Confocal Fluorescence Imaging of sfGFP in *E. coli*

Materials

Reagent

Recombinant *E. coli* strain expressing sfGFP
LB medium
Ampicillin, 100 mg/mL stock solution
IPTG, 1 M stock solution
Phosphate-buffered saline (PBS), pH 7.4
Microscope slides
Coverslips

Equipment

Confocal laser scanning microscope equipped with a 488 nm laser and a high-numerical-aperture 63× or 100× oil-immersion objective; immersion oil, as required

Procedure

Cell Culture and IPTG Induction

1. Pick a single colony from a fresh LB agar plate containing 100 μg/mL ampicillin and inoculate it into 5 mL LB medium supplemented with ampicillin to a final concentration of 100 μg/mL.
2. Incubate at 37 °C and 200 rpm for 12–16 h.
3. Inoculate 150 μL of the overnight culture into each of two 15 mL LB cultures containing ampicillin, designated as the induced and non-induced groups.
4. Incubate both cultures at 37 °C and 200 rpm until the OD_{600} reaches approximately 0.4.
5. Add IPTG to the induced group to a final concentration of 0.4 mM.
6. Continue incubation at 37 °C and 200 rpm for 4 h.
7. Measure and record the final OD_{600} of each culture.
8. Collect a volume of each culture corresponding to 1 A_{600} unit. Calculate the required sampling volume using the following equation:

$$\text{Sampling volume (mL)} = \frac{1}{OD_{600}}$$

Cell Harvesting and Slide Preparation

1. Centrifuge the collected cells at 4,000 × g for 2 min and discard the supernatant.
2. Add 1 mL PBS and gently resuspend the cell pellet.
3. Centrifuge at 4,000 × g for 2 min and discard the PBS.
4. Resuspend the cell pellet in 50–100 μL PBS.
5. Place 5–10 μL of the cell suspension onto a microscope slide and gently cover with a coverslip.
6. Observe the cells as soon as possible, preferably within 15–30 min after sample preparation.

Confocal Laser Scanning Microscopy

1. Turn on the confocal microscope and image-acquisition software according to the instrument operating instructions. Place the freshly prepared slide on the microscope stage.
2. Locate bacterial cells using transmitted-light (bright-field) imaging at low magnification. Switch to a high-numerical-aperture 100× oil-immersion objective, if available; a 63× oil-immersion objective is also suitable for imaging GFP distribution in *E. coli*.
3. Select the GFP/FITC fluorescence channel for sfGFP detection. Use the 488 nm laser for excitation and set the fluorescence detection window to approximately 500–550 nm, or select the nearest available GFP detection preset.
4. Focus on the mid-plane of individual bacterial cells and set the confocal pinhole to approximately 1 Airy unit, if adjustable.
5. Begin with low laser power and adjust detector gain and other acquisition settings until cellular fluorescence is clearly visible without pixel saturation. Minimize photobleaching during focusing and scanning.
6. Acquire sfGFP fluorescence images and corresponding transmitted-light images of the same field of view. Choose a scan format and pixel size that provide adequate sampling of individual bacterial cells.
7. Image both IPTG-induced and non-induced controls. For comparisons across constructs or conditions, keep the objective, laser power, detector settings, pinhole and image-processing parameters consistent whenever feasible.
8. Capture multiple representative, non-overlapping fields of view for each sample. Save the original image files and record the microscope model, objective magnification/numerical aperture, excitation and emission settings, and acquisition parameters.
9. Examine the cellular fluorescence pattern (e.g., peripheral enrichment versus diffuse cytoplasmic fluorescence). Interpret peripheral fluorescence as consistent with membrane/periplasm-associated targeting rather than as definitive proof of periplasmic localization.