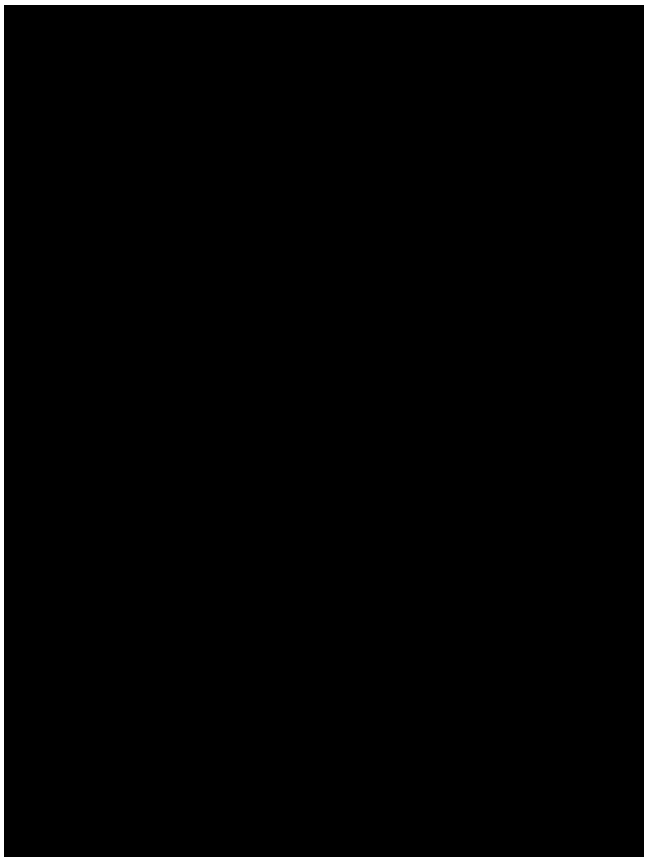
Leia Summer 7/11-7/15

MONDAY, 7/11/2022

Checked GGA ampD Plates:

IMG-1364.jpg



- Not sure what to do now... Keaton says we need to double check that the ampD homologies are correctly ligating

Plan for this week:

- Make more ampD PCR product?
- Run GGA gel again, if it doesn't work, review homologies
- Try Jeffrey's DNA or Keaton's DNA in cells that I grow to see fi transformation is working

GGA ampD (left):

- [x] 2 µL of 10X T4 DNA ligase buffer
- [x] 1 µL BsaI-HF
- [x] 1 µL of T7 DNA ligase
- [x] 250 ng pBTK622 plasmid (250/35.9 = 7 uL)
- [x] 150 ng 5'flank homology (150/54.1 = 2.77 = 2.8 uL = 3 uL)
- [x] 150 ng 3'flank homology (150/30.4 = 5 uL)
- [x] dH2O to 20μL rxn total volume (20-2-1-1-7-3-5 = 1 uL)

GGA Keaton's ACIAD2049 (right):

- [x] 2 µL of 10X T4 DNA ligase buffer
- [x] 1 µL BsaI-HF
- [ ] 1 µL of T7 DNA ligase

- [x] 250 ng pBTK622 plasmid (250/35.9 = 7 uL)
- [x] 150 ng 5'flank homology (150/39.8 = 3.8 uL)
- [x] 150 ng 3'flank homology (150/29.4 = 5.1 uL)
- [x] dH2O to 20μL rxn total volume (20-2-1-1-7-3.8-5.1 = 0.1 uL)

Inoculate culture 5 mL media + 2 uL ADP1 (+DNA, -DNA)

Plan for Tuesday:

- GGA gel + transform in the morning
- Plate in the afternoon

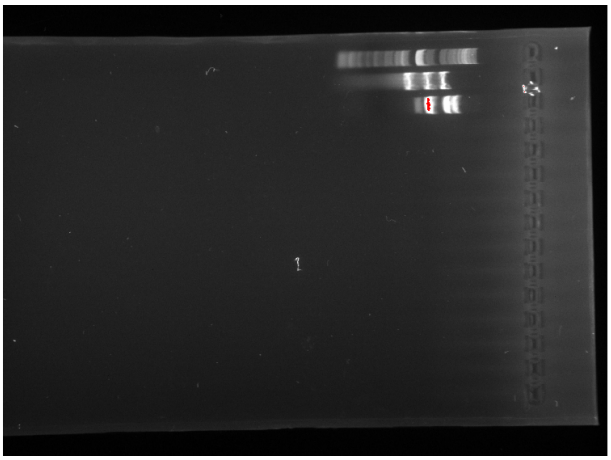
TUESDAY, 7/12/2022

Lab not open... GGA gel + transform at around noon

Gel results:

- Ran the gel for 30 min instead, gel made by Samer
- Smudgy?
- Leia's: No product :(... means there's something wrong with my homologies although people have gotten the transformation to work without any band on the gel
- Keaton's: Product shows up

image (7).png



Transform 500 uL media + 35 uL culture + 20 uL GGA (Keaton's, Leia's, No DNA (0 uL))

Plan for Wed:

- Plate anyway in morning
- Inoculate culture, then next day miniprep, then PCR marionette?

WEDNESDAY, 7/13/2022

Plating 50 uL of 100x saline + stock (1000 uL saline, 10 uL transformed cultures)

GGA ampD again:

- [x] 2 µL of 10X T4 DNA ligase buffer
- [x] 1 µL BsaI-HF
- [x] 1 µL of T7 DNA ligase

- [x] 250 ng pBTK622 plasmid (250/35.9 = 7 uL)
- [x] 150 ng 5'flank homology (150/54.1 = 2.77 = 2.8 uL = 3 uL)
- [x] 150 ng 3'flank homology (150/30.4 = 5 uL)
- [x] dH2O to 20μL rxn total volume (20-2-1-1-7-3-5 = 1 uL)

Plan for Thurs:

- PCR with 1-2 uL GGA product as template and UpF/DownR primers in morning, then gel in afternoon to see if assembly works at all
- PCR ampD upstream
- PCR YFP marionette

THURSDAY, 7/14/2022

PCR GGA:

- Mastermix reactions (5 for up, 5 for down):
  - [x] Phusion buffer (50 uL each MM)
  - [x] dH2O (152.5 uL each)
  - [x] Primer Forward (12.5 uL each)
  - [x] Primer Reverse (12.5 uL each)
  - [x] dNTPs (5 uL each)
  - [x] DMSO (10 uL each)
  - [x] Phusion polymerase (2.5 uL each) ADD LAST
- Each PCR tube:
  - [x] Template (1 uL, NOT IN - CONTROLS)
  - [x] H2O (1 uL in - controls)

GGA ampD: -ctrl, 56, 58, 60
ampD Up: -ctrl, 56, 58, 60
YFP LacI Up and Down: -ctrl, 56, 58, 60
YFP VanR Up and Down: - ctrl, 56, 58, 60
(More like -56, 57, 59, 61)

Plan for Fri:

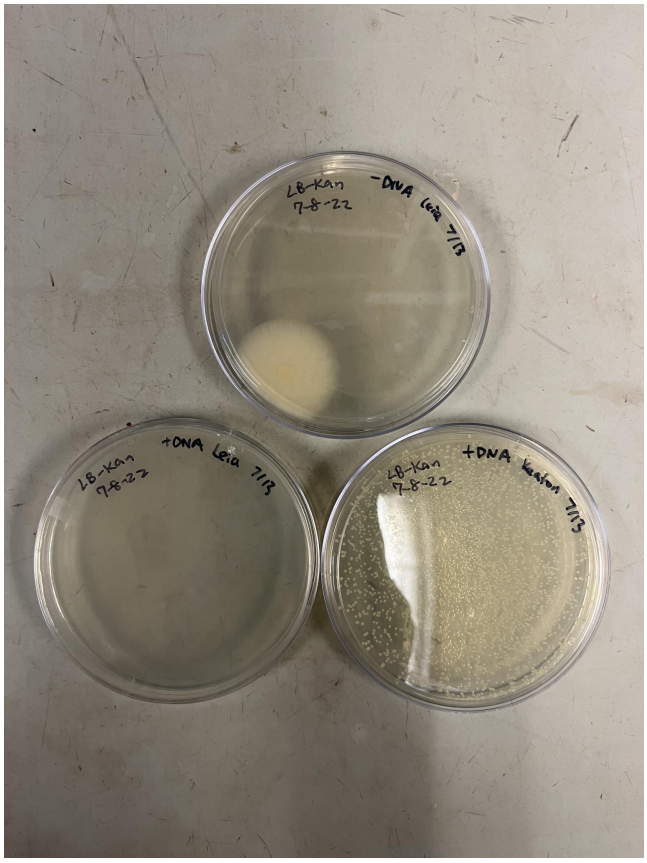
- Check plates!
- Gels!

FRIDAY, 7/15/2022

Plates:

- Have some contamination on -ctrl, probably bc I forgot to check plates yesterday
- Keaton's grew fine! This means nothing's wrong w my technique
- Still nothing grew for my +DNA

IMG_1389.jpg



Gels:

- GGA ampD: Ladder, GGA -ctrl, 56, 58, 60, ampD -ctrl, 56, 58, 60
- YFP LacI Up and Down: Ladder, LacI -ctrl, 56, 58, 60, VanR -ctrl, 56, 58, 60
- (More accurate temps -56, 57, 59, 61)