STANDARD OPERATING PROCEDURE
STAN MAYFIELD BIOREFINERY PILOT PLANT

TITLE: Secondary Propagator 3A VS-3203A

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APPROVALS: Process Change Committee **DATE: July 31st, 2013**

A. Scope

This SOP describes the procedure to clean, sterilize, and operate the Secondary Propagator 3A VS-3203A during normal operation in order to propagate the seed and inoculate the fermentors.

B. Safety and Training Requirements

Refer to UF lab safety policies regarding equipment listed in section D below before starting any process work.

Refer to UF Biosafety guidelines 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.

When performing any work above 6feet from the ground, make sure to properly use a harness to prevent injury in case of a fall.

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

- Safety Goggles
- Protective Gloves
- Hard Hat

C. Related Documents and SOPs

1. Experimental Plan
2. Ethanol Concentration SOP-0500
3. Sugar, Organic Acids and Inhibitors Concentration SOP-0505
4. Viable Plate Count SOP-0507
5. Sampling SOP-0511
6. Optical Density Measurement with Spectrophotometer SOP-0513
7. pH Measurement SOP-0514
8. Plant pH Probe Calibration SOP-0519

9. C5 Hydrolysate Storage SOP-2135
10. Media Preparation SOP-2155
11. Primary Propagator 2A SOP-3210
12. Primary Propagator 2B SOP-3215
13. FermentationTank A SOP-3230
14. Fermentation Tank B SOP-3235
15. Fermentation Tank C SOP-3240
16. Trace Elements Storage SOP-3250
17. Beer Well SOP-4000
18. Decanter Operation SOP-4905
19. Phosphoric Acid System Operation SOP-8110
20. Clean In Place (CIP) SOP-8205
21. Antifoam System Operation SOP-8310
22. Lime Slurry System Operation SOP-8405
23. Base B System Operation SOP-8565
24. Refrigeration System Operation SOP-9210
25. Steam Supply System Operation SOP-9305
26. Air System Operation SOP-9405
27. UV Water System Operation SOP-9555
28. Hot Water System Operation SOP-9605
29. Potable Water System SOP-9705

D. Detailed Procedure

D.1 Vessel Preparation

1. At the HMI ensure that the Secondary Propagator 3A is clean, empty, at atmospheric pressure, and ready for operation.
2. Make sure that the Air System is ready according to Air System SOP-9405.
3. Make sure that the Potable Water System is ready according to Potable Water System SOP-9705.
4. Make sure that the Steam Supply System is ready according to Steam System SOP-9305.

5. Make sure the cooling water is operational according to the Refrigeration System SOP-9210.
6. Make sure the hot water is operational according to the Hot Water System Operation SOP-9605.
7. Instrument Calibration:
   a. Pressure Measurement
      i. Verify that the local indicator is reading ambient conditions (PIT-3203A-33)
         1. NOTE: Ambient pressure readings vary per indicator. It's critical to obtain an understanding of what value equals ambient pressure before proceeding with operation. The values can be found on a sheet in the control room.
      ii. On the HMI, verify the pressure indicator (PI-3203A-33) is working properly and that the pressure value is the same as local indicator.
   b. Temperature Measurement
      i. On the HMI, verify that temperature probe (TIC-3203A-10A) is reading current ambient conditions.
      ii. Verify that the reading of the local probe (TI-3203A-18) is the same as displayed on the HMI.
   c. Vessel Level Measurement
      i. On the HMI, verify that level indicator LI-3203A-03 is reading zero level.
      ii. Configure the following valves to the next position to ensure there is no liquid in the vessel:
         1) Close valves: 3203-V-26 and V-33.
         2) Open valves: 3203-V-52, V-56, V-34, V-30 and V-29.
   d. pH Measurement
      i. Calibrate pH probes AE-3203A-01A and AE-3203A-01B according to the Plant pH Probe Calibration SOP-0519.
      ii. Verify that the pH readings after calibration are the same as displayed on the HMI tag AIC-3203A-01.
8. Verify that all side ports on the vessel are filled and secure.
9. At the HMI, switch the temperature control TIC-3203A-10B to MANUAL, the OUTPUT to -5 and mode to SIP.
10. Verify that the jacket does not have hot/cooling water flowing through it by ensuring that XV-3203A-29, XV-3203A-30, XV-3203A-31 and XV-3203A-32 are closed.

11. Verify that the jacket is at ambient temperature and pressure using PI-3203A-16 and TI-3203A-17.
12. Verify the initial valve settings according to the Table 1.

Table 1. Initial valve positions settings.

Secondary Propagator 3A System				
Line	**Application**	**Valve**	**Position**	**Check**
CIP Supply	Slurry line cleanse	3203-V-01	Closed	
	Slurry line cleanse	3203-V-02	Closed	
	Spray Ball Valve	3203-V-53	Closed	
	Spray Ball Valve	3203-V-54	Closed	
	Vent line cleanse	3203-V-57	Closed	
	Tank cleanse	3203-V-61	Closed	
UV Sterilizer	To propagator 3A	3203-V-04	Closed	
Primary Propagator 2A	To tank inlet	3203-V-07	Closed	
	To tank inlet	3203-V-26	Closed	
Primary Propagator 2B	To tank inlet	3203-V-09	Closed	
C5 Pump	To tank inlet	3203-V-16	Closed	
Phosphoric Acid	To tank inlet	3203-V-62	Closed	
Vent	Vent valve	3203-V-52	Open	
	To Beer Well	3203-V-56	Closed	
Hydrolyzate pH Adjust	To tank inlet	3203-V-03	Closed	
Base B	To tank inlet	3203-V-58	Closed	
Trace Metals	To tank inlet	3203-V-17	Closed	
	To tank inlet	3203-V-18	Closed	
Sterile Air	Pressure indicator	3203-V-10	Open	
	To tank inlet	3203-V-11	Closed	
	To the Vent line	3203-V-12	Closed	
	Vacuum relief valve	3203-V-14	Closed	
	Vacuum relief valve	3203-V-15	Closed	
	To tank inlet	3203-V-19	Closed	
	To tank inlet	3203-V-21	Closed	
Vessel Jacket	High Point Vent	3203-V-49	Open	
	Recirculation	3203-V-25	Closed	

	Recirculation	3203-V-50	Closed
	Recirculation	3214-V-01	Open
	Recirculation	3214-V-02	Open
	Recirculation	3214-V-04	Open
	Pressure indicator	3203-V-24	Open
	Drain	3203-V-27	Open
	Drain	3203-V-28	Open
Low Pressure Steam	Pressure Indicator	3203-V-47	Open
	To the Jacket	3203-V-48	Closed
	Tank bottom inlet	3203-V-35	Closed
	Spare valve	3203-V-44	Closed
	To Sample Port	3203-V-46	Open
	To Sample Port	3203-V-20	Closed
	Spare Valve	3203-V-62	Closed
	Pressure Indicator	3203-V-45	Open
Cooling / Hot water	Cooling / Hot Water Return	3203-V-51	Closed
Vacuum Pump	Vacuum System	3203-V-55	Closed
Transfer line	Bottom Drain	3203-V-29	Open
	Bottom Drain	3203-V-30	Open
	Bottom Drain	3203-V-34	Open
	To Fermentors	3203-V-33	Closed
	To Fermentor A/B	3203-V-40	Closed
	To Fermentor A	3203-V-42	Closed
	To Fermentor B	3203-V-41	Closed
	To Fermentor C	3203-V-38	Closed
	Spare line	3203-V-36	Closed

D.2 Sterilization (SIP)

1. Make sure the Secondary Propagator 3A (VS-3203A) jacket has been drained through the valves 3203-V-27 and V-28 by visual inspection.
2. Add Low Pressure Steam to the tank jacket by:
    a. Close the Jacket High Point Vent valve 3203-V-49.
    b. Open the steam supply valve 3203-V-48.
    c. Wait until the steam comes out through drain valve 3203-V-27.

d. Open the steam trap T1-3203-03 and close the drain valve 3203-V-27.
e. Verify steam pressure in PI-3203A-15 is 15 ± 2 PSI. If not, contact supervisor.

3. Add Low Pressure Steam to the tank by:
   a. Open the valve 3203-V-35 and verify the pressure in PI-3203A-20 is 27 ± 2 PSI.
   b. Close valve 3203-V-29 and open steam trap T1-3203-04.
   c. On the HMI, set sterilization temperature on TIC-3203A-10B to 250 °F (121 °C), and set MODE to AUTO.
4. When the pressure inside the tank reaches 5 PSI, remove the residual air using the Vacuum Pump by:
   a. On the HMI, stop steam to the tank by setting MODE to MANUAL on TIC-3203A-10B and OUTPUT to -5.
   b. Close the tank drain valve 3203-V-34.
   c. Open the vent valve to the vacuum pump (PV-3201) 3203-V-55.
   d. At the HMI, open solenoid valve XV-3201-01 and turn on the vacuum pump (PV-3201).
   e. At the HMI, monitor the pressure on PI-3203A-33 until it reaches -10 PSI.
   f. At the HMI, turn off the vacuum pump (PV-3201) and close the solenoid valve XV-3201-01.
   g. Close the vacuum line valve 3203-V-55.
   h. On the HMI, set MODE back to AUTO on TIC-3203A-10B.
   i. Once the pressure on PI-3203A-33 is above 0 PSI, open the tank drain valve 3203-V-34.
5. Once the tank temperature reaches 250 °F (121 °C), maintain the temperature for 60 minutes.
6. After sterilization is completed, cool the tank by:
   a. At the HMI, stop steam to the tank by setting MODE to MANUAL on TIC-3203A-10B and OUTPUT to -5.
   b. Close the steam supply jacket valve 3203-V-48.
   c. Relieve pressure in the jacket by slowly opening jacket drain valve 3203-V-27.
   d. Open the jacket high point vent valve 3203-V-49.
   e. After the jacket has been drained, close the valves 3203-V-27, V-28 and V-49.
   f. Open valve 3203-V-51.
   g. At the HMI, switch temperature control TIC-3203A-10B to NORMAL.
   h. At the HMI, set the temperature control at TIC-3203A-10A to 98.6 °F (37 °C).

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i. At the HMI, set MODE to AUTO on TIC-3203A-10A and monitor the pressure and temperature.
j. At the HMI, monitor the tank pressure and temperature at PI-3203A-33 and TIC-3203A-10A respectively.
k. When the pressure drops below 10 PSI, open the sterile air valves 3203-V-14 and V-15 to add air to the tank via vacuum breaker.
l. Once the tank temperature reaches 98.6 °F (37 °C):
    i. Open valves 3203-V-25 and V-50 to Secondary Propagator 3A Jacket Pump.
    ii. At the HMI, start Secondary Propagator 3A Jacket Pump (PT-3213).

7. Begin normal operation.

D.3 Operation of Secondary Propagator 3A

D.3.1. Startup

1. Make sure the Base B system is functioning properly according to SOP-8565.
2. Make sure the Phosphoric Acid system is functioning properly according to SOP-8110.
3. Make sure the Antifoam system is functioning properly according to SOP-8310.
4. Make sure the UV Water system is functioning properly according to SOP-9555.
5. Make sure the Trace Elements Storage system is functioning properly according to SOP-3250.
6. Make sure the C5 storage system is ready according to the C5 Hydrolysate Storage SOP-2135.
7. If needed, make sure the Lime Slurry System Operation is functioning properly according to SOP-8405.
8. Turn on the air sparger by:
    a. Open valve 3203-V-11, V-19 and V-21.
    b. Set the desired sterile air flow rate according to the Experimental Plan using the rotameter FI-3203A-26.
    c. Monitor the air pressure in PI-3203A-08; should be approximately 15 PSI.
9. Close the valves to the Vacuum Breaker 3203-V-14 and V-15.
10. Start Antifoam control by:

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a. Ensure the antifoam is ready to transfer according to the Antifoam System Operation SOP-8310.
b. On the HMI, turn on antifoam control by switching the controller to AUTO.

11. Make sure main drain valve 3203-V-34 is closed.
12. Open the valve 3203-V-56 to the Beer Well vent line.
13. Add the specified amount of C5 hydrolysate according to the Experimental Plan by:
    a. Make sure C5 hydrolysate is ready to transfer according to C5 Hydrolysate Storage SOP-2135.
    b. Open the valves 3203-V-16 and 2103-V-18 to open the transfer line.
    c. Start adding C5 hydrolysate according to the C5 Hydrolysate Storage SOP-2135 and the Experimental Plan.
    d. At the HMI, set the flow rate of the C5 Pump(PM-2108) according to the Experimental Plan using SIC-2108-04 and switch to AUTO.
    e. At the HMI, monitor the tank level using LI-3203A-03 and stop the addition of C5 hydrolysate when it reaches the desired level as determined by the Experimental Plan.
14. Add UV Water necessary for operation by:
    a. Open the valve 3203-V-04 to add UV water through the side of the tank.
    b. Once the volume reaches 20%, start the tank agitation by turning on XS-3203A-05 and setting SIC-3203A-05 to 100% in MANUAL.
    c. On the HMI, monitor the liquid level using LI-3203A-03.
    d. Close the UV Water valve 3203-V-04 when the desired level is reached as determined by the Experimental Plan.
15. Close valve 3203-V-16 to close the C5 line going into the tank.
16. Turn on the pH control by:
    a. Open valves 3203-V-58 and V-62.
    b. On the HMI, set the pH to 9.0 in AIC-3203A-01 and switch to AUTO.
    c. Once the pH is at set point, turn off the pH control at the HMI by switching to MANUAL and setting the OUTPUT to -5 in AIC-3203A-01.
    d. Take a sterile sample using sample valve SSP-3203-01 and steam valve 3203-V-20 according to Sampling SOP-0511.
    e. Measure the pH of the sample according to pH Measurement SOP-0514 to corroborate the field measurement.
17. Maintain the conditions in the tank for 22 ± 2 h.

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18. Open the valves 3203-V-17 and V-18 for the addition of the trace metals and magnesium sulfate/sodium metabisulfite solutions.
19. Add the specified amount of Trace Metals and Magnesium Sulfate/Sodium Metabisulfite solution from the 2 gal Trace Metals Tank (TS-3201) and the 2 gal $MgSO_4$ Storage Tank (TS-3202) respectively according to the Trace Elements Storage SOP-3250 and the Experimental Plan by:
    a. At the HMI, set the speed of the pump using SIC-3203-02 (according to the experimental plan), switch the pump controller SIC-3203-02 to AUTO, and turn on the Trace Metals Pump 2 (PT-3203).
    b. At the HMI, monitor the pressure in PI-3203-01 and PI-3203-04.
    c. At the HMI, turn off the Trace Metals Pump 2 (PT-3203) when the transfer is completed.
20. Close the valves 3203-V-17 and V-18 in the Secondary Propagator 3A.
21. Start pH control by:
    a. On the HMI, turn on the pH control by setting the pH to 6.3 in AIC-3203A-01 and switching to AUTO.
    b. Once the pH reaches the set-point, take a sterile sample using sample valve SSP-3203-01 and the steam valve 3203-V-20 according to Sampling SOP-0511.
    c. Measure the pH of the sample according to pH Measurement SOP-0514 to corroborate the field measurement.
    d. If the pH is not 6.3 $\pm$ 0.20, contact supervisor.
22. Close drain valve 3203-V-30.
23. In order to inoculate the Secondary Propagator 3A using Primary Propagator 2A:
    a. Make sure Primary Propagator 2A is ready to provide the culture seed according to Primary Propagator 2A SOP-3210.
    b. Open the valves 3203-V-07, V-26 and V-34.
    c. Transfer the contents of the Primary Propagator 2A to Secondary Propagator 3A according to Primary Propagator 2A SOP-3210.
    d. Close the valves 3203-V-07, V-26 and V-34 after inoculation.
    e. Record date and time of inoculation in propagator log book.
24. In order to inoculate the Secondary Propagator 3A using Primary Propagator 2B:
    a. Make sure Primary Propagator 2B is ready to provide the culture seed according to Primary Propagator 2B SOP-3215.
    b. Open the valves 3203-V-09, V-26 and V-34.

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c. Transfer the contents of Primary Propagator 2B to Secondary Propagator 3A according to Primary Propagator 2B SOP-3215.
d. Close the valves 3203-V-09, V-26 and V-34 after inoculation.
e. Record date and time of inoculation in propagator log book.

25. Take a sterile sample using sample valve SSP-3203-01 and the steam valve 3203-V-20 according to the Sampling SOP-0511.
    a. Measure sugars, organic acids, and inhibitors concentration according to SOP-0505.
    b. Measure ethanol according to Ethanol Measurement SOP-0500.
    c. Measure the Optical Density according to SOP-0513.
    d. Perform viable plate counts according to SOP-0507.

D.3.2. Operation

1. On the HMI, monitor the temperature, pH and agitation speed regularly in TIC-3203A-10A, AIC-3203A-01 and SIC-3203A-05 respectively.
    a. If the temperature, pH or agitation speed is out of normal ranges (as defined by the Experimental Plan), contact the supervisor.
2. Take sterile samples using the sample port valve SSP-3203-01 and the steam valve 3203-V-20 according to the Sampling SOP-0511 and the Experimental Plan.
    a. Measure sugars, organic acids and inhibitors concentration according to SOP-0505.
    b. Measure ethanol concentration according to SOP-0500.
    c. Measure the Optical Density according to SOP-0513.
    d. Perform viable plate counts according SOP-0507.
3. Allow for fermentation to last 24 hours before proceeding to transfer the broth to the Fermentors A, B, or C.

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D.3.3.Broth Contamination or Disposal

1. In case that the Propagation is not carried out forward to Fermentation, transfer the contents to the Beer Well by:
    a. Make sure the Beer Well is ready to receive the broth according to the Beer Well SOP-4600.
    b. On the HMI, turn off the temperature control TIC-3203A-10A and pH control AIC-3203A-01 by switching to MANUAL and setting the OUTPUT to -5.
    c. Turn off antifoam by switching the antifoam control to OFF.
    d. Make sure valves 3203-V-36, V-38, and V-41 are closed.
    e. Make sure valves 3205-V-05, V-07, V-08, V-09, V-11, V-31, V-32, V-36, V-39, V-40, V-41 and V-43 are closed in Fermentor A.
    f. Open valves 3203-V-34, V-33, V-40, and V-42 in order to transfer the broth to the pipes of Fermentor A and then to Beer Well.
    g. Open valves 3205-V-06, V-29, V-35, V-38 and V-42 in Fermentor A.
    h. At the HMI, turn on the Fermentor A Pump (PC-3204A) on XS-3204A-21.
    i. Monitor the pressure in PI-3204A-22.
    j. Transfer the broth to the Beer Well, and when the transfer is complete, turn off the Fermentor A Pump (PC-3204A) on the HMI (XS-3204A-21).
    k. Set the valves back to their original positions.
2. Dispose of the material through the Beer Well and Decanter according to the SOP-4600 and SOP-4905 respectively.

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D.3.4. Transfer to Fermentors A, B or C

1. On the HMI, turn off the temperature control TIC-3203A-10A and pH control AIC-3203A-01 by switching to MANUAL and setting the OUTPUT to -5.
2. Turn off antifoam by switching the antifoam control to OFF.
3. Open the valves 3203-V-33 and V-34.
4. Make sure Fermentors A, B or C are ready to receive the broth according to the Fermentation Tank A SOP-3230, Fermentation Tank B SOP-3235, or Fermentation Tank C SOP-3240 respectively.
    a. In order to transfer the broth to Fermentor A, open the valves 3203-V-40 and V-42.

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b. In order to transfer the broth to Fermentor B, open the valves 3203-V-40 and V-41.
c. In order to transfer the broth to Fermentor C, open the valve 3203-V-38.

5. When the transfer is complete, close the valves 3203-V-33 and V-34.

D.4 Cleaning (CIP)

1. Make sure that the CIP system is ready according to CIP system SOP-8205.
2. Make sure the valves are set according to the Table 2.

NOTE: Before configuring these valves, ensure the CIP system is not supplying pressure to the CIP header but only to the CIP loops. If the CIP header is being pressurized, you must wait for the person who is using the CIP system to finish before you can continue.

Table 2. CIP initial valve positions settings

Secondary Propagator 3A System				
Line	**Application**	**Valve**	**Position**	**Check**
CIP Supply	Slurry line cleanse	3203-V-01	Closed	
	Slurry line cleanse	3203-V-02	Open	
	Slurry line cleanse	3203-V-03	Closed	
	Spray Ball Valve	3203-V-53	Closed	
	Spray Ball Valve	3203-V-54	Closed	
	Vent line cleanse	3203-V-57	Open	
	Air Sparger	3203-V-61	Open	
UV Sterilizer	Slurry line cleanse	3203-V-04	Closed	
Primary propagator 2A	To tank inlet	3203-V-07	Closed	
	To tank inlet	3203-V-26	Closed	
Primary propagator 2B	To tank inlet	3203-V-09	Closed	
C5 Pump	To tank inlet	3203-V-16	Open	
Phosphoric Acid	To tank inlet	3203-V-62	Closed	
Vent	Vent valve	3203-V-52	Open	
	To Beer Well	3203-V-56	Closed	
Trace Metals	To tank inlet	3203-V-17	Closed	

	To tank inlet	3203-V-18	Closed
Base B	To tank inlet	3203-V-58	Closed
Sterile Air	Pressure indicator	3203-V-10	Open
	To tank inlet	3203-V-11	Closed
	To the Vent line	3203-V-12	Closed
	Vacuum relief valve	3203-V-14	Closed
	Vacuum relief valve	3203-V-15	Closed
	To tank inlet	3203-V-19	Open
	To tank inlet	3203-V-21	Closed
Low Pressure Steam	Pressure Indicator	3203-V-47	Open
	To the Jacket	3203-V-48	Closed
	Tank bottom inlet	3203-V-35	Closed
	Spare Valve	3203-V-44	Closed
	To Sample Port	3203-V-46	Closed
	To Sample Port	3203-V-20	Closed
	Drain Valve	3203-V-62	Closed
	Pressure Indicator	3203-V-45	Open
Vacuum Pump	Vacuum System	3203-V-55	Closed
Transfer line	Bottom Drain	3203-V-30	Closed
	Bottom Drain	3203-V-29	Closed
	Bottom Drain	3203-V-34	Open
	To Fermentors	3203-V-33	Open
	To Fermentor A	3203-V-40	Open
	To Fermentor A	3203-V-42	Closed
	Spare line	3203-V-36	Closed
	To Fermentor B	3203-V-41	Closed
	To Fermentor C	3203-V-38	Closed

3. Clean Secondary Propagator 3A (VS-3203A) by:
    a. If the broth was transferred to Fermentor A:
        i. Verify valves 3205-V-06 and V-29 are open, and 3205-V-11 and V-32 are closed from Fermentor A.
        ii. Open transfer valves 3205-V-35, V-38 and V-41 in Fermentor A.
        iii. Make sure valves 3205-V-36, V-39, V-40, V-42 and V-43 are closed in Fermentor A.
        iv. Allow for transfer from Secondary Propagator 3A to Fermentor A pump by opening valves 3203-V-42.
    b. If the broth was transferred to Fermentor B:

i. Verify valves 3206-V-06 and V-29 are open, and 3206-V-11 and V-32 are closed from Fermentor B.
ii. Open transfer valves 3206-V-35, V-38 and V-41 in Fermentor B.
iii. Make sure valves 3206-V-36, V-39, V-40, V-42 and V-43 are closed in Fermentor B.
v. Allow for transfer from Secondary Propagator 3A to Fermentor B pump by opening valve 3203-V-41.

c. If the broth was transferred to Fermentor C:
i. Verify valves 3207-V-06 and V-29 are open, and 3207-V-11 and V-32 are closed from Fermentor C.
ii. Open transfer valves 3207-V-35, V-38 and V-41 in Fermentor C.
iii. Make sure valves 3207-V-36, V-39, V-40, V-42 and V-43 are closed in Fermentor C.
vi. Allow for transfer from Secondary Propagator 3A to Fermentor C pump by closing valve 3203-V-40 and opening valve 3203-V-38.

4. Set CIP system to deliver Rinse Water to drain by:
a. Make sure that the valves 8201-V-04 and V-30 are closed in the CIP system.
b. Make sure that the valve 8201-V-31 is open in the CIP system.
c. Slowly open valve 8201-V-20 and then close valve 8201-V-21.
d. Turn on the respective fermentor pump (according to step D.4.3) at the HMI.
e. To rinse out the C5 line, vent line and air sparger line, let the CIP system run with the current valve configuration for 5 min.
i. Monitor the tank levelusing LI-3203A-03.
ii. If the level exeeds 15%, open valve 8201-V-21 and then close valve 8201-V-20 until the level of the tank reaches 0.1%.
iii. Slowly open valve 8201-V-20 and then close valve 8201-V-21 to continue with the rinse until the 5 min rinse is completed.
f. Open CIP supply spray ball valves 3203-V-53 and V-54.
g.
h. Close CIP supply valves 3203-V-02, V-57 and V-61.
i. Open vent line valve 3203-V-56.
j. Run the rinse cycle through the sprayballs for an additional 15 min.
k. Open valve 8201-V-21 and then close valve 8201-V-20 and turn off the respective fermentor pump at the HMI.

5. Set CIP system to deliver Caustic solutionby:
a. Close CIP supply spray ball valves 3203-V-53 and V-54.

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Lab comp 5/8/2013 3:16 PM
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Lab comp 5/8/2013 3:16 PM
Moved up [2]: Open CIP supply spray ball valves 3203-V-53 and V-54.
Turn on the respective fermentor pump (according to step D.4.3) at the HMI.

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Deleted: <#>After 10 minutes, open valve 8201-V-21 and then close valve 8201-V-20. Turn off the respective fermentor pump.

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Deleted: <#>Open CIP supply spray ball valves 3203-V-53and V-54. ... [2]

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b. Close vent valve 3203-V-56.
c. Open CIP supply valves 3203-V-02, V-57 and V-61 to allow CIP solution into the C5 line, vent line and air sparger line respectively.
d. Close 8201-V-31 and open 8201-V-04 to return CIP solution to the Dilute Caustic Tank.
e. Slowly open valve 8201-V-13 and then close 8201-V-14.
f. Turn on the respective fermentor pump (according to step D.4.3) at the HMI.
g. To clean the C5 line, vent line and air sparger line, let the CIP system run with the current valve configuration for 5 min.
    i. Monitor the tank level using LI-3203A-03.
    ii. If the level exeeds 15%, open valve 8201-V-14 and then close valve 8201-V-13 until the level of the tank reaches 0.1%.
    iii. Slowly open valve 8201-V-20 and then close valve 8201-V-21 to continue with the rinse until the 5 min rinse is completed.
h. Open CIP supply spray ball valves 3203-V-53 and V-54.
i. Close CIP suppy valves 3203-V-02, V-57 and V-61.
j. Open vent line valve 3203-V-56.
k. Run the CIP system for an additional 15 min.
l. Open valve 8201-V-14, then close valve 8201-V-13.
m. Turn off the respective fermentor pump at the HMI.

6. Set CIP system to deliver UV water by:
    a. Close CIP supply spray ball valves 3203-V-53 and V-54.
    b. Close vent valve 3203-V-56.
    c. Open CIP supply valves 3203-V-02, V-57 and V-61 to allow UV Water going into the C5 line, vent line and air sparger line respectively.
    d. Close valve 8201-V-04 and open valve 8201-V-30 to return UV water to the Rinse Tank.
    e. Slowly open valve 8201-V-12.
    f. Turn on the respective fermentor pump (according to step D.4.3) at the HMI.
    g. To rinse the the C5 line, vent line and air sparger line, let the UV Water run with the current valve configuration for 5 min.
        i. Monitor the tank levelusing LI-3203A-03.
        ii. If the level exeeds 15%, close valve 8201-V-12 until the level of the tank reaches 0.1%.

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Lab comp 5/8/2013 3:29 PM
Deleted: <#>After 10 minutes, open valve 8201-V-14 and then close 8201-V-13.

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Moved up [3]: <#>Open CIP supply spray ball valves 3203-V-53 and V-54.

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iii. Slowly open valve 8201-V-12 to continue with the rinse until the 5 min rinse is completed.

h. Open CIP supply spray ball valves 3203-V-53 and V-54.

i. Close CIP suppy valves 3203-V-02, V-57 and V-61.

j. Open vent line valve 3203-V-56.

k.

l. Run the CIP system for an additional 15 min.

m. Close valve 8201-V-12 and turn off the respective fermentor pump at the HMI.

q. Close the CIP supply spray ball valves 3203-V-53 and V-54.

r. Once the tank is empty, close the valve 3203-V-33.

7. Close the valve 3203-V-19.
8. Close main drain valve 3203-V-34.
9. Secondary Propagator 3A is ready for sterilization.

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Deleted: <#>Close valve 8201-V-12.

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Moved up [4]: Open CIP supply spray ball valves 3203-V-53 and V-54.

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Deleted: <#>Re-set CIP system to deliver UV water by slowly opening valve 8201-V-1 ... [4]

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E. Data Archival and Analysis

Date of inoculation:
Time of inoculation:
Experimental Plan Number:

Date	Time of Day	Time Elapsed	Temp (°F)	pH	Agitation (%)	Air flow rate (L/min)	Comments

Take notes of all calculations and measurements. Store the data obtained in the appropriate Log Book.