Version 3

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Chlamydomonas reinhardtii nuclear transformation by electroporation. V.3

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1 *Works for me* Share

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DISCLAIMER

ABSTRACT

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

Here you can find a video following the protocol.

DOI

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EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0192433

PROTOCOL CITATION

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WHAT'S NEW

Better description of vector preparation

KEYWORDS

Microalgae, Recombinant, electroporation, plasmid, Chlamydomonas

LICENSE

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53124

GUIDELINES

Cell density for harvesting is important to overall transformant yields. It should be aimed to mid-log phase cells.
*Transformation tested from 1-6 x 10^6 cells/mL - Worked.

MATERIALS TEXT

MATERIALS

MAX Efficiency™ Transformation Reagent for Algae **Thermo**

Fisher Catalog #A24229 Step 2

SAFETY WARNINGS

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

DISCLAIMER:

BEFORE STARTING

- Prepare a ice bucket
- Separate cuvettes, keep them on ICE
- Allow linearized vectors to melt
- Keep transformation buffer on ICE/Fridge
- Prepare 50 mL centrifugal tubes with 10 mL TAP medium for recover stage

DNA Preparation 6h 30m

1 Digest a large enough amount of plasmid. The goal is to have a concentrated digested sample in the range of 250-700 ng/uL. 6h 30m

1. Select the appropiate enzymes for linearization. Usually, restrictions sites in flanking position to the expression cassete.
2. Mix all components for digestion **40 µg uncut vector** . Digest for **06:00:00** at **37 °C** .
3. Column purify digestion (Avoid gel purify, since vector backbone may helps to prevent intracelular DNAses action).
   **Use a PCR purification kit to purify the digestion reaction.*
4. Quantitate by absorbance measurement (i.e. Nanodrop).

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Component	Amount
10X Cutsmart NEB	6.0 uL
XbaI | NEB 20 U/uL	3.0 uL
KpnI HF | NEB 20 U/uL	3.0 uL
Plasmid 1219.9 ng/uL	40 uL
ddH20, Molecular grade	8.0 uL

Typical reaction setup

Result example

0.641 µg/µl Cutted vector

30 µl Final elution volume

19.230 µg total mass

Cells preparation

2 1. Aseptically inoculate **250 mL 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 **25 °C** , under constant shaking (~150-180 RPM) and light (60-80 µmols de photons/m^2s) until a cell density from **3 x 10^6 cells/mL** to **6 x 10^6 cells/mL** is reached.
3. Pellet cells in centrifuge tubes. Separate culture in sufficient amount of sterile 50mL centrifuge tubes or larger volume tubes, and centrifuge for **2000 x g, 25°C, 00:10:00** .

Pellet Cells

4. Genttly ressupend cells at **3-6-10^8 cells/mL** in Transformation Buffer.
MAX Efficiency™ Transformation Reagent for Algae **Thermo Fisher Catalog #A24229**

Culture at 3x10^6 cells/mL usually yield 12-13 transformations.

Transformation

3 1. Add cutted vector to the bottom of the electroporation cuvette. Typically from **250 ng cutted vector** to

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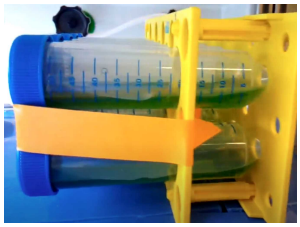
1000 ng cutted vector

2. Add **250 µl ressuspended cells** (at approximatelly **3 x 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 (Elution buffer or water).

Cell suspension ready for electroporation

3. Incubate cells with DNA **On ice** for **00:10:00**
4. Wipe cuvette (to remove condensated water) and electroporate (Table Electroporation).
5. Let it recover for **00:10:00** on the cuvette
6. Add cells to **10 mL TAP/40mM sucrose, pH 7.0** inside sterile 50mL centrifuge tubes. Gently transfer cells from cuvette to TAP/40 mM sucrose. Rinse cubette with TAP/40 mM sucrose to transfer any remaining cells.
7. Incubate at **Room temperature** on rocker or shaker at 50 rpm **Overnight** ambient light.



Recover step of cells in shaker, low mixing.

8. Pellet cells by centrifuging for **2000 x g, 25°C, 00:10:00**
9. Aseptically poor off supernatant. Add **300 µl TAP/40mM sucrose** to pelet. Gently re-suspend cells and pipette onto 2 plates with appropriate antibiotics. ie. **200 µl cells ressuspended** per plate, and let it dry a,septically without plate cover.
10. Spread cells evenly over the plate with a innoculation loop. Avoid spreading to the borders.
11. Use parafilm to block evaporation and place plates under constant light (60 µmols de photons/m^2s), **25 °C** . Colonies should be visible in 5-7 days.

Table Electroporation - Settings

Voltage	800 V
Time Constant	20 ms
Cuvette gap	4 mm

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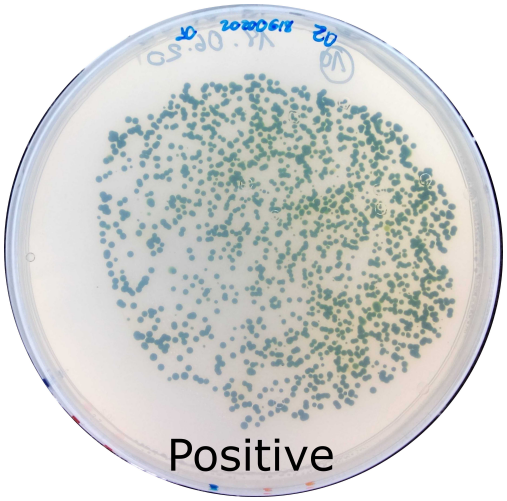
Gene Pulser Xcell Electroporation Systems
Electroporator

Biorad 1652660

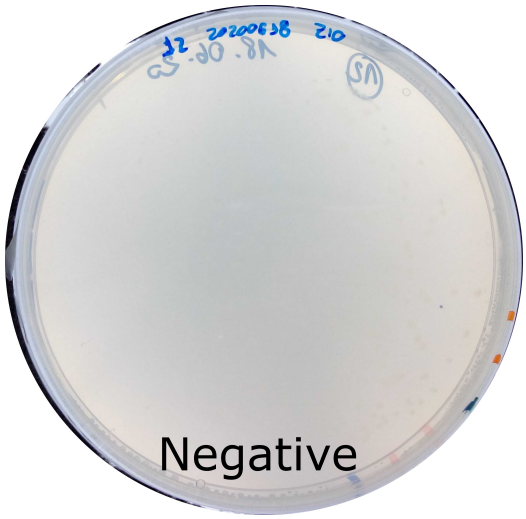
Typical output after electroporation

Time constant (ms)	Voltage (V)	Capacitance (uF)	Resistance (Ohms)
20.1	788	50	650
20.4	789	50	600
19.8	789	50	550

Green colonies should appear in the plate as in the pictures below.



Positive result



Negative control

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