2018 University of Iowa International Genetically Engineered Machine Team
Dr. Craig Ellermeier's Lab, Microbiology and Immunology Department
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iGEM Tube Labeling Key

ID	Description	Date	Concentration
A	Strain 2978 Miniprep	26June2018	304 ng/uL
B	Strain 2979 Miniprep	26June2018	235.2 ng/uL
C	*P. denitrificans* hpdH Clean-up	21June2018	2.9 ng/uL
D	*P. denitrificans* mmsA Clean-Up	21Jun2018	2.3 ng/uL
E	*P. putida* mmsA Clean-up	21June2018	5.6 ng/uL
F	*P. putida* hpdH Clean-up	21June2018	9.2 ng/uL
G	*P. denitrificans* mmsR Eco/Pst	14June2018	18 ng/uL
H	741 Miniprep	13July2018	108.3 ng/uL
I	733 Miniprep	13July2018	36.3 ng/uL
J	*P. denitrificans* hpdH Gel Purify	17July2018	71.1 ng/uL
K	*P. denitrificans* mmsA Gel Purify	17July2018	48.3 ng/uL
L	*P. putida* hpdH Gel Purify	17July2018	61.6 ng/uL
M	*P. putida* mmsA Gel Purify	17July2018	42.8 ng/uL
N	*P. denitrificans* mmsA Miniprep (from liquid culture A)	19July2018	145 ng/uL
O	*P. denitrificans* mmsA Miniprep (from liquid culture D)	19July2018	81.9 ng/uL
P	*P. denitrificans* hpdH Miniprep (from liquid culture A)	19July2018	119.2 ng/uL
Q	*P. denitrificans* hpdH Miniprep (from liquid culture C)	19July2018	105.3 ng/uL
R	*P. denitrificans* hpdH (from plate J #2) Miniprep	26July2018	199.9 ng/uL
S	*P. denitrificans* mmsA Miniprep (from plate #2 K)	26July2018	243.5 ng/uL
T	*P. denitrificans* hpdR Miniprep (from B)	26July2018	263.6 ng/uL

U	*P. denitrificans* hpdH Miniprep (from culture E)	27July2018	149.0 ng/uL
V	*P. denitrificans* mmsA Miniprep (from culture A)	27July2018	162.0 ng/uL
W	*P. denitrificans* hpdR Miniprep (from culture #5)	27July2018	78.1 ng/uL
X	*P. denitrificans* mmsR Miniprep (from culture #1)	27July2018	94.1 ng/uL
Y	*P. denitrificans* hpdH Miniprep (from culture F)	01Aug2018	165.7 ng/uL
Z	*P. denitrificans* hpdH Miniprep (from culture #6)	01Aug2018	296.1 ng/uL
#	*P. denitrificans* hpdH Miniprep (from culture H)	01Aug2018	268.7 ng/uL
*	*P. denitrificans* hpdR Miniprep (from culture #7)	01Aug2018	89.6 ng/uL
(triangle)	*P. denitrificans* hpdR Miniprep (from culture #8)	01Aug2018	79.6 ng/uL

12June2018:

Objective #1: We were given a plate prepared from plasmid strains ECE 741 and ECE 733 by Dr. Ellermeier. We subbed both plasmids onto a new LB+AMP plate and prepared overnight cultures.

Results: Both plasmids showed growth. More growth with the ECE 741 plasmid. ECE 733 had very few colonies. Liquid cultures were very turbid.

13June2018:

Objective #1: Prepare ECE plasmids for transformation via mini-prep. 2mL of liquid cultures were used. Remaining 3mL received glycerol and were frozen.

Results: Plasmids were kept in the freezer. The 741 plasmid with concentration of 118 ng/uL were used for restriction digest.

Objective #2: Run a PCR to evaluate the *P. denitrificans* and *P. putida* mmsR and hpdR genes used in 2017.

Results: PCR products run on a gel on 14June2018.

14June2018:

Objective #1: Run yesterday's regulator PCR products on a gel.

*These were split into two gels because the first was not properly loaded.

Results: Only bands for the two *P. denitrificans* genes. Consistent with earlier experiments done by Dr. Ellermeier. *P. putida* genes were thrown out before gel extraction.

Objective #2: Extract DNA from the PCR gel run previous. Will be used for a restriction digest.

Results: *P. denitrificans* hpdr: 40.8 ng/uL

P. denitrificans mmsr: 40.6 ng/uL

Objective #3: Digest plasmids and regulator genes for ligation. (50uL Digest)

Objective #4: Purify DNA from restriction digest via cleanup.

Results: Plasmid 741: 25.8 ng/uL

P. denitrificans hpdR: 37.5 ng/uL

P. denitrificans mmsR: 18 ng/uL

15June2018:

Objective #1: Run a gel to ensure restriction digest worked.

Lane #1: 100bp Ladder

Lane #2: 5uL Plasmid 741 Digest + 2uL Loading Dye

Lane #3: 5uL hpdR Digest + 2uL Loading Dye

Lane #4: 5uL mmsR Digest + 2uL Loading Dye

Lane #5: 5uL mmsR Digest + 2uL Loading Dye

*possible contamination in Lane #3

Results: Lanes 3-5 had bands. Plasmid 741 did not. Will have to be redone.

Objective #2: Restriction digest of both plasmids 741 and 733. (50uL Digest)

Objective #3: Digest cleanup of plasmids 741 and 733.

Results: Plasmid 741: 23.7 ng/uL

Plasmid 733: 1.9/2.5 ng/uL

Objective #4: Run plasmid digest out on gel.

Lane #1: 100bp Ladder

Lane #2: 5uL Plasmid 733 Digest + 2uL Loading Dye

Lane #3: 5uL Plasmid 741 Digest + 2uL Loading Dye

18June2018:

Objective #1: Redo mini-prep for both Plasmids 741 and 733.

Results: Apparently wrong protocol. Start over.

Objective #2: Redo mini-prep for both Plasmids 741 and 733.

Results: Plasmid 741: 187.7 ng/uL

Plasmid 733: 288 ng/uL

Objective #3: 10uL Restriction Digest on Plasmids 733 and 741.

Objective #4: Run Plasmid digest products on gel.

Lane #1: 100bp Ladder

Lane #2: 5uL Plasmid 741 + 2uL Loading Dye

Lane #3: 10uL Plasmid 741 Digest + 2 uL Loading Dye

Lane #4: 5uL Plasmid 733 + 2uL Loading Dye

Lane #5: 10uL Plasmid 733 Digest + 2uL Loading Dye

Results: 1kB band dropped, redo.

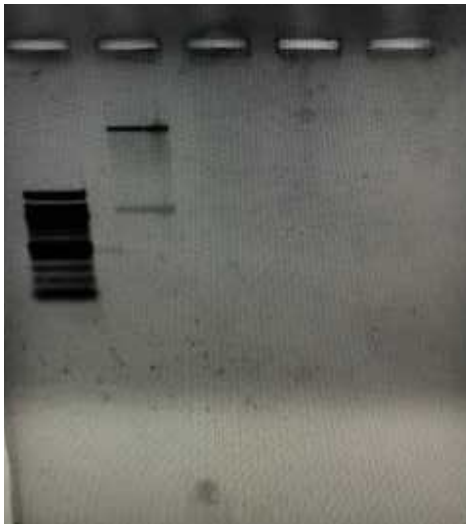
Objective #5: 40uL Digest on Plasmids 733 and 741.

19June2018:

Objective #1: Cleanup digest on plasmids from yesterday.

Results: Plasmid 741: 31.5/22/21.6 ng/uL

Plasmid 733: 9.9/8.6/9.3 ng/uL



Objective #2: Run cleanup digests on gel.

Lane #1: 100bp Ladder

Lane #2: 5uL Plasmid 741 Digest + 1uL Loading Dye

Lane #3: 5uL Plasmid 733 Digest + 1uL Loading Dye

Results: 1kb Band for 741.

Objective #3: Ligation of *P. denitrificans* hpdR and mmsR into Plasmid 741.

Vector: 10x Master Mix +2uL Plasmid 741 + 0uL Insert Gene + 6.5uL H2O + .5 Ligase

hpdR: 10x Master Mix + 2uL Plasmid 741 + .4uL hpdR + 6.1uL H2O + .5 Ligase

mmsR: 10x Master Mix + 2uL Plasmid 741 + .8uL mmsR + 5.7uL H2O + .5 Ligase

Objective #4: Ligation of *P. denitrificans* hpdR and mmsR into Plasmid 741.

Vector: 3uL Plasmid 741 + 0uL Insert Gene + 2uL H2O + 5uL Master Mix

hpdR: 3uL Plasmid 741 + .6uL hpdR + 1.4uL H2O

mmsR: 3uL Plasmid 741 + 1.2uL mmsR + .8uL H2O

Objective #5: PCR of promoters *P. denitrificans* and *P. putida* mmsA and hpdH.

Objective #6: Transformation of *P. denitrificans* mmsR and hpdR into *E. coli* competent cells.

60uL E. coli competent cells + 5uL DNA

Use LB after heat shock

Plate on LB+AMP

20June2018:

Objective #1: Gel to evaluate promoter PCR products.

Lane #1: 100bp Ladder

Lane #2: 5uL *P. putida* hpdH + 2uL Loading Dye

Lane #3: 5uL *P. denitrificans* hpdH + 2uL Loading Dye

Lane #4: 5uL *P. denitrificans* mmsA + 2uL Loading Dye

Lane #5: 5uL *P. putida* mmsA + 2uL Loading Dye

Objective #2: Redo transformation. Not enough ligated product. Repeat ligation.

Vector: 3uL Plasmid 741 + 0uL Insert Gene + 2uL H2O + 5uL Master Mix

hpdR: 3uL Plasmid 741 + .6uL hpdR + 1.4uL H2O

mmsR: 3uL Plasmid 741 + 1.2uL mmsR + .8uL H2O

Objective #3: Repeat transformation of P. denitrificans mmsR and hpdR into E. coli competent cells.

60uL E. coli competent cells + 5uL DNA

Use LB after heat shock

Plate on LB+AMP

21June2018:

Objective #1: Count colonies from transformation growth.

Vector Plate #1: 18 White Colonies

Vector Plate #2: 64 White Colonies

P. denitrificans hpdR Plate #1: 60 White Colonies

P. denitrificans hpdR Plate #2: Over 200 White Colonies

P. denitrificans mmsR Plate #1: 7 White Colonies

P. denitrificans mmsR Plate #2: 45 White Colonies

Objective #2: Pick 8 white colonies from each transformation, restreak.

Objective #3: Cleanup on promoter PCR products.

Results: *P. putida* mmsA: 5.6 ng/uL

P. putida hpdH: 9.2 ng/uL

P. denitrificans mmsA: 2.3 ng/uL

P. denitrificans hpdH: 2.9 ng/uL

22June2018*:*

Objective #1: Pick white colonies from transformation plates from 21June2018. Restreak.

Objective #2: Redo Promoter PCR.

23June2018:

Objective #1: Start overnights of white colonies from mmsR transformation plates from 22June2018.

Results: *P. denitrificans* hpdR transformation plates unsuccessful.

Objective #2: Run Promoter PCR products from 22June2018 on a gel. Look for 500bp Bands.

Objective #3: Gel extraction and cleanup of Promoter PCR products.

25June2018:

Objective #1: Miniprep *P. denitrificans* mmsR transformant from liquid culture.

Results: *P. denitrificans* mmsR: 26.2 ng/uL

Objective #2: Perform 10uL digest on *P. denitrificans* mmsR.

Objective #3: Run mmsR digest on a gel next to uncut plasmid.

Lane #1: 100bp Ladder

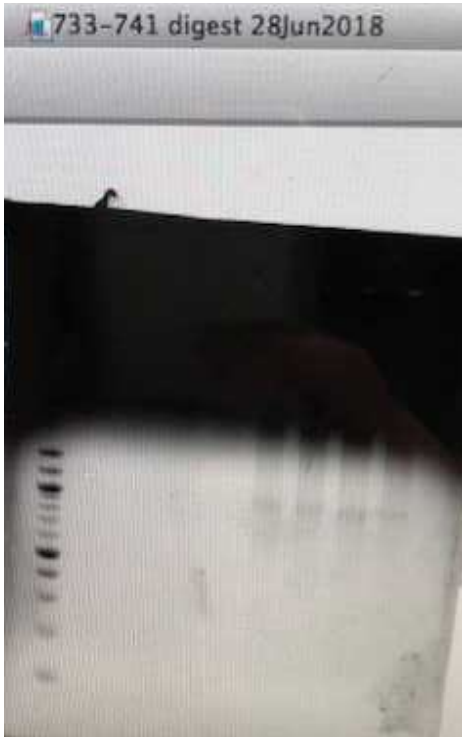
Lane #2: *P. denitrificans* mmsR digest

Lane #3: Uncut Plasmid 741

Objective #4: Prepare new liquid cultures of Plasmids 741 (strain #2979) and 733 (strain #2978).

Objective #5: Restriction digest of *P. denitrificans* hpdR (10uL digest).

Objective #6: Run *P. denitrificans* hpdR digest on gel.



Objective #7: Restriction digest of both Plasmids 733 and 741 (50uL digest).

Objective #8: Run gel of Plasmids digest.

Lane #1: 100bp Ladder

Lane #2: 15uL Plasmid 733 Digest + 4uL Loading Dye

Lane #3: 15uL Plasmid 733 Digest + 4uL Loading Dye

Lane #4: 15uL Plasmid 733 Digest + 4uL Loading Dye

Lane #5: 15uL Plasmid 733 Digest +4uL Loading Dye

Lane #6: 15uL Plasmid 741 Digest + 4uL Loading Dye

Lane #7: 15uL Plasmid 741 Digest + 4uL Loading Dye

Lane #8: 15uL Plasmid 741 Digest + 4uL Loading Dye

Lane #9: 15uL Plasmid 741 Digest + 4uL Loading Dye

Objective #9: Gel extraction of plasmids digest.

Objective #10: Miniprep Plasmids 741 and 733.

Results: Plasmid 741 (done by Katie): 161.1 ng/uL

Plasmid 733 (done by Katie): 82 ng/uL

Plasmid 741 (done by Kyleakin): 106.5 ng/uL

Plasmid 733 (done by Kyleakin): 89.9 ng/uL

Objective #11: Restriction digest on all minipreps of Plasmids 741 and 733. (50uL Digest)

Objective #12: 10uL Digest on white cultures of mmsR and hpdR.

Results: hpdR: 163.6 ng/uL

hpdR: 74.6 ng/uL

mmsR: 40.7 ng/uL

mmsR: 39.4 ng/uL

Objective #13: Not-1 Digest of *P. denitrificans* mmsR transformant.

Objective #14: Gel of Not-1 *P. denitrificans* mmsR transformant.

27June2018:

28June2018:

29July2018:

11July2018:

Objective #1: Miniprep Plasmids 741 and 733 from overnight cultures.

Results: Plasmid 741 A: 115.4 ng/uL

Plasmid 741 B: 112.5 ng/uL

Plasmid 733 A: 88.2 ng/uL

Plasmid 733 B: 88.9 ng/uL

Objective #2: Restriction digest all Plasmid minipreps.

Objective #3: Run Plasmid minipreps on a gel.

Lane #1: 100bp Ladder

Lane #2: Plasmid 741 A

Lane #3: Plasmid 741 A

Lane #4: Plasmid 741 B

Lane #5: Plasmid 741 B

Lane #5: Plasmid 741 B

Lane #6: Plasmid 733 A

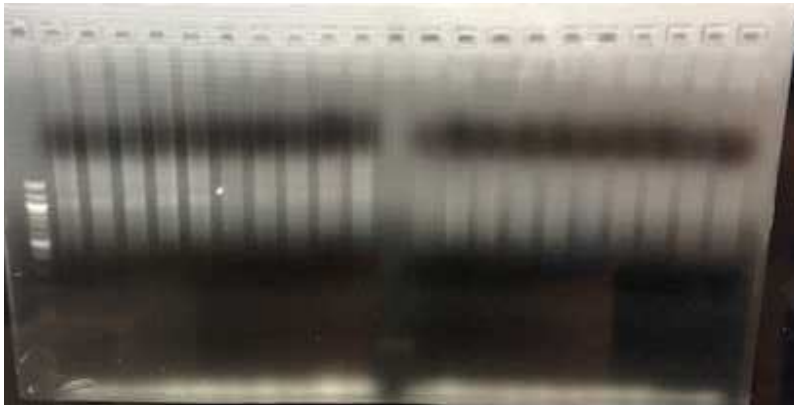
Lane #7: Plasmid 733 A

Lane #8: Plasmid 733 B

Lane #9: Plasmid 733 B

Lane #10: Plasmid 733 A

Lane #11: Plasmid 733 B



Objective #4: Measure nanospecs of digested Plasmids.

Results: Plasmid 733: 40 ng/uL

Plasmid 733: 41.4 ng/uL

Plasmid 741: 62 ng/uL

Plasmid 741: 58.7 ng/uL

Objective #5: Run a gel of digested plasmids and regulators.

Lane #1: 100bp Ladder

Lane #2: Digested Plasmid 741

Lane #3: Dr. Ellermeier's 733

Lane #4: Digested hpdR

Lane #5: Digested mmsR

Results: Plasmid 733 was smeared. Plasmid 741 was okay.

Objective #5: Cleanup of Plasmid 741 Digest.

Objective #6: More nanospecing.

14June2018 mmsR: 37.5 ng/uL

28June2018 hpdR: 163.6 ng/uL

Plasmid 741: 32.6 ng/uL

Objective #7: Gel of 14June2018 mmsR, 28June2018 hpdR and Plasmid 741.

Results: Plasmid 741 was smeared. hpdR and mmsR failed.

Objective #8: Diagnostic gel to find right good hpdR genes. 5uL of DNA + 1uL Loading Dye.

Lane #1: mmsR (?)

Lane #2: hpdR with 2.2 ng/uL

Lane #3: mmsR with 19 ng/uL (?)

Lane #4: hpdR (?)

Lane #5: *P. denitrificans* mmsR with 22 ng/uL

Lane #6: *P denitrificans* hpdR with 22 ng/uL

Lane #7: *P. denitrificans* hpdR with 18 ng/uL **G**

Lane #8: *P. denitrificans* with 74.6 ng/uL

Lane #9: mmsR (?)

Lane #10: hpdR with 37.5 ng/uL

Lane #11: mmsR with 165.6 ng/uL

Results: Only **G** is viable.

13July2018:

Objective #1: Miniprep Plasmids 733 and 741.

Results: Plasmid 741: 108.3 ng/uL **H**

Plasmid 733: 38.3 ng/uL **I**

Objective #2: Ligation of Plasmids and regulator genes.

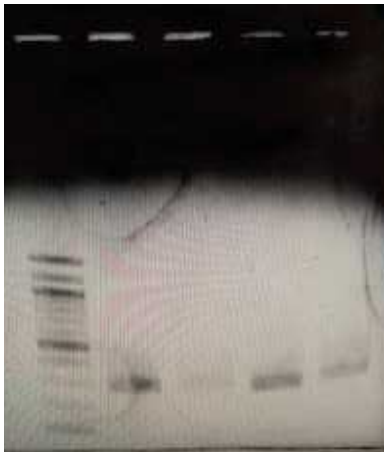
25uL 2x Master Mix + 1.5uL Plasmid 741 + 1uL mmsR

25uL 2x Master Mix + 1.5uL Plasmid 741 + 1uL hpdR

Objective #3: Transform regulator plasmids into E. coli.

16July2018:

Objective #1: Diagnostic digest of mmsR and hpdR transformants. Used Nco1 and PFLF2.



Objective #2: Gel to evaluate diagnostic digest.

Lane #1: 100bp Ladder

Lane #2: mmsR Nco1/PFLF2 Digest #1

Lane #3: mmsR Nco1/PFLF2 Digest #2

Lane #4: hpdR Nco1/PFLF2 Digest #5

Lane #5: hpdR Nco1/PFLF2 Digest #6

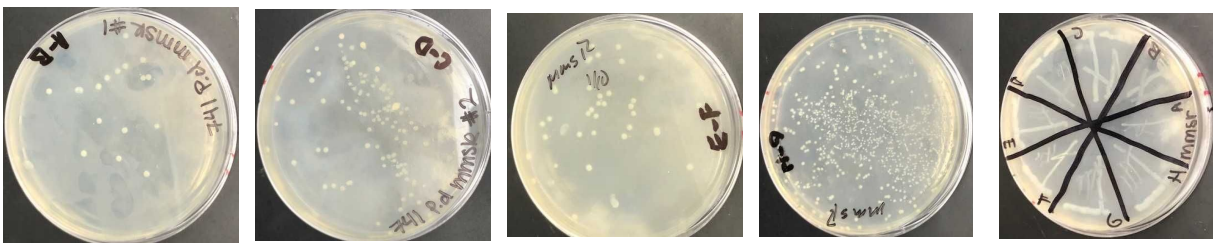
Results: Both mmsR and hpdR were successful.

mmsR #1: 96.6 ng/uL

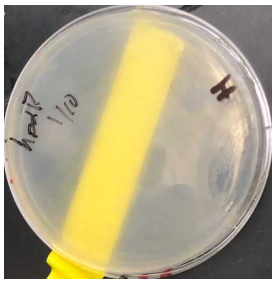
mmsR #2: 79.5 ng/uL

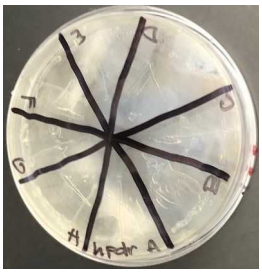
hpdR #5: 116.7 ng/uL

hpdR #6: 79.9 ng/uL



Objective #3: Submit mmsR and hpdR transformants for sequencing. Submit both SEPARATE forward and reverse primers.

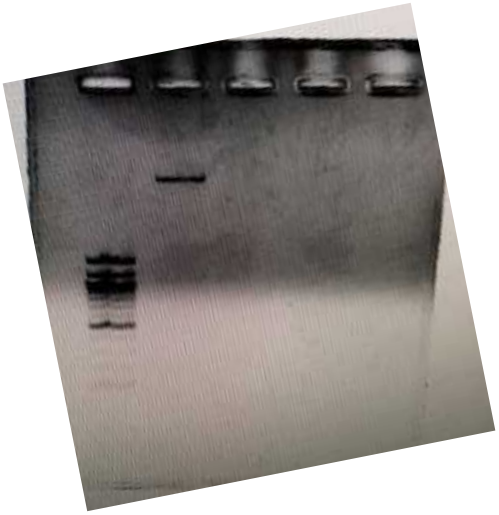




10uL DNA + 1uL Primer Dilution (4uL stock primer to 16uL H2O)

17July2018:

Objective #1: Nanospec gel purified Promoter products.



Results: *P. denitrificans* hpdH: 64.4/71.1 ng/uL

P. denitrificans mmsA: 49.1/48.3 ng/uL

P. putida hpdH: 81.1/61.6 ng/uL

P. putida mmsA: 2/42.8 ng/uL

Objective #2: PCR Cleanup of Plasmid 733 digestion.

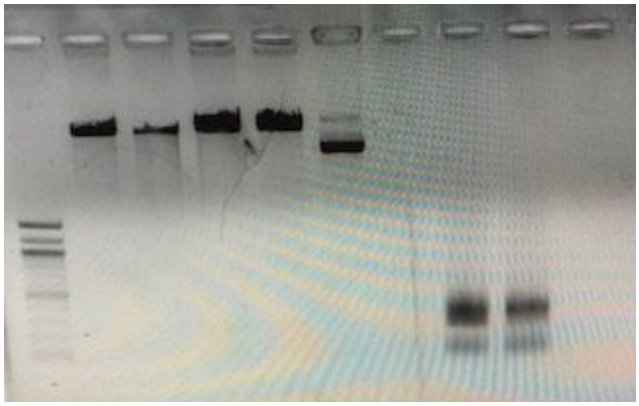
Results: Plasmid 733 Digestion: 14.7 ng/uL

Objective #3: Perform transformation of hpdH and mmsA (the high concentrations on the gel then gel purification)

2.5uL 2x Master Mix + 1.5uL Vector (Plasmid 741) + 1uL Insert (Promoter Genes)

19July2018:

Objective #1: Run a diagnostic PCR and diagnostic digest on hpdH (now known as **J**) and mmsA (now known as **K**) cultures.



Results: Both looked good. Got expected bands. Cleared for sequencing.

20July2018:

Objective #1: Turn in hpdH (**J**) and mmsA (**K**) transformants for sequencing.

23July2018:

Results: Sequencing of regulators showed that we submitted the same mmsR transformant twice. Resubmitted for hpdR.

24July2018:

Objective #1: Issues with sequencing the promoter transformants the first time (wrong protocol).

*Not enough of cultures **J** and **K**, so we redid overnights.

Objective #2: Start overnights on mmsR/Plasmid 741 transformant as well.

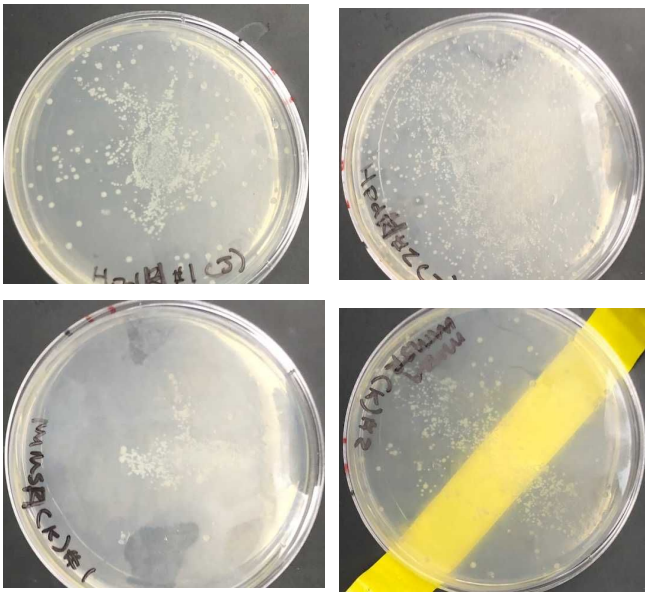
25July2018:

Objective #1: Miniprep **J** (hpdH) and **K** (mmsA), as well as mmsR/Plasmid 741 transformant.

Results: mmsA miniprep: 7.3/11.2 ng/uL

hpdH miniprep: 9.3/20.4 ng/uL

*results too low. Will re-miniprep tomorrow.



26July2018:

Objective #1: Miniprep mmsA, hpdH and hpdH 5 cultures.

Results: mmsA: 243.5 ng/uL

hpdH: 263.6 ng/uL

hpdH 5: 199.9 ng/uL

* these were the wrong genes to prepare liquid cultures.

Objective #2: Prepare overnights from correct mmsA and hpdH stocks.

27July2018:

Objective #1: Miniprep mmsA/hpdH cultures as well as mmsR/hpdR cultures. These were taken from previously grown plates labeled in sections. We took mmsA from section A, hpdH from section E, hpdR from section 5 and mmsR from section 1.

Results: hpdH (E): 149 ng/uL (now known as **U**)

mmsA (A): 167 ng/uL (now known as **V**)

hpdR (5): 60.5 ng/uL (now known as **W**)

mmsR (1): 94.1 ng/uL (now known as **X**)

Objective #2: Sequence hpdH (**U**), hpdR (**W**) and mmsA (**V**).

31July2018:

Objective #1: Begin transformation into *B. subtilis*. Plasmid/gene combos will need the following growth media:

phpdH-lux and PmmsA-lux need MLS plates

pmmsR-xyl need kanamycin plate

Bacillus subtilis transformation:

1.Prepare the following media in a tube: 30uL 1M MgSO4 + 9mL H2O + 1mL 10x MC

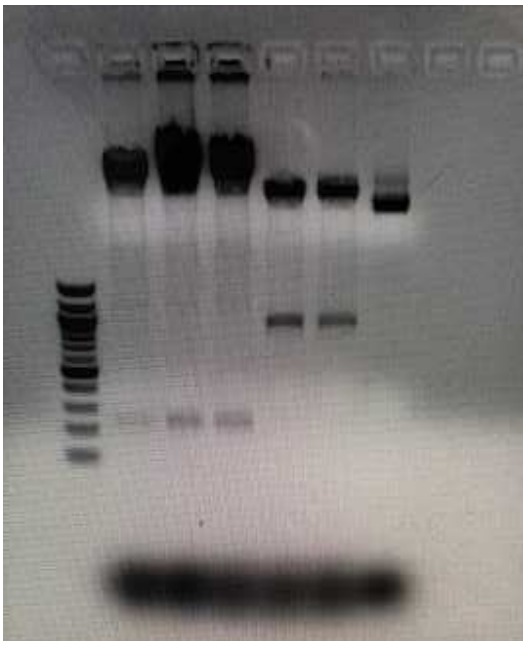
2.Inoculate a single colony of desired recipient into 2mL of the above media. Incubate for 3-5 hours at 37 degrees C.

3.Put 500uL of above culture with 5uL chromosomal DNA. Incubate for 1-2 hours at 37 degrees C.

4.Place on selective media and incubate overnight at 37 degrees C.

*First transformation is PmmsA-lux into py79 strain of *B. subtilis.*

01Aug2018:



Objective #1: Run a diagnostic gel to evaluate our hpdH and hpdR transformants grown up on plate.

Results: hpdH looked okay. hpdR does not.

02Aug2018:

Objective #1: Begin overnights on py79 x PmmsA-lux and py79.

Objective #2: Create frozen stocks of PmmsA-lux, PhpdH-lux, and PmmsR-xyl

500uL of 50% Glycerol in screw-cap tube +1mL Overnight culture (2 tubes per culture)

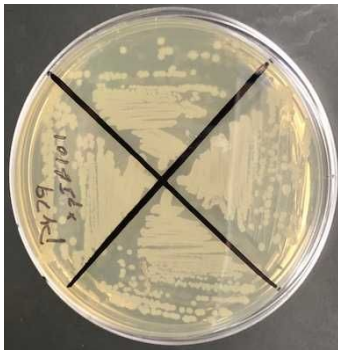
03Aug2018:

Objective #1: Remember that frozen stocks from overnights are from sequenced plates. Overnights restruck and then placed in 5mL LB+AMP.

08Aug2018:

Objective #1: Began overnights for mmsA-lux (strain PIA101), mmsR-xyl (strain PIA102) and hpdH-lux (strain PIA 103) in LB+AMP.

Objective #2: Transformed strain 101 into py79.



09Aug2018:

Objective #1: Remember that py79 x PIA 102 and py79 x PIA 101 x PIA 102 should be plated on Kanamycin. Stored with 600uL pg 50% Glycerol.

Objective #2: Miniprep overnights from 08Aug2018.

Results: PIA 101: 157.6 ng/uL (now known as **AA**)

PIA 102: 76.1 ng/uL (now known as **AB**)

PIA 103: 87.7 ng/uL (now known as **AC**)

10Aug2018:

Results: Transformation of PIA 102 into py79 x 101 looked off. Plate with PIA 103 looked off as well. We will redo transformation of 102 into py79 x 101.

Objective #1: Prepare transformation growth media for *B. subtilis.*

11Aug2018:

Objective #1: Prepare transformation growth media and innoculate one colony. One tube for py79 and one for py79 x 101.

Results: *B. subtilis* cultures did not grow up.

13Aug2018:

Objective #1: Prepare *B. subtilis* transformation cultures py79 and py79 x 101. Transform 102 into py79 x 101.