Oct 06, 2020

Protcol 3: Design on Genious Prime

In 1 collection

1

[1]UCSC

1 Works for me This protocol is published without a DOI.

UCSC BME 22L

Alyssa Ayala

PROTOCOL CITATION

2020. Protcol 3: Design on Genious Prime. **protocols.io**
https://protocols.io/view/protcol-3-design-on-genious-prime-bmd5k286

COLLECTIONS

Protocols for Primer Design

CREATED

Sep 15, 2020

LAST MODIFIED

Oct 06, 2020

PROTOCOL INTEGER ID

42141

PARENT PROTOCOLS

Part of collection
Protocols for Primer Design

BEFORE STARTING

If you already have a primer in mind you, perhaps a primer you saw in a scientific paper, you can manually create that primer. The steps are as follows

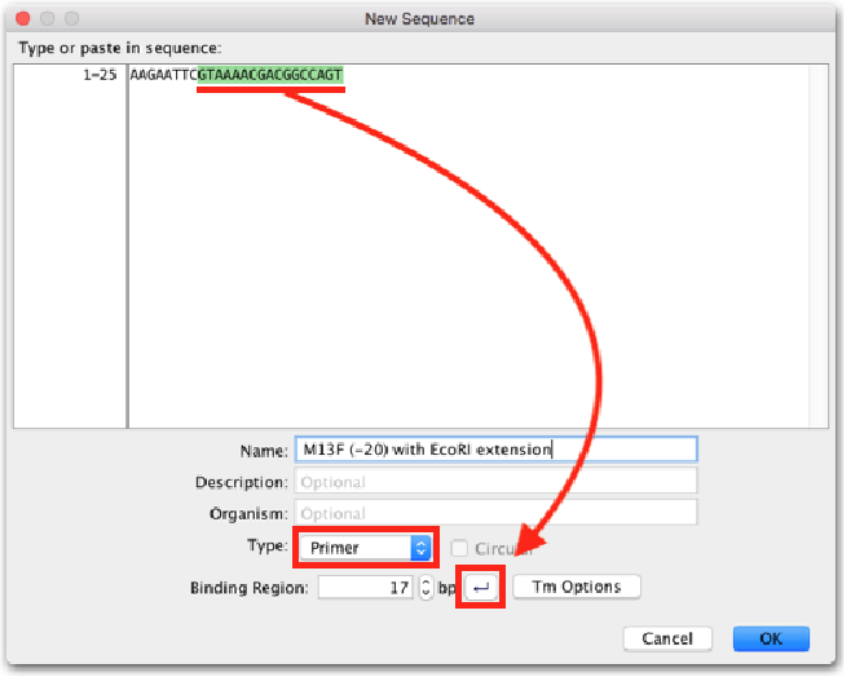
Manual Primer entry

1 Insert desired sequence

Go to file -> New -> Sequence. This will direct you to the new sequence window. From this window, you will be able to enter the sequence manually. You will be asked what type of sequence it is that you are designing. Press the dropdown menu and select the option for primer.

2 Specify binding region

You have the choice of specifying the binding region of the primer as well as specifying the extension of the primer. To designate the binding region of the primer, simply highlight the region of the primer you want to be the binding region, and select the binding region button (keep in mind that the typical binding region of a primer is between 17-27 bp).

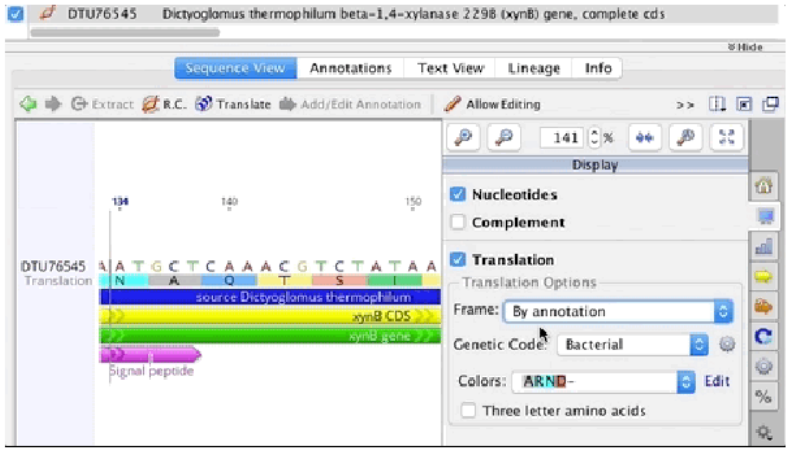


Press ok and the primer will be available.

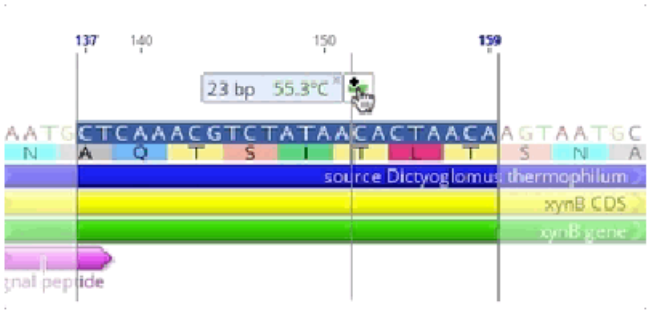
Manual Primer Design

3 If you have a gene you want to amplify and then clone into another vector, Geneious makes this process quite simple. Select the gene that you want to amplify.

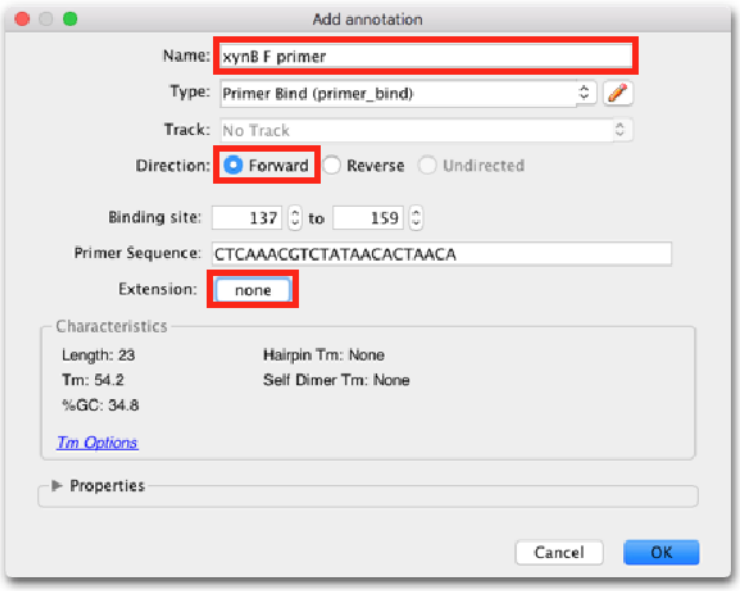
4 Hit the Display button (the TV icon) and make sure that translation is turned on. Also make sure that frame is set to by annotation.



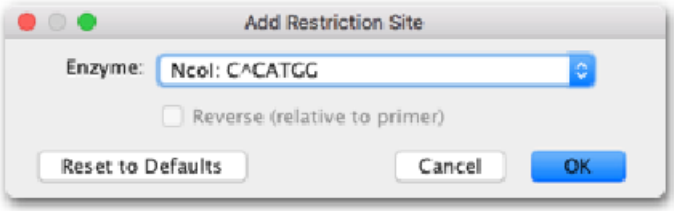
5 Go to the region of the gene you wish the primer to bind and click and highlight over that region. As you highlight, you should see a number indicating the base pairs that you have highlighted over as well as the Tm of that highlighted sequence. Beside that number you will see the add primer button. When you have finished highlighting the desired region, press the add primer button and this will take you to the add annotation window.



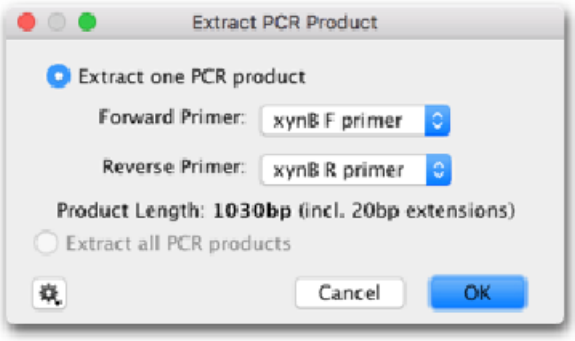
6 Once you are at this add annotation window, you are then given the options of naming the primer. You will also be asked to specify whether this is a reverse or forward primer. You will also be given the option to add an extension to this primer.



7 When cloning into another vector, it is essential to incorporate a restriction enzyme site, as you will need to use it to cut out the region of the gene you want to clone as well as opening up the region of the vector you want to ligate the gene into. Select the extension button and hit the “add restriction site”. This will then take you to a small window where you will be able to choose from numerous restriction enzyme sequences you wish to incorporate into the primer. Make sure that the restriction site you allow the:



8 Once you have finished designing the primer, select the gene and hit the primers button. Once you hit this, you have the option to select the forward and reverse primers. Choose the respective primers that are specific to amplifying the desired region of the gene you want.



9 **Take a screenshot** of the sequence with attached primer blocks with unique names and add the image to the lab notebook.

Storing your primers

10 Theoretically, once you have obtained your oligomers, it is important to know how to store your oligomers. Standard DNA oligos dried down or stored at 5°C in TE buffers or water were found to be stable for long periods; however, better stability is achieved by freezing if the oligos are to be stored for extended periods. Standard DNA oligos stored at 37°C are stable for at least 6 weeks in water, or 25 weeks dried down or in TE. Storage at higher temperatures is not recommended; however, accidents can happen, such as oligos being left on the bench top overnight or freezers failing at the weekend, and it is important to understand that the stability of the oligo should not be affected. Regardless of the storage temperature, TE buffer, rather than water or dried down, is optimal for long-term storage (IDT).