



PROTOCOL

1. Bacterial Culture

Prepare a 5.0 ml culture in the rotating incubator with the appropriate media. See the bacteria culture protocol for specific information.

2. Glycerol Stock Preparation

- Use STERILE pipet tips and sterile microfuge tubes.
- From the 5 ml culture prepared above add 0.50 ml of culture to a sterile 1.5 ml microfuge tube to 0.50 ml of sterile glycerol solution.
- Mix the solution by inversion and quickly place into the -80°C freezer.
- Freeze in the -80°C only. The cells will die if left at -20°C.
- Label the tube with cell line and clone information.
- Revive by scraping off splinters of solid ice with a sterile toothpick or pipet tip (**be careful not to allow contents to thaw**) and streak onto an LB plate with antibiotic.

SOLUTIONS

Glycerol Freezing Solution (100 ml):

- 65 ml glycerol
- M MgSO₄
- 25 mM Tris-Cl pH 8.0
- QS with water to 100 ml.
- Autoclave and store at room temp