Santander Lab
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PROTOCOL TO LABEL BACTERIA WITH 5-([4,6-DICHLOROTRIAZIN-2-YL]AMINO) FLUORESCEIN HYDROCHLORIDE (5-DTAF)

- Grow bacteria o/n.
- Centrifuge (6000-8000 RPM, 10') to concentrate bacteria.
- Resuspend in 500 µL PBS (1X).
- Take 100 µL and to dilute in 900 µL bicarbonate buffer (0.009 M Na_2CO_3 ;
- Take 950 µL and add 50 µL of DTAF solution (100 µg).
- Stain 2 hours with shaking in darkness.
- Wash 3 times in Bicarbonate buffer:
    i. centrifuge 5000 RPM, 5'
    ii. discard s/n
    iii. Add 1 mL of bicarbonate buffer
- After the last wash, resuspend the pellet in PBS (1X).
- Make serial dilutions to plate and count UFC.
- Infect.

Solutions:

Sodium bicarbonate buffer pH 9 (200 mL) Cold Spring Harb Protocols, 2011.

- 1. Prepare a 0.2-M solution of anhydrous sodium carbonate (2.2 g/100 mL).
- 2. Prepare a 0.2-M solution of sodium bicarbonate (Na_2CO) (1.68 g/100 mL).
- 3. Combine 4 mL of carbonate solution from Step 1 and 46 mL of bicarbonate solution from Step 2.
- 4. Bring to 200 mL with H_2O.

DTAF

- 2,5 µL DTAF (50 mg/ml in DMSO)
- 47,5 µL buffer bicarbonate