Notes

Plant Tissue Culture Letters, 8(1), 39~42(1991)

Circuit Diagram and Electrode Chambers for Electric Gene Transfer into Plant Cells

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Electric field-induced gene transfer including electrofusion, electroporation and electroinjection is a useful technique in cell and gene engineering in plants[1-4]. Various commercial devices using this technique are now available. However since optimal electrical and nonelectrical (e. g., medium of the electrode chamber) conditions vary with plant species[1,4] and experimental design, improving circuit design or electrode chambers remains important for optimizing conditions for each type of plant cell. In this communication we describe the electric circuit and electrode chambers which we are using for electrofusion[5-7], electroporation and electroinjection[8-11].

Fig. 1 indicates the circuit for generating the electric pulses needed for the gene transfer. The output of the DC power supply (maximal voltage=2 kv; Atto Co., AE3120) G was connected first charged to capacitor C (Condenser Products Co. and Nihon Condenser Co.) by a high voltage reed switch (Sankyo International Co., TSG DP1A12-5) A. The capacitor size used here was between 0.

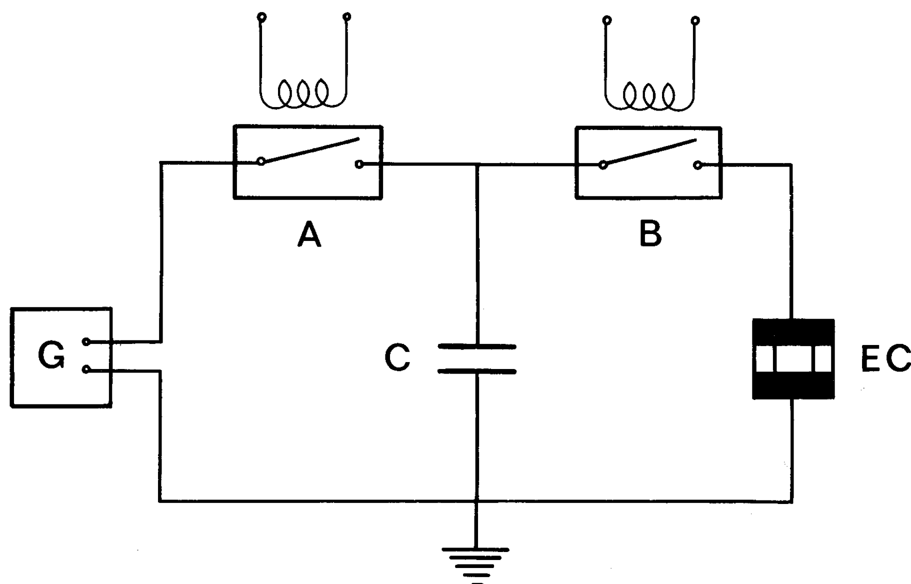

Fig. 1. Circuit diagram for electric gene transfer. G, DC Power supply; A and B, high voltage reed switch; C, high voitage capacitor (working voltage, 4 kv); EC, electrode chamber.

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001 and 10 μF. Next the capacitor was discharged through the electrode chamber EC by first opening switch B and next closing switch A. Alternatively, those two high voltage relay switches could be replaced with a low voltage reed relay switch (Omron, MY2). The exponentially decaying

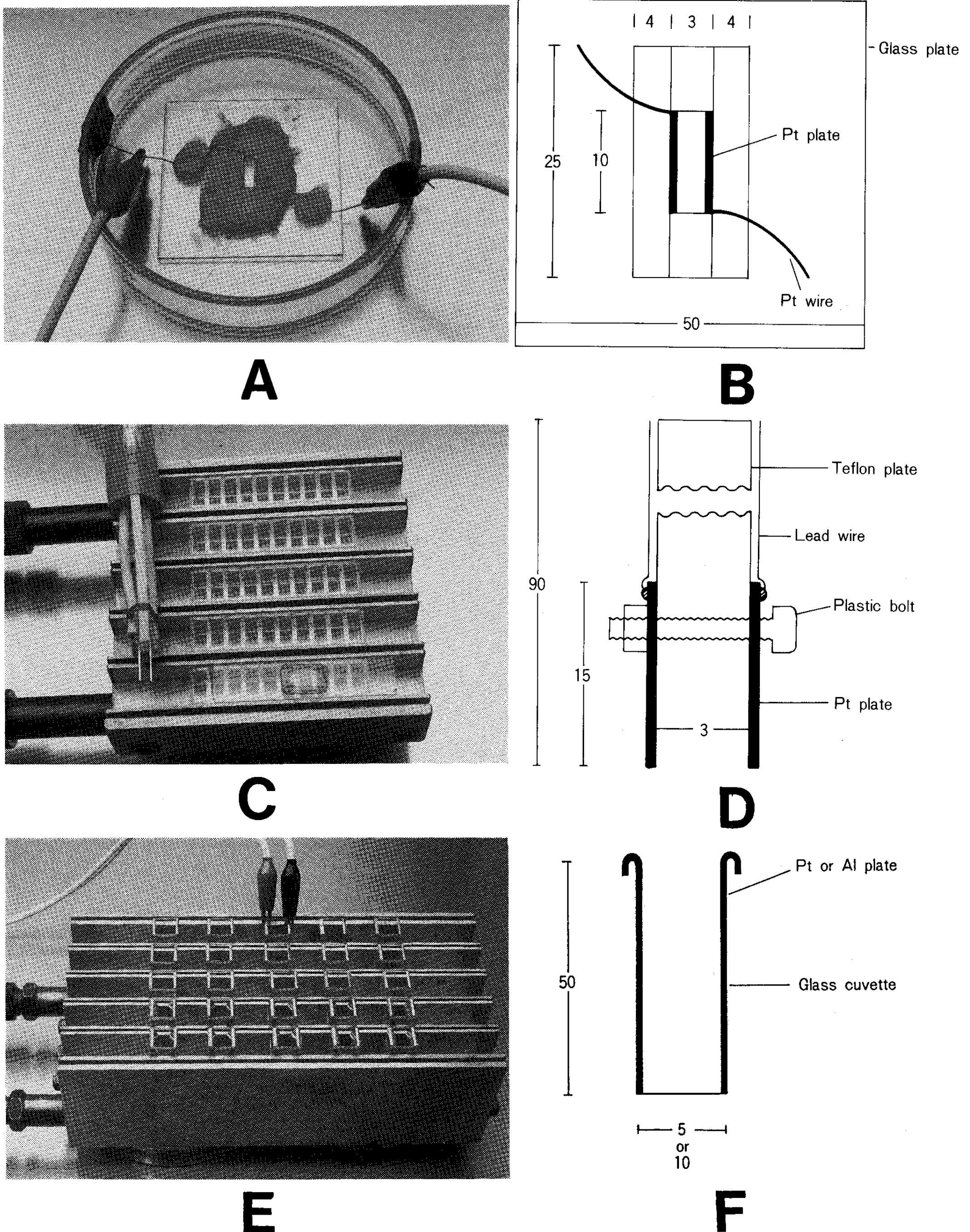

Fig. 2. Microphotographs and diagrams of various electrode chambers. (A) 40-μl chamber with platinum electrodes, (B) schematic top view of 40-μl chamber, (C) 400-μl chambers placed on a cold stage and a transferable platinum electrode device, (D) schematic side view of a transferable electrode device, (E) 2500-μl (bottom two rows) and 5000-μl (top three rows) chambers with aluminum electrodes placed on a cold stage, and (F) schematic side view of a 2500-to 5000-μl chamber. The numbers in the diagrams represent the length of the parts in mm.

pulse was monitored by an oscilloscope (Hitachi, V-134) that was connected in parallel to the electrodes through a voltage divider. The high voltage with which the capacitor was charged was monitored by a voltmeter (Iwatsu Electric Co., SC 7401) through a 1 : 1000 probe (Iwatsu Electric Co., HV-p30). The time constant for charging was less than 100 ms while that for discharging ranged from several μs to several ms depending on the electric resistance between the electrodes.

Fig. 2 shows the design of electrode chambers with different volumes; 40, 400, 2500 and 5000 μl. The forty-microliter chamber was made with; four pieces of glass glued together with epoxide resin on a glass plate (0. 5×50×50 mm) to form a rectangular chamber (3×2×10 mm). A pair of platinum plates (0. 5×2×10 mm) that served as electrodes was then glued inside of the chamber (A and B). Internal size of this chamber was 2×2×10 mm and the volume was 40 μl. The chamber was placed in a petri dish (90 mm i. d.) and autoclaved before use. This chamber could be placed directly on a microscope stage so that the process of protoplast fusion after the electric pulse could be observed under an inverted microscope[7]. This chamber was used for electrofusion[5-7] and electroinjection[8,9].

Fig. 2C shows a 400 μl chamber with a transferable electrode. A transferable electrode was first reported by Watts and King[12]. Quartz plates were fused to form a number of chambers (4×10×10 mm each chamber; C). A pair of platinum plates (0. 5×10×15 mm) was fastened with a plastic bolt and nut on a Teflon plate (3×16×80 mm) (as shown in **Fig. 2D**) and from the transferable device. Usually, 150 to 300 μl cell-DNA/RNA mixture was added to each chamber and the chamber array was placed on a cold stage so that the temperature of the chamber medium could be controlled (usually at 2°C). The transferable electrode device, after sterilization by autoclaving, was inserted into one chamber straddling the wall into the next and pulses of various strength were applied. The temperature of the chamber medium was measured with a temperature transducer (Iwatsu Electric Co., TD-70). This chamber array was used for electric gene transfer into protoplasts and protoplast-derived cells[10].

Fig. 2E and **F** show a 2500-to 5000-μl chambers. Pairs of aluminum or platinum plates (0. 1×10×60 mm) that served as electrodes were glued in glass cuvettes (5×10×50 or 10×10×50 mm; E and F). This type of electrode chamber was first desribed by Potter et al[13]. These chambers allow a large volume of cell-DNA/RNA mixture (500 to 1000 μl) to be used and can also be placed on a cold stage. This type of chamber was used for electric gene transfer into protoplasts and protoplast-derived cells[11]. Electrode chambers similar to **Fig. 2F** but with stainless steel plate electrodes (3. 5×10×10 mm), to each of which a lead wire was soldered, were also used for electric gene transfer. No differences in the cell viability after pulsation and in efficiency of electric gene transfer have so far been observed among aluminum, platinum and stainless steel electrodes.

We are grateful to Prof. E. Stadelmann of University of Minnesota for his critical reading this manuscript and correcting our English. This work was supported by a grant from the Yamada Science Foundation and by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

(Accepted September 12, 1990)

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《和文要約》

電気パルスによる植物細胞への遺伝子導入のための回路と電極室のデザイン

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電気融合，エレクトロポーレーションなど電気パルスによる植物細胞への遺伝子導入のための回路と電極室について実際に組立てるための技術情報を中心に紹介した．回路は放電パルス印加回路について述べた．また，電極室は容量が 40, 400, 2, 500 および 5, 000 μl のものについて述べた．