Soybean Transformation

We are currently using the following 3 different methods for soybean transformation in the laboratory, all targeting embryos or proliferative embryogenic cultures: D20 Transformation, Suspension Culture Transformation, SAAT.

The most user-friendly method is called “D20 transformation”, because the target embryogenic tissues are maintained on a medium containing 20 mg/l 2,4-D. This method (outlined directly below), uses particle bombardment and gives very consistent results, although it is not very efficient. Typically, Three transformation event are obtained for every shot and plant recovery takes 9-12 months. Everyone in the laboratory can easily produce transgenics using D20 transformation.

Transformation of soybean suspension cultures (outlined further below) also uses particle bombardment and is very rapid and efficient, but is technically more demanding. Our laboratory has moved back and forth between D20 transformation and suspension culture transformation because some folks in the lab have a more difficult time with the liquid culture system. It was the first method that was used in the lab and can be very efficient. We have obtained from 1-30 events per shot (average of ~3) although others have reported even higher numbers. Recovery of transgenics can also be rapid but plant recovery still requires 3-6 months post bombardment.

Transformation of both D20 and suspension cultures using SAAT has been obtained. However, we are still working out the details of the protocol. SAAT of embryogenic tissue has the potential to be much more efficient than other soybean transformation methods.

Transformation of proliferative soybean embryogenic tissue can be very efficient as the embryos originate from single cells on the surface of older embryos. These surface cells are accessible to a number of different DNA introduction methodologies. Proliferation of these embryos gives a concentric layering of embryos as the new embryos form.

Histology of proliferative embryogenic tissue showing surface origin (bumps on top) of new

embryos.

D20 Transformation

D20 transformation is a “user-friendly” method for soybean transformation. It targets proliferative embryogenic tissue, maintained on a semi-solid medium. Embryogenic tissue is bombarded with plasmids encoding hygromycin resistance and one or more genes of interest. The genes of interest can either be located on the same plasmid or on different plasmids (co-transformation). After bombardment, tissues can be selection using solid medium or our new hybrid liquid/solid selection.

For selection on solid medium, one week following bombardment, tissues are placed on D20 medium containing 15 mg/l hygromycin. Tissues are transferred to D20 medium containing 15 mg/l hygromycin every 2-4 weeks. Selected tissues are first recovered after 2.5-3 months (it is slow!).

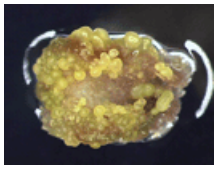
For hybrid liquid/solid selection, tissue can first be placed in liquid culture (FN with 30 mg/l hyg). The medium is replaced every weekly for 4-6 weeks. Hygromycin resistant tissue is removed and placed on solid D20 medium containing 15 mg/l hyg. Liquid selection reduces the selection period from 7-17 weeks to 4-6 weeks.

We proliferate the tissue, perform PCR of putative clones and then regenerate plants. New cultures are generated every 3-6 months and are ready for bombardment after 1-2 months. Sterility using this system has not been a problem. The original paper describing the method came out in late 1999.

Santarém ER, JJ Finer (1999) Transformation of soybean (Glycine max (L.) Merrill) using proliferative embryogenic tissue maintained on semi-solid medium. In Vitro Cellular and Developmental Biology – Plant 35:451-455.

Initiation of proliferative tissue from immature cotyledons

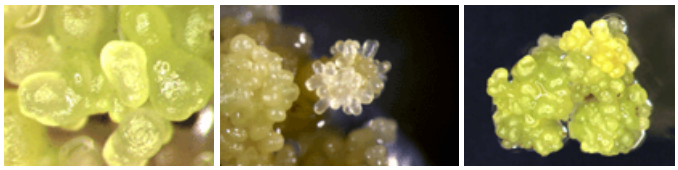
Isolate immature zygotic embryos from pods, 2-3 weeks after flower formation. Immature embryos are 3-4 mm in length. You can see them by backlighting or feel them by slightly squeezing the pods and feeling some resistance. You will have to gauge your vision or feel by opening a few pods to make sure that the size is right. Sterilize pods and remove the immature embryos. Discard the embryo axis. Plate immature cots on D40 for 3-4 weeks at 25°C.



D40
MS salts
B5 vitamins
3% sucrose
40 mg/l 2,4-D
pH 7.0
0.2% Gelrite

Proliferation of D20 tissue

Transfer to D20. Select bright green, globular, proliferative embryos. Subculture every 2-3 weeks under conditions described for induction. Quality is critical!!



D20 tissue

D20

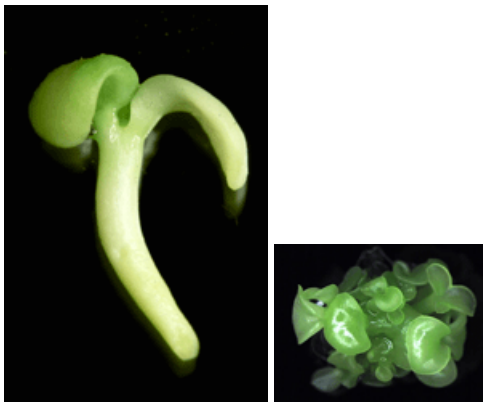
MS salts
B5 vitamins
3% sucrose
5 mM asparagine
20 mg/l 2,4-D
pH 5.7
0.2% Gelrite

Bombardment

Subculture tissue (20-25 clumps/plate) 3-5 days prior to bombardment. Place tissue in the center of dish (containing medium) and dry by uncovering tissue for 15 minutes in the hood. Bombard using the Particle Inflow Gun or another bombardment apparatus 2x on D20. After one week, transfer all clumps to D20 Hyg 15. After 3-4 weeks, select living tissue and transfer every 3-4 weeks thereafter. Maintain on D20 Hyg 15. Keep track of growing sections. When there is enough tissue, extract DNA for PCR analysis.

Development/Maturation

Clones can be regenerated by first placing clumps on M6AC. From 6-9 clumps should be placed in a tall dish (100 x 25 mm) at 23°C. After 3-4 weeks, embryos can be taken directly for germination or separately subcultured to fresh M6AC medium for further development. After 4-6 weeks, embryos are cream-colored and ready for desiccation.



M6AC

MS salts
B5 vitamins

6% Maltose
pH 5.7
0.2% Gelrite
0.5% activated charcoal

Desiccation

Embryos are ready for desiccation once they have turned cream colored. If they have not, leave them on M6AC medium for a little longer. Place 9 embryos in a dry Petri dish, seal with parafilm and place on the shelf for 2-5 days. Embryos should be somewhat flaccid and not crispy-crunchy.

Germinate

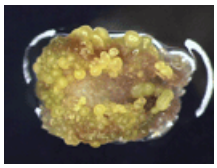
Germination should occur within one week after transfer to OMS (growth regulator-free MS medium). Plants can be transferred to Magenta boxes once they hit the tops of the dishes. Plants can then be placed in soil once there is good root and shoot formation. Rinse OMS from the roots with water, to avoid fungal growth on residual medium on the roots. Keep under 24 hr lights, high humidity. To push flowering and pod formation, plants can be placed directly under 16 hr days. To keep plants vegetative and generate a ton of seed, keep them under 24 hr days until they are 2 ft tall and then transfer to 16 hour or shorter days (to promote flowering).

Suspension Transformation

Soybean suspension culture transformation was first reported by this laboratory in 1991. Suspension culture initiation is not too difficult but maintenance and selection can be problematic for some individuals. Once properly trained in this approach, suspension culture transformation can be very efficient. The starting material is induced as described for D20 tissue but embryo proliferation is performed in liquid culture. Embryogenic tissue is bombarded with plasmids encoding hygromycin resistance and one or more genes of interest. The genes of interest can either be located on the same plasmid or on different plasmids (co-transformation). One week following bombardment, the medium is replaced with fresh medium containing 30 mg/l hygromycin. The medium is replaced with fresh hygromycin-containing medium every week for 3 additional weeks. Selected tissues are first recovered after 3-4 weeks. We proliferate the tissue, perform PCR of putative clones and then regenerate plants. New cultures are generated every 3-6 months and are ready for bombardment after 1-2 months. If cultures are maintained for too long (>6 months) in liquid medium, sterility of regenerants may be a problem.

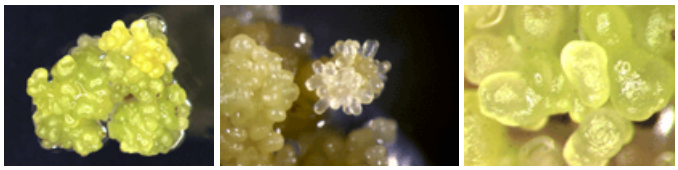
Finer JJ, MD McMullen (1991) Transformation of soybean via particle bombardment of embryogenic suspension culture tissue. In Vitro Cell and Develop Biol – Plant 27P:175-182.

Initiation of proliferative tissue from immature cotyledons (as above)
Isolate immature zygotic embryos from pods, 2-3 weeks after flower formation. Immature embryos are 3-4 mm in length. You can see them by backlighting or feel them by slightly squeezing the pods and feeling some resistance. You will have to gauge your vision or feel by opening a few pods to make sure that the size is right. Sterilize pods and remove the immature embryos. Discard the embryo axis. Plate immature cots on D40 for 3-4 weeks at 25°C.



D40
MS salts
B5 vitamins
3% sucrose
40 mg/l 2,4-D
pH 7.0
0.2% Gelrite

Proliferation of D20 tissue (this step is optional)
Transfer to D20. Select bright green, globular, proliferative embryos. Subculture every 2-3 weeks under conditions described for induction. Quality is critical!!



D20 tissue

D20
MS salts
B5 vitamins
3% sucrose
5 mM asparagine
20 mg/l 2,4-D
pH 5.7
0.2% Gelrite

Establishment of Suspension Cultures
Place a small amount of tissue (10-20 mg) in liquid FN (Finer and Nagasawa) medium (30 ml/125 ml flask). Subculture every 1-2 weeks. Quality is critical – embryogenic tissue should not contain developing embryos; necrotic areas are undesirable, tissues should be yellow to green.

FN
MS salts (minus nitrates)
10 mM NH_4NO_3
30 mM KNO_3
B5 vitamins
3% sucrose
5 mM asparagine
5 mg/l 2,4-D

pH 5.7

Bombardment

Remove tissues from flask and place ~20-25 clumps in a dry dish. Remove excess medium with pipet tip. Uncover in hood and let dry 5-15 min. Seal plates and let rest for 2-4 hours prior to bombardment (pre-bombardment osmotic conditioning). Bombard using the Particle Inflow Gun or another bombardment apparatus 2x. Wrap dishes and let rest for an additional 1-2 hours (post-bombardment osmotic conditioning). Place back in liquid culture. Apply hyg 30 after one week. Replace with fresh medium with antibiotic every week for 3 additional weeks. Clone recovery takes 1-2 months. Clones should be removed from the flask and proliferated separately under selection for an additional 1-2 months. When there is enough tissue, extract DNA for PCR analysis.

Selected suspension culture clone.

Development/Maturation/Germination (as above for D20 tissue)

For additional information on soybean transformation, see the "outputs" link at the "Soybean Tissue Culture and Genetic Engineering Center" site.

Questions???

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