Shooting Maize (and maybe Arabidopsis) Callus (M.Raizada)

(Basic Protocol from P.Lemaux)

<u>In advance:</u>
-read *Methods in Enzymology* article on Optimizing Biolistic Transformation (Alan, I can't find the reference right now - I think it's by J.Sanford - I highly recommend it)
-prepare DNA by CsCl or Qiagen (or by Wizard Maxi, followed by Phenol:Chloroform, ethanol precipitation)
-order rupture discs from Alameda Scientific Instruments (they rupture better than the std discs - I think I gave you their address)
-order fresh spermidine, aliquot 14ul, and freeze at -70C. Prior to use, add 986ul of ddH20 to give a 0.1M concentration.
-gold: 1um spherical particles - store at 60mg/ml in pure ethanol at -20C
-prepare 2.5M CaCl2 - store at 4C

<u>Notes:</u>
-in aqueous solution, the gold, and especially gold+DNA solution will agglomerate: thus, during the procedure, keep the time that the gold is in an aqueous solution down to a minimum (ethanol steps are fine)
-for sterile shooting, I do the DNA precipitation in the hood, with the GUN washed down with ethanol (the inside), also kept in the hood
-for sterile shooting, sterilize the macrocarriers and the metal stop screens in 70% ethanol for about 20minutes (but do not leave longer, since it will cause delamination of the macrocarriers) - I dry these sterilized materials in the hood in petri dishes. I autoclave the metal macrocarrier holders.
-**prior to any shooting, place the rupture discs in isopropanol (less than 20minutes, or instant if not sterile) and load the discs WET onto the gun - this is to get a nice seal to prevent premature/ectopic rupturing (terms I made up, thank you)
- for callus transformation, I reshoot each material 2-3x total, often at a lower pressure (650) and then again at a higher psi (1100), because callus material can very from subculture to subculture in hardness , so this covers all bases, plus increases transformation rates 2x
-to prevent cell injury, definitely pretreat tissues on osmotic media for 4hrs prior to shooting and 16 hrs post-bombardment (0.2M mannitol + 0.2M sorbitol): typically, I place tissues on filters with a fast flow rate, but considerable texture to grip tissue during shooting - I recommend Baxter S/P Filters (5.5cm size, Cat.F2217-55). The filters allow you to quickly move tissues in and off of the different medias
-after the 16 hr post treatment, let the tissues recover for another day on non-selection media without osmoticum, prior to selection - don't break any tissue up again for a few days - this is a recovery period
-in maize, the best time to shoot callus is 4-7 days after the last subculture (out of a 14day cycle), basically when the cells are growing quickly
-in maize, it is recommended that the tissues used for shooting not be broken up the night before - it should be done 4 hours prior to shooting

DNA Precipitation Procedure

Place CaCl2 solution, water, on ice.
To 14ul spermidine, add 986ul sterile H20.

1. Vortex stock gold (60mg/ml in ethanol) for 1min, tube inverted.
   Quickly, withdraw 35ul from middle of tube to new 1.5ml eppendorf tube.
   Spin 2000rpm, 5min, in a Beckman swinging bucket rotor (TJ-6 centrifuge)

Remove ethanol with a pipet.
Replace with 1ml sterile H20 (but don't dislodge pellet too much - this is just a rinse step)

2. Spin 5min, 2000rpm, swinging bucket TJ-6 centrifuge.
Remove all H20 with a pipet.

3. Resuspend gold in 25ul DNA solution (at 1ug/ul - not more). Pipet up and down thoroughly.
Then, one tube at a time, vortexing after each step:
Add: 220 ul sterile ddH20
250ul 2.5M CaCl2
50ul diluted spermide (after spermidine, vortex immediately, but briefly, before DNA precipitates out).

Place on ice 5minutes.
Vortex at room temperature 1-2minutes
Centrifuge 500 rpm for 5 minutes (swinging bucket rotor).
Remove supernatant - should be a smear plus pellet.

4. Resuspend in 600ul ethanol, by pipetting up and down repeatedly - use the pipet tip to scrape down the sides of the tube containing gold. I always feel like the precipitation has occurred when I see clumps which are hard to break up - just try to get them to a uniform size.
Spin 1minutes, full speed in a normal table top microfuge, room temp.

Remove all ethanol.
Resuspend in 36ul fresh ethanol.

Place on ice until shooting (I've let it sit for more than 1 hour without a problem; I think even a few hours would be O.K.).

5. Use 3 shots (x10ul each) from each tube. Vortex briefly, as you withdraw solution to place on macrocarriers.
When placing on macrocarriers, spread around middle with pipet tip as aliquoting out.

The DNA solution can end up irreversibly sticking to the macrocarrier - don't leave out in humid conditions or exposed to air too long. I typically only load up 3 macrocarriers at a time.

Remember to load rupture discs wet with isopropanol to get a good seal.

I wait until the vacuum reaches 27-28psi prior to shooting.

It should take a few seconds for the pressure to build up prior to the burst: ie.it should not happen too quickly - a fast rupture seems to minimize transformation efficiency.

I 've enclosed the GUN diagram. Again, consult the Methods in Enzymology paper on shooting for further tips - they also explain some of the odd details suggested here.

Good Luck!
-Manish