Arabidopsis thaliana

Multiporator/ Eppendorf Eporator ®

Transformation Protocol

Protocol No. 4308 915 548-05-2012

Organism	*Arabidopsis thaliana*
Cell type	Leaf mesophyll protoplasts
Molecules injected	pDONR221 or 207 (Invitrogen) with full length CRF1-8 genes

Enzyme solution	1% cellulose R10, 0.25% macerozyme R10, 0.4 M mannitol, 20 mM MES pH 5.7, (heated to 55° for 10min, than cooled to room temp before adding) 10 mM $CaCl_2$, 1% BSA, 0.45µm filtered
Washing/ Electroporation solution	0.5 M mannitol**,** 4 mM MES pH5.7, 20 mM KCl
Cuvette	1 mm gap width

Reference	Cutcliffe J.W. et al, J Exp Bot. 2011 Oct;62(14):4995-5002

Isolation of leaf mesophyll protoplasts:

1. Protoplasts are prepared from 14-21-day-old plants by slicing leaves into 1mm strips.
2. Strips are placed into enzyme solution for 30-60 min under vacuum and afterwards shaken gently at 40 rpm for 90 min, before shaken more rigid (80-90 rpm) for 10 min to release the protoplasts.
3. Protoplasts are then filtered (40 µM) and gently centrifuged at 150 x g to remove the enzyme solution.
4. Protoplasts are then washed twice with 2 mL washing/electroporation solution and resuspended in final volume (10^6/mL) and stored on ice until use (within 1 hour)

Electroporation of protoplasts:

1. Add 40-50 µg plasmid DNA to ice-chilled 100 µL (~10^5) protoplasts.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation (pulse two times):

Mode (Multiporator only)	prokaryotes “O“
Voltage (V)	300 V
Time constant (t)	5ms

4. Immediately place protoplasts on ice.
5. Incubate in dark at 22ºC for 18 h before examination.

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