Jeffrey Chuong 6/13 - 6/19

MONDAY, 6/13/2022

Meeting Notes

- Gel extraction?
- Talk about most important items first, then side notes/experiments
- Ligation of acrB GGA needs to be plated completely
- Run each fragment pre-GGA on PCR to see expected band sizes

Repeat PCRs for flanking homology of intrinsic AbR genes

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

- Testing 5 degrees less than Tm for annealing temperatures
- Testing a gradient for pbpG downstream homology flanks, 56 to 64 C
- Leia is doing recJ and ampD

Gel 1 - Top Half 16 wells

Ladder, - Upstream control, -Downstream control, Up, Up, Down, Down (*ampD*), space, - Upstream control, - Downstream control, Up, Up, Down, Down (*recJ*)

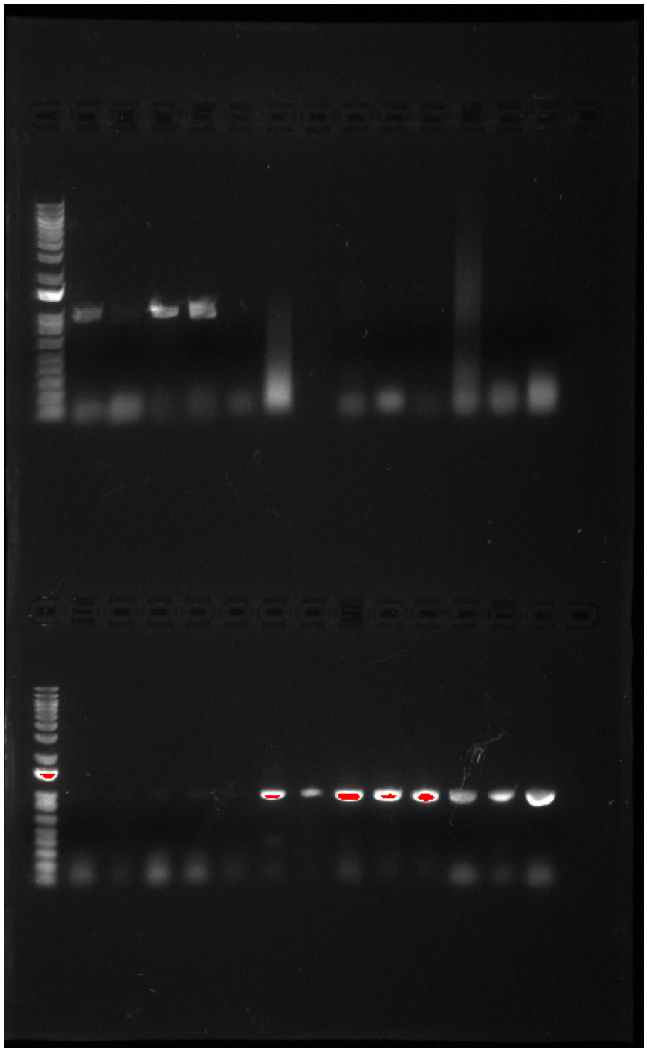
Expected band sizes: 1 kb for homology flanks and no bands for negative control

Gel 1 - Bottom Half 16 wells - *pbpG*

Ladder, - Downstream control 56-64, Downstream 56-64, - Upstream control, Up 58, Up 58

Expected band sizes: 1 kb for homology flanks and no bands for negative control

6-13-22 gel.png



Why does - Upstream control still have a band? Same issue as last PCR

Transformation for BBa_K1825005

- Waited a little bit between heat shocking and putting recovery media because I was adding 10% glucose to SOC
- After recovery, cells seemed to be stuck at bottom
- Plated 10 ul, 50 ul, 500 ul
- Used Dean's BB 190 as a positive control

Repeat Whole Cell PCR for ADP1-ISx *tdk/kan*

- Inoculated a culture of ADP1-ISx *tdk/kan,* a culture of ADP1-ISx for negative control, and LB as a media control

TUESDAY, 6/14/2022

Discussed results of PCRs with Isaac

- Go ahead with the purification of PCR products and GGA
- Maybe reagents, primers have some DNA contamination

Transformation for BBa_K1825005

- Transformation failed, I may have killed the cells in between heat shock and SOC?
- Will repeat again

Whole Cell PCR for ADP1-ISx *tdk/kan*

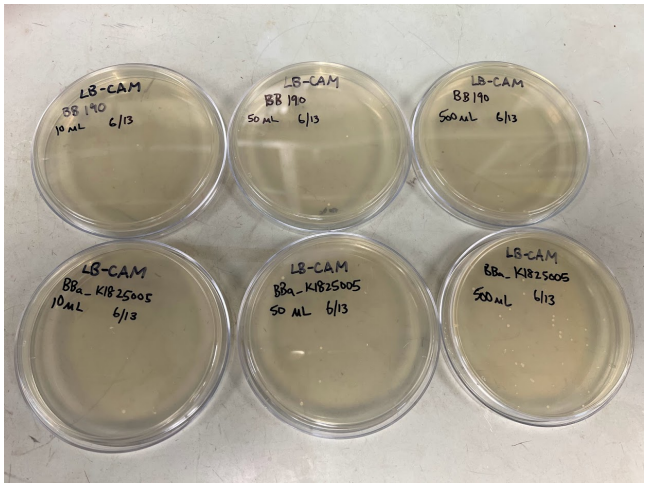
Thermal Cycler steps

94C 10:00
98C 0:30
98C 0:10
58C 0:30
72C 2:00
Goto Step 3, 34x
72C 5:00
12C Forever

Order of Whole Cell PCR products: no DNA negative control, ADP1 control, ADP1 control, tdk/kan, tdk/kan

WEDNESDAY, 6/15/2022

Day 2 BBa_K1825005 Transformation LB-CAM Plates.png



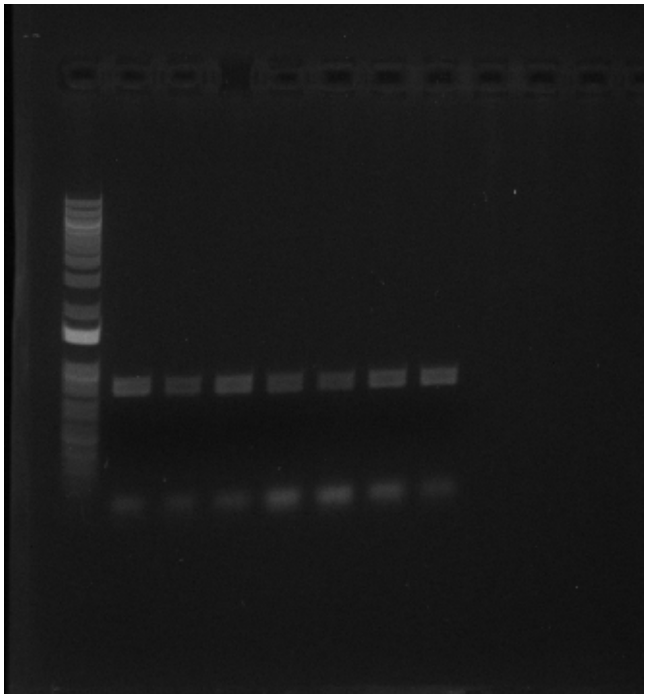
Performing a colony PCR for BBa_K1825005 plates to check if *nptII* gene is present

- Visible colonies took two days to grow. I should have taken photos day 1 (Plates were grown in 30C, maybe that's why it took two days? will put in 37C for the future)
- Calculated NEB Annealing Temperature for BBa_K1825005 primers: 67C, will test 62C
- Added an initial denaturing step of 10 minutes at 98°C to lyse the cells
- Took one colony from each plate and did duplicates for PCR

PCR Order: - Control, 10, 10, 50, 50, 500, 500 (ul of LB-CAM plate)

Growing a liquid culture (30C) for BBa_K1825005 colonies that were used for PCR -> Miniprep and Glycerol Stock

6-15-22 Colony PCR BBa_K1825005.png



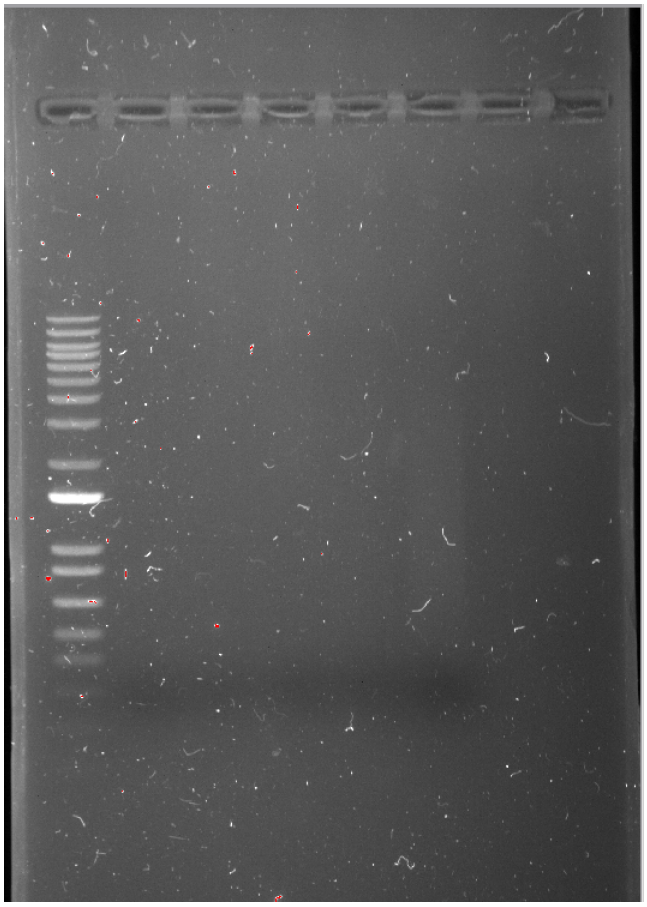
Gel for Colony PCR of BBa_K1825005
Order: Ladder, - control, 10 ul, 10 ul, 50 ul, 50 ul, 500 ul, 500 ul
Expected band sizes: no band for - control, 822 bp for *nptII* CDS

- Got a band for my negative control, contamination?
- Will move on to growing a liquid culture from these colonies (30C) for these BBa_K1825005 colonies -> Colony PCR, Miniprep, and Glycerol Stock

Repeat Transformation for BBa_K1825005, be very careful

- Pelleted cells by centrifuging for 5 min at 3000 RPM and resuspended in 100 uL of SOC, plated entire 100 uL
- Positive Control: BB 190 from Dean

6-15-22 Whole Cell PCR.png



Gel

Ladder, - control, ADP1-ISx, ADP1-ISx, *tdk/kan*, *tdk/kan*

Whole Cell PCR failed - no bands

Expected band size: no band for - control, 4650 bp for ADP1-ISx ACIAD2049 homologies?, 3700 bp for *tdk/kan* cassette and homologies

Repeat Whole Cell PCR

- Inoculated a culture of ADP1-ISx *tdk/kan,* a culture of ADP1-ISx for negative control, and LB as a media control

THURSDAY, 6/16/2022

6/16 iGEM Competition Meeting Notes

- I would like to add more stuff about meeting with Dr. Fuller and Jennifer Smith-Castro to questions 16 and 17
- Add a figure for different circuit designs, maybe to emphasize ADP1 flexibility
- Fail fast
- Highlight importance of WNS, why are bats important, bat death statistics since WNS
- Can start thinking about education and bat importance awareness, spreading WNS unknwowingly
- Looking at the overall environment in sites, not just on actual bats

Whole Cell PCR

- PCR Order: negative control, negative control, ADP1-ISx, ADP1-ISx, *tdk/kan*, *tdk/kan*

BBa_K1825005 from first plates

Miniprep Concentrations: 18.1 ng/ul (10 ul recovery), 14.3 ng/ul (50 ul recovery), 9.57 ng/ul (500 ul recovery)

- Measuring DNA concentrations with Accugreen Broad Range dsDNA Quantitation: 190 ul and 10 ul for standards, 198 ul and 2 ul for DNA samples

Glycerol Stock located in -80 B Lab Freezer in iGEM box, wells 76-78

Whole Cell PCR O/N to Friday, 62C Annealing Temperature, 0:30 Extension Time

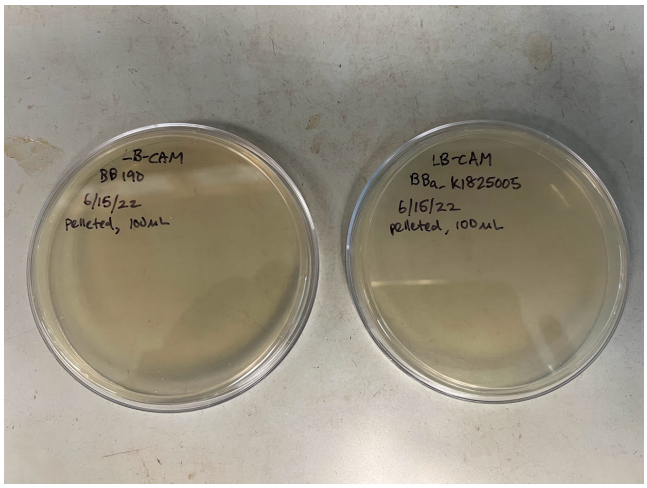
- PCR Order: - control, - control, 10, 10, 50, 50, 500, 500?

Next steps: Check bands on PCR, send for sequencing

Repeated transformation for BBa_K1825005 Plate Results

- No growth on LB-CAM Plates from positive control or BBa_K1825005

Day 1 BBa_K1825005 Failed Transformation LB-CAM Plates, pelleted.png



FRIDAY, 6/17/2022