Biosensors and Bioelectronics 131 (2019) 95–103

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Portoporator©: A portable low-cost electroporation device for gene transfer to cultured cells in biotechnology, biomedical research and education

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ARTICLE INFO

Keywords:
Electroporation
Transformation
Dh5α
3D printing
Arduino

ABSTRACT

Electroporation has been a widely established method for delivering DNA and other material into cells in vitro. Conventional electroporation infrastructure is typically immobile, non-customizable, non-transparent regarding the characteristics of output pulses, and expensive. Here, we describe a portable electroporator for DNA delivery into bacterial cells that can quickly be reconstructed using 3D desktop printing and off-the-shelf components. The device is light weight (700 g), small (70 × 180 × 210 mm) and extremely low-cost (< EUR 130). We provide the electrical circuitry and a detailed parts list for rebuilding the device. We characterize the properties of generated pulses and apply the system for gene delivery into bacterial Dh5α cells. We analyze the transformation efficiency based on the optical density of cell suspensions at 595 nm and on quantitative analysis of images obtained from bacterial cell-grown agar plates using colony forming units as well as confluence as indicators. We demonstrate time-dependency of the transformation efficiency using single pulses of 500 V between 1 and 1000 ms duration and we show that commercially available electroporation cuvettes of 1 mm gap size reveal higher transformation rates compared to cuvettes with 2 mm gap. We benchmark the transformation efficiency obtained using our platform with data from a heat shock-based transformation protocol and with data from a commercially available electroporator and show that our system reveals comparable results as the other techniques in the applied configurations. While this work focuses on genetic manipulation of bacterial cells, the device may also be applicable for delivery of genetic material small molecule or nanomaterials into other cell types, including mammalian cells.

1. Introduction

Genetic modification of biological organisms has been a widely established method and is being applied in various fields of research. There are different methods for genetic modification being used, which can be generally differentiated into chemical, biological, or physical methodologies (Kim and Eberwine, 2010). A widely applied physical approach to facilitate genetic modification is called electroporation. This method is based on exposure of cells to a strong electric field established by e.g. two electrodes of a electroporation cuvette or another hardware configuration through application of a short external pulse of nano- to millisecond duration and high voltage (HV) in the range of a few hundred up to several thousand volts (Dower et al., 1988; Kandušer and Miklavčič, 2008; Lessard, 2013; Teissie et al., 2005). Depending on the amplitude and duration of the electric pulse, either a tolerable size and/or number of pores or irreversible permeable membrane defects are formed, resulting in a marked increase in cell membrane permeability and to allow charged molecules such as DNA to be driven across the membrane through the pores in a manner comparable to electrophoresis (Gehl, 2003; Kandušer and Miklavčič, 2008; Rems and Miklavčič, 2016; Shigekawa and Dower, 1988; Wagstaff et al., 2016). Irreversible permeable membrane defects are inevitably followed by cell death through, e.g. osmotic effects whereas sub-'critical' electroporation preserves cellular viability and allows for subsequent evaluation of e.g. recombinantly expressed proteins (Kandušer and Miklavčič, 2008; Luft and Ketteler, 2015).

Electroporation has successfully has been applied in a variety of fields of research including microbiology (Dower et al., 1988; Wipf et al., 2017), developmental biology (Betters et al., 2018; Blodorn et al., 2018; Li et al., 2018; Qin and Wang, 2019; Tanihara et al., 2018), neurosciences (Au - Li et al., 2018), cancer research (Choromanska et al., 2017; Ritter et al., 2018) and cancer therapy (Campana et al., 2019; Marty et al., 2006; Miklavcic et al., 2014), immunology (Byun et al., 2018; Montoya and Ansel, 2017) and many others (Kotnik et al., 2015; Rosazza et al., 2016). Modified specimen range from human and other mammalian culture (Byun et al., 2018; Choromanska et al.,

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https://doi.org/10.1016/j.bios.2019.02.024
Received 16 November 2018; Received in revised form 3 February 2019; Accepted 10 February 2019
Available online 19 February 2019

2017), primary (Au - Li et al., 2018), blood (Montoya and Ansel, 2017), sperm (Blodorn et al., 2018) and stem cells (Li et al., 2018) or tissues (Ritter et al., 2018), over blastocysts, zygotes and embryos (Betters et al., 2018; Qin and Wang, 2019; Tanihara et al., 2018), to plant cells (Asavasanti et al., 2012; Lu et al., 2013), as well as further multi and mono-cellular organisms, including bacterial cells (Wipf et al., 2017) or protozoa (Keller et al., 2018).

The main advantage of electroporation is its versatility with respect to its applicability to various biological specimen. In addition, as electroporation is a simple and rapid method, it can be used to treat a large number of individual cells within a short time. The main disadvantage of electroporation however, is cell death caused by HV pulses and only partially successful membrane repair, requiring the use of larger quantities of cells compared to other methods for gene delivery. Thus, optimization of the pulse parameters towards a large number of modified cells versus low effects on the cell fitness, is an important aspect to consider when using electroporation for genetic manipulation of biological specimen.

Conventional infrastructure used for in vitro electroporation, on the one hand, offers a large spectrum of applications but, on the other hand, is also limited for a number of reasons. First, commercially available electroporation platforms are mostly complicated to use and tie up highly skilled staff for application and maintenance. Second, conventional electroporation devices are often heavy, bulky, immobile and require permanent installation and thus, consume substantial lab space. Third, manufacturers of commercial electroporation devices commonly provide pre-defined electroporation protocols promising maximum efficiency and at the same time optimal cellular viability, but increasingly do not provide pulse characteristics, including the pulse length or amplitude (Pirc et al., 2017). Fourth, conventional devices are mostly cost intensive and not readily applicable to a broad range of labs, for scientific studies in low-resource settings or for educational purposes, e.g. in primary or secondary schools or universities.

To overcome the limitations of conventional and commercially available technology described above, we aimed to develop an electroporator that is simple to operate, portable, i.e. of handy size to fit on any lab bench, low cost and affordable to a broad range of research labs and educational institutions and at the same time resource efficient. To this end, we aimed to use low-cost open-source microcontroller architecture, off-the-shelf electronic components as well as 3D-desktop printing, all being more and more prominent in the development of devices for use in scientific, academic or educational field (Schneidereit et al., 2016, 2018; Walzik et al., 2015). We further aimed to evaluate the device with regard to the characteristics of generated pulses and its applicability to genetic modification of bacterial cells. In order demonstrate the applicability of our device to biomedical research, we intended to conduct a case study with bacterial cells and to compare data obtained with our system with data from conventional technology and approaches for bacterial transformation.

2. Material and methods

2.1. Electroporator

The portable system was constructed from a total of approx. 60 parts, including a housing, an Arduino Nano board (Arduino, Italy), a liquid-crystal display, 3D printed parts, power sources, purpose-designed printed circuit boards, transistors, resistors, cables, switches, buttons, potentiometers, etc., during a period of approx. six months. A detailed parts list is included in the Supplements. HV pulses are generated using a capacitor that requires a preparatory lead time of approx. 2–3 min due to charging. The power supply used for charging the capacitor is a high voltage DC-DC boost converter, available at any online market place, providing 300–1200 V and a current of 1–20 mA (maximum 50 mA) at 5–12 V input voltage.

2.2. Usage of the electroporator

For using the electroporator, in a first step, the HV capacitor is charged. To this end, the 'HV power ON/OFF switch' is turned to the 'ON' position while the 'charge capacitor switch' and the 'HV out ON/OFF switch' are in the 'OFF' state. Afterwards, the HV capacitor is charged by flipping the 'charge capacitor switch' in the 'ON' position. Subsequently, the voltage reaches the set electroporation voltage of approx. 500 V and the desired pulse length is adjusted through the 'pulse length potentiometer' in the range between 1 and 1000 ms. Upon successful charging, delivery of a pulse into a cell-laden cuvette is prepared by flipping the 'charge capacitor switch' into the 'OFF' position and the 'HV out ON/OFF switch' into the 'ON' position. To trigger pulse delivery, the 'pulse trigger push button' is pressed. During pulse generation, the blue LED lights up. In a last step, to prevent unintentional pulse generation, the 'HV out ON/OFF switch' is turned back into the 'OFF' position.

2.3. Cuvette carrier

The cuvette carrier as shown in Fig. 2A and F was purpose-designed in CAD software (Autodesk Inventor 2017, Autodesk, Inc., USA) and manufactured from black acrylonitrile butadiene styrene (ABS, MakerBot Industries, USA) using a 3D printer (MakerBot Replicator, MakerBot Industries, USA). To ensure electrical contact between carrier and the cuvette, pieces of sheet metal made of aluminum were inserted into the carrier to fit the dimensions of a conventional and commercially available electroporation cuvette and were connected with the electronics of the electroporator using a bifilar cable.

2.4. Reagents

Reagents were purchased from Sigma-Aldrich (USA) or Carl Roth (Germany) if not stated otherwise.

2.5. Cell line

Dh5α (*E. coli*) cells were purchased from Thermo Fisher Scientific (USA) and were stored at − 80 °C until the day of the experiment.

2.6. Cell culture

All experiments were performed on Dh5α bacterial cells cultured in sterile lysogeny broth (LB) medium, containing Trypton/Pepton (10 g/l), yeast extract (5 g/l) and NaCl (10 g/l). For transformation experiments, LB medium was supplemented with 50 µg/ml ampicillin. Cells were cultured at 37 °C in a shaking incubator at 180 rpm according to standard procedures.

2.7. Agar plates

For production of agar plates, LB medium was supplemented with agar-agar (20 g/l) and was autoclaved according to standard procedures. Sterile LB-agar was supplemented with 50 µg/ml ampicillin and was distributed into 9 cm dishes (20 ml/dish) (Carl Roth, Germany). Prepared agar plates were wrapped into plastic foil and stored at 4 °C until used for experiments.

2.8. Molecular construct

For validation of the suitability of the portable electroporator for gene delivery to bacterial cells as well as for evaluation of the transformation efficiency obtained with our device and other approaches, DH5α cells were transformed using the vector pBS SK(+) (Addgene, USA). The plasmid carries the ampicillin resistance gene for the selection with the antibiotic ampicillin. Thus, only successfully transformed

DH5α cells are able to proliferate when cultured in ampicillin-containing growth medium or agar plates.

2.9. Preparation of electroporation experiments

Electroporation experiments were prepared as described in the Supplements. See workflows in Fig. S1 for electroporation experiments and Fig. S2 for transformation using a heat shock. In brief, prior to transformation experiments, frozen electro or chemically competent Dh5α cells were placed on ice for approx. 10 min. Subsequently, 1 µl DNA solution containing 10 ng of the vector pBS SK(+) in water was added to either 40 µl or 100 µl bacterial suspension for electoporation or heat shock-based transformation, respectively, and the mixture was mixed gently by flicking the vial. The mixture was incubated for 1 min or 30 min on ice for electroporation or heat shock-based transformation, respectively, and was then transferred to an electroporation cuvette or exposed to a heat shock.

2.10. Electroporation with Gene Pulser Xcell™

For gene delivery to DH5α cells using the conventional and commercially available system Gene Pulser Xcell™ (Bio-Rad Laboratories GmbH, Germany), a pre-set and pre-optimized protocol provided by the manufacturer ('Bacterial 2′) was used. Pulses generated using this protocol follow an exponentially decaying time course with a maximum amplitude of 2.5 kV. The duration and time constant (τ) of pulses generated using the Gene Pulser Xcell™ are highly dynamic as they dependents on environmental conditions, including e.g. temperature and conductivity of the cell suspension. Due to variations of those variables in the course of this study, the time constants varied between 5.0 ms and 5.3 ms. Upon delivery of the electroporation pulse, 960 µl of pre-warmed LB medium (37 °C) was added to the electroporation cuvette and was gently mixed with the cell suspension. This mixture was then incubated at 37 °C and 180 rpm for one hour and further processed as detailed in Fig. S1 in the Supplements for assessing the transformation efficiency based on the optical density of bacterial suspensions at 595 nm as well as for image-based analysis of the CFU and confluence.

2.11. Electroporation with the portable electroporator

For electroporation of DH5α cells using the in house-built device, experiments were prepared as described above and the cell-laden cuvette was transferred to the 3D-printed cuvette carrier. Electroporation was carried out by delivering an HV pulse of 500 V amplitude and 1–1000 ms into the suspension. Immediately after electroporation, 960 µl of pre-warmed LB medium (37 °C) was added to the electroporation cuvette and was gently mixed with the cell suspension. This mixture was then incubated at 37 °C and 180 rpm for one hour and further processed as detailed in Fig. S1 in the Supplements for assessing the transformation efficiency based on the optical density of bacterial suspensions at 595 nm as well as for image-based analysis of the CFU and confluence.

2.12. Heat shock transformation

For genetic modification of chemically competent DH5α cells using a heat shock, the prepared cells were exposed to 42 °C for 20 s and were subsequently placed on ice for 2 min. 900 µl of pre-warmed LB medium (37 °C) was added to the electroporation cuvette and was gently mixed with the cell suspension. This mixture was then incubated at 37 °C and 180 rpm for one hour and further processed as detailed in Fig. S2 in the Supplements for assessing the transformation efficiency based on the optical density of bacterial suspensions at 595 nm as well as for image-based analysis of the CFU and confluence.

2.13. Imaging of agar plates

For quantification of the colony forming units (CFU) as well as the area of the agar plate covered with bacteria (confluence), as individual measures of the transformation efficiency, agar plates were placed into a Fusion FX imager (Vilber Lourmat GmbH, Germany) and were imaged at 365 nm and at constant exposure time (920 ms) to ensure comparability of the image data. We chose to image plate in UV light as the colonies appear bright at a dark background, facilitating automated image analysis.

2.14. Quantitative image analysis

Images of 9 cm agar plates were quantitatively analyzed using a modified version of DetecTiff software (Gilbert et al., 2009). In brief, images were segmented using an iterative size and intensity-based thresholding algorithm. For assessment of the colony forming units (CFU), the number of colonies per plate were counted and for quantification of the confluence, the area of identified cells was calculated as the percentage of the overall plate size. A representative analysis result is shown in the Supplements in Fig. S3.

2.15. Data analysis and visualization

Data were annotated in MS Excel and analyzed as well as displayed using Origin 7G (OriginLab Corporation).

3. Results

We have developed Portoporator©, a low-cost portable electroporator that is suitable for the generation of pulses of 400–600 V amplitude and varying duration between 1 ms and 1000 ms. We describe the electrical circuitry of the device, characterize the properties of generated pulses, and we demonstrate the applicability for biomedical research by electroporation of bacterial cells using different commercially available cuvette types and varying pulse durations. We compare the transformation efficiency obtained using our platform to data obtained using a standard heat shock-based transformation protocol as well as data generated from electroporation using a commercially available high-end electroporator and validate its applicability for gene delivery to bacterial cells.

The device is made up of a module for generation of HV pulses, a microcontroller for hardware control and an electroporation-cuvette carrier constructed using 3D desktop printing. The system was developed using off-the-shelf components including a liquid crystal display and push buttons/switches for parametrization. The platform has the shape of a cigar box (70 × 180 × 210 mm, H × L × W) and a total weight of 700 g. The total cost of the system's components is about < EUR 130, excluding any other costs such as staff and labor costs. The device is shown in Fig. 2. A detailed list of electronic parts is included in the Supplements (see Table S1).

3.1. Electroporator

As a first step towards developing a portable low-cost electroporator, we modified an electrical circuit previously described in (Grenier, 2006) for operation with an Arduino Uno microcontroller and generation of rectangular voltage pulses with an amplitude of 500 V and a duration of 1–1000 ms. A schematic drawing of the electrical circuit is shown in Fig. 1 and can be distinguished into five functional compartments. 1. HV circuit (orange), for generation of HV pulses. 2. Cuvette circuit (green) as part of the HV circuit for electroporation of biological specimen using commercially available electroporation cuvettes. 3. Protective circuit (blue) for separation of the controlling infrastructure from the HV circuit and protection of the microcontroller from damage by high voltages. 4. CPU and user interface (red) for

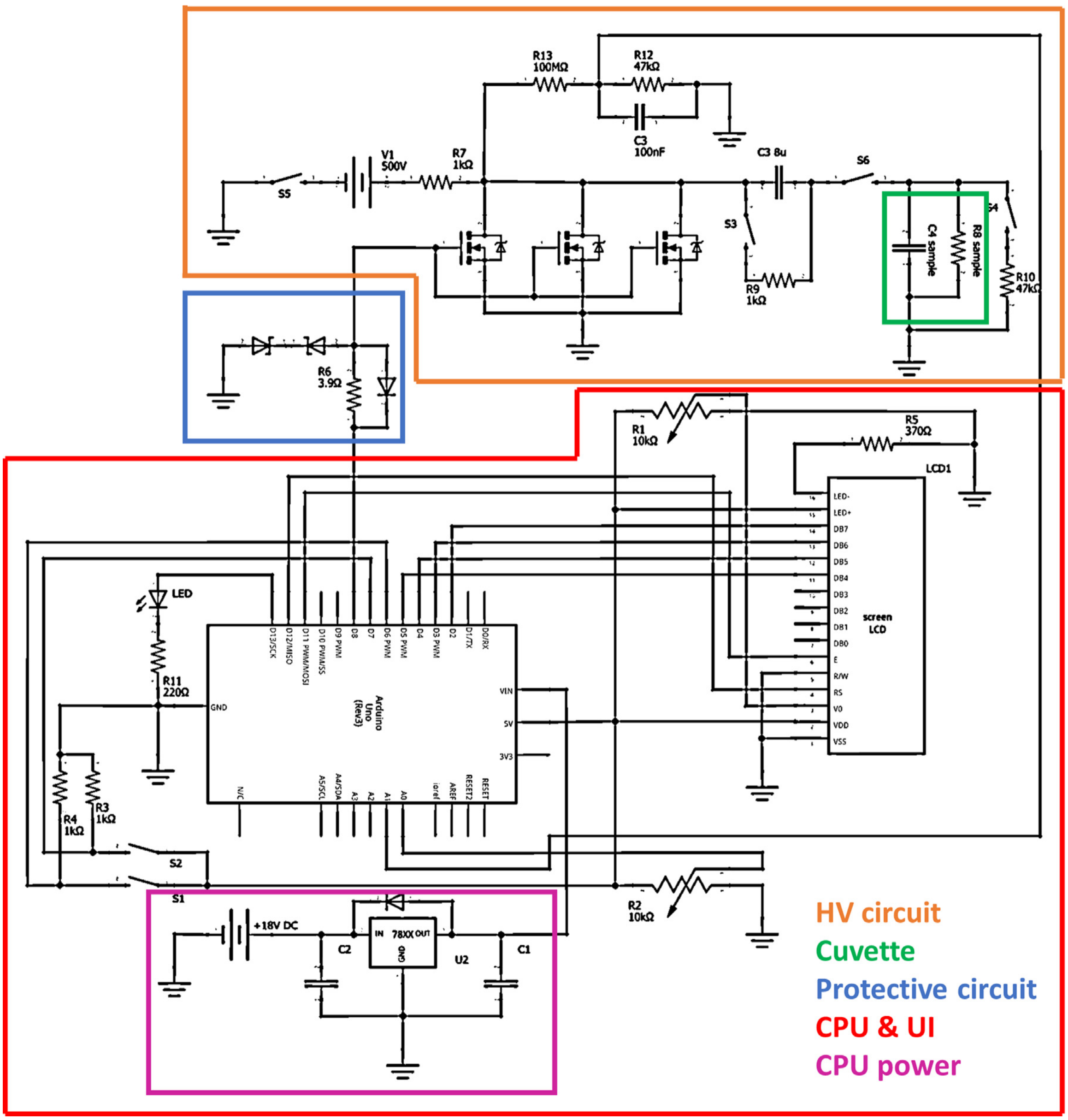

Fig. 1. Electric circuit of the portable electroporator. The circuit is made up of five separate but interconnected sub-circuits for generation of high-voltage pulses ('HV circuit', orange), for connection with a standard and commercially available electroporation cuvette ('Cuvette', green), for protection of the controlling infrastructure from generated high-voltage pulses ('Protective circuit', blue), for communication with the microcontroller and connected push buttons and a LCD display allowing for system parametrization ('CPU & UI', red) as well as for power supply ('CPU power', pink).

system parametrization. 5. CPU power circuit (pink) providing power to the microcontroller.

In a next step, based on the modified electric circuit, we assembled the portable device as shown in Fig. 2. For system parametrization and for triggering HV pulses, the device is equipped with various potentiometers, switches, push buttons and an LCD display as shown in Fig. 2A and B, and as described in the schematic drawings in Fig. 2C and D. A detailed list of all components used for the device is provided in Tab. T1 in the Supplements. For delivering HV pulses into a cell suspension using an electroporation cuvette, we designed a cuvette carrier as described in the methods section and 3D printed it with black ABS plastic. 3D CAD models and the finally assembled cuvette carrier are shown in Fig. A and F, respectively.

3.2. Characteristics of HV pulses

Upon assembly of the electroporation device, we aimed to characterize the electrical properties of HV pulses generated without and with load, i.e. with short circuited cuvette and simulated presence of a cell suspension within the electroporation cuvette. To this end, we measured time courses of the voltage during pulse generation as described in the methods section with short-circuited cuvette and with a simulated cell suspension mimicked using a 47 kΩ resistor. Fig. 3 shows time courses of the voltage for pulses of 1, 3, 10, 30, 100, 300 and 1000 ms duration and 500 V amplitude with short-circuited cuvette (left) and with load (right), indicating that the device is suitable for generation of pulses of varying duration and constant amplitude. Furthermore, these data show that the characteristics, in particular the amplitude, of the pulses is not affected by the presence of a resistor.

3.3. Pulse time dependence of transformation efficiency

To demonstrate that the device is suitable to electroporation-based genetic modification of bacterial cells as well as that the transformation

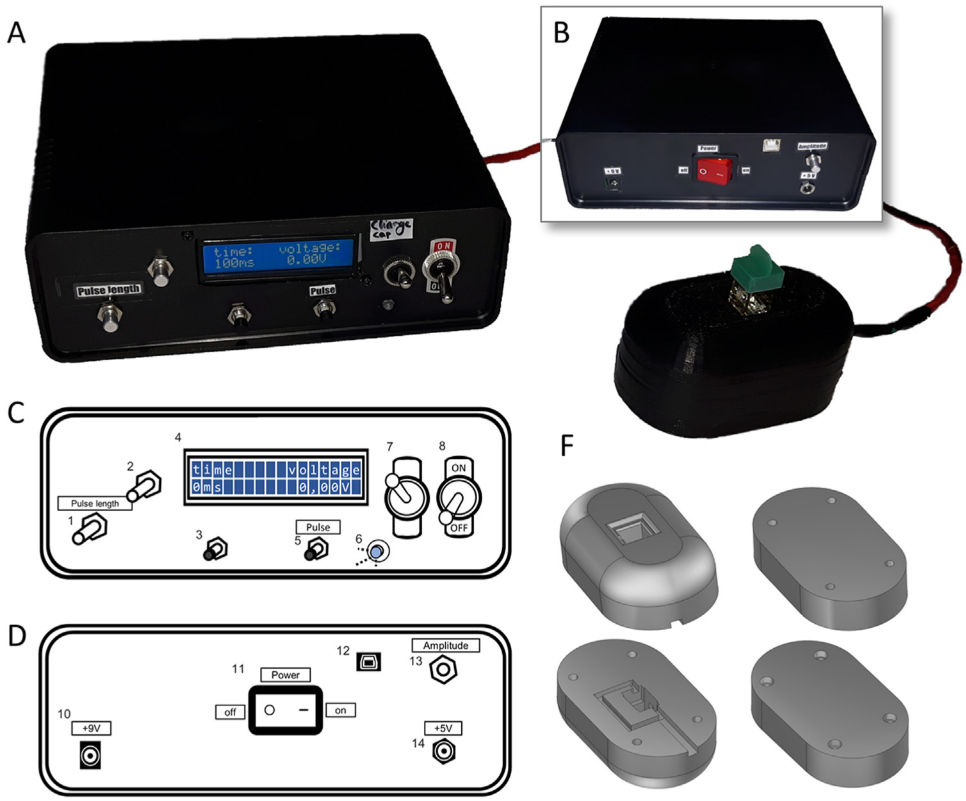

Fig. 2. The Portoporator©. A, B. Front (A) and rear view (B) of the assembled device, with 3D printed cuvette hold and inserted cuvette. **C, D.** Schematic drawings of front and rear panel elements for parametrization of the device and pulse generation. 1. Pulse length potentiometer, 2. Potentiometer for setting LCD brightness, 3. Push button, not in use, 4. LCD, 5. Pulse trigger push button, 6. Capacitor discharge indicator LED, 7. Charge capacitor switch, 8. HV out OF/OFF switch, 9. Discharge capacitor switch (not visible), 10. Main power IN, 11. HV power ON/OFF switch, 12. USB connector, 13. Pulse amplitude potentiometer, 14. HV power IN. **F.** 3D CAD models of the cuvette carrier. Left column: top and bottom view of the upper component. Right column: top and bottom view of the base component.

efficiency obtained using our system depends on the duration of applied high voltage pulses, we exposed Dh5α cells to HV pulses (500 V) of varying duration between 1 ms and 1000 ms and subsequently quantified the transformation efficiency based on three different indicators. As commonly employed measures of the transformation efficiency, we assessed first, the optical density (OD) of bacterial suspensions, measured at 595 nm, second, the colony forming units (CFU) and third, the area covered by colonies (confluence in %) on agar plates. Quantification of CFU and the confluence was conducted by automated analysis of images taken from agar plates under constant conditions, i.e. illumination and exposure time (see *Methods and Supplements* for details). The histograms in Fig. 4A–C show a comparison of the transformation efficiency as indicated by the OD (A) the colony forming units (B) and the confluence (C). Data for pulses of 1, 3, 10, 30, 300 and 1000 ms duration were obtained from a single experiment each (N = 1), whereas data for 100 ms pulse duration were calculated from a total of three biological replicate experiments (mean ± SD). These data indicate that the transformation efficiency for short pulses between 1 ms and10 ms depends on the electroporation pulse duration. For longer pulses, the OD shows strong variation, presumably due to the small number of measurements taken. However, for data obtained from agar plates, CFU and confluence show comparable values at pulse durations greater than 10 ms, indicating good replicability of the results and also imply that the transformation efficiency cannot be further increased by increasing the pulse duration. Representative images of 9 cm agar plates used for image-based analysis of the transformation efficiency are shown in Fig. 4D and depict the time dependence of the transduction efficiency on the pulse duration.

3.4. *Evaluation of the cuvette gap size of the transformation efficiency*

To assess the influence of the cuvette gap size on the transformation efficiency, we quantified the transformation efficiency upon electroporation using pipettes with 1 and 2 mm gap size (see Methods for details). Fig. 4E, F, and G show the OD, CFU and confluence, measured in suspensions of Dh5α cells cultured overnight and on agar plates, respectively, upon electroporation using cuvettes with 1 and 2 mm gap size, respectively. Values represent mean values ± SD (N = 3). The data indicate highest transformation efficiency for cuvettes with 1 mm gap size compared to 2 mm cuvettes. Thus, 1 mm cuvettes were used in all following experiments, also including the evaluation of the time dependence of pulse duration on transformation efficiency.

3.5. *Benchmarking of the portable device with conventional approaches*

In order to evaluate how our platform compares with conventional transformation approaches, we quantified the transformation efficiency of gene delivery using a standard heat shock protocol, a commercially available electroporator, and the portable system as described in the Methods section. Fig. 5A–C show the OD, CFU and confluence, observed upon transformation using the heat shock protocol (HS), the commercially available electroporator (GenePulser, GP), and the portable system (PS), respectively. These data clearly indicate comparable - if not superior - transformation efficiency for the portable system versus the conventional approaches and further validate the suitability of the platform for biomedical research. Fig. 5D depicts images of agar plates for quantification of CFU and confluence associated with the different transformation approaches heat shock (top), electroporation using a commercial device (middle) and electroporation using the in house-built system (bottom).

4. Discussion

We have developed a platform that is advantageous for several reasons. First, with a footprint of 180 × 210 mm (× 70 mm height), and a mass of 700 g the device is at least 10 times smaller and lighter compared to conventional technology (310 × 300 × 280 mm, 11.6 kg) allowing for mobile application. Also, the system requires no additional computer for parametrization, reducing the space required for application. Second, with < EUR 130 costs for material, the portable device

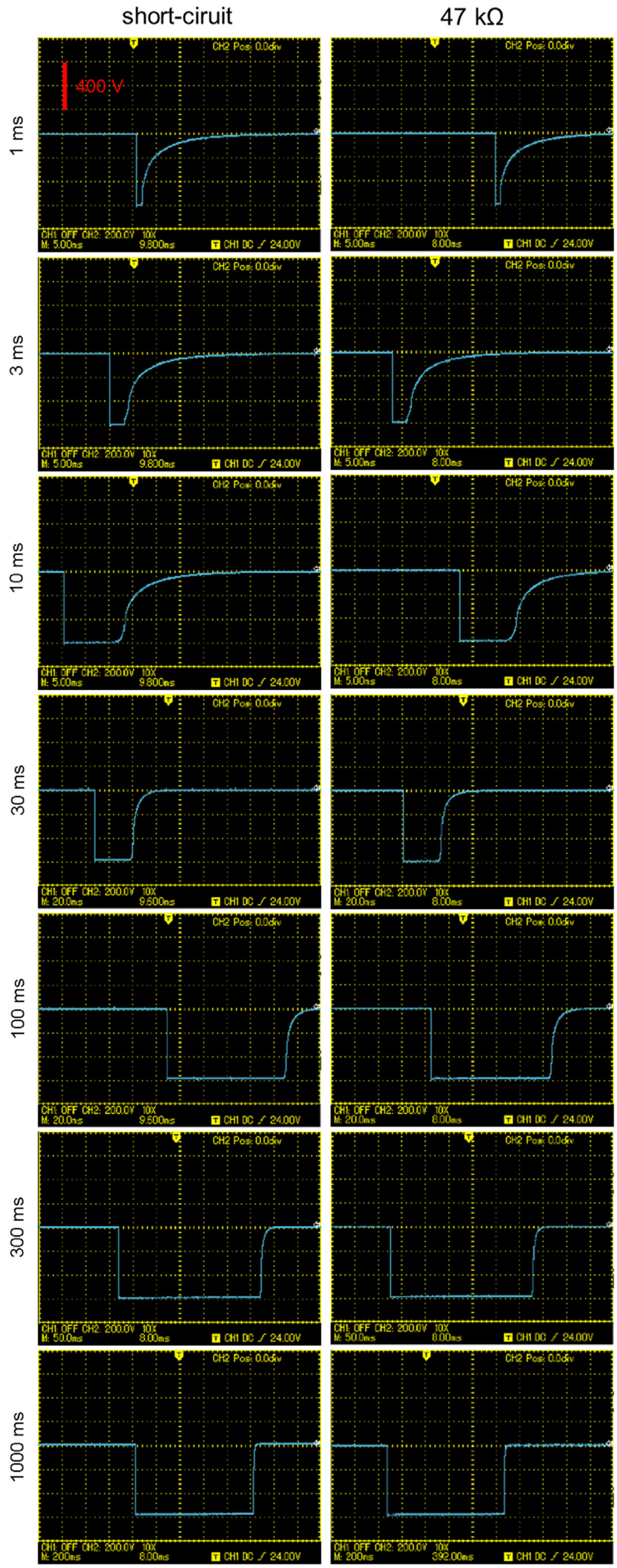

Fig. 3. Pulse characteristics of the electroporator. Time courses of generated pulses with short-circuited cuvette (left) and with simulated load (right, 47 kΩ) indicating the suitability of the controlling infrastructure and HV circuitry to generating pulses of various lengths between 1 and 1000 ms.

is approx. 75 times less expensive compared to commercially available devices (e.g. the reference system used in this work) and is readily applicable to a broad range of laboratories in various fields of research and also educational institutions. The overall costs could be further reduced by production in large numbers. Third, the device was developed based on a 'Makers' approach, i.e. by using off-the-shelf components, 3D desktop printing, and an open-source microcontroller prototyping board thus, it can easily be re-built or modified by individuals. Fourth, the described device is straightforward, simple to use and does not require highly skilled staff for application and maintenance, further highlighting the usability also in the educational field.

The portable platform in its current configuration is equipped for generation of rectangular pulses of 400–600 V amplitude and 1–1000 ms duration. The duration could easily be modified for generation of shorter or longer pulses by changing the Arduino code accordingly. A broad Arduino user community, providing various example codes and detailed support, enables even the unexperienced user to modify or extend the code for the intended purpose. Also the amplitude of the pulse may be further increased by including another or a second step-up booster. We intentionally equipped the circuit with components allowing for integration of a second step-up booster to achieve amplitudes > 1000 V.

We characterized the properties of generated pulses with short-circuited cuvette and with 47 kΩ load and observed no differences between both scenarios. The resistance of the employed load is approx. 9 times higher than what has been reported for the resistance of cell suspensions in the literature (Dower et al., 1988). We thus assume that the resistance of a real suspension does not affect the characteristics, in particular the amplitude, of the generated pulses.

We assessed whether the duration of the pulse affects the transformation efficiency and clearly demonstrated that the transformation rate increases along with increasing pulse length within a range of 1–10 ms. This observation is somewhat expected as it has been previously reported in a number of publications (Calvin and Hanawalt, 1988; Dower et al., 1988; Farinha and Kropinski, 1990) and demonstrates the suitability of the low-cost system for genetic modification of bacterial cells. Data obtained from OD measurements show strong variation at pulse lengths > 10 ms. This is probably due to inhomogeneous distribution of the cell suspension in the cuvette, contamination of the cuvette, inhomogeneous distribution of bacterial cells within the suspension or agglomeration of cells. However, this observation has not been investigated in more detail. On the other hand, agar plate-derived data show very little variation, indicating high reproducibility of our observations. Pulse durations > 10 ms did not further increase or even reproducibly decrease the transformation efficiency. Based on previous publications reporting an increase in irreversible damaging permeability along with increasing pulse length or amplitude, one would expect increasing cell death as well as a decreasing number of transformed cells (Yarmush et al., 2014). This is not the case for our data, either because irreversible damaging permeability in electroporated cells does not increase along with increasing pulse length or due to the fact that the pulse amplitude of 500 V is not high enough to irreversibly and extensively affect the membrane integrity of the cells. In fact, it has recently been reported that voltages up to 600 V did not affect cryo-preserved bovine spermatozoa, whereas higher voltages increasingly affected the cells at increasing voltages > 600 V (Blodorn et al., 2018). The observed phenomenon of saturating transformation efficiency at pulse durations longer than 10 ms may be explained by the amount of DNA used being limiting or by the fact that all cells have successfully been transformed. However, we can only speculate regarding the observed phenomenon as neither the permeability in electroporated cells nor the ratio of transformed versus non-transformed cells has been evaluated in the course of this study.

We compared transformation efficiencies obtained with cuvettes of different gap sizes and observed reproducibly higher values for cuvettes with the smaller gap of 1 mm. This result is somewhat expected as the

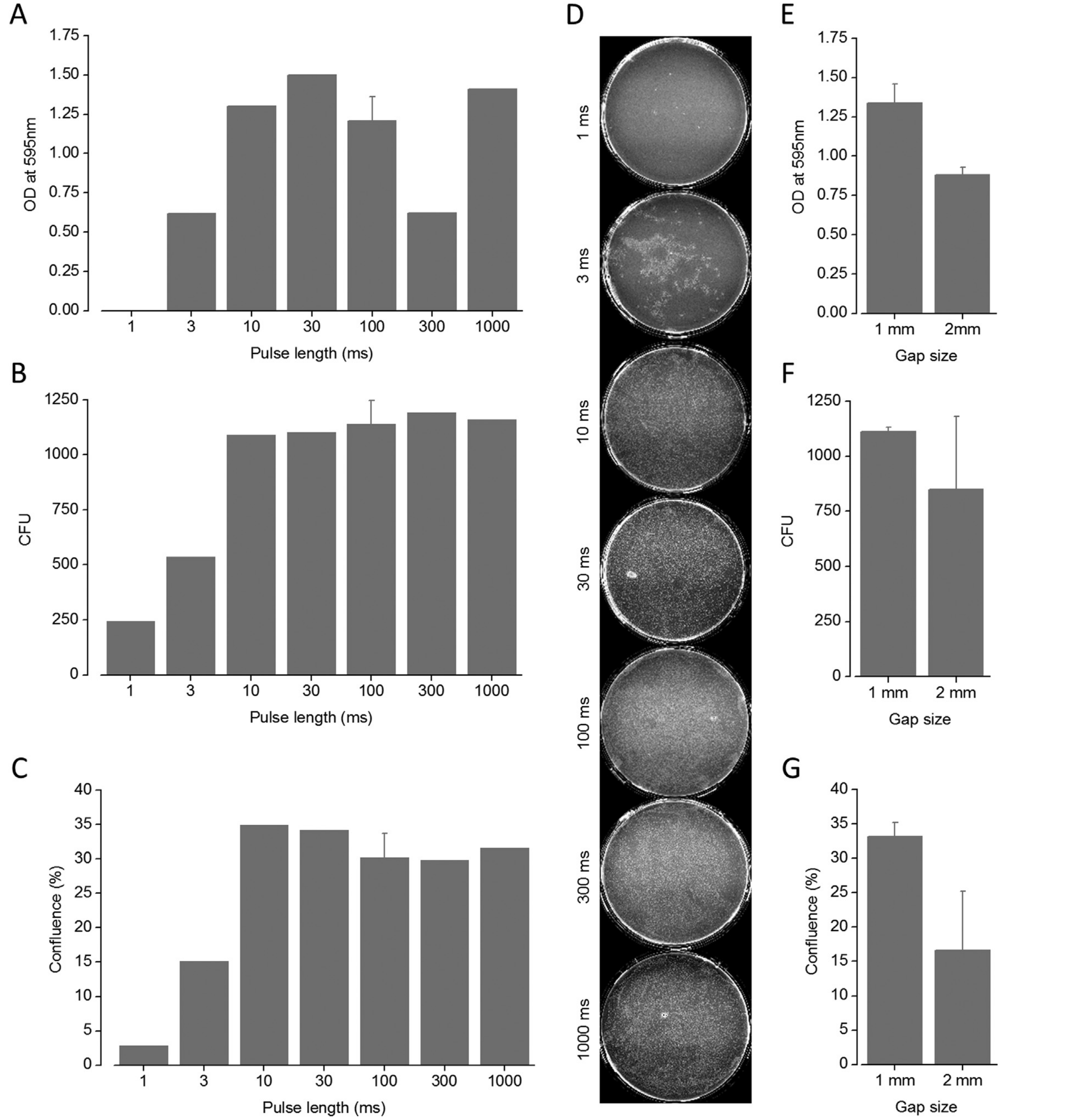

Fig. 4. Evaluation of the pulse time dependence of transformation efficiency and the transformation efficiency obtained with different cuvette types. A, B, C. Pulse time dependence of transformation efficiency. Dh5α cells were exposed to pulses (500 V) of 1, 3, 10, 30, 100, 300 and 1000 ms duration and the transformation efficiency was assessed in overnight cultures by measuring the optical density (OD) at 595 nm (A) as well as by assessing the colony forming units (B) and the plate area covered by colonies (C), respectively, in images taken from agar plates as shown in (D). These data clearly demonstrate the time dependence of the electroporation pulse duration on the transformation efficiency and indicate saturation of the efficiency at values greater than 10 ms for data obtained from agar plates. Data for pulses of 1, 3, 10, 30, 300 and 1000 ms duration were obtained from a single experiment each (N = 1), whereas data for 100 ms pulse duration were calculated from a total of three biological replicate experiments (mean ± SD). D. Representative images of 9 cm agar plates, inoculated with bacteria transformed by electroporation with pulses of varying duration and used for quantification of the colony forming units as well as the confluence in %, shown in (B), (C) and (F), (G), respectively. E, F, G. Comparison of the transformation efficiency upon electroporation using cuvettes with 1 and 2 mm gap size, respectively. Values represent mean values ± SD (N = 3). The data indicate highest transformation efficiency for cuvettes with 1 mm gap size compared to 2 mm cuvettes. These data demonstrate the applicability of the portable ultra-low-cost electroporator for genetic modification of bacterial cells.

gap size affects the strength of the electrical field, with increasing field strength along with decreasing gap size. However, as a strong electrical field may also result in a decreased transformation rate through increased cell death, e.g. triggered by an increasing temperature inside the cuvette or by flash discharge, we aimed at comparing the transformation rates using two cuvette types with gap sizes commonly used with a large variety of biological specimen. The observed differences have not been investigated in more detail.

We benchmarked the transformation efficiency obtained using our device with data generated using a commercially available high-end electroporator as well as heat shock transformation and showed that our device performs as good as the other methods, highlighting the suitability of the portable electroporator to biomedical research. It is important to mention that electroporation and heat shock experiments

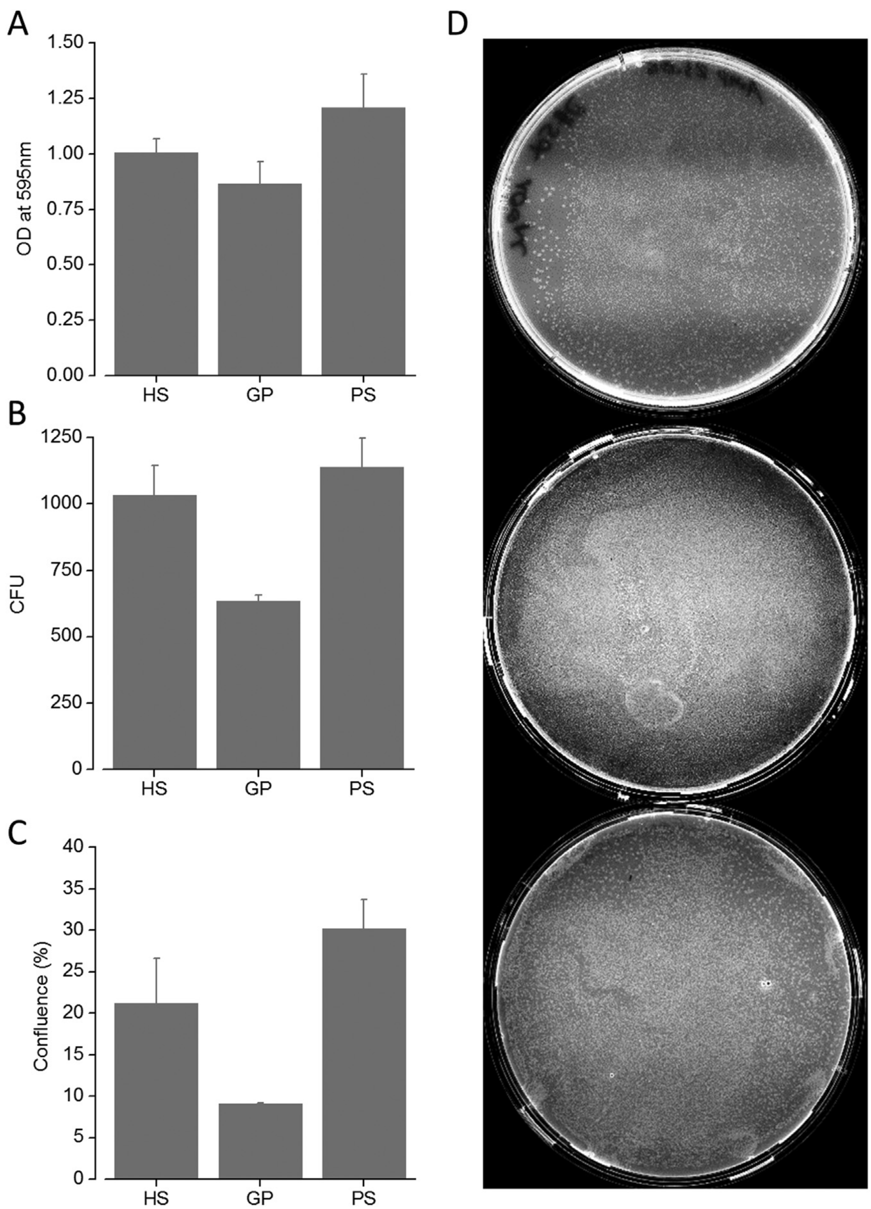

Fig. 5. Benchmarking of the portable device with conventional approaches. A, B, C. Comparison of the transformation efficiency as indicated by the optical density (OD) at 595 nm (A) the colony forming units (CFU, B) and the area (confluence in % of the overall plate area) covered by colonies (C), measured in suspensions of Dh5α cells cultured overnight and on agar plates, respectively, upon transformation using a standard heat shock protocol (HS), a commercially available electroporator (GenePulser, GP, 100 ms, 500 V), and the portable system (PS, 100 ms, 500 V). All values indicate mean values ± SD (N = 3). Data shown in (B) and (C) were obtained by quantitative analysis of images taken from 9 cm agar plates as shown in (D). **D**. Images used for quantification of CFU and confluence associated with the different transformation approaches heat shock (top), electroporation using a commercial device (middle) and the in house-built system (bottom). These data clearly indicate comparable transformation efficiency for the portable system as compared to conventional approaches.

have been conducted with different DNA amounts (electroporation: 10 ng/40 µl cell suspension; heat shock: 10 ng/100 µl cell suspension) and may have resulted in a higher transformation efficiency in the heat shock experiment when the same DNA amount would have been used.

Although the system is currently set up for use with commercially available electroporation cuvettes, the cuvette carrier could easily be modified for application with custom or purpose designed cuvettes or containers within a short time and with little effort. 3D CAD files in STL or STEP format of the cuvette carrier as well as Arduino code and PCB layouts for operation and construction of the system, respectively, will be provided upon request. General protective and safety precautions should be taken when handling high voltage electrical components. Due to the fact that the device requires 5 V and 9 V power supply only, the device could also be operated using a battery pack, further supporting portability of the platform. While the presented platform is based on an Arduino Nano microcontroller, the system could also be realized with any other rapid prototyping board, including raspberry Pi or ESP32, allowing for integration with other technology, e.g. for automated electroporation and high-throughput screening applications. Although our system is low-cost compared to commercially available technology, there is still potential for further decreasing the overall costs, e.g. by replacing the used through-hole electronic parts by surface mounted device (SMD) technology that is much cheaper but that also requires specific soldering infrastructure for production of circuit boards. Also, some of the pre-assembled components such as the step-up-booster may be replaced by open source high-power supply technology as recently reported by Schlatter et al. (2018), further reducing the overall costs. Although this work focuses on genetic modification of bacterial cells, the device may also be applicable for delivery of genetic material, small molecule or nanomaterials into other cell types, including mammalian cells.

5. Conclusions

To our knowledge, we present first anywhere in the world a portable and low-cost electroporator that can be built by anyone from scratch within very short time and with less than 130 €. In contrast to any other commercially available system, our device can be modified and custom modified to fit any other infrastructure. The electroporation technology presented in this article improves conventional technologies in terms of portability, user-friendliness and cost and provides an example of technology that is compatible with fast and resource-efficient system development in the era of rapid prototyping. Altogether, this work contributes to expanding the applicability and availability of commercially viable electroporation devices for use in biotechnology, biomedical research and education.

Acknowledgements

We thank C. Detmers for helpful discussions.

Conflict of interest statement

The authors declare no conflict of interest.

Author contributions

D.F.G. acquired funding and provided resources for the project. D.F.G. conceived, administered and supervised the project. M.A.S. created 3D CAD models, electric circuits and software, conducted experiments and curated the data. M.A.S. and D.F.G. analyzed and visualized the data and wrote the paper. O.F. provided resources and discussions and contributed to editing of the final manuscript. All authors agreed on the final version of the manuscript.

Supplementary information

The document *Supplements.pdf* includes step-by-step protocols or workflows employed in the course of this study for electroporation of electro-competent Dh5α bacterial cells (Fig. S1) and heat shock-based genetic modification of chemically competent Dh5α cells (Fig. S2). The Supplementary information also includes a representative image depicting a segmentation result for quantification of colony forming units and the confluence (Fig. S3) as well as a detailed parts list (Table S1.)

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.bios.2019.02.024.

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