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BSCI:414 Lab2--Create PCR Reaction

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PROTOCOL CITATION

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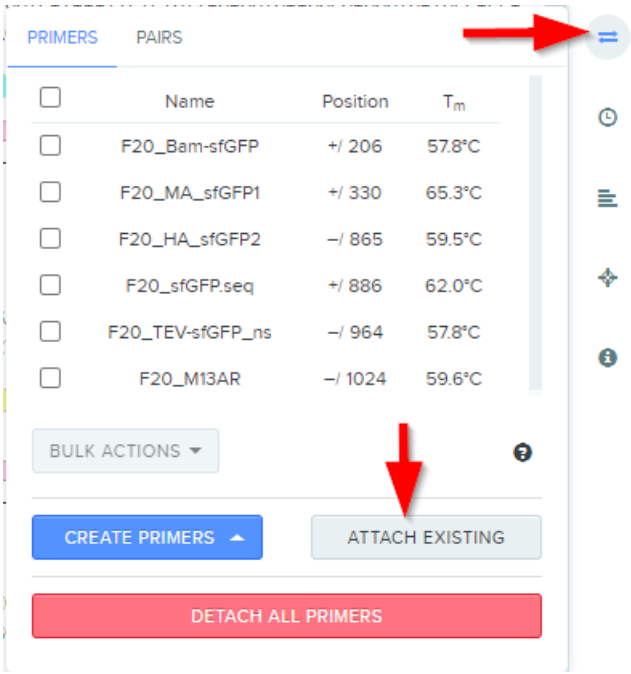
Copy Plasmid to New Folder in Benchling

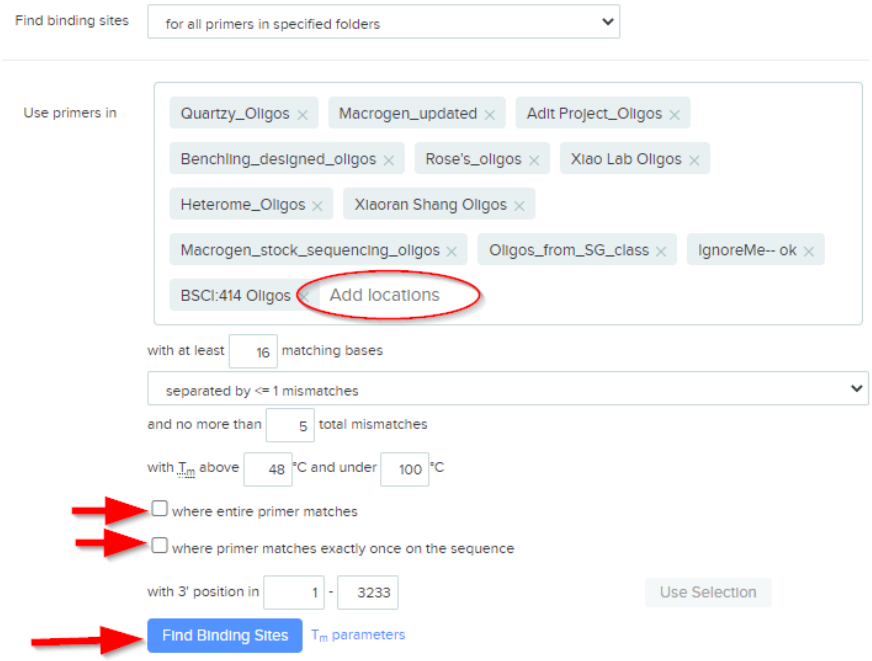
1 In the "BSCI:414 Plasmids" under the class root folder, find plasmid "superfolderGFP/pUC57". Copy this plasmid to a new folder you create under "BSCI:414 Lab 2" using your name e.g. "F20_HarleyKing_Lab 2". Also give the plasmid a new name like "F20_HarleyKing_superfolderGFP/pUC57".

Attach Exisiting Primers to Plasmid in Benchling

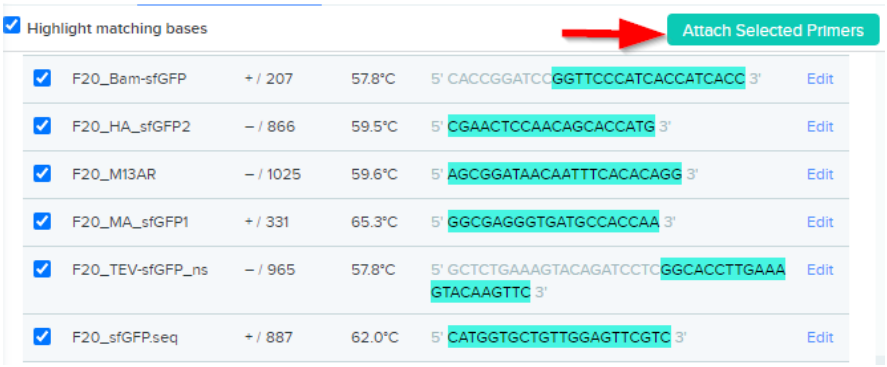
2
1. Select the primers icon from the right side. Select "Attach Existing". Next click "Add locations". In the popup, select "BSCI:414 Oligos" under the class root folder. Ensure a green checkmark is beside it and that it has been added to the "Use Primers in" field. Deselect "where entire primer matches" and "where primer matches exactly once on the sequence" checkmark boxes. When finished, select "Find Binding Sites".
2. Six primers should be found. Put a checkmark next to all primers and then select "Attach Selected Primers."
3. Click the "SEQUENCE MAP" tab and see if you can locate the positions of all six primers.



Attach existing primers.



Find primers in "BSCI:414 Oligos" location.



Attach six primers.

Create PCR Protocol in Plasmid Description

3 Select a forward and reverse primer from the six primers you added. Right click on the un-selected primers and select "Detach Primer". Make sure to keep two primers, a forward and a reverse.

4 Use the "DESCRIPTION" tab next to the "SEQUENCE MAP" tab to make a PCR schedule for your PCR reaction. Use the NEB Q5 protocol: https://www.neb.com/protocols/2013/12/13/pcr-using-q5-high-fidelity-dna-polymerase-m0491

Calculate the reagents necessary for a 20ul reaction. Use can use the template here: https://benchling.com/s/seq-sbXULtIlBpW1dHuMFhIE

This plasmid will serve as template for amplification with two primers using the NEB Q5 protocol. Instead of a 25ul reaction, a 20ul reaction is calculated.

F20 NEB Q5 PCR Sch...

NEB Q5 protocol can be found here.

F20 NEB Q5 PCR Schedule for superfolderGFP/pUC57

	A	B	C	D
1	COMPONENT	20 ul REACTION	25 µl REACTION	FINAL CONCENTRATION
2	5X Q5 Reaction Buffer	X	5 µl	1X
3	10 mM dNTPs	X	0.5 µl	200 µM
4	10 µM Forward Primer	X	1.25 µl	0.5 µM
5	10 µM Reverse Primer	X	1.25 µl	0.5 µM
6	Template DNA	0.5	variable	< 1,000 ng
7	Q5 High-Fidelity DNA Polymerase	0.2	0.25 µl	0.02 U/µl
8	5X Q5 High GC Enhancer (optional)	0	(5 µl)	(1X)
9	Nuclease-Free Water	X	to 25 µl	
10	TOTAL	0.7	50 ul	

PCR Schedule for 20ul

The primers I have chosen are:
Forward: F20_sfGFP.seq
Reverse: F20_M13AR
Expected Size: 182bp

Copy this schedule into your own plasmid "DESCRIPTION".

Add Primers to Collaborative Spreadsheet

5 Below the PCR schedule, @-mention the forward and reverse primers you have chosen. Calculate the expected PCR fragment length after amplification.

The primers I have chosen are:
Forward: ⇆ F20_sfGFP.seq
Reverse: ⇆ F20_M13AR
Expected Size: 182bp

6 Add the forward and reverse primer names to our collaborate spreadsheet, https://docs.google.com/spreadsheets/d/19MUe1tEnCBquPwgs5MkIgvgpck5mXkiXufY6J56CPcw/edit?usp=sharing

Update Lab Notebook

7 Find and open your Lab Notebook. Update your notebook to include the PCR schedule. You can copy/paste.

8 Somewhere in the lab notebook, @-mention the name of your Lab 2 plasmid. This links the work you performed today to your plasmid.