Supplementary information

DNA preparation process

The plasmid pHyg3, carrying the hpt gene (*aph 7''*), were amplified in E. coli strain JM109 and purified by a mini-prep plasmid kit. Then the DNA was cutted by HindIII, purified and quantified by nanodrop (Thermo Scientific). Then the number of DNA fragment including hyg gene was calculated by the equation 1 and was used for transformation.

Equation 1:

DNA number (copies/ml) = [6.02×10^{23}(copies/mol)×DNA conc. (g/ml)] / [DNA molecular weight (g/mol)].

PCR detection

The colony grew in the selective TAP solid medium was picked up and cultured in the selective TAP liquid medium including 15 mg/ml hygromycin B. Then the cells were collected by centrifugation and DNA was extracted by SDS-EB/ phenol method. DNA concentration was checked by nanodrop and the presence of transgene was detected by nested PCR. The PCR condition was shown in Table S1. The sequences of PCR primers was shown in Table S2.

Table S1. The nest-PCR condition for algal cell transgenic detection

10 µl volume	External round	Internal round	The same program for the external & internal round: 94°/20s **25 cycles (**94°/10s, **60**°/30s, 72°/30s**)** 72°/5 min
PCR mixer 2×	5 µl	5µl	
Primer 1	P1 3 pmol	P1 3 pmole	
Primer 2	1197R 4 pmol	1023R 4 pmole	
DMSO	0.5 µl	0	
DNA	Sample: 1 µl (Positive control: hpt DNA of 3.68×10^{5} copies/µl)	1µl (external PCR product)	

Table S2. The PCR primers for nest-PCR detection

External round	Internal round
P1: ATG ACA CAA GAA TCC CTG TTA C	
1197R: CAC GAA GAT GTT GGT CCC G	1023R: CGG GAA GAC CTC GGA ATC

(a) (b)

Figure S1. (a) the image of colonies on the solid selective media, (b) PCR detection of transgene from transformed algal cells by electroporation on mirofluidic chip. −, PCR negative control without DNA template; U, genomic DNA template from untransformed cc-400; +, positive control; M, DNA marker; 1~4, genomic DNA templates from transformed colonies.

Cell viability test

SYTOX® green nucleic acid stain (Invitrogen) was used to test the cell viability. Cells in droplets were electroporated and collected. And some cells were taken out and SYTOX® green was added to the cell sample to generate a final concentration of 1 µM. After incubation for 10 min, the cells were checked under the fluorescent

microscope to obtain the percentages of red fluorescent cells (live cells) among the total cells (dead cells (green fluorescent cells) and live cells) which was defined as the cell viability.

The maximum transmembrane potential

= +/- 1.5 r E = +/- 1.5×5 um×393 V/cm = +/- 295 mV

The diameter of the spherical *C. reinhartdii* cell : 10 μm

The applied electric field for on-chip Electroporation : 393 V/cm.

Table S3. Effect of channel shape on transformation efficiency, performed with 1.58×10^{7} cells/mL of CC-400 cell-loading concentration with 1000 DNA/cell ratio, and five pairs of paralleled electrodes.

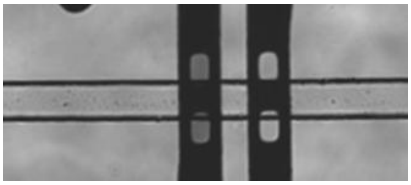
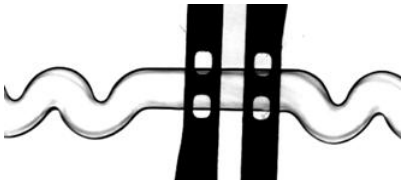
	Channel type	Transformation efficiency
Straight		$2.45\pm0.18\times10^{-4}$
Serpentine		$8.14\pm0.20\times10^{-4}$

Table S4. Effects of repeated electric shocks on the transformation efficiency by on-chip electroporoation, performed with 1.58×10^{7} cells/mL of CC-400 cell-loading concentration, 1000 DNA/cell ratio, and serpentine channel with different pairs of parallel electrodes.

Electrode pairs	Transformation efficiency	Cell viability (%)
1	$8.37\pm0.33\times10^{-7}$	-
2	$1.12\pm0.31\times 10^{-6}$	-
3	$5.19\pm0.27\times10^{-5}$	95
4	$8.88\pm0.21\times10^{-5}$	94
5	$8.14\pm0.20\times10^{-4}$	81

Table S5. Comparative transformation efficiency of cell walled strain, CC-124, by droplet microfluidic and bulk phase electroporation, performed with 1.58×10^{7} cells/mL of CC-124 cell-loading concentration, and serpentine channel with five pairs of parallel electrodes.

Electroporation mehtod	Ratio of DNA to cell	Transformation efficiency
On chip	1000	$1.51\pm0.32\times10^{-5}$
Bulk phase	1000	$6.73\pm0.35\times10^{-8}$