UNIVERSITY OF CAMBRIDGE
Department of Plant Sciences

Biolistic Bombardment / Gene Shooting Protocol for Bio-Rad Gun

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I. Stock Solution Preparation (made in advance)

1. *Calcium Solution*—How to making a 2.5M $CaCl_2$ Solution:
   -Dissolve 3.68g of $CaCl_2$ $2H_2O$ in 10mL of H_2O.
   -Filter sterilize the solution and aliquot into 1mL tubes. Store at -20°C.
2. *Spermidine Solution*—How to make a 0.1M Spermidine Solution:
   Note: Wear gloves, Spermidine is corrosive.
   - Dissolve 1g Spermidine (S0266, Sigma) in 3mL sterile H_2O.
   -Take volume up to 6.9mL H_2O.
   -Make 0.1M aliquots, from diluting the 1M stock solution. Add 100µL of 1M Spermidine Solution to 900µL of H_20. Store at -20°C for up to 5 years.

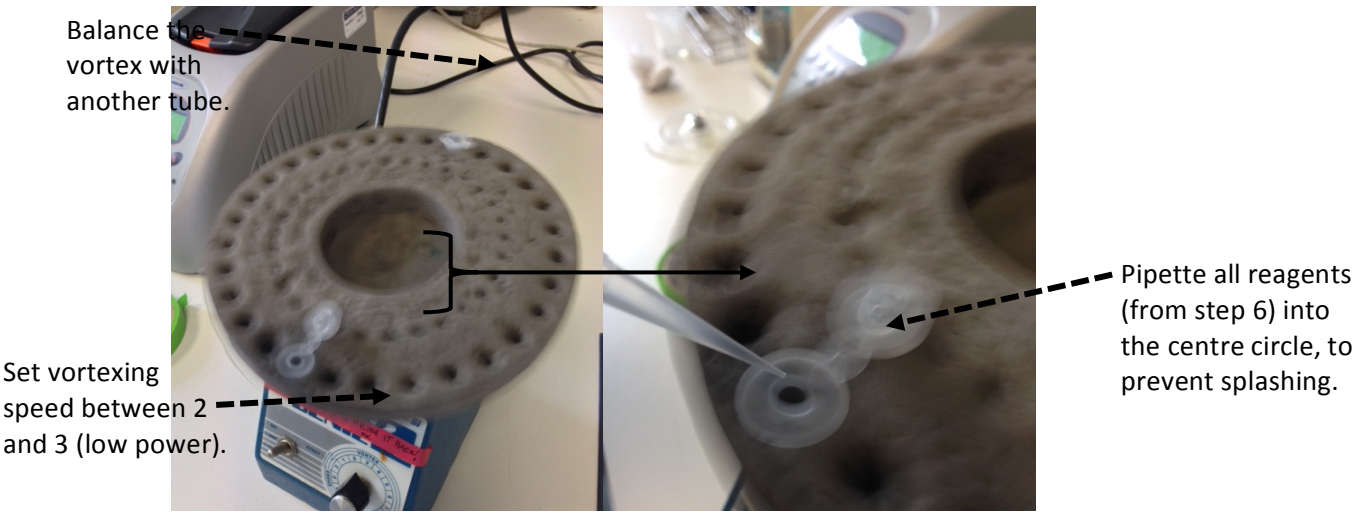
   Alternatively, you can:
   -Melt the Spermidine at 65°C in a water-bath for more than 10mins (do not microwave).
   -For 1M stock: take 15.8µL of Spermidine (free base) and add that to 984.2µL of distilled sterile H_20.
   -Make 0.1M aliquots, from diluting the 1M stock solution. Add 100µL of 1M Spermidine Solution to 900µL of H_20. Store at -20°C for up to 5 years.

3. *Gold/tungsten Particle Solution*—How to wash and make a 100mg/mL particle solution:
   -Weigh out 100mg of gold or tungsten powder (1.1 µm) in an Eppendorf tube.
   -Suspend in 1mL of 100% Ethanol, vortex, spin down (2 quick bursts) in a centrifuge, pipette off liquid. Take care not to disturb the pellet.
   -Resuspend in 1mL of 100% Ethanol, vortex, spin down (2 quick bursts).
   -Resuspend in 1mL of 70% Ethanol, vortex, spin down (2 quick bursts).
   -Resuspend in 1mL of 50% Glycerol (filter sterile).
   -Aliquot as needed into sterile Eppendorf tubes. Store at -20°C.

II. Procedure for precipitating DNA onto particles (day of shooting)

1. Get the 100mg/mL gold or tungsten solution (described above) from the -20°C freezer and vortex well.
2. Pipette 35µL of 100mg/mL gold solution into Eppendorf tubes (one tube for each construct that will be tested).
3. Spin tubes down in a centrifuge (2 seconds at full speed) to pellet the particles.
4. Pipette the 50% Glycerol supernatant (~35µL) off. This should leave a small gold or tungsten pellet.
5. Resuspend in 35µL sterile H_2O.
6. Whilst continually vortexing (see vortex set up on next page), add the following reagents into the tube containing the particles in rapid succession and in this order:
   -35µL of particles (from section II. step 5)
   -10µL of plasmid DNA (see plasmid purification protocol)
   -50µL of 2.5M $CaCl_2$ (from section I. step 1., add slowly, dropwise to avoid splashing out of tube)
   -20µL of 0.1M Spermidine (from section I. step 2., add immediately after $CaCl_2$)
   After adding these components, allow the tube to vortex for at least another minute.

Vortex set up for this step:

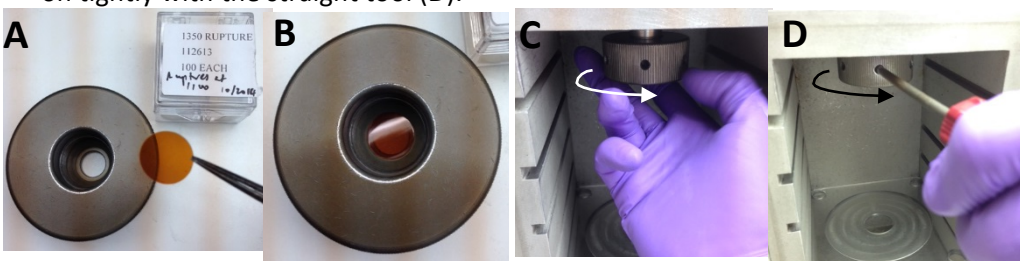


7. Keep tubes on ice for at least 10 minutes. Vortex or flick tubes every few minutes to keep particles suspended.
8. Spin tubes down (2 seconds at 6000rpm) in a centrifuge and pipette off the supernatant.
9. Wash particles with 250µL of 100% Ethanol (just pipette it in, and suck it right off, no need to flick or vortex the tube). Discard the Ethanol.
10. Resuspend the particles in 100µL of 100% Ethanol. Keep tubes on ice.
11. Load 10µL at a time for bombardment (details in the next section).

III. Operating the Gene Gun (day of shooting)

1. To switch on the Bio-Rad Gene Gun, please follow these steps:
   -Open door of gun and clean inside with 70% Ethanol.
   -With door open, open valve on top of the helium cylinder (anti-clockwise).
   -Turn the regulator clockwise until a pressure of 100psi above operating pressure is reached (~1600psi or 110bar). This is the left pressure gauge.
   -Switch gun on.
   -While door is still open, turn on vacuum pump (on the floor).

2. Loading samples
   a. Loading the rupture disk:
      -Get a 1350psi rupture disk (**A**) and place it into the rupture disk holder (**B**)
      -Screw the rupture disk holder onto the screw inside the top of the gun chamber (**C**). Screw on tightly with the straight tool (**D**).



b. Preparing DNA

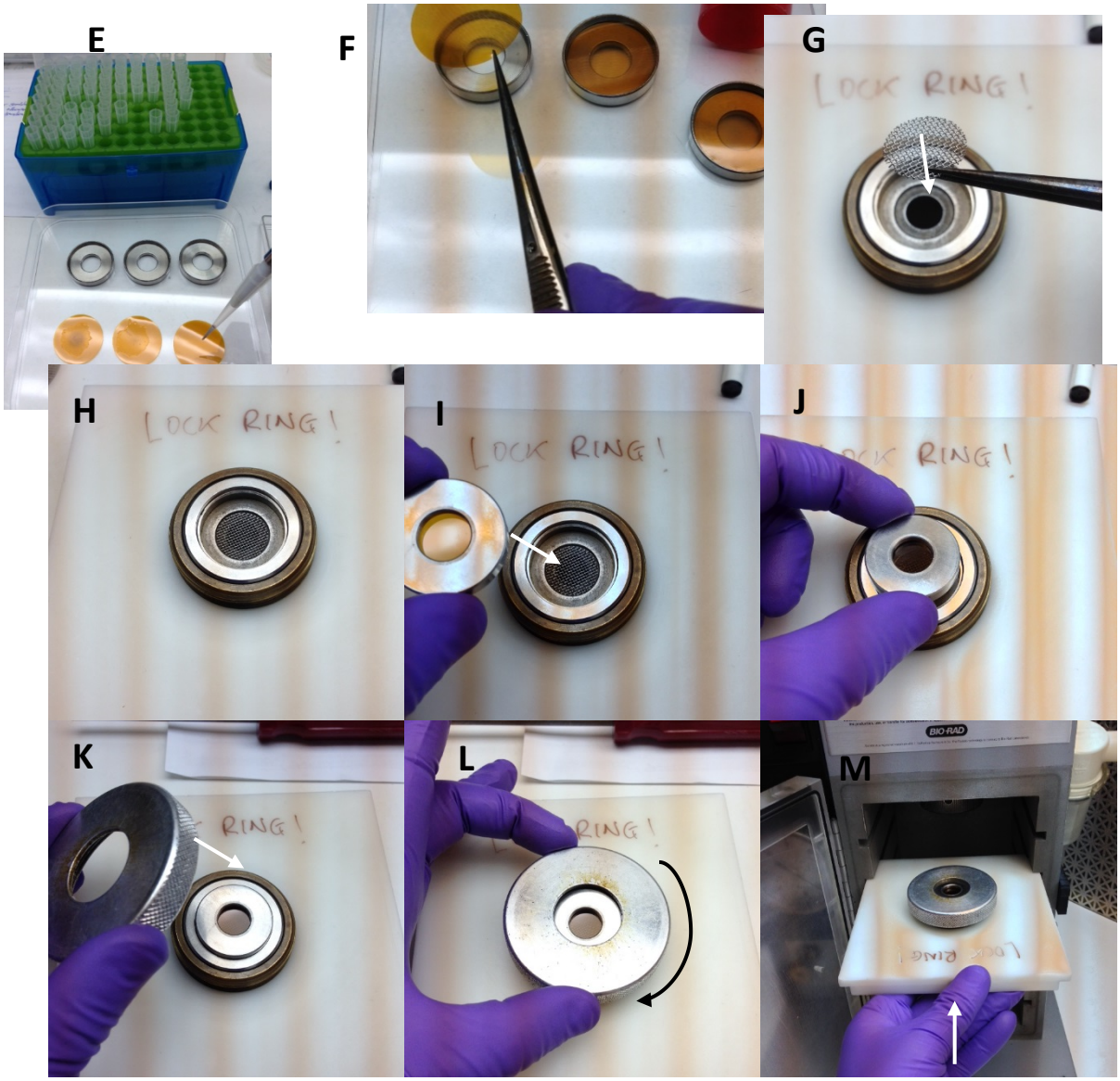
-Get out a carrier disk and place it down so that it is like a bridge (note: the carrier disks are curved like a dome) (**E**).

-Pipette 10µL of the DNA particle solution (from section II. step 10 and 11) onto the surface of the disk (**E**). Try to discharge the sample all at once to ensure an even spread of the sample.

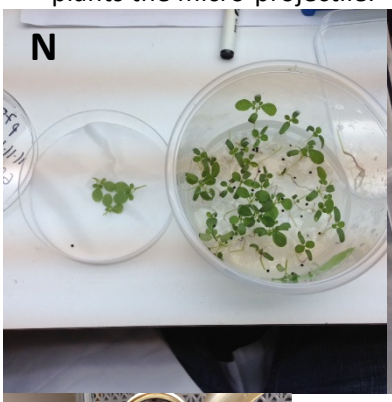
-Once the Ethanol has evaporated off the disks, they can be loaded into the carrier ring. Use the red plastic piece to push down the carrier disk into the carrier ring (**F**). It should fit perfectly under a lip in the carrier ring.

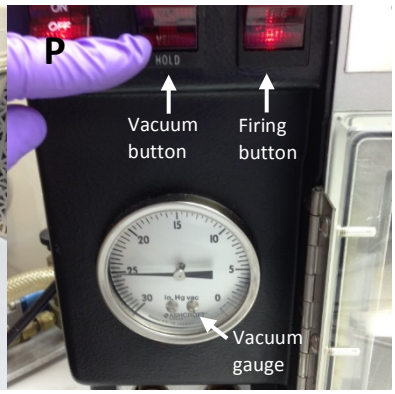
-Load a new wire mesh into the sample block (**G**, **H**), and then place the carrier ring with the carrier disk inside upside-down over the wire mesh (**I**, **J**).

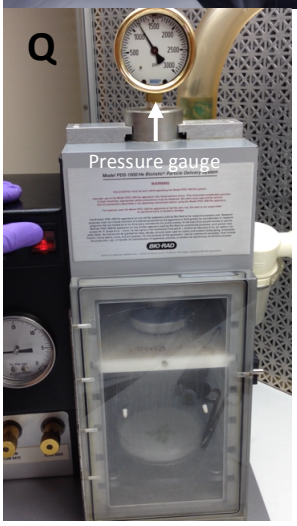
-Fasten the screw cover over the carrier ring (**K**, **L**) and load it into the gun (second shelf down from the top) with the "lock ring" message facing towards the gun door (**M**).



c. Loading plants and firing the gun.
-Prep plants by arranging small seedlings in the centre of a petri dish on a damp filter paper (**N**).
-Load plants on 5th shelf down from the top (**O**).
-Close the gun door and build a vacuum (to 25 in. Hg) by holding the "vacuum" button (flip up), once the dial reads 25 in. Hg then flip the button down to "hold" the vacuum (**P**). Once a vacuum is reached, the firing button will light up red.
-Hold down the firing button by pushing up, to begin building pressure in the rupture disk chamber (**Q**). Once the pressure exceeds the rupture disk (~1350psi), it will bombard the plants the micro-projectile.







3. To switch off the gun, please follow these steps:
-Generate a vacuum in gun chamber.
-Turn off valve on top of helium cylinder (clockwise).
-Depress firing button to remove residual helium in the lines; continue until main cylinder and regulator pressure dials go to zero.
-Turn regulator to closed position (anti-clockwise).
-Vent the gun chamber to remove vacuum.
-Open gun chamber door.
-Turn off vacuum pump.
-Switch gun off.
-Clean inside of the gun and remove any debris.