Kluyveromyces lactis

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.516 – 12/2001

Microorganism	*Kluyveromyces lactis* AWJ137
Cell type	Yeast
Molecules injected	DNA
Growth medium	Not given
Washing solution	YPD, 10 mM DTT, 40 mM HEPES (pH 8.0)
Electroporation solution	2.5 mM Tris/HCl, 1 mM $MgCl_2$, (pH 8.0)
Outgrowth medium	YPD with 1 M sorbitol
Cuvette	4 mm gap width

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Making electrocompetent cells:

1. Grow a 50 ml cell culture of *Kluyveromyces lactis* to a density of 5 x 10^7 cells/ml.
2. Harvest by centrifugation (5 min., at 4,000 x g)
3. Resuspend cells in 5 ml washing solution and incubate for 5 min.
4. Wash twice with electroporation solution and resuspend in 0.25 ml of the same solution. Incubate for 1 hour on ice.

Electroporation of cells:

1. Add 5 µl DNA (1 µg/µl) to 100 µl of electrocompetent cells. 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)	2,500 V
Time constant (τ)	5 ms

4. Add 1 ml YPD medium with 1 M sorbitol, incubate for 1 hour at 30 °C.
5. Centrifuge cells 30 sec., resuspend in 0.4 ml sterile water, plate in aliquots on selective plates.

Expected results:

Transformation efficiency up to 200 transformants/µg of DNA.