Lab Notebook Week 16

Project: NU iGEM 2017 Shared Project
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Dates: 2017-10-03 to 2017-10-05

TUESDAY, 10/3/17

Overnight cultures started:
PelB (2 colonies picked)

iPCR for construct correction:
pC92 and pC94

Primers used:
P139 and P140 for pC92
P140 and 141 for pC94

PCR protocol (2 tubes used)
98 deg C for 30s
Repeat 10x:
98 deg C for 15s
54.6 deg C for 30s
72 deg C for 2:20 mins
Repeat 25x:
98 deg C for 15s
65.6 deg C for 30s
72 deg C for 2:20 mins
72 deg C for 4 minutes
4 deg C for inf

Gel Results:
pC92: successful
pC94: unsuccessful

PCR for cjCas9 insertion

Plasmids

	Plasmid
1	pK95
2	pK96

Primers

	A	B	C	D
1	P144	AATTAAAGAGGAGAAAGGTCATGGGCAGCAGCCATCATCA	FW	59.8, 66.2
2	P145	TAGTGGTGATGGTGATGATGAGGTCCGGACTTTTTGAAGTC	REV	55.0, 65.6

PCR protocol (2 tubes used)

98 deg C for 30s
Repeat 10x:
98 deg C for 15s
58.0 deg C for 30s
72 deg C for 1 mins
Repeat 25x:
98 deg C for 15s
68.6 deg C for 30s
72 deg C for 1 mins
72 deg C for 4 minutes
4 deg C for inf

Insert successfully amplified.

WEDNESDAY, 10/4/17

PCR wash (Do while gel runs)

- Add 250uL of PB and mix well with each PCR reaction
  - Used the one in the Qiagen kit
- Pipette everything into a column from the miniprep kit and spin for 1 minute
- Wash with CWC (500 uL) and spin for 1 minute
- Elute into a steril tube using 30uL of ddH2O

Digestion with DpnI (Prepare while gel runs)

- Add DpnI (1uL(/50uL)) to the purified PCR product and incubate for 1-4 hours (37 degC)
  - Incubated for 60 minutes

Nanodrop results (nanodrop after PCR wash and DpnI treatment):

Nanodrop results - His addit…

	A	B
1	**Plasmid**	**Concentration (ng/uL)**
2	**pC91 A**	
3	**pC91 B**	
4	**pC92 A**	
5	**pC92 B**	

Ligation

Procedure:

1. Calculate the volume of the DpnI-treated PCR product containing 50-150 ng and fill in the table below.
2. Calculate the volume of water to make up to 10 µL.
3. Mix the components below:
4. Incubate for 120 min at room temperature (~22°C).

Reaction Volumes for Ligat…

	A	B	C	D	E
1	**Reagent**	**pC91 A**	**pC91 B**	**pC92 A**	**pC92 B**
2	ddH2O	3	2	3	3
3	Purified PCR product (50 ng)	5	6	5	5
4	10x reaction buffer	1	1	1	1
5	T4 DNA ligase, 5U/uL	1	1	1	1
6	**Total:**	10	10	10	10

Perform Transformations:

Volumes for iPCR Transformations

	A	B	C
1	30 mL C-cells	5 uL iPCR ligated product	35 uL rescue media

THURSDAY, 10/5/17

PCR Wash

Introduction

This protocol outlines the procedure to perform a PCR cleanup.

Materials

- PCR Product
  - Take straight from PCR after removing ~3 uL to run a gel
- 250 uL Buffer PB
  - This is typically found in the Qiagen miniprep kit, this is a binding buffer
- 400 uL CWC
  - This is an ethanol wash, found in the Promega miniprep kit
- 30 uL NF Water
- Spin minicolumn and collection tube
- 1.5 mL microcentrifuge tubes
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Procedure

Procedure

1. Transfer PCR product to a microcentrifuge tube and add 250 uL buffer PB. Vortex to mix.
2. Pipette everything into a minicolumn and collection tube setup and spin at 14000 rpm for 1 min
3. Empty the colection tube and place minicolumn back into the same collection tube.
4. Add CWC and spin for 1 min
5. Transfer minicolum to a fresh 1.5 mL microcentrifuge tube
6. Add 30 uL NF water. Let stand for 1 minute
7. Spin for 30 seconds to elute DNA

Transformation Protocol (Chelsea's)

Introduction

Transformation protocol as recommended by Chelsea Hu.

Materials

- Competent cells
- DNA
- 2 mL microcentrifuge tube
- Agar plates (with right antibiotic resistance if needed)
- Ice (in bucket)

Procedure

1. Thaw comp cells (more than 50 uL) on ice
2. 10 uL of competent cells in each tube (20 uL for JC8031)
3. Remove agar plates (containing the appropriate antibiotic) from storage at 4°C and let warm up to room temperature
4. .5 uL of 1 pg-10 ng of each plasmid DNA in tubes, do not disturb in anyway (not even flick)
    a. Dilute stocks of 50 ng to 10 ng
5. Sit on ice for 20 mins
6. Heat shock at 42 C for 60 secs
7. Chill on ice for 5 mins
8. Add 10 uL (20 uL if using JC8031) of SOC (no antibiotics) and rescue for an hour at 37 C in shaker
9. Plate on appropriate antibiotic -agar plates