Supporting Information

Exogenous Gene Integration for Microalgal Cell Transformation Using a Nanowire-Incorporated Microdevice

Sunwoong Bae, [†] *Seunghye Park,* [‡] *Jung Kim,* [§] *Jong Seob Choi,* [†] *Kyung Hoon Kim,* [†] *Donguk Kwon,* [§] *EonSeon Jin,* [‡] *Inkyu Park,* [§] *Do Hyun Kim,* [†] *Tae Seok Seo* [*,†]

[†]Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

[‡]Department of Life Science, Research Institute for Natural Sciences, Hanyang University, Seoul 133-791, Republic of Korea

[§]School of Mechanical, Aerospace and Systems Engineering, Division of Mechanical Engineering, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

Corresponding author

*Tel.: +82-42-350-3973; Fax: +82-42-350-3910; E-mail: seots@kaist.ac.kr

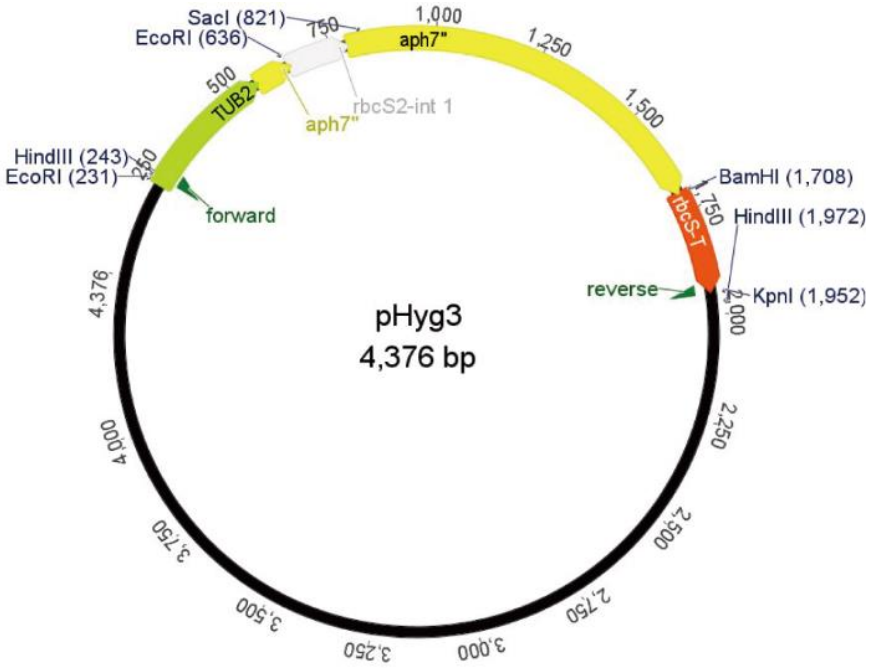

Figure S1. Vector map of pHyg3.[1] The intron 1 of rbcS2 gene were fused with aph7" (amyniglycoside phosphorylase). The forward and reverse refer to the primer binding sequence for amplifying Hyg3 gene.

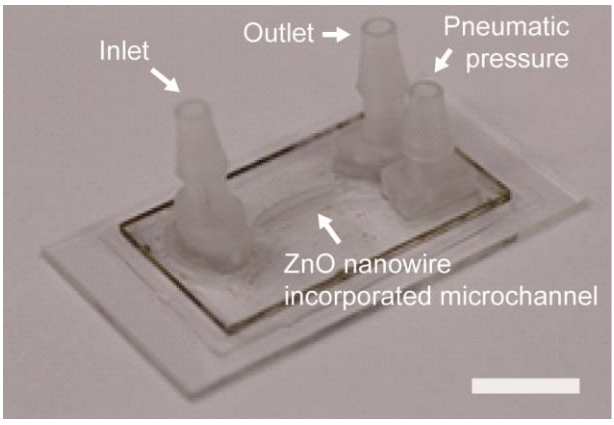

Figure S2. Digital image of the integrated microdevice. Scale bar: 1 cm.

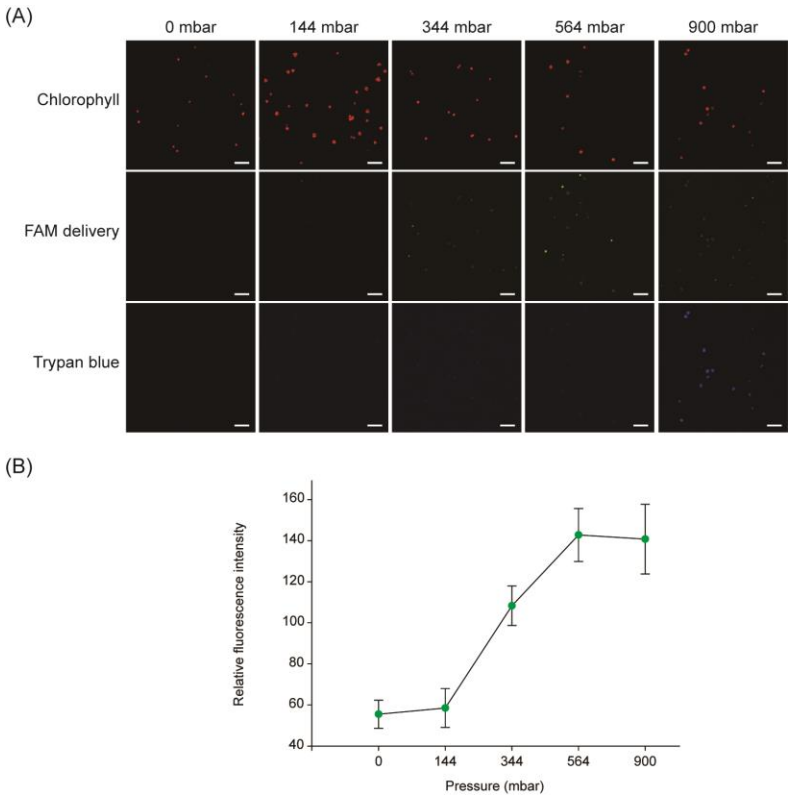

Figure S3. Verification of the fluorescence DNA delivery performance of microdevice and algal cell viability assay under five different types of PDMS compression condition. (A) Fluorescence images of *in vivo* chlorophyll of *C. reinhardtii*, uptaken green fluorescence DNA, and trypan blue staining for live and dead cell assay (Scale bar: 50 μm). (B) Relative fluorescence intensities of FAM labeled ssDNA in *C. reinhardtii* under 0, 144, 344, 564, and 900 mbar of pressurization condition.

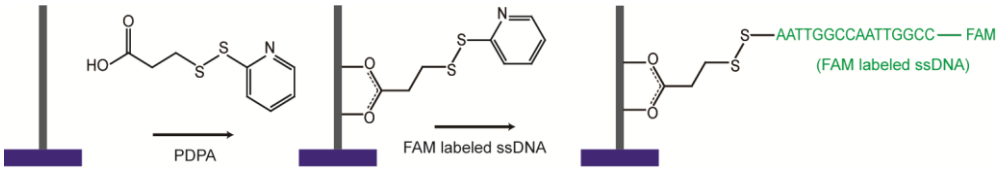

Figure S4. Schematics for covalent chemical bonding of the fluorescence DNA onto ZnO nanowires.

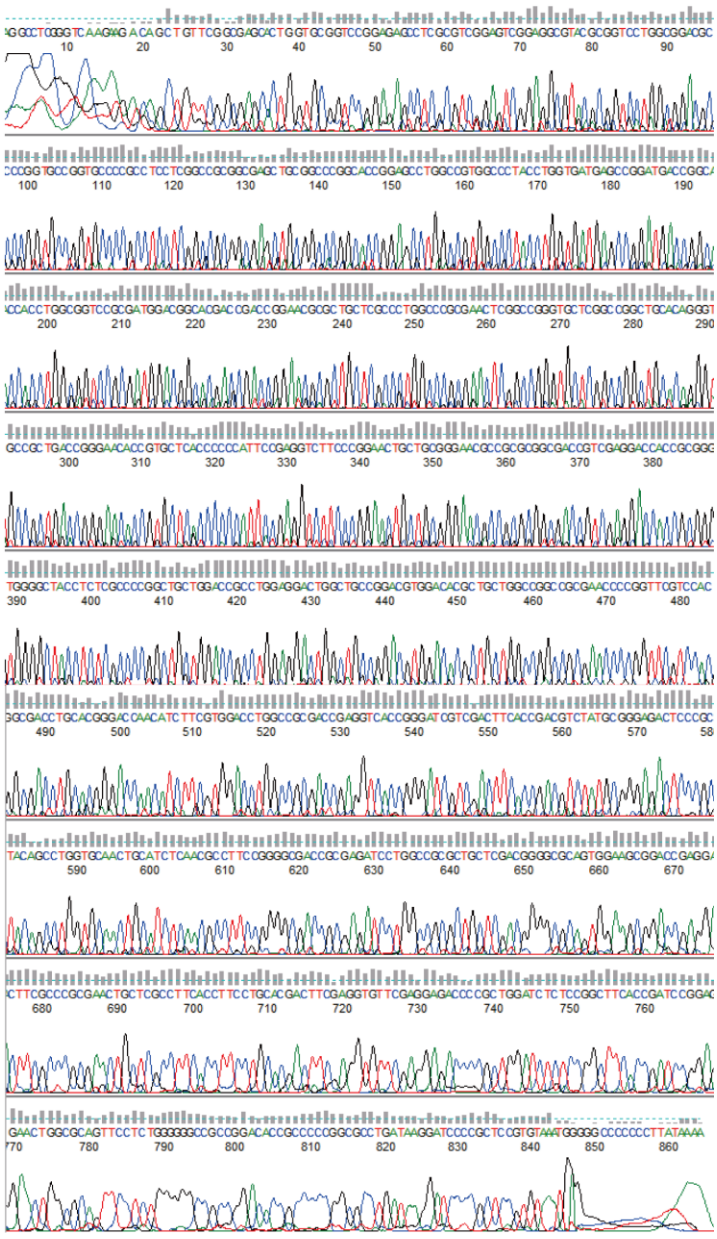

Figure S5. Sequencing data for the identification of the inserted Hyg3 gene amplicon (844 bp).

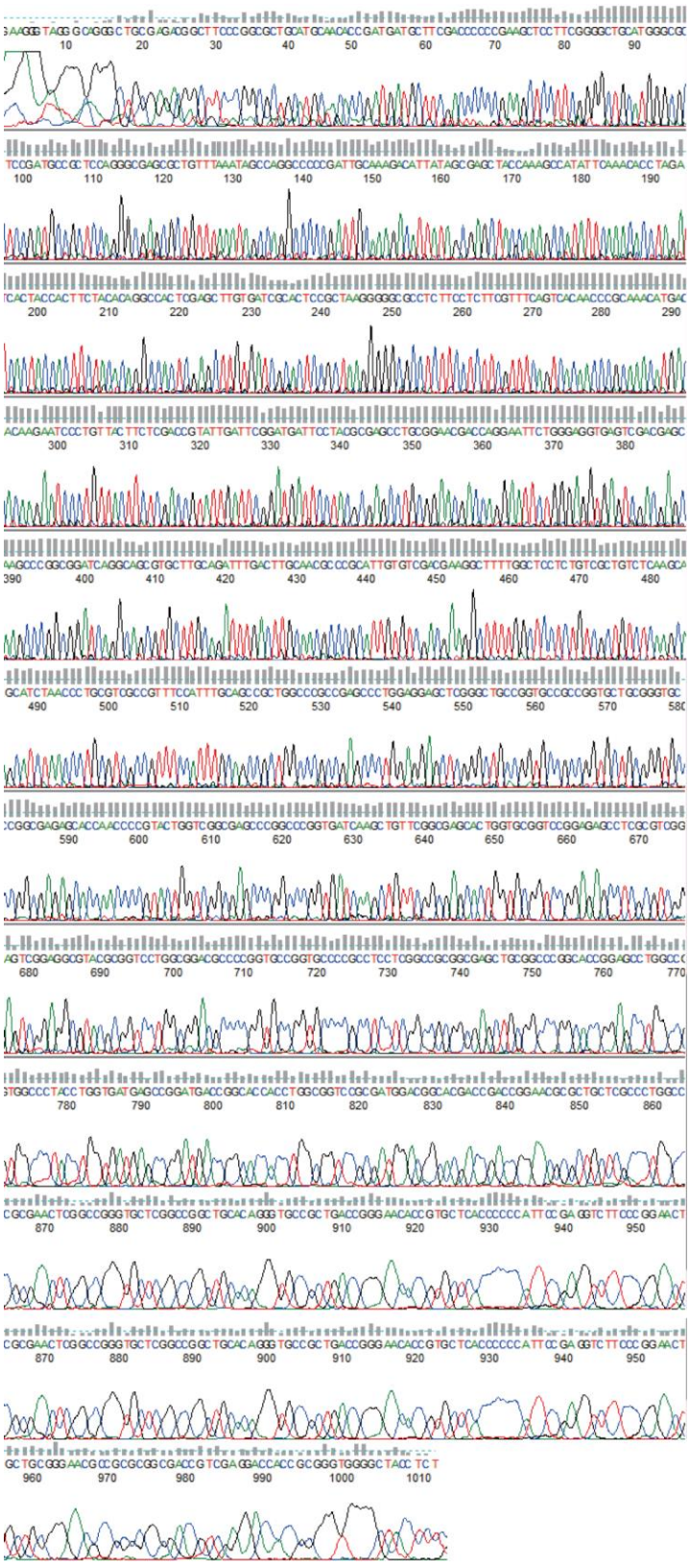

Figure S6. Sequencing data for the identification of the inserted Hyg3 gene amplicon (1,620 bp).

Table S1. Sequence of Hyg3 gene cassette (amplicon). The whole sequence is available at http://www.biologie.uni-regensburg.de/Genetik/Mages/. Primer binding sequences for amplification were underlined.

β2-tubulin promoter (TUB2) (312 bp)	CTTTCTTGCGCTATGACACTTCCAGCAAAAGGTAGGGCGGGCTGCGAGACGGCTTCCCGGCGCTG CATGCAACACCGATGATGCTTCGACCCCCCGAAGCTCCTTCGGGGCTGCATGGGCGCTCCGATGC CGCTCCAGGGCGAGCGCTGTTTAAATAGCCAGGCCCCCGATTGCAAAGACATTATAGCGAGCTA CCAAAGCCATATTCAAACACCTAGATCACTACCACTTCTACACAGGCCACTCGAGCTTGTGATCG CACTCCGCTAAGGGGGCGCCTCTTCCTCTTCGTTTCAGTCACAACCCGCAAAC
aph7" (87 bp)	ATGACACAAGAATCCCTGTTACTTCTCGACCGTATTGATTCGGATGATTCCTACGCGAGCCTGCG GAACGACCAGGAATTCTGGGAG
Intron 1 of rbcS2 gene (145 bp)	GTGAGTCGACGAGCAAGCCCGGCGGATCAGGCAGCGTGCTTGCAGATTTGACTTGCAACGCCCG CATTGTGTCGACGAAGGCTTTTGGCTCCTCTGTCGCTGTCTCAAGCAGCATCTAACCCTGCGTCG CCGTTTCCATTTGCAG
aph7" (912 bp)	CCGCTGGCCCGCCGAGCCCTGGAGGAGCTCGGGCTGCCGGTGCCGCCGGTGCTGCGGGTGCCCG GCGAGAGCACCAACCCCGTACTGGTCGGCGAGCCCGGCCCGGTGATCAAGCTGTTCGGCGAGCA CTGGTGCGGTCCGGAGAGCCTCGCGTCGGAGTCGGAGGCGTACGCGGTCCTGGCGGACGCCCCG GTGCCGGTGCCCCGCCTCCTCGGCCGCGGCGAGCTGCGGCCCGGCACCGGAGCCTGGCCGTGGC CCTACCTGGTGATGAGCCGGATGACCGGCACCACCTGGCGGTCCGCGATGGACGGCACGACCGA CCGGAACGCGCTGCTCGCCCTGGCCCGCGAACTCGGCCGGGTGCTCGGCCGGCTGCACAGGGTG CCGCTGACCGGGAACACCGTGCTCACCCCCCATTCCGAGGTCTTCCCGGAACTGCTGCGGGAAC GCCGCGCGGCGACCGTCGAGGACCACCGCGGGTGGGGCTACCTCTCGCCCCGGCTGCTGGACCG CCTGGAGGACTGGCTGCCGGACGTGGACACGCTGCTGGCCGGCCGCGAACCCCGGTTCGTCCAC GGCGACCTGCACGGGACCAACATCTTCGTGGACCTGGCCGCGACCGAGGTCACCGGGATCGTCG ACTTCACCGACGTCTATGCGGGAGACTCCCGCTACAGCCTGGTGCAACTGCATCTCAACGCCTTC CGGGGCGACCGCGAGATCCTGGCCGCGCTGCTCGACGGGGCGCAGTGGAAGCGGACCGAGGAC TTCGCCCGCGAACTGCTCGCCTTCACCTTCCTGCACGACTTCGAGGTGTTCGAGGAGACCCCGCT GGATCTCTCCGGCTTCACCGATCCGGAGGAACTGGCGCAGTTCCTCTGGGGGCCGCCGGACACC GCCCCCGGCGCCTGA
3' UTR of rbcS2 (rbcS-T) (243 bp)	TAAGGATCCCCGCTCCGTGTAAATGGAGGCGCTCGTTGATCTGAGCCTTGCCCCCTGACGAACGG CGGTGGATGGAAGATACTGCTCTCAAGTGCTGAAGCGGTAGCTTAGCTCCCCGTTTCGTGCTGAT CAGTCTTTTTCAACACGTAAAAAGCGGAGGAGTTTTGCAATTTTGTTGGTTGTAACGATCCTCCG TTGATTTTGGCCTCTTTCTCCATGGGCGGGCTGGGCGTATTTGAAGCG

Table S2. Comparison of the fluorescent intensity of the ZnO nanowire array before and after delivery of the FAM labeled ssDNA into the microalgae.

Initial concentration (μM)	Relative fluorescence intensity		
	Before gene transfer	After gene transfer	Transfer efficiency
10-3	22.1 ± 1.8	4.3 ± 0.1	0.75
10-2	57.7 ± 14.5	23.7 ± 0.9	0.61
10-1	222.1 ± 21.8	105.4 ± 13.4	0.53
1	337.9 ± 23.6	163.8 ± 35.1	0.52
2	395.2 ± 17.1	190.2 ± 31.6	0.52
5	422.7 ± 11.1	197.6 ± 12.6	0.53
10	453.2 ± 21.1	232.6 ± 9.4	0.49

REFERENCES

1. Berthold, P.; Schmitt, R.; Mages, W., An Engineered Streptomyces hygroscopicus aph 7″ Gene Mediates Dominant Resistance against Hygromycin B in Chlamydomonas reinhardtii. *Protist* **2002,** *153* (4), 401-412.