Staphylococcus aureus

Multiporator/Eppendorf Eporator®

Transformation Protocol

Protocol No. 4308 915.533 – 01/2002

Microorganism	*Staphylococcus aureus*
Cell type	Bacteria, gram positive
Molecules injected	Plasmid DNA (pC194)
Growth medium	Basic medium (BM: 1% peptone, 0.5% yeast extract, 0.1% glucose, 0.5% NaCl, 0.1% K_2HPO_4)
Washing solution	10% glycerol (in distilled water)
Electroporation solution	10% glycerol (in distilled water)
Outgrowth medium	SMMP50 medium (5.5 parts SMM buffer (1 M sucrose, 0.04 M maleic acid, 0.04 M $MgCl_2$, pH 6.5), 4 parts 7% Panassy broth, 0.5 parts 10% BSA)
Cuvette	2 mm gap width

Reference Augustin J. and Götz F. ♦ 1990 ♦ FEMS Microbiology Letters 66 ♦ 203-208

Making electrocompetent cells:

1. Inoculate a flask of BM with a fresh overnight culture. Grow at 37 °C with shaking to an $O.D._{578}$ of about 0.5 to 0.65.
2. Wash in one volume of 10% glycerol at room temperature, followed by washes with 1/2, 1/20 and 1/50 volumes.
3. Resuspend to final volume of 1-5 x 10^{10} cells/ml in 10% glycerol. Aliquote and freeze at –70 °C.

Electroporation of cells:

1. Thaw cells for 5 min at room temperature. Add 1-2 µl plasmid DNA (100-500 ng) to 50 µl of electrocompetent cells. Homogenize by gently mixing with pipette several times. Incubate at room temperature for 30 minutes.
2. Transfer mixture into a cuvette and insert the cuvette into the device.
3. Electroporation:

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

4. Add 1 ml SMMP50 medium to the cells and transfer to sterile tube. Incubate 90 minutes at 37 °C with moderate shaking.
5. Plate on selective BM plates. Incubate 20 hours at 37 °C.

Expected results:

Transformation efficiency up to 1.5 x 10^5 transformants/µg of DNA.

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