

Direct Gene Transfer into Mature Seeds Via Electroporation

For Seeds of Rice Plant, Wheat and Barley, Silk Worm, Yeast, etc using

Vacuum & Electrical Pulse

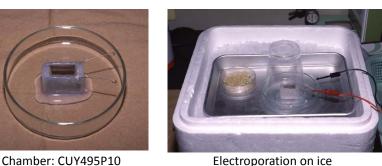
- 1. Starting materials are mature seeds.
- 2. Transformed plants are obtained in relatively short
- 3. Plant tissue culture is not necessary.
- 4. Possibly applicable to animal cells and microorganisms.



Vacuum Chamber & CUY21EDIT

Basic Method 1. Soak mature seeds in water overnight.

- 2. Transfer the seeds into electroporation buffer.
- 3. Vacuum treatment.
- 4. Electroporation on ice.
- 5. Incubate the seeds on ice for a few hours.
- 6. Grow the plants under the favorable conditions.
- 7. Select the transformed plants using antibiotics.

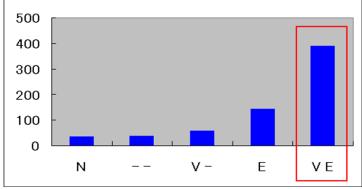


Electroporation on ice

Fertile transgenic plants were regenerated and self-fertilized seeds were obtained in rice and wheat. Transgene integration was confirmed by Southern hybridization. Transmission of the transgene into the next generation (T₁) was indicated by PCR analysis. Transient gene expression was observed in several plant species and silk worm eggs.

No Need for Tissue Culture No Particular Know-How or Skill required Large reduction in handling and labour

- Simple
- **Efficient**
- Low-Cost



N: Negative control, --: Buffer only, V: Vacuum, E: Electroporation The level of CUS gene expression by fluorescence assay (Wheat)



GUS gene expression reference (Wheat)



Not Transformed npt II gene introduced Geneticine Selection of Rice Seeds and/or Seedings



Next Generation (T1) Individual (Rice Plant)

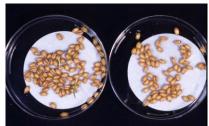


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Production of Transgenic Wheat

Gene transfer into Silk Worm Egg









GUS Gene Expression

Selection by Geneticin

Transformed Plants

Transient GUS Gene Expression









Rice (japonica)

Rice (indica)

Brassica

Soybean

GFP Expression (Fluorescence)

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^{*}Improvement research is in progress in Dr. Hagio's laboratory.