Clean the Barrel between Shots in GDS-80 to Prevent Cross-Contamination

INTRODUCTION

Deliver gene with GDS-80 can perform with variety samples range from animals to plants. Plasmid DNA that used to deliver is also diverse. Sometimes user may encounter with using of more than one target of plasmid DNA to deliver within the same experiment. How to clean the barrel to prevent the DNA cross-contamination would be the most consideration part that users would interest in. Within this articles, take onion as an example to illustrate the operation with four different DNA samples.

EQUIPMENTS AND MATERIALS

- Whole set of GDS-80 with 4.5 mm barrel, including pressure regulator and hose assembling (Wealtec)
- Laminar flow
- 3 cm target spacer (Wealtec)
- Gas cylinder with Helium gas (99.999%) over than 1000 psi.
- Samples: Onion epidermis.

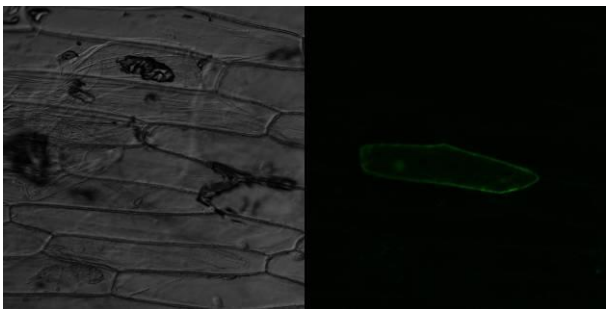
PROCEDURES

1. Setup the GDS-80 system according to the standard procedure 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 whole GDS-80 system.
4. Set the deliver pressure at 50 psi and the gas flow rate 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. Cut the onion epidermis in proper size (3 cm x 3 cm x 2 mm) and sterilized before use.

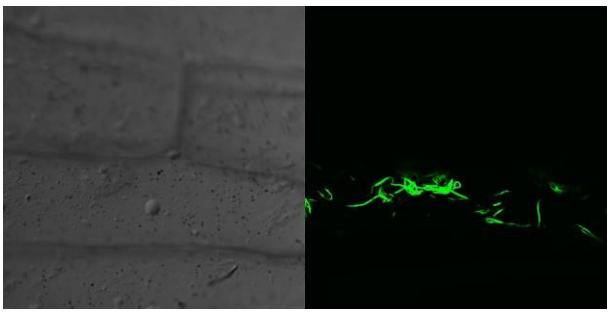
7. Aliquot 10 μL DNA sample solution into the sample loading hole.
8. Perform the bombardment toward the onion with the help of 3 cm target spacers.
9. Between each DNA samples, wash the barrel with following steps:
    - Apply with 20 μL sterilized distilled water and pull the trigger for three times.
    - Repeat the previous step for twice more.
    - Change to 20 μL 100% EtOH and pull the trigger for three times.
    - Repeat with the previous step for two times.
    - After cleaning steps, the GDS-80 is ready to apply with different DNA sample.
10. Incubate the sample for 6 hours and observe the result under the microscope.

RESULTS

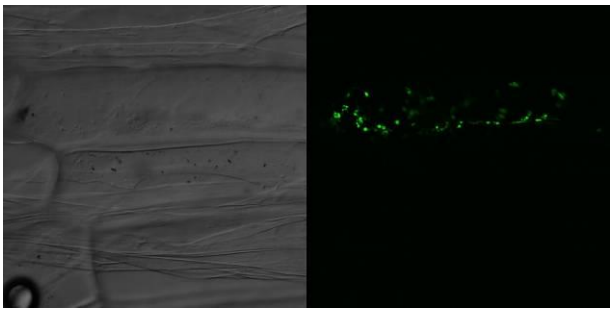
(a) Control plasmid:



(b) pas2-1320



(c) pas6-1320



(d) Nuclear membrane located protein

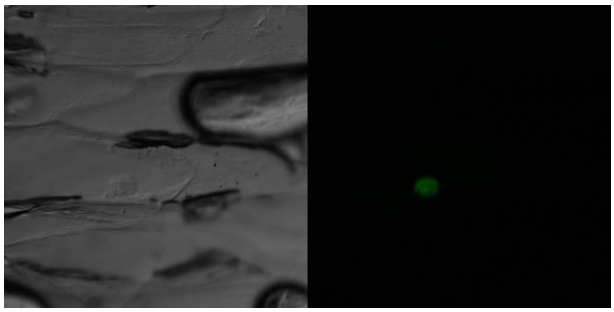


Figure 1. *Deliver of plasmids which GFP constructed with different glycoprotein gene into onion epidermis via GDS-80. Within all pictures, on the left are normal light source observation and fluorophores excitation on the right.*

DISSCUSION

As in the figure 1(a), the glycoprotein gene can be expressed all over the whole cell which was used as a control sample. Onion epidermis cells that were transferred by the control sample would have GFP gene all around the cell. While transferring with the other three DNA samples, GFP protein would be found in different place inside one cell. In figure 1(d), the GFP gene was presented on the nucleic membrane. Gene expression in figure 1(b) and (c) are also successfully expressed on the desired glycoprotein, respectively. And within the same onion sample, there have only one phenotype of the GFP glycoprotein expression. No DNA cross contamination was found.

In the onion glycoprotein located research in this article, it is proved that GDS-80 is a powerful tool for gene transfection. Sample preparation and the operation with multi samples are both very convenient in the GDS-80 system. Most important point is that using of the recommended method to clean the barrel between each DNA samples indeed prevents the DNA cross contamination, so that users can precede the experiment in a very easy way.

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