

Jeffrey Chuong 7/18 - 7/24

MONDAY, 7/18/2022

Meeting Notes

- diversifying HP if we go towards the foundational advances narrative
- collaboration A&M: 9/24 FURS presentation, communicating results to each other
- Transformation efficiency
- putting in a gene that where just one gene in *E. coli*, make it harder for ADP1 to transform

recJ

7-18-22 Transformation of iGEM_014 with ligated rec J flanks Day 3.jpg

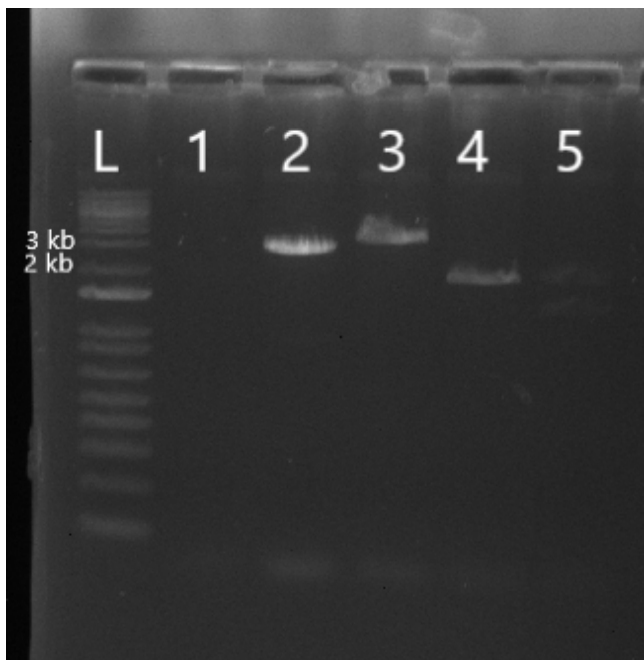


Next steps: Inoculate 3 colonies and make glycerol stocks. Confirm deletion with WC-PCR or colony PCR
Photos taken after 60 hours of 37C incubation

pbpG

Gel from gDNA as template

7-18-22 gDNA PCR ISx pbpG, pbpG tdk kan, KO pbpG 57.png



Gel Order: -control, ADP1-ISx, *tdk/kan*, Δ *pbpG*, Δ *pbpG*

- crop out lane 5

Nate and Sai ran PCRs for YFP transcriptional units

- gradient from 58 to 66, 59.9 to 64.5 middle four rows

TUESDAY, 7/19/2022

recJ

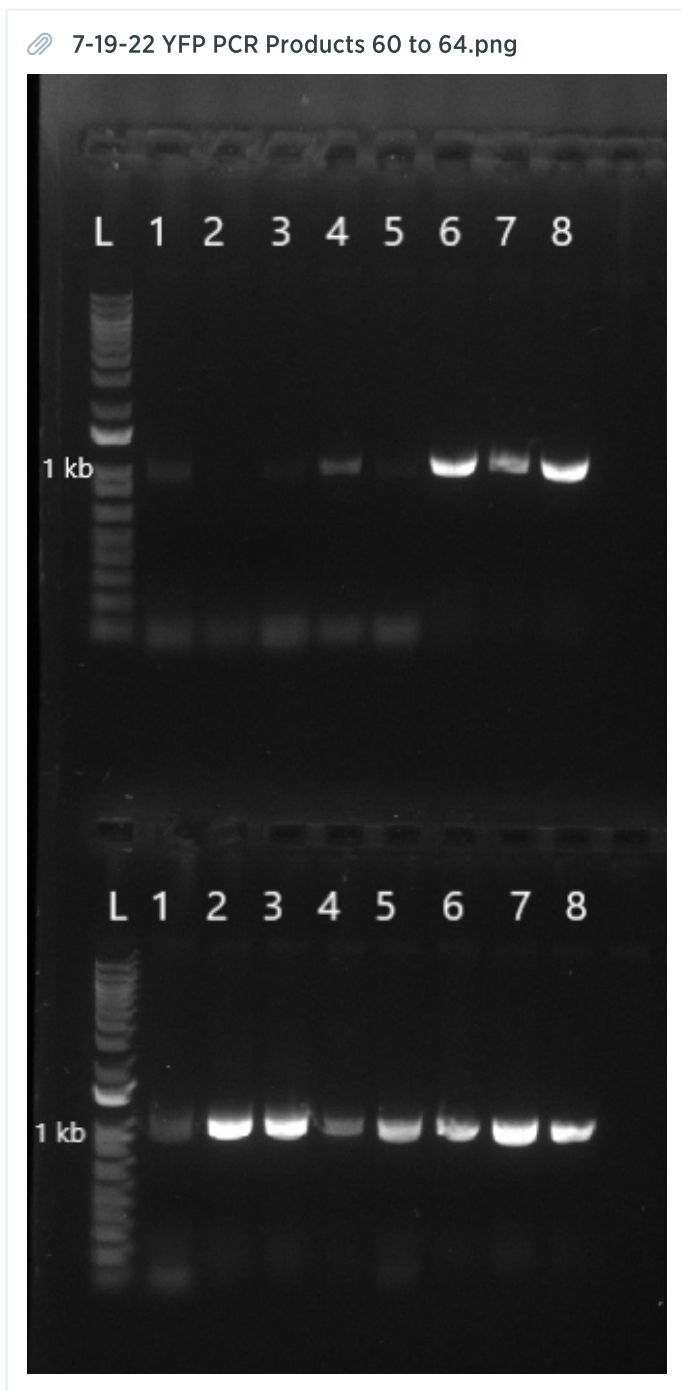
- -LB control contamination, will inoculate the same 3 colonies in LB, *recJ tdk/kan* (strain is from well 69 in -80 freezer) in LB-Kan, and ADP1-ISx in LB
- Next: Glycerol stocks and WC-PCR to confirm deletion

pbpG

- -LB control contamination, but both $\Delta pbpG$ liquid cultures did not show growth in LB-Carb, +control ADP1-ISx showed growth in LB-Carb
- will re run

Nate re-ran PCRs for YFP transcriptional units

- gradient from 58 to 66, 59.9 to 64.5 middle four rows
- noted which column has which Marionette and confirmed negative control -> triplicates started in row F -> C



Top gel: Ladder, -control, *BetI* triplicates, -control, *CymR* triplicates

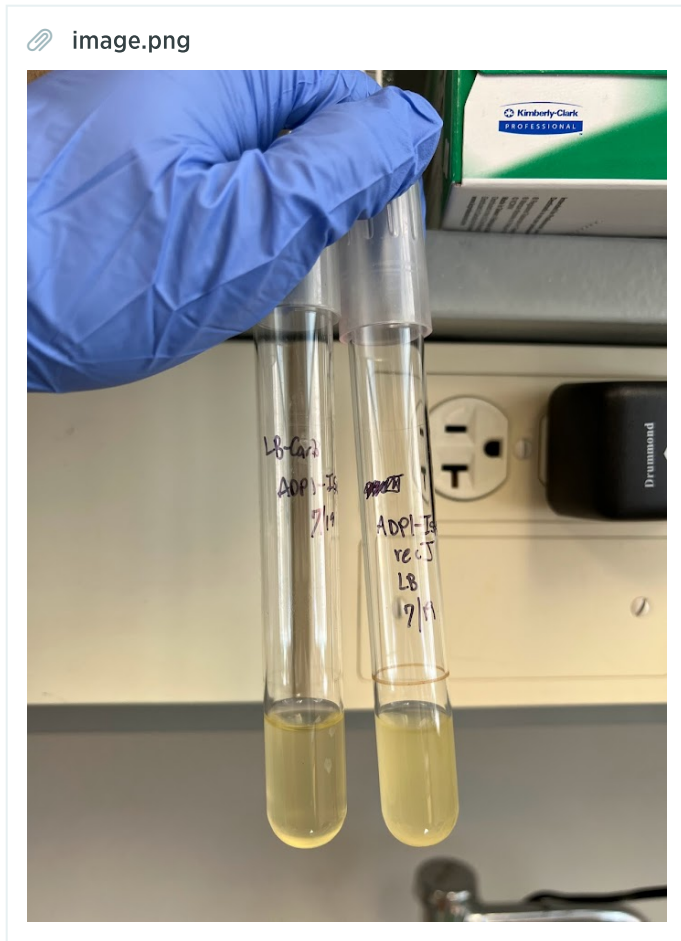
Bottom gel: Ladder, -control, *Lacl* triplicates, -control, *VanR* triplicates

WEDNESDAY, 7/20/2022

Made glycerol stocks of $\Delta recJ$ 1, 2, 3 (B Lab freezer -80 wells 58 to 60)

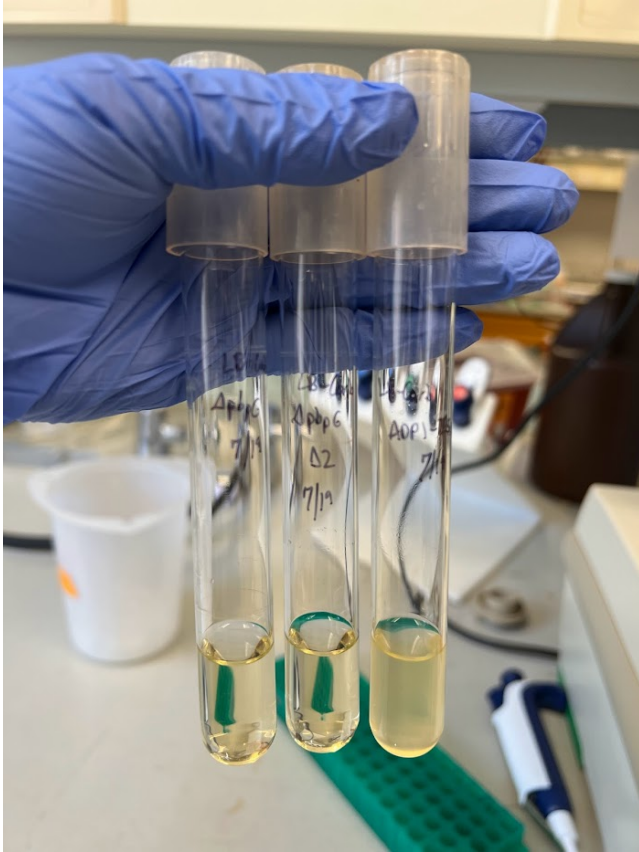
WC-PCR Order: -control, ISx, *tdk/kan*, $\Delta recJ$ 1, $\Delta recJ$ 2, $\Delta recJ$ 3

- Annealing Temperature: 55
- Expected bands ISx: 3.7 kb, *tdk/kan*: 3.8 kb, KO: 2kb
- *recJ* gene size is about 1.7 kb



Interesting to see clear OD difference in growth between ADP1-ISx in LB vs LB-CARB

image.png



- Preliminary data showing knockout *pbpG* strains, ADP1-Isx +control strain (LB-CARB)