Jeffrey Chuong 6/6 - 6/12

MONDAY, 6/6/2022

Meeting Notes:

Gel Images: Label gel lanes, important ladder sizes, note the expected band sizes, copy a clearer gel image from the computer, and put all negative controls before experimental samples

BBa_K1825005 backbone is a pSB1C3 (Chloramphenicol resistance, not Kanamycin)

Using Golden Transformation for PCR products to increase concentrations?, PCR for GGA Assemblies to confirm band sizes?

Try 5 degrees lower than the Tm when calculating Annealing Temperature in PCRs

Created an iGEM22 Primers Database

TUESDAY, 6/7/2022

Gel for Whole Cell PCR of *tdk*/kan cassette insertion into ADP1-ISx

- Standard 50 mL gel: 0.5 g of agarose, 50 mL 1x TAE, heat for 1:30
- Add 2.5 µl of SYBR Safe

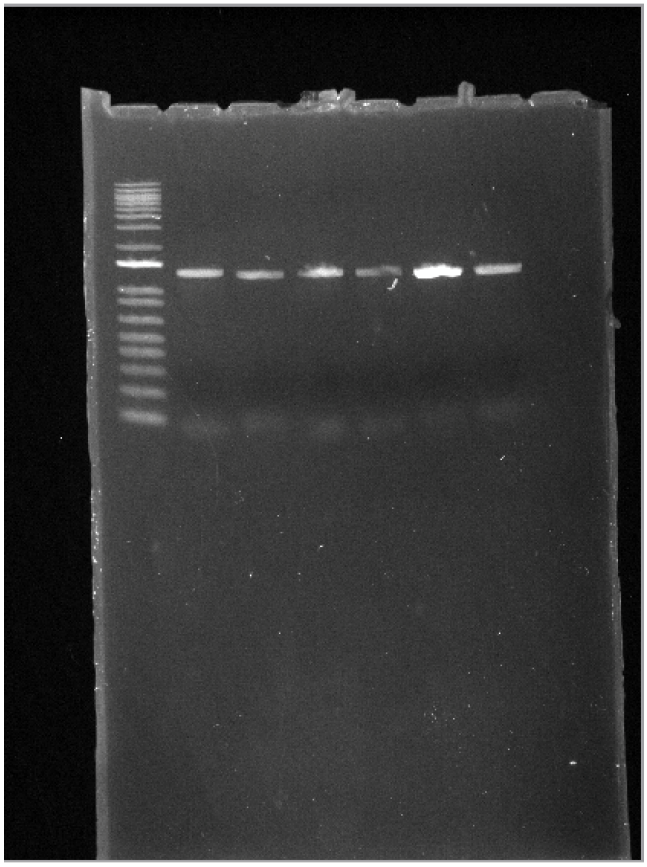
Gel 1

Ladder, Upstream Negative Control, Downstream Negative Control, Up, Up, Down, Down

Expected band size: ≈1200 bp for homology flanks on both ADP1-ISx controls and ADP1-ISx *tdk/kan*

Used the wrong primer pairs

6-7-22 Whole Cell PCR.png



Need to redo with correct primers - iGEM22_001 (UF) and iGEM22_004 (DR), change to 2 minute extension time and try

Expected band size: 3685 bp long for *tdk/kan* cassette and homology flanks, ≈1200 bp for homology flanks only

Preparing LB-Cam plates for BBa_K1825005

- Plates located at entrance of C lab
- Antibiotic stocks located in Glaceon B lab fridge
    - Chloramphenicol Stock Solution: 1000x, 1 µl in 1 mL EtOH
- LB Stock Solutions located in top shelf of A lab
    - Microwave solid LB-Agar for 25 minutes on power level 2, swirl, continue for 15-20 minutes, place in 55C water bath (B lab) for 30 minutes
    - Add appropiate antibiotic
- Let plates cool overnight to detect contamination

WEDNESDAY, 6/8/2022

Preparing Chemically Competent Cells of *E. coli* strain

- Cameron will grow a 10 mL culture of NEB 5alpha overnight
- Autoclaved a 2 L flask for tomorrow

No LB-Cam plates contaminated overnight, put in Wailord A lab fridge

Repeat PCR for flanking homology of *pbpG* gene

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

- pbpG:
    - Upstream: 65 C
    - Downstream: 64 C
- Will test 59/61 for upstream and 58/60 degrees

THURSDAY, 6/9/2022

Preparing Chemically Competent Cells of *E. coli* strain

- Miscommunication with Cameron - I will grow a 10 mL culture of NEB 5alpha today
- Inoculated a 10 mL culture of NEB 5alpha and a negative control of LB media
- Autoclaved cell culture tubes have small amounts of water
- Use larger flasks for better aeration

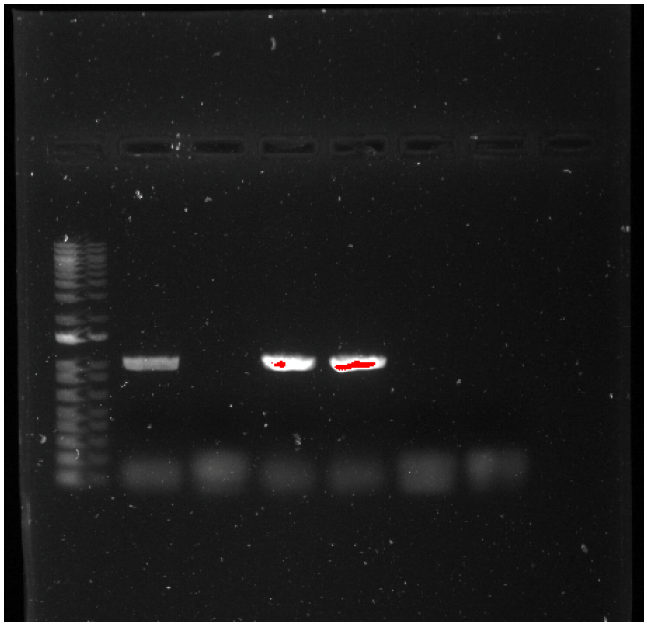
Gel for PCR of flanking homology of *pbpG* gene

Gel 1

Ladder, Upstream Negative Control, Downstream Negative Control, Up 59, Up 61, Down 58, Down 60

Expected band size: 1 kb bands for homology flanks, no bands for negative controls

6-9-22 pbpG upstream, downstream.png



- not sure why my control has a band, downstream flanks continue to fail

Autoclaved 2 L flask with tin foil wrapped over cover. Autoclaved 1 L of LB. Use carts to transport materials, autoclaves located on 2nd and 3rd floors.

FRIDAY, 6/10/2022

Preparing Chemically Competent Cells of *E. coli* strain

- Set aside one mL fresh LB to blank the spectrophotometer.
- Transfer 5 mL of O/N culture into a new flask with 500 mL LB in the 37C Incubator. Started at 9:30 AM. Incubate until reaching OD600 between 0.4 and 0.6. OD600 (1 PM) =
- Make glycerol stocks with the rest of the O/N culture (Name: iGEM22 NEB 5alpha cells, located in iGEM box of -80 freezer in B lab, box numbers 55-58)

(Check primers for pbpG downstream, continues to fail)