Whole Bacteria Preparation for Label-Free MST

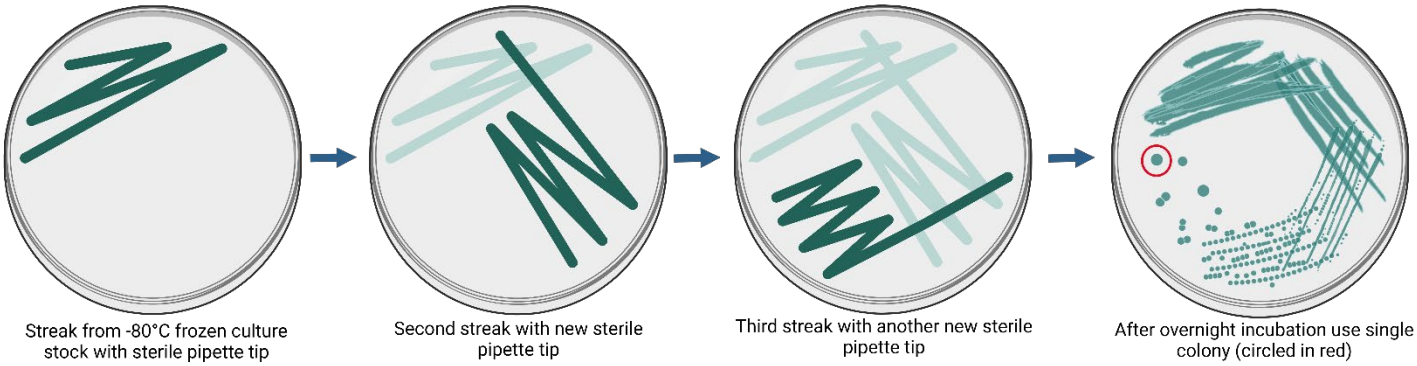
Materials:

- LB (sterile)
- 14 ml bacterial culture tubes with lids
- Falcon tubes 15 ml
- multichannel pipette & pipette tips
- DPBS -$CaCl_2$, -$MgCl_2$ (Gibco Cat. No. 14190-094)
- F-127 buffer (50 mM Tris-HCl (pH= 7.4), 150 mM NaCl, 10 mM $MgCl_2$, 0.2% Pluronic F-127)
- ROTI®Histofix (4% Formaldehyde, ready-to-use, phosphatgepuffert, pH= 7) (Carl Roth GmbH (Art.No.: P087.6))
- *E. coli* ORN 178
- Eppendorf Bio Photometer
- Plastic cuvettes 1 ml

Work under sterile conditions

Method:

Day 1 → Streak out (single-colony method) bacterial strain (*E. coli* ORN178) on LB Agar plate as shown below and allow to incubate overnight (ON) at 37°C.



Day 2 → Prepare bacterial culture by inoculating 5-7mL LB with single colony from plate. Prepare negative control of only LB (3mL). Grow shaking 180-250 rpm at 37°C and allow to grow to mid-exponential phase (OD_{600} 0.5) (approx. 3 hours, measuring OD every 30 min after 2 hours mark).

Centrifuge at 3000 xg for 5 min. Discard supernatant and resuspend by vortexing in 5mL DPBS. Centrifuge at 3000 xg for 5 min. Discard supernatant and resuspend by vortexing in 5mL Histofix. Allow to react at room temperature shaking conditions for 60 min. Centrifuge at 2000 xg for 5 min. Discard supernatant and resuspend by vortexing in 5mL DPBS. Centrifuge at 3000 xg for 5 min. Discard supernatant and resuspend by vortexing in 3mL F-127 buffer. Adjust OD_{600} to 0.1 (Equivalent to 1nM) and handle on ice, store at 4°C. Can be used for up to 5 days.