Electroporation of Aurantiochytrium limacinum (ATCC MYA-1381)

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Abstract

Electroporation strategy used in the transformation of Aurantiochytrium limacinum (ATCC MYA-1381; Stramenopile/ Heterokont, Thraustochytrid). This protocol is following the guidance of Ono et al. 2011. US Patent # 7,888,123 B2.

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Materials

Gene Pulser Electroporation Cuvettes, 0.2 cm gap 1652086 by Bio-rad Laboratories

Protocol

Grow cells

Step 1.

Start a preculture 96 -120 h (4-5 days) prior to electroporation by inoculating 4 ml of GPY (0.5% Yeast Extract, 1% Peptone, 3% D+-Glucose, 1.8% instant ocean) with a colony of *Aurantiochytrium limacinum* (ATCC MYA-1381). Incubate overnight at 28 C in the outermost ring of spinning drum.

Use preculture to inoculate 46 ml of GPY in 250 ml flask. Culture for 3 to 4 days at 28 C, 171 rpm.

Prepare reagents for Electroporation

Step 2.

Make GPYS media (3% glucose, 0.6% peptone, 0.2% yeast extract, 50mM sucrose, 1.8% instant ocean)

Make GPYS ampiciln (100ug/ml) plates (2%agar)

Make GPYS zeocin (100ug.ml) and ampiciln (100ug/ml) plates (2%agar)

Make 1X BSS (10 mM KCl, 10 mM NaCl, and 3 mM CaCl2)

Make 50 mM sucrose solution

Sterilize by autoclave or filter sterilization as appropraite.

Prepare DNA for electroporation

Step 3.

For electroporation reactions in which linear DNA plasmid will be used:

Linearize 10ug of plasmid for each electroporation reaction and clean using PCR purification (QIAGEN) eluting to 12 ul*.

For electroporation reactions in which circular DNA plasmid will be used:

Concentrate DNA to 1ug/ul by PCR purification (QIAGEN) eluting to 12 ul*.

NOTES

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*~2 to 3 ul elution buffer will be bound to spin column, elution of 12 - 13 ul will result in final volume ~10ul.

Prepare cells for electroporation

Step 4.

Count cells.
Dilute 100 ul of culture in 900ul and load 10 ul on hemocytometer. Allow cells to settle for 2 - 5 min. Calculate cell count using recommended hemocytometer practice. Cell density should be 5 x10^-4 cells/ul (5 x10^-7 cells/ml).

Pellet and rinse cells.

Add 1.5 ml of cell culture to a microcentrifuge tube for each electroporation reaction. Centrifuge 5 min, 4 C, 11000rpm.

Decant. Add 500 ul chilled 1X BSS (10 mM KCl, 10 mM NaCl, and 3 mM CaCl2). Centrifuge 5 min, 4 C, 11000rpm.

Decant. Add 500 ul chilled 50 mM sucrose. Centrifuge 5 min, 4 C, 11000rpm.

Decant. Add 500 ul chilled 50 mM sucrose. Centrifuge 5 min, 4 C, 11000rpm.

Decant. Add 100 ul chilled 50 mM sucrose. Resuspend by scraping cell mass off side of tube and vortex.

Add DNA to cells.

Step 5.

Add 10 ul (10ug) DNA to suspended cells. Incubate on ice 5 min. Transfer to chilled electroporation cuvette (0.2cm; BioRad). Keep on ice.

For 'no DNA' control 10 ul elution buffer is added to cells instead of DNA.

Electroporate cells

Step 6.

Dry electrodes upon removing electroporation cuvette from ice. Load into electroporation chamber. Set voltage 0.45 kV, capacitance 125 uF, and resistance 1000 Ω on Gene Pulser (BioRad). Clear surroundings and initiate pulse. Record time constant (τ; 4 to 6 msec). Remove cuvette from chamber upon completion of pulse administration and return cuvette to ice. Proceed to next sample until all have been electroporated.

Outgrowth

Step 7.

Label one microcentrifuge tube for each electroporated sample.

Add 1ml GPYS media to cuvette and transfer entire volume to microcentrifuge tube.

Incubate at 28 C, 1 h. No shaking is necessary.

Plating

Step 8.

Centrifuge cells for 5 min, room temperature, 11000rpm. Remove 1 ml supernatant. Resuspend remaining cells by scraping pellet off side of tube and vortexing. Plate remaining volume (<200ul) on GPYS zeocin (100ug/ml) amp (100ug/ml) plate. Incubate at 28 C.

If 'no DNA' control was used:

For calculating cell survivorship:

Dilute to 7.5x10^3 cells/100 ul and plate 100 ul (expected cells 7500, but usually >80% mortality).

Example dilutions:

(Transformed and pelleted cells, resuspended in approximately 100ul 7.5x10^7 cells)

Take 10 ul cells and dilute in 990ul of1/2 artificial seawater (ASW) or GPYS

(100x dilution 7.5x10^4 cells)

Take 10 ul of 100x dilution in 90 ul 1/2 ASW or GPYS.

(10x dilution 7.5x10^3 cells)

Plate all 100 ul of 10x dilution.

For control on antibiotic selection:

Plate remaining 'no DNA' control cells on GPYS zeocin amp plate as negative control.

NOTES

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Plates will have visible cell mass because of the significant quantity of cells plated. Transformant colonies will appear over the plated cell mass.

Calculate cell survivorship using the plate containing diluted control cells on GPYS plate (no antibiotic selection).

Monitor Transformant Colonies

Step 9.

Colonies of control cells plated on GPYS (no antibiotic selection) will appear in 2 days from electroporation.

Aurantiochytrium limacinum transformant colonies will appear between 3-5 days following electroporation. Streak transformant colonies onto GPYS zeocin/amp plate and monitor for transformation efficiency. Include wildtype for comparison.