STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Strain Storage and Handling

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APPROVALS: Process Change Committee **DATE: March 78th, 2013**

A. Scope

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

B. Safety and Training Requirements

Refer to UF lab safety policies and review the Material Safety Data Sheets (MSDS) for each material listed in section D below before starting any process work.

Refer to UF Biosafety guidelines and the NIH Guidelines for Research Involving Recombinant DNA Molecules whenever handling biological cultures/genetically modified organisms.

Review the location of fire extinguishers, fire blankets, safety showers, spill cleanup equipment and protective gear before beginning any process work.

During operations in the lab, the following safety gear will be utilized at all times:

- Lab Coat
- Safety Goggles
- Protective Gloves (nitrile, neoprene)

C. Related Documents and SOPs

1. UF Laboratory Safety Manual
2. UF Biosafety Manual
3. Sterile Air Laminar Flow Hood Manual
4. Strain Performance Verification SOP-0521

D. Preparation/Materials/Equipment

1. Sterile cryogenic vials (1.5 mL)
2. 80% w/v sterile glycerol

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3. Storage box for vials
4. Freezing container, Nalgene 5100-0001
5. 1-propanol
6. Sterile Air Laminar Flow Hood
7. Fresh culture broth (AM1 Media)
8. Sterile pipette tips (1 mL)
9. Pipette (1 mL)
10. Vortex mixer
11. Paper towels

E. Detailed Procedure

1. All vials and culture boxes must be labeled first, using a sharpie marker
    a. Label both tubes and box with the date, bacterial strain, and preparer's initials.
2. Place the freezing container inside the -80°C freezer for at least 1 hour prior to placement of any cryovials within it.
3. Place the strain storage box inside the -80C freezer for at least 30 minutes for pre-chilling.
4. Spray the surface of the Sterile Air Laminar Flow Hood with 70% v/v ethanol and wipe clean (wearing nitrile gloves) using a paper towel.
5. Aseptically, in the Sterile Air Laminar Flow Hood, transfer 750 µL of sterile glycerol and 750 µL of the fresh culture broth into a 1.5 mL cryogenic vial (1:1 ratio of culture and glycerol stock; final glycerol concentration 40%).
6. Replace cap to seal.
7. Ensure mixing by inverting several times, and if possible, vortex on high setting for 10 seconds.
8. Pour approximately 100 mL of 1-propanol into the freezing container.
9. Place cryovial in a -80 °C propanol bath for at least 1 hour to ensure a temperature depression at a rate of -1°C/min.
10. Quickly transfer the cryovial to the pre-chilled cardboard or plastic strain storage box and replace the box into the -80°C freezer.

F. Data Archival and Analysis

Record all fermentation parameters, OD, sugars, inhibitors, and ethanol measurements in strain batch record.