Lab Notebook - Week 11 (8/21/2017-8/27/2017)

Project: NU iGEM 2017 Shared Project
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MONDAY, 8/21/17

Gibson PCR product concentrations

NapA insert 15.7 ng/uL - dilute to 20nM
NapA bb 96.3 ng/uL - dilute to 20nM
YcdB bb 83.7 ng/uL -dilute to 20nM
YcdB ins 18.9 ng/uL -dilute to 20nM

Gibson YcdB

10uL of Gibson MM
1uL of YcdB bb (20nM)
3uL of YcdB ins (20nM)
6uL nf water

Gibson Transformation: YcdB

a. Thaw comp cells (50uL) on ice for 15 minuts
b. Pipette 15uL of Gibson assembly DNA into comp cells
c. Incubate on ice for 20min.
d. Heat shock at 42°C for 60s
e. Incubate on ice for 5min.
f. Add 65uL of SOC medai, rescue for 1:15 min in shaker at 37°C
g. Plate 80uL onto Cm plate and put in incubator at 37°C for 18 hours.

SWAP cjCAS9 and saCAS9

YcbK-cjCas9-His6

PCR backbone:

Primers: P81 (FWD)
P95 (REV)
DNA: YcbK-saCas9-His6

resuspend primers to 100uM
dilute primers from 100uM to 10uM
2.5uL of 100uM P81/P95
22.5uL of nf water each

PCR components:

nf water: 22uL
P81: 1uL
P95: 1uL
DNA Plasmid: 1uL
Phusion HS MM: 25uL

*Dilute YcbK-saCas9-His6 to 10ng/uL

PCR Protocol:

98°C - 30s
10 cycles
98°C - 15s
57.4°C - 30s
72°C - 1:09 min

25 cycles

98°C - 15s

69.5°C - 30s

72°C - 1:09 min

72°C - 5min

4°C - inf.

Test NapA primers

Primers: P89, P67

DNA: SS-Gblock (10ng/uL)

*resuspend primers to 100uM

*dilute primers from 100uM to 10uM

2.5uL of 100uM to 10uM

22.5uL of nf water each.

PCR components:

98°C - 30s

10 cycles

98°C - 15s

60.0°C - 30s

72°C - 15s

20 cycles

98°C - 15s

71.0°C - 30s

72°C - 15s

72°C - 5min

4°C - inf.

NANODROP RESULTS

Table1

	Plasmid	Concentration	260/280	260/230
1	NapA-saCas9-His6 1	378.5 ng/uL	1.86	2.05
2	NapA-saCas9-His6 2	304.0 ng/uL	1.84	1.79
3	NapA-saCas9-His6 3	293.6 ng/uL	1.87	2.26
4	YcdB-saCas9-His6 1	282.5 ng/uL	1.88	2.28
5	YcdB-saCas9-His6 2	380.0 ng/uL	1.87	2.23
6	YcdB-saCas9-His9 3	303.3 ng/uL	1.84	1.81