2. Basic manual primer design

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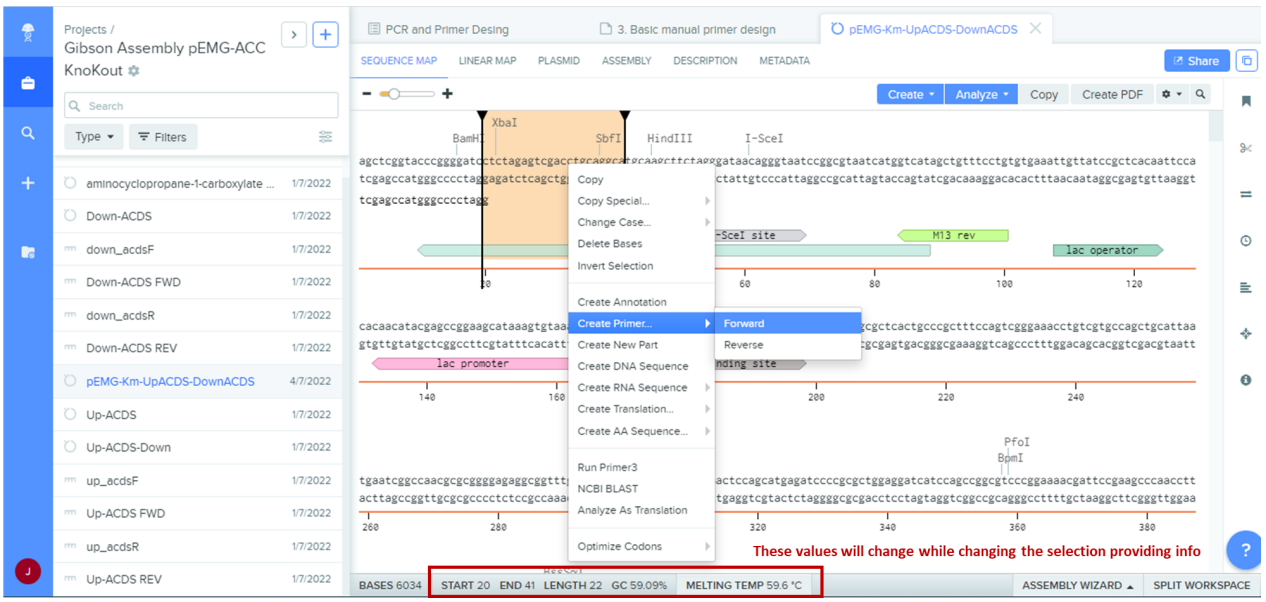
This doc will explain how to create and modify manually primers, check features such secondary structures, melting temperature (Tm), GC content and save them on a destination folder.

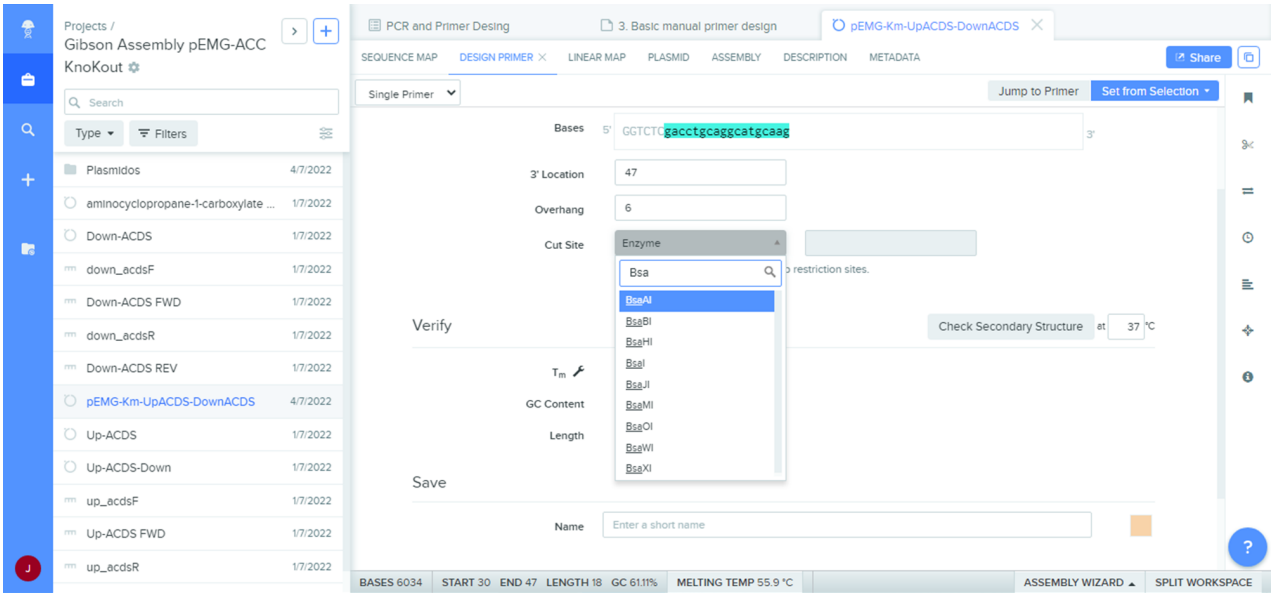
1. **Create a primer from your sequence**
   Open a DNA sequence, go to your "Sequence Map" view and select any region of the sequence. Notice at the bottom how the values change while selecting the sequence. A right click on the selected DNA will open a dropdown menu, select "Create Primer", and select the direction you'd like (**Image 1**)

Image 1



2. **Design Primer**
   A "Design Primer" tab will appear that displays other parameters to assist you in designing your primer. It is here where you can modify the sequence adding cutting sites or additional sequence. For instance you can look for an enzyme on **cut site,** copy the sequence and paste it at the 5′ end of the primers sequence (**Image 2**). Adding additional sequence will create an overhang and you'll need to add the extra number of base pairs to the **overhang box.**

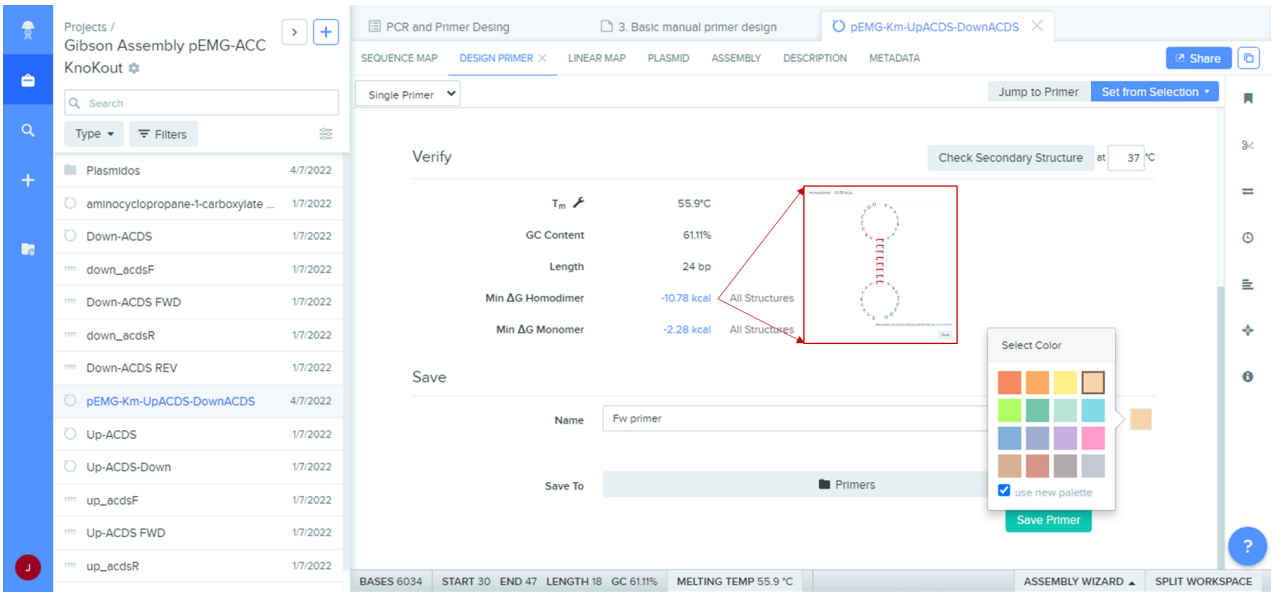
Image 2



3. **Check for secondary structures**

In the Verify section you can specify the temperature if you wish (37C by default) and check the free energy. Make sure all changes in energy are above -9.0 kcal.
Click on the Gibbs Free Energy (ΔG) values to view homodimer and monomer structures.
Check the melting temperature (Tm) and GC content. You can click on the wrench icon next to Tm to modify other thermodynamic parameters for priming (**optional and more advanced**) (**Image 3**)

Image 3



Some guidelines for designing PCR primers include:

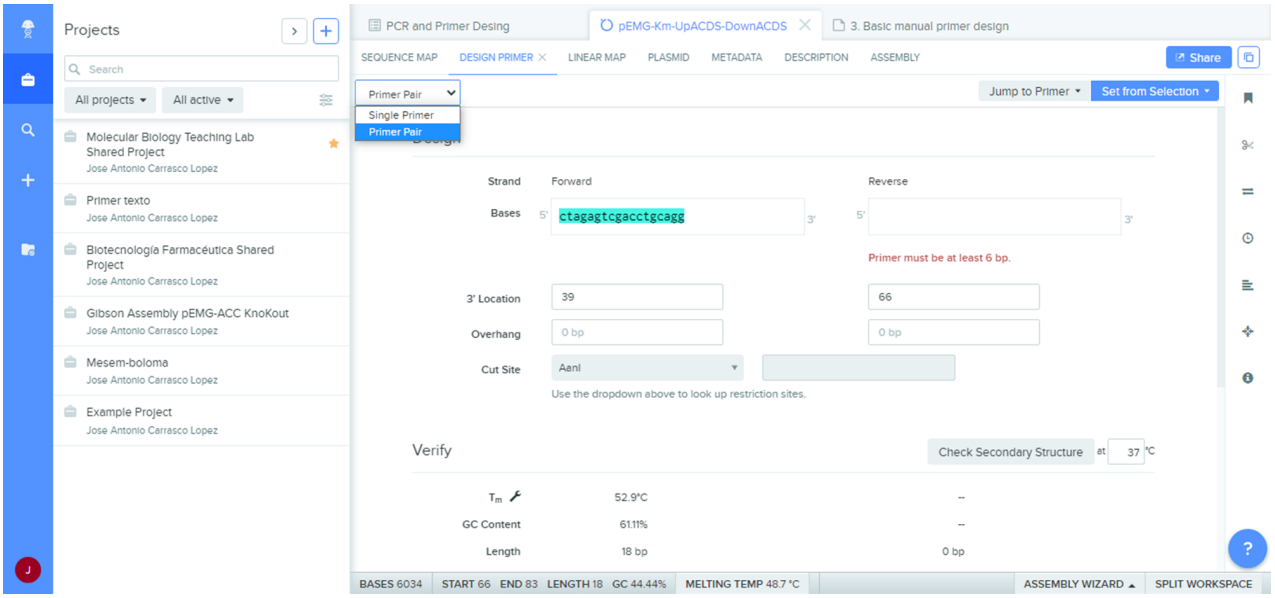
a. Length of **18-24 bases** (Specificity usually is dependent on the length and annealing temperature. The shorter the primers are, the more efficiently they will bind or anneal to the target.)
b. **40-60% G/C** content (Be mindful not to have too many repeating G or C bases, as this can promote primer-dimer formation.)

c. Start and end with **1-2 G/C** pairs (This is known as a **GC Clamp**).
d. Melting temperature (**Tm**) of **50-60°C** (This can depend on your PCR buffer conditions and DNA polymerase as well).
e. Primer pairs should have a **Tm within 5°C of each other**
f. Primer pairs **should not have complementary regions**

4. **Save your work on a destination folder**
   Input a name for your primer and choose the color for it to appear on your sequence. Choose an appropriate Project or Folder to save your primer and hit "Save Primer" (**Image 3**). This **video** will explain the main steps of the process.

5. **Creating pairs of primers**
   Usually primers will be used to amplify target DNA fragments, so we will need pairs of them to do so. To create pairs, instead of saving the work done, you can select "Primer Pair" from the dropdown menu on top of the work space (**Image 4**)

Image 4

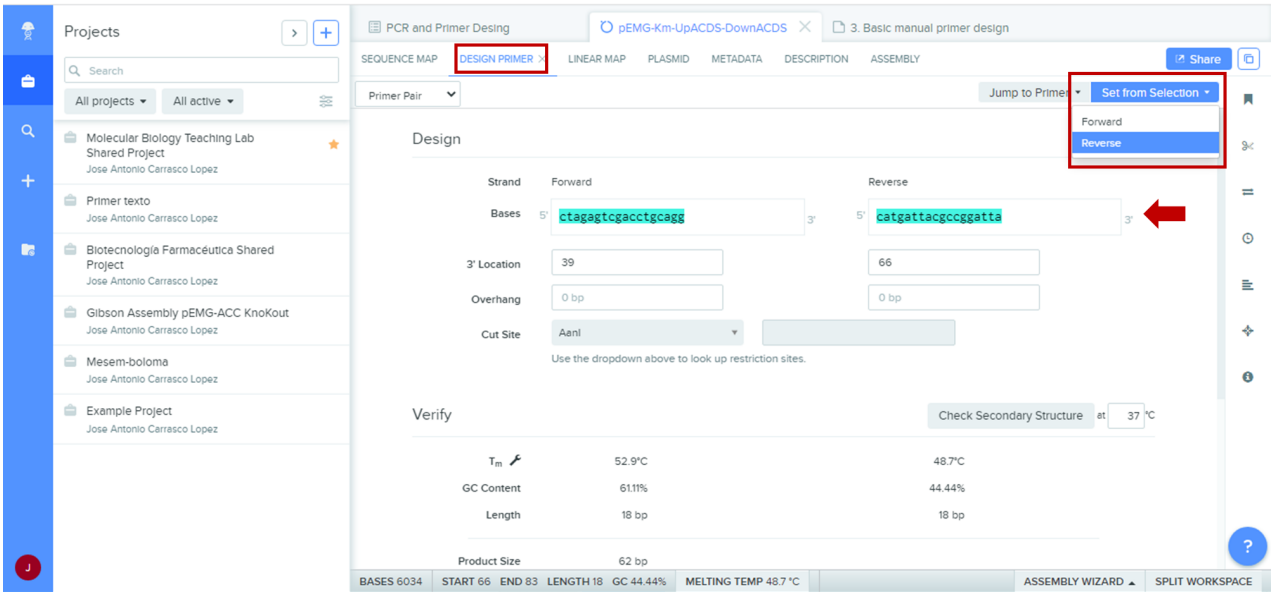


Go back to the sequence map and select the reverse primer sequence of your choosing. (Image 5)

Image 5

Click back to the design tab and set the reverse primer from selection. This will add the sequence in the right sense to the reverse primer box.

Image 6



You can now follow the previous same steps to further design, verify, modify and save your pair of primers on a destination folder. We recommend to save **primers on an specific folder** within every project.