Leia Summer 6/28-6/30

TUESDAY, 6/28/2022

- Clean & concentrate ampD downstream PCR product
  - Qubit:
    - 1.92 ng/uL = .0293 ug/mL
    - Sample out of range
  - Messed up clean and concentrate.... didn't keep resulting solution from first elution step --> had to do clean and concentrate with 1 PCR tube :(((
    - Final result: 1 tube with 21.3 ng/uL = .213 ug/mL :(((((
- Golden Gate for ampD
  - [x] 2 µL of 10X T4 DNA ligase buffer (NEB: M0202S)
  - [x] 1 µL BsaI-HF (NEB: R3535S)
  - [x] 1 µL of T7 DNA ligase (NEB: M0318S)
  - [x] 250 ng pBTK622 plasmid
    - [x] 250 ng / 41.5 ng/uL = 6.024 uL
  - [x] 150 ng 5'flank homology (for ~1000bp) *
    - [x] 150 ng / 73.8 ng/uL = 2.03 uL
  - [x] 150 ng 3'flank homology (for ~1000bp) *
    - [x] 150 ng / 21.3 ng/uL = 7.04 uL
  - [x] dH2O to 20μL rxn total volume
    - [x] - control (no enzymes): 20 - 2 - 6.024 - 2.03 - 7.04 = 2.906 uL
    - [x] Sample (enzymes): 20 - 2 - 1 - 1 - 6.024 - 2.03 - 7.04 = 0.906 uL
  - 1 rxn without enzymes for a control, 1 with everything, 1 with no homologies (shows plasmid backbone) if there is enough
    - 3.7 and backbone (1.6) if successful on gel (5 uL GGA + 1 uL, treat it like PCR product)
- Inoculate ADP1-ISx in LB liquid media
  - 5 mL LB + 2 uL ADP1-ISx, 30 C incubator overnight

Plan for Wed:

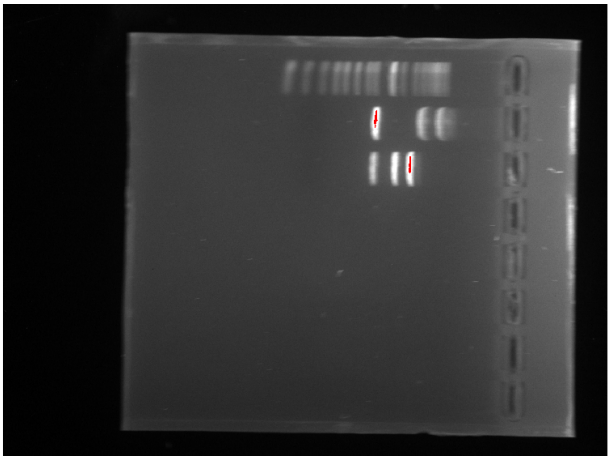
- GGA gel
  - If this works, continue with transformation

WEDNESDAY, 6/29/2022

GGA Gel results:

- Still didn't get any GGA products but there is a faint line... transformed anyway

image (6).png



Plan for Thurs:

- Plating, get Jeffrey and/or Keaton to check plates + continue inoculating + freeze culture if it works

THURSDAY, 6/30/2022

Dilutions + plating:

- Make solution 1: 1000 uL saline 10 uL (10^-2) stock --> plate onto +DNA KAN
  - Make soln 2: 10 uL soln 1^ + 1 mL saline
  - Make soln 3: 632 uLsoln 2^ + 1mL saline (185 colonies/50 uL)
- Didn't do regular 10^-6 LB plates since there were none left
  - Just did 1 plate with 10^-2, 1 plate with 10^-6 (+DNA KAN)
- 50 uL of dilution on each plate
- Incubate at 30 C standing incubator at B lab

FRIDAY, 7/1/2022

- Didn't come in to lab, had Jeffrey take photos of plates
  - Unfortunately no growth on +DNA KAN
  - Probably need to do LB AR plates in the future, even though ADP1 samples are probably fine
  - Not sure what went wrong...
    - 15 uL GGA bc used 5 for gel? No, not an issue

Image from iOS (1).jpg