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Bacterial glycerol stocks

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We use this protocol and it's working

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Abstract

Ward lab protocol for making bacterial glycerol stocks



- 1 Start with bacteria struck for isolation on an LB+agar plate with the appropriate antibiotic (if strain carries an antibiotic resistance marker).
- 2 Pick a single, isolated colony into 5 ml of LB with the appropriate antibiotic. Shake 16 hours at 37°C.
- 2.1 Ensure that sterile 60% glycerol is prepared. Mix 300 ml of 100% glycerol with 200 ml of milliQ water. We typically do this in a 1 L graduated cylinder with a small stir bar added. Mix on a magnetic stir plate. Sterilize by filtering through a Millipore Stericup vacuum filter, or similar vacuum filtration system. Alternatively, transferring to a 500 ml bottle and autoclaving on a liquid cycle also works.
- 3 In a sterile cryovial, add 500 µl of sterile 60% glycerol and 500 µl of the overnight culture. Label the tube and number it. We maintain a database of glycerol stocks where each tube has a unique numeric identifier. Store tube at -80°C.