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TECHNICAL NOTE

Simple electroporation device for gene functional analyses in insects

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Abstract In vivo electroporation is a very powerful technology that enables both gain-of-function and loss-of-function analyses. However, the device required for the method is expensive. Here, we report a simple, inexpensive (under US$85), easily constructed, and one-piece electroporation device for gene functional analyses of insects. Using this simple electroporator, we successfully introduced exogenous fluorescence protein genes into three moth species. This simple low-cost electroporator will render gene function analyses accessible to many non-model insects, including pest species.

Keywords In vivo electroporation · Somatic transformation · *Bombyx mori* · *Ostrinia furnacalis* · *Ostrinia scapulalis*

Introduction

Current high-throughput DNA sequencing technologies and improved gene databases have facilitated the molecular analyses of many non-model insects, including pest species. However, gene functional analyses available for non-model insects are quite limited. RNA interference (RNAi) is an invaluable, widely used tool for gene silencing in various non-model insects (Futahashi et al. 2011; Miyazaki et al. 2014; Ohde et al. 2013). However, the efficiency of RNAi is low or very variable in some insects (Christiaens et al. 2014; Tomoyasu et al. 2008). Genome editing technologies such as TALENs and CRISPR/Cas9 have recently become popular for manipulating target genes in insect genomes (Ma et al. 2014; Watanabe et al. 2012); however, their use is restricted to early embryos. The phenotypic effects should then be checked at particular developmental stages. Therefore, in some species, it is difficult to microinject RNA into the eggs without significantly damaging their growth.

In vivo electroporation introduces plasmid DNA encoding a gene of interest or short-hairpin RNA into various tissues, enabling both gain-of-function and loss-of-function analyses. This technique is useful for the rapid functional analyses of non-model insects (Ando and Fujiwara 2013; Golden et al. 2007; Kunieda and Kubo 2004). In vivo electroporation is very powerful technology; however, a special and expensive device system (around US$10,000 in general) is required for the method. Ando and Fujiwara (2013) reported a low-cost (under $200) handmade electroporator. However, it is not easy for non-experts to construct it because it requires a program with C language to control voltage pulse duration, and an additional special power supply such as that used in electrophoresis.

To overcome these limitations, we have developed a simple, very inexpensive, easily constructed, all-in-one electroporation device for the gene functional analyses of insects. Here, we report the design and features of the device. Using the simple electroporator, we successfully introduced exogenous fluorescence protein genes into silkworm, *Bombyx mori* (Linnaeus), and two important pest species, adzuki bean borer moth, *Ostrinia scapulalis* (Walker) and corn borer moth, *Ostrinia furnacalis* (Guenée).

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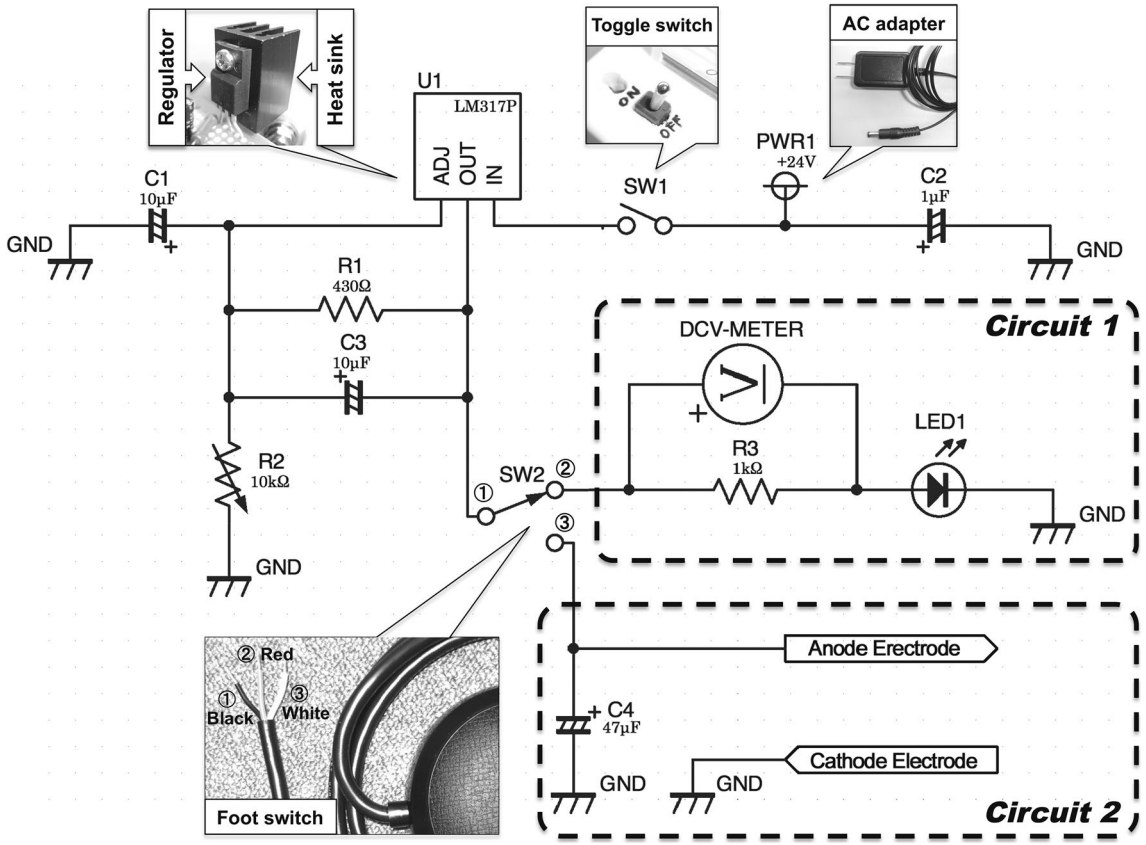

Fig. 1 Circuit diagram of the simple electroporation device. Details of all designators (except GND) are shown in Table 1. The ground (GND) connects to the negative terminal of the PWR1

Materials and methods

Figure 1 is a circuit diagram of the electroporation device. All electronic parts (listed in Table 1) and their connecting wires are soldered onto the circuit board. A schematic of the in vivo electrode of the device is presented in Fig. 2a. When the toggle switch is on, a direct current flows through Circuit 1, turning on the blue LED, and showing a voltage on the voltmeter (Fig. 1). The electrode voltage is controlled by operating the potentiometer, while checking the voltage registered on the voltmeter. Depressing the footswitch reroutes electricity to the electrodes in Circuit 2, switching off the LED and the voltmeter in Circuit 1.

The *B. mori* strain Kinsyu × Showa (Ueda-Sanshu), and field-collected *O. scapulalis* and *O. furnacalis* were maintained on an artificial diet (Silkmate 2 M, Nihon–Nosan) at 23 ± 1 °C in a long-day regime of 16 h light and 8 h dark.

A vector and helper plasmids pPIG-A3GR and pHA-3PIG system allows stable expression of exogenous enhanced-green (*EGFP*) and red fluorescence protein (*DsRed2*) genes in targeted tissues (Ando and Fujiwara 2013; Tamura et al. 2000). Injection of both plasmid solution and electroporation were conducted as described by Ando and Fujiwara (2013) with some modifications. Injection was performed under a dissecting microscope using a glass capillary needle (GD-1, Narishige) made by a glass microcapillary puller (PN-31, Narishige) connected to an air pump (FP15 N, Tokyo Glass Kikai). The capillary was inserted into third instar nymphs of the three moth species that were anesthetized in a stream of carbon dioxide. Approximately 0.5 μl of each donor and helper plasmid solution (1 μg/μl) was co-injected into the body cavity by using air pressure. Immediately after injection, platinum electrodes holding phosphate-buffered saline (PBS) droplets (Fig. 2b) were placed on the larval body: one near the injection site, the other on the opposite side of the body (Fig. 2c). The larvae were loaded with five 15-V pulses (each lasting approximately 250 ms) by manually controlling the simple electroporation device with the footswitch.

All insect images were taken with a digital camera (DFC310 FX, Leica) connected to a fluorescence stereomicroscope (M205FA, Leica).

RNA was isolated from the electroporated sites of *O. scapulalis* and *O. furnacalis* 5 days after electroporation using RNeasy plus Mini Kit (QIAGEN). First strand cDNAs were synthesized using pd(N)6 primers and

Table 1 Parts list

Designator[a]/ function	Description	Quantity	Manufacture name	Part number	Price (Japanese Yen)
Electronic parts for device					
Ul	Voltage regulator, 1.2–37 V 1.5A	1	STMicroelectronics, Geneva, Switzerland	LM317P	50
PWR1	AC adapter, O/P 24 V 0.25 A (12 W), I/P AC 100–240 V	1	Go Fowrward Enterprise Corp., Taichung, Taiwan	GF06-US24025A	650
Rl	Metal-oxide film resistor, 430 Ω 3 W	1	KOA Corp., Minowa, Japan	MOS3C431 J	40
R2	Wirewound potentiometer, 10 kΩ 1.2 W	1	Tokyo Cosmos Electric, Zama, Japan	RA25Y20S B103	665
R3	Metal-oxide film resistor, 1 kΩ 1 W	1	KOA Corp., Minowa, Japan	MOS1C102 J	20
CI	Electrolytic capacitor, 10 μF 50 V	1	Rubycom Corp., Ina, Japan	50PK10MEFC5X11	10
C2	Electrolytic capacitor, 1 μF 50 V	1	Rubycom Corp., Ina, Japan	50PK1MEFC5X11	10
C3	Electrolytic capacitor, 10 μF 50 V	1	Rubycom Corp., Ina, Japan	50PK10MEFC5X11	10
C4	Electrolytic capacitor, 47 μF 35 V	1	Rubycom Corp., Ina, Japan	35PK47MEFC5X11	10
LED1	High-brightness blue LEDs	1	Opt supply, Hong Kong, China	OSUB5161P	80
SW1	Toggle switch (Double pole, single throw)	1	Cosland Corp., Taipei, Taiwan	1MS1-T1-B1-M1-Q-N	70
SW2	Foot switch	1	Kokusai Dengyo Corp., Nagoya, Japan	SFU-1B	2131
DCV-METER	Analog panel voltmeter, DC 30 V	1	DER EE Electrical Instrument Corp., New Taipei, Taiwan	DE-550 DC30 V	1000
Circuit board	Universal board	1	Sunhayato Corp,. Tokyo, Japan	ICB-90	184
Parts for electrodes					
Electrode	Platinum alloy wire, Pt90 %–Ir10 %: φ0.30 mm	10 cm	Nilaco Corp., Tokyo, Japan		2440
Electrode grip	Bamboo stick	2	–		–
Others					
Molded case	Case for circuit and electric parts	1	Teishin Electric, Kawaguchi, Japan	TB-3-IV	300
Bushing	Anti-slip for case	4	Sato Parts Corp., Tokyo, Japan	BU-692A	21
Banana jack	Connection jack of the circuit and the test lead	2	Sato Parts Corp., Tokyo, Japan	TJ-563	121
DC Power jack		1	Marushin Electric Mfg. Corp., Kawasaki, Japan	MJ-14	63
Test lead	Lead for connection of the circuit and electrode	1	Teishin Electric, Kawaguchi, Japan	TLA-26	620
Heat sink	Radiation for voltage regulator	1	Global Electronics Corp., Tokyo, Japan	17PB024	63
Aluminium knob	A volume knob for potentiometer	1	Sato Parts Corp., Tokyo, Japan	k-5424	383
3 mm screw	Screws for fixing the circuit and case	5			–
Lead	Conducting wire	50 cm			–
				Total	8941 (US$ 82.79)

Dollar-based price is calculated at the exchange rate of 107 Yen = 1 US dollar

[a] Designators corresponding to those in Fig. 1are shown in italic text

PrimeScript reverse transcriptase (Takara-Bio). Quantitative reverse transcription-PCR (RT-PCR) was performed as previously described (Kayukawa and Ishikawa 2009) using a Mx3005P QPCR system (Agilent) and THUNDERBIRD SYBR qPCR MIX (Toyobo) with primers EGFP-q-F1 (5′-GGGCATGGCGGACTTGAAGA-3′) and EGFP-q-R1 (5′-ACGGCAAGCTGACCCTGAAG-3′) for *EGFP*, with primers DsRed2-q-F1 (5′-TGCAGGACGGCTGCTTCATC-3′) and DsRed2-q-R1 (5′-TTCAGGGCCTTGTGGGTCTC-3′) for *dsRed2*, and with primers Osef1α-q-F1 (5′-GAC

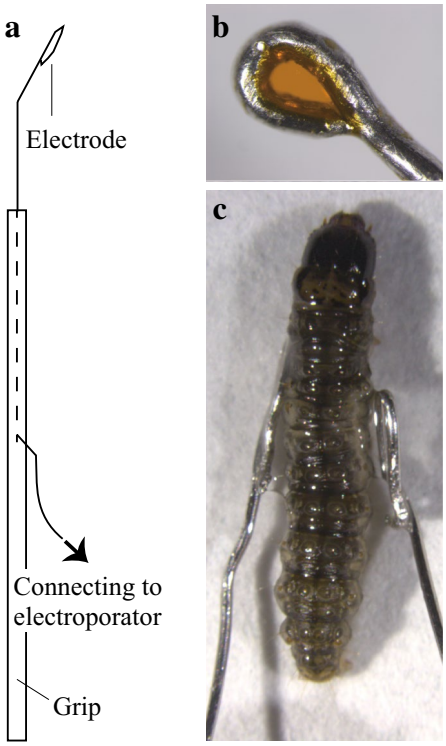

Fig. 2 Electroportation of *O. scapulalis* larva. **a** Schematic illustration of the in vivo electrode. **b** The ring-shaped electrode easily holds phosphate buffered saline (PBS) when dipped in the buffer. PBS colored with an *orange dye* was used in this image for easy understanding. **c** Platinum electrodes holding PBS droplets were placed near the injection site and on the opposite side of the body and were supplied with electric pulses

TCCGGCAAGTCCACCAC-3′) and Osef1α-q-R1 (5′-CCT GGGCCTCCTTCTCGAAC-3′) for *elongation factor 1α* (*ef1α*). The PCR program consisted of 98 °C for 1 min, followed by 50 cycles of 98 °C for 10 s, 62 °C for 15 s, 68 °C for 30 s, with final dissociation curve analysis. The relative expression levels were normalized by the transcript levels of *ef1α*.

Results and discussion

The construction of the simple electroporation device is shown in Fig. S1. The device can be constructed in 3 h from materials costing under US$85 (Table 1), and it requires no additional power supply, unlike the previous device reported by Ando and Fujiwara (2013). Whereas commercially available in vivo electrodes are often inappropriately large for application to small insects, the size and shape of our electrodes can be adjusted to the target insects. Moreover, our ring-shaped electrode readily holds the PBS (Fig. 2b) that prevents epidermal burn injury. The potentiometer controls the electrode voltage output from

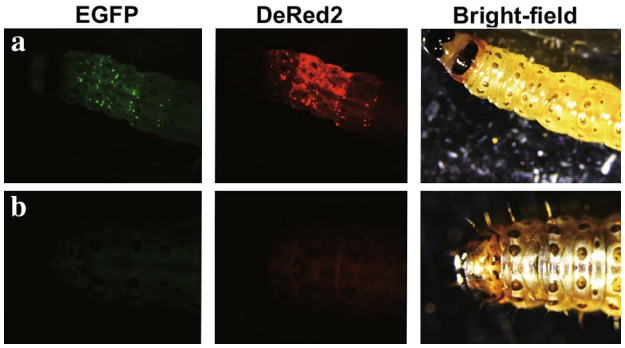

Fig. 3 Fluorescence microscopy analysis of overexpressed exogenous *EGFP* and *dsRed2* in *O. scapulalis* epidermis. **a** 9 days after electroporation and **b** non-electroporated controls injected with the donor and helper plasmids

1.5 to 22.5 V. When the more than 32 V AC adaptor is used, a maximum voltage of 30.5 V can be outputted. Appropriate adaptors can realize a 100–240 V input, compliant with the power supplies of many countries.

Using this simple electroporation device, we first confirmed the successful expression of exogenous fluorescence proteins in the larval epidermis of *B. mori* by fluorescence microscopy (Fig. S2). We then applied the same method to two important pest and model species in chemical ecology, *O. scapulalis* and *O. furnacalis* (Ishikawa et al. 1999; Lassance 2010). The larval epidermis of both species electroporated with *EGFP* and *dsRed2* exhibited green and red fluorescence (Figs. 3a and S3a). *DsRed2* fluorescence was more strongly observed in both *Ostrinia* species. Expressions of the exogenous fluorescent genes were confirmed by quantitative RT-PCR analyses in the electroporated sites in both *Ostrinia* species (Fig. S4). As expected, no fluorescence was observed in the no-injection control or in the negative control with distilled water (data not shown). The insects injected with the donor and helper plasmids, but not treated with an electric pulse, showed no fluorescence (Figs. 3b and S3b) and very low mRNA expressions of *EGFP* and *DsRed2* were detected (Fig. S4). Twenty-five days post-treatment, the survival rates were not significantly lower in electroporated insects [*O. scapulalis*, 69.2 % (18/26); *O. furnacalis,* 65.2 % (15/23)] than in non-electroporated controls [*O. scapulalis*, 86.7 % (13/15); *O. furnacalis,* 80.0 % (13/15)]. These results indicate the effectiveness of our electroporation device for the somatic transformation of the examined moth species.

The simple electroporation device presented in this study has several good properties: e.g. low cost, easily constructed and universally applicable. A previous study indicated that various insect tissues are successfully electroporated by 20–30 V pulses; namely, the larval epidermis and pupal wing of *B. mori*, and the larval epidermis of the

Asian swallowtail butterfly, *Papilio xuthus* (Linnaeus) and the red flour beetle, *Tribolium castaneum* (Herbst) (Ando and Fujiwara 2013). This voltage range is within the usable range of our electroporator, suggesting that our device is applicable not only to the moths but also to diverse insect species. Thus far, electroporation methods that enable gain-of-function analyses have required expensive equipment. This simple low-cost electroporator will render gene function analyses accessible to many non-model insects, including pest species.

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