Journal of open hardware

A Low-Cost Electroporator for Genetically Modifying Social Amoeba Dictyostelium Discoideum

HARDWARE METAPAPER

MICHAEL C. CAUCHY
ALI A. KHAN
YULIA ARTEMENKO
DAVID A. DUNN
*Author affiliations can be found in the back matter of this article

]u[ubiquity press

ABSTRACT

The social amoeba *Dictyostelium discoideum* is a commonly used eukaryotic model organism for the study of cell division, chemotaxis, differentiation, phagocytosis, and other cellular processes. Electroporation is an effective and efficient method for delivering plasmid DNA into *D. discoideum*, an invaluable tool for studying intracellular processes. The technology is readily available but often prohibitively expensive. Although several custom-built electroporation devices have been developed, none deliver the specific 8.5kV/cm exponentially decaying waveform required for *D. discoideum* transformation. The present study examined whether a simple, inexpensive device can be built to produce this waveform through a simple resistor-capacitor (RC) circuit. A pulse generator RC circuit was built incorporating inexpensive electronic components and a 3D printed cuvette chamber. All four possible combinations of custom-built and commercial pulse generators and custom-built and commercial cuvette chambers were used to transform *D. discoideum* cells with a plasmid encoding green fluorescent protein (GFP). There were no significant differences in the number of surviving cells immediately following or 24 hours post-transformation between the systems. All combinations of custom-built and commercial systems achieved comparably high transformation efficiency shown by percent of cells expressing GFP six days after the transformation. Since the waveform-specific electroporation system we present here can be built by non-experts with easily obtainable materials and 3D printing, we envision this device to benefit investigators in areas with low research budgets and educators in multiple STEM fields.

CORRESPONDING AUTHORS:

Dr. David A. Dunn

Dept. of Biological Sciences at the State University of New York at Oswego, Oswego, NY USA

david.dunn@oswego.edu

KEYWORDS:

3D printing; genetic engineering; GMO; open-source; transformation

TO CITE THIS ARTICLE:

Cauchy, MC, Khan, AA, Artemenko, Y and Dunn, DA. 2023. A Low-Cost Electroporator for Genetically Modifying Social Amoeba Dictyostelium Discoideum. *Journal of Open Hardware*, 7(1): 5, pp. 1–13. DOI: https://doi.org/10.5334/joh.52

METADATA OVERVIEW

- Main design files: https://dicty-electroporator.github.io/
- Target group: Biological and biochemical researchers; high school, undergraduate, graduate students, post-doctoral fellows, and faculty; especially those located at non-research-intensive institutions, high school science departments, foreign and domestic undergraduate institutions.
- Skills required: 3D printing – easy; Hardware assembly – standard pulse generator: intermediate (soldering), modular pulse generator: easy, with precautions; high-voltage testing and electroporation – easy, with precautions; *D. discoideum* culture – easy, requires timely attention to detail
- Replication: Basic, existing lab equipment (electrophoresis); readily available, inexpensive electrical components; open-source plans and documentation (https://dicty-electroporator.github.io/).

(1) OVERVIEW

INTRODUCTION

Genetic manipulation of model organisms is a powerful tool in biological research. The insights provided by this exploration have led to a revolution in understanding basic and pathologic biological processes, from molecular to organismal scales. Additionally, genetic engineering has played an integral role in the development of a variety of biotechnology applications, including the generation of animal and tissue culture models for pharmaceutical research and development, as well as the testing and manufacture of improved crop varieties. Several methods exist for introducing recombinant DNA into a host, including direct microinjection, infection with viral vectors, chemical transformation, and electroporation. This process is often referred to as transfection, transformation, or transduction depending on the cell type and method used.

D. discoideum is a commonly used eukaryotic model organism for the study of cell division, chemotaxis, differentiation, phagocytosis, and other cellular processes (Schaap 2011; Müller-Taubenberger, Kortholt & Eichinger 2013; Dunn et al. 2017; Artemenko, Lampert & Devreotes 2014; Stuelten, Parent & Montell 2018). As a model organism, *D. discoideum* is relatively easy and inexpensive to grow and maintain, is easy to manipulate genetically, poses very low biosafety risk, and multiple mutant strains are readily available at low cost from the Dicty Stock Center (Fey et al. 2013). Electroporation, which involves delivering an electrical field to cells resulting in the opening of temporary pores through which DNA can enter, is the method of choice for the transformation of *D. discoideum* cells (Escoffre et al. 2009; Gaudet et al. 2007). Electroporation allows for efficient delivery of plasmids that can, for example, encode fluorescently-tagged biosensors or deliver components for CRISPR/Cas9-mediated gene disruption, among other functions. Transformation of *D. discoideum* is routinely carried out via two identical pulses, five seconds apart in which a 25μF capacitor charged to 850V is discharged through a 0.1cm cuvette containing an electrolytic solution, plasmids, and *D. discoideum* cells (Gaudet et al. 2007).

Commercially available electroporation devices vary in the number and types of electrical parameters they can deliver, which determines which organisms and cell types they can electroporate, as well as their cost. Commercial electroporation equipment can cost in excess of $10,000 (USD), which precludes many researchers from having access to this technology. There has been increasing interest in building inexpensive custom-built electroporators, each with different advantages and drawbacks (Rodamporn et al. 2007; Borges et al. 2013; Bullmann et al. 2015; Schmitt, Friedrich & Gilbert 2019; Byagathvalli et al. 2020). Several custom-built devices have been designed for electroporation of mammalian cells or embryos, which require relatively low voltages (between 10 and 160V) delivered as a square pulse (Rodamporn et al. 2007; Borges et al. 2013; Bullmann et al. 2015). These conditions are not suitable for electroporation of many microorganisms, which require high voltages and an exponential decay waveform (Chassy et al. 1988; Dower et al. 1988). Two recent devices, the Portoporator and Electropen, have achieved the delivery of high voltage exponential decay waveforms, although

both have only been tested with bacterial cells. Although the Portoporator has been tested at 400-600V, its design allows it to be modified for pulses greater than 1000V (Schmitt, Friedrich & Gilbert 2019). The Electropen, which uses piezoelectricity, was able to deliver 2000V pulses (Byagathvalli et al. 2020). However, despite its elegant design, the Electropen performance is dependent on the availability of the appropriate lead zirconate titanate crystal, which is difficult to readily or reproducibly obtain. Thus, none of the current devices offer the required conditions or have been tested for the transformation of *D. discoideum*. This prompted us to develop an affordable device that would deliver the necessary waveform specific to this model organism.

The device described here is uniquely suited to deliver a precise, uniformly repeatable waveform using an inexpensive and adaptable collection of off-the-shelf and 3D printed components that are easy to assemble and utilize. Modules required to build this device include a power supply, pulse generator, and cuvette chamber, as depicted in Figure 1. As a standalone solution for transforming *D. discoideum*, its performance is equivalent to commercial devices, and at an exceptionally low cost. Additionally, our design is easily adaptable to different parameters suitable for other cell types, thus greatly expanding the potential utility of the device.

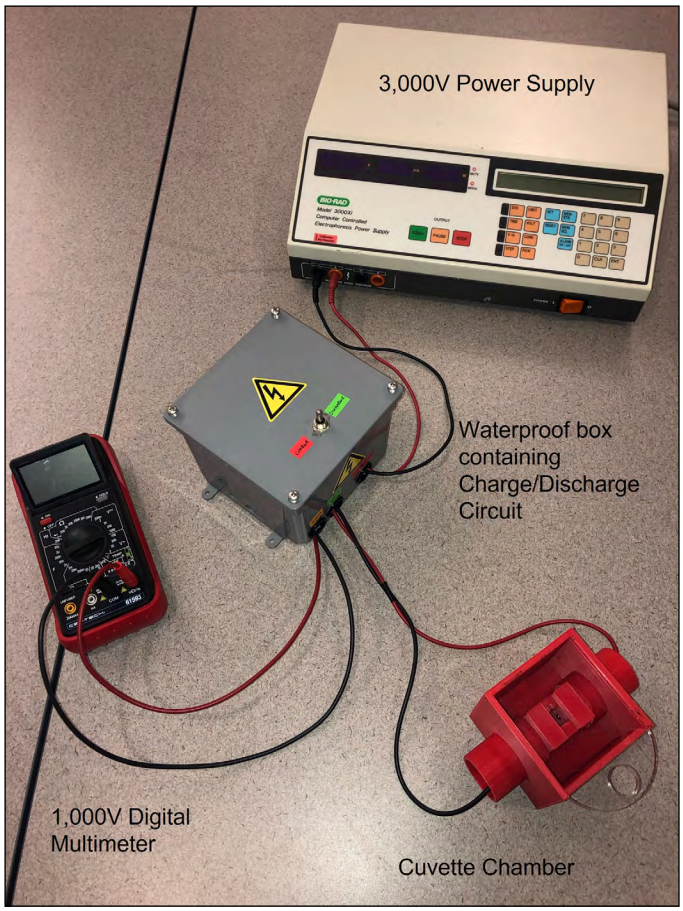

Figure 1 Complete electroporation apparatus with all functional modules and peripherals.

OVERALL IMPLEMENTATION AND DESIGN

Pulse Generator: General Considerations

Exponential decay electroporation is commonly used to transform *D. discoideum,* bacteria, and other organisms. The waveform is commonly produced by a circuit in which a charged capacitor is discharged into the biological sample. The rate at which voltage drops during discharge is exponential. We have chosen to use a protocol by Gaudet et al. (2007) that is specific to *D. discoideum*. This protocol recommends two pulses, five seconds apart, with a field strength of 8.5kV/cm, each featuring an exponential decay waveform of 850V through a 0.1cm cuvette from a 25μF capacitor. The protocol also states that there is no need for an additional resistor and should yield a time constant (tau or τ) of approximately 0.6msec. In resistor-capacitor (RC) circuits, tau is defined as the time taken for the voltage across a discharging capacitor to drop to ~36.8% (1/e) of its initial charge. Tau (units = seconds) is calculated by resistance (R; units = ohms or Ω) multiplied by capacitance (C; units = Farads or F) in the discharge circuit: [τ = RC].

Therefore, the total resistance in our discharge circuit should equal the quotient of the time constant and the capacitance: R = τ/C = 600µsec / 25µF = 24Ω.

To ensure the production of a waveform that fits the above requirements, we measured the sample resistance of the cell suspension to be electroporated. Resistance measurements of live cells in H-50 (the electroporation buffer) with plasmid DNA were taken from three homogenous samples in triplicate with a mean ± SD of 26.22 ± 2.39Ω (yielding τ = 656µsec). The similarity of this measured value to the calculated resistance value of 24Ω required to produce the desired waveform provided confidence in the pulse generator circuit design.

The circuit design for the pulse generator is depicted in Figure 2. In general, the circuit functions to charge a 25µF capacitor to 850V and is then switched to deliver that charge to a cuvette as described above. Any number of alternate exponential waveforms can be created by using the above equations, along with the resistance value of the sample, to determine what values for capacitance and resistance would replace those specific to *D. discoideum*. Alternatively, square waveforms can be manufactured using a microcontroller and an optically isolated power circuit for electroporation of other cell types such as mammalian.

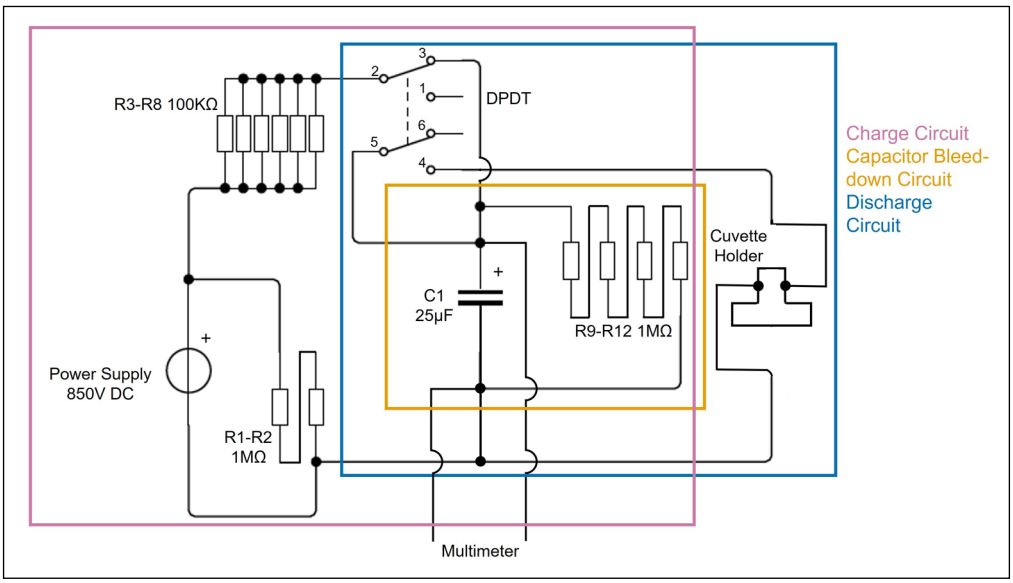

Figure 2 Circuit Diagram: Charge circuit outlined in reddish purple; capacitor bleed-down circuit outlined in orange; discharge circuit outlined in blue.

We used Bio-Rad PowerPac 1000 and model 3000xi electrophoresis power supplies to provide the voltage necessary to charge our circuit. These have several important safety features, such as current limiting, that influenced the pulse generator design. While lower voltage sources (e.g. batteries) could have been stepped up to produce the required voltage, most biology labs have access to electrophoresis power supplies with built-in safety features, similar to above. The connection to the supply's output was recessed into the unit and required power supply-specific male banana plugs that were especially long.

We present here two designs for the pulse generator, which we will refer to as 'standard' and 'modular'. The standard pulse generator was designed with durability in mind. Its soldered construction requires care and attention to detail: soldering tools should be grounded and will become extremely hot. This design includes a soldered padboard and components with high power ratings.

The modular pulse generator is designed to maximize the ease and rapidity of build (no soldered junctions), is easily modified by swapping components for alternative waveforms, and is intended for a small number of electroporations before disassembly (not routine or repeated use). One potential application of this device would be in an educational setting where supervised students construct the device and conduct test electroporations. The device could be modified, based on appropriate calculations, to produce alternative waveforms suitable for other cell species, which could then be tested. In these instances, we recommend the replacement of resistors and breadboards before subsequent course sections. In educational contexts, we also recommend strict attention to ensuring electrical safety, as described

below. Another application of the modular device would be in a research context where an investigator is developing and testing new electroporation protocols. These potential uses are made possible by the use of a solderless breadboard.

Pulse Generator: Charge circuit design

The power supplies used in this project are manufactured for electrophoresis of protein and nucleic acid samples by researchers with little to no knowledge of electrical safety procedures. Their designs include robust safety features to prevent accidental shock. The charge-discharge circuit was designed to take some of these safety features into account.

One feature interrupts the power supply output when a pre-programmed current is exceeded. To maintain current within preset power supply tolerances, six 100kΩ resistors were wired in parallel between the power supply and the switch (R3-R8 in Figure 2). This resistor array yields an effective resistance of 16,667Ω. Thus, charge tau = RC = 16,667Ω · 25μF = 417mS to reach 61.4% capacity, 5 · tau = 2.09 seconds to >99% charge. This charge timing is well within the five-second pulse interval described by the protocol (Gaudet et al. 2007). To determine minimum component power requirements, the average energy dissipation of the charging circuit resistors was calculated by dividing the total power consumption – CV^2 / 2 by the five-second duty cycle. Average power was calculated by ((25μF · 850^2V) / 2) / 5 = 9.03J / 5 seconds = 1.8W / 6 resistors = 0.3W per resistor. Six 8W resistors and a soldered padboard were used in the standard build while 2W resistors and a solderless breadboard were used in the modular build.

Another safety feature of the power supply cuts output if the current flow drops too low. To resolve this issue, two 1MΩ resistors were connected across the power supply leads to ensure a constant low current, keeping the power supply on regardless of switching position. 2W resistors were tested in the standard build and 500mW resistors were used in the modular circuit.

Pulse Generator: Discharge circuit design

With the capacitor charged the circuit is switched to deliver the stored charge to the cuvette. Switching is accomplished via a manual double-pole, double-throw (DPDT), 20A, on-off-on toggle switch that is commercially available. As depicted in Figure 2, pins 1 and 6 of the switch are left unconnected, 2 is connected to the positive lead of the power supply, positions 3 and 5 to the capacitor, and position 4 is connected to the cuvette chamber. Optionally, the voltage on the capacitor can be monitored using a digital multimeter connected to each pole of the capacitor (Figure 2).

To minimize the potential hazard of leaving a 25μF capacitor (labeled 'C1' in Figure 2) charged to 850V we incorporated a separate bleed-down circuit across the capacitor terminals. Four 1MΩ resistors were wired in series across the capacitor (4MΩ) allowing discharge of the circuit to a voltage (<50V) deemed safe by the United States Occupational Safety and Health Administration (OSHA, 2015) in approximately 5 minutes. This was calculated using the equation: $V_C = V_S \cdot e^{-(t / \tau)}$, where V_C = safe capacitor voltage, 50V; V_S = source voltage, 850V; τ = R · C = 4MΩ · 25μF = 100sec. Thus, $50V = 850V \cdot e^{-(t / 100)}$. Solving for t indicates a discharge from 850 to 50 volts through 4MΩ in 4.72 minutes. Four 1MΩ resistors in series were therefore connected across the plates of the capacitor (see orange box in Figure 2). These bleed-down resistors dissipate 9.03J / 4 = 2.26J over 5 minutes (300 seconds) = 0.0075 J/s or watts. Power ratings for these resistors were 500mW in our modular circuit and 2W in the soldered circuit.

Cuvette Chamber

Electroporation of cells occurs in a commercially available sterile single-use 'Potter-style' electroporation cuvette as shown in Figure 3A (Potter, Weir & Leder, 1984). The cuvette features a 0.1cm gap between two aluminum plate electrodes where the electroporation suspension is placed. This precise gap yields an electric field of 8.5kV/cm from the 850V capacitor discharge. A 3D printed polylactic acid (PLA) cuvette chamber that allows safe and secure delivery of the electroporation pulse to the cuvette is presented here. The cuvette chamber is designed to accept an inserted cuvette only in the proper orientation to the banana plugs (Figure 3B). At the insertion points for the banana plugs, the cuvette chamber has an orifice for each of the two plugs (Figure 3D). Each orifice was designed with varying diameters at different depths (Figure 3E, also visible in 3D). This takes advantage of the spring-like friction fit of the male banana plug

to ensure that contact between the circuit and cuvette is maintained throughout electrical pulse delivery. In early iterations of the cuvette chamber with a cylindrical orifice, the high voltage across the cuvette during discharge forced the banana plugs away from the cuvette. The final design ensures continuous contact. A collar protrudes from each side of the cuvette chamber to safely shield the banana plugs (circled in Figure 3C). Cuvette chamber dimensions were dictated by precise measurements of standard commercially available electroporation cuvettes as well as 4-mm banana plugs. This design features a lid that slides in and out of the cuvette chamber. The lid can be 3D printed or laser cut from a sheet of clear acrylic or other material.

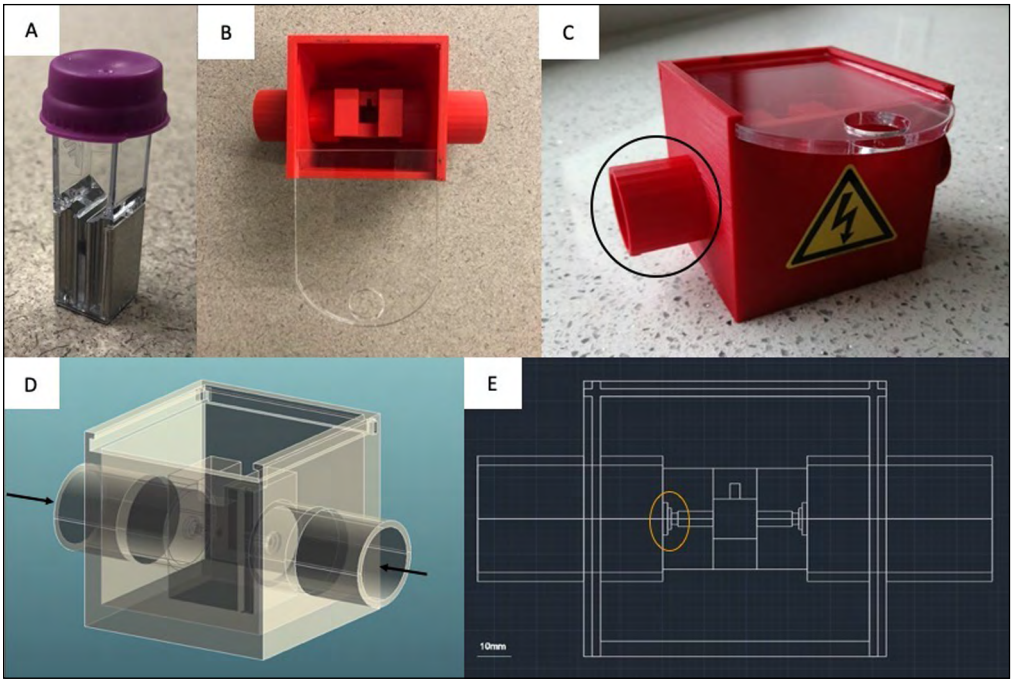

Figure 3 (A) Potter-style 0.1cm cuvette. **(B)** 3D printed cuvette chamber. **(C)** Cuvette chamber with protective collar depicted in circle. **(D)** Cuvette chamber rendering. **(E)** Top view rendering of cuvette chamber. Varying diameters of banana jack sockets depicted in circle.

Enclosure

Protection from electrical components of the pulse generator was provided by a plastic junction box (gray box in Figure 1). Holes were drilled for mounting the switch and to provide wire connections to the power supply, cuvette chamber, and multimeter.

(2) QUALITY CONTROL

SAFETY

Hazards present during device construction are related to electronics fabrication and are easily mitigated by proper safety precautions. Attention to detail and the use of personal protective equipment (PPE) may help decrease the risk of personal injury resulting from exposure to sharp edges and hot soldering equipment.

Hazards present during device usage are related to electric shock and biosafety. To avoid risk of electric shock a rubber floor mat and dry working space should be employed during all assembly procedures. Soldering irons and any other device using mains electricity should make use of a 3-prong grounded cord. Inexpensive electric shock hazard stickers were applied to the pulse generator enclosure and cuvette chamber. In the modular circuit components, including capacitor terminals, are directly exposed when the enclosure lid is off and so the enclosure lid should not be removed without disconnecting the power supply and waiting five minutes as the capacitor bleeds down. Care should be taken to ensure the lid is always attached during operation to prevent shock. Particular attention should be paid to ensure that accidental capacitor discharge does not occur. While a safety voltage bleed-down circuit is in place to ensure the capacitor does not retain a charge indefinitely, care should be taken by assuming that the capacitor may have a residual charge. This could occur if there was a compromise of the discharge resistor array circuit. Safely discharge the capacitor before handling (Scott, 2019).

Arcing presents a risk if buffers or cell suspensions are prepared incorrectly or if the cuvette is not wiped dry before electroporation. This condition might lead the cuvette top (purple

in Figure 3a) to loudly pop off. The cuvette chamber completely surrounds the cuvette and features a lid that slides into place providing protection should this occur (Figure 3b).

Personal protective equipment and laboratory safety protocols consistent with biosafety level 1 (BSL1) standards should be utilized while working with *D. discoideum*. Electroporation cuvettes with removable tops should be utilized and eye protection worn during their removal.

GENERAL TESTING

Ensuring the correct function of the circuit in real-time was difficult to visualize with a tau of 0.6msec. While observation of the waveform might be accomplished using a high bandwidth/sample-rate oscilloscope with a high voltage probe, many users will not have access to these resources. Therefore, in order to evaluate circuit performance in a way that we could visualize, we altered the circuit to produce a waveform with a longer time constant. We accomplished this by increasing the resistance in the circuit by removing the cuvette from the cuvette chamber. This enabled rough measurements of the circuit's performance to be taken with an off-the-shelf digital multimeter and a stopwatch. Using stackable banana jacks at the cuvette chamber of the modular build, we connected a 1,000V multimeter and set it to read DC voltage. In this configuration, resistance in the discharge circuit results from the parallel combination of the bleed-down resistors (4MΩ) and the internal resistance of the multimeter set to the 1,000V range (~9.3MΩ). This total resistance is calculated at 2.8MΩ. With this resistance, the new time constant is calculated to be ~70sec (25μF · 2.8MΩ = 70sec). After charging the capacitor to 850V, we toggled the switch to discharge and began a stopwatch while observing voltage using the multimeter. Voltage dropped through 312V (equivalent to the drop expected in 1 time constant) in approximately 70 seconds. In normal operation of the standard pulse generator device, we connected the multimeter to the capacitor to monitor the charge on the capacitor live (Figures 1 and 2).

Tau was measured in the standard circuit by capturing the waveform using a 50W – 25Ω resistor to represent a sample, a high voltage probe, and iWorx TA data acquisition device (Figure 4). The software was set to capture the exponential decay (the rate of capacitor discharge decreases as it discharges) waveform at 100k samples per second. Testing voltage was set to discharge 500V (500V being the maximum detectable voltage in our setup—tau is not voltage dependent) from the wave generator circuit through a 50W, 25Ω resistor and testing wires. A measured output voltage of 489.9V reached 179.7V (≅ 36.8% of 489.9V) in 0.69msec, as indicated by the time between the two red lines. The measured tau of 0.69msec is slightly greater than our expected 0.625msec (25μF · 25Ω = 0.625msec). This is likely due to additional resistance of the testing wires and high voltage probe.

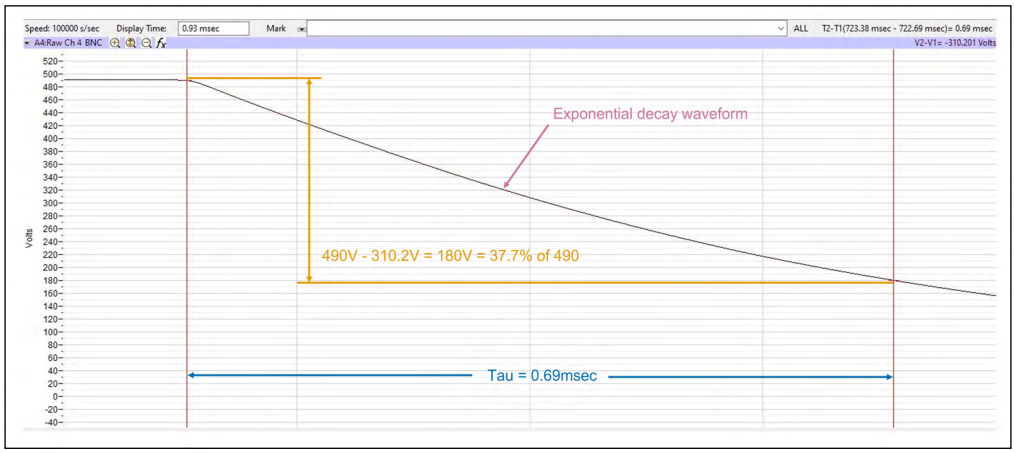

Figure 4 Tau measurement in the standard pulse generator circuit. The output voltage decayed exponentially through the circuit and reached ~36.8% of its starting value in 0.69msec, as indicated by the time between the two red lines. The exponential decay of voltage was due to the rate of capacitor discharge decreasing as it discharged.

(3) APPLICATION

USE CASE

The two electroporator components described here: the pulse generator and the cuvette chamber are designed to carry out electroporation in tandem. However, each is also designed to be compatible with hardware from other suppliers. As proof of concept for this interchangeability, and to test our custom components independently, we electroporated

recombinant plasmid DNA encoding green fluorescent protein (GFP) into the commonly used axenically growing Ax2 strain of *D. discoideum* using four hardware combinations:

1. Custom pulse generator (modular) with custom cuvette chamber
2. Custom pulse generator (modular) with Bio-Rad ShockPod cuvette chamber
3. Bio-Rad Gene Pulser Xcell pulse generator with custom cuvette chamber
4. Bio-Rad Gene Pulser Xcell pulse generator with Bio-Rad ShockPod cuvette chamber

Cells were transformed according to Gaudet et al. (2007): $5 \cdot 10^6$ *D. discoideum* cells in a total volume of 0.1ml electroporation buffer (H-50) were transformed with 2µg plasmid DNA in an electroporation cuvette with a 0.1cm gap. Electrical pulses were delivered as described above. Transformants were selected with 20µg/mL antibiotic G418, which was added 24 hours after electroporation.

To compare the performance of our device to the commercially available Bio-Rad system, we assessed cell survival immediately following electroporation using the four hardware configurations above, as well as cell doubling during the first 24 hours (Table 1). There was a 50 to 60% reduction in cell number immediately following the pulse. One-way ANOVA analysis detected no significant difference between cell numbers in any of the groups ($F(3, 8) = 0.42$, $p = 0.74$). Cells that survived the electroporation process proliferated over the next 24 hours, with a two to three-fold increase in cell number. Again, there were no differences between the four conditions tested ($F(3,8) = 0.71$, $p = 0.57$).

Pulse generator	**Custom**	**Custom**	**Commercial**	**Commercial**
Cuvette chamber	**Custom**	**Commercial**	**Custom**	**Commercial**
Cells surviving electroporation (%)	46 ± 20[a]	41 ± 1	32 ± 15	53 ± 12
Fold increase in cell number over 24 hours	2.4 ± 0.6	2.3 ± 0.3	3.2 ±1.4	1.5 ± 0.5

Table 1 Comparison of survival and growth of *D. discoideum* cells following electroporation using combinations of custom and commercial pulse generator and cuvette chamber components.

[a]Data shown as mean ± SE of 3 independent experiments

Transformation efficiency was measured 6 days post-electroporation, when cells reached confluency, by counting the proportion of cells expressing GFP via fluorescence microscopy (Figure 5). For all four hardware combinations above, approximately 85% of cells had detectable levels of GFP ($F(3, 8) = 0.22$, $p = 0.88$). Another experiment in which three similar electroporations were performed with the standard pulse generator and the custom cuvette chamber yielded similar GFP expression levels (data not shown).

REUSE POTENTIAL AND ADAPTABILITY

The electroporation design described here is for *D. discoideum*, although it could be used for other organisms requiring similar electroporation parameters. Other electroporation modalities are capable of transforming fungi (Delorme 1989; Margolin, Freitag & Selker 1997), multiple species of bacteria (Dower, Miller & Ragsdale 1988; Smith & Iglewski 1989; Schenk & Laddaga 1992), cultured mammalian cells of various types (Potter, Weir & Leder 1984; Chu, Hayakawa & Berg 1987), plants (Fromm, Taylor & Walbot 1985; D'Halluin et al. 1992), mouse embryos (Wefers et al. 2017), chicken embryos (Sato et al. 2007), as well as other biological models. Adaptation of this device to function in many of those protocols using exponential decay waves would be simple to achieve with minimal changes to the device, e.g., voltage and capacitor.

(4) BUILD DETAILS

AVAILABILITY OF MATERIALS AND METHODS

A detailed bill of materials is provided in the GitHub online repository (https://dicty-electroporator.github.io/). All materials are either readily available from multiple suppliers or can be 3D printed using the design and print files in the online documentation (cuvette chamber).

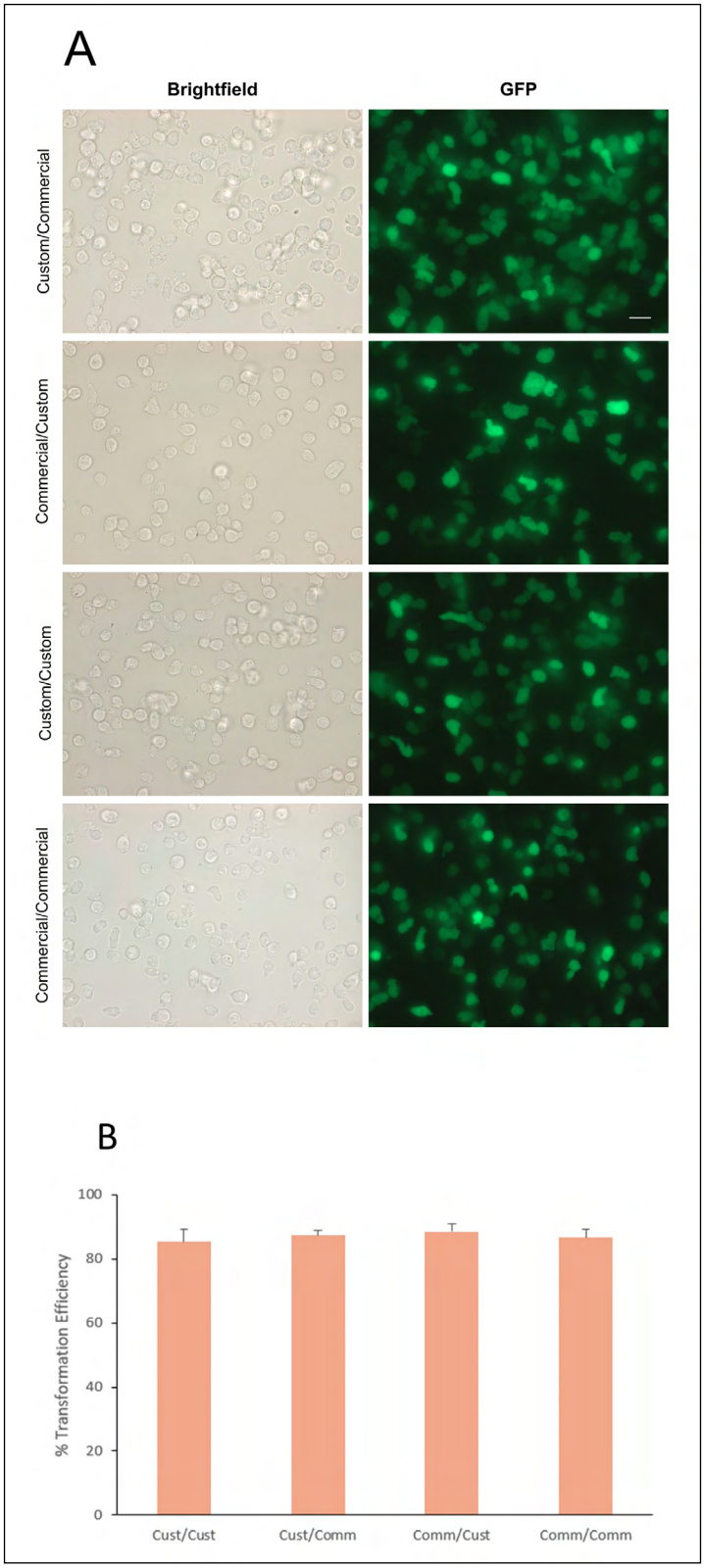

Figure 5 Transformation efficiency following electroporation using different combinations of custom and commercial (labeled as Cust and Comm, respectively) pulse generator/cuvette chamber. *D. discoideum* cells were transformed with a plasmid encoding cytosolic GFP and imaged after 5 days of selection with antibiotic (6 days after electroporation). **(A)** Brightfield and fluorescence images were acquired at 630X magnification. Representative images are shown. Scale bar, 20µm. **(B)** Transformation efficiency was calculated as the number of cells with detectable levels of GFP compared to the number of cells in corresponding brightfield images. Data were collected from five fields for each condition (between 150 and 750 cells per condition) over three independent experiments and is shown as mean ± SE. The variation in cells per condition was due to the random settling of cells once pipetted into the eight-well microscope slides – as well as the random selection of fields.

Any power supply capable of providing 1kV DC can be used. In our hands, the Bio-Rad PowerPac 1000 served as the power supply to the modular pulse generator and the Bio-Rad 3000xi for the standard pulse generator. They are basic power supplies designed for use in electrophoresis. Similar electrophoretic power supply devices are present in many biology labs, but used units are available inexpensively from used laboratory equipment suppliers and several online outlets. It will be useful to search the internet for 'used electrophoresis power supply'. There are many used devices to be found costing $100–200 including shipping.

Consumable products include reagents and materials needed for *D. discoideum* culture, electroporation buffer, and electroporation cuvettes. They are available from multiple scientific suppliers.

EASE OF BUILD

Electrical connections in the modular pulse generator have been designed to not require soldering – which also facilitates easy modularity for other waveforms. Most connections are formed by 24AWG – 3.5A solid breadboard jumper wires, 26AWG – 2.2A flexible jumper wires, and resistors plugged directly into a solderless breadboard or terminal block. Other connections make use of banana plugs or crimping wires onto terminals (switch, capacitor). While these connections have not failed in our hands, soldered connections, as seen in the standard pulse generator, yield a more permanent connection. Step-by-step build instructions are available in GitHub (https://dicty-electroporator.github.io/) and Zenodo (https://doi.org/10.5281/zenodo.7348874) repositories.

DEPENDENCIES

Detailed protocols and materials for culturing and electroporating *D. discoideum* are required to prepare for and carry out usage of the device. We utilized methods described in Gaudet et al. (2007).

Charging the device requires a power supply capable of providing 1kVDC. Several commercially available power supplies exist. We chose the closed source Bio-Rad PowerPac 1000 and 3000xi electrophoresis power supplies. In early versions of our build, we accessed deeply recessed terminals in the power supply using the lid of a gel electrophoresis chamber as an adapter between the power supply and terminals of our device. Later iterations made use of commercially available power supply banana plugs. Circuit testing requires a multimeter capable of measuring 1kVDC.

Hardware documentation and files location:

Name: *Dictyostelium discoideum* Electroporator

Persistent identifiers: https://dicty-electroporator.github.io/; https://doi.org/10.5281/zenodo.7348874

License: CERN OHL v1.2

Publisher: David A. Dunn

Date published: September 23, 2022

(5) DISCUSSION

CONCLUSIONS

A major guiding principle in our design was to present a safe, functional electroporator for *D. discoideum* that was 1) low-cost, 2) easy to build, and 3) easy to use. In this paper, we present two versions of an open-source, low-cost DNA delivery device for the genetic modification of *D. discoideum* which we built and tested. This device is composed of obtainable parts, both 3D printed and available from several commercial sources. Components of the device are easy to assemble, using either solderless or soldered connections. The device can, with some alterations, be tailored to deliver pulses for other cell types. Our device, in comparison with a commercial electroporator, showed similar transformation efficiency.

The total cost of prototyped devices for either the modular or standard build was under $400 (USD) and all hardware design files are publicly available. We hope that the adoption of this device will lower the cost and difficulty of developing genetically modified strains of *D. discoideum* for researchers and educators. Our use of a solderless breadboard in the modular pulse generator facilitates constructing any number of modified circuits to produce and test optimal waveforms of numerous organisms/cell types by swapping out the capacitor and/or resistors to produce novel exponential decay waveforms. Similarly, swapping out the switch for a microcontroller and bypassing the capacitor would allow for easy production of square waves.

We also constructed and tested a more durable device, intended for long-term use, with a minimal increase in cost. This device features a soldered breadboard with higher power components. Assembly of the standard pulse generator circuit is straightforward and can be constructed by a beginner with access to soldering equipment.

3D design and print files are available on the Github and Zenodo repositories linked herein. Note that we chose PLA, a biodegradable 3D print media. While our PLA cuvette chamber has held up well over >150 electroporations, other cost effective and more durable print media, including Acrylonitrile butadiene styrene (ABS) and Polyethylene terephthalate glycol (PETG) are available.

FUTURE WORK

We envision multiple future design innovations. An inexpensive DC power supply may be integrated into the pulse generator, further decreasing the already low cost of the device. A printed circuit board could simplify and standardize many connections in the device. Controlling pulse timing could be accomplished with a microprocessor (Arduino, Raspberry Pi, or similar). Switching in the device presented here is performed manually. While this is not difficult and requires moderate attention to detail to achieve two pulses five seconds apart, automation of this task might decrease opportunities for human error.

ADDITIONAL FILE

The additional file for this article can be found as follows:

- **Supplemental Methods.** Detailed procedures for *D. discoideum* culture, transformation and statistical analysis. DOI: https://doi.org/10.5334/joh.52.s1

ACKNOWLEDGMENTS

We thank Francisco Feito, Gigi Niu, Timilehin Ogunjana, Dr. Sungeun Kim, and Dr. Hui Zhang for helpful discussions.

FUNDING STATEMENT

This work was supported by a SUNY Oswego Faculty-Student Challenge grant to A.K. and D.D., by a SUNY Oswego Student Scholarly and Creative Activity Grant to A.K., Y.A., and D.D., and by NSF-RUI grant no. 1817378 to Y.A.

COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

Michael Cauchy designed the device, cultured *D. discoideum* cells, performed test electroporation and gene expression (fluorescence microscopy) experiments, and wrote the manuscript.

Ali Khan designed the device, cultured *D. discoideum* cells, performed test electroporation and gene expression (fluorescence microscopy) experiments, and wrote the manuscript.

Yulia Artemenko designed the device, advised *D. discoideum* culture and microscopy experiments, and wrote the manuscript.

David Dunn conceived the idea to build an open-source electroporator, designed the device, advised device design experiments, and wrote the manuscript.

AUTHOR AFFILIATIONS

Michael C. Cauchy orcid.org/0000-0001-7085-7788
Dept. of Biological Sciences at the State University of New York at Oswego, Oswego, NY, USA
Ali A. Khan orcid.org/0000-0003-0337-6735
Dept. of Biological Sciences at the State University of New York at Oswego, Oswego, NY, USA
Yulia Artemenko orcid.org/0000-0001-7456-8924
Dept. of Biological Sciences at the State University of New York at Oswego, Oswego, NY, USA
David A. Dunn orcid.org/0000-0001-9275-4798
Dept. of Biological Sciences at the State University of New York at Oswego, Oswego, NY, USA

REFERENCES

Artemenko, Y., Lampert, T. J., & **Devreotes, P. N.** 2014. Moving towards a paradigm: common mechanisms of chemotactic signaling in Dictyostelium and mammalian leukocytes. *Cellular and molecular life sciences: CMLS*, *71*(19), 3711–3747. DOI: https://doi.org/10.1007/s00018-014-1638-8

Borges, R.M., Horne, J.H., Melo, A., Vidal, J.T., Vieceli, F.M., Melo, M.O., Kanno, T.Y., Fraser, S.E., Yan, C.Y. 2013. A detailed description of an economical setup for electroporation of chick embryos *in ovo*. *Brazilian Journal of Medical and Biological Research*, 46(9): 752–757. DOI: https://doi.org/10.1590/1414-431X20133232

Bullmann T., Arendt T., Frey U., Hanashima C. 2015. A transportable, inexpensive electroporator for in utero electroporation. *Development Growth and Differentiation*, 57(5): 369–377. DOI: https://doi.org/10.1111/dgd.12216

Byagathvalli, G., Sinha, S., Zhang, Y., Styczynski, M.P., Standeven, J., & **Bhamla, M.S.** 2020. ElectroPen: An ultra-low–cost, electricity-free, portable electroporator. *PLoS Biology*, 18(1): e3000589. DOI: https://doi.org/10.1371/journal.pbio.3000589

Chassy, B., Mercenier A., Flickinger, J. 1988. Transformation of bacteria by electroporation. *Trends in Biotechnology*, 6(12): 303–309. DOI: https://doi.org/10.1016/0167-7799(88)90025-X

Chu, G., Hayakawa, H., Berg, P. 1987. Electroporation for the efficient transfection of mammalian cells with DNA. *Nucleic Acids Research*, 15(3): 1311–1326. DOI: https://doi.org/10.1093/nar/15.3.1311

Delorme, E. 1989. Transformation of *Saccharomyces cerevisiae* by electroporation. *Applied and Environmental Microbiology*, 55(9): 2242–2246. DOI: https://doi.org/10.1128/aem.55.9.2242-2246.1989

D'Halluin, K., Bonne, E., Bossut, M., De Beuckeleer, M., Leemans, J. 1992. Transgenic maize plants by tissue electroporation. *The Plant Cell*, 4: 1495–1505. DOI: https://doi.org/10.1105/tpc.4.12.1495

Dower, W.J., Miller, J.F., Ragsdale, C.W. 1988. High efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Research*, 16(13): 6127–6145. DOI: https://doi.org/10.1093/nar/16.13.6127

Dunn, J.D., Bosmani, C., Barisch, C., Raykov, L., Lefrançois, L.H., Cardenal-Muñoz, E., López-Jiménez, A.T., Soldati, T. 2017. Eat prey, live: *Dictyostelium discoideum* as a model for cell-autonomous defenses. *Frontiers in Immunology*, 8: 1906. DOI: https://doi.org/10.3389/fimmu.2017.01906

Escoffre, J., Portet, T., Wasungu, L., Teissié, J., Dean, D., Rols, M. 2009. What is (still not) known of the mechanism by which electroporation mediates gene transfer and expression in cells and tissues. *Molecular Biotechnology*, 41(3): 286–295. DOI: https://doi.org/10.1007/s12033-008-9121-0

Fey, P., Dodson, R.J., Basu, S., Chisholm, R.L. 2013. One stop shop for everything Dictyostelium: and the Dicty Stock Center in 2012. *Methods in Molecular Biology*, 983: 59–92. [dictybase.org: Last accessed 23 September 2022]. DOI: https://doi.org/10.1007/978-1-62703-302-2_4

Fromm, M., Taylor, L.P, Walbot, V. 1985. Expression of genes transferred into monocot and dicot plant cells by electroporation. *Proceedings of the National Academy of Sciences USA*, 82(17): 5824–5828. DOI: https://doi.org/10.1073/pnas.82.17.5824

Gaudet, P., Pilcher, K.E., Fey, P., Chisholm, R.L. 2007. Transformation of *Dictyostelium discoideum* with plasmid DNA. *Nature Protocols*, *2*(6): 1317–1324. DOI: https://doi.org/10.1038/nprot.2007.179

Margolin, B.S., Freitag, M. Selker, E.U. 1997. Improved plasmids for gene targeting at the his-3 locus of *Neurospora crassa* by electroporation. *Fungal Genetics Reports*, 44: 13. DOI: https://doi.org/10.4148/1941-4765.1281

Müller-Taubenberger, A., Kortholt, A., Eichinger, L. 2013. Simple system – substantial share: the use of *Dictyostelium* in cell biology and molecular medicine. *European Journal of Cell Biology*, 92: 45–53. DOI: https://doi.org/10.1016/j.ejcb.2012.10.003

OSHA 2015, Occupational Safety and Health Administration, *Guarding requirements for 50 volts or more DC*. Available at: https://www.osha.gov/laws-regs/standardinterpretations/2015-09-04 [Last accessed 23 September 2022]

Potter, H., Weir, L., Leder, P. 1984. Enhancer-dependent expression of human kappa immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation. *Proceedings of the National Academy of Sciences USA*, 81(22):7161–7165. DOI: https://doi.org/10.1073/pnas.81.22.7161

Rodamporn, S., Beeby, S.P., Harris, N.R., Brown, A.D., Chad, J. 2007. Design and construction of a programmable electroporation system for biological applications. *Proceedings of the Thai BME*, 234–238.

Sato, Y., Kasai, T., Nakagawa, S., Tanabe, K., Watanabe, T., Kawakami, K., Takahashi, Y. 2007. Stable integration and conditional expression of electroporated transgenes in chicken embryos. *Developmental Biology*, 305: 616–624. DOI: https://doi.org/10.1016/j.ydbio.2007.01.043

Schaap, P. 2011. Evolutionary crossroads in developmental biology: *Dictyostelium discoideum*. *Development*, 138: 387–396. DOI: https://doi.org/10.1242/dev.048934

Schenk, S., Laddaga, R.A. 1992. Improved method for electroporation of *Staphylococcus aureus*. *FEMS Microbiology Letters*, 94: 133–138. DOI: https://doi.org/10.1111/j.1574-6968.1992.tb05302.x

Schmitt, M.A., Friedrich, O., Gilbert, D.F. 2019. Portoporator©: a portable low-cost electroporation device for gene transfer to cultured cells in biotechnology, biomedical research and education. *Biosensors and Bioelectronics*, 131:95–103. DOI: https://doi.org/10.1016/j.bios.2019.02.024

Scott, M.A. 2019. Working safely with hazardous capacitors. *IEEE Industrial Applications Magazine*, 25(3): 44–53. DOI: https://doi.org/10.1109/MIAS.2018.2875130

Smith, A.W., Iglewski, B.H. 1989. Transformation of *Pseudomonas aeruginosa* by electroporation. *Nucleic Acids Research*, 17(24):10509 DOI: https://doi.org/10.1093/nar/17.24.10509

Stuelten, C.H., Parent, C.A., Montell, D.J. 2018. Cell motility in cancer invasion and metastasis: insights from simple model organisms. *Nature Reviews Cancer* 18(5): 296–312. DOI: https://doi.org/10.1038/nrc.2018.15

Wefers, B., Bashir, S., Rossius, J., Wurst, W., Kühn, R. 2017. Gene editing in mouse zygotes using the CRISPR/CAS9 system. *Methods*, 121-122: 55–67. DOI: https://doi.org/10.1016/j.ymeth.2017.02.008

TO CITE THIS ARTICLE:
Cauchy, MC, Khan, AA, Artemenko, Y and Dunn, DA. 2023. A Low-Cost Electroporator for Genetically Modifying Social Amoeba Dictyostelium Discoideum. *Journal of Open Hardware*, 7(1): 5, pp. 1–13. DOI: https://doi.org/10.5334/joh.52

Published: 08 May 2023

Journal of Open Hardware is a peer-reviewed open access journal published by Ubiquity Press.