1. Procedure summary

This procedure describes the steps necessary to inoculate a carboy from flask culture.

1.1. Related Procedures

Carboy media filter	LC-01-001-004
Carboy assembly	LC-01-001-007
Carboy Environment	LC-01-001-009
Washing Dishes	LC-06-003-004
Autoclave Usage	LC-01-001-019
Laminar Flow Hood Use	LC-01-001-018

1.2. Procedure impacts and concerns

Safety	Proper PPE for this procedure: safety glasses, safety toe shoes and gloves. Nitrile gloves should be worn when handling pond samples. The MSDS/SDS for chemicals used in this SOP should be reviewed.
Quality	Improper maintenance of the carboy environment can result in compromised culture health.
Delivery	N/A
Environmental	N/A
Cost	N/A
Compliance	Compliance with OSHA's Hazardous Waste Operations and Response, and Hazardous Communication Standard in addition to the Sapphire Energy, Inc. Chemical Hygiene Plan is required. See 29 CFR 1910.120 and 1200. An authorized user list, MSDS's and label information will be available for easy reference in a binder in the administration building.

1.3. Responsibilities and owners

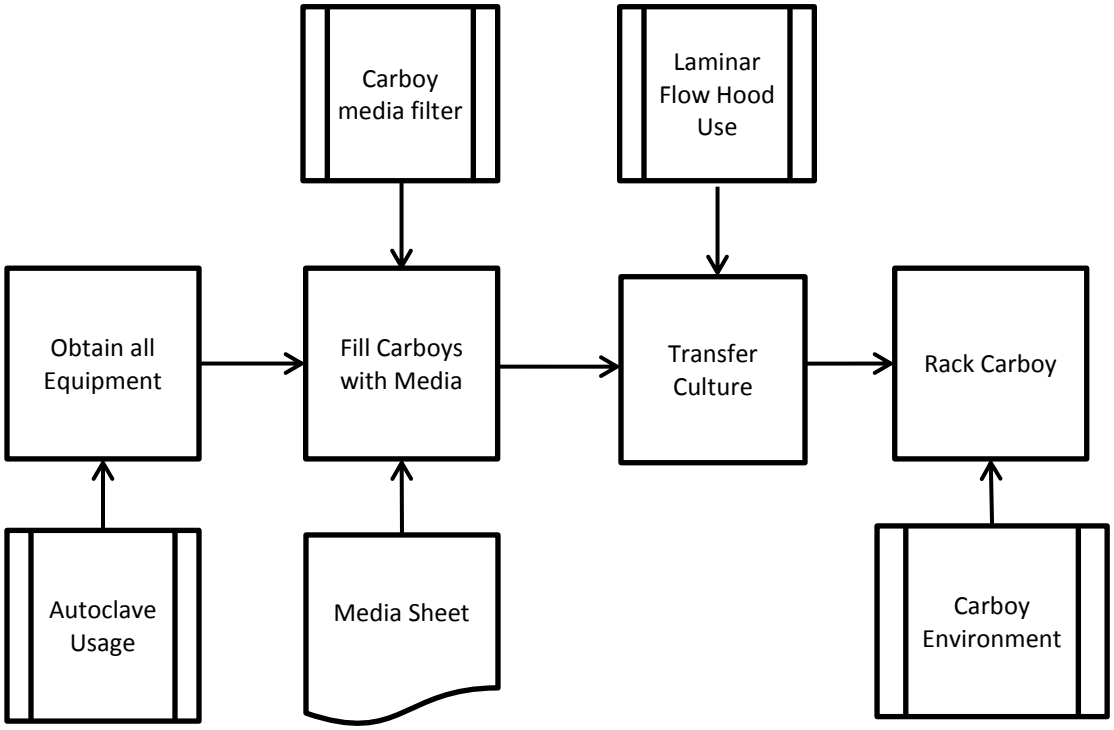
Document Owner	Manage content and distribution	Thomas A. Holguin
Process Owner	Responsible for content and process validation	Abraham Anderson
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

Equipment for the flask to carboy transfer needs to be cleaned and appropriately autoclaved. Carboys need to be filled with sterilized media. The flask transfer tops will replace the regular flask lids. Then culture can be transferred using a pressure gradient. The inoculated carboy can then be placed on the rack and cultivated.

2.2. Process diagram: Work Instruction



2.3. Process steps

2.3.1. Set Up.

2.3.1.1. Obtain a carboy that has already been assembled and fill with the requested media. See Culture Request Form for media details. LC-01-001-004

2.3.1.2. Remove the parent flask of culture that is to be transferred and place on table near carboy from **2.3.1.1.**

2.3.1.3. Obtain transfer lids that have been autoclaved.

2.3.1.4. You will need to input data for the culture transfer being performed. The culture room transfer database is in the Culture room folder; create a new tab for the current month and fill in all other data according to column title. **See Figure 1**.

								Culture
ID	Start Dat	Inoculum origin	Culture ID	Strain	Type of culture	Volume of medium	Media	In
SE00107	1/5/2014	SE00107-141222-08	SE00107-150105-01	SE00107	E10	200mL	IABR-10AL3-101	
SE61268	1/5/2014	SE61268-141222-24	SE61268-150105-02	SE61268	E10	200mL	IABR-10AL3-101	
SE61289	1/5/2014	SE61289-141222-83	SE61289-150105-03	SE61289	E10	200mL	IABR-10AL3-101	
SE61330	1/5/2014	SE61330-141222-62	SE61330-150105-04	SE61330	E10	200mL	IABR-10AL3-101	
SE61333	1/5/2014	SE61333-141222-65	SE61333-150105-05	SE61333	E10	200mL	IABR-10AL3-101	
SE00004	1/5/2014	SE00004-141222-02	SE00004-150105-06	SE00004	E10	200mL	IABR-6AL3-101	
SE70210	1/5/2014	SE70210-141222-68	SE70210-150105-07	SE70210	E10	200mL	IABR-6AL3-101	
SE70215	1/5/2014	SE70215-141222-74	SE70215-150105-08	SE70215	E10	200mL	IABR-6AL3-101	
SE70218	1/5/2014	SE70218-141222-80	SE70218-150105-09	SE70218	E10	200mL	IABR-6AL3-101	
SE00087	1/5/2014	SE00087-141222-51	SE00087-150105-10	SE00087	E10	200mL	ErdINST-303	
SE50416	1/5/2014	SE50416-141222-55	SE50416-150105-11	SE50416	E10	200mL	ErdINST-303	
TR00002	1/5/2014	TR00002-141125-11	TR00002-150105-12	TR00002	E10	500mL	TRITON HSM	
TR00002	1/5/2014	TR00002-141125-11	TR00002-150105-13	TR00002	E10	500mL	TRITON HSM	
TR00002	1/5/2014	TR00002-141125-11	TR00002-150105-14	TR00002	E10	500mL	TRITON HSM	

Figure 1- Culture Room Transfer Database.

2.3.1.5. Print labels of the daughter carboy using a label maker. See Figure 2 and 3.

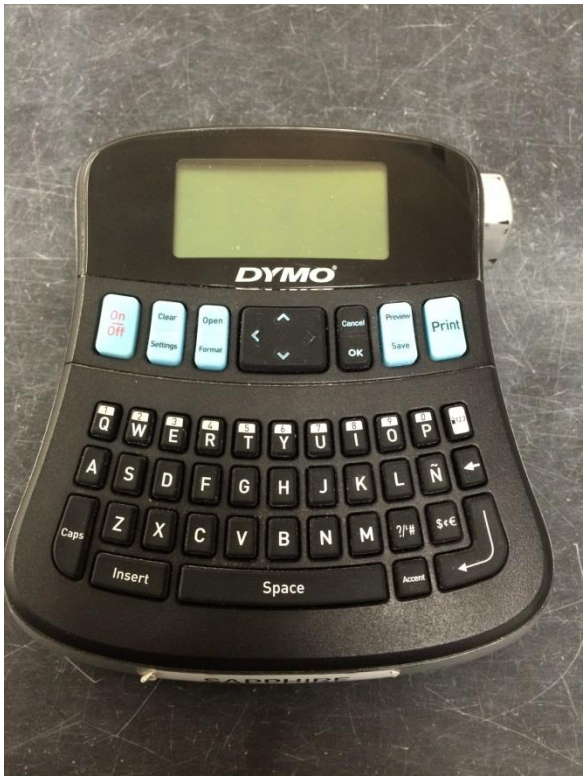


Figure 2 – Label Maker.

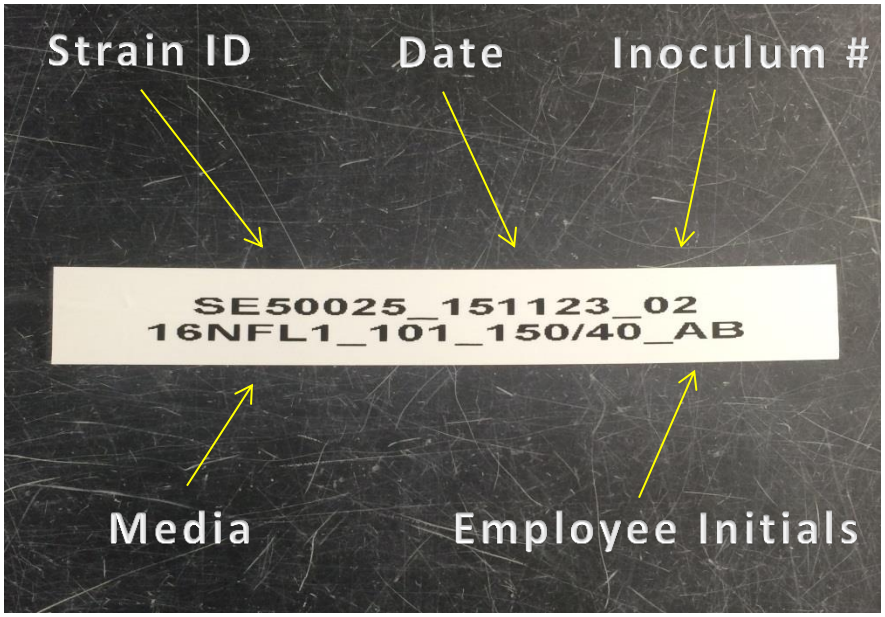


Figure 3– Proper Label format.

2.3.2. Transfer culture

2.3.2.1. All transfer tops should be packaged, wrapped, and taped in autoclaved bags. **DO NOT USE A TRANSFER TOP THAT IS NOT AUTOCLAVED!!** Take the autoclaved transfer tops and the flasks into the laminar flow hood. See figure 4.

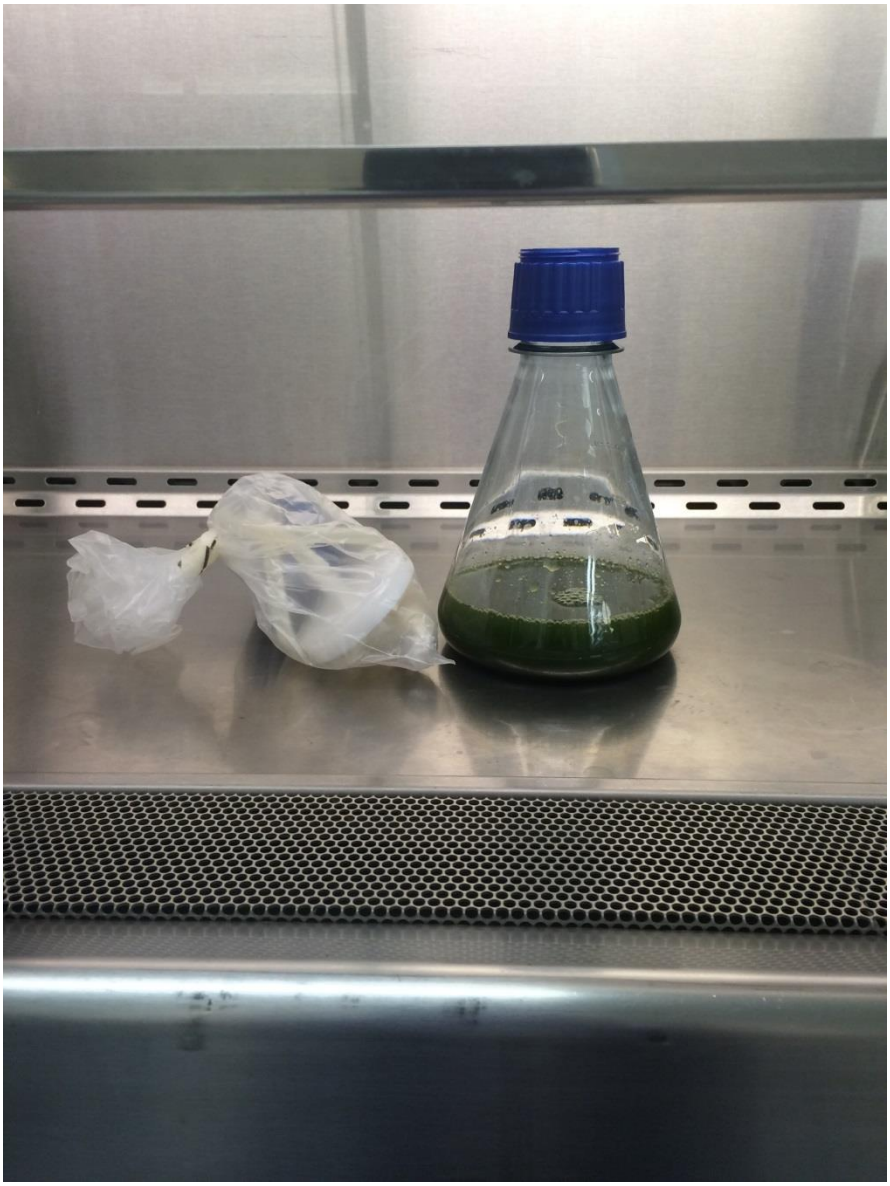


Figure 4 – Autoclaved transfer lid and a flask ready for assembly. Do not open autoclaved instruments or flasks with culture until inside sterile Laminar Hood; perform assembly of transfer top to flask inside the Laminar Hood.

2.3.2.2. Remove one flask lid at a time and replace it with a transfer top. See figure 5.

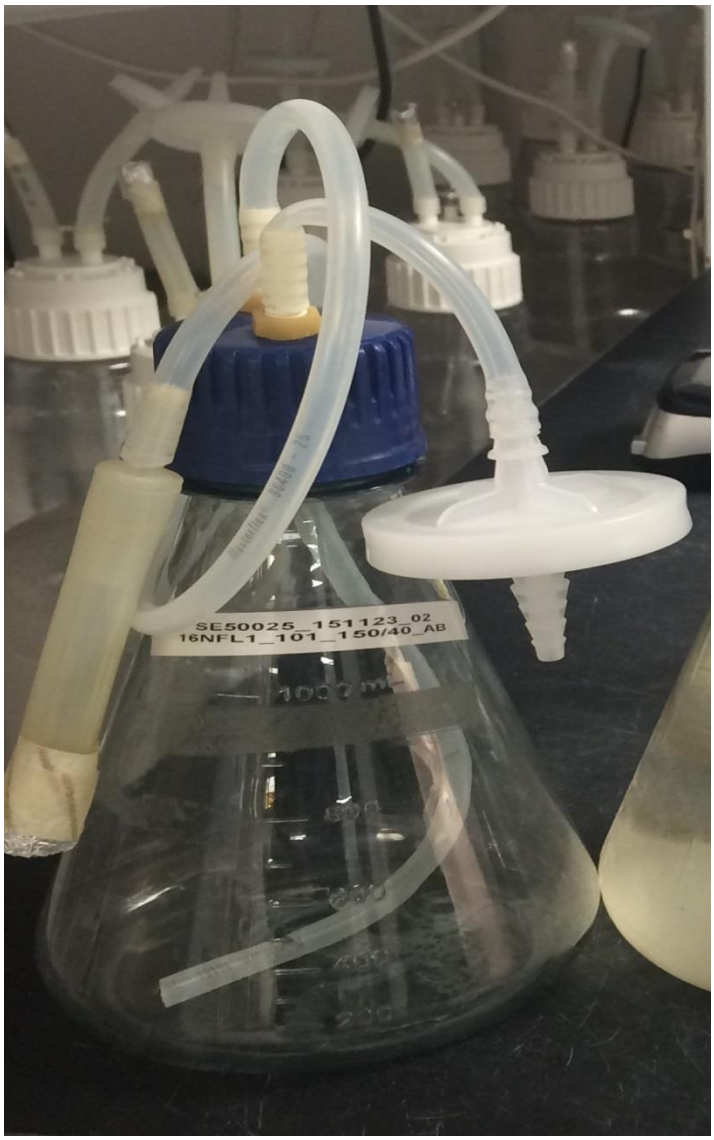


Figure 5 – Flask with transfer top.

2.3.2.3. Remove the flasks with transfer lids from the flow hood.
2.3.2.4. Spray area around intake filter of carboy with 70% ethanol. Remove intake filter from carboy; place tubing end of filter in alcohol bath. There is 70% Ethanol and 70% Isopropanol stored in bulk in the culture room flammables cabinet. You can use a small flask or small beaker to pour the ethanol for this procedure. ***Note: Ethanol is the better solution for the spray bottle; Isopropanol is a better solution for soaking the tubing ends.***

Figure 6 – Flask with transfer top, IPA Bath, and spray bottle with 70% ethanol.

2.3.2.5. Connect the long outlet hose of the transfer top to the inlet of the carboy lid (filter side).
2.3.2.6. Connect the filter of the transfer top to an air source and let the culture transfer/flow completely to the carboy. See figure 7.

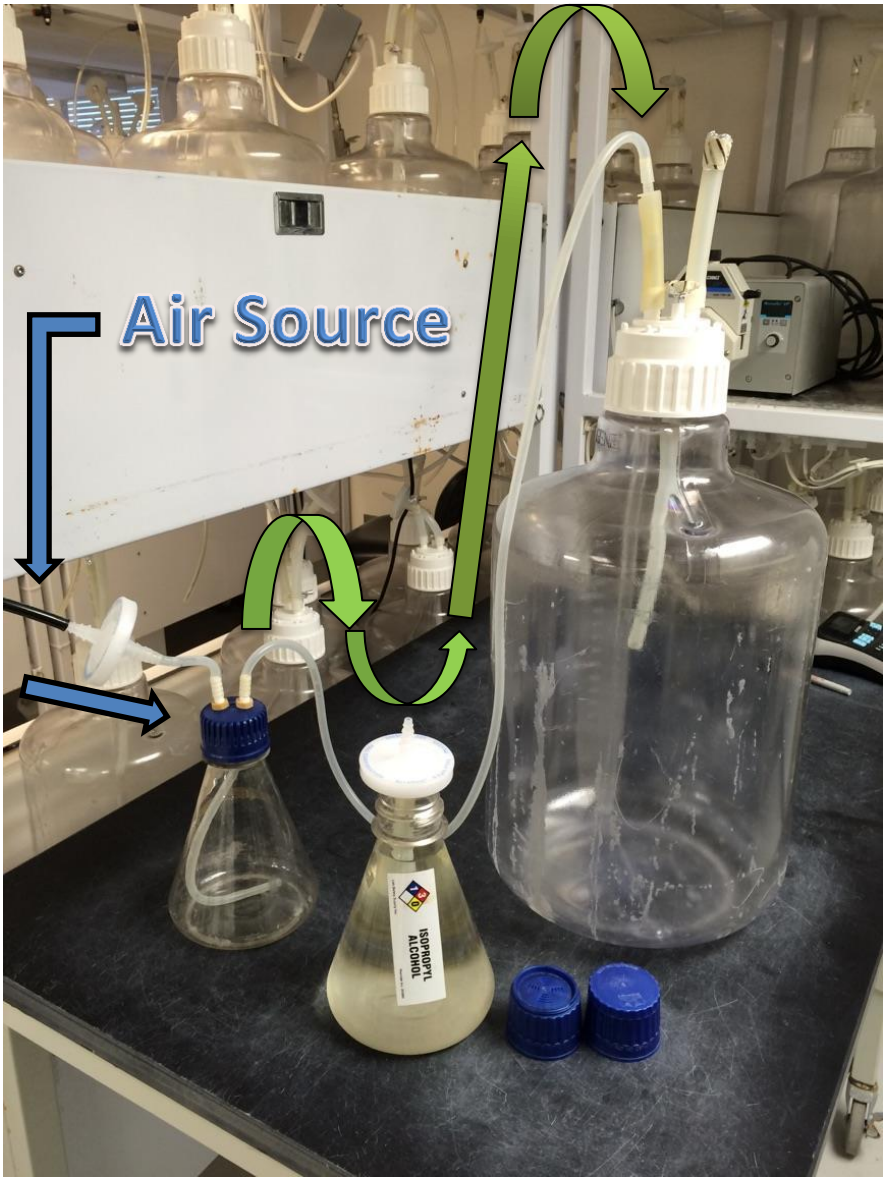


Figure 7 – Set up of air flow to transfer the culture into carboy.

2.3.2.7. Spray area around intake filter of carboy with 70% ethanol. Remove the transfer hose from the carboy and reattach the filter.

2.3.3. Rack Carboy.

2.3.3.1. Place carboy(s) on the designated carboy racks that are prepped and ready for carboy environment.

3. Required documents

3.1. Input documents
Media Recipe Sheets
Culture Request Form

3.2. Output documents
NA

4. Document control

4.1. Revision history
R0 – Thomas A. Holguin 12/3/2015

4.2. Document approval
Becky Ryan 1/29/2016

4.3. Document reviewers
Abe Anderson 12/3/2015

5. Risk analysis
NA