Modified protocol for Gene Gun cartridge preparation

(Helios manual/Hussey lab, Durham, UK/V. Žárský and F. Cvrčková)

Reagents and materials required:

100 % water free ethanol (pure ethanol incubated 3x over dessicated Sigma M-9882 beads[1], kept in sealed bottle in a dessicator)
Fresh 0.05 M spermidine free base (dilute immediately before use[2])
1M $CaCl_2$
Plasmid DNA at approx. 1 ug/ul (known concentration) in 10 mM Tris pH 8
Eppendorfs, falcons, pipettes, tips.
PVP m.w. 360 000[3] and The Tubing from the kit.

1. Particle preparation.

1. Weight gold into an eppendorf tube (15-25 mg gold per tubing length, which will produce 50 cartridges). 0.6 um pellets are OK for onion transformation. Resuspend in 70 % ethanol, leave for 15 min. Pellet at low speed (5000 rpm 15 sec), discard ethanol.
2. Wash 3x in water (pellet 30 sec to 1 min at 7000 rpm), resuspend carefully each time.
3. Resuspend in 500 ul 50 % glycerol. Divide the suspension equally into separate tubes for each construct; leftovers can be kept in the fridge for further use.
4. Wash 2x in water as in step 2, remove supernatant.
5. Resuspend each aliquot (= for 1 tubing length) in 100 ul of 0.05 M spermidine. Wortex for 2 seconds, sonicate 2x at most 30 sec in the water bath (NO CLUMPS!)
6. Add plasmid DNA at no more than 1 ug per cartridge, i.e. 50 ug per coating (may be less – down to 10 ug – but not much more because of clumps)[4], in a volume of less than 100 ul (if DNA is dilute, scale up the spermidine and $CaCl_2$ – there should be more spermidine than DNA). Vortex the suspension for 5 seconds.
7. Carefully and slowly add 100 ul of 1M CaCl2 (the same volume as the spermidine), using a gel-loading tip. Vortex after adding each drop. Let it sit for 10 min at RT to precipitate.
8. Spin as in step 1, discard supernatant. Wash the pellet 3x with 100 % ethanol (spin 5 sec 5000 rpm at each wash).
9. Resuspend in 0.05 mg/ml PVP in 100 % ethanol and transfer into a 10 ml plastic tube (you will have to rinse the tube several times with a small aliquot to gety all gold out). Use 0.12 ml PVP per mg gold, i.e. total volume 1.8 to 3 ml.
10. Continue to coating, or store the suspension well sealed (parafilm) at –20 ^{o}C up to 2 months.

2. Coating (have someone show it to you the first time!)

1. Attach the nitrogen hose to the tubing prep station. Measure and cut the tubing (using razor, NOT scissors) and insert into the machine. Pre-dry by blowing nitrogen at 0.3 to 0.4 l/min tor 15 min. (To open gas flow: check that all is closed, open bottle valve to see pressure in regulator, open black valve to 0.4, finely tune flow on the machine. Close black valve only when transiently stopping, bottle valve first, then the rest, when finishing.)
2. Take the tubing out and attach a syringe with a piece of soft tubing to one end. Resuspend the gold (by sonication if clumpy) and immediately suck the suspension into the tubing using syringe, leaving several cm on the ends. NO BUBBLES!!! Immediately turn the tubing horizontal and re-introduce it into the machine, with syringe still on.
3. Leave for 5 min, carefully connect to the other attached syringe, and suck out the ethanol VERY slowly and carefully (1.5-3 cm/sec, 30 to 45 sec to empty the tubing), so that gold stays in place.
4. Briefly press the switch to II, to rotate tubing by 180^{o}. Remove the syringe, wait for 4 sec, switch to I and let the tubing rotate.
5. After 30 sec, start nitrogen flow at 0.35 to 0.4 l/min. Dry for 5 min, then stop rotation and gas flow and remove the tubing.
6. Trim the ends using the tubing cutter and remove any large obviously empty areas. Cut the good parts to cartridges using the tubing cutter. Store bullets at 4 ^{o}C in selaed cartridges with a dessicant pellet.

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[1] Wash fresh beads with water 5 x to remove dust,dry at 150 ^{o}C for 3 hours; dry recycled beads the same way.
[2] Keep pure spermidine, which is liquid and 6.37 M, in aliquots at –80 ^{o}C. 7.8 ul spermidine + 1 ml water OR 10 ul spermidine + 1264 ul water gives 0.05 M.
[3] Make a stock of 20 mg/ml PVP in 100 % ethanol, keep in dessicator under parafilm. Dilute an aliquot to 0.05 mg/ml in 100 % ethanol before use (0.12 ml per mg gold).
[4] If using multiple plasmids, mix them before and do not use more than 50 ug of the mix.