Targetting viral diversity

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Using publicly available data

In order to design primers we must have some knowledge of the viral diversity we intend to capture. One way of gaining this *a priori* knowledge is to utilise publicly available sequence data. If available, public sequences can be used as references to guide primer design.

NCBI

Let's start by going to one of the most comprehensive public repositories for DNA sequence data: NCBI GenBank

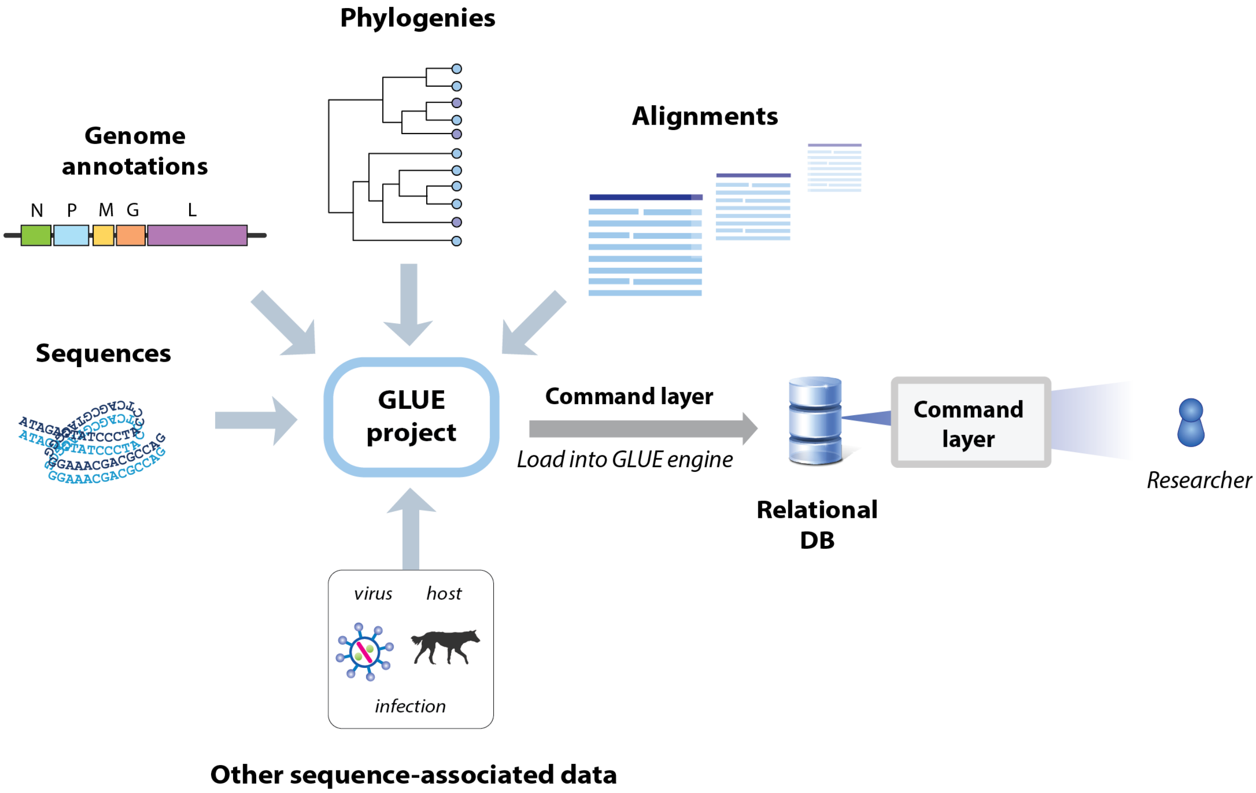
Let's say we want to start sequencing canine rabies virus in Kenya…

Search for "rabies virus kenya"
- How many results do we see?
- Where is the associated metadata?

Now let's try refining the search to find only genome sequences from dogs
- How many results are there?
Try genome sequences from canis
- How many results are there?

It becomes increasing difficult to refine searches in GenBank without using command line based tools. Even then, the tools can only extract from the information in the GenBank file. As we have seen, there is no enforced standardisation of the metadata e.g. a sequence from a domestic dog might be labelled as "dog", "canis lupus familiaris"… etc

Introducing RABV-GLUE



GLUE is an open, integrated software toolkit that provides functionality for storage and interpretation of sequence data.

RABV-GLUE is a rabies specific implementation of the GLUE framework. The web GUI has a number of useful functionalities:
- It curates sequences from GenBank, cleaning-up and standardising the metadata
- Presents results as a data table
- Easy to filter data
- Classifies rabies virus into major and minor clades

Let's attempt the same refined search we tried in GenBank.

Group practical

Getting a dataset from RABV-GLUE

Go to RABV-GLUE's curated database.

Task 1.
Choose a country or global region to investigate. Use filters to search for whole genome sequences from this country/region.

Questions:

1. What clade assignment do these sequences have?

2. Do you think these sequences are suitable for designing primers? Are there any potential issues that you can see?

Click on the accession id of the sequence with host “Mus”, then click to view the **NCBI Nucleotide DB Entry**. Scroll down to the Features section. Note the isolation source.

3. Why might this sequence be a problem?

Navigate back to the RABV-GLUE table (it will still be an open tab).

Task 2.
Open the Filter tab and add another filter to exclude host “Mus”. Download the sequences, changing the default filename to something suitable (keep the “.fasta”). When the file is ready to download **you must click on the link** to get the file, don’t hit ok until the file has downloaded!

Note: imposing a filter on host automatically excludes entries with no host information.

primalscheme

primalscheme is a tool for designing primer panels for multiplex PCR. Go to the primalscheme webpage. It is pretty simple: upload a fasta file, choose an amplicon length and a scheme name, then press enter! However, there are some important things to note before you upload any data (scroll down to instructions).

Check data

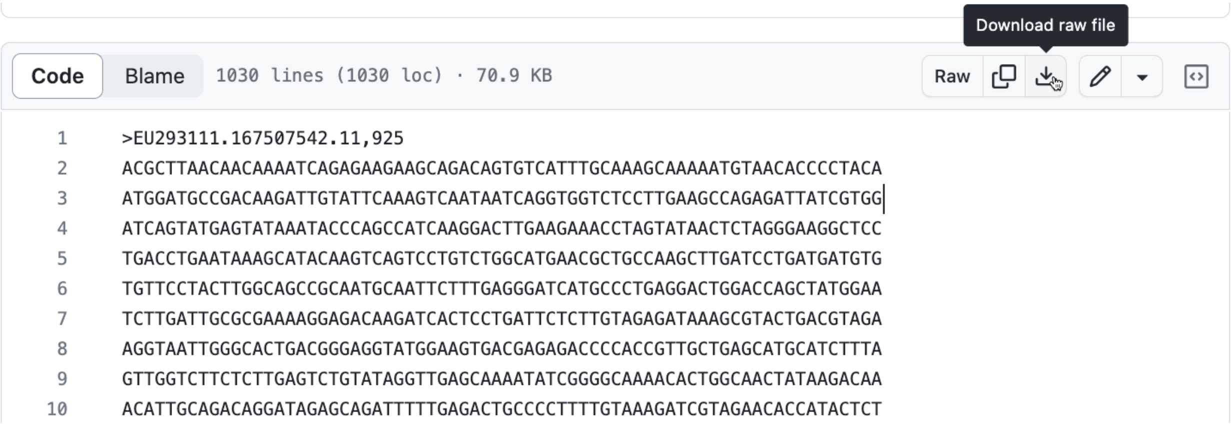
It advises checking your sequences in the tool Clustal Omega, specifically to use the sequence identity matrix to check how closely related your sequences are. We won’t do this here but the identity matrix for your sequences is shown below.

4. Can you see any problematic sequences?

```
#  Percent Identity  Matrix - created by Clustal2.1
   1: LC717423    100.00   97.86   97.76   98.34   96.13   96.87   96.61   96.78   96.75
   2: LC717424     97.86  100.00   98.36   98.96   96.02   96.75   96.46   96.62   96.59
   3: EU293111     97.76   98.36  100.00   98.97   95.93   96.67   96.39   96.55   96.52
   4: LC717422     98.34   98.96   98.97  100.00   96.46   97.17   96.90   97.08   97.05
   5: MN075931     96.13   96.02   95.93   96.46  100.00   98.27   97.99   98.16   98.15
   6: LC717426     96.87   96.75   96.67   97.17   98.27  100.00   99.16   99.27   99.27
   7: LC717425     96.61   96.46   96.39   96.90   97.99   99.16  100.00   99.65   99.64
   8: LC717427     96.78   96.62   96.55   97.08   98.16   99.27   99.65  100.00  100.00
   9: LC717428     96.75   96.59   96.52   97.05   98.15   99.27   99.64  100.00  100.00
```

Prune data

Sequences with >99% identity should be removed. Based on the matrix, we will remove sequences 7-9. A pruned sequence data file has been provided for you here. Download it.



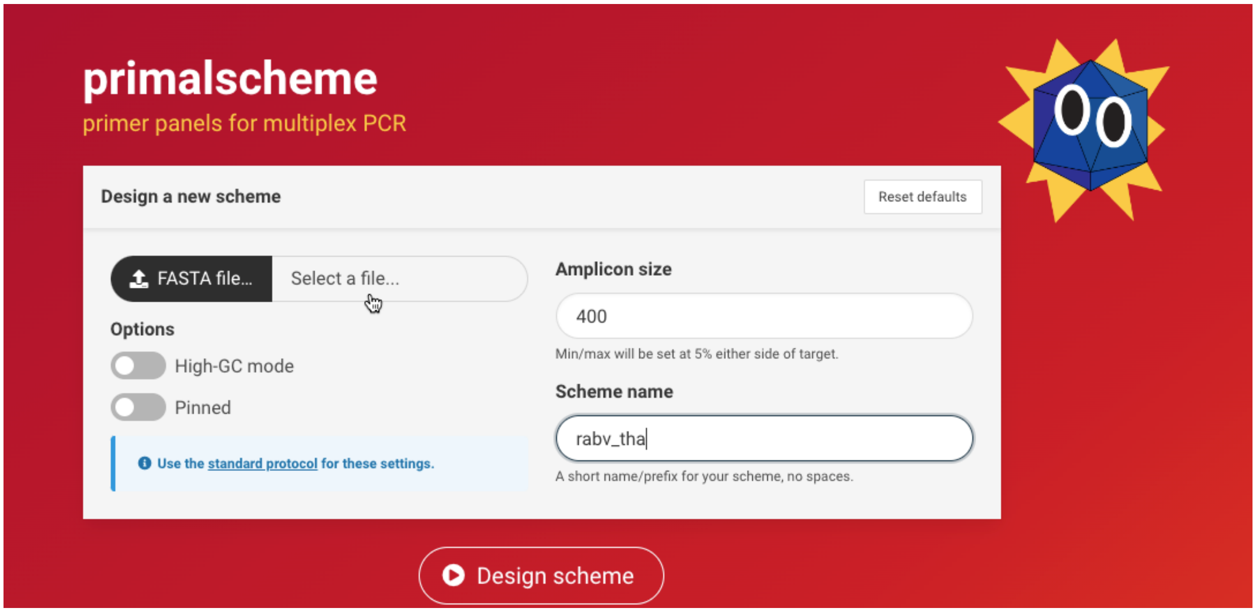
1030 lines (1030 loc) · 70.9 KB

```
>EU293111.167507542.11,925
ACGCTTAACAACAAAATCAGAGAAGAAGCAGACAGTGTCATTTGCAAAGCAAAAATGTAACACCCCTACA
ATGGATGCCGACAAGATTGTATTCAAAGTCAATAATCAGGTGGTCTCCTTGAAGCCAGAGATTATCGTGG
ATCAGTATGAGTATAAATACCCAGCCATCAAGGACTTGAAGAAACCTAGTATAACTCTAGGGAAGGCTCC
TGACCTGAATAAAGCATACAAGTCAGTCCTGTCTGGCATGAACGCTGCCAAGCTTGATCCTGATGATGTG
TGTTCCTACTTGGCAGCCGCAATGCAATTCTTTGAGGGATCATGCCCTGAGGACTGGACCAGCTATGGAA
TCTTGATTGCGCGAAAAGGAGACAAGATCACTCCTGATTCTCTTGTAGAGATAAAGCGTACTGACGTAGA
AGGTAATTGGGCACTGACGGGAGGTATGGAAGTGACGAGAGACCCCACCGTTGCTGAGCATGCATCTTTA
GTTGGTCTTCTCTTGAGTCTGTATAGGTTGAGCAAAATATCGGGGCAAAACACTGGCAACTATAAGACAA
ACATTGCAGACAGGATAGAGCAGATTTTTGAGACTGCCCCTTTTGTAAAGATCGTAGAACACCATACTCT
```

Create a scheme

Having checked and corrected the input data we will now proceed to create a scheme.

Task 3.
Click on **select a file** to upload the pruned fasta file as input, leave the amplicon length as 400 and call the scheme "rabv_tha". Then press **Design scheme**.



Design a new scheme

Options

○ High-GC mode

○ Pinned

Use the standard protocol for these settings.

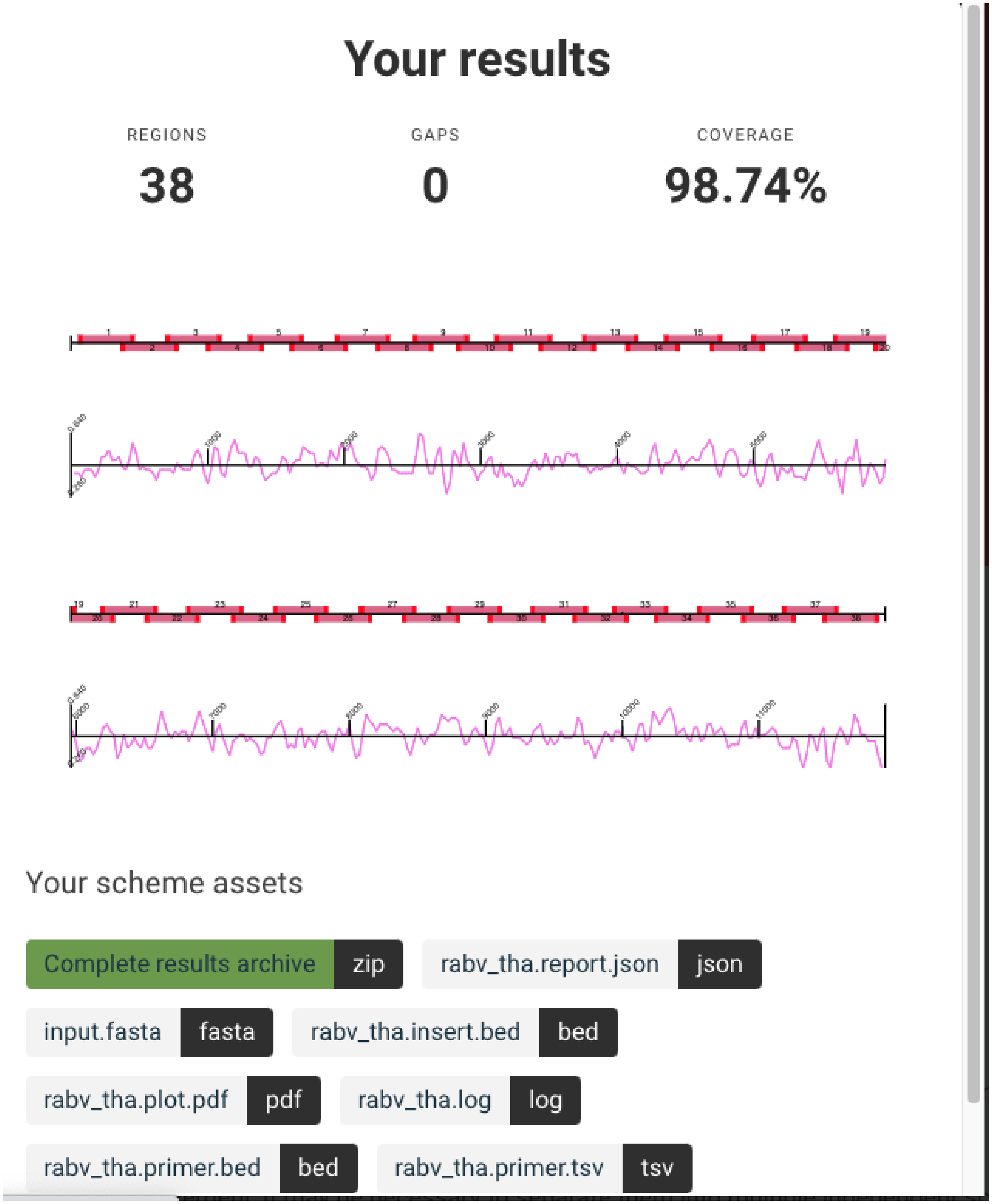
Amplicon size: 400

Min/max will be set at 5% either side of target.

Scheme name: rabv_tha

A short name/prefix for your scheme, no spaces.

You will get an on-screen output like below (note you may have to scroll down to see the files):



Your results

REGIONS
38

GAPS
0

COVERAGE
98.74%

Your scheme assets

The image shows an overview of the primer/amplicon positions on the reference genome and indicates the level of coverage they provide (98.74% of the genome).

You can choose to download the Complete results archive to look at all the output files. The rabv_tha.primer.tsv file provides a list of primer names, sequences and the multiplex pool they should be combined in. You can use the info in the file to order your primers! *Note:* It is important that the original names (e.g. rabv_tha_3_RIGHT) are kept as this informs downstream bioinformatics pipelines.

More complicated scenarios

It isn't always as easy as this! Have a look at the supporting material in our Jove manuscript for additional advice to help with primer design and optimisation:
Bautista, C., Jaswant, G., French, H., Campbell, K., Durrant, R., Gifford, R., Kia, G. S. N., Ogoti, B., Hampson, K., Brunker, K. Whole Genome Sequencing for Rapid Characterization of Rabies Virus Using Nanopore Technology. J. Vis. Exp. (198), e65414, doi:10.3791/65414 (2023).