

Tech-Trends

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Plant Protoplast Fusion

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Protoplast fusion has many uses in plant research. This technique is used to create hybrids between plant species that cannot be crossed sexually.

Electrofusion is a non-toxic technique that minimizes damage of the target protoplasts and results in significantly higher fusion frequencies than obtained by chemical fusion. Isolated protoplasts are placed into a fusion chamber and aligned into close physical contact (often referred to as "pearl chains") by application of a low intensity, high frequency, alternating electric current—a process known as dielectrophoresis. The alternating electric field causes pores to form on the protoplast membrane due to a large electric potential at the two poles of the protoplast (Chang et al. 1992). Before these pores are able to "reseal," the protoplasts are induced to fuse through application of anywhere from one to several short direct current square pulses.



ECM® 2001 Protoplast Fusion PROTOCOL

Protoplast Preparation:

Digest protoplast with a solution of (w/v)
Macerase, 2%(w/v) Cellulysin in 50 mM
Mes-NaOH buffer (pH 5.7) with 0.6 M
Mannitol

Electroporation Settings:

Alignment Amplitude:	3-5 V
Time:	10-15 sec
Field strength:	60-100V/cm
Electroporation Amplitude:	3—150V
Pulse Width:	40-60 µsec
Number of pulses:	1
Electrode gap:	BTX Microslide 0.5 mm gap
Field Strength:	0.63 – 3 Kv/cm

Electroporation Procedure:

Volume: 20ul (1.5 –
3x10⁵protoplasts/ml)

Microslide Attachment:

1. Sterilize microslide with ETOH, dry
2. Place Microslide on microscope stage
3. Attach Micrograbber cables to posts and tape cable to stage to stabilize
4. Focus microscope
5. Pipette solution between microslide bars
6. Press Automatic Star to activate pulse sequence. Alignment will occur followed by an electroporation pulse. Observe rounding-off of hybrids through microscope.

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