Citrullus colocynthis

Multiporator/ Eppendorf Eporator ®

Transformation Protocol

Protocol No. 4308 915 549-05-2012

Organism	*Citrullus colocynthis*
Cell type	cotyledone protoplasts
Molecules injected	pEarlyGate 103 [1]

Enzyme solution	2% cellulose R10, 0.5% macerozyme R10, 0.5% driselase, 2.5% KCl, 0.2% $CaCl_2$, pH 5.7, 0.45 µm filtered
Washing/ Electroporation solution	0.5 M mannitol**,** 4 mM MES pH5.7, 20 mM KCl
Cuvette	1 mm gap width
Reference	Ying Si et al J Exp Bot. 2010 Jun;61(6):1635-42

Isolation of cotyledone protoplasts:

1. Cotyledons from soil-grown plants are excised and cut into 1mm strips.
2. Strips are immediately placed into enzyme solution for overnight digestion in the dark.
3. After overnight incubation, strips are shaken gently at 40 rpm for 30 min to release the protoplasts.
4. Protoplasts are then filtered (40 µM) and gently centrifuged at 150 x g to remove the enzyme solution.
5. Protoplasts are then washed twice with 2 mL washing/electroporation solution and resuspended in final volume for electroporation.

Electroporation of protoplasts:

1. Add 40-50 µg plasmid DNA to ice-chilled 300 µL (1-2 x 10^5) protoplasts.
2. Wipe moisture from the cuvette and insert the cuvette into the device.
3. Electroporation:

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

4. Immediately place protoplasts on ice.
5. Incubate overnight in dark before examination.

[1] Earley K et al Plant J. 2006 Feb:45 (4),616–629

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Eppendorf AG · 22331 Hamburg · Germany · Phone +49 40-5 38 01-0 · Fax +49 40-5 38 01-556
e-mail: eppendorf@eppendorf.com · Internet: www.eppendorf.com