Stab Agar Protocols: how to store strains for shipment

Creating Stab Agar

A stab Agar should have 0.6% agar, using our premixed powders add 14.4g of LB-Agar and 10.8g LB Medium powders to 900mL of milliQ water. Stir mix with a fish-magnet and immediately autoclave. Alternatively, can create stab agar by mixing LB-agar from powder mix which contains 15g agar with LB medium from powder mix to get a final concentration of 6g per liter. Example: for 1200µL stab agar (0.6% agar) in the cryotube, 720µL LB-agar standard mixed with 480µL LB.

Fill cryotube 2/3 full (1200µL) with 0.6% agar LB medium under sterile conditions. Close and place in refrigerator to solidify quickly. Take out of refrigerator and let come to room temperature. Inoculate the stab agar with single colonies by collecting most of the colony on a pipette tip/loop/toothpick and then repeatedly stab/poke the instrument into the solidified agar in the cryotube. Close the cryotube loosely and place in the 37°C incubator to grow for 8 to 12 hours (depending on bacteria maybe longer). Cloudy tracks of bacteria should be visible and demonstrate growth. Then seal the vials tightly and store them in a cool (15°C to 22°C), dark place until shipping/transport.

Reviving bacteria from Agar Stabs

You are provided with *E. coli* ORN 178 and E. coli ORN 208 (which is a ORN178 derivative with a *fimH* gene deletion). The strains have been placed in stab culture and should be refrigerated once received (4°C), this way they can be stored up to two weeks. For long term storage, a stock culture should be created. Place a sterile toothpick, loop or pipette tip inside the puncture in the stab culture (Fig 1) and move the tool around until a gobbet of bacteria-laden agar is stuck onto the toothpick/loop/pipette tip. Then proceed to smear the bacteria onto an LB plate using the single colony streak out method (Fig 2), close the plate, place upside-down (lid side down) in the incubator and allow to incubate overnight (ON) at 37°C.

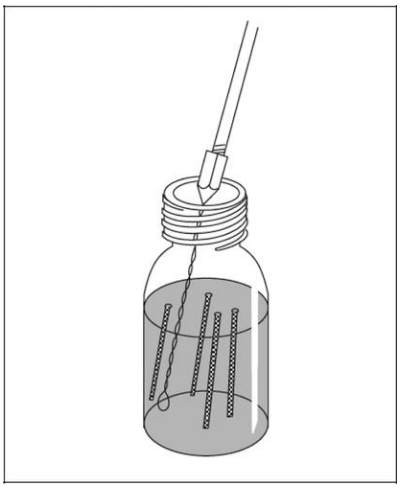

Figure 1: Agar stab with puncture marks and loop.

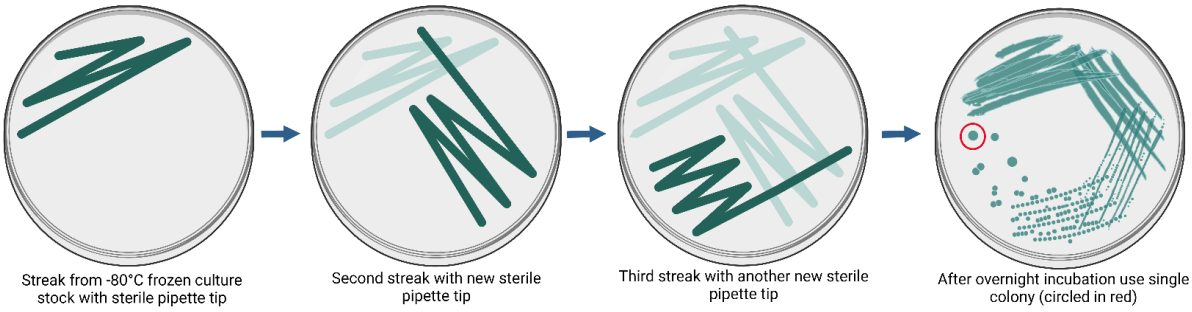

Figure 2: Single-colony streak out method.

Prepare bacterial culture by inoculating 5mL 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 overnight. Confirm that after overnight the negative control LB is still clear and sterile. Make 1 mL storage culture of bacterial strain by adding 930µL of bacterial culture and 70µL DMSO or 500µL of bacterial culture and 500µL of 50% sterile glycerol solution, mix vigorously or vortex for 3 seconds and place in the -80°C freezer for long-term storage.