6. After the vacuum treatment, leave seeds in the bleach for another 15–20 min, swirling occasionally. By the end of the treatment, most of the green will have been bleached from the pericarp.
7. Transfer the flask to a tissue-culture hood; spray the flask and all materials introduced into the hood with 70% EtOH. Wash hands and forearms with Betadine and use aseptic technique for subsequent manipulations.
8. Decant the bleach, discarding any seeds that float (these tend to have undersize embryos), and distribute 75–100 seeds per 100 × 25-mm tissue-culture dish. Add 25–30 mL sterile water to each culture dish and gently shake until all the seeds are submerged. Thoroughly decant the water and repeat this wash for a total of at least three washes.
9. Transfer approximately five seeds to a sterile 100 × 15-mm tissue-culture dish lid or bottom.
10. Hold a seed with the tips of one forceps and use the other to carefully peal the pericarp from the embryo end of the seed. Puncture the seed coat (testa and exosperm layers) and dislodge the embryo from the endosperm.
11. Place the embryo on LS2.5 medium so that the flat side (plumule and radicle side) is in contact with the medium and the scutellum is up.
12. Repeat **steps 9–11**, arranging 40–60 embryos in a rectangular pattern centered in the dish.
13. Incubate overnight at 26°C in the dark.
14. Examine for contamination and, if present, subculture axenic embryos onto fresh plates. Be careful not to mistake endosperm starch granules for contaminants.

3.2. Preparation for Biolistics

It is assumed anyone using a PDS1000/He biolistics system for bombardment of rice tissues has access to the Bio-Rad operation manual. Therefore, details of the basic operation of the system will not be covered here. Rather, the following protocol will only discuss details and variables, as needed, to clarify the rice transformation process.

All the following steps (until the plants are put into soil) must be done with aseptic technique in a laminar flow or biocontainment hood. It cannot be overemphasized that one of the primary reasons for failure of an experiment is contamination.

Preparation of microcarriers can be done several days in advance and, as indicated above, embryo isolation should be done 1–2 d in advance, in order to detect and minimize contamination. The microcarriers should not be coated with DNA until just prior to bombardment.

1. Thoroughly spray the biolistics apparatus and the interior of the hood with 70% EtOH. If possible, also irradiate the contents of the hood with a germicidal lamp.
2. Distribute the burst disks, stop screens, macrocarrier holders, macrocarriers, and macrocarrier loader into 100 × 25-mm culture dishes and cover them with 70% EtOH.