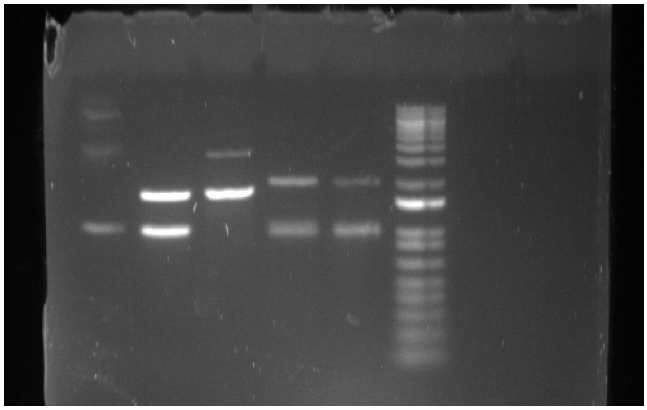
Jeffrey Chuong 6/27 - 7/4

MONDAY, 6/27/2022

6-27-22 GGA pBTK622 tdk kan ligation with recJ flanks + pbpG flank cassette.png



Lane 1: *tdk/kan* ligation with *recJ* flanks (Expected band size: 3.7 kb ligation and other background, but got weak/bad bands)
Lane 2: Control with no ligase (Expected band size: 1 kb flank, 1.7 kb *tdk/kan* cassette)
Lane 3: Control with no flanks (Expeected band size: 1.7 kb *tdk/kan* cassette, 3.3 kb plasmid/ligated plasmid?)
Lane 4-5: *pbpG* flanks, first has an extra ul of T7 Ligase (Expected band size: 2 kb ligation, 1 kb part)

pbpG
Transformation plates
+DNA Plates: Expected growth with cells that have *pbpG* knockout

- AZT should kill the cells that still contain the *tdk/kan* cassette insertion
- no growth on 6/27

Going to repeat setting up a GGA rescue ligation cassette (*pbpG* flanks only) and inoculating LB-Kan liquid culture with *tdk/kan* + *pbpG* flanks strain iGEM22_012

- 42C with BsmBI GGA mix

recJ
Transformation plates
+DNA Plates: Expected growth with cells that have *tdk/kan* insertion with *recJ* flanks

- no growth on 6/27

Going to repeat setting up a GGA ligation pBTK622 *tdk/kan* with *recJ* flanks and inoculating LB liquid culture with ADP1-ISx

- 37C with BsaI-HF
- need more DNA for flanks

BBa_K1825005

- test a PCR with my miniprep product from BBa_K1825005

PCR order: -control, *nptII*, *nptII*, *nptII*, *nptII*

- first 2 are from 30.9 ng/ul sample, last 2 are from 34.9 ng/ul sample (SHOULD BE identical)
- annealing temp gradient 58-64
- expected band size 800 bp

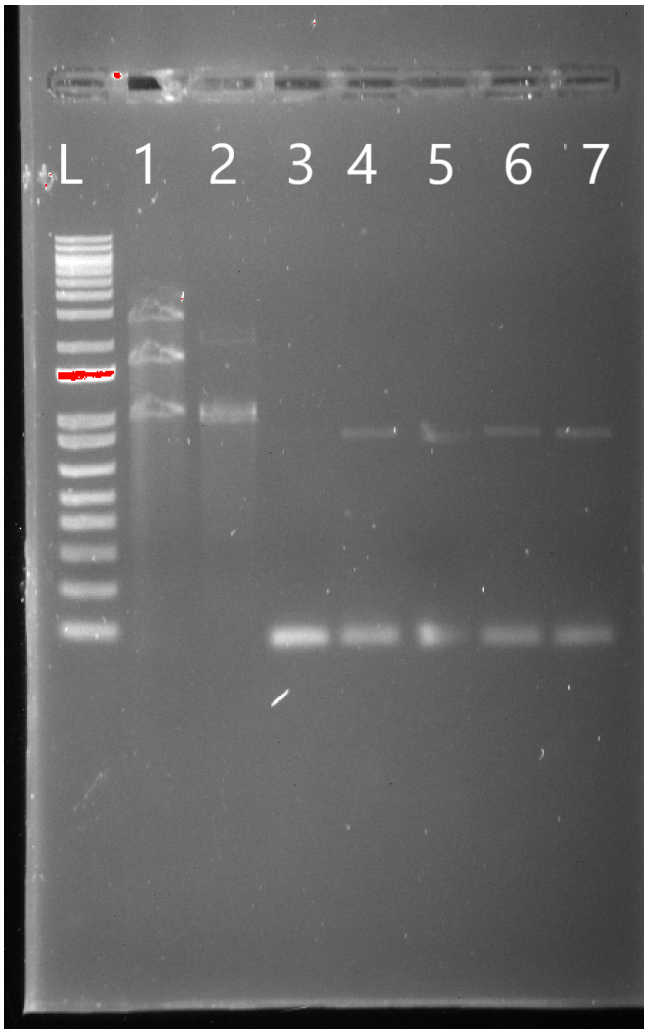
TUESDAY, 6/28/2022

Overnight Transformations for *recJ* and *pbpG*

https://barricklab.org/twiki/bin/view/Lab/ProtocolsAcinetobacterGoldenTransformation

- probably won't work based on gel product?? Victor says even low amount of product can still work, will still try plating

6-28-22 GGA pBTK622 tdk kan ligation with recJ flanks, pbpG flanks, BBa_K1825005 nptII product.png



Lane 1: *tdk/kan* ligation with *recJ* flanks (Expected band size: 3.7 kb ligation and other background, but got weak/bad bands)

Lane 2: *pbpG* flanks (Expected band size: 2 kb ligation, 1 kb part)

Lane 3-7: -control, replicates of BBa_K1825005 *nptII* gene (Expected band size: 800 bp)

- such weak bands?
- Will purify and send for sequencing

WEDNESDAY, 6/29/2022

Golden Transformation plates for recJ and pbpG

- Diluted each transformation 100x in sterile saline.
- Plated 50 ul of diluted *recJ* transformation mix onto LB-Kan plate.
- Plated 50 ul of diluted *pbpG* transformation mix onto LB-AZT plate.
- Forgot to grow a -DNA control in liquid cultures, so don't have any -control plates.

THURSDAY, 6/30/2022

Made new LB plates and LB-CAM plates

Sequencing

- give sequencing info to Dr. Mishler, he will print out a sheet for you to put into ziploc baggie for Eton dropbox

6-30-22 Transformation of ADP1-ISx with GGA ligation of pBTK622 tdk kan and recJ homology.png

recJ plate: pBTK622 *tdk/kan* ligation with *recJ* homology flanks

- Grow a liquid LB-Kan culture, make a glycerol stock of these tomorrow

6-30-22 pbpG rescue cassette transformation with strain iGEM_012.png

pbpG plate: no growth

- Need to repeat a GGA to ligate *pbpG* flanks. Transformation efficinecy is probably low - AZT is killing everything with *tdk/kan*
- try putting in all 20 ul of GGA - don't run a gel to check ligation product
- I may try using 1 ul of GGA DNA as template to run a PCR to amplify the 2 kb region?

FRIDAY, 7/1/2022

GGA overnight for *recJ* rescue cassette

- Inoculated two new cultures from another colony in *recJ tdk/kan* insertion in liquid LB-Kan culture so that it's fresh
- Isaac mentioned potential mutation from an overnight liquid LB-Kan culture

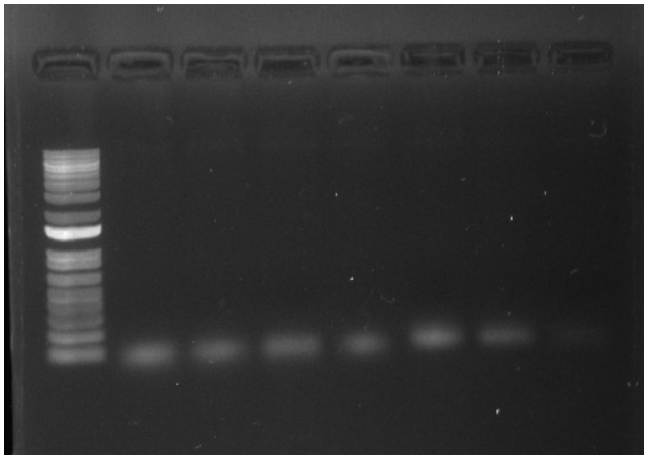
Worked with Keaton to figure out Amp dilutions for his *acrB* deletion
Stored LB-CAM plates and LB plates in 4C deli fridge in A lab

SATURDAY, 7/2/2022

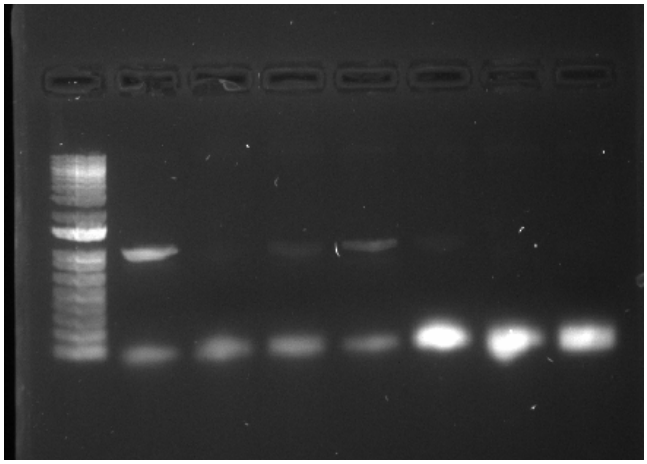
recJ and *pbpG* PCR flanks, combine for purifications

- Annealing temperatures, 57C for *recJ*, 60C for *pbpG*
- all garbage

7-2-22 pbpG upstream and downstream flanks 60C.png



7-2-22 recJ upstream and downstream flanks 57C.png



Inoculated cultures from last night's *recJ tdk/kan* insertion had growth in -LB media control

- will test contamination for that LB media stock again tonight
- will repeat process and incoulate two new cultures from *recJ tdk/kan* insertion LB-Kan plate into liquid LB-Kan culture

Set up a GGA for *pbpG* rescue cassette and a GGA for *recJ* rescue cassette

- Inoculate a culture from iGEM_012 strain stock for *pbpG*

SUNDAY, 7/3/2022

Need to focus on making more DNA for flanks - figure out PCR troubleshooting, not enough DNA to do GGA rescues