Serratia liquefaciens

Multiporator/Eppendorf Eporator ®

Transformation Protocol

Protocol No. 4308915.550

Microorganism	*Serratia liquefaciens*
Cell type	Bacteria, gram negative
Molecules injected	Plasmid DNA pDsRed
Growth medium	LB medium
Washing solution	10% glycerol
Electroporation solution	10% glycerol
Outgrowth medium	LB medium with antibiotics agar plates
Cuvette	1 mm gap width
Reference	**Lilian Pilares and Jose Ramos Vivas, IFIMAV, Santander, Spain.**

Making electrocompetent cells:

1. Dilute an overnight culture of *S. liquefaciens* in LB, into fresh media (1:100). Grow at 37 ºC with shaking until reaching an $O.D._{600}$ of 0.5.
2. Chill cells on ice for 10 minutes and transfer to a pre-chilled centrifuge tubes to harvest cells. Wash three times with cold sterile 10% glycerol. The first time in 25 ml, the second time in 12,5 ml, the third and fourth times in 2 ml.
3. Resuspend in 10% glycerol at a concentration of approx. 10^9 cells/ml and store at –70 ºC until needed.

Electroporation of cells:

1. Add 1 µg plasmid DNA to 50 µl (~10^9) of electro competent cells. Homogenize by gently mixing with pipette several times. Transfer mixture into a pre-chilled cuvette (-20ºC).
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

Voltage (V)	1,800 V
Time constant (t)	5ms

4. Immediately add 1 ml of pre-warmed LB medium and incubate at 37ºC for 1 hour with strong shaking.
5. Plate on selective agar plates (ampicillin) and incubate al 37ºC for 24 h.

Expected results:

Transformation efficiency from 10^5 to 5×10^7 transformants/µg of DNA (strain-dependent differences).