4/16/22 - 4/24/22

SATURDAY, 4/16/2022

Objectives:

- Amplify flanking regions for deletion of target gene flanks
- Confirm insertion of tdk/kanr casette into ADP1-ISx replacing ACIAD2049

ACIAD2257 Upstream Annealing Temp- 63°
ACIAD2257 Downstream Annealing Temp- 62°
PCR- Master Mix

- [x] 30 µL of Phusion HF buffer
- [x] 3 µL of mM dNTPs
- [x] 7.5 µL Forward Primer
- [x] 7.5 µl Rverse Primer
- [x] 1.5 µL Phusion DNA polymerase
- [x] 97.5 nuclease free water

SUNDAY, 4/17/2022

Whole cell PCR setup

- Dilute cells one thousand folf
- 1µl of DNA
- 1000µL of

- [x] 30 µL of Phusion HF buffer
- [x] 3 µL of mM dNTPs
- [x] 7.5 µL Forward Primer
- [x] 7.5 µl Rverse Primer
- [x] 1.5 µL Phusion DNA polymerase
- [x] 85.5 µL water
- [x] 5 µL Cell's

15-150= 135
Length =3685

Gel Electrophoresis
Left d1 d2 cd u1 u2 cu ladder right
length= 3685
extension time= (3685)/1000 * 15 =56s
No Bands produced... Next time add 1.5ml of DMSO to each PCR

699+ 2000

SUNDAY, 4/24/2022

Top Gel=1
Bottom Gel =2

Keaton's Gel Electrophoresis

- Left side of gel 1

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

Sai's Gel Electrophoresis

- Right side of gel 1
- Leftv : Ladder-C1-U1-U2-C2-D1-D2 : Right

Marisa's Gel Electrophoresis

- Left Side Gel 2
- Left : Ladder-C1-U1-U2-U3--D1-D2-D3-C2 : Right

Whole Cell PCR