Saccharomyces cerevisiae

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.531 – 12/2001

Microorganism	*Saccharomyces cerevisiae*
Cell type	Yeast
Molecules injected	Plasmid DNA
Growth medium	YEPD (1% yeast extract, 2% bactopeptone, 2% dextrose)
Washing solution	Ice-cold, sterile water; ice-cold 1 M sorbitol
Electroporation solution	Ice-cold 1 M sorbitol
Outgrowth medium	1 M sorbitol
Cuvette	2 mm gap width

Reference Dr. Robert Sclafani ♦ University of Colorado ♦ Health Science Center ♦ Denver, CO ♦ USA

Making electrocompetent cells:

1. Inoculate 500 ml YEPD with an aliquot of an overnight culture or a colony from a plate and grow to an $O.D._{600}$ of 1.3-1.5 (about 1 x 10^8 cells/ml).
2. Harvest by centrifugation at 4,000 x g for 5 min. at 4 °C.
3. Wash in 500 ml ice-cold sterile water, centrifuge (4,000 x g, 5 min., at 4 °C), repeat washing step with 250 ml water.
4. Resuspend in 20 ml ice-cold sorbitol and centrifuge as above. Resuspend in 0.5 ml of 1 M sorbitol to a final volume of 1.0 to 1.5 ml, keep on ice.

Electroporation of cells:

1. Add <5 µl (0.1 µg) DNA to 65 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Incubate 5 min on ice. 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)	1,500 V
Time constant (τ)	5 ms

4. Immediately add 1 ml of cold sorbitol. Plate various aliquots onto selective plates containing 1 M sorbitol.

Expected results:

Transformation efficiency up to 10^4 to 10^5 transformants/µg of DNA.

Note:

It is possible to scale this protocol down by starting with a culture volume of 50 ml.

eppendorf

Eppendorf AG · 22331 Hamburg · Germany · Phone +49 40-5 38 01-0 · Fax +49 40-5 38 01-556

e-mail: eppendorf@eppendorf.com · Internet: www.eppendorf.com