3.2.3. Loading Macrocarriers

1. For each construct to be bombarded, insert four sterile macrocarriers into macrocarrier holders, using the macrocarrier loader. Rotating the loader 90–180 degrees, while pressing down, helps in seating the macrocarrier. Place each set of 4 into a labeled sterile culture dish.
2. Place a set of four macrocarriers to be loaded with particles on sterile lab wipes in the rear of a laminar flow hood.
3. With the DNA-coated particles evenly suspended by vigorous vortexing or pipeting, remove a 9-µL aliquot and quickly spread it over the central portion of a macrocarrier, i.e., the area directly above the opening in the holder. The ethanol will quickly evaporate (*see* **Note 3**).
4. Repeat **step 3**, loading the other three macrocarriers with the particles coated with the same construct. Once the ethanol has evaporated from the last macrocarrier, place all four back into the labeled culture dish.
5. Repeat **steps 2–4** for particles coated with each of the other constructs.

3.2.4. Particle Gun Parameters

1. Rupture disk to macrocarrier gap: one-eighth in.
2. Macrocarrier travel distance: 6 mm.
3. Target distance: approx 11 cm, i.e., target platform in second slot from bottom.
4. Helium pressure: We routinely bombard each set of embryos four times; twice using 1100-psi burst disks, then twice using 1300-psi burst disks.
5. Chamber vacuum: 26 in. Hg.
6. Microcarriers: 1.0 µm, Au (*see* **Note 4**).

3.3. Bombardment of Embryos

1. Turn on the PDS1000/He, the vacuum pump, and the helium. Adjust the pressure at the He bottle to 1300 psi.
2. Assemble rupture-disk assembly with an 1100-psi rupture disk.
3. Assemble microcarrier launch assembly: Place a sterile stop screen into position in the stopping screen support, place a loaded macrocarrier (particle side down) in the fixed nest, and screw the macrocarrier cover lid on until snug. Insert launch assembly into the second slot from the top.
4. Center a plate of embryos on the target platform, slide it into the second slot from the bottom, remove the culture plate lid, and close the chamber door.
5. Draw vacuum to approx 26 in. Hg.
6. Press the "fire" button until the rupture disk bursts.
7. Release the vacuum, open the chamber door, put the lid back on the plate of embryos, and remove the plate and target platform from the chamber.
8. Disassemble the microcarrier launch assembly, discarding the spent macrocarrier and stop screen.
9. Disassemble the rupture disk assembly and discard the spent rupture disk.
10. Repeat **steps 2–9** for each additional 1100 psi bombardment. Bombard each plate of embryos twice at 1100 psi, rotating the plates 90° between shots.