Operation of GDS-80 within Laminar Flow

EQUIPMENTS AND MATERIALS

- GDS-80 with 4.5 mm barrel, including pressure regulator and hose assembling (WGB09O001) (Wealtec)
- Laminar flow
- 3 cm target spacer (Wealtec)
- Helium gas 99.999%
- Samples: *Zea mays* L. embryo.

PROCEDURES

1. Adjust the GDS-80 with the needed delivery pressure setting and have the best even spread condition of the performance prior to the experiment. Remember to wash the sample loading sleeve and the barrel with 70% EtOH after adjustment.
2. Clean and sterilize the laminar flow before operation. (Expose to UV light for at least a half-hour)
3. Sterilize the barrel, sample loading sleeve, target spacers, and forceps prior to send them into the laminar flow.
4. Spray with 70% EtOH onto the GDS-80 main body and hose assembly before putting them into the laminar flow to sterilize.
5. Assemble the barrel and sample loading sleeve with main body of GDS-80 inside the laminar flow and connect the whole GDS-80 system.
6. Set the pressure regulator at 50 psi and make sure the gas flow rate is around 10~15 L/min.
7. Prepare the plasmid DNA/ gold particle solution prior to perform the bombardment. (0.5 ug DNA/ 0.148 mg Gold)
8. Perform the bombardment toward the samples with the help of 3 or 6 cm target spacers, and transfer samples onto the MS medium plate after bombardment.
9. Incubate the sample for at least two days and stain with GUS stain solution for 1 day.
10. Observe the result under the microscope.

RESULTS

(a) (b)

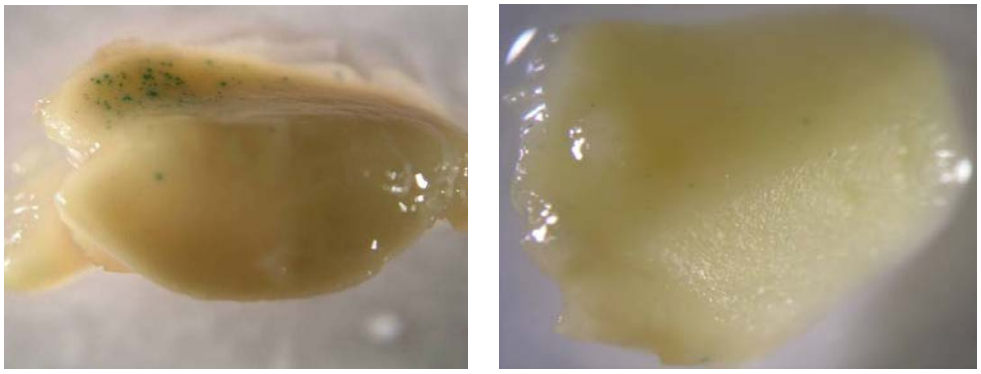

Figure 1. *Deliver of GUS gene into maize embryo with 50 psi with the help of 3 cm target spacer. (a) Normal condition without contamination. (b) In the contaminated medium.*

DISSCUSION

While operating within the laminar flow, there are many things that need to be noticed at all times. Otherwise, contamination might occur seriously and affect the observation of experiment result. (*fig.* 1) If there is contamination inside the plates, the expression quantity of GUS protein would become too low to be found even though the sample was not infected. Contaminations are commonly caused from the following situations as well as some observations that help operators to avoid them:

a. Contamination from the bombardment equipments

While operating the GDS-80 within the laminar flow, users easily forget to disassemble the barrel and sample loading sleeve for sterilization. They would only spread the 70% alcohol outside the sample loading sleeve. Although the DNA samples used in delivery are prepared in the 100% alcohol, it's hard to remove those micro-organisms that attach on the barrel and sample loading sleeve. During the delivery, the bacteria will be pushed and spread onto the samples at the same time. Use of the GDS-80 in the plant targeting experiment would need some accessories to

assist in the performance in the experiment. For example, using target spacers to manipulate the tissue experiment in the Petri-dish or using UTS-10 to perform the experiment with callus cells. All the accessories that are used in the experiments could have easily come into contact with the samples or even be used as a supporter to the samples. Therefore, it should be fully sterilized to prevent contamination. Sterilization of all the experimental equipments can be performed with the autoclave at 121 ^{o}C for 30 minutes to ensure complete sterilization. The other way to sterilize is to immerse the barrel and the loading sleeve into 70% EtOH for over 20 minutes and dry out before use in the laminar flow.

b. Contamination from samples itself.

Plant samples from the field are exposed to many microorganisms. If the sample is not sterilized, it will easily get contaminate in the medium. Thus, samples need to be sterilized prior to experiments. Due to the characteristic of the samples being different, the sterilization methods will be different as well. Most commonly used method is to immerse the sample in the prepared solution with proper ratio of bleach and Tween-20 for at least 20 minutes. Then wash with sterilized distilled water. Spread the samples on the clean Petri-dish to dry before the experiment.

c. Contamination from unfamiliar operation within the laminar flow.

While operating experiment inside the laminar flow, most people might overlook some important points. Before doing the experiment in the laminar flow, make sure to completely wash both hands and sterilize with 70% EtOH. Once get both hands into the laminar flow, do not let your hands out of the machine before finishing the experiment. If it is needed to take something in or out of the laminar flow, sterilize both hands again with 70% EtOH again before returning to the inside of the laminar flow. While operating the Petri-dish, do not open the cover plate widely and do not let any hands or objects go over the plate while it is opened. Make sure to sterilize the tips prior to sending into the laminar flow. Change the tip every time before picking up delivery samples. Only use the forceps to manipulate the target samples. Also, make sure to sterilize the forceps with fire before each operation. Do not let the forceps touch anything else beside the samples. Do not pick up the sample if dropped on the desk and put back into medium. Make sure all the equipment and materials are properly sterilized before putting them into the laminar flow.

d. Laminar flow is not well maintained.

According to the design of the laminar flow, the filter inside the flow should be changed after using a period of time or the filtrate efficiency toward the microorganism will become lower. Please change the filter inside the laminar flow regularly to maintain the best mo-free environment. Even though the laminar flow is maintained well, operators still need to spray the 70% EtOH to clean the operation area inside the machine before use.

Sterilization of all the experimental material is absolutely needed prior to manipulation of the whole system. Contamination is not allowed within the procedures. Although the laminar flow standard operation procedures (SOP) are different from lab to lab, the main purpose to prevent contamination remains the same. Operators should follow the SOP carefully and notice these points which were discussed in how to avoid microorganism contamination.

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