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# Algae strain maintenance (*Chlamydomonas reinhardtii*)

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Works for me

This protocol is published without a DOI.

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## KEYWORDS

Microalgae, Recombinant, electroporation, plasmid, Media, Algae, *Chlamydomonas reinhardtii*, Strain maintenance, algae, agar plate

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## GUIDELINES

All steps described in this protocol are intended to be conducted in a research laboratory. Follow aseptic procedures.

## SAFETY WARNINGS

Use EPIs at all times.

UV radiation is harmful, protect your skin.

## DISCLAIMER:

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## BEFORE STARTING

Prepare the work place (i.e: Clean and disinfect with ethanol 70%), if in a biological cabinet, follow the UV disinfection protocol.

Plate preparation

- 1 Always follow aseptic techniques
  1. Autoclavate freshly prepared TAP media with **1.5 Mass / % volume bacteriological agar** with a magnetic bar inside
  2. Mix the flask using a magnetic stirrer to evenly dissolve the agar in the solution
  3. For antibiotic containing plates, cool down the flask temperature until **55 °C - 60 °C** or until it is possible to hold it without hurting the skin.
  4. Add antibiotics in the desired amount, and mix using a magnetic stirrer to evenly dissolve the antibiotic
  5. Pour the molten media in sterile petri dishes. (Keep volume constant among plates).
  6. Wait for media to solidify
  7. Stock at **4 °C - 8 °C** until use. (Storage for 2 months was used without noticeable difference)

#### Innoculation

- 2
  1. Keep agar plates in the bench until they reach **Room temperature**
  2. From a plate or liquid culture containing the algae sample, collect a portion and apply it to the fresh plates. From a dense culture ( **1 x 10<sup>7</sup> cells/mL** ) **50 µl** to **100 µl** suffice. From an agar plate, scratch the lawn with the inoculation loop until the loop is full.
  3. Spread the cells in the new plate
  4. Wrap the plate with parafilm, and place it at **25 °C** , 60-80 µmol photons/m<sup>2</sup>s for **120:00:00** (5days).

#### Storing

- 3 Place plates with the newly grown cells at a dark environment and **Room temperature** After . (Dark is needed to avoid overgrowth of the cells, due to photosynthesis. Media contains acetate which keeps the cells viable over a month).

#### Re-stocking

- 4 After **672:00:00** (1 month), cells should be passed to a new plate. Longer periods are possible, but increase the chance of losing the strain.