



PROTOCOL

1. Bacterial Culture

Prepare a 1.0 to 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 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. This is often done for 24 hrs in a closed Styrofoam box to control freezing rate.
- 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.

SOLUTION

Glycerol Freezing Solution (100 ml):

- 50 ml glycerol
- 50 ml water
- Autoclave and store at room temp – may need bring the volume back to 100 ml after autoclaving using sterile water.