Chlamydomonas reinhardtii nuclear transformation by electroporation.

João Vitor Molino,Beth Rasala,Stephen Mayfield

Abstract

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

Citation: João Vitor Molino,Beth Rasala,Stephen Mayfield Chlamydomonas reinhardtii nuclear transformation by electroporation.. **protocols.io**
dx.doi.org/10.17504/protocols.io.kfkctkw
Published: 24 Oct 2017

Guidelines

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

Before start

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

Protocol

DNA preparation

Step 1.

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

AMOUNT
5 µg Additional info: uncut vector
TEMPERATURE
37 °C Additional info:

Cells preparation

Step 2.

1. Aseptically inoculate 250 mL of TAP media with wild type cells. Either by scrappeing cells of a plate with a inocculating loop or from a previous cultured cells.
2. Incubate at 25ºC, under constant shaking (150-180 RPM) and light (60 µmols de photons/m^2s) until a cell density of 3-6 x 10^6 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. Genttly ressupend cells at **3-6-10^8 cells/mL** in **TAP/40mM sucrose**. (250 mL of culture usually yields 10 transformations)

AMOUNT
250 ml Additional info: TAP media
TEMPERATURE
25 °C Additional info: wild type cells culture

NOTES
João Vitor Molino 24 Oct 2017

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

Transformation

Step 3.

1. Add 250 µL of ressuspended cells (at 3-6-10^8 cells/mL) to each cuvette. Pippet 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 condensated water) and electroporate.
    - Voltage = 800 V
    - Capacitance = 25 µF
    - Resistance = infinity (∞)
    - Gap = 4 mm
5. Let recover for 5-10 min on cuvette
6. Add 10 mL of Tap/40mM sucrose into one clean 50mL falcon tube per electroporation
7. Gently transfer cells from cuvette to TAP/40 mM sucrose. Rinse cubette with TAP/40 mM sucrose and transfer remaining cells to Falcon tuve
8. Incubate at room temperature on rocker overnight
9. Pellet cells by centrifuging for 10 min at 2000xg
10. Aseptically poor off supernatant. Add 600 µL of TAP/40 mM sucrose to pellet. Gently re-suspend cells and pipette onto 2 plates with appropriate antibiotics. ie. 400 µL of cells per plate, and let it dry aseptically without plate cover.
11. Parafilm and place plates under constant light (60 µmols de photons/m^2s), 25 ºC. Colonies should be visible in 5-7 days.

AMOUNT
600 µl Additional info: TAP 40 mM sucrose to re-suspend cells

Warnings

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