Protocol 0906

ElectroSquarePorator™

ECM® 630 HT 25/96 Multi-Well ELECTROPORATION PROTOCOL

Cell Line: *Sacchromyces cerevisiae*

Transfectant: plasmid DNA

Cell Preparation: *Sacchromyces cerevisiae* were grown overnight to stationary phase (OD_{600} of ~3). An aliquot of the culture was inoculated into 100 ml of YPD media (10 g/l yeast nitrogen base, 20 g/l peptone and 20 g/l D-(+)-glucose) to reach OD_{600} of ~0.3. The cells were grown until OD_{600} reached ~1.6 before collecting by centrifugation. The cell pellet was washed twice with 50 ml of cold water, and once with 50 ml electroporation buffer (1 M sorbitol/1 mM $CaCl_2$). Cells were conditioned in 20 ml of 0.1 M LiAc/10mM DTT by incubation in culture flask with shaking (225 rpm) for 30 min at 30ºC, washed one or more times with 50 ml of electroporation buffer, then suspended in 100-200 µl of the same buffer to reach 1 ml volume, which corresponds to 1.6 x 10^9 cells/ml. (The scale up of culture and washed volumes should be proportional if more electrocompetent yeast suspension is needed.)

Electroporation Settings:

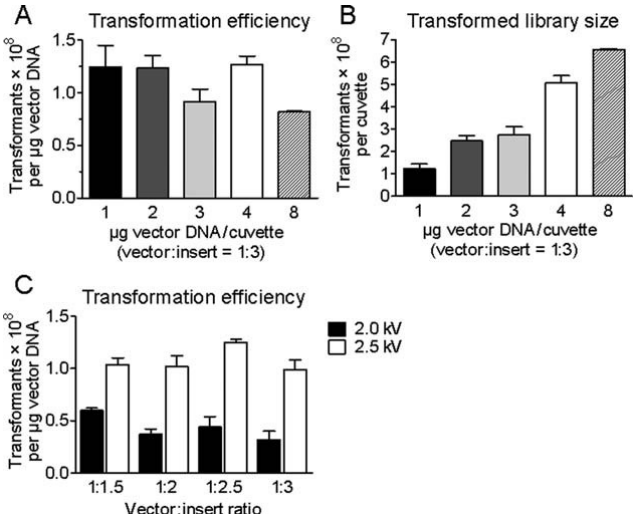
Choose Mode:	HV
Set Voltage:	2500 V
Electrode Gap:	2 mm*
Capacitance:	25 µF
Resistance:	~100 – 200 Ω
Pulse number:	1
Electrode Type:	96 – well plate
Desired Field Strength:	12.5 kV/cm
Time constant:	3 – 4.5 ms

Electroporation Procedure:

Temperature:	4ºC
Total Volume:	250 µl
Transfectant conc:	4 µg vector:12 µg insert (1:3 ratio)

Cell density:	1.6 x 10^9 cells/mL
Pulse:	Press Automatic **Start** to activate Charge and Pulse Sequence
Post Pulse Treatment:	Cells were suspended in 10 ml of 1:1 mix of 1 M sorbitol: YPD media and incubated at 30ºC for 1 h.

Results:



Increased DNA input and high voltage, but not vector to insert ratio, are critical for maximal transformation efficiency. Saccharomyces cerevisiae were treated as described in Fig. 1. Four hundred microliters of yeast cell suspension (1.6 x 10^9 cells/mL) were electroporated using 1, 2, 3 or 4 mg vector DNA per cuvette (vector to insert ratio is always maintained at 1:3). After 72 h the numbers of colonies were determined and represented as (A) transformation efficiency, transformants/mg vector DNA (mean+standard deviation) and (B) transformation yield, total number of transformants per cuvette (mean+ standard deviation). Additionally, (C) 400 ml yeast suspension were electroporated by 2 or 2.5 kV and different vector to insert DNA ratios. The transformation efficiencies are expressed as in (A).

Reference: Benatuil,L. et al., An improved yeast transformation method for the generation of very large human antibody libraries, February 3, 2010, Protein Engineering, Design & Selection vol. 23 no. 4 pp. 155-159

BTX item numbers:
450423 ECM 630 generator, 2 mm gap 2 x 96-well plates, Plate seals and HT-200 plate handler

*Protocol adapted for a 96-well plate.

Compiled: November 1, 2010 – DMerdaa, BTX Technical Support