Electroporation of Aurantiochytrium limacinum (ATCC MYA-1381)

Mariana Rius

Abstract

Citation: Mariana Rius Electroporation of Aurantiochytrium limacinum (ATCC MYA-1381). **protocols.io** dx.doi.org/10.17504/protocols.io.hg6b3ze
Published: 31 Mar 2017

Protocol

Grow cells

Step 1.

Start a culture 72 h before anticipated electroporation date.

Inoculate 50 ml of 790 By+ Medium with a fresh colony of *Aurantiochytrium limacinum* (ATCC MYA-1381).

Incubate at room temperature (25-28 C) for 48 h (non-shaking conditions).

NOTES
Mariana Rius 30 Mar 2017

(https://www.atcc.org/~/media/920FDAC93FF84B79851C29FBB8049862.ashx)

ATCC Medium: 790 By+ Medium

Yeast Extract.....1.0 g

Peptone.....1.0 g

D+-Glucose.....5.0 g

Seawater.....1000 ml

Autoclave at 121ºC.

Grow cells

Step 2.

Create a subculture 24 h before anticipated electroporation date.
Inoculate 50 ml of 790 By+ Medium with 1 ml of previous culture.

Incubate at room temperature (25-28 C) for 24 h (non-shaking conditions).

Grow cells

Step 3.

Count cells for electroporation.
Isolate 100 ul of culture for cell counts. Using a hemocytometer load 10 ul of culture and wait 2 min to allow cells to sink into chamber. Calculate cell count using recommended hemocytometer practice. Estimate whether volume of cells at the calculated density will be sufficient for anticipated elctroporation experiment. If insufficient volume from most recent culture is observed, plan to use older culture as well.

Prepare cells

Step 4.

Pellet cells. Pour 50 ml culture into 50 ml falcon tube. Centrifuge 3000x rpm at 4 C for 15 min. Gently pour out supernatant, avoid disturbing pellet.

Prepare cells

Step 5.
Add 25ml ice cold distilled water. Vortex cells to resuspend in distilled water. Centrifuge 3000x rpm at 4 C for 15 min. Gently pour out supernatant, avoid disturbing pellet.

Prepare cells

Step 6.
Add 15 ml of 25 mM DTT (dissolved in 50mM PBS, pH=7). Vortex to resuspend cells. Incubate on ice for 10 min. Centrifuge 3000x rpm at 4 C for 15 min. Gently pour out supernatant, avoid disturbing pellet.

Prepare cells

Step 7.
Add 25ml ice cold distilled water. Vortex cells to resuspend in distilled water. Centrifuge 3000x rpm at 4 C for 15 min. Gently pour out supernatant, avoid disturbing pellet.

Prepare cells

Step 8.
Add 2 ml (or necessary volume for electropration experiment) of 1 M Sorbitol. Vortex cells to resuspend, place on ice.

Prepare cells

Step 9.

Count cells and dilute to appropriate cell density for electroporation.
Load 10 ul of cells resuspended in sorbitol to hemocytometer. Wait two minutes for cells to sink. Calculate cell density and dilute to appropriate cell density (1-5 x 10 ^6 cells per ml) for electroporation using 1 M sorbitol.

Electroporate cells

Step 10.

Load cuvette. Place cuvette on ice prior to loading. Add 100 - 200 ul cells in sorbitol. Add 2-4 ug circular plasmid and 2-4 ug linear plasmid to cuvette. Pipette up and down gently to evenly mix solution.

Electroporate cells

Step 11.

Dry exterior walls of cuvette before loading into electroporator. Set resistance (1000Ω), voltage (0.45 - 1.8 kV), and capacitance (25-125 u*F*) to desired settings. When surroundings are clear, use two fingers to initiate pulse. Record time constant (τ, 5-20 ms). If administering two pulses, clear surroundings and use two fingers to initiate second pulse. Record time constant. Remove electroporated cuvette from electroporator and maintain on ice until all electroporation reactions are complete.

Outgrowth and plating of cells

Step 12.

Using a small or medium size pipette tip, gently angle the cuvette and pipette electroporated solution into a sterile, labeled, 1.5 ml microcentrifuge tube. Add 200 ul 790 By+ media to cuvette to collect any remaining cells and pipette into microcentrifuge tube. Add 300 ul - 800 ul 790By+ media to microcentrifuge tube (final volume 700ul - 1ml). Incubate at 28 C, 250 rpm for 1 - 24 h.

NOTES

Mariana Rius 30 Mar 2017

The difference in the volume of media added can depend on the number of plates on which the electroporated solution will be plated.

The range of outgrowth period can lead to substantial growth in the nonelectroporated cells at the longer end of the range. This longer incubation period was also associated with the potential of higher risk of contamination, observed in the plating of the electroporated media.

Outgrowth and plating of cells

Step 13.

Plate non-electroporated cells (prior to outgrowth) to measure survivability. According to the cell counts prior to transformation, plate 10 - 50 ul non-electroporated cells on 790 By+ media with agar (add 100mg/ml ampicillin to reduce contamination risk).

NOTES

Mariana Rius 30 Mar 2017

Several survivorship experiments prove that less than 0.1% of cells survive entire cell preparation procedure. This leads to a significant reduction in the electroporated cell mass, and may not be favorable for electroporation reactions with low efficiency.

Outgrowth and plating of cells

Step 14.

Plate electroporated cells on selective media to isolate transformants.

Plate 200 - 300 ul electroporated (and recovered) cells on 30 and 60 ug/ml nourseothricin 790 By+ Media plates (add 100mg/ml ampicillin to reduce contamination risk).

Plate 200 - 300 ul electroporated (and recovered) cells on 200 and 600 ug/ml G418 (geneticin) 790 By+ Media plates.

Plate 200 - 300 ul electroporated (and recovered) cells on 15 and 30 ug/ml zeocin 790 By+ Media plates.

Plate 200 - 300 ul electroporated (and recovered) cells on 100 and 400 ug/ml hygromycin B 790 By+ Media plates.

Outgrowth and plating of cells

Step 15.

Plate electroporated cells on non-selective media to measure survivability. Dilute 30ul electroporated cells in 170 ul half artificial seawater (ASW) and plate on non-selective media (790By+ Media, 100 mg/mlampicillinn can be added to reduce contamination risk).

Outgrowth and plating of cells

Step 16.

Plate 50 - 150 ul of control ('recovered') non-electroporated cells on each of the representative selective plates to compare growth with that of the electroporated cells.