Comparative phage genomics

Marie-Agnès PETIT

Phage team of the MICALIS dept

INRAE, Jouy en Josas, FRANCE

Once all ORF are called and functions are predicted..

We have a Genbank file

- We would like to view globally the genetic map of this phage
- And to compare it to other, similar phages, to try assign it to a species and a genus

```
LOCUS       Lam4C10             49168 bp    DNA     linear   PHG
DEFINITION  Escherichia virus Lambda4C10, complete genome
ACCESSION   Lam4C10
KEYWORDS    .
SOURCE      Escherichia virus Lam4C10
  ORGANISM  Escherichia virus Lam4C10


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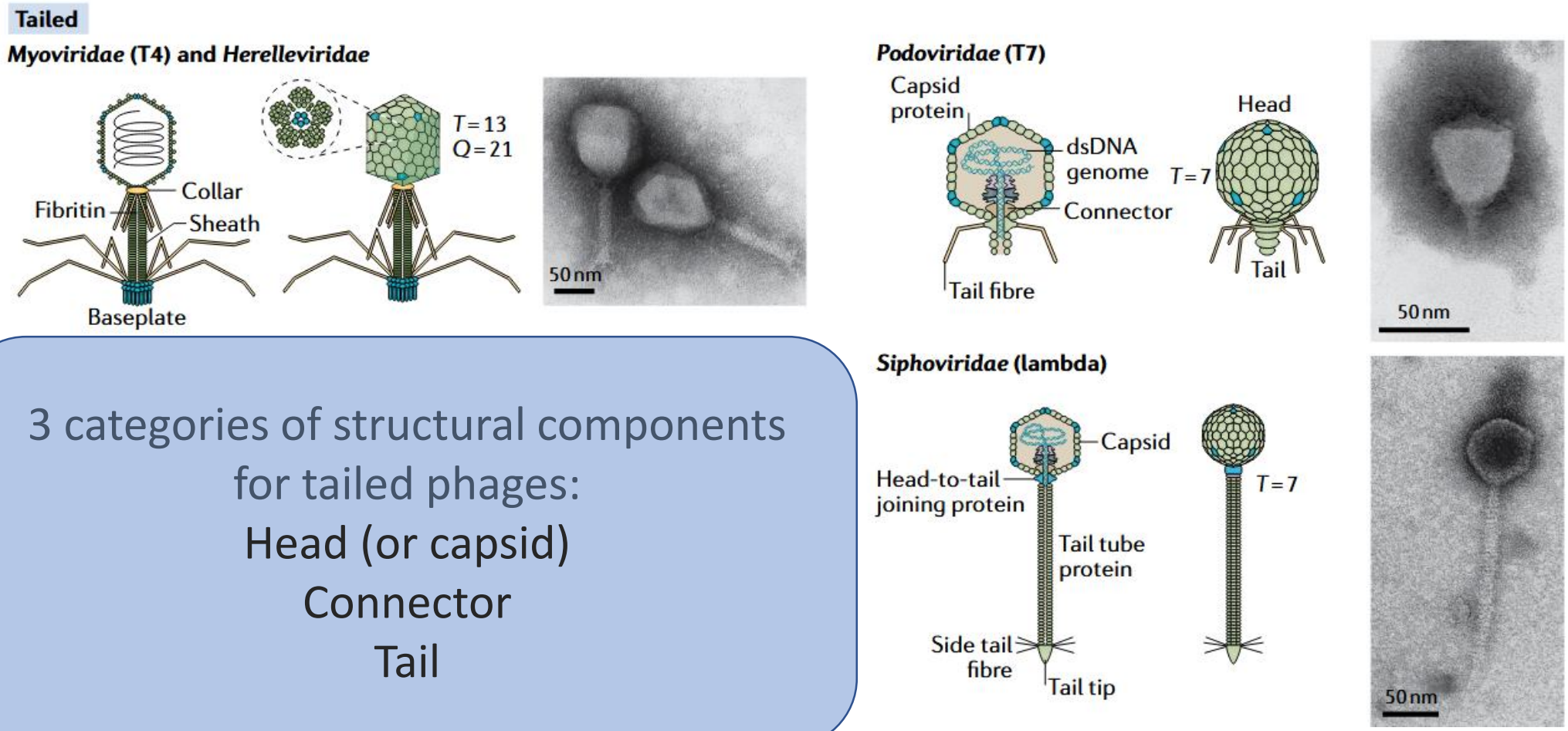
```

The main functions encoded by a phage genome



3 categories of structural components for tailed phages:

Head (or capsid)

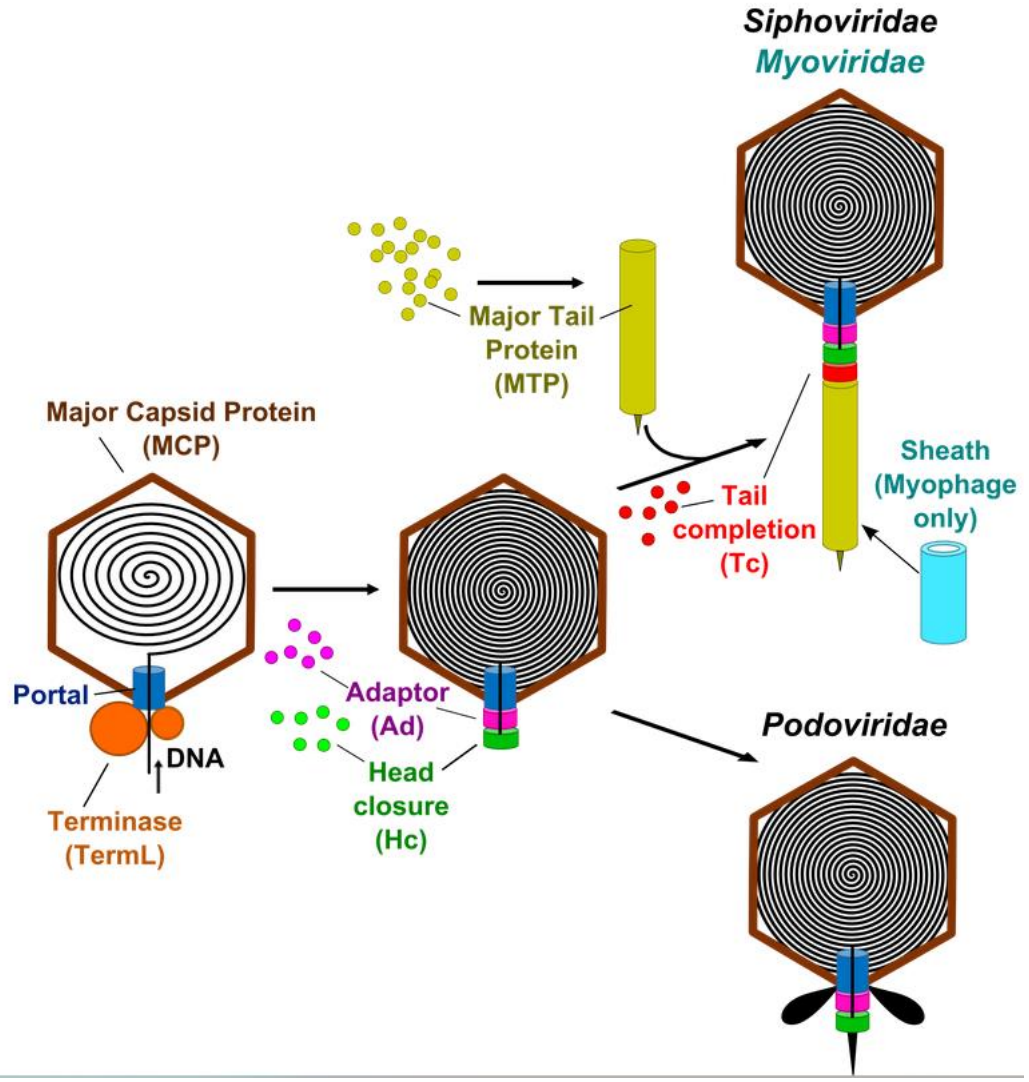
Connector

Tail

Dion et al., Nature Reviews Microbiol. 2020

Of note: Particle shape no longer used for taxonomy, we now say myovirus/siphovirus/podovirus

Conserved functions inside each category



Head:

MCP, decoration, minor head proteins

Portal, Terminase L and S

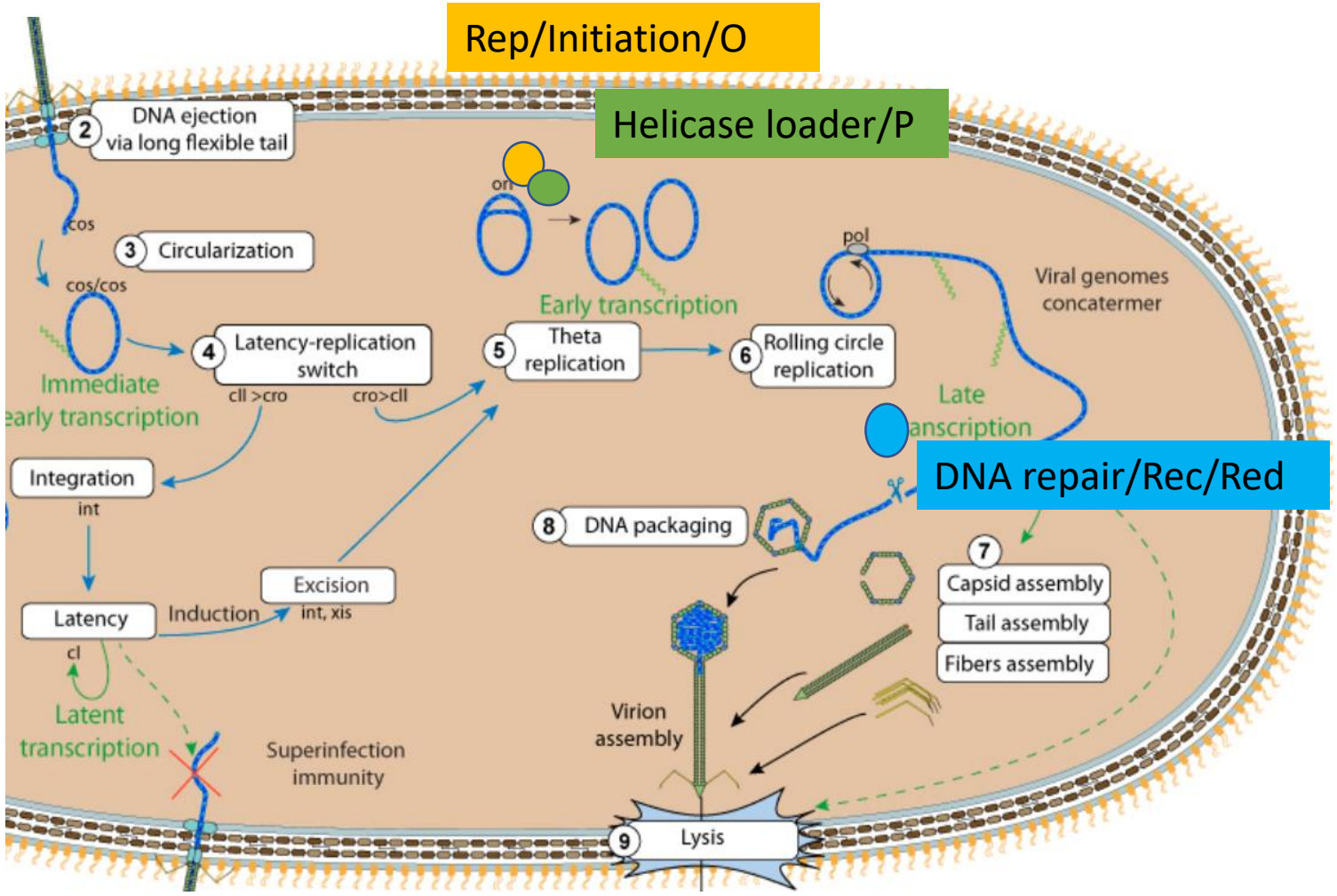
Connector:

Adaptor, head closure, Tail completion (tail terminator/neck)

Tail:

MTP (or tail tube), Sheath, Tail length tape measure, base plate, tail fiber, receptor binding RBP

The replication module



- Replication initiation
- DNA repair

Often taken from the host:

DNA polymerase

Replicative DNA helicase

RNA polymerase

Ribosomes

…

Once all ORF are called and functions are predicted..

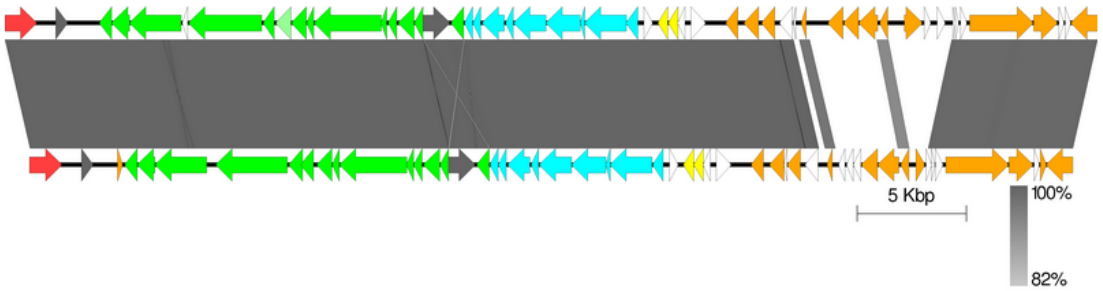
1

- We have a Genbank file
- Global genetic map

→ Use Easyfig

Takes Genbank files as entry

Other softwares: GenoplotR, clinker



Easyfig

Easy genome comparison figures.

Example files

Example 1

Comparison of two ~50 Kb prophage regions from different *Salmonella* genomes. - Easyfig_example1_files.zip

Example 2

Creating a comparison figure of three *Escherichia coli* genomes. - Easyfig_example2_files.zip

---

Windows

Easyfig - Easyfig_2.2.5_win.zip

OSX

Easyfig - Easyfig_2.2.2_OSX.zip

Linux

Easyfig - Easyfig_2.2.2_linux.tar.gz

Preparing the Genbank file

- Create a copy of the gbk file
- Next to each gene with a function predicted, insert a field colour

PHROG functional category	
4	Head
5	Connector
3	Tail
7	DNA, RNA and nt metabolism
2	Lysis (holin, endolysin,…)
10	Integration/excision
6	Transcriptional regulator
12	Morons, AMG, host take-over
	Unknown function (default colour)

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                     GSCQIEKAANESAHFMRFYVPCPHCGEEQYLKFGDDASPFGLKWEKNKPESVFYLCEH
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                     TWVQIVYDWLDALKDPNGLKTFVNTTLGETWEEAVGEKLDHQVLMDKVVRYTAAVPAR
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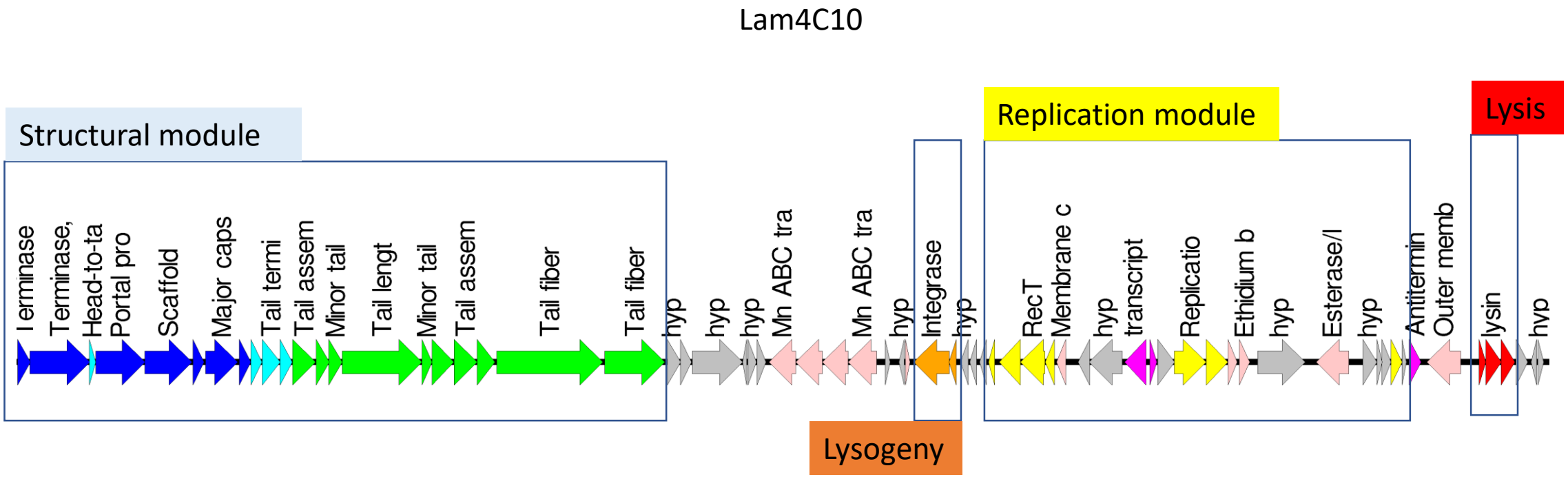
```

Easyfig result



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TAXONOMY?

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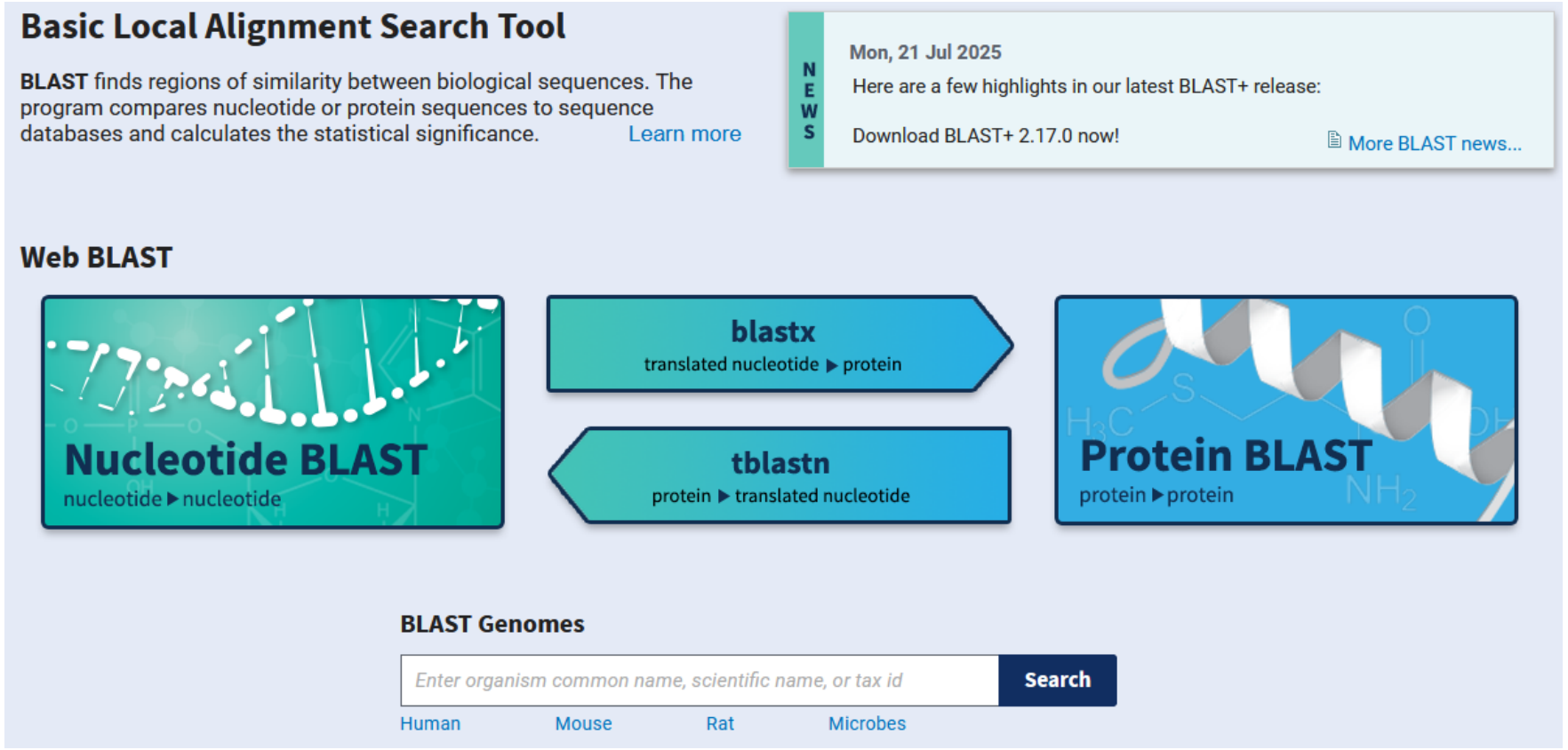
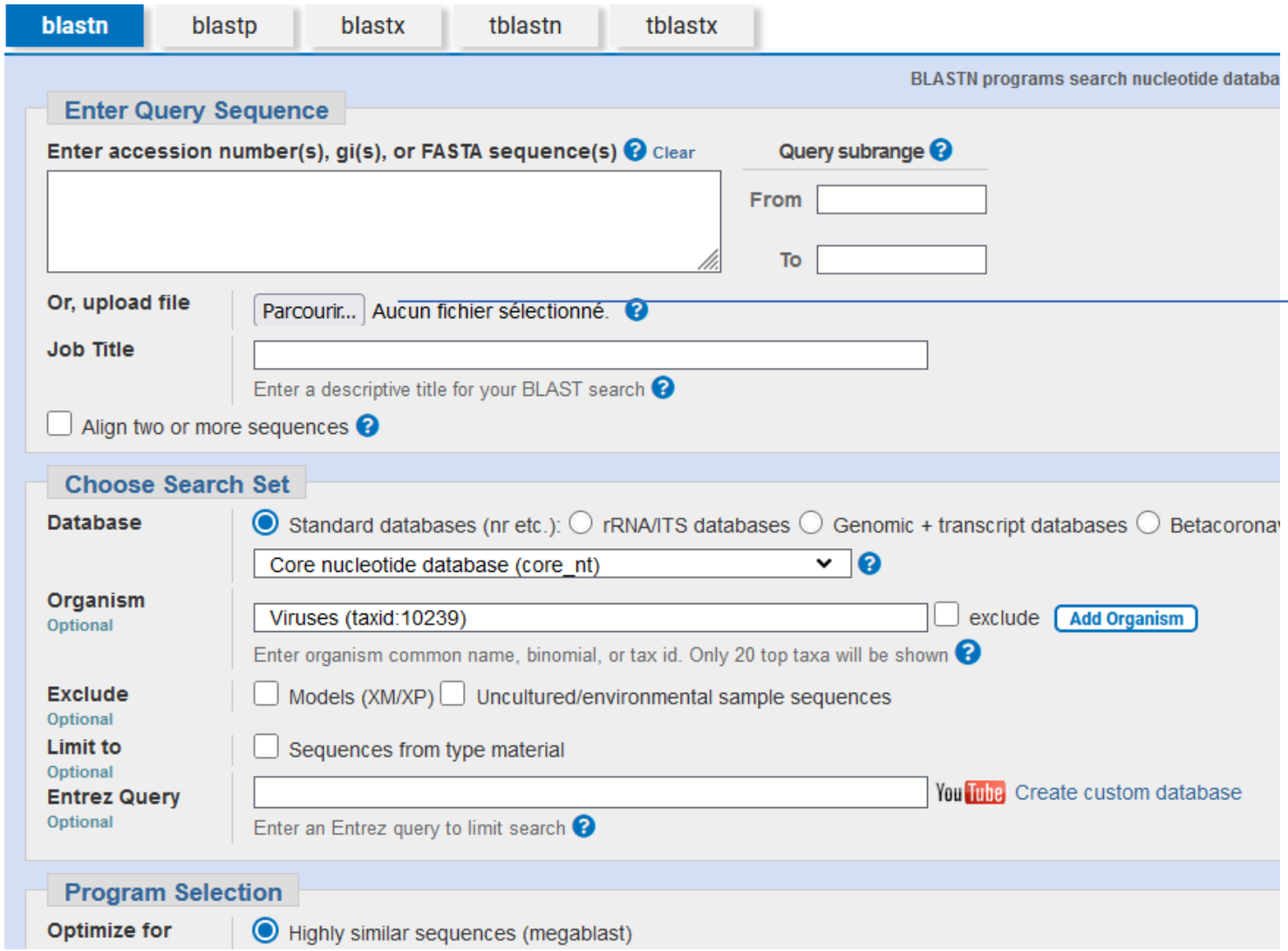
```

A simple and efficient first trial:
whole genome alignment to the NCBI viral database



How to run a nucleotide BLAST



1. Upload a fasta file of your genome:

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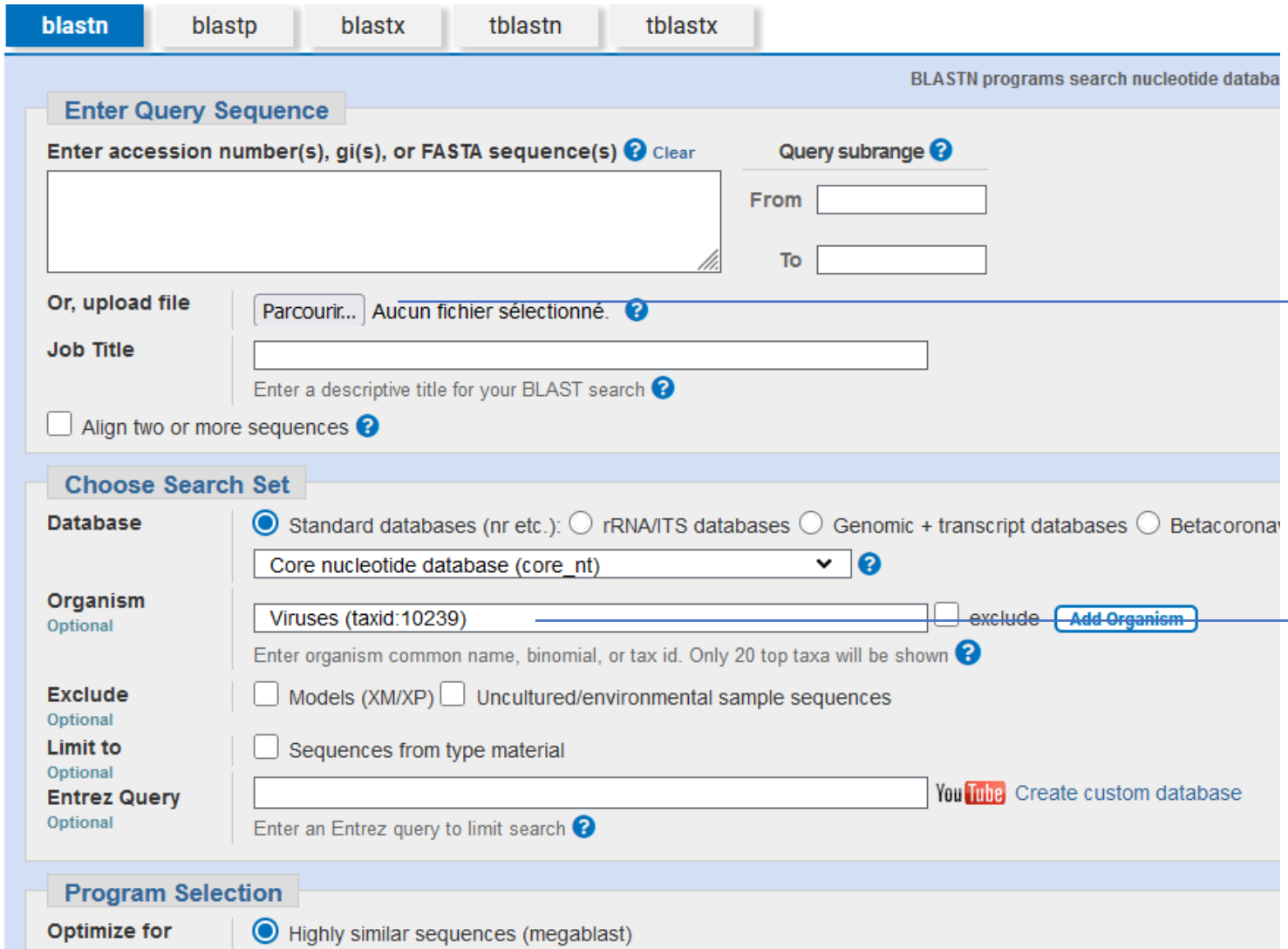
```



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ATGCGCAGAGGGAAACTGATATCGAAAACGAAAAGCTCCGCAAAGAACTG
GACGATTTGCGTGCGGCAGCGGAGTCAGATTTACAACCCGGCACCATTGA
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TGGTGAAAACTGAAATCGCGAAAGCCTCCAATGTTGCAGCTAAGGCCGGT
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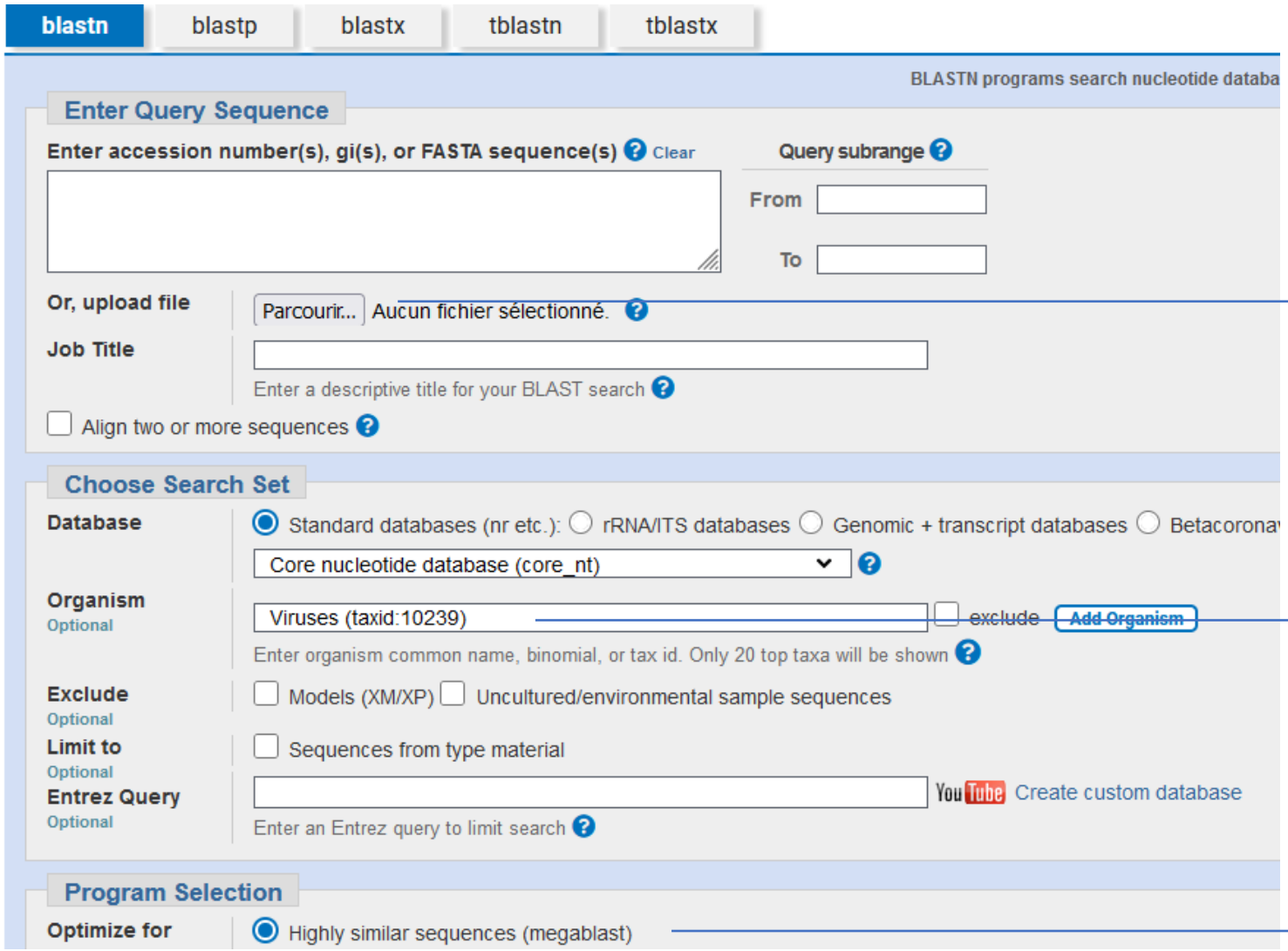
```

2. Align it against all sequenced viral genomes



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TGGTGAAAACTGAAATCGCGAAAGCCTCCAATGTTGCAGCTAAGGCCGGT
GAAAACGTGGGCGGGTGGATCGATGATTTCAGACGCGCAGAAGGCAGCTA
```

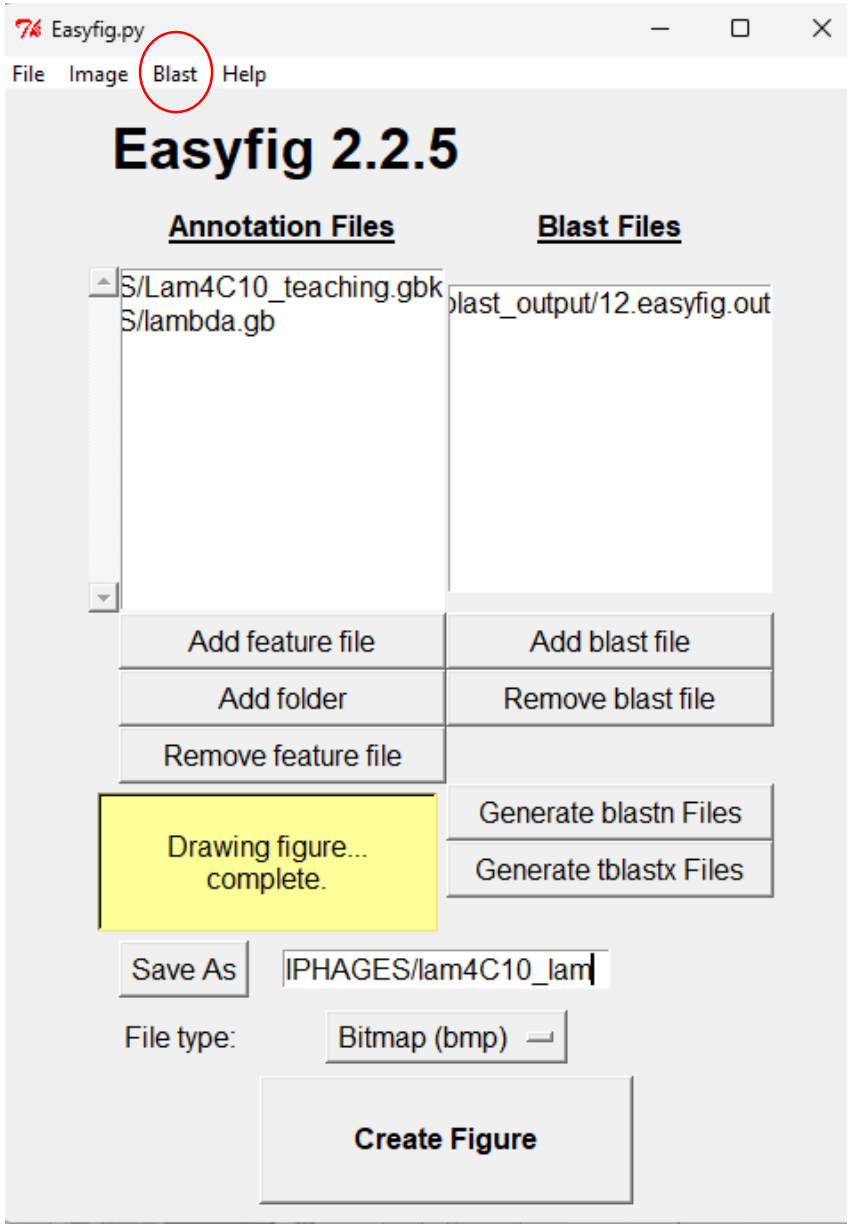
2. Align it against all sequenced viral genomes

3. Megablast is the default, rapid version of Blast

Result

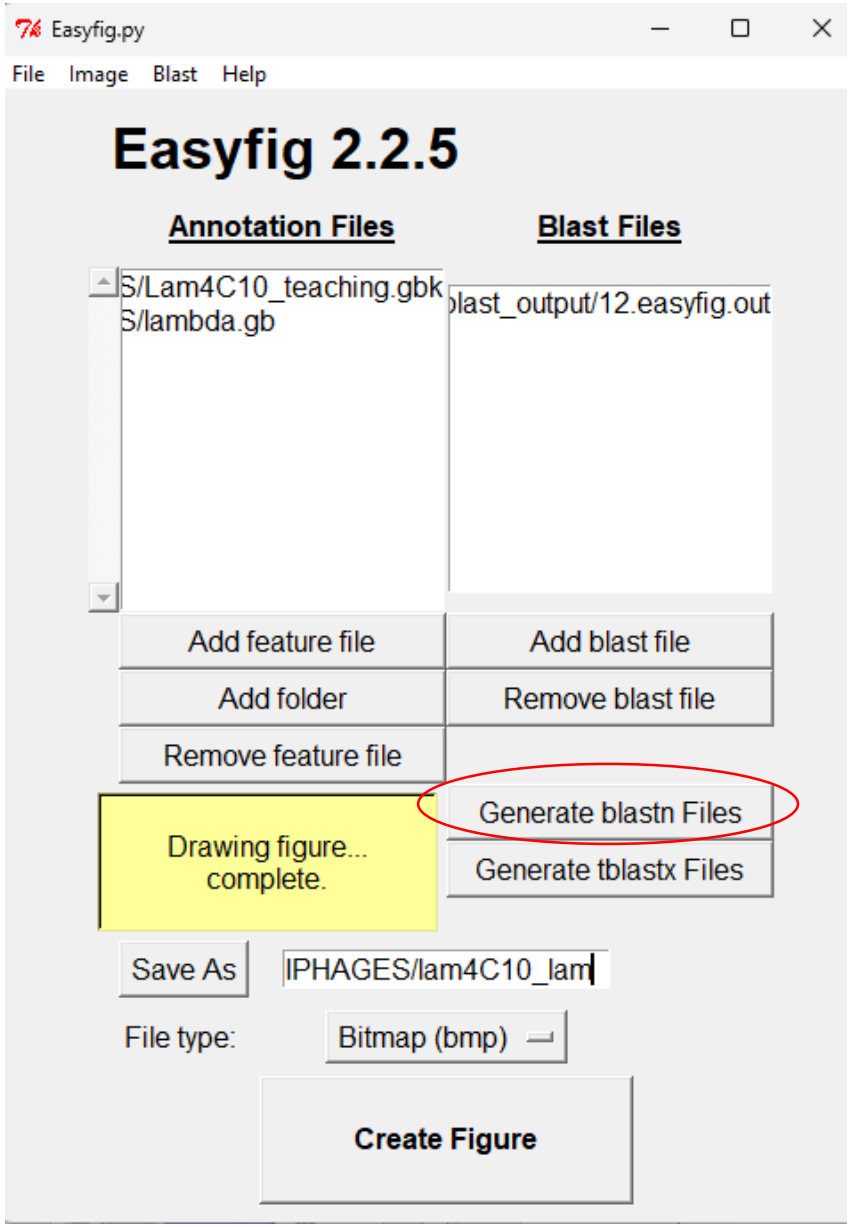
Escherichia phage PhiR63_2, complete genome	Escherichia phage PhiR63_2	30548	61144	73%	0.0	97.47%	48138	PV340574.1
Escherichia phage 21, complete genome	Escherichia phage 21	30531	55753	67%	0.0	97.45%	42931	OL657228.1
Escherichia phage Lambda_ev017 genome assembly, chromosome: 1	Escherichia phage ev017	30348	60912	73%	0.0	97.26%	50126	NC_049948.1
Escherichia phage PhiR57_1, complete genome	Escherichia phage PhiR57_1	30326	60206	72%	0.0	97.24%	49482	PV340572.1
MAG TPA_asm: Bacteriophage sp. isolate ctxLv2, partial genome	Bacteriophage sp.	22432	22811	27%	0.0	97.38%	37726	BK024793.1
Escherichia phage Perceval genome assembly, complete genome: monopartite	Escherichia phage Perceval	21272	60675	71%	0.0	99.84%	50143	OV696612.1
MAG TPA_asm: Caudoviricetes sp. isolate ctm1A1, partial genome	Caudoviricetes sp.	19699	30111	36%	0.0	98.17%	32059	BK032296.1
Escherichia virus Lambda_2G7b genome assembly, chromosome: 1	Escherichia phage 2G7b	17571	33944	43%	0.0	96.35%	47142	NC_049954.1
Escherichia phage PhiR30_1, complete genome	Escherichia phage PhiR30_1	17547	36913	48%	0.0	95.17%	47390	PV340558.1
Escherichia phage PhiR11_1, complete genome	Escherichia phage PhiR11_1	17455	32509	41%	0.0	96.32%	49016	PV340547.1
Escherichia phage PhiR11_1_star, complete genome	Escherichia phage PhiR11_1_star	17455	32509	41%	0.0	96.32%	50509	PV340548.1
Escherichia phage Lambda_ev207 genome assembly, chromosome: 1	Escherichia phage ev207	17350	45613	56%	0.0	96.14%	46685	NC_049949.1
Escherichia phage 434, complete genome	Escherichia phage 434	17341	26291	34%	0.0	96.13%	47993	OL657226.1
Mutant Escherichia phage 434 isolate 434B, complete genome	Escherichia phage 434	17335	29459	39%	0.0	96.12%	47075	OL657227.1
Escherichia phage Turner, complete genome	Escherichia phage Turner	17309	39939	53%	0.0	94.79%	48223	PP925837.1
Escherichia virus Lambda_2H10 genome assembly, chromosome: 1	Escherichia phage 2H10	17237	18020	24%	0.0	94.69%	46288	NC_049950.1
Escherichia phage Lambda h434 imm21, complete genome	Escherichia phage Lambda h434 imm21	17152	24846	32%	0.0	95.81%	43452	OM418627.1
Lambdavirus lambda Lambda_AGU DNA, complete genome	Lambdavirus lambda	17108	24378	31%	0.0	95.80%	48503	LC730321.1
Escherichia phage Lambda imm21, complete genome	Escherichia phage Lambda imm21	17108	24400	31%	0.0	95.80%	46148	OM418625.1
Escherichia phage E26-Berryhill, partial genome	Escherichia phage E26-Berryhill	17102	24605	31%	0.0	95.79%	48850	OM475435.1
Escherichia phage Lambda imm434, complete genome	Escherichia phage Lambda imm434	17102	24367	31%	0.0	95.79%	47326	OM418626.1
Enterobacteria phage HK629, complete genome	Escherichia phage HK629	17102	17485	23%	0.0	95.79%	47288	NC_019711.1
Escherichia phage TPL-02, complete genome	Escherichia phage TPL-02	17102	17102	22%	0.0	95.79%	170231	PQ338057.1
Enterobacteria phage O276, complete genome	Enterobacteria phage O276	17102	24411	31%	0.0	95.79%	41969	NC_049951.1
Escherichia phage 4W, partial genome	Escherichia phage 4W	17102	24604	31%	0.0	95.79%	48634	OM475432.1
MAG: Caudoviricetes sp. isolate 61, complete genome	Caudoviricetes sp.	17102	17102	22%	0.0	95.79%	28800	MN855675.1
Escherichia phage Lambda, complete genome	Escherichia phage Lambda	17102	24486	31%	0.0	95.79%	48507	PV340536.1

- Hit to a well known temperate phage: the phage lambda
- Let us compare lam4C10 to lambda with Easyfig



Comparing the two genomes with Easyfig

1. Download locally the Blast package



Comparing the two genomes with Easyfig

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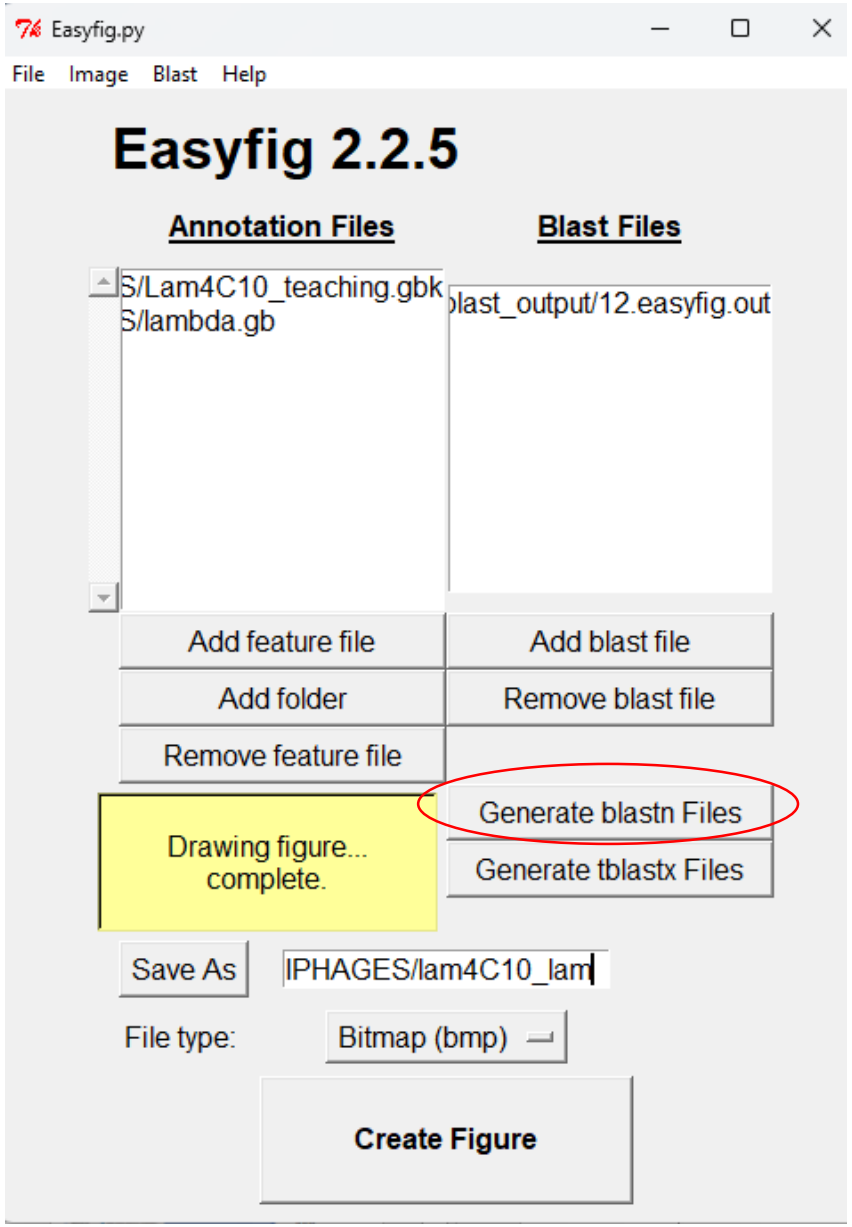
2. Indicate where to store the blastn result, and generate blastn files



Comparing the two genomes with Easyfig

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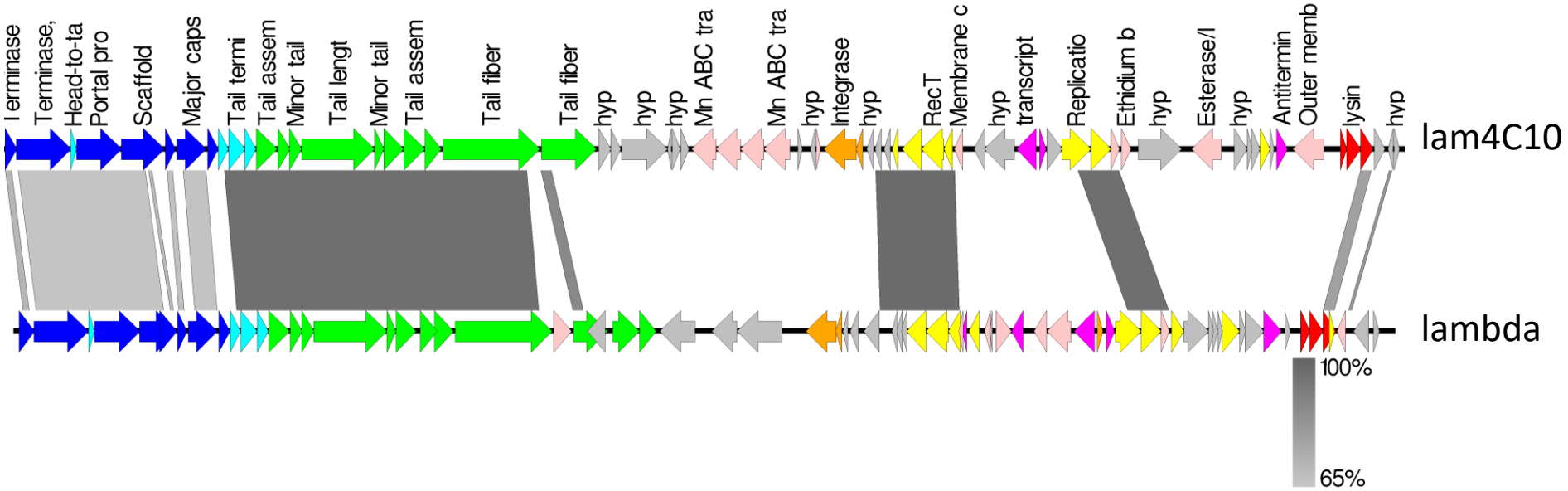
2. Indicate where to store the blastn result, and generate blastn files
3. Push the Create figure button

Result



Conclusions:
Few regions shared: our genome is not the same species/genus as lambda

Definitions:
→ 2 genomes belong to the same **species** if, over more than 80% of their genomes, they share >**95%** identity
→ ‘ ‘ ‘ **genus** ‘ ‘ ‘ **>70%**

lam4C10 defines a new genus, named **Bievrevirus,** and gave its name to the species: **bv4C10**
All new genomes sharing >95% identity with lam4C10 will be of the species « *Bievrevirus bv4C10* »
This is the classical bi-nominal way to name species, we say *Escherichia coli* or *Ratus norvegicus*

To conclude

- Starting from a phage genome in a fasta file format, it is nowadays easy to define

1. Its open reading frames and protein function predictions. This will be practised this afternoon, using the genome of the phage Ecoha you tested this week, and Pharokka
2. Its functional modules, and its overall genomic similarity to other phage genomes, to propose a taxonomic classification at the genus and species level. If we have time, this will also be done this afternoon for Ecoha.