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Lemna minor (duckweed) sterilization protocol V.2

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is designed to sterilize fronds of the common duckweed (*Lemna minor*) but should also be applicable to other members of the *Lemnoideae*. It involves both the surface sterilization of plants in bleach and testing for the presence of bacteria on plants through culturing in enriched media as a test for successful sterilization.

Duckweed Sterilization : Preparation

1 In a tea-strainer, strain duckweed plants under running water (doesn't have to be sterile). This step gets rid of some surface algae.

2 Move surface-rinsed duckweed into a water bath; remove up to 50 plants from water bath and place in 50 mL Falcon tube approximately half full with autoclaved Phosphate Buffered Saline (PBS).

3 Vortex or shake intensely for 5 minutes.

Duckweed Sterilization : Sterilization

4 All of the subsequent sterilization steps should take place in a safety cabinet.

5 Strain plants into a tea-strainer reserved for bleaching. Plants stuck to the side of the Falcon tube can be dislodge with distilled water.

6 Place in (1% sodium hypochlorite) bleach for 60 seconds.

7 Remove from bleach; rinse tea-strainer thoroughly by moving around in jar of autoclaved distilled water.

8 Soak in autoclaved distilled water for 5 minutes or more. Repeat this step 3 times, in a new jar of fresh autoclaved distilled water each time.

9 Open tea-strainer; pour autoclaved medium (e.g. Hoagland's, Krajncic medium) over tea-strainer to dislodge plants. This should be done over a clean, autoclaved jar (I use 500 mL Mason jars). Seal with lid and move to environmental chamber.

Checking Duckweed Sterility

10 Wait at least 3 days to give plants a chance to recover from the bleaching.

11 In the safety cabinet (or other sterile location), transfer plants to autoclaved Mason jar approximately half full of your ENRICHED media of choice. I add 1 g yeast extract and 10

g sucrose per L to make it enriched.

12 In as little as 2-3 days, you will know if your duckweed remains contaminated with bacteria — in this case, the media will become opaque as microorganisms replicate. If the media remains clear, congratulations!!! You have successfully sterilized your duckweed plants.

13 If the media is cloudy, wash plants and transfer to Mason jar with autoclaved, unenriched media (in safety cabinet). After plants have recovered (ie. look green and healthy, after a week or more) you can re-sterilize them until all bacteria are removed.