# Transient assay using onion epidermal cells

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Particle gun bombardment with IDERA GIE-III (TANAKA Co.,Ltd., Sapporo, Japan) is used to introduce GFP (or other fluorescent protein) fusion plasmids into onion epidermal cells. Tungsten particles (2μm) were coated with the respective plasmid. Bombarded onion epidermal cells were examined under a fluorescence microscope.

1) Washing particle
   - ☐ Add sterile water to Tungsten particles (2μm, TANAKA Co.,Ltd., Sapporo, Japan) in 1.5ml tube.
   - ☐ Sonicate the particles for several seconds.
   - ☐ Spin down and remove supernatant.
   - ☐ Wash with sterile water similarly.
   - ☐ Wash with ethanol.
   - ☐ Vacuum dehydration and store in desiccator.

2) Coating particle with DNA
   - ☐ Prepare several mg of Tungsten particles in 1.5ml tube.
   - ☐ Add 20μl TE(10mM Tris-HCl, 1mM EDTA, pH8.0) per mg Tungsten particles.
   - ☐ Sonicate for several seconds.
   - ☐ Add 20μl(1mg) Tungsten particles to 2μg DNA in 1.5ml tube
   - ☐ Add TE up to 50μl
   - ☐ Add 5μl of 3M NaOAc(pH5.2)
   - ☐ Add 110μl of 100% ethanol and mix well.
   - ☐ Leave at -20℃ for 10 mins.
   - ☐ Spin down and remove supernatant.
   - ☐ Wash with 70% ethanol.
   - ☐ Add 160μl of 100% ethanol
   - ⌊ Briefly sonicate particles.

3) Preparing samples
   - ☐ Cut a piece of onion bulb into a square measuring 5 cm on each side.
   - ☐ Peel epidermis.
   - ☐ Put the epidermis turning onion core side up on solid MS (Murashige snd Skoog) medium in

a 9cm diameter Petri dish.

4) Bombarding particles

☐ Open the valve of Helium and regulate pressure to 4.0kgf/cm$^2$.

☐ Put 4μl(6.25μg) of DNA coated particles on 1.3cm filter folder.

☐ Set the onion sample at 9cm.

☐ Vacuum of 600mmHg and bombardment at pressure of 4.0kgf/cm$^2$for 0.025 second.

☐ Release to normal pressure.

5) Incubation and obsarvation

☐ Incubate the bombarded samples in darkness at 26°C for 17~20h.

☐ Observe the samples under a fluorescence microscope.

Fig.1 Onion epidermal cell visualized cytosol.

GFP were localized in cytosol.

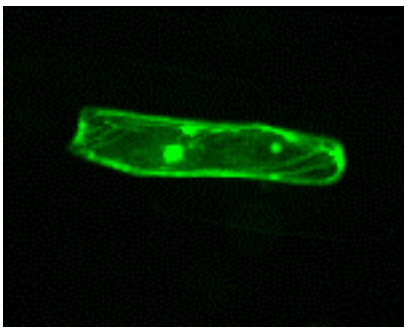

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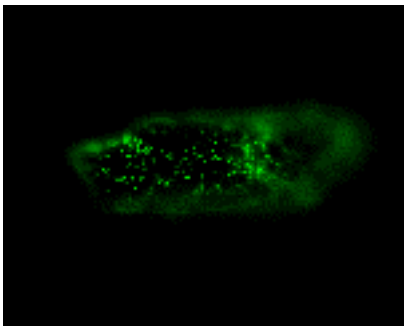
Fig.2 Onion epidermal cell visualized peroxisomes.

GFP fused pumpkin catalase (Cat1) were localized in peroxisomes.



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