

Protocol 0013

Electro Cell Manipulator™ ECM® 600/630 ELECTROPORATION PROTOCOL

E. coli DH5 α , DH1

DNA: pUC12

Cell Preparation:

Growth Medium: L-Broth (10 gm bacto tryptone, 5 gm bacto yeast extract, 5 gm NaCl per liter)
Temperature: 37°C
Cell Density: 0.5 - 1.0 OD₆₀₀ (~ 10¹⁰ cell/ml)
Temperature: Chill on ice
Washing procedure: Pellet 1 L of culture at 4000 x g at 4°C 15 min
Wash 1: Resuspend 1 volume sterile cold H₂O
Pellet 4000 x g at 4°C 15 min
Wash 2: Resuspend 0.5 volume sterile cold H₂O
Pellet 4000 x g at 4°C 15 min
Wash 3: Resuspend 0.5 volume sterile cold H₂O
Pellet 4000 x g at 4°C 15 min
Wash 4: Resuspend 0.02 volume sterile cold H₂O
Pellet 4000 x g at 4°C 15 min
Final Dilution: Resuspend 0.002 - 0.003 volume sterile cold 10% Glycerol (filter sterilized)
(aliquots may be frozen at -70°C, thaw on ice 5-10 min)

Electroporation Settings:

Choose Mode: **T** HV
Set Capacitance: **C** Default setting is 50 μ F in HV
Set Resistance: **R** R5 (129 ohms)
Chamber Gap: BTX Disposable Cuvette P/N 610 (1 mm gap)
Set Charging Voltage: **S** 1.3 - 1.5 kV
Desired Field Strength: **E** 13.0 - 15.0 kV/cm
Desired Pulse Length **t** 5-6 msec

Electroporation Procedure:

Sample Volume: 40 μ l
Transfectant Volume: 1 μ l plasmid (1 ng/ μ l in H₂O, low salt TE or ligation mix diluted 1:5 in H₂O)
Handling: "Flick" cuvette to mix and settle cell mixture (Note: suspension must touch both side walls of cuvette)
Operating Temperature: 0°C - chill filled cuvette on ice for 1 min prior to electroporation
Pulse: Press **A** to activate Automatic Charge & Pulse sequence
Dilution Media: Immediately after green Charging light goes out, add 960 μ l LB or SOC
This step is critical - Do Not Delay!
Briefly and gently pipet up and down to mix thoroughly
Incubate: Transfer to polypropylene tube and hold at 37°C for 1 hr (shaking at 225 rpm may improve recovery)
Plating: Transfer 1 - 100 μ l to appropriate agar plate
Selection Method: Antibiotic resistance

Results:

$1 \times 10^8 - 1 \times 10^9$ transformants/ μg DNA

Reference:

Personal communication with David Sherman, Ph.D, Washington University, St. Louis, MO