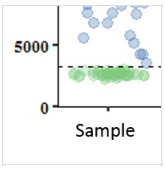

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Fluorescent mutants screening in 96 well plates - Chlamydomonas reinhardtii

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1 *Works for me* This protocol is published without a DOI.


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ABSTRACT

This protocol is intended for screening experiments with algae cells. Nevertheless, with modifications, it can be used for other microrganims.
It is possible to use this protocol with deep-well plates, with modifications.

PROTOCOL CITATION

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KEYWORDS

96 well plate, Chlamydomonas reinhardtii, growth, High-throughput screening (HTS)

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GUIDELINES

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

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BEFORE STARTING

Separate all material needed for the protocol

Plate preparation 10m

10m

1 For 96 well plates, the usual max volume is ~ **330 µl** .

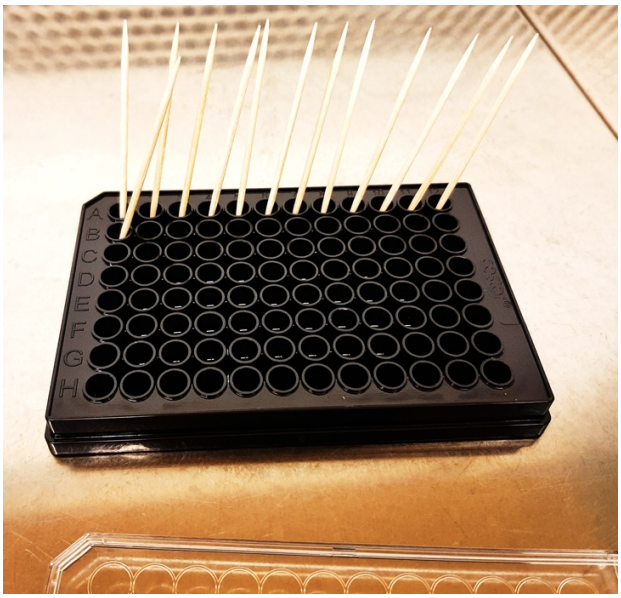
1. Clean and desinfect a biological cabinet
2. Place all materials inside. (*e.g: sterile tips, media, sterile 96 well plates with lids, pipettes*)
3. Add **160 µl TAP media** per well (Or another media) on the choosen plate. (*For fluorescent experiments, black plates are recommeded. Clear bottom allows to simultaneously check absorbance*)

Colony picking 15m

15m

2
1. Use sterile tooth picks to collect individual colonies.
2. Place tooth pick into the well
3. Proceed to the next colony
4. After collecting the desired number of colonies, mix the tooth pick in the media by spining it
5. Remove and discard the used tooth picks
6. Visually inspect the presence of green material inside the wells.

Sterile toothpicks

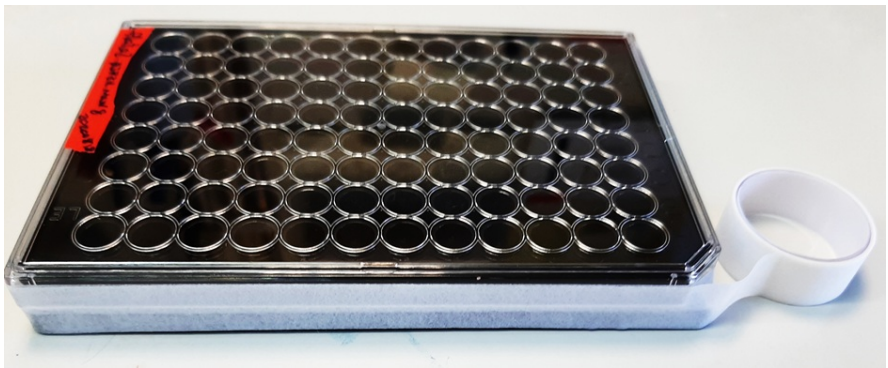


Colony picking with toothpick

Plate wraping 2m

2m

3
1. Add the lid to the plate
2. Wrapp it with a porous tape (Microporous), 3 laps around the plate, taping together the lid and plate.
3.



Growth 5d

5d

4 1. Add plates to a microplate shaker

2. Set the shaker to continuous mode, **900 rpm**
3. Illuminate with 60-80 µmols de photons/m^2s, at **25 °C**
4. Grow the cells for **120:00:00** (5 days) *(Important to let cells grow enough to make the reads, 5-7 days have been tested)*

Reading 20m

5 20m

1. Centrifuge the plates **2000 x g, 25°C, 00:03:00** to remove any condensation to the lid
2. Add the plates to the microplate shaker
3. Set the shaker to **00:10:00** , 900 RPM
4. Place plates in the Plate reader with the desired reading settings.
5. Analyse the results