Preparation of Glycerol Stocks for Plasmid Storage

Introduction

This protocol creates glycerol stocks to store the pSender and pReceiver plasmids sent to us by the 2016 Imperial College of London iGEM team in a -80°C freezer for long-term storage. The glycerol stocks prevent damage to the plasmid cell membrane.

Reagents

- Glycerol 100% v/v
- Milli-Q H_20
- Imperial Samples
- Dry ice
- Ethanol

Equipment

- Small cylindrical flasks
- 10 mL graduated cylinder
- Pipette
- Modified pipette tips
- 1.5 mL snap-cap tubes
- Bucket

Procedure

1. Creation of a 50% v/v glycerol solution was necessary, so 5 mL of glycerol was mixed with 5 mL of Milli-Q H_20 in a small cylindrical flask.
    a. *We highly recommend that you measure out the glycerol in a small graduated cylinder, as the viscosity of the glycerol will make working with pipette tips nearly impossible. Mixing by pipette can be performed by cutting a 1000 µL pipette tip approximately 1 cm from the bottom to widen the hole at the end of the tip.*
2. 500 µL of each Imperial sample was added to various 1.5 mL snap-cap tubes. 500 µL of the 50% glycerol stock solutions was added to the snap-cap tubes and mixed thoroughly.
3. Dry ice and ethanol were added to a small bucket to create a cold liquid solution. Finished samples placed in snap-cap tubes were placed in this solution to rapidly cool them down to -80°C.
4. Samples were placed in a -80°C freezer for long-term storage.