ethanol to the microcarriers, the aggregates should become dispersed again. If large aggregates persist, it may mean that either DNA was not sufficiently clean or DNA concentration is too high. Presence of the microcarrier aggregates affects the evenness of loading the microcarriers onto macrocarriers.

b. To obtain uniform amounts of microcarriers, all pipeting should be done from a continuously vortexed tube. Pipeting should be done rapidly, because microcarriers settle out quickly, even in the pipet tip. Dispense the microcarriers onto the macrocarriers and allow them to dry only in a vibrationless environment, because vibration enhances the agglomeration of microcarriers. High humidity results in rapid water absorption by the ethanol, resulting in slower drying. This can lead to serious agglomeration of the microcarriers.

11. If the retaining cap is not tightened sufficiently, the rupture disk may slip out of place before it ruptures. This will not properly launch the macrocarrier to accelerate the microcarriers. The rupture disks should not be handled with bare hands at any stage, because the grease and sweat left on the disk prevent it from holding its position properly, even when the cap is tightened. To remove grease or dust, the disk can be soaked in isopropanol or pure ethanol immediately before use.
12. Occasionally, the operator forgets to put the stopping screen in the stopping screen support. When the rupture disk bursts, this will cause the macrocarrier to pass straight through the stopping plate, which destroys the sample. Therefore, always double-check the placement of the stopping screen.
13. A metering valve is installed in the solenoid valve assembly to control the rate of fill of the gas acceleration tube. It should take about 12–15 s to fill to bursting pressure. The gage at the top of the acceleration tube should be observed. A more rapid fill rate may result in what appears to be a lower bursting pressure, because of gage lag. The metering valve is preset, but may be adjusted, if desired.
14. If necessary (e.g., to test transient gene expression), multiple bombardments can be applied to the same sample. More DNA particles will be delivered to the target tissues, but cell damage caused by more particles, multiple shock wave, and vacuum, is more severe. Control samples should also be set up at this stage, i.e. immature embryos bombarded with particles coated with the calf thymus DNA.
15. a. Although *gus*A gene is the most widely used reporter gene for plant transformation test, maize *C1/Lc* genes ***(9,10)*** can also serve as reporter for wheat transformation ***(11)***. The *C1/Lc* genes encode *trans*-factors, which regulate anthocyanin biosynthesis in maize. Delivery and expression of the *C1/Lc* genes into wheat cells results in cell-autonomous coloration (in most cases, red color). The advantages of using such a reporter system are: Gene expression can be easily visualized on target tissues and cells without using a destructive assay; cell-autonomous coloration allows more accurate counting of the gene expression events; it represents a more accurate estimation of DNA delivery events than the *gus*A gene in wheat. But the disadvantage of using this reporter system is that it can only be used as a marker for transient gene expression, because these regulatory genes could impinge upon other cellular and physiological processes. This requires the *C1/Lc* genes to be coated on particles separately from the target genes.