1. Procedure summary

This procedure describes how to transfer algae from a petri plate to liquid transfer (flasks).

1.1. Related Procedures

Laminar Flow Hood Use	LC-01-001-018

1.2. Procedure impacts and concerns

Safety	All proper PPE should be worn including safety glasses, safety toe shoes and appropriate gloves. The MSDS for chemicals used in this SOP should be reviewed.
Quality	NA
Delivery	NA
Environmental	NA
Cost	NA
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 users list, MSDSs and label information will be available for easy reference in a binder in the administration building.

1.3. Responsibilities and owners

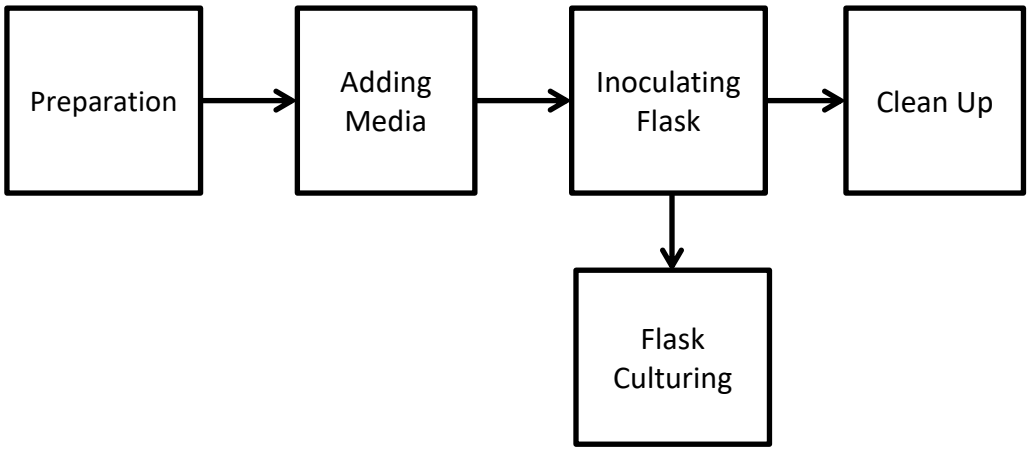
Document Owner	Manage content and distribution	Thomas A. Holguin
Process Owner	Responsible for content and process validation	Phil Lee
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

This process describes the method used to perform a sterile transfer of culture from petri plate to flask. The purpose is to scale up inoculums from petri to flask.

2.2. Process diagram: Work Instruction



2.3. Process steps

2.3.1. Preparation

2.3.1.1. All culturing must be done in a sterile flow hood. See Laminar Flow Hood Use SOP. 01-001-018

2.3.1.2. Spray all supplies with ethanol and immediately place into hood. Refer to Appendix 1 for list of supplies and **Laminar Flow Hood SOP**.

2.3.1.3. Spray gloves with ethanol and rub hands together before placing into hood.

2.3.2. Adding Media to flasks

2.3.2.1. Place empty flask on left side of media bottle.

2.3.2.2. Flask size is usually 125ML, but size can depend on quantity of inoculation.

2.3.2.3. Loosen lids on flasks and media bottle.

2.3.2.4. Using your thumb and index finger from one hand, remove lid from the bottle.

2.3.2.5. While holding the media bottle lid with your thumb and index finger, remove the lid from the flask with your middle finger and palm of hand.

2.3.2.6. Using your hand that is holding the media bottle, pour appropriate amount of media into flask.

2.3.2.7. Replace flask lid then media bottle lid.

2.3.3. Inoculating Flask

2.3.3.1. Remove surgical tape from parenting plate.

2.3.3.2. With sterile inoculating loop remove a full streak of culture from parenting plate.

Note: It is an Aseptic technique to use a small container to hold used inoculating loops and other trash while working inside the Laminar Flow Hood.

2.3.3.3. Keep in mind that you should remove the appropriate amount of culture from the parenting plate to be inoculated with exact measured media.

2.3.3.4. Using thumb and index finger remove lid from flask.

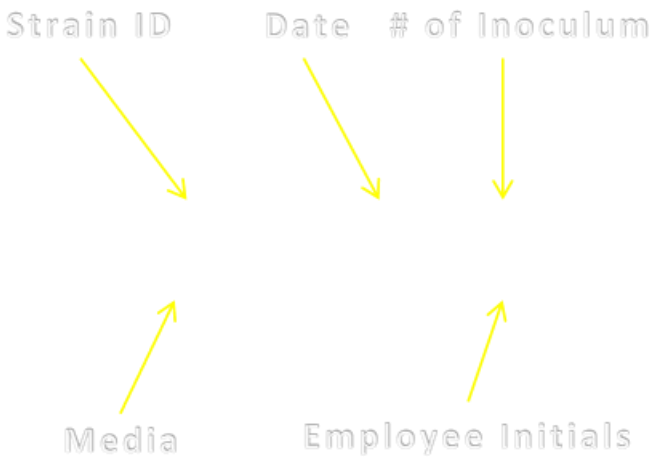
2.3.3.5. Place top of flask between middle and ring finger. Lean flask to the side.

2.3.3.6. Put inoculating loop into media and vigorously swirl the loop. Ensure culture is removed from loop.

2.3.3.7. Remove loop from flask and discard into trash container.

2.3.3.8. Replace lid on flask.

2.3.3.9. Properly label flask with culture ID, date, and sample number. See Figure 1.



Date is written year/month/day.
At the start of each day the sample number starts at one.
Each plate gets its own sample number.

Figure 1- Labeling format for plates, flasks, and carboys.

2.3.3.10. Place flask on shaker table

2.3.3.11. Enter information in culture room transfer database found here: N:\Production\Culture Room\Culture Room\Culture Room Notebook Transfers, under the tab for the current month. Print this page and paste into culture room notebook.

2.3.4. Flask Culturing

2.3.4.1. Set the shaker table rotational speed to 150 rpm

2.3.4.2. Turn on CO_2 and adjust flow to 1L/min

2.3.4.3. Turn on overhead lights

2.3.4.4. Flask cultures should be disposed of if not used within 30 days

2.3.5. Clean Up

2.3.5.1. Remove all supplies and trash from hood.

2.3.5.2. Spray inside of hood with 70% Ethanol and wipe down

2.3.5.3. Close hood and turn UV light on.

3. Required documents

3.1. Input documents

NA

3.2. Output documents

NA

4. Document control

4.1. Revision history

R1- Thomas A. Holguin	7/02/2015

4.2. Document approval

Becky Ryan	???

4.3. Document Reviewers

Abe Anderson	7/19/2013
Eric Ruvacalba	7/24/2013

5. Risk analysis

NA

6. Appendix 1. Supplies needed for petri to flask transfer

1. Parent petri plates
2. Sterile inoculating loops
3. Autoclaved 1L flasks with venting lid
4. Sterile desired liquid media
5. Label maker
6. 70% ethanol in spray bottles
7. Surgical tape
8. Culture room notebook
9. Culturing environment (Shaker table, Box, lights, CO_2)