Glycerol Stock

Aim of the Experiment

This experiment can be used for the production of a glycerol stock of a bacterial colony useful for save long time storage at -80 °C. This set up glycerol stock can be used to reliably set up overnight cultures.

Materials

- LB-Medium (Carl Roth, Germany)
- 100 % Glycerol (Carl Roth, Germany)
- overnight culture of bacterial colony of interest (at least 1 ml)
- Bunsen burner

Procedure

1. Mix 100 % glycerol solution 1:1 with LB-Medium and autoclave the new 50 % (v/v) glycerol/LB-Medium solution to make sure it is sterile
2. Use Bunsen-burner and 70 % ethanol solution to set up sterile working area (too prevent contamination of glycerol stock, let ethanol fully evaporate before lighting the Bunsen burner)
3. Mix bacterial overnight culture 1:1 with 50 % (v/v) glycerol/LB-Medium solution in sterile working area (typically in 1 ml of culture mixed with 1 ml of glycerol/LB-Medium)
4. Fully freeze culture medium mix as quickly as possible (-80 °C freezer or liquid N_2)
5. For long time storage keep stock at -80 °C and keep thawing periods as short as possible (especially for culture set up)

Safety note

Remember to keep track about the full information (full vector DNA sequence and insert DNA sequences, bacterial strain) of all bacterial glycerol stocks as you are legally obligated to do so for all GMOs kept for long time storage.

Possible follow up protocols

The following protocols are the next steps of a possible cloning cycle after a glycerol stock:

1. Sequencing
2. Overnight culture