherichia phage Peacock (taxid:2591100) – **huge number of frameshifts**



What are the problems

- ❑ scientists with little knowledge and experience working with phages
- ❑ individuals not taking advantage of free expertise in the International Committee on Taxonomy of Viruses (ICTV)

- ❑ Nanopore versus Illumina sequencing technology

```
COMMENT     ##Assembly-Data-START##
            Assembly Method       :: Canu v. 1.7.1; Flye v. 2.9; Racon v.
                                     1.4.13
            Coverage              :: 24.22x
            Sequencing Technology :: Oxford Nanopore Technology
            ##Assembly-Data-END##
FEATURES            Location/Qualifiers
```

End of Part 1

Questions?