Microprojectile Bombardment Protocol

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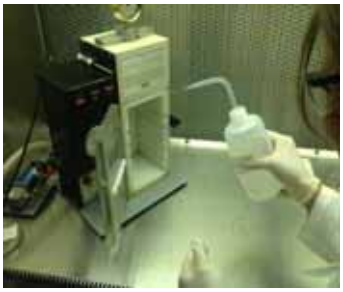
Microprojectile bombardment was developed by John Sanford and Ted Klein in the mid-1980's. Bombardment then proved as the method for the first achieved transformation of maize and other monocots resulting in fertile transformants. It was subsequently replaced by enhanced *Agrobacterium*- mediated protocols. However, this method is still important for several reasons and applications. First microprojectile bombardment, serves as a delivery method for transient expression assays. Second, this protocol has become increasingly important to the development of genome editing delivery.

Gun Preparation



1. Surface sterilize the laminar flow hood working surfaces and the gene gun (inside and out) with 70% ETOH.

2 Autoclave removable metal gun parts, stopping screens, macrocarrier holders and forceps.

3. Sterilize macrocarriers (henceforth called flyers) in 70% ETOH for 1 hour. After surface sterilization in 70% ETOH for 1 hour, use sterile forceps to move the flyers to a sterile petri dish placed toward the back of the hood to dry. Note: because they were sterilized in 70% ETOH the flyers may take 20-30 minutes to dry. The may be left in the bombardment hood until needed. *Ensure these and all gun parts are completely dry.*

DNA Precipitation

1. Reagents: Prepared prior to procedure

    2.5 M $CaCl_2$ $2H_2O$ (100ml):
    Dissolve 36.76g in ~50ml ultrapure H_2O
    Bring to a final volume of 100 ml
    Filter sterilize or autoclave solution

0.1 M Spermidine (1ml): (is solid at 2-8C, right)
Mix 15.7 ul spermidine with 984.3ul ultra pure water
Filter sterilize

Note: Spermidine when properly stored (2-8°C) is a solid. Melt spermidine in 65°C water. Spermidine will oxidize and turn yellowish and should therefore be stored under N_2. In addition, keep bottle and a desiccant pack in a 50 ml Falcon tube. Mix a fresh 1 ml working solution each bombing experiment.

2. Prepare a gold stock solution. This stock can be used for many microprojectile bombardment experiments events but should be discarded when the volume drops below 250µl.

Transfer 60 mg of 0.6 µ gold to a 1.5 ml tube with 1 ml of 100% ETOH.
Note: 100% ethanol will hydrate quickly becoming 90-95%. So, splurge and open a new bottle of ETOH. Using old ETOH is likely to cause clumping of gold particles and decrease the efficiency of the transformation.

3. Prepare working solutions of gold particles and DNA. To maintain uniformity in bombardment parameters, only 3 bombardments should be performed per solution. Also, keep experiments under six to eight tubes.

a. Vortex the gold stock (60 mg/ml) for 1 min. to break up any gold aggregates.

b. Sonicate the gold for 15-20 seconds. *Caution: Although it is important to find the "active spot" in the sonicator, leaving the gold in the sonicator for more than 20 seconds will overheat the gold particles.*

c. Invert the tube between sampling to prevent settling of gold particles, withdraw 35 µl of suspended gold from the middle of the 1.5 ml Eppendorf tube and dispense it to the bottom of a sterile 1.5 ml tube. Invert the tube between each sample to ensure a uniform sample concentration of the gold, remember gold particles will settle quickly

d. Using a centrifuge with a swinging bucket rotor, pellet the gold for 5 minutes at 500 RPM.

Note: This is a good time to prepare the spermidine. Take an aliquot of meted spermidine from the stock and filter sterilize it into a sterile microfuge tube.

e. Remove the ETOH and without disturbing the pellet, then carefully add 1 ml of sterile water without disturbing the pellet. If pellet is not disturbed skip to step g.

f. Spin for 5 minutes at 500 RPM.

g. Carefully, without disturbing the pellet, remove the H_2O (as close to close to dryness as pipeting will allow).

h. Resuspend the microprojectiles in 9µl of DNA solution [1µg/µl]. Pipette up and down to facilitate re-suspension. *The microprojectiles should go into solution readily and not be clumpy. Multiple constructs can be added. Co-transformation can occur up to 70% of the time.*

i. Add 220 µl of sterile H_2O, and mix well.

j. Add 250 µl of sterile $CaCl_2$, and mix well. *Calcium chloride salts the DNA out of solution.*

k. Add 50 µl of sterile spermidine, mix well and place on ice. *Spermidine tends to stabilize the DNA.*

l. Using a Genie 2 vibrating mixing pad (left) mix this solution in refrigerator (4C) for 10 minutes. *A small amount of foam on the top of the solution indicates a good rate of vortexing.*

Note: This is a good time to start surface sterilization of the rupture discs in 70% ETOH for at least 20 minutes. The sterilized flyers should now be dry (see step 3 under "Gun preparation).

m. Centrifuge in a swinging bucket rotor for 5 minutes at 500 RPM in a refrigerated centrifuge *(if available, this may help to reduce DNase activity.)*

n. Remove the supernatant from the DNA/gold precipitate (as much as possible) and carefully add 600 µl of 100% ETOH. It may be necessary to break the pellet apart with a pipet tip.

o. Spin for 5 minutes at 500 RPM in a refrigerated centrifuge, remove ETOH and re-suspend pellet in 38 µl of 100% ETOH. The precipitate should readily go into solution. the particles are now ready for bombardment.

"Bombs Away" *The ideal maximum number of plates per experiment to bomb is approximately 18 or 21 to accomplish in a reasonable time period*

1. Soak rupture disks (1100psi) in 70% ETOH for at least 20 minutes prior to use. *Oversoaking may affect the integrity of the mylar material and rupture setpoint.*

2. Open helium tank supply valve, and adjust line pressure to ~1200 psi (slightly higher than rupture disc setpoint.)

3. Turn on vacuum pump.

4. Place flyers in holders. Resuspend DNA/gold by sonicating for 10 seconds and place 10 µl of solution on center of flyer. Allow the flyer to dry thoroughly. *Do several of these at a time. (Multiples of 3-number of bombs per tube.)*

5. Place stopping screen in the microcarrier launch assembly.

6.. Invert the flyer holder and insert into microcarrier launch body. Then screw flyer holder cap screw onto micrcarrier launch body. *Note: the flyer holder can be inserted in different positions pending the placement of spacers. Start with the middle location unless a different location has been optimized for the specific application.*

7. Unscrew rupture disk retainer cap from inside of the gun, and insert a wet rupture disk which has been soaking in 70% ETOH until use.. Screw retainer cap back on and tighten firmly. Rupture discs are available with different rupture setpoints, and can be used in pairs to achieve desired setpoints.

8. Inset loaded microcarrier launch body into the first slot from the top with the hole to the front. (this distance may and is determined experimentally for each system)

9. Insert Petri dish platform into the fourth slot from the top.

10. Insert plated tissue on Petri dish platform.

11. Close chamber door. *Operator should wear safety goggles.*

12. Begin drawing a vacuum by pressing upward on the vacuum button . Watch as the gauge goes up until it indicates 27-28”Hg. Press down the button rapidly to the ‘Hold” position . Press up on the “Fire position and monitor the gauge on top of the device. When the pressure exceeds rupture disk setpoint (in this case 1100 psi) the gun should fire. Note the actual fire pressure on the gauge located on the top of the gun Close chamber door.

13. After the gun fires, release fire button and vent chamber.

14. Remove and cover sample.

15. Remove microcarrier launch body and place on a sterile surface. Unscrew cap and remove flyer holder. Unscrew rupture disk retainer cap and remove spent rupture disk. Return to step 4 for additional transformation attempts.

16. When finished clean gun parts with 70% ETOH, autoclave metal gun parts, stopping screens, macrocarrier holders and forceps for next bombing campaign. Shut helium supply valve and vent supply line by drawing a vacuum and holding the fire button with a rupture disc installed. Shut off vacuum pump. Leave chamber vacuum switch on vent.


Shutting down the Gun Apparatus

Lastly, after all the bombardments have been completed,:

1. Shut off the valve to the Helium tank.
2. draw a vacuum on the gun chamber by closing the chamber door, and pressing the vacuum button.

3. Next press the vent button to deplete the helium from the line. This procedure should be repeated until the pressure reaches zero.
4. Vent the chamber and open the door.
5. Turn off the vacuum pump last (*Caution : DO NOT leave the chamber under vacuum and then turn off the pump as the pump oil will be sucked into the chamber)*