Name: Asma, Kennex, Krithika, Chiara

Date: 08/07/2019

Goals:

1. Transform mCherry into BL21 and NEBa cells
    a. Repeat transformation of mCherry with control
2. PCR on DinoIII-rfp colonies from transformations done on 8/6/19
3. Restriction Digest codon optimized RFP 200 µL reaction
    a. Enzymes: XbaI & BglII
4. Overnight Culture on Ligation of DinoIII and RFP

Name: Chiara Brust
Date: 8/7/19

Goal:

1. Restriction Digest codon optimized RFP 200 µL reaction
    a. Enzymes: XbaI & BglII
    b. Used C.O. RFP sample # 3 from 7/24/19 midiprep

Protocol:

Restriction Digest

60 µL Fast Digest Restriction Digest

1. Prepared a Fast Digest concentration cocktail with the following proportions: 2 µL Restriction Enzyme XbaI, 2 µL Restriction Enzyme BglII, 6 µL of 10X Fast Digest Buffer, and 30 µL of diH2O.
2. Add 40 µL of this cocktail to a clean 1.5 Eppendorf tube and then add 20 µL DNA
3. Incubate at 37° C for 1 hour

Name: Kennex Lam and Krithika Karunakaran
Date: 8/7/19

Goal:

1. Overnight on Ligation of DinoIII and RFP

Protocol:

1. 15 mL of LB + 15 uL of Ampicillin was added into 6 falcon tubes.
2. An individual colony was picked and a tip was dropped in a separate tube.
3. The tubes were left to shake at 220 rpm at 37° C overnight.

Results:

Conclusion:
We are going to measure to concentration of DinoIII with RFP and perform a restriction digest on the plasmid in order to linearize it before transforming the ligated DinoIII into O. marina.

Name: Asma Khimani

Date: 08/07/2019

Goals:

1. PCR Colony on DinoIII-rfp colonies from transformations done on 8/6/19

Protocol:

Colony PCR Protocol

20 µL Reaction
1. Prepared a PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL PCR Mastermix, 1 µL of the forward primer, and 1 µL of the reverse primer.
2. Added 19 µL of the concentration cocktail into a PCR tube.
3. Using a 10 µL micropipette, touched the tip onto the selected colony and swirled around in the PCR tube.
4. Placed PCR tube in the thermocycler at the following generic settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. 52° C for 1:00 minute *Annealing temperature varies depending on primer
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

    Lid Temperature: 105° C

Conclusion:
We will run the gel tomorrow.