Jeffrey Chuong 7/11 - 7/17

MONDAY, 7/11/2022

recJ

Sandy ran a colony PCR for two duplicates from each plate

- tested two different dilutions in 10ul and 250ul
- Expected band size: 3.8 kb for *tdk/kan* ligated with flanks
- Inoculated cultures from picked colonies, also tried inoculating cultures with diluted colonies in water (both worked)

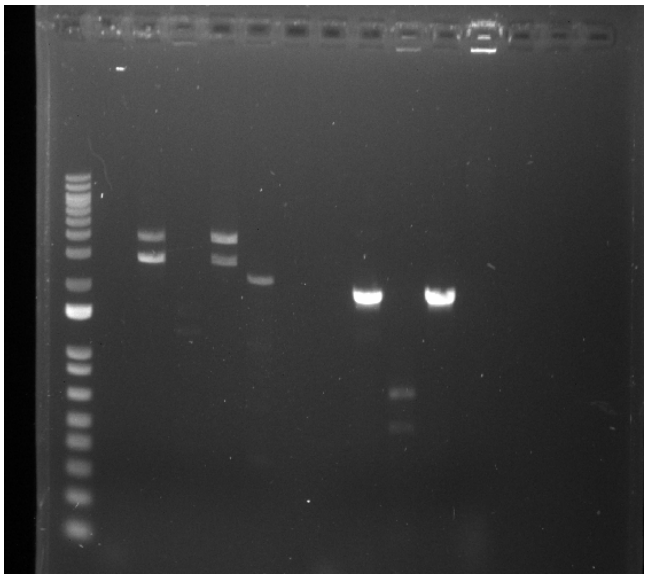
pbpG

Sandy ran a colony PCR for two duplicates from each plate

- tested two different dilutions in 10ul and 250ul
- Expected band size: 2 kb for flanks
- Inoculated cultures from picked colonies

Gel order: Ladder, (recJ) R1, R2, R3, R4, blank, (pbpG) P1, P2, P3, P4

7-12-22 Colony PCR recJ ligated with tdk kan, pbpG knockout.png



Extra bands in *recJ* may be due to too low of an annealing temperature?

TUESDAY, 7/12/2022

Marionette strains

key: plasmid repressor - cloning vector

MH101728 cymR - pJAM657
MH101731 lacI - pJAM336
MH101734 betI - pJAM683
MH101730 vanR - pJAM773

Talked to Beth about GGA and stitching the promoter/RBS with repressor CDS and terminator - definitely doable with BsmBI cut sites but will need to design new primers for the CDS/Terminator -> final product is entire repressor with Type 6 sticky ends

- read up on designing a part plasmid

Made *recJ* glycerol stocks - new strain: iGEM22_014 with *recJ* knockout and *tdk/kan* insertion
Next steps: Inoculate a culture of *recJ* knockout with *tdk/kan*, Set up rescue GGA O/N with *recJ* flanks only

Made *pbpG* glycerol stocks - new strain: iGEM22_012B with *pbpG* knockout
Next steps: Inoculate a culture of *pbpG* knockout, *pbpG* knockout with *tdk/kan*, and ADP1-ISx to run a WC-PCR

- Expected band sizes: 2 kb, 3.8 kb, 3.1 kb

WEDNESDAY, 7/13/2022

Design gBlocks for Marionette repressors (Promoter/RBS, CDS, Terminator)
Marionette strains
key: plasmid repressor - cloning vector

Miniprep concentrations
MH101728 cymR - pJAM657: ng/ul
MH101731 lacI - pJAM336: ng/ul
MH101734 betI - pJAM683: ng/ul
MH101730 vanR - pJAM773: ng/ul

recJ
Next steps: Inoculate a culture of *recJ* knockout with *tdk/kan*, Set up rescue GGA O/N with *recJ* flanks only

pbpG
Next steps: Inoculate a culture of *pbpG* knockout, *pbpG* knockout with *tdk/kan*, and ADP1-ISx to run a WC-PCR

- Expected band sizes: 2 kb, 3.8 kb, 3.1 kb

THURSDAY, 7/14/2022

pbpG
WC-PCR: Annealing temperature 57C, 2:00 min extension time for all reactions, maybe too long for 2 kb? (Could cause smearing in gel)

- Order: ADP1-ISx, *tdk/kan*, Δ*pbpG,* Δ*pbpG*, -control
- Purified gDNA from O/N cultures to re-run a PCR since this WC-PCR will most likely not work

Expected band sizes: 3.1 kb, 3.8 kb, 2 kb, 2 kb

recJ

- Made two tubes with 500 ul LB and 35 ul O/N culture of iGEM22_014, one tube contains +GGA DNA, one tube contains no DNA
- Made a -control LB media tube
- Plate tomorrow on LB-AZT

Meeting Notes

- Streamlined narrative for selecting our best candidate sequences, avoid talking about troubleshooting - Sai and Neil
- Talk about how we want to design our rescue cassette (with no DNA in between, 10 bp to 1000 bp in between?) - Jeff
  - GGA schematic slide
- Overall ADP1 biosensor and reporter schematic (Type 1 through 8) - Jeff
  - Indicate which parts we still need (Use check marks, question marks, x marks via slide transitions?)
- Repressor construct design: YFP reporter gene, promoter/RBS, CDS, Terminator + GGA sticky ends - Jeff
  - Locations within ADP1 genome
  - gBlock designs on Benchling

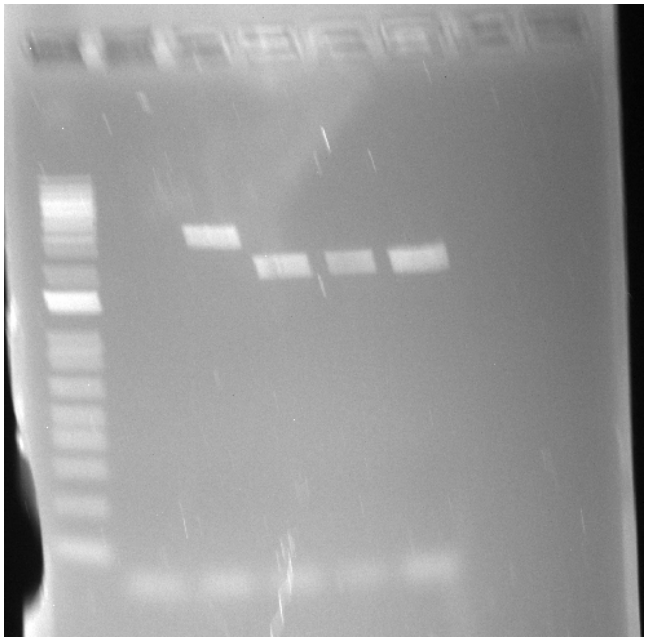
FRIDAY, 7/15/2022

recJ

- Diluted transformations 100x in sterile saline. Plated 50 ul of -control DNA and 50 ul of +control DNA (duplicates) on LB-AZT, 37C O/N

pbpG

7-15-22 WC-PCR ISx pbpG, pbpG tdk kan, KO pbpG 5 7.png



Gel Order: -control, ADP1-ISx, *tdk/kan*, Δ*pbpG*, Δ*pbpG*
pros: WC-PCR worked, Δ*pbpG* (2 kb) is basically confirmed, and no temp gradient needed
cons: really ugly gel, *tdk/kan* band should be 3.8 kb (potentially mixed with Δ*pbpG*?)

Re-running a PCR O/N with purified gDNA from each strain

- a little messy setting up PCR because I only plan on running a couple of samples that each have different template concentrations -> no master mixes
- PCR Order: -control, ADP1-ISx, *tdk/kan*, Δ*pbpG*, Δ*pbpG*