1. Procedure summary

This procedure describes how to transfer culture between San Diego and the Las Cruces Test Site (LCTS).

1.1. Related Procedures

Sequencing for Identification	LC-06-001-006

1.2. Procedure impacts and concerns

Safety	Proper PPE and caution should be used when handling Algae and Media, including safety glasses and gloves.
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.

1.3. Responsibilities and owners

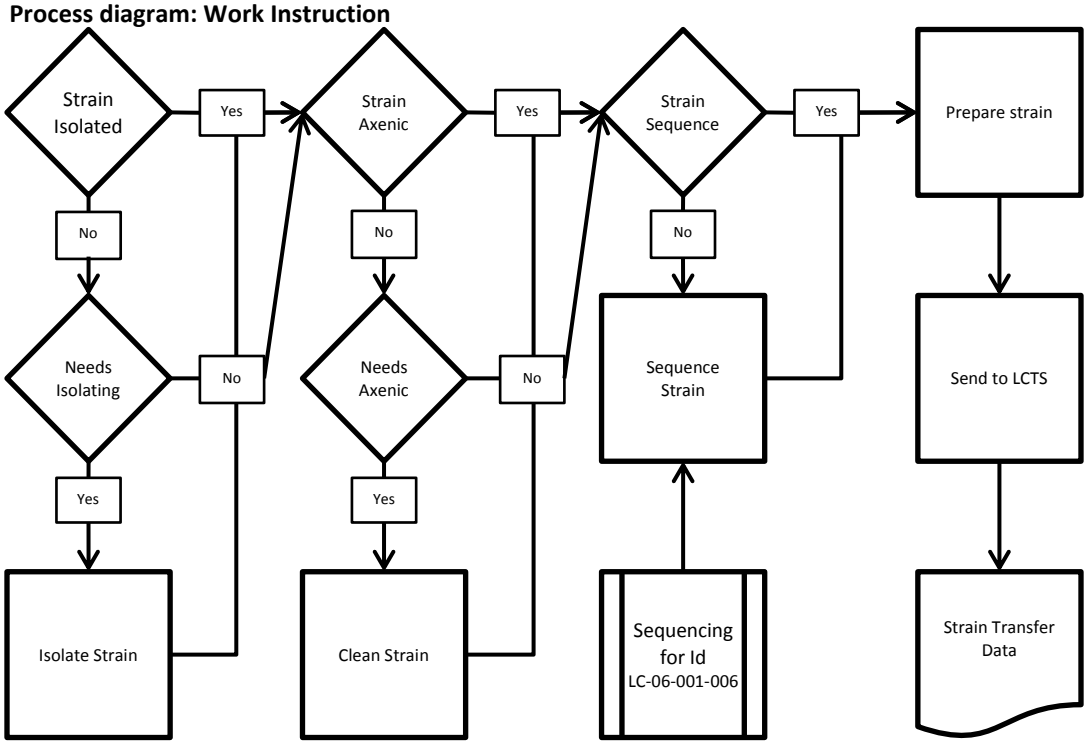
Document Owner	Manage content and distribution	Alex Diffley
Process Owner	Responsible for content and process validation	Becky Ryan
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

The process describes the procedure to be followed when transferring culture between Sapphire San Diego and Sapphire Las Cruces Test Site (LCTS). It includes quality controls that need to be carried out prior to shipping a strain, as well as detailing information that needs to accompany strains that are shipped to the LCTS.

2.2. Process diagram: Work Instruction



2.3. Process steps

2.3.1. Strain Status.

Determine if the strain is isolated. This is typically done by passing the culture through a single cell bottleneck (this can be done a number of ways, flow sorter, micro dissection, plating etc…). Isolation can be verified by sequence analysis. Phenotypic validation is not ideal, but can give an immediate indication of possible contamination: It should be used for indicating contamination, but not for ruling it out.

2.3.1.1. If the strain is not isolated, and needs to be isolated this should be done by which ever means most appropriate for the strain.

2.3.1.2. If the strain is not isolated and does not need to be isolated, then proceed to the next step. A strain does not need to be isolated if sufficient effort and appropriate technique has been exerted to isolate the strain, with no success. A strain also may not need to be isolated if there is appropriate indication that performance of the strain is not harmed by other microalgae present in the sample, and that there is no reasonable risk of losing dominance of the strain of interest in the sample. It should be noted that SD and LCTS often handle strains in different ways or systems, and the strain of interest must remain dominant in the sample during handling at either site.

2.3.2. Strain Axenic.

Determining whether the strain is clean can be done in a number of ways. Streaking on relevant media (what it grows on) + YA or TAP-YA plates, followed by incubation at 32^{o}C for 24-48 hours, is the simplest way to verify whether a culture has bacterial contamination or not and is the minimum requirement of this procedure.

2.3.2.1. If the culture is contaminated, and needs to be cleaned (this is the purview of the experiment owner and the resource holder at LCTS) this can be done either by sorting or by growing in selective media.

2.3.1.2. If the strain is not axenic and does not need to be axenic, then proceed to the next

step.

2.3.2.3. A strain does not need to be axenic if sufficient effort and appropriate technique has been exerted to "clean" the strain, with no success, and if there is appropriate indication that performance of the strain is not harmed by other organisms present in the sample.

2.3.3. Strain Identification.

Prior to a strain being shipped, the strain needs to have been sequenced. The region for eukaryote strains is the ITS1+2 regions, and the region for cyanobacteria is the 16S region.

2.3.3.1. If the strain does not have a sequence, it should be sequenced. Refer to the **Sequencing for Identification SOP** for directions on how to generate this sequence.

2.3.4. Preparing strains for shipment.

The strains should either be shipped on plates or in liquid culture. The media should be the relevant media for culturing the strain.

2.3.4.1. Plates with visible colonies free from contamination should be sealed with parafilm, and shipped in an appropriate container overnight to the LCTS Monday through Wednesday. An appropriate container is properly resistance to plates moving and overheating, and will not leak.

2.3.4.2. Liquid culture should be sealed in an appropriate container and shipped overnight to the LCTS Monday through Wednesday. An appropriate container is fully sealed and leak proof, and also wrapped with parafilm or similar to guard against any leakage at the closure. An appropriate container is not a culture flask with breathable lid, common for growth of strains in the lab – this lid is not water tight.

Do not ship samples on Thursday to account for shipping delays that may cause samples to arrive on weekend.

2.3.5. Deposit Strain in the Sapphire Culture Collection (SCC)

If a strain is being sent to the LCTS then it has demonstrated a phenotype of interest and must be deposited into the SCC. Reference the SCC Protocol for Depositing a Strain into the SCC. **Sapphire strain ID number will be assigned upon submission to the SCC.**

2.3.6. Filling out Strain Transfer Data Sheet

The Strain transfer sheet is available in the SOP documents folder (N:\Cultivation Group\SOP's\Active Documents). It should be filled out with each strain transfer. Here is an elaboration on each of the fields that need to be populated. All fields need to be populated.

Strain Identification Number: All strains shipped to LCTS must have a Strain ID number (see 2.3.5).
Proposal ID: This will be issued once the proposal has been accepted
Algae genus: What Genus is the algae (e.g. Scenedesmus, Arthrospira). This information will come from blasting the strain sequence to a relevant database.
Sequence: What region was sequenced for identification?
Sequence link: Where is the sequence located on the company drive?
Culture condition: What is the culture condition (e.g. Axenic, unialgal, etc.)?
Transport media: What media are the strain(s) transported in?
Liquid media: What liquid media should the strains be grown in?
Solid media: What solid media should the culture be grown on?
Optimal pH: What pH should the culture be scaled at?
Optimal temperature: What temperatures ranges should the culture be scaled at?
Plates: What plate media are the culture transported on?
Flasks: What liquid media are the culture transported on?
Ship date: What date are the strains shipped?
Carrier: What shipping carrier is being used?

Tracking number: What is the tracking number?
Prepared by: Who prepared the strains?
Special notes: Are there any special notes? E.g. Is the strain is particularly light sensitive? Does the strain have an unusual growth phenotype? Are there special C02 conditions needed for the strain? Who grew these strains in SD? Etc.
Strain image: Image of the strain at 40x objective lens magnification in bright field as well as what conditions the culture that is being photographed was propagated in (stationary phase, high light etc…)

2.3.7. Shipping strains to LCTS.

All strains should be shipped overnight to the LCTS. Each shipment should be accompanied by an email notifying LCTS that the shipment has departed, and should have as a completed **Strain Transfer Data Sheet LC-01-001-017.R1** attached. Each package should be accompanied by a physical copy of the Strain Transfer Data Sheet in the box.

2.3.8. Exceptions

Any exceptions to this SOP must be approved in an email by the LCTS resource holder. A printout of the email must accompany the Strain Transfer Sheet. The strain transfer sheet must also contain notes in the Special Notes section regarding the reasoning for the exception.

3. Required documents

3.1. Input documents

NA

3.2. Output documents

Strain Transfer Data sheet	LC-01-001-017.R3

4. Document control

4.1. Revision history

R0 – Initial Release – Rob McBride	03-10-2012
R1 – Robert McBride	05-01-2012
R2 – Amanda Provencio	07-12-2013
R3 – Bruce Miles	11-08-2013

4.2. Document approval

Becky Ryan	11-08-2013

4.3. Document reviewers

Abe Anderson	11-08-2013
Eric Ruvalcaba	11-08-2013

5. Risk analysis

NA