Microfluidic Transfection of Parabodo caudatus with plasmids Version 3

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Abstract

We recently developed a continuous flow system to transform microorganisms in high throughput in a microfluidic device (Garcia et al., 2017). This system employs microfluidic channels that contain a bilateral constriction between the inlet and outlet electrode connections (*length* = 3.0 mm, $width_{min}$ = 50 µm, $width_{max}$ = 2.0 mm, and *height* = 100 µm). The constriction amplifies the electric field under an applied voltage between the inlet and outlet electrodes to levels sufficiently high to induce electroporation. During *P. caudutus* transformations, the cells were driven through the microfluidic device at flow rates of 50 µL/min and 500 µL/min, which correspond to residence times (i.e. pulse durations) of 20 ms and 2 ms, respectively. Square wave pulses with, for example, 5 ms ON and 5 ms OFF cycles (50 % duty cycle) are applied to the microchannel through the dispensing needle. Therefore the cell viability cannot be accurately evaluated since only 50 % of the cells experience the electric field. The pulses are delivered from electrodes with alternating polarity between the pulses to reduce electrolytic effects at the electrode-buffer interface (Fig. 1). After flowing through the microchannel, each 200 µL cell sample is added to a 1.5 ml Eppendorf tube containing 1 ml of fresh growth media for cell recovery. The applied voltages we evaluated had amplitudes of 250 V (E_{max} = 1,500 V/cm), 375 V (E_{max} = 2,250 V/cm), and 500 V (E_{max} = 3,000 V/cm) for each polarity. The non-uniform constriction in the microfluidic devices generates a variable electric field that is capable of transfecting cells while minimizing exposure to the highest electric field.

Figure 1: Electric field waveforms employed for transient and stable transfection of *Bodo caudatus*. Three independent electroporation systems were used for reproducible transfection including a) our microfluidic electroporation platform (Garcia et al., 2017), the NEPA21 square-wave transfection system (BulldogBio), and the MicroPulser™ exponential decay electroporator (Bio-Rad). Additionally, the b) signature waveforms for the NEPA21 square wave transfection system of ‘poring’ and ‘transfer’ pulses for electroporation are shown. Note: The time scale in Fig. 1a is a zoomed-in version of the red-dashed box from Fig. 1b.

Garcia, P.A., Ge, Z., Kelley, L.E., Holcomb, S.J., and Buie, C.R. (2017) High efficiency hydrodynamic bacterial electrotransformation. *Lab Chip* (**17**): 490-500.

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Protocol

Soft Lithography Protocol for Microfluidic Device Fabrication

Step 1.

Soft lithography is employed in order to fabricate devices with microscale features. This process creates a master stamp from photomasks that can be used to create devices repeatedly. The photomasks are designed in AutoCAD 2014 (Autodesk, San Rafael, CA) with bilaterally converging geometries, and are printed by Fine-Line Imaging, Inc. (Colorado Springs, CO). The microchannels are microfabricated using soft lithography techniques described by Garcia *et. al* (Whitesides et al., 2001; Garcia et al., 2016). Briefly, SU-8 (SU-8 2050, Micro-Chem, Westborough, MA) molds are patterned on silicon wafers with standard photolithography. Afterwards, the surfaces of the SU-8 master mold are treated for 2 hours with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (Sigma Aldrich, St. Louis, MO) under vacuum before being used for molding. Next, the SU-8 master mold polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI) was used at a 10:1 ratio after 2-hour vacuum for removal of air bubbles in the polymer. The PDMS devices are bonded to a glass substrate after a 45 second plasma treatment and placed overnight in an oven at 75 °C prior to subsequent experiments.

Garcia, P.A., Ge, Z., Moran, J.L., and Buie, C.R. (2016) Microfluidic Screening of Electric Fields for Electroporation. Sci Rep (6):21238.

Whitesides, G.M., Ostuni, E., Takayama, S., Jiang, X.Y., and Ingber, D.E. (2001) Soft lithography in biology and biochemistry. Annual Review of Biomedical Engineering (3):335-373.

Cell Growth

Step 2.

Parabodo caudatus (ATCC 50361) was used in this study. Initially, P. *caudatus* was grown in 50 % ATCC seawater 802 media. Subsequently, seawater was replaced with distilled water in order to reduce the high electrical conductivity during the electroporation. Briefly, this is a cerophyl-based media enriched with 3.5 mM sodium phosphate dibasic (Na_2HPO_4) and with *Klebsiella pneumoniae* added as a food source. Cultures were incubated at 22°C and sub-cultured weekly in fresh T-25 vented tissue culture flasks (Falcon brand, Fisher Scientific) containing 30 ml of fresh media.

Plasmid selection and preparation

Step 3.

Three plasmids were obtained from Addgene. pEYFP-Mitotrap was a gift from Margaret Robinson (Addgene plasmid # 46942; (Robinson et al., 2010)); pEF-GFP and pUB-GFP were gifts from Connie Cepko (Addgene plasmid # 11154 and # 11155, respectively; (Matsuda and Cepko, 2004)). Plasmids were purified from 100 mL cultures grown overnight in standard Luria Bertani liquid medium (2006) with the appropriate selection marker. Purification was done according to the manufacturer's protocol for the Plasmid Midi Kit (Qiagen, Germantown, MD), with the following modifications: 1) Each 100 mL

culture was split into two 50 mL volumes and centrifuged at 4,500 rpm for 20 min at 4°C to pellet bacterial cells; 2) Each half went through the lysis steps separately, and the lysate was pooled after neutralization; 3) Pelleting of precipitated DNA was done by centrifugation at 4,600 rpm for 60 min at 4°C; 4) Each 2 mL volume of pellet (in 70 % ethanol wash) was split into two 1 mL volumes, centrifuged at 15,000 X g for 10 min at 4°C, and the supernatant decanted; and 5) Dried DNA pellets were re-suspended in 50 µL of nuclease-free water, and the two 50 µL volumes were combined for each sample. Purified plasmid DNA was quantified using the Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA) and stored at -20°C until use.

Matsuda, T., and Cepko, C.L. (2004) Electroporation and RNA interference in the rodent retina in vivo and in vitro. *Proc Natl Acad Sci U S A* (**101)**:16-22.

Robinson, M.S., Sahlender, D.A., and Foster, S.D. (2010) Rapid inactivation of proteins by rapamycin-induced rerouting to mitochondria. Dev Cell (18): 324-331.

Cell preparation prior to electroporation

Step 4.

P. caudatus cells at logarithmic growth phase (approximately $1x10^7$ cells/ml) were harvested by centrifugation at 5000 X g for 30 seconds, re-suspended in 200 µl cytomix (50 % in distilled water) and mixed with 20 to 40 µg of plasmid and then transferred into an electroporation cuvette, 0.2 mm gap, for electroporation with the exponential decay system. For the microfluidic system, cells in cytomix buffer were aspirated into 1/16 inch tygon tubing (McMasterr-Carr) prior to being delivered into the microchannel. We carried out hundreds of electroporation trials using the three platforms; however, only the successful transformation parameters are summarized in Tables 1. Electroporation parameters that were not successful are included in Table S1 for the exponential decay system and Table S2 for the microfluidic system.

Parabodo caudatus transfection efficiency post microfluidic electroporation

Step 5.

The microfluidic electroporation system resulted in the highest transfection efficiencies ranging from 20 % to 50 % (Figure 3). We demonstrated successful *P. caudatus* transfection employing electric fields of 1,500 V/cm, resulting in transfection efficiencies of 30-40 %, and 2,250 V/cm (40-50 %) using 5 ms pulse durations in MilliQ water using the bilaterally constricting geometry. Additionally, by decreasing the electric field to 1,000 V/cm and employing longer 20 ms pulses we achieved 20-30 % transfection efficiencies in 50 % cytomix buffer using the straight channel constriction. These results demonstrate that different geometric constrictions can be used successfully to modulate the electric field that the cell is exposed to for successful transfection.

Figure 3: Microfluidic electroporation of *Parabodo caudatus*. Panel a) *P. caudatus* (brightfield) b) stable pEYFP-Mitotrap transfection at 250 V (E_{max} = 1,500 V/cm), c) transient pUB-GFP transfection using 375 V (E_{max} = 2,250 V/cm), d) autofluorescence control for *P. caudatus*, e) transient transfection using pEF-GFP and 313 V (E_{max} = 1,000 V/cm) in the straight channel, and f) merged image of

brightfield and fluorescence image from e) for visualizing cell morphology.