

**STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT**

TITLE: Transfer Vessel

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A. Scope

This SOP describes the procedure to sterilize and operate the transfer vessel in order to inoculate the Primary Propagator 2A (VS-3202A) and Primary Propagator 2B (VS-3202B).

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment 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.

Refer to UF Biosafety guidelines and the NIH Guidelines whenever handling biological cultures/genetically modified organisms.

During operations in the laboratory, the following safety gears will be utilized at all times:

- Lab Coat
- Safety Goggles
- Protective Gloves

During operations in the plant, the following safety gears will be utilized at all times:

- Hard Hat
- Safety Goggles
- Protective Gloves

C. Related Documents and SOPs

1. Autoclave Operation SOP-0504
2. Primary Propagator 2A VS-3202A SOP-3210
3. Primary Propagator 2B VS-3202B SOP-3215
4. Primary Flask Culture SOP-0509
5. ESCO Airstream Horizontal Laminar Flow Clean Bench Manual (2008)

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D. Preparation/Materials/Equipment

1. Transfer Vessel
2. Laminar flow clean bench
3. Graduated cylinder (1000 mL)
4. Aluminum foil
5. Portable peristaltic pump
6. Transfer Vessel hose
7. Hose clamps
8. Autoclave gloves
9. Plastic funnel
10. 70 % Ethanol
11. 0.6% bleach solution (10% dilution of commercial bleach)

E. Detailed Procedure

1. Ensure Transfer Vessel is clean and empty.
2. Connect the Transfer Vessel hose to the barbed fitting on outlet and clamp the hose.
3. Connect an air filter to the vent port of Transfer Vessel and cap the filter end with aluminum foil.
4. Ensure the screw top and the other ports are closed.
5. Sterilize the Transfer Vessel and plastic funnel in the autoclave according to the Autoclave Operation SOP-0504.
6. Turn on the laminar flow cabinet and wipe the work surface using a paper towel and 70% Ethanol.
7. Take out all the material from the Autoclave when the sterilization cycle is completed using the autoclave gloves and put it on the laminar flow clean bench.
8. Wait for the material to cool before starting to work in the laminar flow clean bench.
9. Once seed culture is ready according to the Primary Seed Flask Culture SOP-0509, pour 1.2 gallons (4.54 L) of the culture into the Transfer Vessel by opening the screw top and using the sterile plastic funnel and the graduated cylinder. This volume will be used to inoculate a Primary Propagators at 3 % (v/v) with a total volume of 40 gallons (151.4 L).
10. Close the screw top and make sure the other ports are closed.
11. Take the Transfer Vessel upstairs in order to inoculate the Primary Propagators VS-3202A or VS-3202B by:
 - a. Put the transfer hose of the Transfer Vessel in the cartridge of the portable peristaltic pump.
 - b. Connect the transfer hose end to the respective inoculation port in the Primary Propagator to be inoculated.
 - c. Ensure that the pressure in the Propagator vessel is less than 5 PSI.

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- d. Open the inoculation port on the Propagator.
 - e. Turn on the portable peristaltic pump at full speed to start inoculum transfer.
 - f. After inoculation is complete, turn off the portable peristaltic pump and clamp the transfer hose of the Transfer Vessel.
 - g. Close the inoculation port of the respective Primary Propagator.
 - h. Disconnect the transfer hose from the inoculation port of the respective Primary Propagator.
 - i. Take out the transfer hose of the Transfer Vessel from cartridge of the portable peristaltic pump.
12. In the case of any spill of the seed culture in the plant or laboratory, refer to Biohazard Spill Emergency Procedures.
13. Wash and dry the Transfer Vessel immediately.
14. Sterile the Transfer Vessel with all the hoses used according to the Autoclave Operation SOP-0504.
15. Transfer Vessel is now clean and empty to be used to inoculate another Primary Propagator.