

Leia Summer 7/19-7/22

MONDAY, 7/18/2022

(no lab)

TUESDAY, 7/19/2022

Inoculated cultures in morning:

- 5 mL LB + 2 uL ADP1 and a - ctrl, pipetted the 2 uL twice bc forgot to touch the media w tip

Plan for Wed:

- Transform in morning, plate in afternoon

WEDNESDAY, 7/20/2022

Transformation:

- 500 uL LB + 35 uL ADP1 culture + 20 uL GGA (3 tubes), and a - ctrl

Make LB KAN plates

- Microwave 500 mL agar for 40 min @ Power 2
- 55 C water bath for 30 min
- 500 uL KAN stock in 1 agar bottle
- Bunsen burner, 20 mL agar per plate

Plan for Thurs:

- Plate

THURSDAY, 7/21/2022

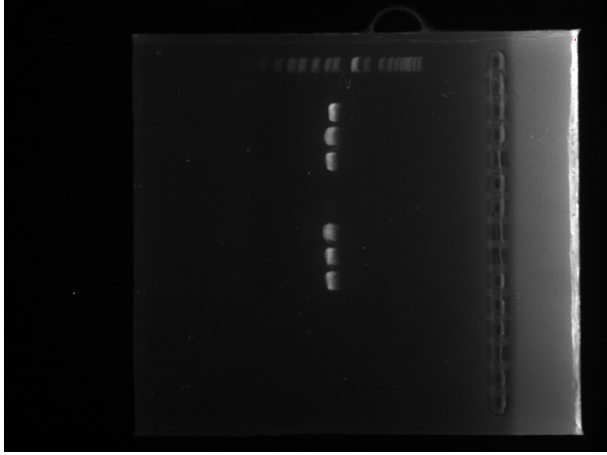
acrB up and down homologies PCR, gradient 56 58 60 62:

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

Plating:

- 100x dilution in saline, 50 uL on LB KAN plates, - ctrl

Gel:

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Purify

- acrB Up = 98.2 ng/uL
- acrB Down = 125 ng/uL

Plan for Fri:

- Check plates
- Run Jeffrey's WC PCR and VanR PCR gels?

FRIDAY, 7/22/2022

Plates:

- :(something wrong with the KAN