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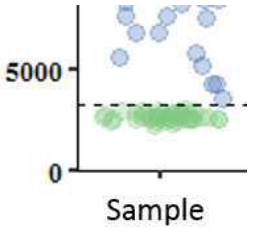
Fluorescent mutants screening in 96 well plates - Chlamydomonas reinhardtii



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

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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.

Guidelines

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

Before start

Separate all material needed for the protocol

Plate preparation

10m

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

10m

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

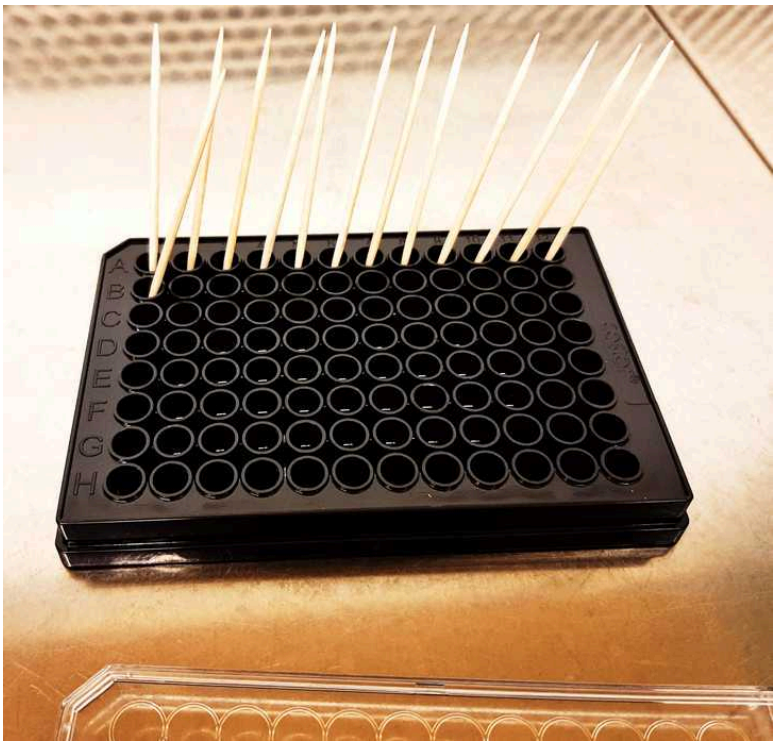
15m

2

15m

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

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.

2m

Growth

5d

4

5d

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