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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 Existing 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.

PRIMERS PAIRS

<input type="checkbox"/>	Name	Position	T _m
<input type="checkbox"/>	F20_Bam-sfGFP	+/- 206	57.8°C
<input type="checkbox"/>	F20_MA_sfGFP1	+/- 330	65.3°C
<input type="checkbox"/>	F20_HA_sfGFP2	-/- 865	59.5°C
<input type="checkbox"/>	F20_sfGFP.seq	+/- 886	62.0°C
<input type="checkbox"/>	F20_TEV-sfGFP_ns	-/- 964	57.8°C
<input type="checkbox"/>	F20_M13AR	-/- 1024	59.6°C

BULK ACTIONS ▼

CREATE PRIMERS ➤ ATTACH EXISTING

DETACH ALL PRIMERS

Attach existing primers.

Find binding sites for all primers in specified folders ▼

Use primers in

Quartz_Oligos ×
Macroen_updated ×
Adit Project_Oligos ×

Benchling_designed_oligos ×
Rose's_oligos ×
Xiao Lab Oligos ×

Heterome_Oligos ×
Xiaoran Shang Oligos ×

Macroen_stock_sequencing_oligos ×
Oligos_from_SG_class ×
IgnoreMe-- ok ×

BSCI:414 Oligos ×
Add locations

with at least matching bases

separated by mismatches ▼

and no more than total mismatches

with T_m above °C and under °C

☐ where entire primer matches

☐ where primer matches exactly once on the sequence

with 3' position in -

Find Binding Sites T_m parameters Use Selection

Find primers in "BSCI:414 Oligos" location.

<input checked="" type="checkbox"/>	Highlight matching bases					Attach Selected Primers
<input checked="" type="checkbox"/>	F20_Bam-sfGFP	+ / 207	57.8°C	5' CACCGGATCCGGTCCCATCACCATCACC 3'	Edit	
<input checked="" type="checkbox"/>	F20_HA-sfGFP2	- / 866	59.5°C	5' CGAACTCCAACAGCACCATG 3'	Edit	
<input checked="" type="checkbox"/>	F20_M13AR	- / 1025	59.6°C	5' AGCGGATAACAATTTACACAGG 3'	Edit	
<input checked="" type="checkbox"/>	F20_MA-sfGFP1	+ / 331	65.3°C	5' GCGAGGGTGATGCCACCAA 3'	Edit	
<input checked="" type="checkbox"/>	F20_TEV-sfGFP_ns	- / 965	57.8°C	5' GCTCTGAAAGTACAGATCCTCGGCACCTTGAAA GTACAAGTTC 3'	Edit	
<input checked="" type="checkbox"/>	F20_sfGFP.seq	+ / 887	62.0°C	5' CATGGTGCTGTTGGAGTTCGTC 3'	Edit	

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 ul 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/19MUe1tEnCBquPwgs5Mklgvqpc5mXkiXufY6J56CPcw/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.