

**STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PLANT**

TITLE: Culture Transfer 10 L to 140 L Fermentor Operating Procedure

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A. Scope

This procedure describes how to transfer a 10 L seed culture from a New Brunswick Scientific Bioflo 3000 fermentor into a 140 L Applikon Fermentor.

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.

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 plant, the following safety gear will be utilized at all times:

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

Avoid inhalation of vapors and wear nitrile or neoprene rubber gloves. Contain spills by using spill kits next to fermentors. The ammonium hydroxide must be tightly sealed at all times to avoid escape of vapors.

C. Related Documents and SOPs

1. 10 L seed culture SOP
2. Applikon 140 L SOP
3. UF Biosafety manual
4. MSDS sheets for chemicals listed in section D

D. Preparation/Materials/Equipment

The equipment used in this SOP is listed below:

1. Manostat Varistaltic Pump (solid state model)
2. Autoclave aluminum foil covered Saint-Gobain Masterflex Norprene tubing (06402-16)
3. Autoclaved Pyrex 400 ml bottle with screw-cap

E. Detailed Procedure

1. Open sampling port at the top of the 10 L fermentor.
2. Place one end of the autoclaved Norprene tubing into the bottom of the fermentor.
3. Connect the exit end of the tubing to one of the spouts on the top left side of the 100 L fermentor (V-14.51/V-14.41/V-14.31) after removing aluminum cap on the spout.
4. Open the spout by pressing the side buttons of the spout while pushing the spout out (toward you).
5. Flip pump controller switch to “forward” direction.
6. Flip pump controller switch on to begin pumping culture into 100 L fermentor.
7. Flip pump controller switch off when all of the culture has been transferred out of the 10 L fermentor.

8. Take the tube out of the 10 L fermentor and place it into a sterile Pyrex 400 ml bottle containing 100 ml of sterile deionized water.
9. Flip pump controller switch on to begin pumping the water into the 100 L fermentor. This procedure is used to flush the culture remaining in the tubing out into the fermentor.
10. Flip pump controller switch off when all of the water has been transferred.
11. Disconnect tubing from the spout and place the aluminum cap back on the spout.
12. Close the spout by pressing the side buttons in while pushing the spout in (away from you).