Protein secretion efficiency of construct in Chlamydomonas reinhardtii with fluorescent protein

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Abstract

This protocol describe how to asess secretion efficiency of diferrent constructs in *Chalmydomonas reinhardtii*, using a fluorescent protein. Colonies are picked from transformation plate and inoculated on TAP media for growth. A high number of colonies is picked to avoid bias on colonie picking.

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Guidelines

Cell cultures in 96 well plates should ideally be cultivated at high orbital shaking (>800RPM) to avoid suboptimal growth.

Before start

- Separate 96 well plates
- Prepare sufficient media
- Separate gas exchangable seal

Protocol

Cell growth

Step 1.

1. Pippete 500 µL of TAP media to each well of a 96 deep-well plate
2. Pick single colonies with autoclavated pippete tips or teeth cleaning twigs. Add to the wells
3. Cover plate with a gas exchangeable seal (Breathe-Easy®, Sigma-Aldrich®)
4. Place plates on a rotary shaker (150 RPM) under constant illumination (50 µmol photons/m^2s), at 25 ºC for 7 days

AMOUNT
500 µl Additional info: TAP media per well

TEMPERATURE

25 °C Additional info: growth temperature

NOTES

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Low orbital shaker speed are sufficient for cell growth. Nevertheless, microtiter plate shakers can be used and enhance cell growth.

Cell and supernatant separation

Step 2.

1. Remove seal and transfer 100 µL of culture to fluorescence reading in a clear bottom 96-well plate (Corning Costar, Tewksbury, MA, USA).
2. Centrifuge the 96 deep-well plate at 3000xg for 10 min
3. Transfer 100 uL of supernatant to fluorescence reading in a clear bottom 96-well plate (Corning Costar, Tewksbury, MA, USA).

AMOUNT

100 µl Additional info: sample size for each material

Fluorescence reading

Step 3.

1. Read fluorescence in the bottom of the plates at fluorescent protein specific wavelenghts in a plate reader

2. Ex: mCherry fluorescent protein

    - Excitation: 575/9 nm
    - Emission: 608/20 nm
    - Gain: 200
3. Read fluorescence in the bottom of the plates at chlorophyll specific wavelenghts in a plate reader

4. Ex: Chlorophyll

    - Excitation: 440/9 nm
    - Emission: 680/20 nm
    - Gain: 100

NOTES

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*Compare results of the whole culture fluorescence and supernatant fluorescence to estimate secretion efficiency.

**Check chlorophyll fluorescence to surveil for contaminations or cell growth failure.

Absorbance reading

Step 4.

1. Read cell density by absorbance at 750 nm

NOTES

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Cell density can be used to normalize protein fluorescence results, and obtain a estimate of each colony productivity.