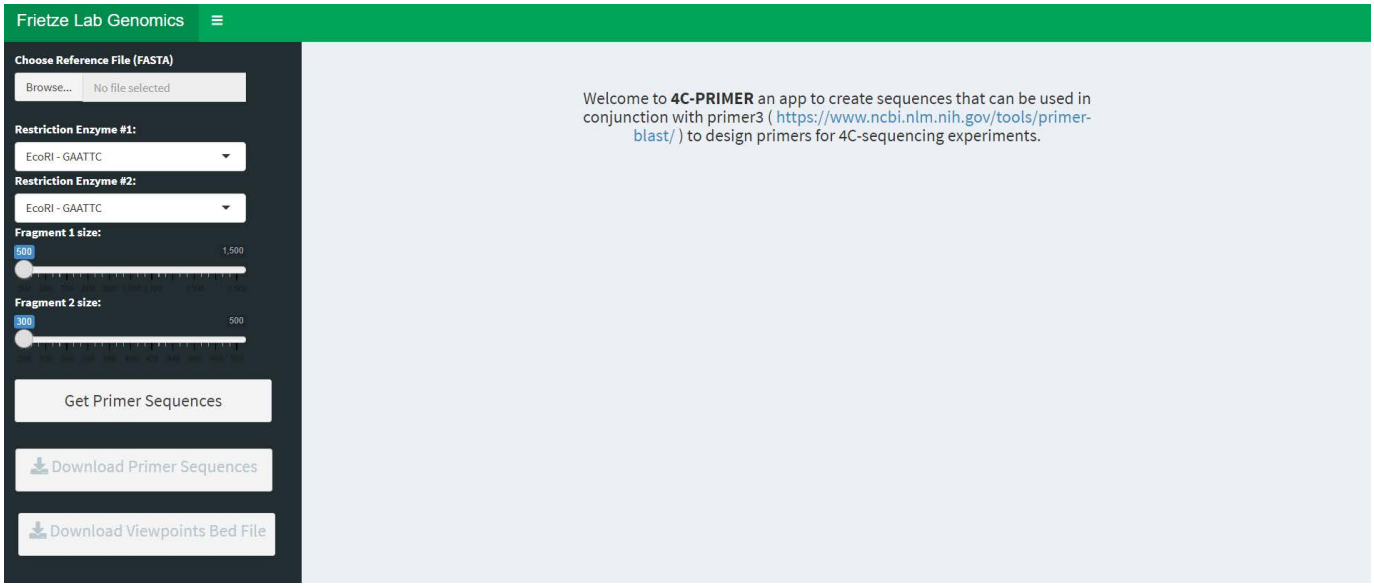
4C Primer

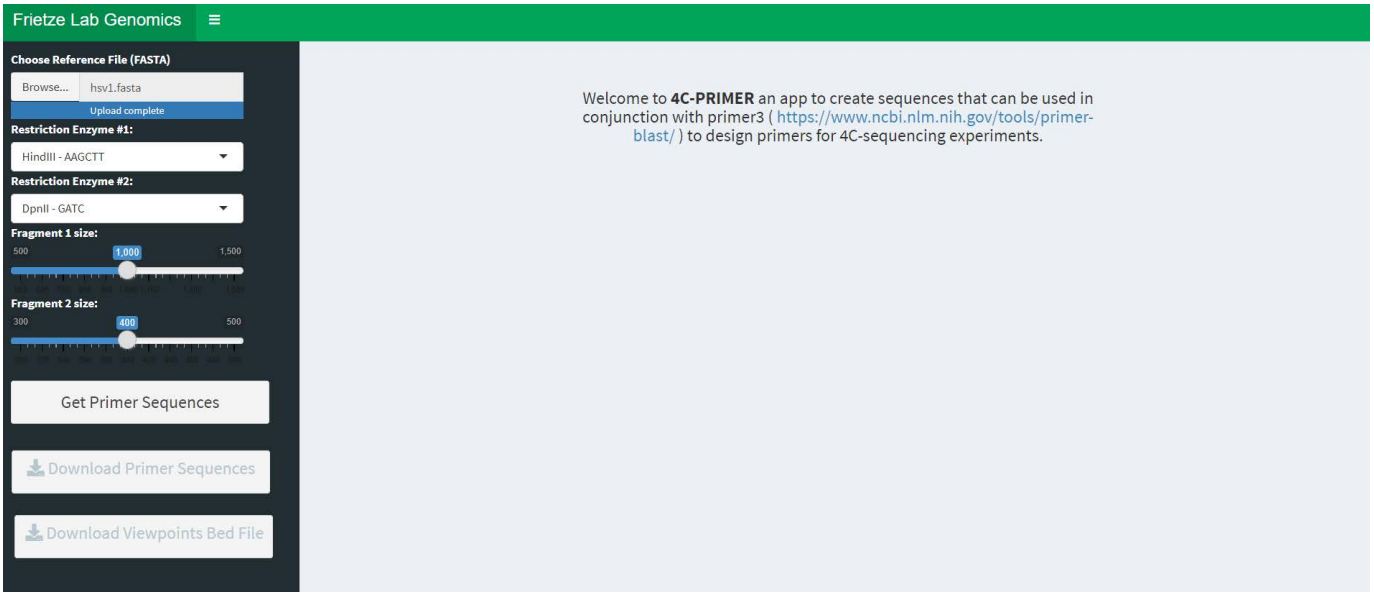
A SHINY App for facilitating the design of inverse 4C primers

Created by Michael Mariani, PhD (2021)

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

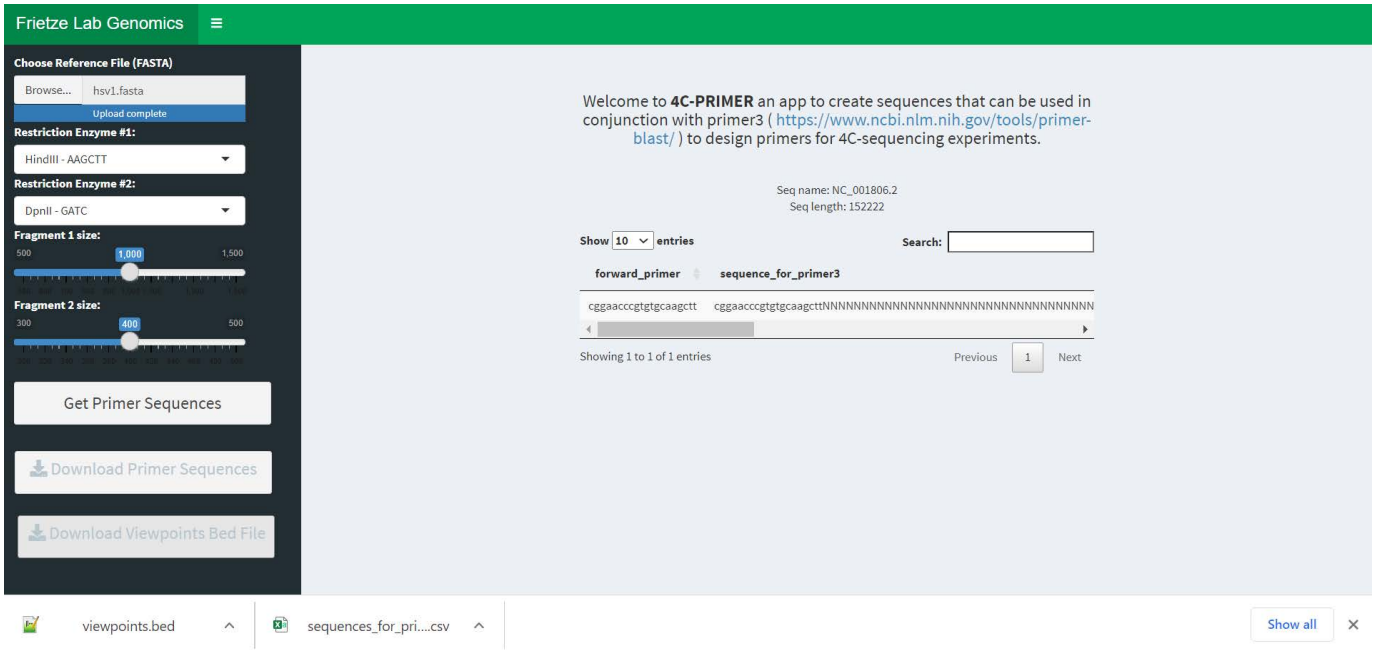


First, upload the .fasta reference file of choice. Then select the two restirciton endonucleases that you will be using to prepare your libraries. Finally, select the desired maximum size of the circular dna fragments after the first and second rounds of digestion, rspecitvely - using the sliding bars.

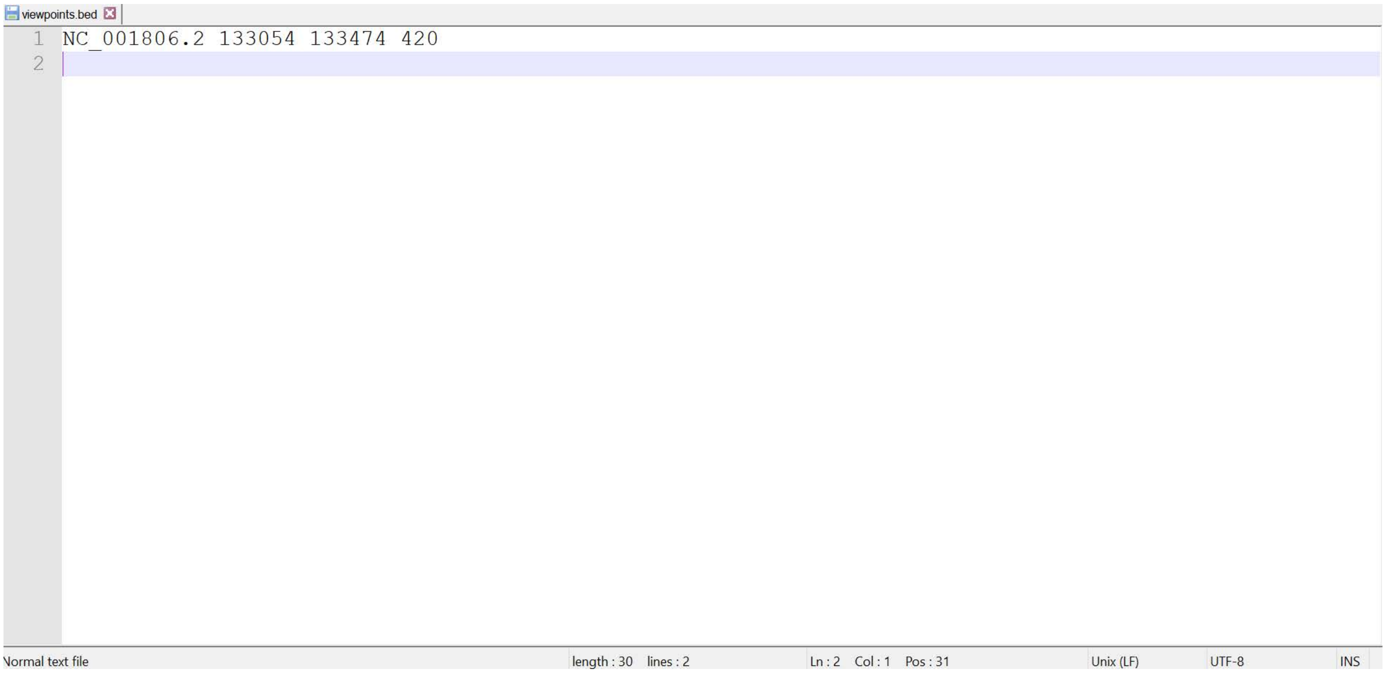


Next you simply hit the "Get Primer Sequences" button and the forward viewpoint sequence(s) and reverse viewpoint sequence(s) (for input to Pimer3) will appear. Note that it is possible you may not find any sequences depending on your genome and choice of endonucleases - some exploration may be required. We can see below that using the selected parameters, only one viewpoint region was identified for the HSV-1 genome.

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



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 wnat 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.