Particle bombardment of Hi II Type II callus and recovery of transgenic maize plants

Frame, B. et al. 2000. Production of transgenic maize from bombarded Type II callus: effect of gold particle size and callus morphology on transformation efficiency. In Vitro Cell. Dev. Biol-Plant. 36:21-29.

Materials

Plasmid

A plasmid containing a selectable marker (*bar* gene, Spencer et al., 1990) and a screenable marker (*uid*A or *gus* gene) or gene of interest (GOI). For example, pAHC25 (Christensen and Quail, 1996).

Plant material

Ear of maize Hi II plant (A188xB73 origin, see Armstrong et al., 1991). Greenhouse ears are collected 10-13 days after pollination from greenhouse grown plants when embryos are about 1.2-1.8 mm in size. Immature zygotic embryos are dissected from the ear to N6E medium for initiation of Type II callus.

Biolistic Gun

PDS 1000/He biolistic gun and all disposables from Bio-Rad (Hercules, CA).

Media

N6E (callus initiation):

4 g/L N6 salts (Chu et al., 1975), 1 ml/L (1000X) N6 vitamin stock, 2 mg/L 2,4-D, 100 mg/L myo-inositol, 2.76 g/L proline, 30 g/L sucrose, 100 mg/L casein hydrolysate, 2.5g/L gelrite, pH 5.8 (100x15 mm petri-plates). Filter sterilized silver nitrate (25 µM) added after autoclaving.

N6OSM (osmotic medium):

4 g/L N6 salts, 1 ml/L N6 vitamin stock, 2 mg/L 2,4-D, 100 mg/L myo-inositol, 0.69 g/L proline, 30 g/L sucrose, 100 mg/L casein hydrolysate, 36.4 g/L sorbitol, 36.4 g/L mannitol, 2.5g/L gelrite, pH 5.8 (100x15 mm petri-plates). Filter sterilized silver nitrate (25 µM) added after autoclaving.

N6S (selection):

4 g/L N6 salts, 1 ml/L N6 vitamin stock, 2 mg/L 2,4-D, 100 mg/L myo-inositol, 30 g/L sucrose, 2.5g/L gelrite, pH 5.8 (100x15 mm petri-plates). Filter sterilized bialaphos (2 mg/L) and silver nitrate (5 µM) added after autoclaving.

⌘ Regeneration Medium I:

4.3 g/L MS Salts (Murashige and Skoog, 1962), 1 ml/L (1000X) MS vitamin stock, 100 mg/L myo-inositol, 60 g/L sucrose, 3 g/L gelrite, pH 5.8 (100x25 mm petri-plates). Filter sterilized bialaphos (3 mg/L) added after autoclaving.

⌘ Regeneration Medium II:

4.3 g/L MS Salts, 1 ml/L MS vitamin stock, 100 mg/L myo-inositol, 30 g/L sucrose, 3 g/L gelrite, pH 5.8 (100x25 mm petri-plates).

N6 based media for Hi II is after Songstad et al., 1996.
Media for regeneration after is Armstrong and Green, 1985.

Methods

⌘ Embryo Dissection for Callus Initiation

1. Dehusk ear. Cut off and discard top 1 cm of ear and insert a straight nosed forceps into the tip end of the ear. This "handle" facilitates aseptic handling of the cob during embryo dissection. Place impaled ear and forceps into a sterilized mason jar in laminar flow bench. If necessary, sterilize up to 4 ears in one mason jar.

2. Add ~ 700 ml of sterilizing solution (50% commercial bleach (5.25% hypochlorite) in water + 1 drop of surfactant Tween 20) to cover ear. During the 20 minute disinfection, occasionally swirl the ears and tap the mason jar on the surface of the flow bench to dislodge air bubbles for thorough surface sterilization of ear. Holding on to the forceps, pour off bleach solution and rinse the ears three times in generous amounts of sterilized water. The final rinse is drained off and the ears are ready for embryo dissection.

3. In a large (150x15mm) sterile petri-plate, cut off the kernel crowns (the top 1-2 mm) with a sharp scalpel blade. Use sterilizing ovens for intermittent re-sterilizing of utensils throughout this protocol.

4. Excise the embryos by inserting the narrow end of a sharpened spatula between the endosperm and pericarp at the basipetal side of the kernel (towards the bottom of the cob) popping the endosperm out of the seed coat. This exposes the untouched embryo which sits at the top-side of the kernel, close to the kernel base. The embryo is gently coaxed onto the spatula tip and plated with the embryo-axis side down (scutellum side up) onto the N6E media (30 embryos/plate).

5. Wrap the plate with vent tape and incubate at 28°C in the dark for 2 weeks.

⌘ Initiation of Type II callus lines for bombardment

1. After 2 weeks, friable, rapidly growing embryogenic callus can be seen proliferating from the embryo scutellar tissue of ~100% of the immature zygotic embryo explants. This tissue is sub-cultured to fresh N6E medium and plates are wrapped with parafilm (28°C, dark)

2. Callus lines, each originating from an independent immature zygotic embryo source are developed for bombardment over a 6-8 week period by weekly sub-culturing of this material to fresh N6E medium. Lines are discarded 4 months after initiation.

⌘ Gold particle preparation

1. See protocol "Gold Particle Preparation".

⌘ Micro-projectile bombardment

1. Draw a 3.5 cm diameter circle on the bottom of a plate of osmotic medium (N6OSM), Vain, P., et al. 1993. This defines the target area to which callus pieces are loaded for bombardment.

2. Four hours prior to the bombardment, use a microscope to transfer 30 callus pieces (4 mm) from a friable, rapidly growing callus line to the target area.

3. Load 650 psi rupture disk.

4. Assemble the macro-carrier launch assembly by first laying in place a stopping screen followed by an inverted, pre-loaded macro-carrier holder (see Gold Particle Preparation Protocol) which is held in place by screwing on the launch assembly lid.

5. Slide the launch assembly into place immediately below the helium nozzle, and set the gap distance (6 mm).

6. Slide a 150 micron mesh screen (McMaster Carr, Elmhurst IL), which is sterilized by autoclaving, onto the shelf directly below the launch assembly. This screen is supported on a second plexi-glass stage (like the one that holds the petri plate at bombardment) with a 3.8 cm diameter hole cut in the middle of it. Provided the construct being bombarded does not change, we re-use this screen for 8-10 shots before discarding.

7. Slide the opened petri dish containing the target tissue onto the shelf at a selected distance from the stopping screen (6 cm).

8. The vacuum chamber is closed, a vacuum pulled, and the gun fired in time for the rupture disk to break as soon as the vacuum reaches 28 inches of Hg.

9. The chamber is vented, the plate containing the bombarded tissue removed, and the gun prepared for the next bb by replacing the spent rupture disk, macro-carrier and stopping screen (disposables). All plasmid waste is disposed of in biohazard bags for autoclaving.

10. Repeat step 3-9 for each shot.

11. The bombarded callus is left on N6OSM medium for 1 hour after bombardment and then transferred to N6E medium maintaining the integrity of the each bombarded piece. Plates are then wrapped with vent tape (28°C, dark).

⌘ Selection for stable transformation events

1. After 7-10 days on initiation medium (N6E) the bombarded callus pieces are transferred to N6S selection medium (2.0 mg/L bialaphos) to begin the recovery of transformed cells.

2. Three weeks later, individual callus pieces are again transferred to fresh N6S medium. Within 6-8 weeks of bombardment, bialaphos resistant clones emerge from selected callus pieces.

⌘ Regeneration of transgenic plants

1. Regeneration of transgenic Type II callus (friable, stocked somatic embryos present) is accomplished by transferring about 15 small pieces (approximately 4 mm) of highly embryogenic callus to Regeneration Medium I and incubating for 2-3 weeks at 25°C in the dark. Petri-plates are wrapped with vent tape.

2. After 2-3 weeks, matured somatic embryos are transferred to the light on Regeneration Medium II for germination and again the plates are wrapped with vent tape. Plantlets sprout leaves and roots on this medium.

⌘ Plant Acclimatization

1. Acclimatization of regenerated plants to soil is accomplished as described in Frame et al., 2000. Also see protocol "Greenhouse".

References

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