High Efficiency Helios Gene Gun Protocol and Onion bombardment (Adapted from Bio-Rad.com and Dr. Liu's lab at University of Maryland at College Park)

Making bullets
Bullets are good for up to 6 months, but may begin to decrease in transfection efficiency after 2-3 months. Bullets should be stored at 4°C in the presence of desiccant pellets. Always let the scintillation vials, in which bullets are stored, warm to room temperature before opening the vial.
Before getting started have ready:

- Spermidine (0.05M)
- $CaCl_2$ (1M)
- EtOH (100%-high grade-unopened bottle)
- Autoclaved H_20
- DNA to be transfected
- Polyvinylpyrrolidone (PVP-20 mg/ml stock)
- 2 X 15 ml conical (2/bullet set)
- Tubing (cut into 1 X ~ 30 inches/ bullet set)
- Scintillation vials (1/bullet set)
- Desiccation pellets
- 10 ml syringe w/tubing on the end

Note: Students will do the steps from 3 to 14, and TA will do 1 to 2 and 15 to 26

1. Cut a length of tubing long enough for all samples that will be prepared. Wash tubing with fresh 100% ethanol at least 3 times.
2. Start drying gold-coat tubing with 0.4-0.5 L/s Nitrogen for 30 minutes. While drying, continue to next step.
3. Dilute 100 ul frozen stock of spermidine from 0.5 to 0.05 M with 900 ul dH2O.
4. Weigh gold and put into microfuge tube.
    a. both 1.0 um and 0.6 um sized gold work for onion.
    b. 12.5 mg gold/ prep (0.25mg/shot) is plenty for onion

5. Prepare 20 mg/ml PVP solution in a small screw cap tube using fresh unopened ethanol. Very little is needed, make smallest volume possible

6. Using fresh ethanol prepare 3.5 ml of 0.05mg/ ml PVP solution for each length of 30” tubing
    a. 8.75 ul of the 20 mg/ml solution for every 3.5 ml ethanol
    b. Keep tightly sealed in a screw cap tube.

7. In a new tube, prepare 100 ul plasmid solutions containing 50 ug total of your DNA.
8. Add 100 ul 0.05M spermidine to tube with gold then vortex and sonicate (3-5s).
9. Add plasmid DNA to gold. Vortex (High speed) 5s
10. Vortex at a very slow speed with cap opens and add 100 ul (sterile) 1M CaCl2 drop-wise.
11. Let sit at room temperature for 10 min
12. Spin to pellet gold (-30s, 13000rpm) and discard supernatant.
13. Vortex pellet and then thoroughly resuspend with 1 ml fresh ethanol. Spin to pellet, discard supernatant, and wash two more times (3total) with 1 ml 100% ethanol.
14. After final wash, resuspend pellet with 1 ml the 0.05mg/ ml PVP solution. Transfer to a 15 ml screw cap tube. Wash original microfuge tube with 1 additional ml of 0.05mg/ml PVP then transfer to the 15 ml tube. Bring final volume of gold/ PVP solution up to 3 ml with 0.05 mg/PVP. Cap suspension tightly.

Note: the following step will be done by TA.

15. Bring sample, cortex, and sonicator to prep station. Turn off nitrogen removing dry tubing room prep station and cut 30” length.
16. Vortex, sonicate, and then invert the 3ml gold/ plasmid solution . Immediately draw it up into the tubing using a syringe with the adapter tubing. Avoid generating bubbles in tubing.
17. Flip tubing horizontally immediately after filled, and insert into prep station. Do not detach syringe from tubing (if you do, the fluid will flow into the prep center)

18. Allow gold to settle (15 min for 0.6 um gold, sooner if 1.0 um gold is used)
19. Quickly detach loading syringe, and attach 10 cc syringe with adapter tubing (clamped to prep station) for removing ethanol, this syringe, is for removing ethanol, should have plunger already slightly pulled out, so the start of the removal won't be jerky
20. Remove ethanol with syringe at a rate of 0.5 / second.
21. Remove syringe and immediately rotate tubing in prep station 180 degree and leave it there for 3-4 seconds
22. start continuously rotating tubing and allow to smear for 20-30s
23. Turn on Nitrogen flow from prep. Station until 0.35-0.4 LPM is reached. Dry with gas on and rotating for 5 minutes
24. Remove tubing, cut into 0.5" pieces with tubing cutter, avoiding regions of uneven spreading. Obtaining cartridges with an even coat is crucial. Discard all band ones.
25. Cartridges can be used fresh or stored at 4 degrees in tightly sealed scintillation vial containing a Dry cap desiccant and wrapped with parafilm. The desiccant vial should be blue or dark purple if good. It turns bright pink when saturated with water.
26. When using cartridges from 4 degrees, acclimate vial to room temperature for 30 minutes before opening. This is important, as it will prevent moisture from accumulating in cartridges and vial.

Method for Shooting Onion samples: (Done by the students)

a. Cut 1 cm square chunk of onion from inner layers of onion bulb
b. Insert battery and fresh barrel liner into gun. Make sure that barrel liner has an o ring in it.
c. Insert empty cartridge holder cylinder into gene gun, with printed number 12 on top
d. Advance cartridge holder once or twice to make sure cartridge is inserted correctly.

e. Attach gun to helium tank. Check that regulator knob is closed , then open main valve on tank. Next, adjust pressure to 150 to 200 psi using regulator knob.
f. Fire gun a few times with empty cartridge holder to clean out chamber of gun and to make sure pressure is stable.
g. Remove empty cartridge holder and replace with holder containing 'bullets' (#12 has to be on top to insert holder)
h. Advance cartridge holder so that the cartridge you want to fire is lined up.
    a. When firing, cartridge that gets shot is actually the one opposite of the one on top. If number 12 is lined up on top, cartridge in hole number 6 will be fired.
i. Reset barrel liner on Petri dish lid with onion sample in the middles and inner epidermis side face up. Fire gun (hold down side button then pull trigger-)
j. Transfer onion to petri dish containing moist filter paper or kim wipes. Cover, wrap with parafilm to prevent drying.
k. Incubate at room temperature for 16-48 hours.
    a. You may want shoot several replicates, so you can check a few at different time points.
l. When you are done firing all samples, remove battery, cartridge holder, and barrel liner from gene gun. Next remove used cartridges from cartridge holder.
    a. Cartridge will still be coated with gold, but if you fire same cartridge twice onto parafilm, no gold will be shot the second time.
m. Clean barrel liner, and diffusion screen if used, by submerging in a beaker of soapy water, and then sonicate the beaker for 20 minutes in ultrasonic cleaner, rinse all parts well with water, and then soak in 70% ethanol for an hour.
    a. Liner and cartridge holders can be autoclaved if sterility is needed.