TheFinerPrimerDesigner USER GUIDE

Summary

TheFinerPrimerDesigner is an application that allows the user to design DNA primers for polymerase chain reaction (PCR). There are three tabs: the input tab, the design tab, and the results tab. When the program first starts, the user is able to upload a DNA sequence that will act as the template sequence on the input tab. When the program receives a valid input, the program switches to the design tab on which the inputted sequence is displayed graphically. The user can then interact with this graphical representation by highlighting portions of the sequence. From there, the user can either use the selected portion as the amplicon from which the program will automatically generate the best primer pairs, or the user can create a forward or reverse primer from the selected region. Any primer pairs generated from either method will then be displayed on the results tab in an interactive and graphical way.

Background on PCR

PCR is a molecular biology technique to amplify a region of interest on a DNA sample called the template strand. Please see this website for the necessary background information on what primers are and why it is important they are designed correctly. It is also important to see what factors must be considered when designing a PCR primer.

The Input Tab

How to input a template DNA sequence:

This is what the user sees when the program first begins. To input a DNA sequence, click on the button that says INPUT DNA SEQUENCE. The following stage will pop up.

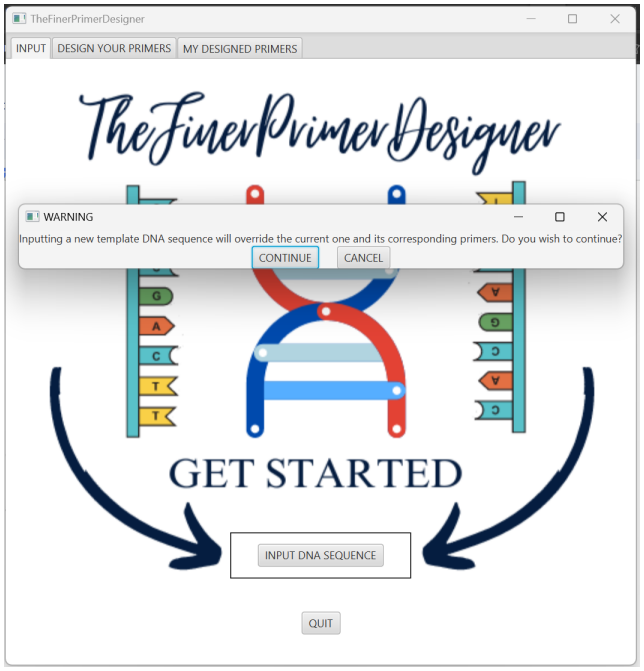
Inside this text field, the user can then input the template DNA sequence and select OK. The program will then check to see whether the input is a valid DNA sequence above the minimum

length or not. Here are some sample inputs and outputs that indicate the functionality of the program:

- Input: zyxwvut → Output: “Not a valid DNA sequence.” (Program checks for the nucleotides a, c, t, and g).
- Input: acgt → Output: “Input sequence too short.” (Program checks whether primers of the correct length can even be designed).
- Input: acgtacgtacgtacgtacgtacgtacgtacgtacgt → Output: Valid sequence! Displays sequence on the display tab.
- Input: acgTacgTacgTacgTacgTacgTacgTacgTacgT → Output: Valid sequence! Displays sequence on the display tab. (Program is case insensitive)

Otherwise, if the user selects the “Cancel” button, then the stage requesting input will close.

Note: if the user inputs a DNA sequence then proceeds to go back to the input tab and input another DNA sequence, a warning message will pop up telling the user that uploading a second DNA sequence will overwrite the first one as shown below. From here, the user can either continue or cancel the action.

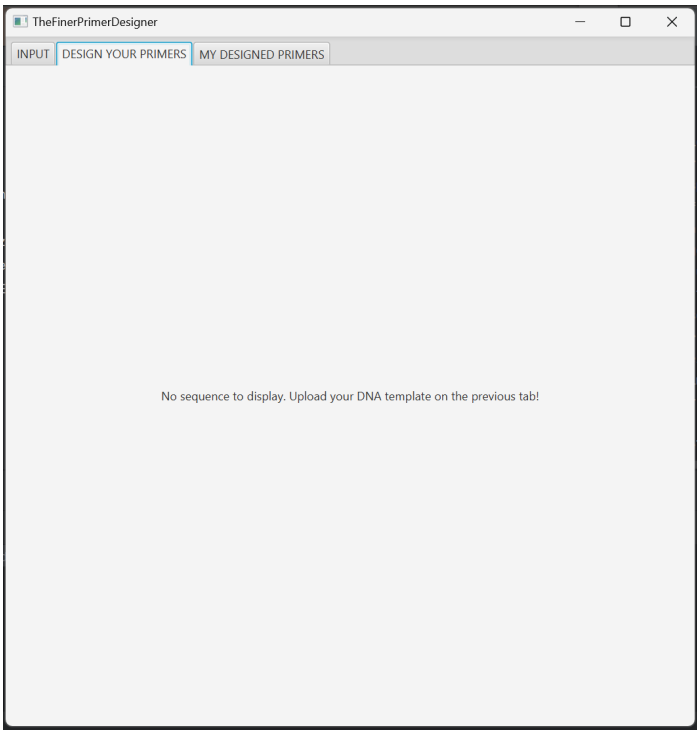


How to quit the program:

The user can quit the program by selecting the QUIT button at the bottom of the screen. Once they do that, a warning message will pop up, warning the user that any progress will not be saved as shown below. From here, the user can confirm that they want to quit or not confirm.

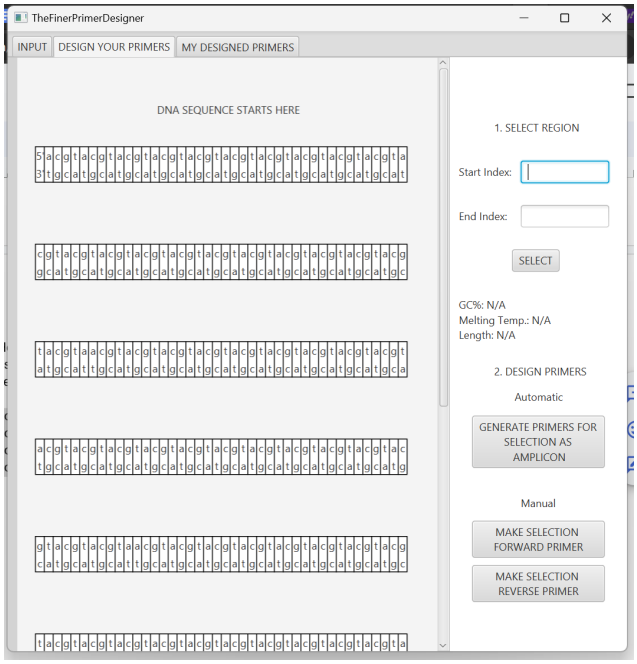
The Design Tab

When the program first starts, the design tab will be empty because there is no input sequence to display as shown below.



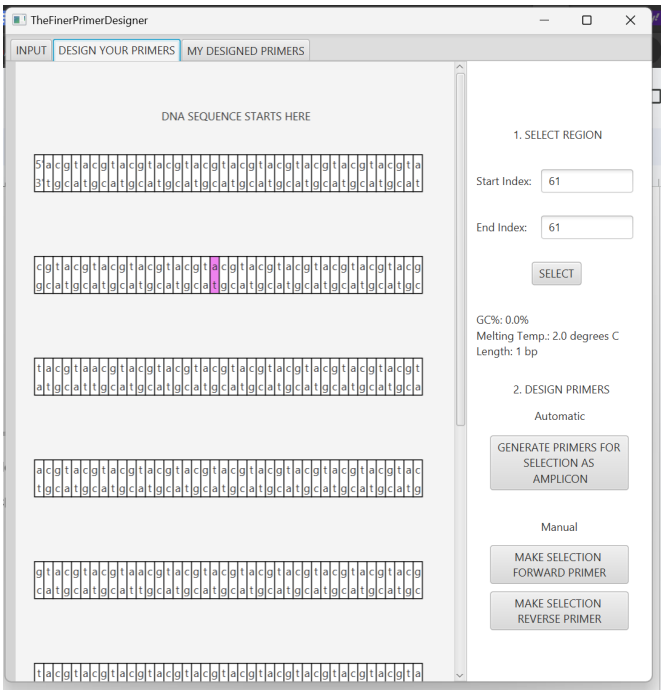
However, after inputting a valid DNA sequence, this tab will display the sequence, which the user can scroll through. The below screen is what happens when the user inputs the DNA sequence

acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgta
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtaacgt
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtaacgtacgt
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgta

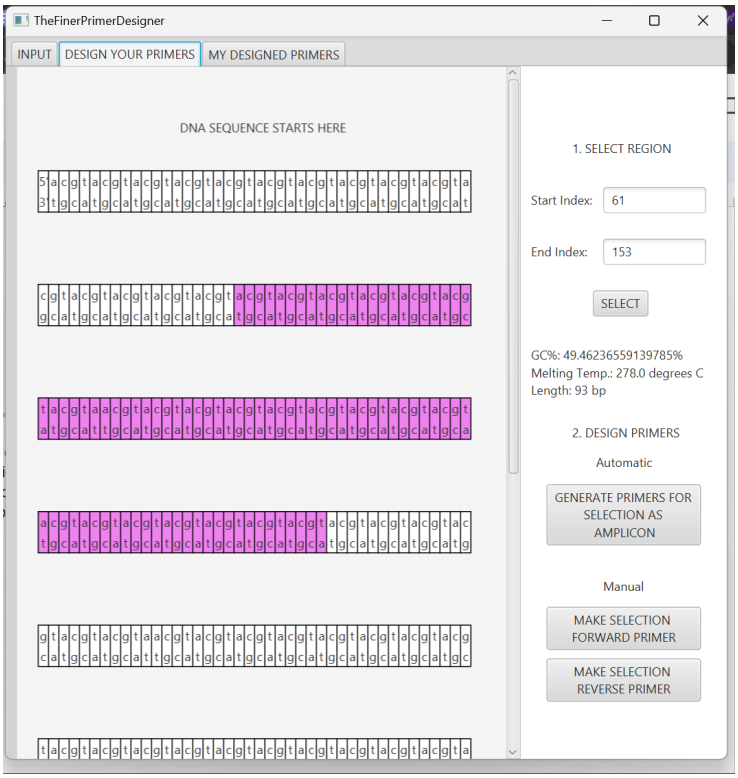


How to select a DNA sequence via mouse clicking:

Click on a nucleotide within the DNA sequence. That nucleotide will turn violet as shown below. Note that the panel on the side will update to include various information about your selection.

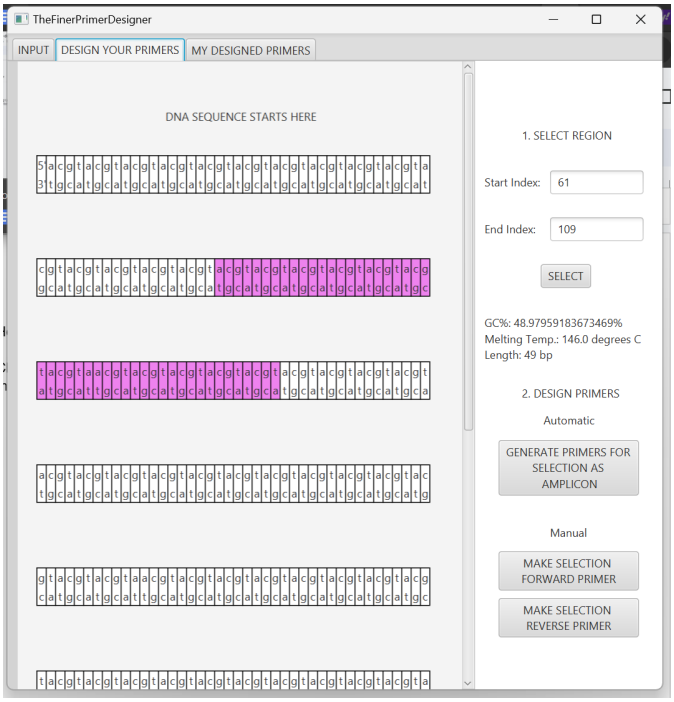


Next, select another nucleotide that isn't currently selected. The program will automatically highlight every nucleotide in between the two selected nucleotides to keep the selection continuous. After all, amplicons must be continuous sequences of DNA, resulting in the following.



How to De-select a region via mouse clicking:

Click on a nucleotide that is already selected. For example, if you click on a nucleotide in the middle of the selected region in the previous figure, the following will occur.

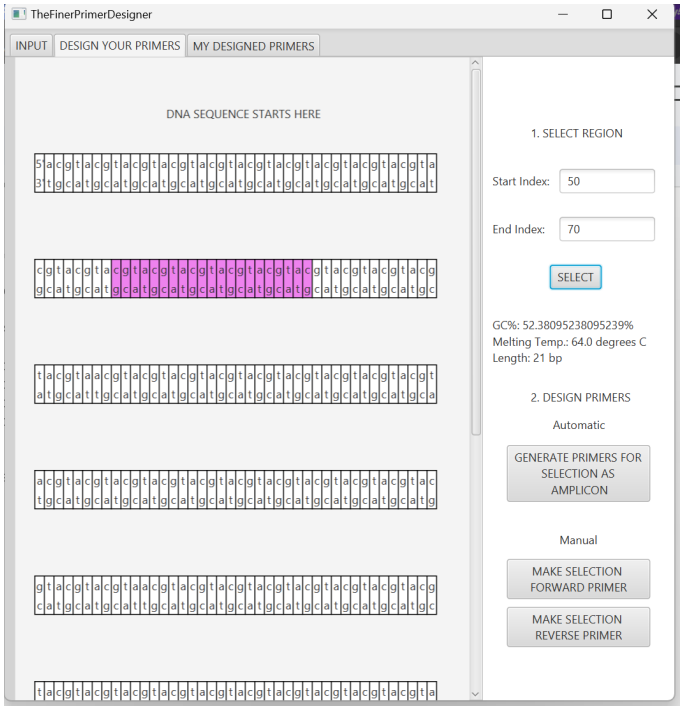


How to select a region using the text fields in the rightward pane:

Using the input sequence

acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgta
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtaacgt
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtaacgtacgt
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgta

input 50 as the start index and 70 as the end index and hit the SELECT button. This will make nucleotides 50 through 70 selected as shown.



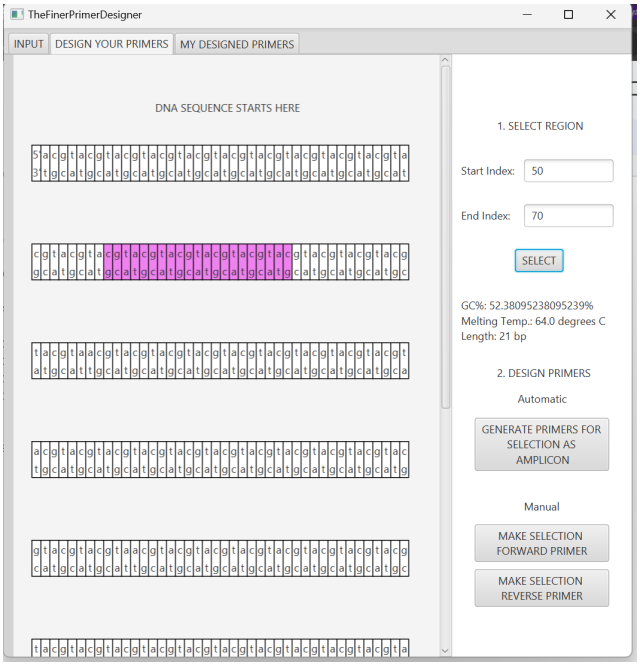
Note: Inputting blanks into the start and end index will deselect everything. In addition, the program will error check by making sure that the start index is less than the end index and that the inputs are integers.

How to automatically design primers for using a selected region as an amplicon:

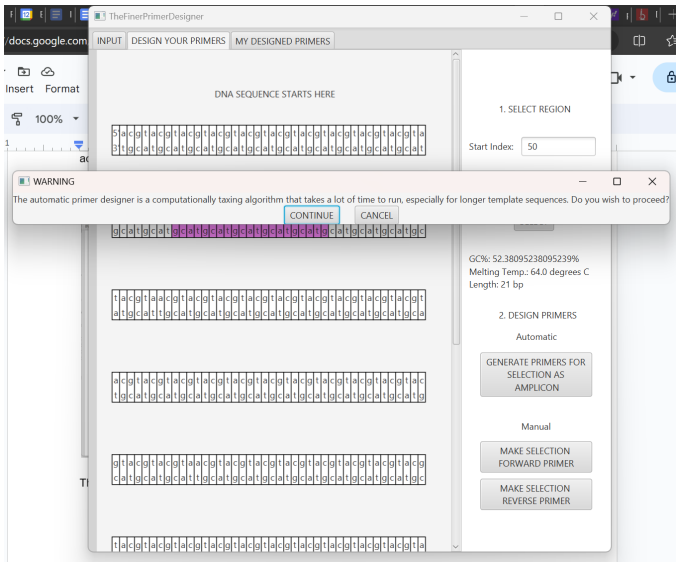
After inputting the sequence

acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgta
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtaacgt
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtaacgtacgt
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgta

and selecting nucleotides 50 through 70, the screen will look like this as shown below.



Then, click on the button that says GENERATE PRIMERS FOR SELECTION AS AMPLICON. A warning message will appear that asks whether the user wants to go through with running the computationally-taxing algorithm as shown.



Continuing will run the algorithm and design primers that show up on the results tab.

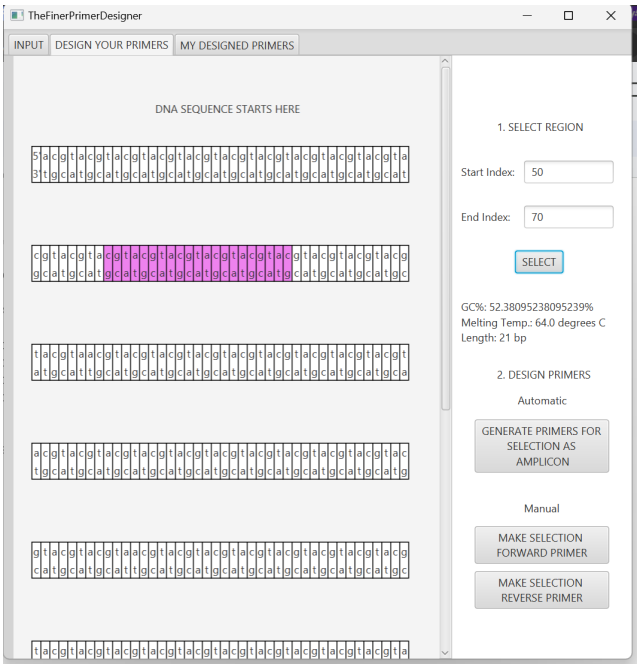
Note: The automatic primer designing algorithm performs error checking as well. For example, if nucleotides 2 through 50 are selected instead, then there will be an error as no primers can be generated.

How to manually design primers:

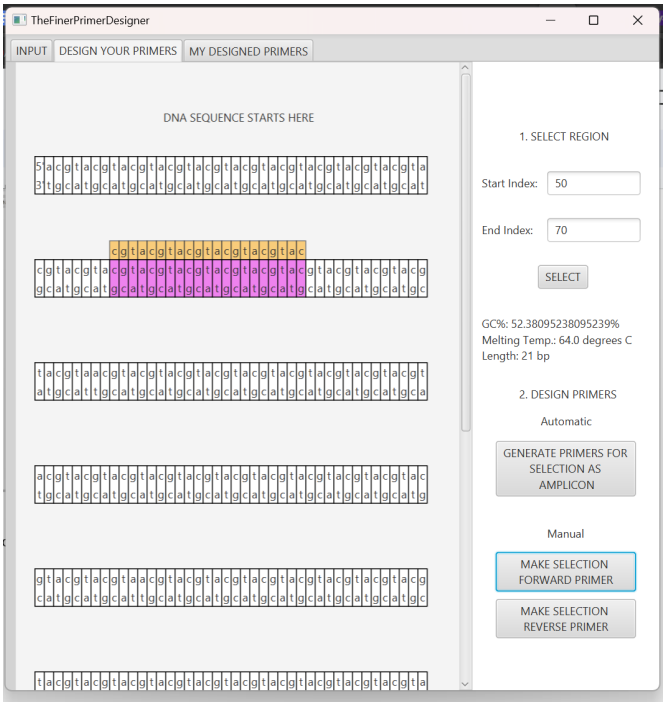
After inputting the sequence

acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgta
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtaacgt
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtaacgtacgt
acgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgtacgta

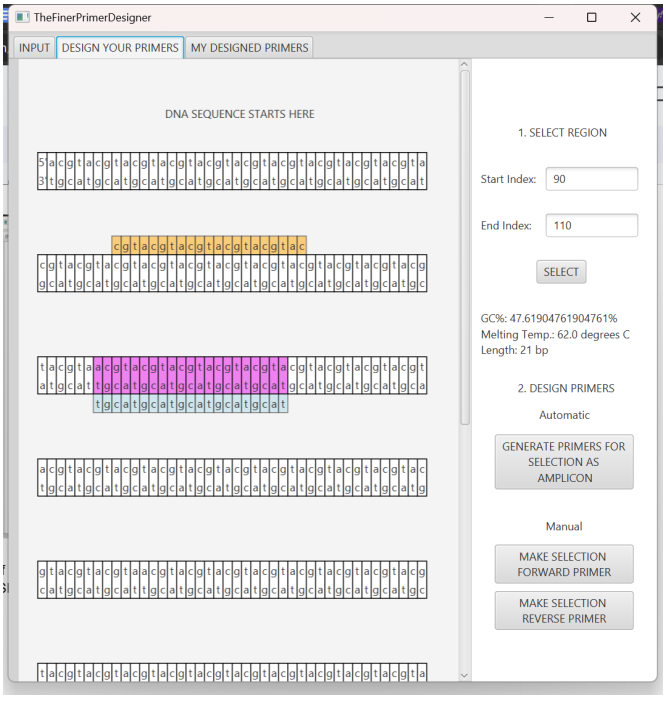
and selecting nucleotides 50 through 70, the screen will look like this as shown below.



If the user then selects MAKE SELECTION FORWARD PRIMER, then a forward primer will be added graphically.

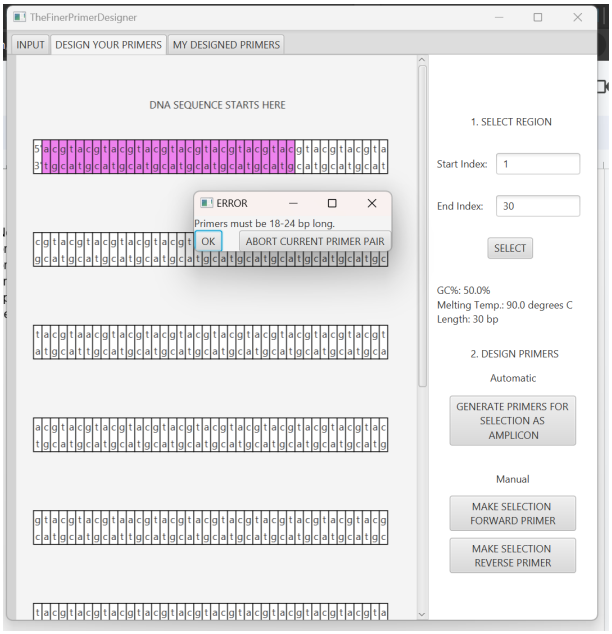


If the user then selects nucleotides 90 through 110, for example, and then selects MAKE SELECTION REVERSE PRIMER, a reverse primer will appear below the selection graphically.



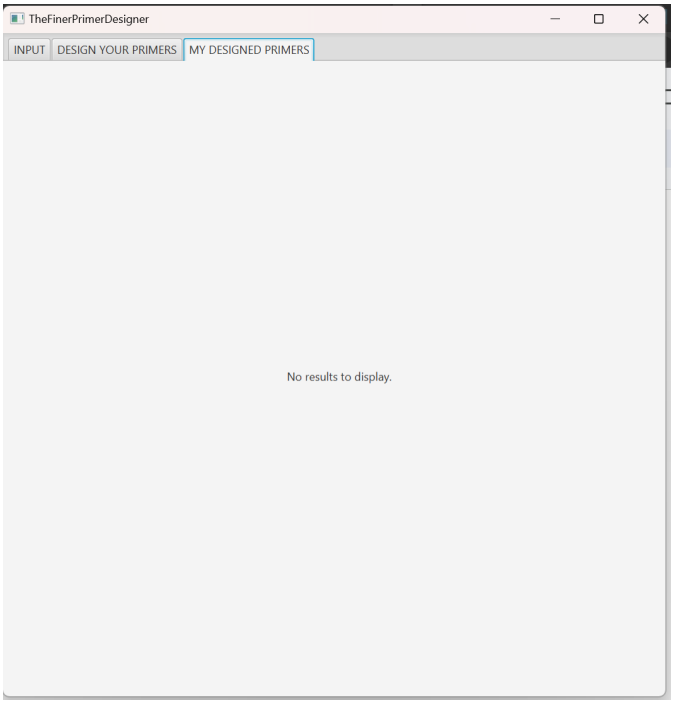
The designed primer pair will then appear on the results tab automatically.

Note: Stringent error checking is performed during manual primer designing. If the proposed primer is too short or two long, or if the forward primer is downstream of the proposed reverse primer, or if the reverse primer is upstream of the proposed forward primer, or if the user tries to create multiple forward primers without first creating a reverse primer or vice versa, then a special error message will appear that allows the user to abort the current primer pair being designed. It looks like below.

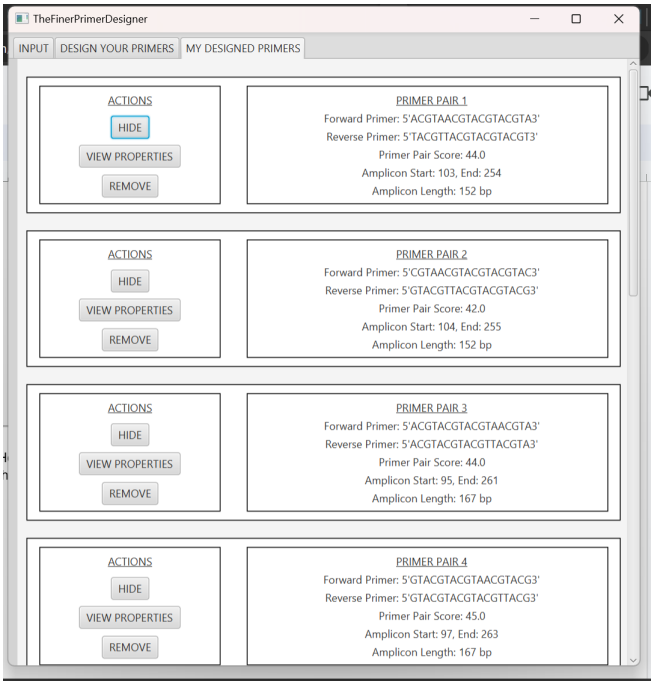


Results Tab:

When the program begins, the results tab will be empty and look like this.



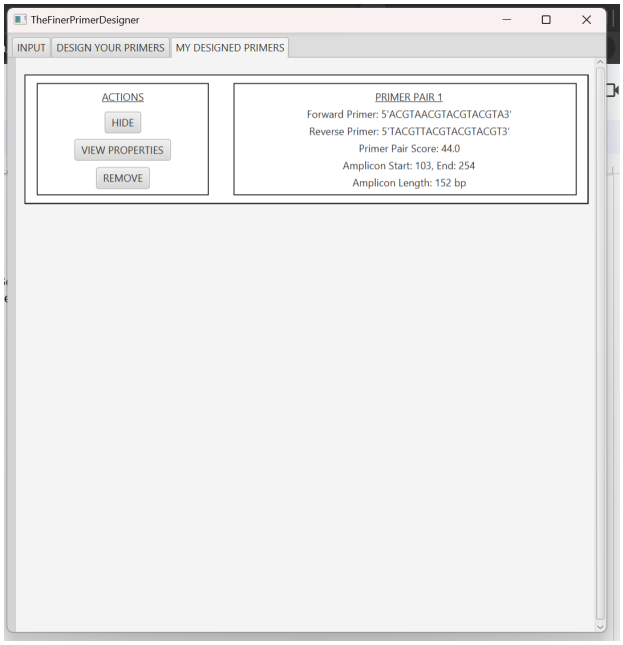
However, after populating the tab with designed primer pairs, then the results tab will look like this below.

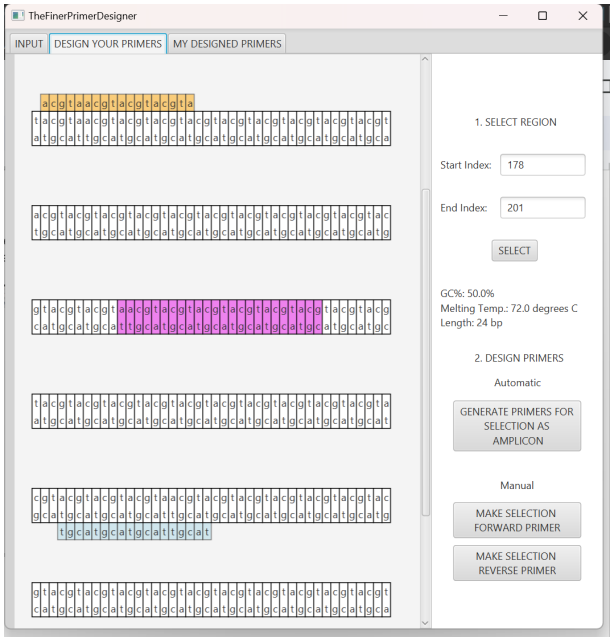


How to show/hide a primer pair:

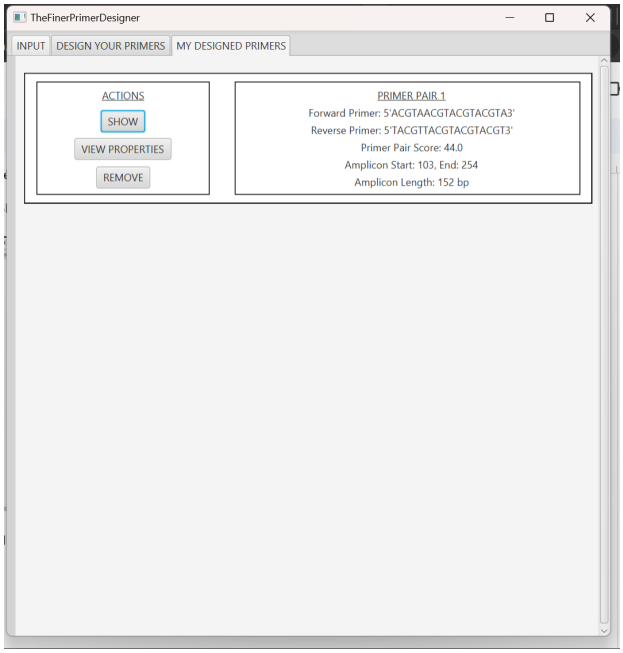
Selecting the HIDE button on a primer pair will hide it on the display tab. Compare the difference between

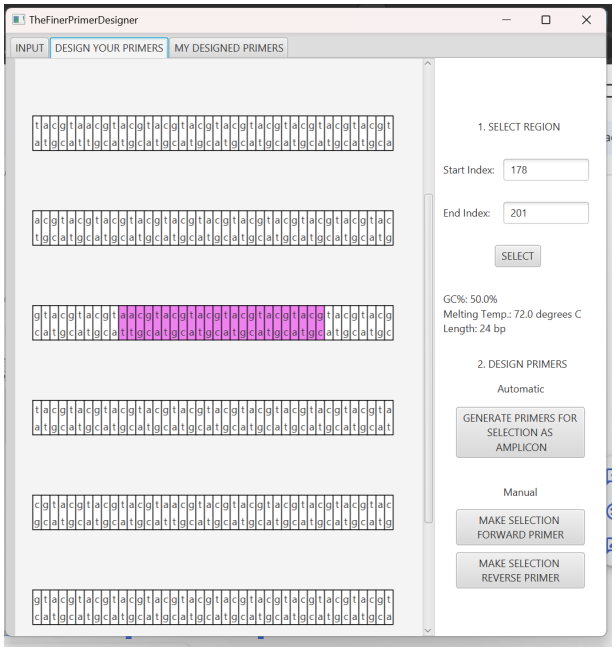
SHOWN:





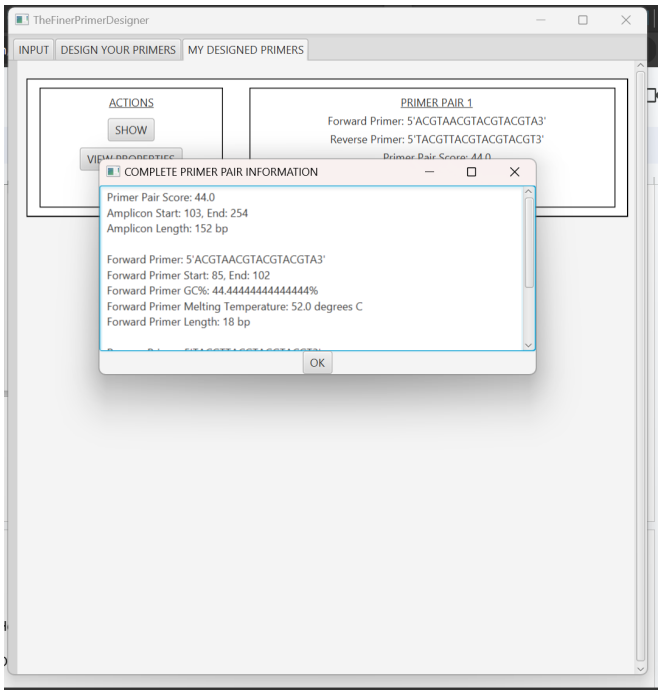
HIDDEN:





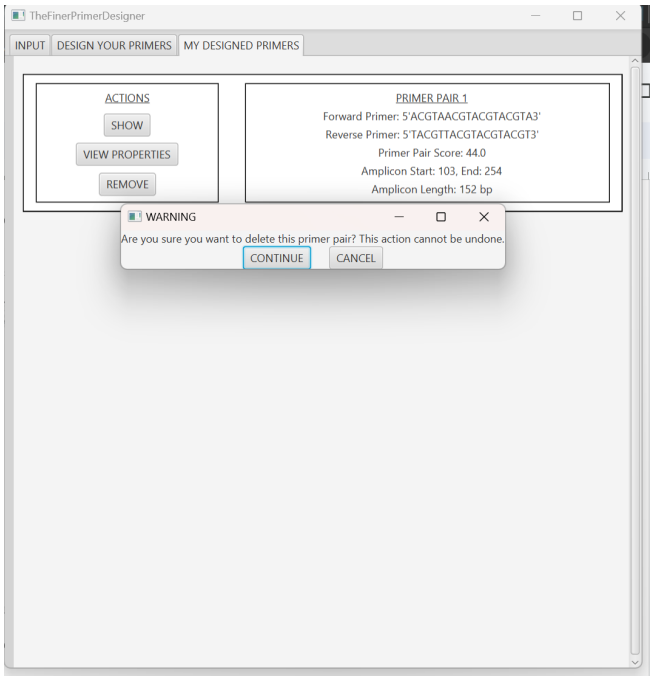
How to get more information on a primer pair:

On the results page, clicking the VIEW PROPERTIES button will bring up a new stage that displays more information about the primers the user has designed.



How to remove a designed primer pair:

To remove a designed primer pair, the user should click on the REMOVE button. A stage will pop up asking the user to confirm the action.



When the user clicks CONTINUE, the primer pair will be removed graphically from the design tab and from the list of results.