Lab Notebook - Week 13 (9/4/2017 - 9/10/2017)

Project: NU iGEM 2017 Shared Project
Authors: Lulu
Dates: 2017-09-04 to 2017-09-08

MONDAY, 9/4/17

Labor day - No experiments performed

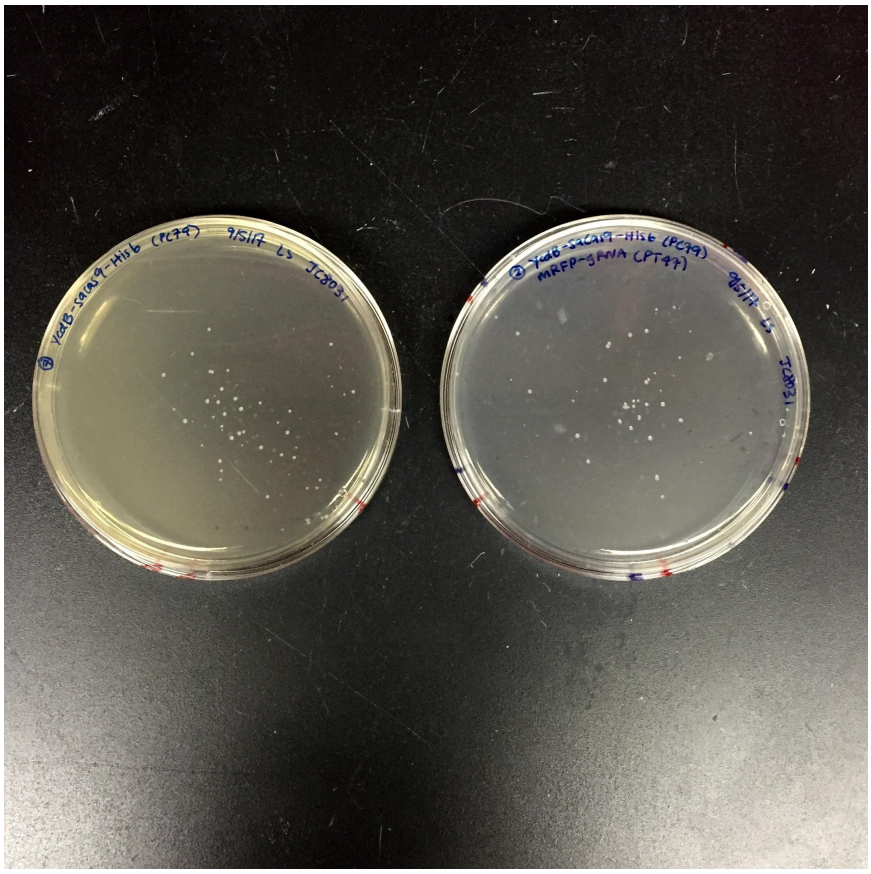
TUESDAY, 9/5/17

Transformations perfored according to protocol (transformation with gibson product)

Samples

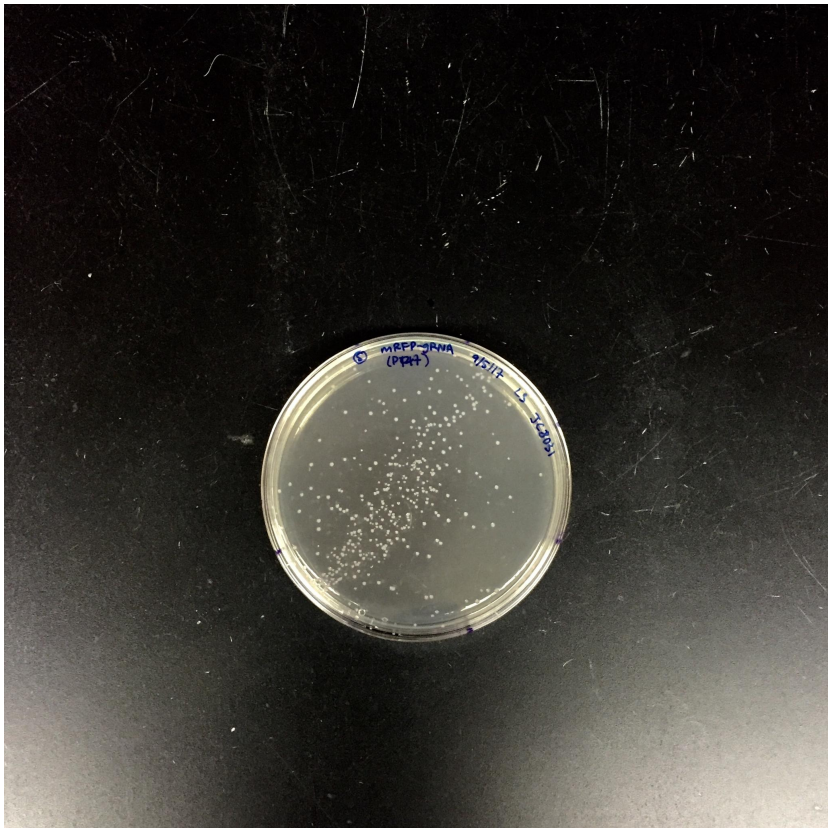
	A	B	C	D
1	**Sample #**	**DNA #1**	**DNA #2**	**Resistance**
2	1	YcbK-saCas9-His6	mRFP-gRNA	Tet+Cam
3	2	YcdB-saCas9-His6	mRFP-gRNA	Tet+Cam
4	3	NapA-saCas9-His6	mRFP-gRNA	Tet+Cam
5	4	saCas9-His6	mRFP-gRNA	Tet+Cam
6	5	mRFP-gRNA		Tet
7	6	YcbK-saCas9-His6		Cam
8	7	YcdB-saCas9-His6		Cam
9	8	NapA-saCas9-His6		Cam

Ycdb-saCas9-His6 (JC8031) neg ctrl and w/mRFP-gRNA



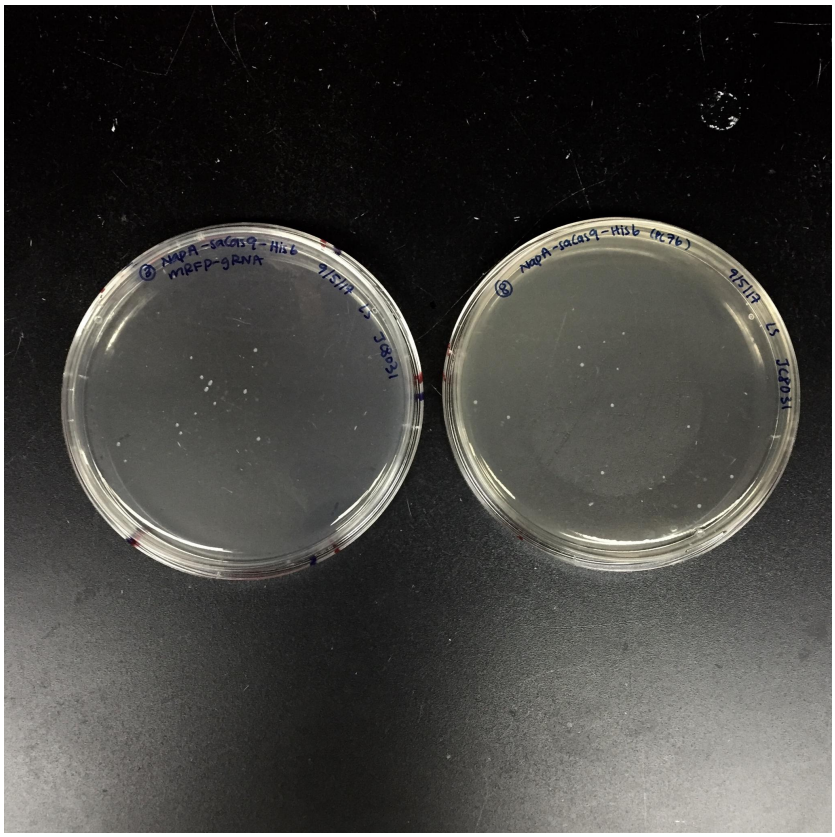
Negative control (left)
Co-transformation (right)

mRFP-gRNA in JC8031



Looked pink, could have put in a higher concentration of DNA

NapA-saCas9-His (JC8031) w/mRFP-gRNA and neg ctrl



Co-transformation (left)
Negative control (right)

YcbK co-transformation and negative controls failed to grow

Started overnight cultures (3) of JC8031 (no antibiotic) for OMV fusion experiments
Attempted to PCR amplify cjCas9 with restriction site overhangs but failed

WEDNESDAY, 9/6/17

OMV Purification

Total time for completion: ~ 4 hours
Uses ion exchange chromatography (highly charged surface of OMVs)
Load no more than 100mg of protein per 1mL of resin into the column
As a rough estmate, one reaction of 200uL of the resin is sufficient for 10mL of the clasified bacterial supernatant.

1. Prepare clarified supernatant from bacterial culture
    a. Culture the bacteria in its growth medium overnight at 37 deg.
    b. Spin down bacteria at 5000xg for 15 minutes at 4 deg C
        I. 4000xg for 20 minutes

2. Pack the column/bind OMVs
    a. Pipette 200 mL of the resin onto the column
    b. Equillibrate by adding 1mL of the Binding Buffer and allow the solution to flow through. Discard the flow through
    c. Place the yellow cap onto the bottom of the column
    d. Add 10mL of the clarified bacterial supernatant (prepared in step 1) to the resin and incubate on a rotating rack at 4 deg C for 3-4 hours to allow for OMV binding
        I. Use centrifuge at a very low speed
3. OMV Elution
    a. Place the column onto a rack and allow the resin/supernatant to flow through (collect the flow through for analysis if desired)

b. Wash the resin with 10mL Binding Buffer 2 times. Discard the flow through.

c. Add 500uL Elusion Buffer and collect in in 1.5ml Eppendorf tube
    I. Repeat the elusion step for a total of 5 times in separate tubes

4. Analysis
    a. Perform downstream analysis of the five separate elutions (or pool if desired)

3 tubes (600uL each) eluded and stored at +4 deg C

PRC for addition of saCas9-YcbK-His6 in pBAD vector

BACKBONE PCR:

Primers:

plasmid pC37-pC39 should work but should dilute

PCR Primers - Backbone

	A	B	C
1	**Primer Name**	**Sequence**	**Melting Temperature**
2	P112 (FW)	tgcaccaccatcatcatcatTGGCTGTTTTGGCGGATGAG	58.4, 68.2
3	P118 (REV)	TTCGCATCAAATTTATCCATCGAATTCGCTAGCCCAAAAA	54.2, 64

PCR Materials:

- nf water 22 uL
- 1 uL 10uM P112
- 1 uL 10uM P118
- 1 uL plasmid (~1 ng/uL)
- 25 uL Phusion HS Flex 2xmm
- PCR tube

Total volume = 50 uL

PCR protocol (4 tubes used)

98 deg C for 30s

Repeat 10x:

98 deg C for 15s

57.2 deg C for 30s

72 deg C for 2:30 mins

Repeat 25x:

98 deg C for 15s

67 deg C for 30s

72 deg C for 2:30 mins (125 s)

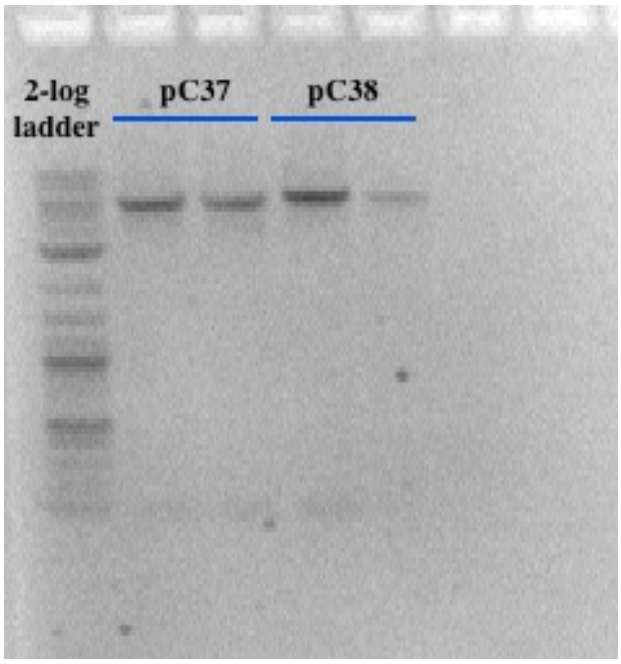
72 deg C for 5 minutes

4 deg C for inf

pBAD BACKBONE

	A	B	C	D
1	**Lane**	**PCR Tube**	**Expected**	**Results**
2	1	2-log ladder	-	-
3	2	pC37 (A)	5kB	Success
4	3	pC37 (B)	5kB	Success
5	4	pC38 (C)	5kB	Success
6	5	pC38 (D)	5kB	Success

pBAD Backbone gel results



INSERT PCR:

Primers:

Plasmid: pC75: 238.4 ng/uL --> need to dilute

PCR Primers - Insert

	A	B	C
1	**Primer Name**	**Sequence**	**Melting Temperature**
2	P119 (FW)	CCATTAGCTTTCTCCTCTTTTTCGCTAGCCCAAAAAAACG	51.9, 64
3	P115 (REV)	CTCATCCGCCAAAACAGCCAatgatgatgatggtggtgca	54.4, 68.2

PCR Materials:

- nf water 22 uL
- 1 uL 10uM P115
- 1 uL 10uM P119

- 1 uL plasmid (~1 ng/uL)
- 25 uL Phusion HS Flex 2xmm
- PCR tube

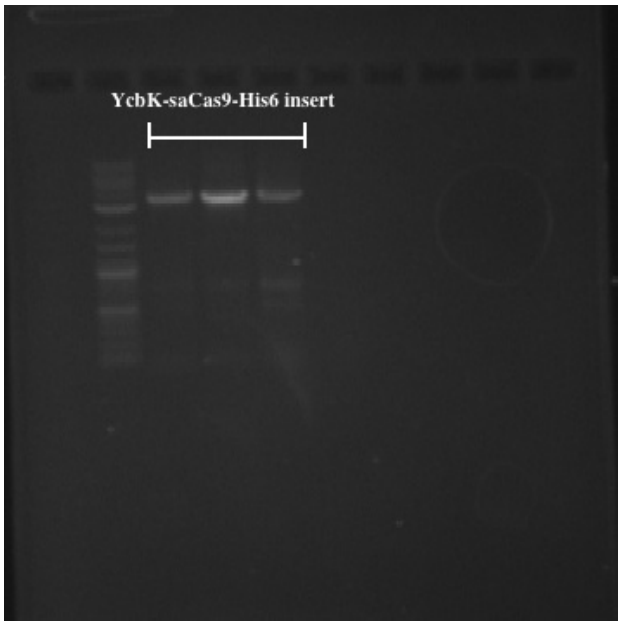
Total volume = 50 uL

PCR protocol (3 tubes used)
98 deg C for 30s
Repeat 10x:
98 deg C for 15s
54.9 deg C for 30s
72 deg C for 1.7 mins
Repeat 25x:
98 deg C for 15s
67 deg C for 30s
72 deg C for 1.7 mins
72 deg C for 5 minutes
4 deg C for inf

PCR Insert layout

	A	B	C	D
1	**Lane**	**PCR Tube**	**Expected**	**Results**
2	1	2-log ladder	-	-
3	2	pC75 (1)	~3kB	Successful
4	3	pC75 (2)	~3kB	Successful
5	4	pC75 (3)	~3kB	Successful

pBAD PCR Insert gel results



THURSDAY, 9/7/17

GEMSTONE

- Resuspend 200 - 500ug protein equivalent of OMVs in 500uL PBS
    - 150 uL for each one of the two dyes
- Add 1uL of the 500X or 2.5uL of the 250X labeling dye to the OMV preparation and incubate at 37degC with shaking for 20 minutes
- Add 167uL ExoQuick-TC to the solution and incubate at 4deg C for 2h-overnight
- Spin the eppendorf tube at 10000rpm for 10 minutes
- Carefully aspirate the supernatant from the corner of the tube
- Resuspend the OMV pellet in 500 uL PBS and proceed with downstream applications

Gibson assembly for YcbK-saCas9-His6 into pBAD backbone:

DPNI Digest (1uL added) of backbone and insert at 37deg C for 1 hour

PCR wash: Backbone, Insert

- Add 250uL of PB and mix well with each PCR reaction
- Pipette everything into a column from the miniprep kit and spin for 1 minute
    - Dump out PB into miniprep waste
- Wash with CWC (500 uL) and spin for 1 minute
    - Dump out CWC into miniprep waste
- Spin dry column for 1 minute
    - Dump out any elution
- Add 30uL of nf water into sterile 2mL tube, spin for 1 minute to elute

Gibson concentrations

	A	B	C	D	E
1		**ng/uL**	**bp length**	**nmol/uL**	**nM**
2	pBAD Backbone	26.5	6103	7.14841e-6	7.148406117
3	pBAD Insert	112.3	3159	5.85221e-5	58.52206003

Gibson Volumes

	A	B	C
1		**YcbK Assembly**	**Negative control**
2	BB	3 uL	1 uL
3	Insert	1 uL	3 uL
4	nf water	6 uL	16 uL
5	Gibson MM	10 uL	---

Place Gibson mix into PCR machine, 15 minutes @ 50°C
Placed in +4 for completion the following day

FRIDAY, 9/8/17

Transformation of Gibson product
Protocol given by Chelsea

Materials:

- 50 uL competent cells per Gibson assembly DNA
- 15 uL of GIbson assembly DNA
- 65 uL SOC media
- Agar plates

Transformation protocol

- Place 50 uL of competent cells into a microcentrifuge tube (Or use original vial, if it already has 50 uL)
    - Cells used: JC8031
- Keep on ice
- Pipette 15 uL of Gibson assembly DNA into each tube
- Incubate on ice for 20 minutes
- Heat shock tubes in water bath at 42°C for 60s
- Incubate on ice for 5 minutes
- Add 65 uL of SOC media, rescue for 1 hour & 15 minutes at 37°C in shaker
- Plate 80 uL onto plate with correct antibiotics (Cm)

Transformation with Gibson Product

Introduction

Transformation into competent cells after Gibson assembly
Protocol given by Chelsea

Materials

- 50 uL competent cells per Gibson assembly DNA
- 15 uL of GIbson assembly DNA
- 65 uL SOC media
- Agar plates

Procedure

Transformation protocol

1. Place 50 uL of competent cells into a microcentrifuge tube

   (Or use original vial, if it already has 50 uL)
   Keep on ice
2. Pipette 15 uL of Gibson assembly DNA into each tube
3. Incubate on ice for 20 minutes
4. Heat shock tubes in water bath at 42°C for 60s
5. Incubate on ice for 5 minutes
6. Add 65 uL of SOC media, rescue for 1 hour & 15 minutes at 37°C in shaker
7. Plate 80 uL onto plate with correct antibiotics