Pichia pastoris

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.545 –03/2004

Microorganism	*Pichia pastoris*
Cell type	Yeast
Molecules injected	Linear plasmid DNA
Growth medium	YPD (1% yeast extract, 2% bactopeptone, 2% dextrose)
Washing solution	Ice-cold, sterile bidistilled water; ice-cold sterile 1 M sorbitol
Electroporation solution	Ice-cold sterile 1 M sorbitol
Outgrowth medium	Ice-cold sterile 1 M sorbitol
Outgrowth plates	YPDS (1% yeast extract, 2% bactopeptone, 2% dextrose, 1 M sorbitol, 2 % agar)
Cuvette	2 mm gap width

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Making electrocompetent cells:

1. Streak *Pichia* cells from a glycerol stock (30 % glycerol) onto YPD plates to grow single colonies.
2. Inoculate 10 ml YPD with one colony and grow 12-14 h with shaking at 30 °C to an $O.D._{600}$ of ~3.0. It is important to have healthy log phase cells.
3. Inoculate 250 ml YPD with an aliquot of the overnight culture to reach an $O.D._{600}$ of 0.005 and grow ~12 h to an $O.D._{600}$ of 1.0-1.3.
4. Harvest by centrifugation at 1,500 x g for 5 minutes at 4 °C.
5. Gently resuspend in 250 ml ice-cold ddH_2O using a glass pipette, centrifuge as above.
6. Repeat washing step with 125 ml ice-cold ddH_2O.
7. Resuspend in 20 ml ice-cold sorbitol and centrifuge as above. Resuspend in 0.5 ml of 1 M sorbitol and keep on ice. Use the cells that day.

Electroporation of cells:

1. Add 10 µl DNA (5-10 µg) to 80 µl of electrocompetent cells. Gently mix with a blue-tip pipette several times. Transfer mixture into a prechilled cuvette and incubate on ice for 5 minutes.
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 ice-cold sorbitol to the cuvette. Transfer sample to sterile 15 ml tube and incubate for 2 hours without shaking at 30 °C.
5. Spread 200 µl aliquots on YPDS plates and incubate for 3 days at 30 °C until colonies appear. Number of colonies is increased about tenfold by adding 1 ml YPD and shaking for 3 hours before spreading thus incubating for a total of 5 hours.

Expected results:

Transformation efficiency up to 2 x 10^2 transformants/µg of DNA.