Streptococcus thermophilus

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.535 – 12/2001

Microorganism	*Streptococcus thermophilus* St11
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA (pGK12)
Growth medium	Complex medium with 20 mM DL-threonine
Washing solution	272 mM sucrose, 1 mM EDTA, 7 mM HEPES (pH 6.5), 15% glycerol
Electroporation solution	272 mM sucrose, 1 mM EDTA, 7 mM HEPES (pH 6.5), 15% glycerol
Outgrowth medium	Complex medium
Cuvette	1 mm gap width

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Making electrocompetent cells:

1. Grow cells at 40 °C to a cell density of $O.D._{620}$ of 0.2 to 0.4.
2. Wash twice in ice-cold washing solution.
3. Resuspend in ice-cold electroporation solution to an $O.D._{620}$ of 1.7 to 1.8. Keep on ice.

Electroporation of cells:

1. Add 0.25 µg plasmid DNA 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)	2,000 V
Time constant (τ)	5 ms

4. Add 1 ml ice-cold complex medium. Incubate 5 hours at 40 °C.
5. Resuspend in 4 ml soft agar and plate on selective chloramphenicol plates; incubate 2 days at 40 °C.

Expected results:

Transformation efficiency up to 3.2×10^4 transformants/µg of DNA.