Name: Chiara Brust, Asma Khimani, Amirah, Rehmat Babar, Yilin Lu, Zeshi Wang, Jia Deng, Xuecheng Ye, Zexi Guo, Xinyi Liu

Date: 8/20/19

Goal:

1. Start overnight cultures for glycerol stocks of mCherry, GFP, RFP, and HCG B3 loop in pGEX
2. Streak plates of glycerol stocks of mCherry, GFP, RFP, and HCG B3 loop in pGEX
3. Gel extraction
    a. DinoIII-GFP
    b. DinoIII-RFP
4. Ethanol precipitate the gel extractions

Name: Asma Khimani

Date: 8/20/19

Goal:

1. Start overnight cultures for glycerol stocks of mCherry, GFP, RFP, and HCG B3 loop in pGEX
    a. mCherry: Plate 1 Colony 9

Protocol:

1. Labeled eight 50ml falcon tubes: RFP+LB+AMP, RFP+LB+CHLOR, GFP+LB+CHLOR, GFP+LB+AMP, MCHERRY+LB+CHLOR, MCHERRY+LB+AMP, HCG+LB+CHLOR, HCG+LB+AMP
2. Added 10 ml of LB and 10 ul of respective antibiotic and scraped the top of each respective glycerol stock with a micropipette tip.
3. Placed samples in the shaking incubator overnight (3pm 08/20/2019)

Conclusion:

Use plate reader in NSC to measure fluorescence tomorrow.

Name: Asma Khimani

Date: 8/20/19

Goal:

1. Streak plates of glycerol stocks of mCherry, GFP, RFP, and HCG B3 loop in pGEX
    a. mCherry glycerol stock: Plate 1 Colony 9

Protocol:

1. Obtained 4 LB+CHLOR plates and 4 LB+AMP
2. Used sterile technique to streak each glycerol on an AMP plate and CHLOR plate.
3. Placed in 37℃ Incubator overnight

Conclusion:

Check to see if colonies grew to check the validity of glycerol stocks.

Name: Yilin Lu, Zeshi Wang, Jia Deng, Xuecheng Ye, Zexi Guo, Xinyi Liu.

Date: 8/20/19

Goal: Dilute the overnight culture and measure the zero time point abs600 and make overnight culture for another measurement.

Protocol:

1. Made a 1:10 dilution of each overnight culture in LB/YPD/YM/TB+Chloramphenicol (0.5mL of culture into 4.5mL of LB+Chlor)
2. Measured Abs 600 of these 1:10 diluted cultures
3. Recorded the data
4. Diluted the cultures further to a target Abs 600 of 0.02 in a final volume of 12 ml LB/YPD/YM/TB medium + Chloramphenicol in 20 mL tube
5. Incubated the remainder of the cultures at 37°C and 220 rpm for overnight.

Result:

YPD	**Colony 1 OD 600**	**Colony 2 OD 600**
Positive control	0.059	0.05
Negative control	0.072	0.057
Device 1	0.062	0.067
Device 2	0.068	0.071
TB		
Positive control	0.105	0.079
Negative control	0.08	0.109
Device 1	0.056	0.063
Device 5	0.094	0.041
YM		
Positive control	0.043	0.041
Negative control	0.034	0.033

Device 1	0.039	0.031
Device 5	0.037	0.009
LB		
Positive control	0.046	0.038
Negative control	0.101	0.039
Device 1	0.053	0.066
Device 5	0.056	0.047

Table 1: the original data measured from the 10 times diluted overnight culture

YPD	**Colony 1 OD 600**	**Colony 2 OD 600**
Positive control	0.051525	0.1328
Negative control	-0.03	0.067368
Device 1	0.029677	-0.00239
Device 5	-0.00824	-0.02479
TB		
Positive control	-0.14629	-0.06278
Negative control	-0.067	-0.1556
Device 1	0.075714	0.022857
Device 5	-0.1166	0.249756
YM		
Positive control	0.219535	0.249756
Negative control	0.383529	0.407273
Device 1	0.283088	0.459355
Device 5	0.32	2.56
LB		
Positive control	0.17913	0.301053

Negative control	-0.13624	0.283077
Device 1	0.102642	0.003636
Device 5	0.075714	0.166809

Table 2: the original overnight culture volume that need to add into the 20ml tube (ml).

Excel calculation formula: =0.0222*12/('original data'!DATA*10)-0.4

YPD	**Colony 1 OD 600**	**Colony 2 OD 600**
Positive control	7.948475	7.8672
Negative control	8.03	7.932632
Device 1	7.970323	8.002388
Device 5	8.008235	8.024789
TB		
Positive control	8.146286	8.062785
Negative control	8.067	8.155596
Device 1	7.924286	7.977143
Device 5	8.116596	7.750244
YM		
Positive control	7.780465	7.750244
Negative control	7.616471	7.592727
Device 1	7.716923	7.540645
Device 5	7.68	5.44
LB		
Positive control	7.82087	7.698947
Negative control	8.136238	7.716923
Device 1	7.897358	7.996364
Device 5	7.924286	7.833191

Table3: the pure medium volume that need to add into the 20 ml tube (ml).

Excel calculation formula: =12-4-DATA

Result :

1. eGFP Fluoresein ZF

YPD	Positive	Negative	Test Device1	Test Device5	Blank with media
Colony1	18412	18234	21249	18805	18523
Colony1	18429	18541	22289	19156	18935
Colony2	18352	18704	20969	19796	
Colony2	19416	19810	21164	20319	
TB					
Colony1	23511	24265	25560	24608	25889
Colony1	24606	24586	30303	26024	25329
Colony2	25210	25408	30649	29323	
Colony2	25550	25789	30276	29840	
LB					
Colony1	18181	18231	18016	18218	18189
Colony1	18304	19214	19306	18502	20233
Colony2	17861	18043	17943	18093	
Colony2	18418	18942	18992	19702	
YM					
Colony1	6145	6357	9460	6381	6271
Colony1	6155	6333	9523	6520	6562
Colony2	6160	6275	9412	9549	
Colony2	6505	6620	9117	10096	

2. Abs600

YPD	Positive	Negative	Test Device1	Test Device2	Blank with media
Colony1	0.058	0.056	.057	.058	.052
Colony1	0.062	.06	.061	.059	.053
Colony2	.058	.057	.056	.057	
Colony2	.060	.061	.059	.060	
TB					
Colony1	.067	.065	.066	.064	.061
Colony1	.071	.067	.074	.067	.058
Colony2	.067	.065	.063	.066	
Colony2	.067	.066	.066	.067	
LB					
Colony1	.08	.080	.077	.077	.070
Colony1	.079	.081	.083	.079	.076
Colony2	.081	.080	.078	.079	
Colony2	.083	.082	.081	.085	
YM					
Colony1	.05	.049	0.05	.053	.045
Colony1	.051	.05	0.052	.054	.05
Colony2	.049	.048	.052	.056	
Colony2	.051	.050	.052	.062	

Name: Chiara
Date: 8/20/19

Goal:

1. Gel extract DinoIII GFP

Protocol:

Gel Extraction

Qiagen QIAEX II Gel Extraction Kit

1. Ran a restriction digest on the targeted DNA part using restriction enzymes and ran an agarose gel overnight
2. Cut the targeted DNA sequence out using a razor blade, made sure to get as much DNA while limiting the amount of agarose extracted
3. Pre-weighed an empty 50 mL falcon tube before adding the gel exicisions.
4. Added the gel extracts to the Eppendorf tubes and weighed again.
5. Calculated the mass of the gel using the difference of the two measurements.
    - 3.6 g
6. Multiplied the mass by a factor of 3 to get the volume of Buffer QG needed.
7. Added the respective amounts of Buffer QG to each of the tubes
    - 1000 µL
8. Vortexed the tubes until all the gel had dissolved
9. Added 1:1 ratio of Gel mass: Isopropanol
    - 3.6 g gel: 3.6 mL Isopropanol
10. Once dissolved, added the solution into spin columns and centrifuged for 30 second at 13,000 rpm and carefully removed the supernatant.
    - One column can only handle .4 grams of agarose
11. Added 750 µL of Buffer QG to the tubes and resuspend by vortexing
12. Resuspend the pellet in 750 µL of Buffer PE and centrifuged for 30 seconds. Removed supernatant and repeated the centrifugation twice.
13. Air dried pellet for 30 minutes
    a. Until it became white
14. Eluted the DNA by adding 50 µL EB
15. Centrifuge for 30 seconds into an eppendorf tube
16. Measured and recorded the concentrations.

Results:

Sample	**[DNA]**	**260/280**
DinoIII-GFP	27.5 ng/μL	2.571

Conclusion:

The concentration is good. We will now do an ethanol precipitation to concentrate and purify the DNA.

Name: Amirah
Date: 8/21/19

Goal:

1. Gel extract DinoIII-RFP

Protocol:

Gel Extraction

Qiagen QIAEX II Gel Extraction Kit

1. Ran a restriction digest on the targeted DNA part using restriction enzymes and ran an agarose gel overnight
2. Cut the targeted DNA sequence out using a razor blade, made sure to get as much DNA while limiting the amount of agarose extracted
3. Pre-weighed an empty 50 mL falcon tube before adding the gel exicisions.
4. Added the gel extracts to the Eppendorf tubes and weighed again.
5. Calculated the mass of the gel using the difference of the two measurements.
    - 3.6 g
6. Multiplied the mass by a factor of 3 to get the volume of Buffer QG needed.
7. Added the respective amounts of Buffer QG to each of the tubes
    - 1000 µL
8. Vortexed the tubes until all the gel had dissolved
9. Added 1:1 ratio of Gel mass: Isopropanol
    - 3.6 g gel: 3.6 mL Isopropanol
10. Once dissolved, added the solution into spin columns and centrifuged for 30 second at 13,000 rpm and carefully removed the supernatant.
    - One column can only handle .4 grams of agarose
11. Added 750 µL of Buffer QG to the tubes and resuspend by vortexing
12. Resuspend the pellet in 750 µL of Buffer PE and centrifuged for 30 seconds. Removed supernatant and repeated the centrifugation twice.
13. Air dried pellet for 30 minutes
    b. Until it became white
14. Eluted the DNA by adding 50 µL EB
    Accidentally added 500ul eb into some of the tubes
15. Centrifuged for 30 seconds into an eppendorf tube
16. Measured and recorded the concentrations.
    Too low because I added 500ul instead of 50ul of eb
17. Ethanol precipitation
    - Added 3M sodium acetate to dna to get to 0.3M sodium acetate
    - Added cold (freezer) 100% etoh
    - Centrifuged for 30 mins

- Removed supernatant
- Added 200ul of 70% cold (freezer) etoh
- Centrifuged for 15 mins
- Removed supernatant
- Allowed to air dry (overnight

Name: Chiara
Date: 8/20/19

Goal:

1. Ethanol precipitate DinoIII-GFP

Protocol:

1. Added 1:10 ratio of Sodium Acetate: Gel extraction volume
2. Added chilled ethanol in a ratio of 2 times the volume of the gel extraction
3. Centrifuged at 13,000 rpm for 30 minutes
4. Removed supernatant, being careful not to disturb the clear pellet
5. Resuspended in 200 µL of 70% chilled ethanol
6. Centrifuged for 15 minutes at 13,000 rpm
7. Removed supernatant
8. Air dried iunder hood for 30 minutes
9. Resuspended in 100 µL of EB
10. Measured the concentration

Results:

Sample	**[DNA]**	**260/280**
DinoIII-GFP	170 ng/µL	1.789

Conclusion:

We now have 20.75 µg of DinoIII-GFP ready to transform.