4/8/2022 - 4/12/2022

FRIDAY, 4/8/2022

Day 1 Objective

-Our goal today is to amplify the upstream and downstream regions of the ACIAD2049 ADP1 gene

-We also identified a gene within ADP1 to delete and use the genome to design a primer for that gene.

PCR Reactions

Thermocycler Settings

-50 µL

-Denaturing- 98

-Annealing- 58

-Elongation- 72

- [x] 10 µL of Phusion HF buffer
- [x] 1 µL of mM dNTPs
- [x] 2.5 µL Forward Primer
- [x] 2.5 µl Rverse Primer
- [x] .5 µL Phusion DNA polymerase
- [x] 1.5 µL DMSO
- [x] 31 nuclease free water
- [x] 1 µL template DNA

Primer Design

Golden Gate

gc 40-60%

melting temp 55-60

forward and reverse withing 5 degrees of each other

Forward part creation primer 5′ extension

SATURDAY, 4/9/2022

Day 2 objective-

1.) First we are running gel electrophoresis on each of the 6 PCR products to determine if DNA was successfully made and if we made the correct dna sequence

2.) Finally, we're eluting DNA from the PCR products with the PCR cleanup kit and then determining the [DNA] with the Quibit

Agarose Gel Electrophoresis

- [ ] Dissolve agarose in TE buffer
- [ ] Add SYBR Safe dye
- [ ] Set Up casting tray with 15 well comb
- [ ] Pour molten agar into tray, allow to solidify for 25-30 minutes
- [ ] Transfer gel into electrophoresis unit
- [ ] Load ladder into one lane of the gel
- [ ] Mix 1 µL of dye with 5 µL of PCR product. Load all 6 products into a lane
- [ ] Make sure to label each lane
- [ ] Start electrophoresis running at 130 volts for 20 minutes\
- [ ] Visualize gel, upload to benchling

PCR Cleanup-

-Follow instructions in DNA clean and Concentration kit.

-For elution step, add 20 µL of buffer, incubate at room temp for 1 min befor centrifuging

-Quantify DNA of final elutionwith quibit (see week 1 procedure)

DNA Concentrations-

TUESDAY, 4/12/2022

Colony Counts

DNA Control 1 10^{-2} LB+AR+KAN

- Positive Control- 1024
- Negative Control- 0
- Transformation Efficiency = (1024+0)/2 * 10^{6}= 5.12E8

DNA Control 1 10^{-5} LB+AR

- Positive Control- 556
- Negative Control- 540
- Transformation Efficiency- (556+540)/2 * 10^{6}= 5.48E8

DNA Control 2 10^{-2} LB+AR+KAN

- Positive Control- 608
- Negative Control 0
- Transformation Efficiency- (608)/2 * 10^{6}= 3.04e8

DNA Control 2 10^{-5} LB+AR

- Positive Control- 444
- Negative Control- 512
- Transformation Efficiency- (512+444)/2 * 10^{6}=4.78E8

2.2e9 for tdkkan