card the supernatant; resuspend the microcarriers in 60 μL of 100% ethanol (*see* **Note 10a**).
4. Pipet 6 μL of the microcarrier suspension onto the center of the macrocarrier already installed in the macrocanier holder.
5. Let microcarrier/macrocarrier dry in a low-humidity and vibration-free environment for about 1 min. To obtain the best results, use the prepared macrocarriers as soon as possible *(see* **Note 10b**).

3.3. Microprojectile Bombardment

Caution: Safety glasses should be worn by everyone in the area when the PDS-1000/He or a different biolistic device is being operated. The procedures described below are for the use of the PDS-1000/He.

1. Set the helium pressure at 1300 psi (or another pressure 200 psi above the burst pressure of the selected rupture disk) by turning the regulator adjusting-screw clockwise.
2. Place an 1100-psi rupture disk in the rupture disk retaining cap, screw, and tighten the cap onto the gas acceleration tube (*see* **Note 11**).
3. Place a sterile stopping screen on the stopping screen support (*see* **Note 12**). Install the macrocarrier holder with macrocarrier on the top rim of the fixed nest. The microcarriers should be facing down toward the stopping screen. Place the macrocarrier cover lid on the assembly and turn clockwise until snug. Place the macrocarrier launch assembly in the second slot from the top in the sample chamber.
4. Place the precultured wheat embryos (in a Petri dish without the lid) at a distance of 13 cm from the stopping plate and close the sample chamber door tightly.
5. Turn the vacuum pump on. Set the VACUUM switch on the PDS-1000/He to the VAC position to evacuate the sample chamber to 26–28 in. of mercury. Put the vacuum switch in the HOLD position.
6. Press and hold the FIRE switch until the rupture disk ruptures (*see* **Note 13**); release the FIRE switch immediately after the disk ruptures.
7. Release the vacuum in the sample chamber by setting the VACUUM switch to the VENT position.
8. Open the chamber door; remove and cover the Petri dish.
9. Reassemble rupture disk and microcarrier launch assembly for next bombardment (*see* **Note 14**).

3.4. Recovery of Bombarded Embryos and Assessment of DNA Delivery

1. Rearrange the treated embryos by placing 10 of them in a Petri dish containing fresh MS2 medium; grow them in darkness at 25°C for 2 d.
2. If the *gus*A gene is used as a reporter (*see* **Note 15a**), take a few embryos for GUS histochemical staining using the x-gluc solution *(7)*. Incubate the embryos in the x-gluc solution for 12–18 h at 37°C, then transfer the embryos into 70% ethanol for fixation. As a result of delivery and expression of the *gus*A gene, blue spots