Leia Summer 6/20-6/24

MONDAY, 6/20/2022

Meeting Notes:

- Longer extension time for whole-cell PCR, maybe extra MgCl2, ask Isaac
- When plating, - control has water instead of DNA
  - + control can be DNA/GGA that worked for someone already
- AZT plating later to verify deletion/rescue cassette
- recJ helps integration of small DNA (see paper!!!)
- Repressors - lacI, some others
- White Nose
  - Destructans file BLAST w NCBI
  - Design homology flanks

- Redesign recJ primers
- Golden Gate for ampD
  - [x] 2 µL of 10X T4 DNA ligase buffer (NEB: M0202S)
  - [x] 1 µL BsaI-HF (NEB: R3535S)
  - [x] 1 µL of T7 DNA ligase (NEB: M0318S)
  - [x] 250 ng pBTK622 plasmid
    - [x] 250 ng / 66.8 ng/uL = 2.2455 uL
  - [x] 150 ng 5'flank homology (for ~1000bp) *
    - [x] 150 ng / 84.1 ng/uL = 1.78 uL
  - [x] 150 ng 3'flank homology (for ~1000bp) *
    - [x] 150 ng / 22.1 ng/uL = 6.787 uL --> changed to 5 uL bc didn't have enough
  - [x] dH2O to 20μL rxn total volume
    - [x] - control: 20 - 2 - 1 - 1 - 2.25 - 1.8 - 5 = 6.95 uL --> round to 7 uL
    - [x] Sample: 20 - 2 - 2.25 - 1.8 - 5 = 8.95 uL --? round to 9 uL
      - ^accidentally switched the two?...
  - 1 rxn without enzymes for a control, 1 with everything, 1 with no homologies (shows plasmid backbone) if there is enough
    - 3.7 and backbone (1.6) if successful on gel (5 uL GGA + 1 uL, treat it like PCR product)
- Inoculate ADP1-ISx in LB liquid media
  - 5 mL LB + 2 uL ADP1-ISx, 30 C incubator overnight

Plan for Tuesday:

- GGA gel
  - ampD PCR to get more downstream product if this doesn't work
- ADP1-ISx Transformation (follow GGA protocol)
- Ask about recJ primer design:
  - Too many secondary structures? Test 2ndary structures at melting temp?

TUESDAY, 6/21/2022

recJ primer design:

- Use Oligo Calc, primers are ordered!
- Up Forward: gtgcgatcctgcacaagt
  Basically no structures, 3' @ 3416735

Up Reverse: ATGCGGTCTCACGTTCGTCTCAGACCcggtatgatgcacccactga
5 strucutres, 3′ @ 3417684

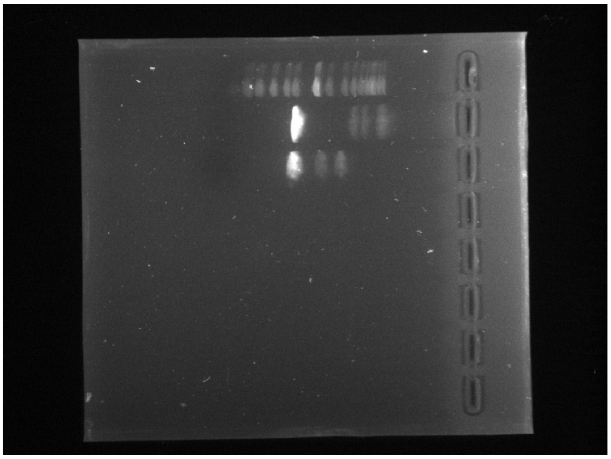
Down Forward: GCATGGTCTCAGCTGCGTCTCAGGTCttatggcatcagtcgttgca
No structures, 3′ @ 3419484

Down Reverse: caacgggtgatgccattattg
No structures, 3′ @ 3420448

GGA Gel Results:

- GGA tubes warped in the thermocycler??
- Lane 1 has 1 kb+ ladder
- Lane 2 has GGA w no enzymes
- Lane 3 has good band at around 3.7 kb

image (4).png



Transformation:

- 500 uL media + 35 uL overnight ADP1-ISx culture in both tubes, then 20 (actually 15 bc used 5 uL for gel) uL GGA in tdk/kan tube in 30 C incubator

Plan for Wednesday:

- Dilutions + plating
  - 100x dilution in sterile saline, or more?
  - How much culture to take?
  - Where to get saline? Sterile beads? LB-KAN plates?

WEDNESDAY, 6/22/2022

Dilutions + plating:

- Make solution 1: 1000 uL saline 10 uL (10^-2) stock --> plate onto +DNA KAN
  - Make soln 2: 10 uL soln 1^ + 1 mL saline
  - Make soln 3: 632 uLsoln 2^ + 1mL saline (185 colonies/50 uL)
- 3 plates with 10^-6
  - - DNA KAN, - DNA LB, + DNA LB
- 1 plate with 10^-2
  - + DNA KAN
- 50 uL of dilution on each plate

- Incubate at 30 C standing incubator at B lab

Plan for Thursday:

- See if tdk/kan colonies grew; if yes, colony counts + efficiency calculations
  - Trans Eff = (avg CFU plates) * 10^-6 = Ans cfu/ug
- Liquid culture in LB-KAN
  - 5 uL KAN to 50 mL LB in 2 tubes but inoculate only 1
- ampD downstream PCR? Maybe Friday

THURSDAY, 6/23/2022

- recJ primers arrived, Jeffrey taking over

tdk/kan didn't integrate!:

IMG_1249.jpg

- Looked at GGA gel picture again... weird results
  - Lane 3 actually doesn't have 3.7 segment, the segments are smaller than they're supposed to be
  - Lane 2 unsure what the bigger fragments are... should only have 2 bands since no enzymes

So... have to redo GGA. First need more ampD downstream product:

- Gradient again
- Mastermix 6 reactions for downstream
  - [x] Phusion buffer (70 uL each MM, 1 downstream)
  - [x] dH2O (227.5 uL each)
  - [x] Primer Forward (17.5 uL each)
  - [x] Primer Reverse (17.5 uL each)

- [x] dNTPs (7 uL each)
- [x] DMSO (10.5 uL each)
- [x] Phusion polymerase (3.5 uL each) ADD LAS
- [x] Each PCR tube:
  - [x] Template ADP1 ISx (1 uL, NOT IN - CONTROLS)
  - [x] H2O (1 uL in - controls)

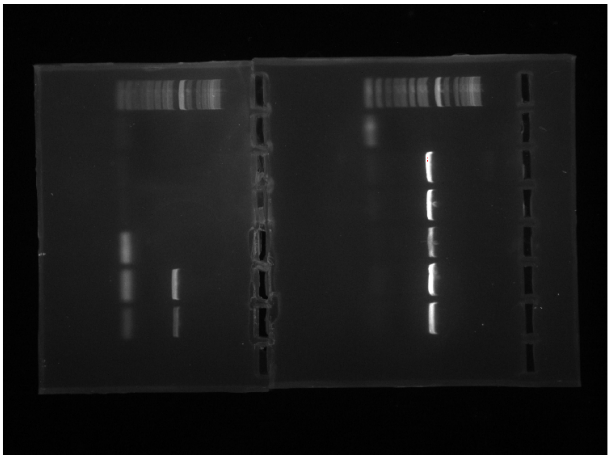
recJ handled by Jeffrey with new primers:

- Both NEB TM temperatures at 62 C (https://tmcalculator.neb.com/#!/main)

PCR results:

- Very nice! Top row D- at all temperatures (56-64)

image (5).png



Plan for Friday:

- GGA ampD
- Clean and concentrate ampD downstream

FRIDAY, 6/24/2022

- Didn't come in :(maintenance issue