Cpx Dimerization

Measuring of eYFP Fluorescence

1. From a glycerol stock, streak some bacteria on a LB Agar plate (+ antibiotics, if applicable) and incubate overnight 37ºC.
2. Pick a single colony and grow it in 2mL LB (+ antibiotics) overnight at 37ºC, 250rpm.
3. Inoculate a 5mL culture with 100µL from the preculture and grow overnight at 37ºC, 250rpm. Induce the cells with 0.2% arabinose 3 hours after inoculation.
4. The next day, spin down the cells for 5 minutes at 4,700x g and resuspend in 1mL PBS.
5. Leave the cells at 4ºC for 3 hours to allow for fluorophore maturation.
6. Spin down the cells at 4,700x g for 5 minutes and resuspend again in 1mL PBS.
7. Take 100µL per sample and transfer to a 96-wells plate (flat, black bottom).
8. Measure fluorescence using the SynergyMx plate reader using the following settings:
    a. Shake plate for 10 seconds
    b. Measure OD_{600}
    c. Measure fluorescence (ex: 513/9.0nm, em:532/9.0nm)

Cpx Dimerization, BifC measuring

1. Streak some bacteria from the glycerol stock on a LB Agar plate (+ antibiotics, if applicable) and grow overnight at 37ºC.
2. Inoculate a 3mL saltless LB starter culture with a single colony and grow overnight at 37ºC, 250rpm.
3. Inoculate a 25mL saltless LB culture with 100µL of the starter culture. Use a 250mL Erlenmeyer and incubate at 37ºC, 180rpm until an OD_{600} of ~0.6 has been reached.
4. Transfer cells to 3mL of fresh, saltless LB until an OD_{600} of ~0.2 has been reached. Subsequently induce them with 0.2% L-arabinose.
5. Transfer 100µL samples, in triplo, to a black bottom 96-wells plate and measure fluorescence using SynergyMx plate reader using the following settings:
    a. Set temperature at 30ºC, use continuous shaking and measure initial fluorescence (ex: 513/9.0nm, em:532/9.0nm) and cell density. Use a re-read interval of 10 minutes.
    b. After 20 minutes, activate the Cpx pathway by adding KCl (25mM to 100mM final concentration). Measure OD_{600} and fluorescence as before. Use a re-read interval of 15 minutes, in a timespan of 6 hours.

Cpx Dimerization after IPTG Induction

1. Make liquid pre-cultures by inoculating 5mL M9 (+antibiotics) in 15mL Greiner tubes from either a glycerol stock or directly from a transformant colony. Grow overnight at 37 °C, shaking at 180 rpm.
2. Add 100µL into a Greiner tube, containing a fresh 5mL of M9 (+antibiotics). Grow this culture for 5 hours at 37°C, shaking at 180 rpm.
3. Induce the culture with IPTG (0.2mM final concentration) and incubate overnight at 37°C, shaking at 180 rpm.
4. Spin down the culture at 4,700rpm for 5 minutes. Discard the supernatant and resuspend the pellet in 1mL PBS.
5. Store for 2 hours at 4°C and spin the cell suspension down at 4,700rpm for 5 minutes afterwards.
6. Discard the supernatant and wash the cells by resuspending in 1mL PBS again.
7. Transfer 100µL samples to a black bottom 96-wells plate and measure fluorescence using SynergyMx plate reader using the following settings:
    a. Set temperature at 27ºC, use continuous shaking and measure initial fluorescence (ex: 513/9.0nm, em:532/9.0nm) and cell density.

Cpx Dimerization after Spheroplasting

1. Inoculate 10mL LB (+antibiotics) in 50mL Greiner tubes with either a glycerol stock or a transformant colony and incubate overnight at 37°C, 180 rpm.
2. Add 100µL of the overnight culture to a 50mL Greiner tube, containing 10mL fresh LB (+antibiotics) and grow at 37°C, 180 rpm until an OD_{600} of 0.5-0.7 has been reached.
3. Transfer 10µL of this culture to 90µL fresh LB (NO antibiotics) in a black, clear-bottomed 96-wells plate.
4. Grow for 1.5 hours at 37°C, 180 rpm and induce the system by adding IPTG to a 0.02mM final concentration. Incubate for another hour under the same conditions.
5. Harvest the cells by centrifuging at 4,700rpm for 5 minutes.
6. Start the spheroplasting procedure by adding the following components (in this order):
    - Resuspend pellet in 10µL 0.8M Sucrose.
    - Add 6µL 0.1M TRIS-HCl pH8.
    - Add 4.8µL 50 mg/mL lysozyme.
    - Add 1.2µL 0.5mg/mL DNase.
    - Add 1.2µL 12.5mM EDTA-NaOH pH8
    - Incubate for 20 minutes at room temperature.
    - Finish by adding 2µL spheroplasting STOP solution.
7. Spin the culture down at 3000x g for 3 minutes and add 100µL LB to the cell pellet.
8. Measure the RFP fluorescence in the plate reader.
9. Grow for an additional hour in the plate reader at 37°C, shaking at 180rpm, while measuring the OD_{600} and RFP fluorescence every 5 minutes.