

# Leia Summer 7/5-7/10

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TUESDAY, 7/5/2022

Redoing GGA ampD:

- 2  $\mu$ L of 10X T4 DNA ligase buffer
- 1  $\mu$ L Bsal-HF
- 1  $\mu$ L of T7 DNA ligase
- 250 ng pBTK622 plasmid
  - 250 ng / 41.5 ng/uL = 6.024 uL round 6.1
- 150 ng 5'flank homology (for ~1000bp) \*
  - 150 ng / 73.8 ng/uL = 2.03 uL round 2.1
- 150 ng 3'flank homology (for ~1000bp) \*
  - 150 ng / 21.3 ng/uL = 7.04 uL round 7.1
- dH<sub>2</sub>O to 20 $\mu$ L rxn total volume
  - 20 - 2 - 1 - 1 - 6.1 - 2.1 - 7.1 = 0.7 uL

And again for a second sample:

- 2  $\mu$ L of 10X T4 DNA ligase buffer
- 1  $\mu$ L Bsal-HF
- 1  $\mu$ L of T7 DNA ligase
- 250 ng pBTK622 plasmid
  - 250 ng / 41.5 ng/uL = 6.024 uL round 6.1
- 150 ng 5'flank homology (for ~1000bp) \*
  - 150 ng / 73.8 ng/uL = 2.03 uL round 2.1
- 150 ng 3'flank homology (for ~1000bp) \*
  - 150 ng / 21.3 ng/uL = 7.04 uL round 7.1
- dH<sub>2</sub>O to 20 $\mu$ L rxn total volume
  - 20 - 2 - 1 - 1 - 6.1 - 2.1 - 7.1 = 0.7 uL

Inoculate ADP1-ISx in LB liquid media

- 5 mL LB + 2 uL ADP1-ISx, 30 C incubator overnight
- Also made one with Jeffrey's successful pbpG tdk/kan insertions

Plan for Wed:

- Transformation morning, plating afternoon
  - Jeffrey's tdk/kan sample should grow on +DNA +KAN

WEDNESDAY, 7/6/2022

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Transformation:

- 500 uL LB + 35 uL overnight culture (mine or Jeffrey's), +DNA 1 and 2 with 20 uL GGA product

Dilutions + plating:

- Make solution 1: 1000 uL saline 10 uL ( $10^{-2}$ ) stock --> plate onto +DNA KAN
- +DNA1, +DNA2, +Jeffrey's DNA, -DNA (all  $10^{-2}$ , all KAN)
- 50 uL of dilution on each plate

Plan for Thurs:

- Help Sai w PCR + ampD U and D PCR

**THURSDAY, 7/7/2022**

Check plates:

- Jeffrey's grew but nothing else did
- Maybe PCR concentrations are wrong, gonna redo that today

Couldn't run Sai's PCR for marionette strains bc no template

- Resuspended primers 31-46

ampD PCR:

- 58 and 60
- Mastermix reactions (5 for up, 5 for down):
  - Phusion buffer (50 uL each MM)
  - dH<sub>2</sub>O (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 ADP1 ISx (1 uL, NOT IN - CONTROLS)
  - H<sub>2</sub>O (1 uL in - controls)

Plan for Fri:

- ampD gel + purify in morning
- Miniprep Sai's marionette plasmids? If Cameron found them?

**FRIDAY, 7/8/2022**

PCR Gel:

- ampD upstream didn't work, downstream did

(pic)

- Clean and concentrated ampD downstream:
  - 25.5, 30.4, 22.7

GGA ampD:

- 2  $\mu$ L of 10X T4 DNA ligase buffer
- 1  $\mu$ L Bsal-HF
- 1  $\mu$ L of T7 DNA ligase
- 250 ng pBTK622 plasmid ( $250/35.9 = 7$  uL)
- 150 ng 5'flank homology ( $150/54.1 = 2.77 = 2.8$  uL = 3 uL)
- 150 ng 3'flank homology ( $150/30.4 = 5$  uL)
- dH<sub>2</sub>O to 20 $\mu$ L rxn total volume (20-2-1-1-7-3-5 = 1 uL)

**SATURDAY, 7/9/2022**

Transformation:

- 500 uL LB + 35 uL overnight culture (1 mine, 1 Jeffrey's) with 20 uL GGA product
  - Weird issue with thermocycler smushing the GGA tube... see pic --> may not have gotten the full 20 uL of GGA product

**SUNDAY, 7/10/2022**

## Dilutions and Plating:

- Make solution: 1000 uL saline 10 uL ( $10^{-2}$ ) stock --> plate onto +DNA KAN
  - +DNA, +Jeffrey's DNA, -DNA (all  $10^{-2}$ , all KAN)
  - 50 uL of dilution on each plate (new KAN plates made by Jeffrey, 20 mL)