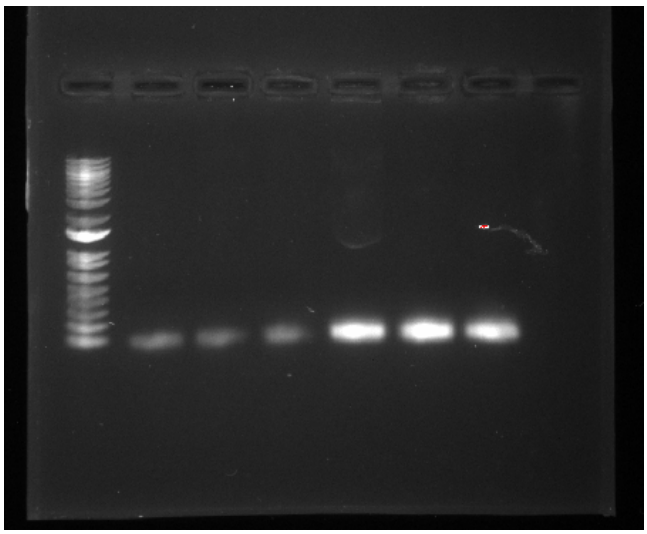
Jeffrey Chuong 7/5 - 7-10

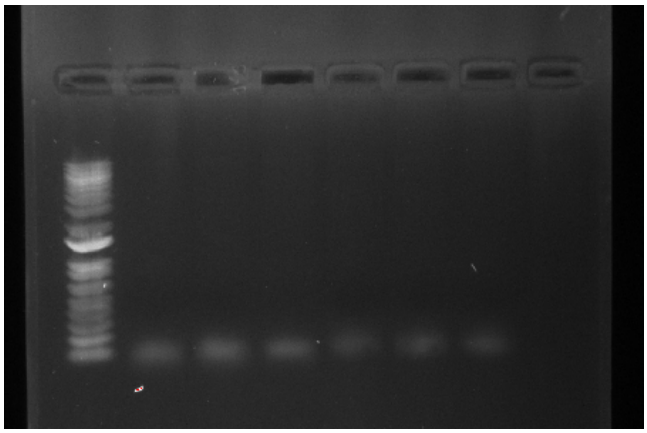
TUESDAY, 7/5/2022

7-5-22 recJ 55.8.png



recJ PCR: Annealing temp 55.8C for upstream and downstream
recJ Gel: -, Up, Up, -, Down, Down

7-5-22 pbpG 57.4.png



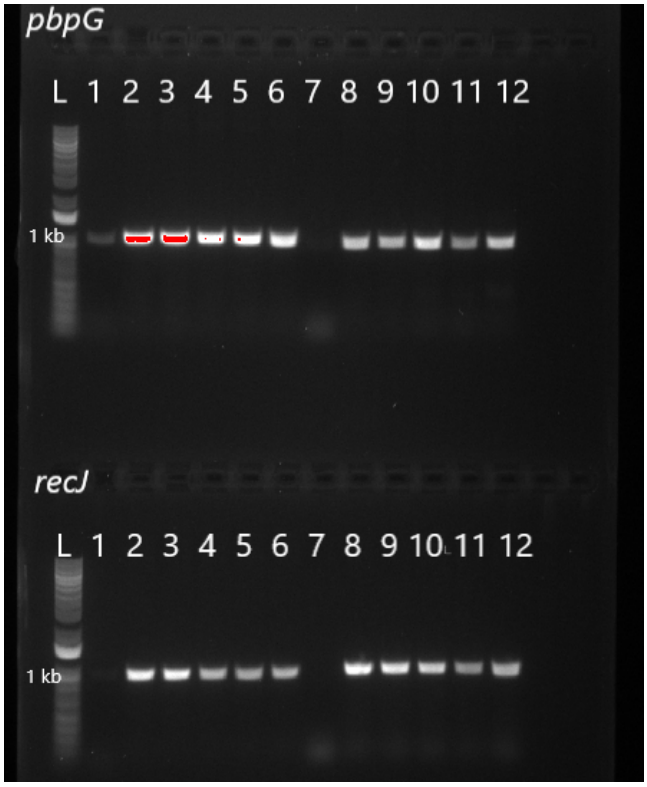
pbpG PCR: Annealing temp 57.4C for upstream and downstream
pbpG Gel: -, Up, Up, -, Down, Down

WEDNESDAY, 7/6/2022

REPEAT PCR - made new primer stocks

- 53 to 60 for both annealing temperatures

7-6-22 pbpG and recJ gradient 53-60.png



Sequencing results analysis: Matt thinks the Sanger sequencing by Eton is just bad, I shouldn't trust the results - will talk to Dr. Mishler

Started GGAs for *recJ* and *pbpG* - *recJ* homologies ligated with *tdk/kan*, *pbpG* homologies ligated together

THURSDAY, 7/7/2022

recJ - made 500 ul transformations with 35 ul O/N culture ADP1-ISx and 20 ul GGA ligation of *recJ* homologies with pBTK622 *tdk/kan*

pbpG - made 500 ul transformations with 35 ul O/N culture iGEM22_012 and 20 ul GGA ligation of *pbpG* homologies

Incubated O/N 30C 200 RPM

FRIDAY, 7/8/2022

Made new LB-KAN plates

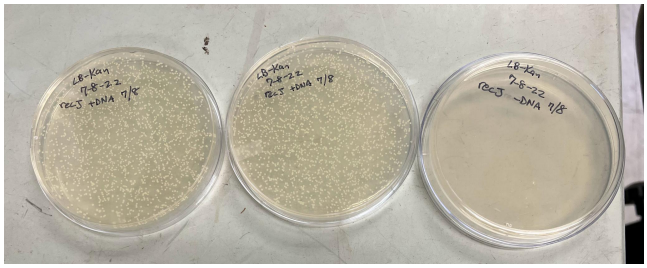
- old LB-KAN plates were faulty or only 10 mL, could have been causing the issues for Leia?
- suggestions to defeat condensation

recJ plating +DNA and -DNA transformations on LB-Kan - diluted 100x in sterile saline, plated 50 ul

pbpG plating +DNA and -DNA transformations on LB-AZT - diluted 100x in sterile saline, plated 50 ul

SATURDAY, 7/9/2022

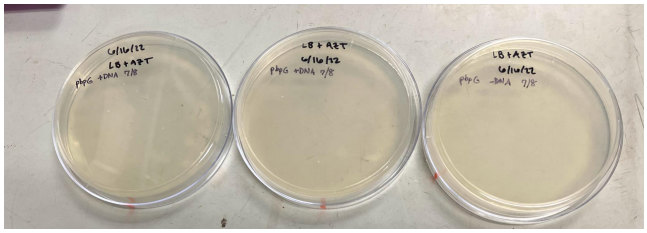
7-9-22 Transformation of ADP1-ISx with GGA ligation of recJ flanks with pBTK622 tdk kan.jpg



recJ

- Inoculated two colonies from each successful +DNA plate, may have messed up on some cultures, liquid LB-Kan cultures marked with an * are good
- May try whole cell PCR to confirm *tdk/kan* insertion with *recJ* upstream forward and downstream reverse primers (3.7 kb)
- Make a glycerol stock tomorrow

7-9-22 Transformation of iGEM_012 with ligated pbpG rescue flanks Day 1.jpg



pbpG

- Did not inoculate colonies due to very little growth, may try leaving it an extra day and try with the colonies that show up
- I need to check primer restriction sites on *pbpG* primers