Pediococcus spp.

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.540 – 07/2002

Microorganism	*Pediococcus* spp.
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA (pGK12 and pPN-1)
Growth medium	MRS-G with 0.5 M sorbitol, 3 % glycine and 40 mM DL-threonine
Washing solution	0.5 M sorbitol, 10 % glycerol
Electroporation solution	0.5 M sorbitol, 1 mM K_2HPO_4, 1mM $MgCl_2$, pH 7.0
Outgrowth medium	MRS with 0.5 M sorbitol, 20 mM $MgCl_2$ and 2 mM $CaCl_2$
Cuvette	1 mm gap width
Reference	Caldwell, S. L. et al ♦ 1996 ♦ Applied and Environmental Microbiology 62, No. 3 ♦ 936-941

Making electrocompetent cells:

1. Cultivate cells by adding 12 ml overnight preculture (grown in MRS-G with 0.5 M sorbitol) to 800 ml growth medium. Incubate for 2 to 4 hours at 37 °C to a cell density of $O.D._{600}$ of 0.4-0.6.
2. Harvest by centrifugation.
3. Wash twice in 25 ml washing solution.
4. Resuspend in 1 ml electroporation solution.

Electroporation of cells:

1. Add 400-600 ng plasmid DNA to 80 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into cuvette.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Mode	Prokaryotes “O“
Voltage (V)	1,800 V
Time constant (τ)	5 ms

4. Immediately add 2 ml outgrowth medium and keep on ice for approx. 5 min. Incubate for 2 h at 37 °C.
5. Plate aliquots onto selective agar plates; incubate 2-5 days at 37 °C.

Expected results:

Transformation efficiency up to 4.6 x 10^3 transformants/µg of DNA.

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