Name: Rehmat Babar, Chiara Brust, Jiayi Lan, Yujie Huang, Xinyi Liu, Yilin Lu, Xuecheng Ye, Zeshi Wang
Date:

Goal:

1. Transform pcb302 into A. Tumefaciens
    a. Use DNA extracted from E. Coli transformations Plate B Colony 4
2. Gel extraction on Dino III GFP
3. Gel extraction on Dino III RFP
4. Ethanol precipitate on gel extraction samples Dino III GFP

Name: Jiayi Lan, Yujie Huang, Xinyi Liu, Yilin Lu, Xuecheng Ye, Zeshi Wang

Date: 08/16/2019

Goal:

1. Do the miniprep of the overnight culture.
2. Interlab part: Do the calibration of the OD600 Reference, Particle Standard Curve and fluorescence standard curve.

Protocol:

MINIPREP

a. Centrifuged 3 mL of bacterial overnight culture in two separate Eppendorf tubes (1.5 mL in each) at 8,000 rpm for 3 minutes at room temperature.
b. Discarded the supernatant and resuspended pelleted bacterial cells in one tube with 250 µL Buffer P1 and transferred to the other and resuspended until one eppendorf tube contained the pelleted cells resuspended in 250 µL Buffer P1.
c. Added 250 µL of Buffer P2 and inverted 5 times.
d. Added 350 µL of Buffer N3 and immediately mixed by inverting 5 times.
e. Centrifuged for 10 minutes at 13,000 rpm.
f. Micropipetted 800 µL of the clear supernatant into a spin column and centrifuged for 60 seconds and discarded the excess liquid.
g. Added 500 µL of PB and centrifuge the spin columns for 60 seconds. Discarded the flow through.
h. Added 750 µL of PE to the spin columns, centrifuged for 60 seconds, and discarded the flow through.
i. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discarded the flow through.
j. Transferred the spin columns to a clean eppendorf tube and added 50 µL of EB to the center of the spin column to elute the DNA.
k. Allowed the spin column to stand for one minute and then centrifuge for one minute.
l. Recorded the concentrations for each sample.

CALIBRATION:
Calibration 1: OD600 Reference point - LUDOX Protocol

Materials:
1ml LUDOX CL-X (provided in kit)
ddH20 (provided by team)
96 well plate, black with clear flat bottom preferred (provided by team)

Method
❏ Added 100 µl LUDOX into wells A1, B1, C1, D1
❏ Added 100 µl of dd H2O into wells A2, B2, C2, D2
❏ Measured absorbance at 600 nm of all samples in the measurement mode you plan to use for cell measurements
❏ Recorded the data in the table below or in your notebook
❏ Imported data into Excel sheet provided (OD600 reference point tab)

Calibration 2: Particle Standard Curve - Microsphere Protocol
Materials:
300 µL Silica beads - Microsphere suspension (provided in kit, 4.7 x 10^8 microspheres)
ddH20 (provided by team)
96 well plate, black with clear flat bottom preferred (provided by team)

Method:
Prepare the Microsphere Stock Solution:
❏ Obtained the tube labeled “Silica Beads” from the InterLab test kit and vortexed vigorously for 30 seconds. NOTE: Microspheres should NOT be stored at 0°C or below, as freezing affects the properties of the microspheres. If you believe your microspheres may have been frozen, please contact the iGEM Measurement Committee for a replacement (measurement at igem dot org).
❏ Immediately pipetted 96 µL microspheres into a 1.5 mL eppendorf tube
❏ Added 904 µL of ddH2O to the microspheres
❏ Vortexed well. This is your Microsphere Stock Solution.

Calibration 3: Fluorescence standard curve - Fluorescein Protocol

Materials:
Fluorescein (provided in kit)
10ml 1xPBS pH 7.4-7.6 (phosphate buffered saline; provided by team)
96 well plate, black with clear flat bottom (provided by team)

Method
Prepare the fluorescein stock solution:
❏ Spun down fluorescein kit tube to make sure pellet was at the bottom of tube.

❏ Prepared 10x fluorescein stock solution (100 μM) by resuspending fluorescein in 1 mL of 1xPBS. [Note: it is important that the fluorescein is properly dissolved. To check this, after the resuspension you should pipette up and down and examine the solution in the pipette tip – if any particulates are visible in the pipette tip continue to mix the solution until they disappear.]
❏ Diluteed the 10x fluorescein stock solution with 1xPBS to make a 1x fluorescein solution with concentration 10 μM: 100 μL of 10x fluorescein stock into 900 μL 1x PBS

Prepare the serial dilutions of fluorescein:
Accurate pipetting is essential. Serial dilutions will be performed across columns 1-11. COLUMN 12
MUST CONTAIN PBS BUFFER ONLY. Initially you will setup the plate with the fluorescein stock in column 1 and an equal volume of 1xPBS in columns 2 to 12. You will perform a serial dilution by
consecutively transferring 100 μl from column to column with good mixing.

RESULT:

colony	**concentration（ng/ul）**	**A230**	**A260/A280**	**A260/A230**
P1 C3	**57.5**	**0.013**	**1.769**	**1,769**
P1 C8	**80**	**0.017**	**1.778**	**1.882**
P1 C9	**50**	**0.011**	**1.818**	**1.818**
P2 C2	**85**	**0.015**	**1.789**	**2.125**
P2 C4	**67.5**	**0.011**	**1.8**	**2.077**
P2 C5	**62.5**	**0.013**	**1.786**	**1.786**
P2 C6	**62.5**	**0.01**	**1.667**	**2.273**
P2 C9	**67.5**	**0.013**	**1.929**	**2.077**
P2 C10	**77.5**	**-0.011**	**1.722**	**NA**

Table1: the concentration of the miniprep product.

	LUDOX	H2O
REPLICATE 1	0.056	0.029
REPLICATE 2	0.056	0.03
REPLICATE 3	0.059	0.03
REPLICATE 4	0.057	0.03
ARITH. MEAN	0.057	
CORRECTED ABS600	0.027	
REFERENCE OD600	0.03	
OD600/ABS600	1.111	

Table 2: OD600 reference point tab

0.056	0.029	0.041	0.038	0.038	0.038	0.038	0.038	0.038	0.038	0.039	0.038
0.056	0.030	0.038	0.039	0.039	0.039	0.039	0.039	0.039	0.042	0.040	0.041
0.059	0.030	0.039	0.041	0.045	0.040	0.039	0.039	0.039	0.039	0.039	0.039
0.058	0.030	0.039	0.039	0.040	0.040	0.039	0.040	0.039	0.039	0.039	0.039
0.077	0.069	0.050	0.040	0.035	0.035	0.031	0.031	0.031	0.031	0.031	0.031
0.085	0.072	0.051	0.041	0.036	0.033	0.032	0.031	0.031	0.031	0.031	0.030
0.076	0.070	0.049	0.042	0.037	0.033	0.032	0.033	0.031	0.031	0.031	0.031
0.077	0.072	0.050	0.040	0.035	0.032	0.031	0.032	0.031	0.031	0.031	0.036

Table 3: absorbance at 600 nm of all samples in the measurement mode/particle standard curve tab

2386327	1466351	823801	431696	211025	126581	60245	30489	15470	7917	4368	691

2382837	1493554	837579	428990	242372	119867	58597	30045	16420	8292	4209	694
3144333	1509056	381757	444484	233120	111181	59854	31056	16105	8639	4947	671
2361109	1498347	799197	450601	244903	121437	61485	31054	15375	7702	3981	748
577	526	581	578	506	508	545	530	542	542	559	550
570	517	565	591	605	553	541	561	762	496	543	549
531	566	563	516	525	550	584	559	564	504	567	579
603	581	579	598	542	552	529	609	543	533	561	580

Table 4: Fluorescence standard curve

Name: Chiara
Date: 8/16/19

Goal:

1. Transform pcb302 into A. Tumefaciens
    a. Use DNA extracted from E. Coli transformations Plate B Colony 4

Materials:
Agrobacterium tumefaciens LBA4404

Protocol:

Electroporation of Agrobacterium tumefaciens

1. Thawed Agrobacterium tumefaciens cells on wet ice
2. Combined 1 µL of pCB302-gfp-MBD plasmid DNA and 20 µL of cells in an Eppendorf Tube
3. Pipetted the cells into a cuvette and electroporated at 2 kV
4. Added 1 mL of YM media and transferred to a 15 mL falcon tube
5. The tubes were incubateXDSQ1`1`1Q1``d at 30°C at 200 rpm for 3 hours
6. 400 µL of each culture was streaked onto a LB kanamycin plate.
7. 300 µL of cultures 1 & 3 was streaked onto a YM kanamycin plate.
8. 300 µL of culture 2 was streaked onto a LB kanamycin plate.
9. 200 µL of each culture was also streaked onto a LB kanamycin plate.
10. The plates were incubated at 30°C for 48 hours

Name: Rehmat
Date: 8/16/19
Goal: Gel extraction on Dino III GFP and Dino III RFP

Protocol:

Gel Extraction Dino III GFP

1. Dissolved 4.6 grams of agarose in 14 mL Buffer QG
2. Added 2.3 mL isopropanol
3. Added 750 µL of the solution to 12 different spin columns, centrifuged for 1 minute and discarded the flow through, repeated until all of the solution had run through
4. Added 750 µL of Buffer QG to each of the spin columns, centrifuged for 1 minute, and discarded the flow through
5. Added 750 µL Buffer PE to each of the spin columns, centrifuged for 1 minute, and discarded the flow through
6. Centrifuged the spin columns empty to remove the residual buffer.
7. Added 50 µL Buffer EB to each of the spin columns and eluted the DNA into two different clean eppendorf tubes.

Gel Extraction Dino III RFP

8. Dissolved 3.6 grams of agarose in 11 mL Buffer QG
9. Added 1.8 mL isopropanol
10. Added 750 µL of the solution to 12 different spin columns, centrifuged for 1 minute and discarded the flow through, repeated until all of the solution had run through
11. Added 750 µL of Buffer QG to each of the spin columns, centrifuged for 1 minute, and discarded the flow through
12. Added 750 µL Buffer PE to each of the spin columns, centrifuged for 1 minute, and discarded the flow through
13. Centrifuged the spin columns empty to remove the residual buffer.
14. Added 50 µL Buffer EB to each of the spin columns and eluted the DNA into two different clean eppendorf tubes.

Results

Sample	**Concentration**
Dino III RFP	20 ng/µL
Dino III RFP	17 ng/µL
Dino III RFP	15 ng/µL
Dino III GFP	25 ng/µL
Dino III GFP	56 ng/µL

Name: Rehmat
Date: 8/16/19

Goal:

1. Ethanol precipitate on gel extraction Dino III GFP samples

Protocol:

Ethanol Precipitate Dino III GFP

1. Added 30 µL of Sodium Acetate
2. Added 600 µL chilled ethanol
3. Centrifuge at 13,000 rpm for 30 minutes, removed as much ethanol as possible
4. Added 200 µL of 70% ethanol from freezer
5. Spinned that down at 13,000 rpm for 15 minutes, removed as much ethanol as possible, allowed to air dry and resuspended in 100 µL EB

Results:

Sample	**Concentration**
Dino III GFP	50 ng/µL
Dino III RFP	