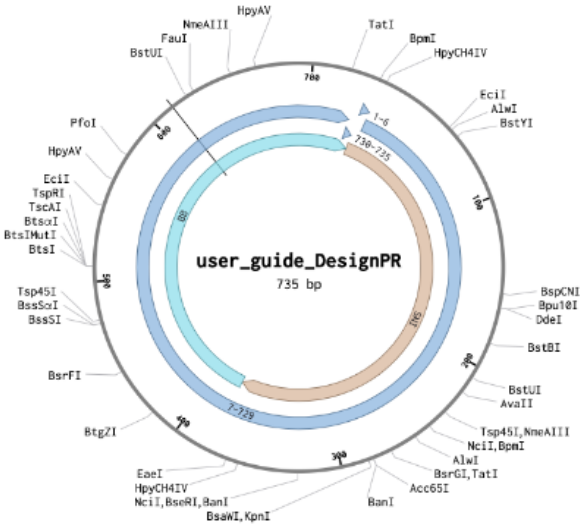
Step-by-step guide: DesignPR

For the purpose of this user manual, I have generated a fake DNA plasmid that is the target of our primer design. It is composed of two fragments: an insert and backbone sequence.

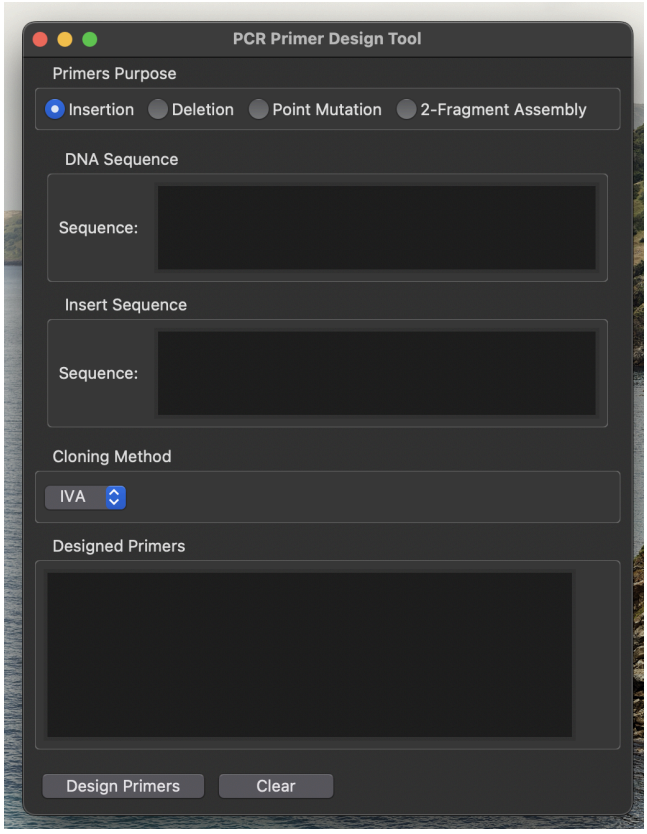
The INS sequence is annotated yellow and the BB sequence is annotated blue.

We want to use DesignPR to generate primers to amplify the BB sequence and the INS sequence such that they can assemble into this plasmid.



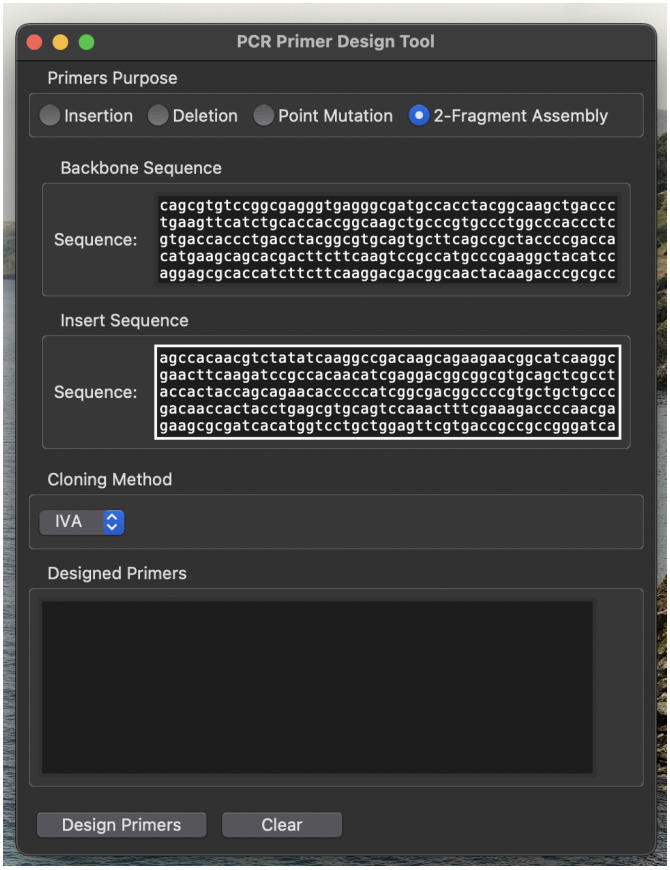
Step #1: pip install DesignPR and run the following command “run_designPR”

The GUI interface should appear in the default settings



Step #2: input user information

Select “2-Fragment Assembly”
Input BB sequence and INS sequence into the appropriate entry boxes



IMPORTANT: input sequences starting at the assembly site and following the 5’ → 3’ rule.

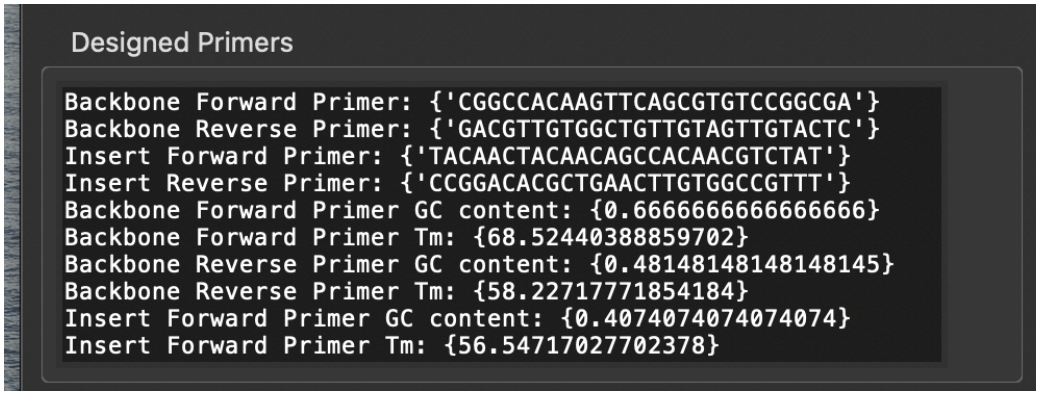
Note:

If an entry box is left empty, an error message will appear.

If there is non-DNA character in the sequence entries, an error message will appear.

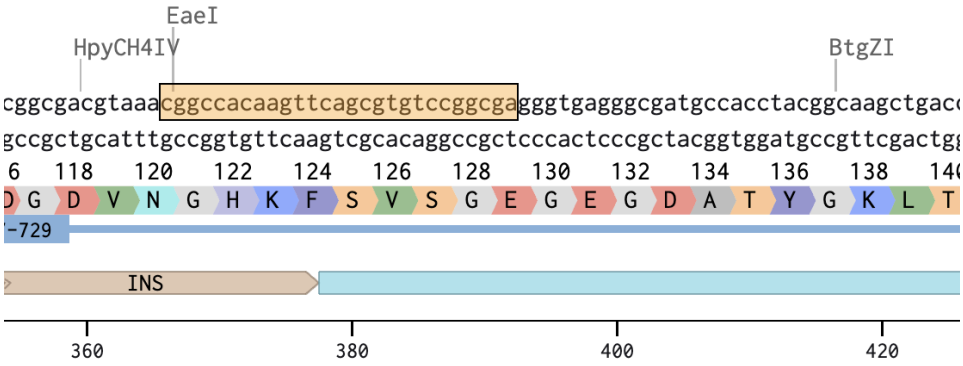
Step #3: Press Design Primers

The following output should appear in the “Designed Primers” box

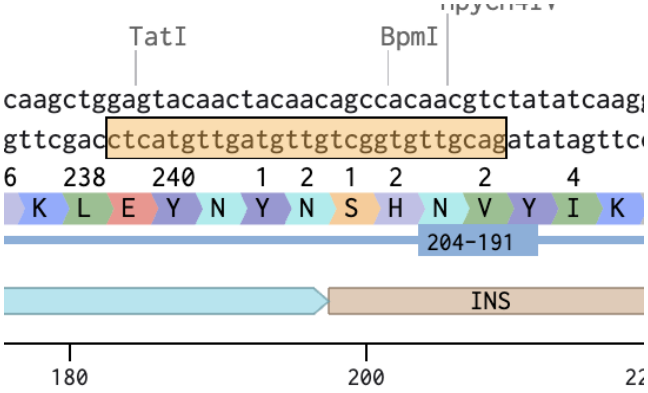


Testing the primers, we can see that they anneal at the correct sites. They also include both a template binding and overlap region with the other fragment (important for IVA cloning).

Backbone forward primer



Backbone reverse primer



Step #4: Press Clear and continue