

Neurospora crassa

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.542 – 08/2003

Microorganism	<i>Neurospora crassa</i>
Cell type	Filamentous fungus, conidia
Molecules injected	Supercoiled plasmid DNA (pCSN44)
Growth medium	VM agar with supplements
Washing solution	Sterile water; ice-cold 1 M sorbitol
Electroporation solution	Ice-cold 1 M sorbitol
Outgrowth medium	Ice-cold 1 M sorbitol
Cuvette	2 mm gap width
Reference	Borkovich, Katherina A. • University of Texas-Houston Medical Center • Dept. of Microbiology and Molecular Genetics • Houston, Texas Vollmer, Kimberly • Brinkmann™ Instruments Inc. • BioSystems Application Lab • Westbury, New York

Treatment of cells:

1. Culture a *Neurospora crassa* wild type strain in VM agar in a 250 ml Erlenmeyer flask. Grow at 30 °C for 3 days, then transfer to room temperature and expose to fluorescent light for an additional 5 days. It is necessary to use conidia that are at least eight days old.
2. Harvest conidia by using sterile water. Transfer suspension to a 50 ml conical tube and centrifuge at 2,500 rpm for 5 min. to pellet the conidia.
3. Wash in 25 ml sterile water, vortex and centrifuge (2,500 rpm, 5 min.). Repeat washing step three times with 25 ml ice-cold 1 M sorbitol.
4. Resuspend in 0.5 ml of 1 M sorbitol and transfer to a 2 ml microcentrifuge tube. Adjust the concentration to 2.5×10^9 conidia/ml.

Electroporation of cells:

1. Add 1 µg DNA to 40 µl of electrocompetent cells on ice. Homogenize by gently mixing with pipette several times. Transfer mixture into a prechilled cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes "O"
Voltage (V)	2,000 V
Time constant (τ)	5 ms

4. Immediately place cuvette on ice and add 960 µl of cold sorbitol. Mix gently using a pasteur pipette, then transfer into a 2 ml tube. Plate 100 µl of each sample on a selection plate. Incubate at 30 °C for 2 days.

Expected results:

Transformation efficiency up to 370 transformants/µg of DNA.

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