

Diversity of Plant Applications with GDS-80 Accessories

INTRODUCTION

Designed according to the aerodynamic theory for the barrel, GDS-80 can perform the gene delivery with a very low gas pressure requirement. Setting of gas pressure below than 80 psi can perform as well as setting over hundreds or even thousand pressures in the previous system because of the advance design principle. Also according to the different theories, GDS-80 can be easily applied on diverse samples with the help of different accessories. Followed are some typical plant samples in the GDS-80 gene delivery experiments. Different samples were performed with the help of different accessories according to its characteristics. Usage of the accessories is chosen according to the sample characteristics. Mainly, there have two types of accessories including target spacers and universal target spacers (UTS-10).

Universal target spacer can be used in different target deliveries by changing the configurations. Mainly UTS-10 can be applied on live plant leaves (so called LC-10) and powder-liked samples (ex: callus and PLBs). Target spacer was a bowl-liked stainless cover with many apertures on it. While calibrating the spread even ability of GDS-80, using target spacer as a fixed distance with 3 or 6 cm, delivery of each shot should be performed evenly. Target spacers also can be applied on sample delivery. With those apertures on the cover, the gas from the GDS-80 can easily passed through the cover without losing the samples.

EQUIPMENTS AND MATERIALS

- GDS-80 (S/N: WGB090001) with hose assembly and pressure regulator (Wealtec)
- Gas cylinder with over than 1000 psi of 99.999% Helium gas
- Universal target spacer, UTS-10 (Wealtec)
- 3 cm and 6 cm target spacers (Wealtec)
- Plant samples from all reference laboratories
- Plasmid DNAs were also provided by reference laboratories.

PROCEDURES

- 1. Setup the GDS-80 system according to the standard procedures in the manual.
- 2. Sterilize whole equipments and materials with proper treatment prior to the experiment.
- 3. Assemble the barrel and sample loading sleeve with main body of GDS-80 inside the laminar flow and connect the GDS-80 system with gas cylinder through hose assembly.
- 4. Set the delivery pressure at required pressure setting and the gas flow rate is around 10~15 L/min.
- 5. Prepare the plasmid DNA/gold particle solutions prior to perform the bombardment. (1 µg DNA/ 0.6 mg Gold)
- 6. Prepare the samples in proper size and sterilized before use.
- 7. (a) For UTS-10, assemble the UTS-10 according to the instruction in the manual. Place the sample in the middle of the tetra-claw leaf clamp or at the bottom of the pollen cup. Select proper distance away from the sample and close the whole system with tetra-claw leaf clamp or lid stopper with pollen shielder.
 - (b) For target spacer, place the samples in the middle of the 10 cm Petri-dish dish.
- 8. Make sure totally to remove the medium or distilled water around the samples.
- 9. Aliquot 10 µL DNA sample solution into the sample loading hole on GDS-80.
- 10. (a) For UTS-10, perform the bombardment with closed system.
 - (b) For target spacer, vertically attached the barrel onto 3 cm or 6 cm target spacers and put the trigger to deliver the DNA samples.
- 11. Transfer the samples in new medium for further incubation.

RESULTS

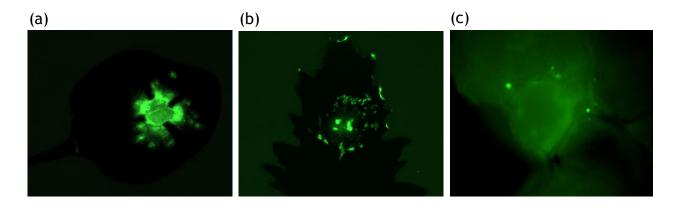


Figure 1. Samples delivered with UTS-10 and LC-10: (a) Tobacco's leaf with GFP gene and with the assist of LC-10. (b) Chenopodium album's leaf with GFP gene and with the assist of LC-10. (c) Rice callus with GFP gene and with the assist of UTS-10.

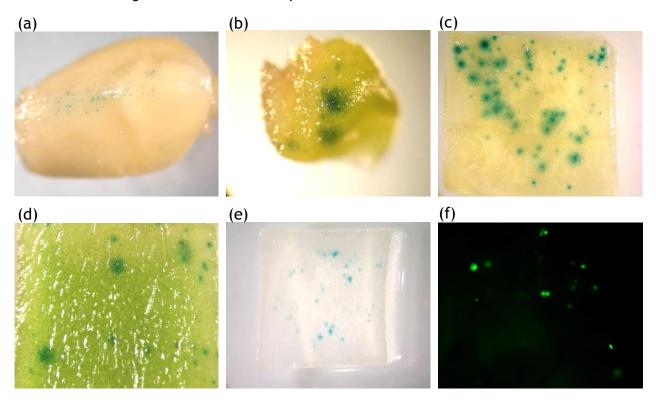


Figure 2. Samples delivered with target spacers: (a) Maize embryo, (b) Orchid PLB, (c) Orchid Petal, (d)

Orchid Leaf, and (e) Onion epidermis transfect with GUS gene, and (f) Rice callus with GFP gene.

DISSCUSION

All samples that listed in this article were performed with GDS-80 which with proper conditions in all Wealtec's GDS-80 Cook Books. Different samples have different delivery conditions. Some of them were bombarded with the assist of UTS-10 (*fig.* 1) and some are with target spacers (*fig.* 2). Each of them was confirmed over and over again, and was promised to have good result with the same conditions. According to the result that had been confirmed so far, and because of the different design principles of GDS-80 which already compared to those on the market. GDS-80 was proved again to have the ability to provide customers an easier way to deliver bio-particles into target cells with very low pressure setting. Needed only range from 30 to 60 psi for the plant targeting samples, GDS-80 does lower down the risk of operating with high pressure instrument and provide more possibility on transferring targets with the help of these two accessories. Users can easily refer to the Wealtec's Cook Book for delivering the bio-particles with the assist of the target spacers or UTS-10.

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