1. Procedure summary

This procedure describes how to ensure that the culture integrity of a pond is assayed in outdoor raceway experiments.

1.1. Related Procedures

Sequencing for Identification	LC-06-001-006

1.2. Procedure impacts and concerns

Safety	Site and Lab safety procedures should be followed while executing this SOP.
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 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

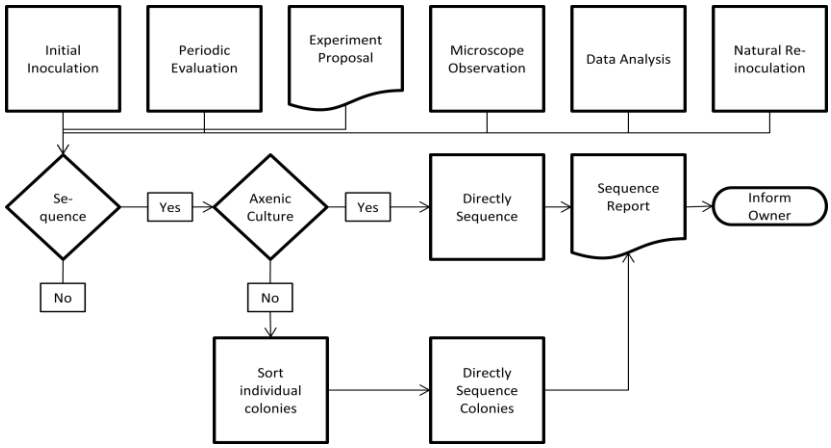
Document Owner	Manage content and distribution	Micheal Burnett
Process Owner	Responsible for content and process validation	Philip Lee
Site Manager	Responsible for implementation and conformance	Becky Ryan

2. Process

2.1. Process description

The process describes when and how to sequence a pond to ensure that the genetic integrity of the pond is intact. For the inoculation, the amount of sequencing depends on the source of the culture. If the culture is from an outdoor raceway, more sequencing may be required to ensure the culture's identity is known, whereas if the culture is from the culture train, less sequencing may be required. Once the experiment is set up, the pond may be sequenced every 3 months, upon conclusion or after culture interruption and natural re-inoculation, to ensure the culture identity is known. Frequency is determined by the Senior Manager of Field Testing, Pond Manager and Experiment Owner. The process also describes when sequencing should be triggered outside of the above mentioned timeframes, specifically when microscope observations, flowcam analysis or review of culture data indicate, as determined by the Senior Manager of Field Testing, Pond Manager and Experiment Owner.

2.2. Process diagram: Work Instruction



2.3. Process steps

2.3.1. Determine if sequencing is necessary

The Senior Manager of Field Testing, the Pond Manager and (if applicable) the Experiment Owner should determine whether sequencing to verify culture integrity is required at the following decision points.

2.3.1.1. Initial Inoculation. The decision to verify culture integrity should be made upon the initial inoculation of an experimental pond.

2.3.1.1.1. If the culture used for inoculation is believed to be axenic (i.e. from the Culture Room), samples can be directly sequenced as described in 2.3.2 at least 2 weeks before inoculation.

2.3.1.1.2. If the culture used for inoculation is not axenic (e.g. it is being drawn from an outdoor pond), samples must be sequenced as described in 2.3.3. at least one month before inoculation.

2.3.1.2. **Periodic Culture Integrity Check.** The decision to sequence to verify culture integrity should be made periodically for all outdoor cultures.

2.3.1.2.1. For experiments of less than 3 months, the decision to sequence should be made at the conclusion of the experiment. If sequence verification is determined to be required, sequencing should proceed as described in 2.3.3.

2.3.1.2.2. For experiments that run for periods greater than three months, the decision to verify culture integrity should be made at 3 month intervals. If sequence verification is determined to be required, sequencing should proceed as described in 2.3.3.

2.3.1.3. **Per Experimental Procedure.** The decision to verify culture integrity should be made as specifically outlined in the Experimental Procedure. If sequence verification is determined to be required, sequencing should proceed as described in 2.3.3.

Some experiments may require sequencing more often e.g. those that evaluate culture transition or to monitor a poly-culture experiment

2.3.1.4. **Microscope Observations**. The decision to sequence to verify culture integrity should be made if microscopic observation or flowcam analysis of the culture indicates significant levels of contamination.

2.3.1.4.1. If during the course of regular pond monitoring microscopic observations or analysis of other pond data

indicate there are contaminant strains, these should be quantified using heamocytometer (3 counts of 100 cells) or the flow cam (3000 particles).

2.3.1.4.2. If the heamocytometer (LC 06-001-003) or flowcam (LC 06-001-004) shows that the average composition of the pond is less than 75% of the expected strain, the Senior Manager of Field Testing, the Pond Manager and (if applicable) the Experiment Owner should determine if the pond should be sequenced as described in 2.3.3.

2.3.1.4.3. If the culture is >25% foreign algae, the strain ID of the culture is changed to reflect the presence of other strains. The Senior Manager of Field Testing and Pond Manager should determine if a novel strain should be analyzed further and if a novel strain should be transferred to the Sapphire Strain Collection as described in the Strain Transfer SOP (LC 06-001-017)

2.3.1.5. Data Review. If the review of assembled pond data indicates a significant change in culturing parameters has occurred, the decision to sequence to verify culture integrity should be made. If sequence verification is determined to be required, sequencing should proceed as described in 2.3.3.

2.3.1.6. Natural Repopulation after Culture Interruption. If a pond repopulates naturally after a culture Interruption, the decision to sequence to verify culture integrity should be made. If sequence verification is determined to be required, sequencing should proceed as described in 2.3.3.

2.3.2. Direct Sequencing of putative axenic culture

2.3.2.1. The Experiment Owner should ensure that samples are submitted along with a completed Sample Submission form. The Submission form should specify expected strain ID, primers to be used and a brief description of the reason(s) for sequencing.

This process requires ≈ 2 weeks to complete.

2.3.2.2. DNA should be extracted directly from the culture and sequenced without cloning. Reference the Sequencing SOP (LC 06-001-006) for details.

2.3.2.4. A sequencing report should be circulated.

2.3.2.5. The Experiment Owner, Senior Manager of Field Testing and Pond Manager should determine if the culture should be used for the proposed experiment, or if additional sequencing is required.

2.3.3. Sequencing of non-axenic culture (via strain isolation)

2.3.3.1. The Experiment Owner should ensure that samples are submitted along with a completed Sample Submission form. The Submission form should specify expected strain ID, primers to be used and a brief description of the reason(s) for sequencing.

This process requires ≈ 1 month to complete.

2.3.3.2. The culture should be sorted or streaked for individual colonies on permissive solid media. The sorting or plating should aim to produce at least 96 individual algal colonies. These plates should be incubated until the appearance of colonies.

Typically, three plates will be sorted, each with 96 colonies. Polyculture ponds may require additional plates.

2.3.3.3. 48 colonies should be sequenced. DNA should be amplified and directly sequenced without cloning. Reference the Sequencing for Identification SOP (LC 06-001-006) for details.

2.3.3.4. A Sequencing Report should be circulated.
2.3.3.5. The Experiment Owner, Pond Manager and Senior Manager of Field Testing should determine whether the experiment should be allowed to proceed or if additional sequencing is required.

3. Required documents

3.1. Input documents

Experiment Proposal
LCTS Sample Submission Form

3.2. Output documents

LC-01-003-011 R1 – Culture Integrity Check - Detailed Sequencing Report
LC-01-003-011 R1 – Culture Integrity Check - Sequencing Report Summary

4. Document control

4.1. Revision history

R0 – Initial Release – Rob McBride	03-10-2012
R1 – Robert McBride	04-27-2012
R2 – Phil Lee, Mike Burnett, Becky Ryan	02-06-2013

4.2. Document approval

Robert McBride	04-27-2012
Becky Ryan	02-06-2013

4.3. Document reviewers

Robert McBride, Phil Lee, Becky Ryan, Mike Burnett	02-06-2013

5. Risk analysis