5/19/22 - 5/24/22

THURSDAY, 5/19/2022

This Week's Objectives

- Perform Deletion of ACIAD-2257 and RecJ

Today Checklist

- [x] Create Master 4 Mixes
- [x] Run PCR
- [x] Make Gel
- [ ] Design Sequences
- [ ] Run Gel

Master Mixes

31 uL Water
10 uL Phusion buffer
2.5 uL Forward Primer
2.5 uL Reverse Primer
1.5 uL DMSO
1 uL mM dNTP's
1 uL Template DNA
.5 uL Phusion Ploymerase

ACIAD 2257 Deletion

- [x] 93 uL water
- [x] 30uL Phusion buffer
- [x] 7.5 uL Forward
- [x] 7.5 uL Reverse
- [x] 4.5 uL DMSO
- [x] 3 uL mM dNTP's
- [x] 1.5 uL Phusion polymerase

ADD TEMPLATE DNA AFTER PUT INTO PCR Tubes

Annealing Temps-

- Up Annealing Temp- 63° C
- Down Annealing Temp- 62° C

Left- Ladder- U1-U2-C1-D1-D2-C2- Right

UNSUCCESSFUL, NO BANDS WERE OBSERVED

- I suspect that my primers are incorrect.
- I do not know why my C2 well produced DNA...
- **Tommorrow-**
  - Run PCR w/ Sai or Vivek's primers to see if the problem is my procedure or my primers
  - I have proof that both Sai and Marissa's Primers were correct meaning that their annealing temps were also correct.

MONDAY, 5/23/2022

Master Mixes

31 uL Water
10 uL Phusion buffer
2.5 uL Forward Primer
2.5 uL Reverse Primer

1.5 uL DMSO
1 uL mM dNTP's
1 uL Template DNA
.5 uL Phusion Ploymerase

AcrB Deletion

- [x] 93 uL water
- [x] 30uL Phusion buffer
- [x] 7.5 uL Forward
- [x] 7.5 uL Reverse
- [x] 4.5 uL DMSO
- [x] 3 uL mM dNTP's
- [x] 1.5 uL Phusion polymerase

ADD TEMPLATE DNA AFTER PUT INTO PCR Tubes
Upstream Annealing- 64
Downstream Annealing- 66

RecJ Deletion

- [x] 93 uL water
    - [x] 30uL Phusion buffer
    - [x] 7.5 uL Forward
    - [x] 7.5 uL Reverse
    - [x] 4.5 uL DMSO
    - [x] 3 uL mM dNTP's
    - [x] 1.5 uL Phusion polymerase

ADD TEMPLATE DNA AFTER PUT INTO PCR Tubes
Upstream Annealing- 61
Downstream Annealing- 61

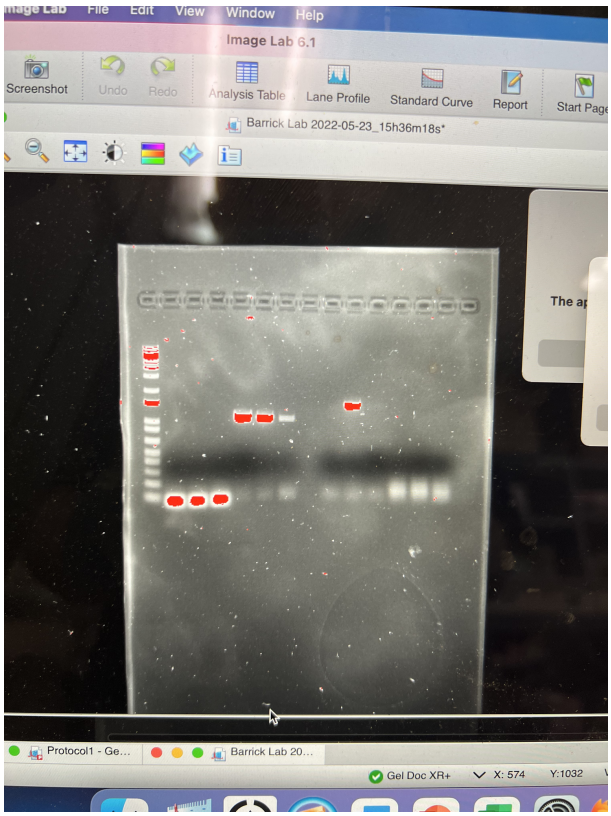
Gel Electrophoresis-

Ladder-AU1-AU2-AC1-AD1-AD2-AC2-Blank-RU1-RU2-RC1-RD1-RD2-RC2

AcrB

Upstream length= 999
Downstream Length= 1011

AcrB Gel Electrophoresis.JPG



Results- AcrB Down 1 and Down 2 were successful

Analysis- AcrB Up1, Up 2 and al of recJ were unsuccessful.

Rec J- The wrong DNA was used

AcrB Up'S- The primers were the only DNA present in the Gel.

Control- The control for AcrB showed the presence of DNA however, at a lower concentration. I think this is due to contamination of the control tube. Meaning, some of the ISX DNA got into the tube.

PCR Clean and Concentrate-

- [x] 45 uL of PCR product
- [x] 9 uL of DNA Binding Buffer
- [x] Transfer to a Zymospin Column in a collection tube
- [x] Centrifuge for 30 seconds @ 10,000
- [x] Discard Flow through
- [x] Add 200 uL DNA wash buffer
- [x] Centrifuge for 30 seconds @ 10,000
- [x] Discard Flow through
- [x] Repeat Wash step again
- [x] Add 20 uL directly to matrix and incubate at room temp for one minute
- [x] Transfer to microcentrifuge tube
- [x] Centrifuge for 30 seconds
- [x] Take flow through, re insert into tube and repeat last two steps

AcrB Deletion

- [x] 93 uL water
- [x] 30uL Phusion buffer

- [x] 7.5 uL Forward
- [x] 7.5 uL Reverse
- [x] 4.5 uL DMSO
- [x] 3 uL mM dNTP's
- [x] 1.5 uL Phusion polymerase

ADD TEMPLATE DNA AFTER PUT INTO PCR Tubes
Upstream Annealing- 64
Downstream Annealing- 66

Tomorrow-

1. Run Gel for AcrB Up PCR
2. If successful clean and concentrate the PCR product
3. Quantify [DNA] w/ quibit for both up and down stream AcrB
4. Continue onto week 5 procedure

TUESDAY, 5/24/2022

Gel Was Unsuccessful, I think the genomic DNA I used was wrong