Streptococcus salivarius

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.541 – 03/2002

Microorganism	*Streptococcus salivarius*
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA
Growth medium	HJGS (Hogg-Jago glucose broth with 0.4 sorbitol)
Washing solution	5 mM potassium phosphate (pH 4.5), 0.4 M sorbitol, 10% glycerol
Electroporation solution	5 mM potassium phosphate (pH 4.5), 0.4 M sorbitol, 10% glycerol
Outgrowth medium	HJGS (Hogg-Jago glucose broth with 0.4 sorbitol)
Cuvette	1 mm gap width
Reference	Buckley, N. D. et al ♦ 1999 ♦ Applied and Environmental Microbiology 65, No. 9 ♦ 3800-3804

Making electrocompetent cells:

1. Cultivate cells by adding 1% overnight preculture to fresh medium. Grow cells at 37 °C without agitation to a cell density of $O.D._{660}$ of 0.5. Add glycine to a final concentration of 10% and incubate again for 1 h at 37 °C.
2. Harvest by centrifugation.
3. Wash twice in ice-cold washing solution.
4. Resuspend in electroporation solution to a 50-fold concentration and freeze in an ethanol-dry ice bath. Store at -80 °C.

Electroporation of cells:

1. Thaw cells on ice. Add 1 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)	1,600 V
Time constant (τ)	5 ms

4. Add 1 ml ice-cold outgrowth medium and incubate for 3 h at 37 °C.
5. Plate aliquots onto selective agar plates; incubate 2-3 days at 37 °C.

Expected results:

Transformation efficiency up to 1 x 10^5 transformants/µg of DNA.

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