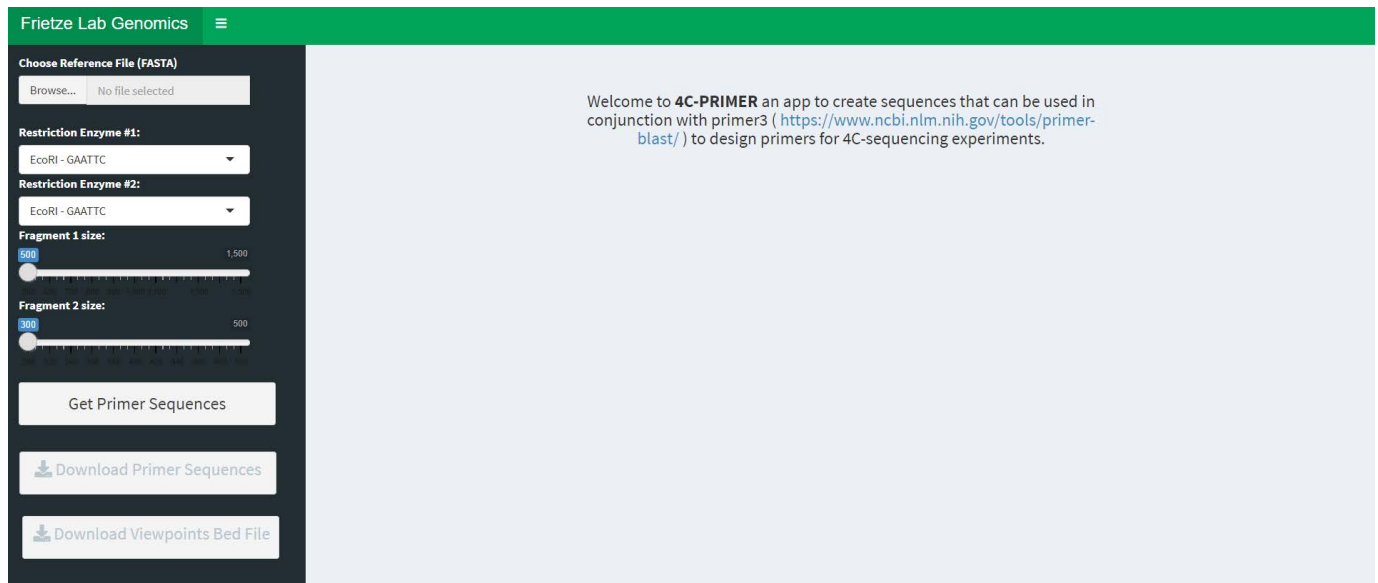


4C Primer

A SHINY App for facilitating the design of inverse 4C primers
Created by Michael Mariani, PhD (2021)

4C primer is easy to use. When launching the APP you should see the screen below.

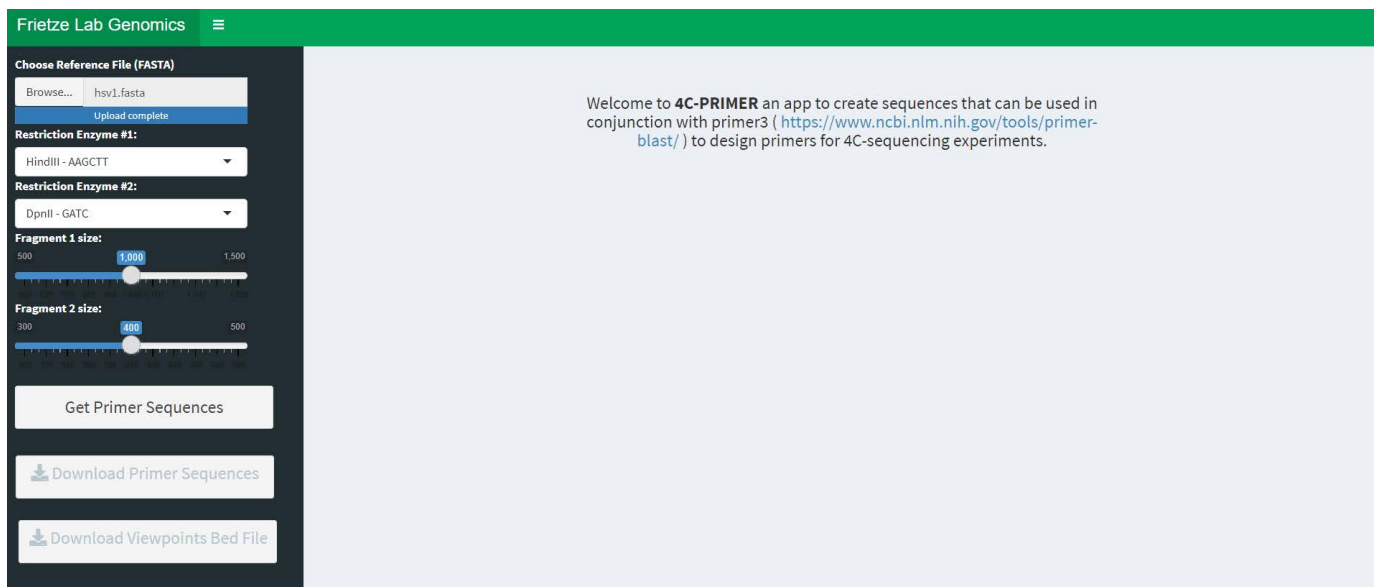


The screenshot shows the initial interface of the 4C-PRIMER app. The left sidebar contains the following controls:

- Choose Reference File (FASTA):** A button labeled "Browse..." and a status "No file selected".
- Restriction Enzyme #1:** A dropdown menu showing "EcoRI - GAATTC".
- Restriction Enzyme #2:** A dropdown menu showing "EcoRI - GAATTC".
- Fragment 1 size:** A slider bar ranging from 500 to 1,500.
- Fragment 2 size:** A slider bar ranging from 300 to 500.
- Buttons:** "Get Primer Sequences", "Download Primer Sequences", and "Download Viewpoints Bed File".

The main panel on the right displays a welcome message: "Welcome to **4C-PRIMER** an app to create sequences that can be used in conjunction with primer3 (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) to design primers for 4C-sequencing experiments."

First, upload the .fasta reference file of choice. Then select the two restriction endonucleases that you will be using to prepare your libraries. Finally, select the desired maximum size of the circular dna fragments after the first and second rounds of digestion, respectively - using the sliding bars.



This screenshot shows the app interface after configuration. The left sidebar controls are now:

- Choose Reference File (FASTA):** A button labeled "Browse..." and a file named "hsv1.fasta" with a status "Upload complete".
- Restriction Enzyme #1:** A dropdown menu showing "HindIII - AAGCTT".
- Restriction Enzyme #2:** A dropdown menu showing "DpnII - GATC".
- Fragment 1 size:** A slider bar ranging from 500 to 1,500, with the value set to 1,000.
- Fragment 2 size:** A slider bar ranging from 300 to 500, with the value set to 400.
- Buttons:** "Get Primer Sequences", "Download Primer Sequences", and "Download Viewpoints Bed File".

The main panel on the right remains the same, displaying the welcome message.

Next you simply hit the "Get Primer Sequences" button and the forward viewpoint sequence(s) and reverse viewpoint sequence(s) (for input to Primer3) will appear. Note that it is possible you may not find any sequences depending on your genome and choice of endonucleases - some exploration may be required. We can see below that using the selected parameters, only one viewpoint region was identified for the HSV-1 genome.

[illegible]

Pressing the "Download Primer Sequences" button or the "Download Viewpoints Bed File" button will download the respective .bed and .csv files

[illegible]

The .bed file contains the standard first three columns. The chromosome name in column 1, followed by the viewpoint start position (0-based as always with bed files) in column 2, and the viewpoint stop region in column 3. The total length of the viewpoint in bp is included as column 4.

The .csv file contains the forward primer sequence in the first column and the reverse primer sequence for Primer3 in the second column. This sequence can be copy and pasted into Primer Blast <https://www.ncbi.nlm.nih.gov/tools/primer-blast/> , to obtain candidates for the reverse primer from which you can choose the most suitable one. Note that you will want to specify "Use my own forward primer (5'->3' on plus strand)" in the Primer-BLAST window with the forward primer produced by 4C Primer.

