increase in the frequency of herbicide-resistant plants that also contain the gene of interest. However, adding more of this DNA may also result in an increase in the number of copies of the gene of interest in each transformant. Consideration of this eventuality is especially relevant, because of the increasing number of reports indicating that multiple copies of an introduced DNA sequence can lead to gene silencing by cosuppression, methylation, and DNA rearrangement and deletion (for reviews of these phenomena, *see* **refs. *19–21***).

3. When done correctly, the particles will be evenly distributed over the center of the macrocarrier. This step is tricky, and, to a large extent, will determine how evenly the particles will be distributed over the target area after bombardment. It is especially important to be fast, since the particles will settle very quickly, even in the pipet tip.
4. We have had significantly more success using gold particles than the much less expensive tungsten particles. However, acid pretreatment has been reported ***(22)*** to greatly increase the transformation frequencies obtained using tungsten particles. We have confirmed this observation in transient expression experiments, and we are currently testing it in stable transformation experiments.
5. Bialaphos is dissolved in water at a concentration of 4 mg active ingredient/mL and is stable for months at –20°C. To the best of our knowledge, unformulated bialaphos is not currently available commercially. We obtain bialaphos through the courtesy of Dr. Hiroyuki Anzai, Meiji Seika Kaisha, Morooka-cho, Kohoku-ku, Yokohama, 222 Japan.
6. Chimeric plants can be obtained, presumably because of crossprotection ***(23)***. Therefore, it is imperative to apply stringent selection for a minimum of 2 mo.
7. If problems are encountered in regenerating plants, subculture the resistant calli onto hormone-free LS2.5 without bialaphos for 7–10 d after the selection regimen, then transfer them to MSD4.
8. Some plants without roots can be transferred, if the aerial portion of the plant is well-developed. They will usually develop roots quickly in the hormone-free medium.
9. To sample plantlets, remove the tissue-culture lid and position the upper 1.5–2 cm of a leaf inside a 1.5-mL microcentrifuge tube. Shear the leaf off by closing the lid while holding the lower portion of the leaf with forceps (to avoid pulling the plant up during sampling). As with all samples collected for PCR analysis, extreme care must be taken to avoid crosscontamination. Store samples at –20°C until they are used for DNA isolation.
10. After the putative transgenic plant has been transplanted to soil and developed 4–5 leaves, a simple way to test for functional expression of the *bar* gene is to dip the end 3–4 cm of a leaf into an Herbiace solution (250 mg Herbiace/100 mL H_2O) and allow the leaf to dry. After 5 d under normal growth conditions, the reaction can be scored (cool, low-light conditions delay the response). If resistant, the plant will not suffer any damage. If sensitive, only the leaf tissue directly exposed to Herbiace will brown and die, but the rest of the plant will remain unaffected. Always include a bialaphos-sensitive control for comparison.