Winter wheat varieties require vemalization treatment at a young seedling stage (4–28 d after germination). The wheat seedlings are placed in cold room or refrigerator at 4°C for 6–8 wk before they are transferred to a greenhouse or growth chamber.

2. To maintain wheat plants free from pests and diseases, spraying insecticide and fungicide is sometimes necessary.
3. Contamination by RNA and/or proteins in DNA samples affects the quality of DNA coating onto the particles. RNase (DNase-free) and protease K can be used to remove RNA and protein contamination, respectively. It is recommended to check the plasmid DNA's purity using agarose gel electrophoresis before coating. The form of DNA can be either supercoiled or linear.
4. If a gunpowder cartridge device is used, installation of a nylon membrane (250-μm meshes) between the stopping plate and the sample plate in the sample chamber is recommended. This could reduce the cell damage caused by the shock wave and gunpowder residues.
5. The choice of particles (gold or tungsten) is a matter of personal preference. No difference at a transient gene expression level was observed after comparison of the two types of particles for DNA delivery into wheat and other cereal cells (Chen and Dale, unpublished data). Electron-microscopic examination showed that gold particles were spherical and uniform, while tungsten particles were less unifomm in size and polygon in shape. Gold particles, however, are about 120 times more expensive than tungsten particles (Bio-Rad).
6. Although most wheat varieties respond to an order of culture on MS2, MS1, and MS0 media by regeneration through somatic embryogenesis, some varieties may require special media for a higher frequency of regeneration. The L3 medium ***(4)*** has been used to promote regeneration.
7. L-phosphinothricin (Sherman) is the active ingredient of the herbicide Basta. Basta can also be used as a selective agent. Proper conversion to obtain the correct concentration of an active ingredient is needed when using Basta.
8. Seeds at different positions along the spike mature at a different rate. Seeds at the central part of the spike normally mature earlier than those at the temminal positions. Therefore, immature embryos harvested 12–14 d after anthesis often show different sizes and colors, which represent different degrees of maturity. Embryos longer than 13 mm, with opaque white color, are too old to be cultured (they will germinate on medium), and embryos less than 10 mm, with total transparency color, are too young to be cultured.
9. Because of the low transformation frequency when using microprojectile bombardment, 600–1000 immature embryos are usually needed to produce one or a few transgenic plants for testing one DNA construct.
10. a. Coating of DNA onto the particles occurs more evenly if the preparation is kept under continuous agitation. This can be achieved by using a multiple-placement platform attachment head on a vortex mixer. Once $CaCl_2$ is added to the mixture, particle-DNA aggregates will form. Continuous vortexing at this stage, and further vortexing after adding spermidine, are very important. After adding