InterLab Protocol

OD_{600} Reference Point

1. Add 100µl LUDOX into 96 well plate.
2. Add 100µl of H_2O into 96 well plate.
3. Measure absorbance 600nm of all samples in all standard measurement modes in instrument.
4. Import data into Excel (OD_{600} reference point tab from Sheet_1, provided by iGEM InterLab study).

Protocol FITC fluorescence standard curve

1. Spin down FITC stock tube to make sure pellet is at the bottom of tube.
2. Prepare 2x FITC stock solution (500µM) by resuspending FITC in 1mL of 1xPBS.
3. Incubate the solution at 42°C for 4 hours.
4. Dilute the 2x FITC stock solution in half with 1xPBS to make a 1x FITC solution and resulting concentration of FITC stock solution 250µM.
5. Prepare the serial dilutions of FITC with 1xPBS, measure fluorescence in all standard measurement modes.
6. Import data into Excel (FITC standard curve from Sheet_1, provided by iGEM InterLab study).

Transformation

1. Thaw 5 tubes of competent cell DH5α on ice. Transfer 1 µl of each plasmids (Positive Control, Negative Control, Test Device 1, Test Device 2, and Test Device 3) into 50µl of competent cell. Stay on ice for 30 min.
2. Heat shock tubes at 42°C for 1 min, immediately put tubes on ice for 3 min. Add 1ml of LB medium into the transformation for recover the cell, incubate at 37 °C for 1 hr with shaking.
3. After incubation, centrifuge for 1 min at 12,000g . Then discard 800µl LB medium, mix the solution by pipetting.
4. Pipette 40µl of the transformation onto appropriately labeled plates, and spread with glass beads. Incubate overnight (14-16hr) at 37 °C, put the plate upside down (let the agar on the bottom).
5. The second day: pick colonies for PCR or inoculate.

Inoculation

1. Set up the medium: pipette 20ml LB broth and 20µl Chloramphenicol in to 50ml falcon tube, mix well by vortex or shaking.
2. Separate medium to 4ml per tube.
3. Pick 4 colonies from both Device 1, Device 2 and Device 3 plates, pick 3 colonies from both Positive Control and Negative Control.
4. Grow the cells overnight (14-16 hr) at 37°C and 220 rpm.

Plasmid Purification

By using "High-Speed Plasmid Mini Kit" from Geneaid company from plasmids purification, so following the protocol provide from the kit.

1. Transfer 1.5ml cultured bacteria cell to microcentrifuge tube, and centrifuge for 30sec at 14-16,000g then discard the supernatant. Repeat this step twice.
2. Add 200µl of PD1 buffer (RNase was added) to the tube, re-suspend the cell pellet by vortex.
3. Add 200µl of PD2 buffer to the tube, mix gently by inverting the tube 10 times (do not vortex!). Stand at room temperature for at least 2min(do not exceed 5min).
4. Add 300µl of PD3 buffer and mix immediately by inverting the tube 10 times (do not vortex!), then centrifuge at 14-16,000g for 3min.
5. Prepare the collection tube, put the PD Column in a 2ml Collection Tube.
6. Transfer all the supernatant to the PD Column. Centrifuge at 14-16,000g for 30sec then discard the flow-through. Put the PD Column back in collection tube.
7. Add 600µl of Wash buffer(ethanol was added) into PD Column. Centrifuge at 14-16,000g for 30sec then discard the flow-through. Put the PD Column back in collection tube. Then centrifuge at 14-16,000g for 3min to dry the column matrix. Place the PD Column in a new 1.5ml microcentrifuge tube.
8. Add 30µl of Elution buffer to the PD column matrix, and stand for at least 2min in room temperature. Centrifuge at 14-16,000g for 2min.

PCR

1. Set up the PCR reaction:

Component	Volume (µl, per tube)
Taq 2X Master Mix Red	10
Forward primer	1
Reverse primer	1
Template plasmid	2
ddH_2O	6
Total	20

2. Set up the PCR machine by following setting:

Step		Temperature (°C)	Time
Initial Denaturation		95	10min
30 cycles	Denaturation	95	30sec
	Annealing	56	30sec
	Extension	72	1min
Final Extension		72	10min

3. Start the PCR cycle.

Cell measurement

The experiment is followed "InterLab Plate Reader Protocol 2016" from iGEM 2016.

1. Set up instrument to read OD600 (as OD calibration setting). Measure OD600 of the overnight cultures.
2. Import data into Excel (normalization tab from Sheet_1, provided by iGEM InterLab study).
3. Dilute the cultures to a target OD600 of 0.02 (see the volume of preloading culture and media in Excel, normalization tab from Sheet_1) in 10 ml LB borth + Chloramphenicol in 50 mL falcon tube.
4. Incubate the cultures at 37°C and 220 rpm.
5. Take 100µL (1% of total volume) samples of the cultures at 0, 1, 2, 3, 4, 5, and 6hr of incubation. Place samples on ice.
6. At the end of sampling point, measure all the samples (OD and Fl measurement).
7. Import data into Excel (cell measurement tab from Sheet_1).