

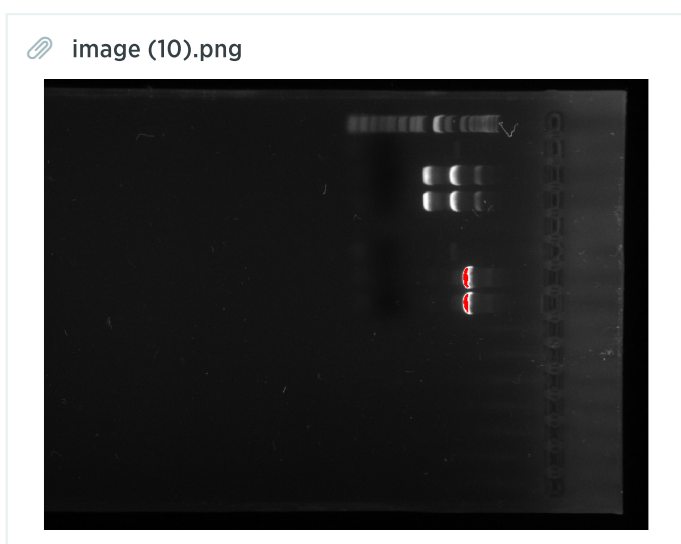
Leia Jiang 8/16-8/21

TUESDAY, 8/16/2022

PCR GGA1 and 2 products, 61 C:

- 3 rxns each MM, - ctrl + 2 PCRs
- ✓ 7.5 uL forward primers
- ✓ 7.5 uL reverse primers
- ✓ 30 uL Phusion buffer
- ✓ 3 uL dNTPs
- ✓ 1.5 uL Phusion polymerase
- ✓ 6 uL DMSO
- ✓ 91.5 uL diH2O

PCR GGA1 and 2 Gel:



- Expected: 2000 bp GGA1, 3000 bp GGA2
 - GGA1 weird.... why 2 bands?
 - GGA2 good!

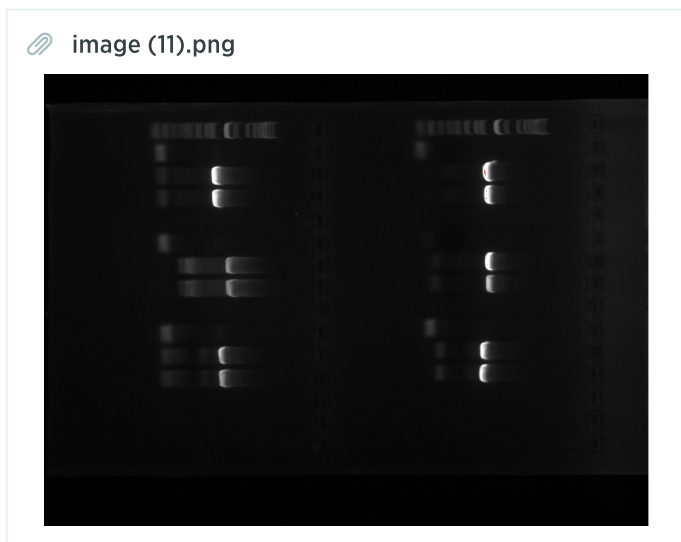
Clean and concentrate?

- Did GGA1 just in case the product is fine

PCR amplifying g-blocks, 60 C (Samer and Adam):

- PD 3a: 74 and 64
- PD 3b: 73 and 65
- All repressors: 71 and 72

PCR g-blocks Gel:



- Products for everything! Pd_3a, Pd_3b, BetI on top, CymR, Lacl, VanR on bottom

Inoculating Interlab plasmids:

- 5 mL LB media + 5 uL CAM + 1 colony per tube
- 2 tubes for each plate

WEDNESDAY, 8/17/2022

Glycerol stocks

- Only 1 tube with purple growth in 12I and 14E, so only these were frozen
- Only 1 turbid tube for 5L frozen
- -80 C freezer Interlab box, the rightmost 2 columns going down in number/alphabetical order
 - Bottom row has 3 tubes

Day 2 Procedures: GGA with 1, 2, and pBTK622

- ✓ 2 uL T4 Ligase Bugger
- ✓ 2 uL NEB GG Mix
- ✓ 100 ng GGA1 product = $100/32.6 = 3.1$ uL
- ✓ 100 ng GGA2 product = $100/44.5 = 2.3$ uL
- ✓ 170 ng pBTK622 = $170/35.9 = 1.8$ uL
- ✓ dH₂O = $20 - 4 - 3.1 - 2.3 - 1.8 = 8.8$ uL
- Overnight culture: 5 mL LB + 2 uL Strain O24A and a - ctrl, shaking at 30C

Miniprep plasmids

- Started new iGEM box for Interlab, currently unlabeled
- Has plasmids

THURSDAY, 8/18/2022

Day 3 Procedures: Transforming construct into O24A

- +DNA has 500 uL LB + 35 uL O24A + 20 uL GGA product
- -DNA has 500 uL LB + 35 uL O24A
- -ctrl has 500 uL LB
- Shaking overnight 30 C

Qubit plasmids

Clean and concentrate g-blocks

FRIDAY, 8/19/2022

Day 4 Procedures: Plating

- 100-fold dilution for KAN +DNA
- 10^{-6} dilution for KAN -DNA and both LB plates
- 30 C standing incubator

Made 25 LB-CAM plates

- Didn't realize half the agar bottle was still solid until after I added CAM... made 4 plates and then reheated for 5 min at power level 10 (oops goeey microwave....)
- Add 400 uL CAM into the rest of the bottle
 - These plates might be iffy... :////

Qubit g-blocks

SATURDAY, 8/20/2022

Day 5 Procedures: Check plates + overnight culture

- P. DESTRUCTANS DETECTOR SUCCESSFUL!!!!!!
- 5 mL LB + 5 uL KAN, 3 tubes inoculated from 3 cultures

Transforming (heat-shocking) DH5 alpha cells

- Plate 10 uL and 100 uL

SUNDAY, 8/21/2022

Glycerol stocks of *P.destructans* detector

- iGEM22_029A, B, C