

## **Glycerol Stock**

Store cells that have been confirmed to have successful ligations in glycerol stocks at  $-80^{\circ}\text{C}$ .

9.1 Grow a 2-mL culture of a particular cell type overnight for each frozen stock that is needed.

9.2 Microwave sterile glycerol for 30s. Do not mix the glycerol so that the top portion remains as hot as possible.

9.3 Cut a 200- $\mu\text{L}$  pipette tip with a clean razor blade to make a larger opening at the tip.

9.4 Label Nunc cryotubes as appropriate and add 150  $\mu\text{L}$  of hot glycerol.

9.5 Allow the glycerol in the tubes to cool at room temperature for 1 min. Add 850  $\mu\text{L}$  of bacterial culture to each tube, making 15% glycerol mixtures.

9.6 After putting the caps on, shake the tubes vigorously to ensure that the glycerol mixes evenly with the bacterial culture.

9.7 Immediately after step 10.6, put the tubes into a  $-80^{\circ}\text{C}$  freezer.