Protocol 0031

Electro Cell Manipulator™

ECM® 600/630 ELECTROPORATION PROTOCOL

Saccharomyces cerevisiae

Cell Preparation:

Growth Medium:	500 ml YPD (1% yeast extract, 2% Bactopeptone, 2% glucose) in a 2-1 flask
Temperature:	30°C with vigorous shaking
Cell Density:	1 x 10^8 cells/ml OD_{600} = 1.3 - 1.5
Harvesting procedure:	1) Pellet by centrifugation and resuspend in 100 ml YPD broth with 2.0 ml sterile 1 M HEPES, pH 8.0 2) Add 2.5 ml sterile 1 M dithiothreitol (DTT) while swirling gently 3) Incubate 15 min at 30°C with gentle shaking 4) Bring to 500 ml with sterile cold Milli-Q H_2O (or equal)
Washing Procedure:	Pellet 4000 x *g* at 4°C 5 min
Wash 1:	Resuspend in 500 ml (original volume) sterile cold Milli-Q H_2O Pellet 5000 x *g* at 4°C 5 min
Wash 2:	Resuspend in 250 ml sterile cold Milli-Q H_2O Pellet 5000 x *g* at 4°C 5 min
Wash 3:	Resuspend in 20 ml sterile cold 1M Sorbitol Pellet 5000 x *g* at 4°C 5 min
Final Dilution:	Resuspend in 0.5 ml sterile cold 1M Sorbitol

Electroporation Settings:

Choose Mode:	**T**	2.5 kV / RESISTANCE High Voltage (HV)
Set Capacitance:	**C**	Default setting is 25μF in HV
Set Resistance:	**R**	R5 (129 ohms) 200 ohms on ECM630
Chamber Gap:		BTX Disposable Cuvette P/N 620 (2 mm gap)
Set Charging Voltage:	**S**	1.5 kV
Estimated Field Strength:	**E**	7.5 kV/cm
Desired Pulse Length:	**t**	~5 msec

Electroporation Procedure:

Sample Volume:	40μl
Transfectant Volume:	<100 ng in 5 μl low TE
Operating Temperature:	Place on ice
Pulse:	Press **A** to activate Automatic Charge & Pulse sequence
Dilution Media:	Immediately add 1 ml cold 1 M Sorbitol and recover the yeast with gentle mixing to a culture tube
Selection Method:	Plate on selection media containing 1 M Sorbitol (outgrowth period not required)

Results:

5 x 10^5 transformants/μg DNA

Reference:

Becker, DM and Guarente L, *Guide to Electroporation and Electro-fusion*, Academic Press, Chapter 31, 1991 (BTX ID #1362)