***Ladder:** 2-Log

*oct:octane

20 June

- Competent Cell Preperation (DH5alpha PRO)
- Performed plasmid isolation(miniprep) for pZA -which is going to be use for plasmid backbone of AlkR- and pGEX that includes sfGFP to use in test constructs.

Sample	**Concentration(ng/ul)**
pZA	191,7
PGEX sfGFP	459,9

Table 1: Nanodrop results

22 June

- PCR was performed for AlkR-pAlkM and sfGFP to ligate with pZA backbone. [Q5]

primer	**sequence**
pAlkM - AlkR FWD(65)	5' GATAGAGATACTGAGCACAGTCGACGATTTGGGTATTAAAGAGGAGAAAGGTAC 3'
pAlkM - AlkR REV(65)	5' GCATGGTACCTTTCTCCTCTTTAATACTGTTTCCTGTGTGAAATGTGC 3'
sfGFP FWD(69)	5' TTTCACACAGGAAACAGTATTAAAGAGGAGAAAGGTACCATGCGTAAAGGCGAAGAGC TGTT 3'
sfGFP REV(69)	5' TTTATTTGATGCCACGCGTCATTTGTACAGTTCATCCATACCATGC 3'
pZA CmR rr12 pL(tetO) - Ag43 FWD (1)	5' GATGAACTGTACAAATGACGCGTGGCATCAAATAAA3'
pZA CmR rr12 pL(tetO) - Ag43 REV (1)	5' CTCTTTAATACCCAAATCGTCGACTGTGCTCAGTAT3'

Table 2: PCR primers

DNA	**Length(bp)**

pZA	1824
AlkR-pAlkm	1204
sfGFP	714

Table 3: Lengths of test construct's parts

- Agarose gel was prepared and samples are loaded into the gel.

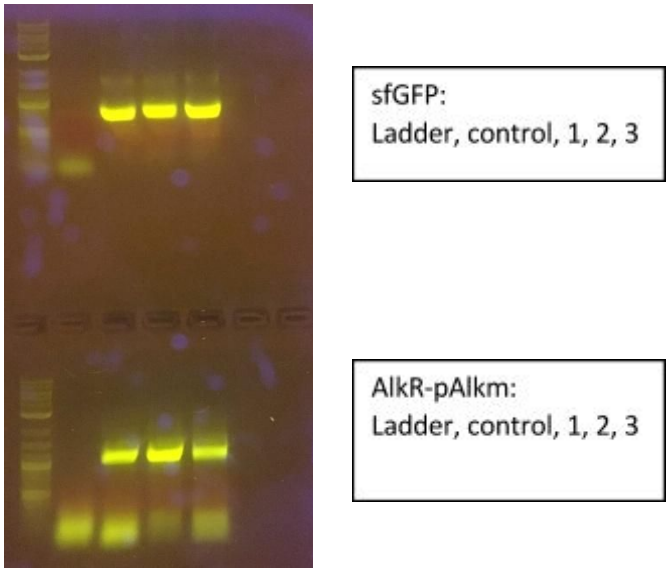


Figure 1: Agarose Gel Results

- Gel extraction was performed.[MN gel extraction kit]

Sample	**Concentration(ng/ul)**
sfGFP	187,8

AlkR-pAlkm	85,9

Table 4: Nanodrop results

Now, we have two insert with targeted primer sequence and plasmid backbone(pZA).

- Gibson Assembly was done to ligate AlkR-pAlkm, sfGFP and pZA.
- Gibson products transformed into PRO competent cells.
- Plates put into incubator (37°C) overnight.

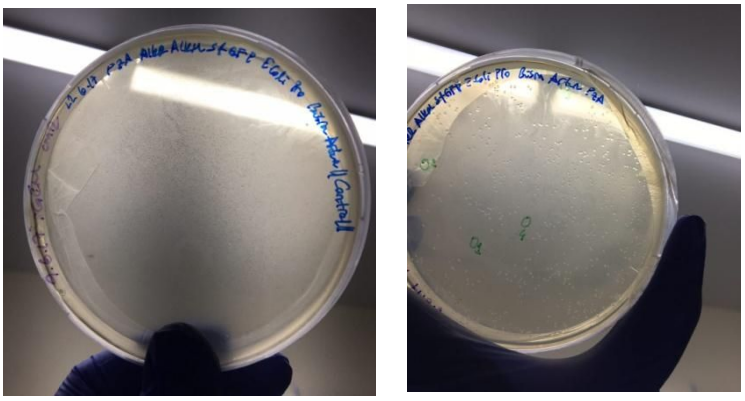
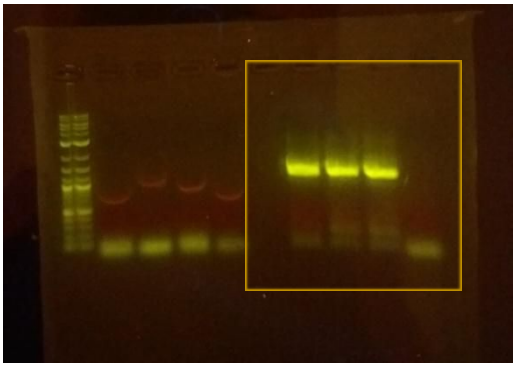
- 

Figure 2: pZA_AlkR-pAlkm_sfGFP transformation results

23 June

- PCR was performed for AlkR-pAlkm to insert into pSB1C3. [Q5]



AlkR-pAlkm:
3,2,1, control(from left to right)

Figure 3: Agarose Gel Results

- After choosing 4 colony from pZA_AlkR-pAlkm_sfGFP plate (Figure 2), colony PCR was performed. [pfu]
- Overnight culture for 4 colonies.
- Glycerol stock was created.

24 June

- Miniprep of 4 pZA_AlkR-pAlkm_sfGFP colony was performed (Elution was done with 40 ul ddH_2O -heated 65°C-).

Sample	**Concentration(ng/ul)**
Colony 1	13,6
Colony 2	67,7
Colony 3	69,7
Colony 4	61,3

Table 5: Nanodrop results

- 4 colonies are sent for sequencing.
- Gel extraction for AlkR-pAlkm which is going to be inserted into pSB1C3 plasmid was done.

Sample	**Concentration(ng/ul)**
pSB1C3_AlkR-pAlkm	107,1
pSB1C3_AlkR-pAlkm	111,2

Table 6: Nanodrop results

- Colony PCR of pZA_AlkR-pAlkm_sfGFP run on the gel.

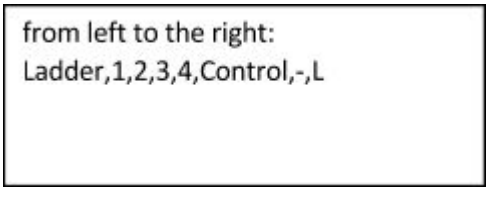


Figure 4: Agarose Gel Results

29 June

- Glycerol stock of positive results of colony PCR products was created.
- Sequencing results show that pZA_AlkR-pAlkm_sfGFP colony 1,2 are verified.

3 July

- Induction: pZA_AlkR-pAlkm_sfGFP colony 2 tested with octane.

Sample preperation:

Dilute overnight culture 1:50 ratio. [18 ml LB + 360 ul o/n culture]

After OD reached 0.4-0.6, seperate it two : uninduced and with aTc-**aTc stock solution is 1000X and its concentration is 250 ug/ul**-. Add aTc 1:1000 ratio. [3 ml was used to measure OD, uninduced : 5ml, with aTc: 10 ml --> add 10 ul aTc]

Incubate them at 37°C 200 RPM for 2.5 hours, then seperate falcon with aTc into 2 different falcon (aTc, aTc+octane). [aTc: 5 ml, aTc+octane : 5 ml] Add octane that concentration would be 10mM.Overnight culture were loaded into 96-well plate.

Plate reader was used to measure OD and fluorescence.

4 July

-

GFP						
empty	uninduced	atc	atc+octane	uninduced	atc	atc+octane
295.874	356.013	364.319	351.603	58.5205253	75.042728	63.165437
291.637	356.618	366.228	353.403			
OD						
empty	uninduced	atc	atc+octane	uninduced	atc	atc+octane
1.046	1.0683	0.9496	0.9299	1	1.28233176	1.07937235
1.0372	1.0697	0.9562	0.9302			

Table7: Plate Reader Induction Results(empty refers to pro competent cells without plasmid)

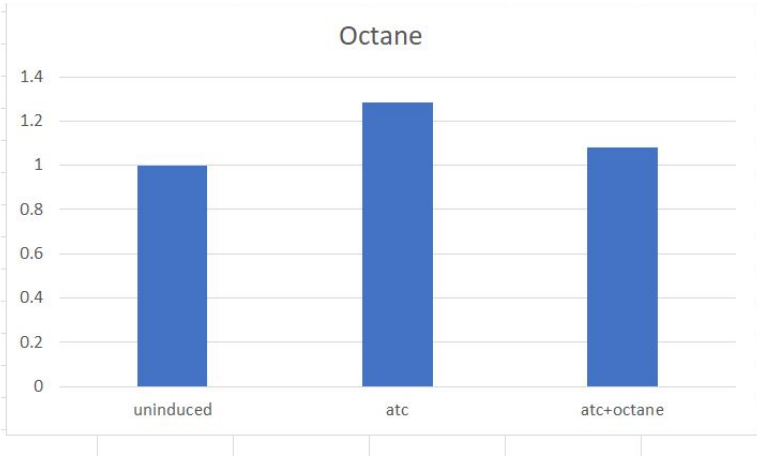


Figure 5: sfGFP Fold Change Graph of Induction

- pSB1C3_AlkR-AlkM plates not grown, PCR, Gibson and transformation are going to be repeated again.

6 July

- Induction is repeated. Same protocol is followed, octane concentration is kept the same (10mM) but aTc concentration is changed as a ratio of 1:25000, 1:10000, 1:5000, 1:2500. Each culture sample is 3 ml.
  Total sample count: 9 [un, 1:25000 aTc, 1:10000 aTc, 1:5000 aTc, 1:2500 aTc, 1:25000 aTc+ oct , 1:10000 aTc+oct, 1:5000 aTc+oct, 1:2500 aTc+oct]
  OD and fluorescence are measured 1-2-3-4th hour and overnight.

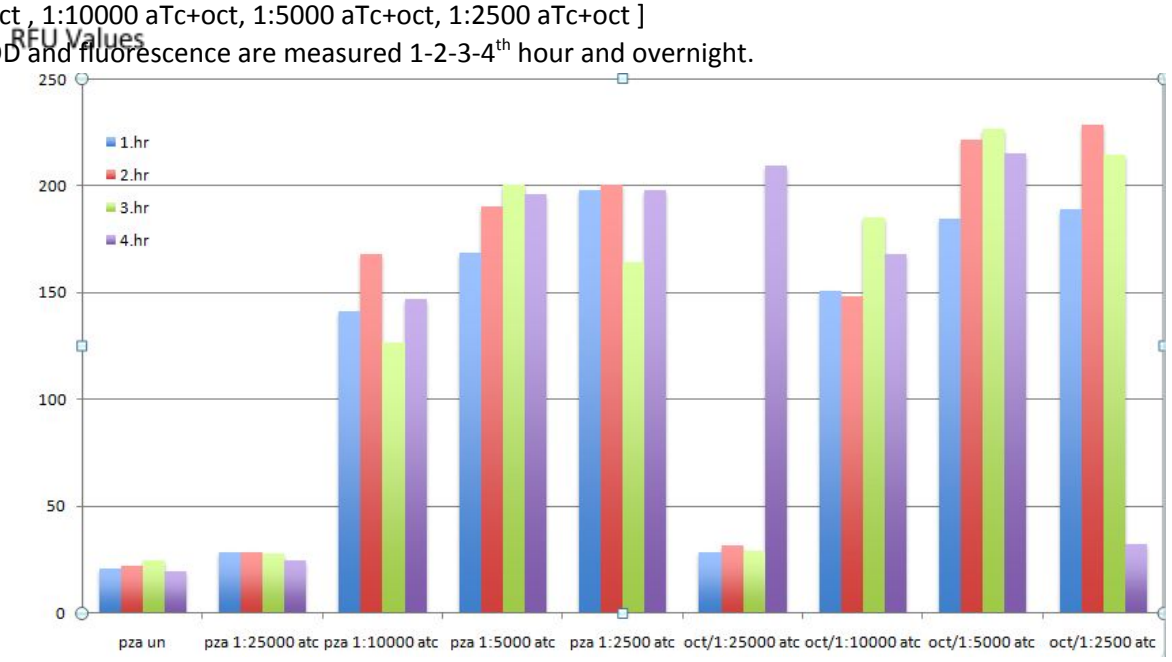


Figure 6: sfGFP Fold Change Graph of Induction. 'pza un' refers to uninduced culture, 'pza ## atc' refers to culture induced with atc only, and 'oct/## atc' refers to culture induced both octane and atc.

Simplified Graph :

RFU Value

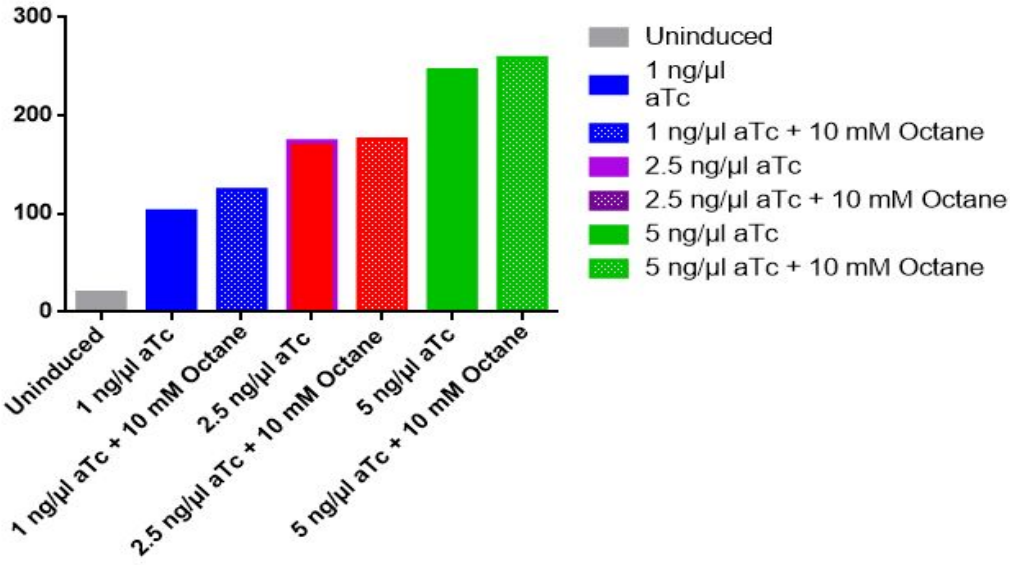
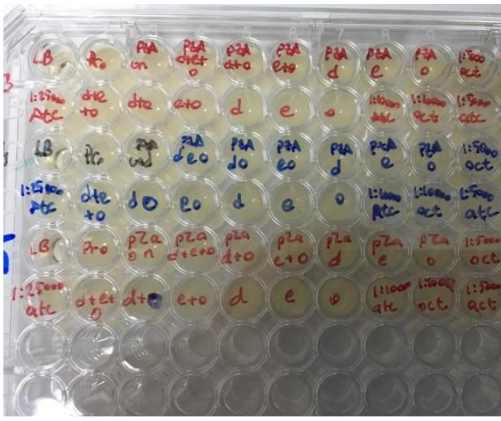


Figure 7: Induction for determining the optimal aTc concentration.

11 July



- Induction:

  Try to dissolve octane in the culture. To get more efficient results, octane is mixed with ethanol and DMSO. As octane concentration will be 10 mM and aTc kept in 1:25000 dilution in the culture, mixture of ethanol and octane (50:50) is prepared.For octane-DMSO mixture, DMSO amount is set %1. Same induction protocol is followed. Apart from that, different aTc concentartions are tested to induce the culture: octane concentration set 10 mM, aTc conc varies as 1:50000 1:25000, 1:10000, 1:5000, 1:2500 dilution ratio.
  Sample count: 23

Figure 8: 96-well plate with induction samples

[Pro, uninduced, DMSO+EtOH+Octane(d+e+o), DMSO+Octane(d+o), EtOH+Octane(e+o), DMSO(d), EtOH(e), Octane(o)
following series induced with 1:25000 aTc :
1:25000 aTc, DMSO+EtOH+Octane(d+e+o), DMSO+Octane(d+o), EtOH+Octane(e+o), DMSO(d), EtOH(e), Octane(o)
following series to observe effects of variable aTc amount on induction:

1:50000 aTc, 1:25000 aTc, 1:10000 aTc, 1:5000 aTc, 1:2500 aTc, 1:50000 aTc+ oct, 1:25000 aTc+ oct, 1:10000 aTc+oct, 1:5000 aTc+oct, 1:2500 aTc+oct

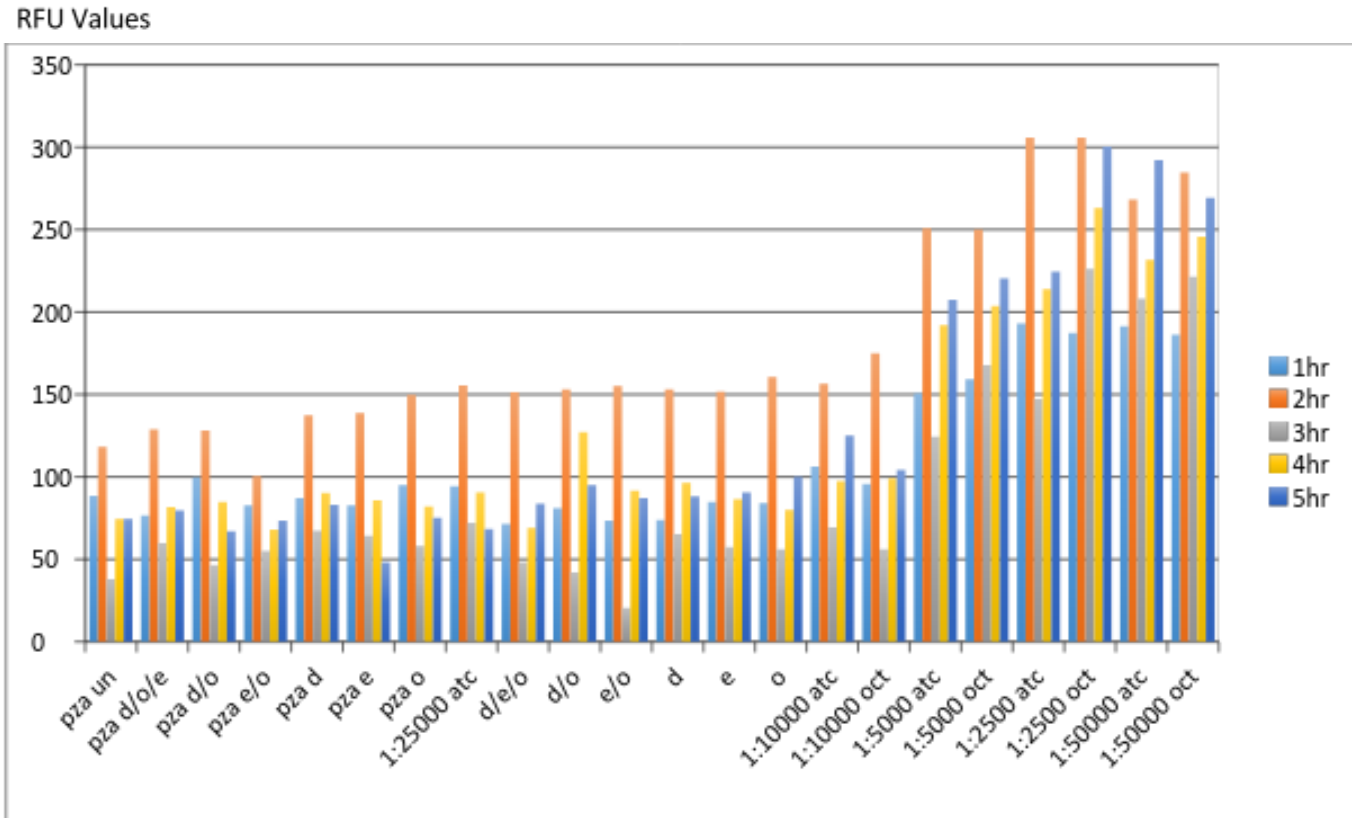


Figure 9: Induction for determining the effect of 1% DMSO and EtOH on effective concentration of octane. d, e, o abbreviations refer for DMSO, EtOH and octane respectively.

17 October

- Induction
  3 replicate per sample is prepared. aTc dilution for each sample is 1:25000. Different octane concentrations are utilized to induce the pAlkM. Overnight culture's OD and fluorescent intensity are measured.

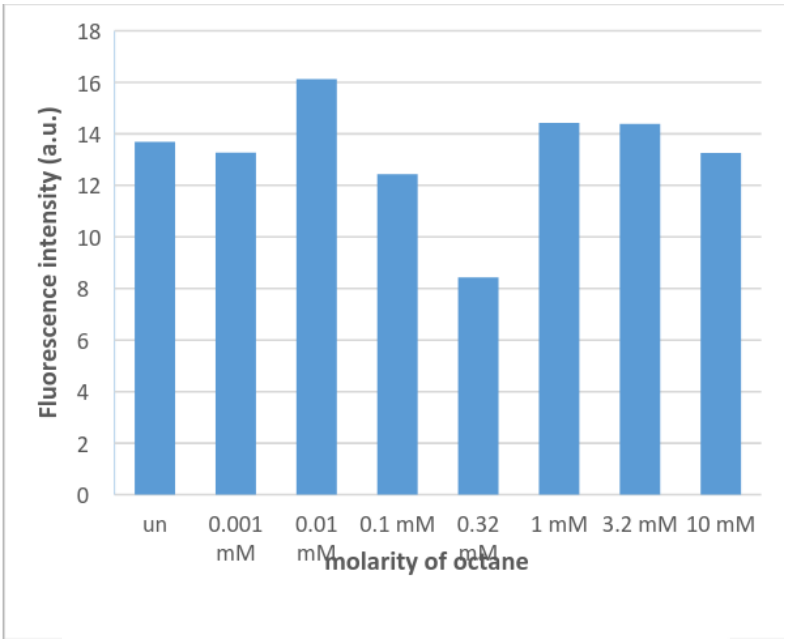


Figure 10: sfGFP Fold Change Graph of Induction