GLYCEROL STOCK PREPARATION

ADAPTED FROM THE BONNET TEAM PROTOCOL REPOSITORY

PRINCIPLE:

Freezing is an efficient way of storing bacteria. Glycerol allows to reduce the harmful effect of ice crystals of bacteria which can damage cells by dehydration caused by a localized increase in salt concentration leading to denaturation of proteins. Additionally, ice crystals can also puncture cellular membranes.

Glycerol as a cryoprotectant depresses the freezing point of bacterial cells, enhancing supercooling. It does so by forming strong hydrogen bonds with water molecules, competing with water-water hydrogen bonding. This disrupts the crystal lattice formation of ice unless the temperature is significantly lowered.

The glycerol final concentration have to be no more than 20% to avoid mutation.

Glycerol stock in cryogenic tube.

MATERIAL:

- Cryogenic 2mL tubes
- Overnight culture or 6h of bacteria in 2mL of LB with or without antibiotic for selection
- Sterile 50 % glycerol
- cryobox 100 position

PROTOCOL:

1. Label a cryogenic tube with sticker label:

Construction name

Short name

Plasmid

Antibiotic resistance

Strain

Book reference

Date and initials

2. In sterile condition, add 300µL of glycerol 50 % and 1.2mL of the liquid culture.

3. Homogenize by inverting and vortexing.

4. Store the tube at -80°C in a labeled plastic cryo-box 100 positions. You can also process a flash freeze in liquid nitrogen before store the glycerol stock at -80°C.

5. Register information