Protocol 0305a

Electro Cell Manipulator™

ECM®399 ELECTROPORATION PROTOCOL

GENERAL OPTIMIZATION PROTOCOL FOR TRANSFORMATION OF BACTERIA

Cell Preparation:

Growth:	Grow to mid log phase in the appropriate non-minimal growth media
Washing Procedure:	Chill cells on ice and perform step wise washes in decreasing volumes of ice cold sterile water or a high resistivity buffer. For 1 L of cells, pellet cells and resuspend well in 1 L sterile water. Repellet and resuspend in 500 ml sterile water. Repellet and resuspend in 250 ml sterile water. Repellet and resuspend in 100 ml sterile water. Repellet and resuspend in 20 ml sterile water.
Electroporation Buffer:	Final resuspension should be in 2-3 ml of 10% ice cold sterile glycerol or other high resistivity buffer (Always filter sterilize glycerol, never autoclave)

Electroporation Settings:

Choose Mode:	HV Mode
Chamber Gap:	BTX Disposable Cuvette P/N 610 (1 mm gap)
Set Charging Voltage:	1.5 kV - 2.5 kV in 0.1 kV increments
Desired Field Strength:	15 - 25 kV/cm
Desired Pulse Length:	Approximately 5 msec

Electroporation Procedure:

Sample Volume:	20 - 70 µl
DNA Amount:	1 ng - 1 µg
Temperature:	Mix Cells and DNA on ice and incubate on ice 10 min
Pulse:	Press Start to activate the Automatic Charge & Pulse Sequence
Optimization:	Choose the appropriate voltage/field strength range or pulse separate samples at 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, and 2.5 kV or until the samples arc at the higher field strengths
Post Pulse Treatment:	Immediately dilute in the appropriate enriched media and grow at the appropriate temperature for 1 - 3 hrs, then plate on selective media. Determine the transformation efficiency and plot versus voltage in order to determine the appropriate voltage for further experimentation.

Results: Bacterial species and strain dependent

Reference: BTX Technical Support, Genetronics, Inc., San Diego, CA