Name: Rehmat, Krithika, Amirah, Jiayi Lan, Yujie Huang, Zeshi Wang, Xinyi Liu, Xuecheng Ye
Date: 8/13/19

Goal:

1. Mass miniprep of Dino III GFP and Dino III RFP 500 mL overnight cultures
2. Characterization of Test Device 1 and 5 and positive/negative controls in LB, TB, YM, YPD
3. Colony PCR on pcb302 A & B
    a. Primers: GFP fwd/Rev & Primers 3/4
4. Run gel on colony PCR
5. 250 mL Overnight Cultures of pcb302

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

Date: 8/13/2019

Goal:

1. Characterization of Test Device 1 and 5 and positive/negative controls in LB, TB, YM, YPD (Cell growth, sampling, and assay)

Protocol:

Sampling Protocol:

1. Made a 1:10 dilution of each overnight culture in LB+Chloramphenicol (0.5mL of culture into 4.5mL of LB+Chlor) (Culture medium depends what was used for the overnight culture)
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 (it all depends) medium + Chloramphenicol in 50 mL falcon tube (amber, or covered with foil to block light).
5. Took 500 µL samples of the diluted cultures at 0 hours into 1.5 ml eppendorf tubes, prior to incubation. (At each time point 0 hours and 3 hours,a sample was made from each of the 2 devices, 4 cultures, two colonies per device, with 500 µL samples per time point, 30 samples total). Placed the samples on ice.
6. Incubated the remainder of the cultures at 37°C and 220 rpm for 3 hours.
7. .Took 500 µL samples of the cultures at 3 hours of incubation into 1.5 ml eppendorf tubes.Placed samples on ice.
8. At the end of sampling point, measured the samples (Abs $_{600}$ and fluorescence measurement), see the below for details.
9. Recorded data in your notebook
10. Imported data into Excel sheet provided (**fluorescence measurement tab**)

Cell measurement protocol:

1. Samples were laid out according to the plate diagram below.
2. Pipetted 100 µl of each sample into each well.
3. From 500 µl samples in a 1.5 ml eppendorf tube,
    a. 4 replicate samples of colony #1 were pipetted into wells in rows A, B, C and D.
    b. Replicate samples of colony #2 were pipetted into wells in rows E, F, G and H.
    c. Made sure to include 8 control wells containing 100uL each of only

LB+chloramphenicol on each plate in column 9, as shown in the diagram below.

4. Set the instrument settings to those that gave the best results in the calibration curves (no measurements off scale).
    a. If necessary, more than one of the previously calibrated settings can be tested to get the best data (no measurements off scale). Instrument temperature should have been set to room temperature (approximately 20-25 C) if your instrument has variable temperature settings.

Results:

0 hr Time point

OD 600

1. LB medium

OD 600 (LB medium)	1	2	3	4	5	6	7	8	9	10	11	12
Positive control, Colony1	0.074	0.067	0.067	0.076	0.070	0.068	0.071	0.068	0.075	0.069	0.075	0.067
Negative control, Colony1	0.076	0.080	0.078	0.075	0.070	0.069	0.074	0.072	0.070	0.067	0.069	0.069
Positive control, Colony2	0.079	0.075	0.073	0.068	0.070	0.069	0.070	0.073	0.070	0.066	0.070	0.065
Negative control, Colony2	0.078	0.073	0.072	0.069	0.067	0.071	0.067	0.069	0.072	0.069	0.066	0.072
Devuce1, Colony1	0.081	0.073	0.071	0.068	0.070	0.072	0.070	0.066	0.068	0.074	0.068	0.068
Device 5, Colony1	0.078	0.075	0.072	0.069	0.066	0.069	0.072	0.079	0.070	0.074	0.069	0.075
blank	0.038	0.039	0.040	0.043	0.040	0.039	0.039	0.041	0.040	0.043	0.044	0.039
blank	0.038	0.039	0.039	0.040	0.039	0.040	0.039	0.039	0.039	0.040	0.040	0.040

2. TB medium

OD 600 (TB medium)	1	2	3	4	5	6	7	8	9	10	11	12
Positive control, Colony1	0.063	0.067	0.053	0.055	0.055	0.052	0.051	0.053	0.049	0.054	0.049	0.048
Negative control, Colony1	0.061	0.058	0.056	0.053	0.056	0.052	0.054	0.053	0.055	0.048	0.052	0.053
Positive control, Colony2	0.064	0.057	0.057	0.057	0.056	0.053	0.053	0.056	0.058	0.052	0.054	0.051
Negative control, Colony2	0.066	0.060	0.057	0.060	0.056	0.055	0.055	0.053	0.057	0.055	0.052	0.054
Devuce1, Colony1	0.074	0.058	0.064	0.060	0.059	0.060	0.058	0.058	0.056	0.057	0.063	0.057
Devuce1, Colony2	0.063	0.057	0.061	0.060	0.060	0.058	0.059	0.058	0.059	0.059	0.058	0.060
Device 5, Colony1	0.058	0.058	0.057	0.064	0.064	0.058	0.062	0.056	0.057	0.106	0.062	0.053
Device 5, Colony2	0.058	0.064	0.065	0.064	0.064	0.059	0.061	0.079	0.058	0.085	0.052	0.046

3. YM medium

OD 600 (YM medium)	1	2	3	4	5	6	7	8	9	10	11	12
Positive control, Colony1	0.047	0.047	0.041	0.044	0.042	0.042	0.043	0.046	0.045	0.043	0.052	0.044
Negative control, Colony1	0.051	0.040	0.064	0.066	0.042	0.048	0.042	0.040	0.045	0.060	0.048	0.048
Positive control, Colony2	0.053	0.048	0.046	0.049	0.046	0.046	0.045	0.046	0.045	0.048	0.044	0.054
Negative control, Colony2	0.058	0.048	0.050	0.049	0.044	0.048	0.044	0.045	0.051	0.042	0.042	0.047
Devuce1, Colony1	0.057	0.063	0.045	0.056	0.048	0.049	0.047	0.050	0.045	0.049	0.044	0.048
Devuce1, Colony2	0.043	0.076	0.051	0.055	0.048	0.048	0.050	0.047	0.046	0.051	0.046	0.049
Device 5, Colony1	0.065	0.036	0.065	0.047	0.048	0.052	0.048	0.049	0.045	0.048	0.045	0.049
Device 5, Colony2	0.053	0.048	0.051	0.045	0.052	0.049	0.048	0.050	0.050	0.048	0.046	0.055

4. YPD medium

OD 600 (YPD medium)	1	2	3	4	5	6	7	8	9	10	11	12
Positive control, Colony1	0.056	0.056	0.054	0.053	0.051	0.053	0.052	0.051	0.051	0.053	0.052	0.086
Negative control, Colony1	0.056	0.060	0.057	0.054	0.061	0.052	0.054	0.053	0.054	0.054	0.055	0.054
Positive control, Colony2	0.058	0.059	0.054	0.055	0.057	0.050	0.056	0.068	0.051	0.055	0.056	0.071
Negative control, Colony2	0.072	0.067	0.060	0.058	0.054	0.055	0.054	0.055	0.053	0.057	0.054	0.055
Devuce1, Colony1	0.058	0.059	0.057	0.059	0.058	0.057	0.054	0.056	0.054	0.056	0.055	0.059
Devuce1, Colony2	0.066	0.063	0.058	0.057	0.057	0.056	0.065	0.057	0.054	0.056	0.054	0.058
Device 5, Colony1	0.059	0.062	0.057	0.058	0.098	0.060	0.060	0.103	0.054	0.054	0.053	0.056
Device 5, Colony2	0.059	0.060	0.056	0.058	0.059	0.061	0.056	0.062	0.056	0.058	0.055	0.051

Fluorescence

1. LB medium

eGFP Fluoresein (LB medium)	1	2	3	4	5	6	7	8	9	10	11	12
Positive control, Colony1	17076	15609	16450	18331	18670	18248	18194	17440	20183	17634	17892	17185
Negative control, Colony1	16498	17985	19734	19905	18614	18557	19359	18613	17697	16946	17521	17020
Positive control, Colony2	17366	17772	17217	16802	17443	17851	18776	18366	18738	16683	17069	15791
Negative control, Colony2	16927	17248	16881	16699	17207	18614	17015	17801	18624	16880	16065	17619
Devuce1, Colony1	24010	19478	17789	17390	17466	18347	17658	16480	17108	18768	17269	16097
Devuce5, Colony1	16507	17689	17081	16535	15790	17442	16931	19768	17903	17905	17191	18507
blank	539	529	568	531	583	561	572	565	579	513	559	540
blank	540	521	577	579	539	543	602	544	528	525	518	537

2. TB medium

eGFP Fluoresein (TB medium)	1	2	3	4	5	6	7	8	9	10	11	12
Positive control, Colony1	21461	23227	21154	24376	24763	22015	21069	19699	19165	19561	17771	17341
Negative control, Colony1	19556	21642	20084	20329	22248	20666	22553	21852	22739	17612	21483	19967
Positive control, Colony2	20053	20939	20659	20477	21979	21873	22416	22795	27590	20339	21953	18269
Negative control, Colony2	21910	21633	21543	22594	22766	22483	24025	21966	23181	22181	18828	21026
Devuce1, Colony1	24622	20801	24614	24629	23515	25783	24858	25378	23296	23556	29019	23873
Devuce1, Colony2	22786	21303	24837	24759	25871	26174	24415	24457	25540	25496	24763	24217
Devuce5, Colony1	16957	19438	21125	26026	28678	24609	24851	23968	24989	50077	27832	19279
Devuce5, Colony2	21119	19904	26654	28020	25711	24351	25468	31633	22893	36292	18108	10449

3. YM medium

eGFP Fluoresein (YM medium)	1	2	3	4	5	6	7	8	9	10	11	12
Positive control, Colony1	5163	5371	3933	5851	5629	5884	6106	5931	5872	5537	7448	5970
Negative control, Colony1	5576	3063	8951	9989	4764	7260	4125	2807	5758	9379	5593	5862
Positive control, Colony2	5975	5434	5670	5504	5821	6277	5878	5868	5634	6052	5553	5361
Negative control, Colony2	6263	5026	5369	5885	5116	6277	5538	6057	7586	3862	3737	5978
Devuce1, Colony1	5989	6727	4582	7471	5374	4803	5603	6142	5671	6177	5052	6066
Devuce1, Colony2	3637	12903	7194	7531	6405	5917	6579	5638	5507	6414	5878	5964
Devuce5, Colony1	9635	782	8854	5928	6364	7086	5581	6170	5961	6009	5771	5918
Devuce5, Colony2	6614	5586	6869	5140	6211	5731	6054	5935	6643	5292	5652	6565

4. YPD medium

eGFP Fluoresein (YPD medium)	1	2	3	4	5	6	7	8	9	10	11	12
Positive control, Colony1	15025	16352	16827	17070	16718	17202	17252	16769	16966	16721	16213	34133
Negative control, Colony1	16406	17178	17261	16782	18340	16476	16781	16369	17140	17167	16077	15419
Positive control, Colony2	17848	17050	15440	17359	18578	14744	17914	26380	16260	16998	16923	24495
Negative control, Colony2	18118	20895	17818	17480	16358	17284	17604	18593	16799	17218	16854	16903
Devuce1, Colony1	19142	16879	16931	18579	18311	17330	17029	17762	17128	17673	17268	17230
Devuce1, Colony2	20521	18947	18514	17011	17110	16984	17021	17874	17544	16928	17149	17741
Devuce5, Colony1	17511	18144	17070	17900	34016	18488	17605	34429	18050	16894	16449	17505
Devuce5, Colony2	21887	19529	17939	18141	16581	17457	16794	17741	17285	16674	17222	12951

3 hr Time point

OD 600

OD 600	positive, Colony1	positive, Colony2	negative, Colony3	negative, Colony4	Device1, Colony1	Device1, Colony2	Device5, Colony1	Device5, Colony2
LB medium	0.247	0.254	0.254	0.247	0.253	0.036	0.283	0.038
TB medium	0.249	0.216	0.226	0.236	0.191	0.170	0.239	0.207
YM medium	0.150	0.141	0.141	0.130	0.212	0.175	0.166	0.172
YPD medium	0.250	0.252	0.246	0.249	0.264	0.270	0.251	0.289
each of fresh medium	0.145	0.118	0.069	0.094	0.037	0.039	0.038	0.039
blank	0.039	0.039	0.040	0.040	0.037	0.035	0.037	0.038
blank	0.038	0.038	0.036	0.038	0.039	0.035	0.038	0.038
blank	0.038	0.038	0.039	0.038	0.039	0.036	0.038	0.039

Fluorescence

eGFP Fluoresein	positive, Colony1	positive, Colony2	negative, Colony3	negative, Colony4	Device1, Colony1	Device1, Colony2	Device5, Colony1	Device5, Colony2
LB medium	41567	42413	34058	34120	89600	592	34766	559
TB medium	50049	50572	45862	46224	82389	75144	46895	82022
YM medium	13637	13544	12397	11980	32497	30520	12383	39968
YPD medium	36307	36846	35485	35981	61164	60323	36131	65939
each of fresh medium	36866	49712	12975	37257	538	540	546	485
blank	575	570	574	607	550	571	545	541
blank	562	534	556	579	581	597	584	536
blank	535	559	526	564	550	554	569	619

Name: Krithika
Date: 8/13/2019

Goal:

1. Colony PCR on pcb302 from 8/12/19 transformations
    a. Papers A and B

Protocol:

Colony PCR

1. Two PCR cocktails were created. The “GFP cocktail” had the following components: 28µL diH2O, 40µL PCR MasterMix, 4µL GFP fwd primer, and 4µL GFP rev primer. And the “3,4 Primer cocktail” had: 28µL diH2O, 40µL PCR MasterMix, 4µL Primer 3, and 4µL Primer 4.
2. 19µL of the GFP cocktail was added to tubes: A1, A2, B1, B2
3. 19µL of the 3,4 Primer cocktail was added to tubes: A3, A4, B3, B4
4. Each colony (4 on plate A, 4 on plate B) was picked and added to separate tubes containing 100µL diH2O.
    a. This e. Coli suspended in H2O was frozen, thawed, frozen, and thawed again to help break open cells and give a cleaner PCR product
5. 2µL of the colony H2O was added to its corresponding PCR tube
6. Placed PCR tube in the thermocycler at the following settings:
    a. 95° C for 3:00 minutes
    b. 95° C for 0:30 minute
    c. 50° C for 0:30 minute
    d. 72° C for 1:00 minute
    e. 30X (Go to Step 2)
    f. 72° C for 5:00 minutes
    g. 4° C for ∞
    h. Lid Temperature: 105° C

Name: Amirah
Date: 8/13/2019

Goal:

1. Gel of pcb302 colony PCR products

Protocol:

Preparing

1. Added 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
2. Heated in the microwave until fully dissolved
3. Allowed the solution to cool until comfortable to touch
4. Added 10 µL GelRed Nucleic Acid Gel Stain and mixed
5. Inserted casting tray, made sure the rubber on the sides was not overlapping
6. Carefully poured the agarose into the tray and placed the comb to create the wells
7. Allowed the gel to solidify
8. Once solidified, changed the orientation of casting tray where the rubber sides were not in contact with the sides of the system.
9. Poured in 1X TBE into the gel electrophoresis system to the fill line, made sure to submerge the gel, and removed the comb

Loading

1. Loaded ~5 µL of the ladder in the first well
2. Prepared samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and loaded

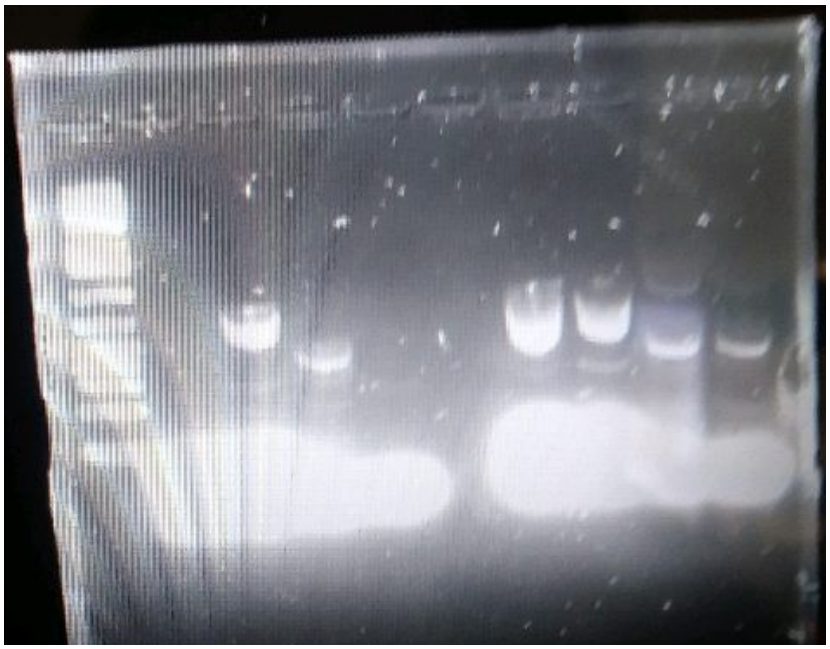
Running

1. Once the gel had been loaded, slid on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Ran for about 45 minutes to an hour

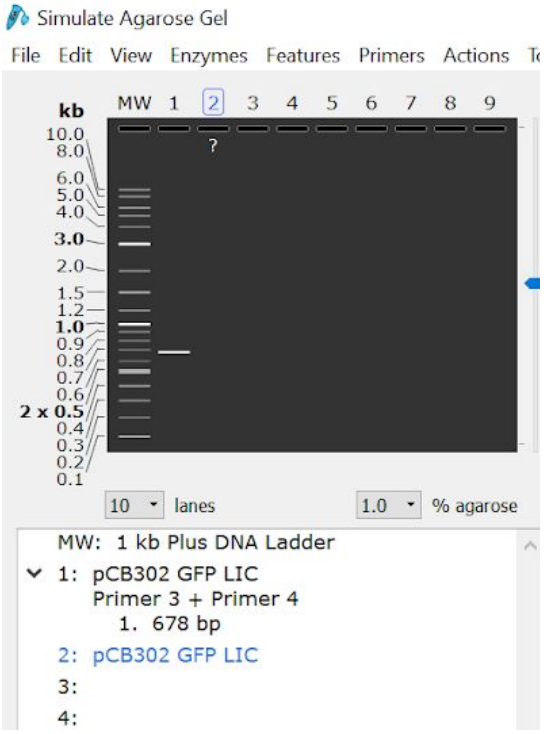
GEL LEGEND

Lane #	1	2	3	4	5	6	7	8
PCR product	A1	A2	A3	A4	B1	B2	B3	B4

Results



Expected Results:



Conclusion

It looks like the pcb302 got amplified and existed in at least some of the colonies

Name: Amirah
Date: 8/12/2019

Goal: 250mL Overnight Cultures of pcb302

Protocol:

Overnight Cultures

1. 250mL of LB and 250µL of Kanamycin antibiotic was added in an erlenmeyer flask
2. A p10 tip was dipped into the colony and dropped into the flask
3. The flask was placed in the back shaker at 37° C at 220 rpm for 16-18 hours
4. Overnight cultures were made for A2-3 and B2-4 because they looked the best on the gel

Name: Amirah
Date: 8/13/2019

Goal: Transformation of mcherry

Protocol:

1. Both electrical and chemical transformations were done
    a. electrical - Rosetta
        i. one pcb302 and one pUC19 control
        ii. both of the electroporations arced
    b. chemical - Oneshot
        i. Two pcb302 and one pUC19 control

Electroporation

1. Combined 40 µL of electrically competent DH5a cells and 1 µL of ligated DNA to an Eppendorf tube.
2. Transferred the contents of the Eppendorf tube to a cuvette and lightly tapped the cuvette on the table to evenly distribute the contents and to get rid of air bubbles.
3. Placed the cuvette into the Bio-Rad MicroPulser and delivered the electric shock.
4. **Immediately after**, added 900 µL SOC medium to the cuvette and micropipette mixed the solution.
5. Transferred the solution from the cuvette to a shaker tube and placed in the shaker at 37°C at 200 rpm for 1 hour.
6. After shaking for 1 hour, streaked 150 µL of the solution onto an agar plate with the respective antibiotics **(ampicillin)**.
7. Incubated plates at 37°C for at least 24 hours.

Heat Shock

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 2 µL of DNA sample into competent cells
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** added 250µL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, streaked 150 µL of the solution onto an agar plate with the respective antibiotics **(ampicillin)**.
8. Incubated plates at 37°C for at least 24 hours.

Name: Amirah
Date: 8/13/2019
Goal: Make LB + Amp plates

Protocol:

1. 1L diH2O (in 2L flask)
2. 40 g LB agar
3. Mixed on spin plate
4. Autoclaved for 45 mins
5. Allowed to cool to touch
6. Added 1ml ampicillin
7. Mixed on spin plate
8. Poured plates under sterile conditions
9. Plates placed in 4 ◦ C

Name: Rehmat
Date: 8/13/2019

Goal: Extract plasmids from the 500 mL cultures of Dino III with GFP and 500 mL cultures of Dino III with RFP

Protocol:

Mini Prep of 500 mL cultures

1. Pelleted the cultures in 50 mL falcon tubes at 5000 rpm for 5 minutes
2. The pelleted cells were resuspended in 2.5 mL of P1 and mixed by vortexing
3. 400 µL of this solution was placed in an Eppendorf tube
4. 400 µL of P2 was added to the tubes and the tubes were inverted about 5 times
5. 600 µL of N3 was added and the tubes were immediately inverted about 5 times
6. Centrifuged at 13,000 rpm for 10 minutes
7. The clear supernatant was carefully removed and added to a spin column until full, centrifuged for 1 minute at 13,000 rpm, and removed the flow through.
8. This was repeated for each spin column three times until all of the clear supernatant had been collected
9. Added 500 µL of PB and centrifuged the spin columns for 60 seconds. Discarded the flow through
10. Added 750 µL of PE to the spin columns, centrifuged for 60 seconds, and discarded the flow through
11. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discarded the flow through
12. 50 µL of EB buffer was added to each of the spin columns and eluted into an Eppendorf tube, centrifuged for 1 minute at 13,000 rpm.
13. The samples were combined so there were 750 µL in two tubes each.

Results

Sample	**Concentration**
Dino III RFP 1	180 ng/µL
Dino III RFP 2	170 ng/µL
Dino III GFP 1	260 ng/µL
Dino III GFP 2	248 ng/µL

Conclusion

We got great concentrations and were able to get about 270 µg of the Dino III with RFP DNA and 375 µg of Dino III with GFP DNA. The next step is to linearize the DNA and gel extract.