

**STANDARD OPERATING PROCEDURE  
DEPARTMENT OF MICROBIOLOGY PILOT PLANT**

TITLE: Ethanol Production Strain Storage

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## 1. Scope

This procedure describes the methods pertaining to the preparation, storage, and thawing of bacterial strains for the production of ethanol.

## 2. Safety and Training Requirements

Ensure proper use of PPE – gloves, lab coats, and safety glasses  
Handling of Biosafety Level 1 genetically modified bacteria.

## 3. Related Documents and SOPs

Before beginning procedure ensure thorough understanding of the UF biosafety manual regarding proper care of recombinant bacterial strains that are classed as Biosafety level 1.

- UF Laboratory Safety Manual
- UF Biosafety Manual
- Biological Safety Cabinet Manual
- SOP-XXXX Ethanol Strain Performance Verification

## 4. Preparation/Materials/Equipment

- A. Sterile cryovials with a working volume of 1.5mL
- B. 80% w/v sterile glycerol
- C. 1mL sterile pipette
- D. Metal cryovial block pre-cooled to -80C
- E. Storage box for vials pre-cooled to -80C
- F. Sterile flow hood

## 5. Procedure

### A Strain Storage

#### A.1 Preparation of Sterile Glycerol Solution (80%)

- A.1.a – Weigh 800g glycerol into a \_\_\_\_ liter bottle
- A.1.b - Add deionized water to 1L total volume
- A.1.c – Sterilize the solution in an autoclave at 121C for 30 minutes
- A.1.d – Store Sterile Glycerol Solution at room temperature for \_\_\_\_ weeks.

All procedures from A.2 to A.4 to be carried out in a biological safety cabinet.

A.2. Aseptically transfer 0.75mL of sterile glycerol into cryovial using 1mL pipette

A.3. Aseptically transfer 0.75mL of bacterial culture into cryovial from A.2

- A.4. Replace cap to seal
- A.5. Ensure thorough mixing by inverting several times
- A.6. Place into pre-cooled cryovial metal block for 20 minutes
- A.7. Place into -80C freezer
- A.8. Transfer to pre-cooled storage box for storage until needed for use.

#### B. Strain Thawing

- B.1. Remove cryovial from -80C freezer and defrost at room temperature for 7-10 minutes.
- B.2. Immediately place desired volume of thawed glycerol stock into desired growth medium.

#### C. Strain Performance Verification

Each strain should be tested for viability and ethanol production performance according to SOP-XXXX at the following intervals:

- C.1. Immediately after freezing in Section 5.A.
- C.2. One month after freezing
- C.3. Six months after freezing
- C.4. One year after freezing

Cultures should be removed from the working stock after \_\_\_\_ months of storage