

Jeffrey Chuong 6/1 - 6/5

WEDNESDAY, 6/1/2022

Set up PCR for flanking homologies of genes pbpG, acrB, recJ, ampD

Annealing Temperatures: (NEB Tm calculator, 250 nM primer concentration, Phusion)

- pbpG:
 - Upstream: 65 C
 - Downstream: 64 C
- recJ:
 - Upstream: 61 C
 - Downstream: 61 C
- ampD:
 - Upstream: 64 C
 - Downstream: 64 C
- acrB:
 - Upstream: 64 C
 - Downstream: 66 C

No negative controls for pbpG and acrB, messed up

Ran gel for PCR products, Mixed 1 ul of Blue loading dye with 5 ul of PCR product, 5 ul Ladder

Gel 1

ladder, recJ up, up, negative, down, down, negative, ladder, ampD up, up, negative, down, down, negative

 6-1-22 recJ and ampD.jpg

Gel 2

ladder, pbpG up, up, up, down, down, down, ladder, acrB up, up, up, down, down, down

 6-1-22 pbpG and acrB.jpg

PCR failed

THURSDAY, 6/2/2022

Repeating PCR for pbpG gene

- Added DMSO, 1.5 ul per 50 ul reaction

Gel 1 (Keaton)

ladder, 58 up, up, control, 60 up, up, control, 62 up, up, control, 64 up, up, control, ladder

 6-2-22 Keaton acrB upstream 58-64 gradient.jpg

Gel 2

ladder, pbpG up, up, control, down, down, control

 6-2-22 pbpG upstream, downstream.jpg

All of Keaton's samples worked! Gradient from 58C to 64C, Annealing Temp 64C

Only one of pbpG upstream worked, all downstream failed

Transforming BBa_K1825005 Biobrick Plasmid containing *nptII* gene of interest into homemade NEB 5alpha competent cells

- Plated on LB-Kan
- Plate contamination? >1 month old

Set up Whole-Cell PCR to confirm *tdk/kan* cassette insertion

1. Inoculate overnight cultures to demonstrate *tdk/kan* cassette insertion in transformed ADP1
 - a. Inoculated 2 ul of frozen stock (Articuno -80) into 5 mL of LB media with 5 ul of kanamycin stock solution.
 - b. Inoculated 2 ul culture of ADP1-ISx (Articuno -80) into 5 mL of LB media without antibiotics (negative control for PCR)
 - c. LB media control tube, uninoculated

FRIDAY, 6/3/2022

Transforming BBa_K1825005 Biobrick Plasmid containing *nptII* gene of interest into commercial NEB 5alpha competent cells

Whole-Cell PCR to confirm *tdk/kan* cassette insertion

Annealing Temperatures: (NEB Tm calculator, 250 nM primer concentration, Phusion)

- ACIAD2049:
 - Upstream: 58C
 - Downstream: 59C

94C 10:00

98C 0:30

98C 0:10

58C 0:30

72C 1:00

Goto Step 3, 34x

72C 5:00

12C Forever

SATURDAY, 6/4/2022

No growth on BBa_K1825005 on LB-Kan Plates

- bad plates? bad transformation?