Oct 05, 2025

Colony Picking

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvw4mo9lmk/v1

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Protocol Citation: igemnthuth 2025. Colony Picking. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvw4mo9lmk/v1

Protocol status: Working
We use this protocol and it's working

Created: October 03, 2025

Last Modified: October 05, 2025

Protocol Integer ID: 228962

Keywords: colony picking isolate, subsequent liquid culture, colonies on culture media, experimental material, reproducibility of experimental material, colony, frozen storage, single strain, strain, frozen stock, material

Abstract

Isolate a single strain from frozen stock or colonies on culture media, then purify and establish subsequent liquid culture or frozen storage to ensure uniformity and reproducibility of experimental materials.

Materials

- Frozen glycerol stock culture or colonies from LB agar plates
- LB broth (liquid medium)
- LB agar plate (or species-specific medium, e.g., *Pseudomonas* isolation agar)
- Sterile PBS (optional, if dilution is required)
- Sterile glycerol (for preparing glycerol stocks, final concentration 20%)
- Sterile inoculation loop
- 50 mL Falcon tube or other liquid culture vessel
- Shaking incubator (30 or 37 °C, 140–220 rpm, depending on optimal growth conditions for the strain)
- Spectrophotometer or microplate reader (for measuring OD_{600})
- Incubator (30 or 37 °C)

Initial Inoculation

1 Take a single colony from a glycerol stock or agar plate.

2 Using a sterile inoculating loop, pick a small amount of the colony and inoculate it into 10 mL LB broth (in a 50 mL Falcon tube).

3 Gently swirl the inoculating loop to ensure the bacteria are suspended.

4 Loosen the cap of the culture tube (to maintain aerobic conditions) and incubate in a shaking incubator at 30 or 37 °C, 140 rpm, for 12–14 hours.

Check Growth Status

5 Observe whether the culture has turned turbid.

6 If necessary, measure OD_{600} = 0.6–0.8 to confirm it is in log phase.

Streaking for Isolation

7 Prepare a fresh LB agar plate (equilibrated to room temperature).

8 Dip a sterile inoculating loop into the overnight culture and streak three parallel lines on the agar.

9 Using a new sterile loop dipped in sterile LB broth, streak three more lines at an angle, making sure the first line crosses the previous streaks.

10 Rotate the plate 90°, use a new sterile loop, and streak three “dry lines.”

11 Rotate the plate another 90° and repeat streaking three more dry lines, with the final streak extending toward the center of the plate.

12 Invert the plate and incubate at 30 or 37 °C for 24 hours.

Inspection and Picking of Single Colonies

13 On the following day, check for single colonies approximately 1–2 mm in diameter.

14 If growth is insufficient: extend incubation for an additional 12–24 hours.

15 If growth is excessive: dilute the culture before streaking (e.g., 1:4 dilution).

16 Using a sterile inoculating loop, carefully pick a single colony and inoculate into 10 mL LB broth.

17 Incubate in a shaking incubator at 30 or 37 °C, 140 rpm, for 12–14 hours.