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Chlamydomonas reinhardtii nuclear transformation by electroporation. V.1

PLOS One

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ABSTRACT

This protocols describe the steps required for nuclear transformation of *Chlamydomonas reinhardtii* by electroporation.

GUIDELINES

Cell density for harvesting is important to overall transformant yields. It should be aimed to mid-log phase cells.

SAFETY WARNINGS

- High voltage is used in the electroporation, use EPIs and avoid contact with electrodes on the electroporator.

BEFORE START INSTRUCTIONS

- Separate cuvettes
- Prepare a ice bath
- Allow linearized vectors to melt

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DNA preparation

1. Digest 3-5 µg of DNA with appropriate enzymes, for 3-5h at 37°C.
2. Column purify digestion (Avoid gel purify, since vector backbone helps to prevent intracellular DNAses action)
3. Quantitate by absorbance measuring (Nanodrop)

🧴 5 µg uncut vector

🕒 05:00:00 Digestion time

🌡️ 37 °C

Cells preparation

2. 1. Aseptically inoculate 250 mL of TAP media with wild type cells. Either by scrappeing cells of a plate with a inoculating loop or from a previous cultured cells.
2. Incubate at 25°C, under constant shaking (~150-180 RPM) and light (60 µmols de photons/m²s) until a cell density of 3-6 x 10⁶ cells/mL is reached
3. Pellet cells in centrifuge tubes. Separate culture in sufficient amount of 50mL tubes (Falcons), and centrifuge for 10 min at 2000xg
4. Gently resuspend cells at **3-6-10⁸ cells/mL** in **TAP/40mM sucrose**. (250 mL of culture usually yields 10 transformations)

🧴 250 mL TAP media

🌡️ 25 °C wild type cells culture

Note

Usually cells will reach desired concentration in 3 days, depending on the amount of inoculum

00:10:00 Centrifugation for pelleting cells

Transformation

- 3
 1. Add 250 μL of resuspended cells (at $3\text{-}6\text{-}10^8$ cells/mL) to each cuvette. Pipette up and down on DNA sample. Flick cuvette to mix DNA and cells. Shake cells to the bottom of the cuvette. Also add no DNA control.
 2. Incubate cells with DNA **ON ICE** for 5-10 min
 3. Wipe cuvette (to remove condensed water) and electroporate.
 4.
 - Voltage = 800 V
 -
 - Capacitance = 25 μF
 -
 - Resistance = infinity (∞)
 -
 - Gap = 4 mm
 -
 1. Let recover for 5-10 min on cuvette
 2. Add 10 mL of TAP/40mM sucrose into one clean 50mL falcon tube per electroporation
 3. Gently transfer cells from cuvette to TAP/40 mM sucrose. Rinse cuvette with TAP/40 mM sucrose and transfer remaining cells to Falcon tube
 4. Incubate at room temperature on rocker overnight
 5. Pellet cells by centrifuging for 10 min at 2000xg
 6. Aseptically pour off supernatant. Add 600 μL of TAP/40 mM sucrose to pellet. Gently resuspend cells and pipette onto 2 plates with appropriate antibiotics. ie. 400 μL of cells per plate, and let it dry aseptically without plate cover.
 7. Parafilm and place plates under constant light ($60 \mu\text{mol photons/m}^2\text{s}$), 25 $^{\circ}\text{C}$. Colonies should be visible in 5-7 days.

00:10:00 incubation on ICE

00:10:00 incubation at Room temperature

⌚ 00:10:00 Centrifugation for pelleting cells

⌚ 18:00:00 Incubate on liquid media for cell recovering

🧪 600 μ L TAP 40 mM sucrose to re-suspend cells