GFP measuring

Measure the GFP and mCherry signal before and after lysis.

Estimated bench time: 30 min

Estimated total time: 4 hours

MATERIALS

- Large cultures
- Photospectrometer
- ddH2O
- Black 3 mm cuvettes
- Centrifuge
- Falcon tubes
- BugBuster
- Benzoase
- Shaking rack
- High speed centrifuge

SETUP & PROTOCOL

1. Put 1.5 mL of each culture into an Eppendorf tube and divide the leftover over falcon tubes.
2. Centrifuge the falcon tubes for 15 min at 8.000 x rpm at room temperature.
3. Discard the supernatant and dissolve pellets in the least amount of PBS buffer (~5 mL).
4. Centrifuge for 15 min at 8.000 x rpm in a table centrifuge at room temperature and discard the supernatant.
5. Add approx. 10mL BugBuster and 10µL of Benzoase per 1L culture.
6. Resuspend the pellet well, by pipetting up and down, and transfer the mixture into a clean Eppendorf tube.
7. Incubate for 20-30 min in a shaking rack at room temperature.
8. Centrifuge at 40.000 x g for 30-40 min in a high-speed centrifuge.
9. Take a picture of the results and transfer the supernatant to a clean Eppendorf tube.
10. Measure the concentrations of GFP and mCherry before and after lysis on the photospectrometer.
    a. Put 70 µL blanco or sample in a black 3 mm cuvette.
    b. Measure the sample with the following settings: GFP: ex 485, em start 495, em end 530, ex slt 5, em slit 5, mCherry: ex587, em start 590, em end 650, ex slit 5, em slit 10. Voltage on medium
    c. Clean the cuvettes with water and ethanol and dry them using air pressure.
11. Save the graphs