[Frontiers in Bioscience 17, 1931-1939, January 1, 2012]

# Application of cell-free expression of GFP for evaluation of microsystems

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## 1. ABSTRACT

Coupled cell-free transcription-translation (CFTT) of green fluorescent protein (GFP) has been applied as a reporter system to microfluidic chip-related technologies. In polymerase chain reaction (PCR)-based biomolecular logic gate system, in which addition of primer set and amplification of PCR product represent input and output signal respectively, GFP gene was inserted in the template DNA, which was then amplified, transcribed and translated to GFP. The green fluorescence reported as if the amplification has occurred or not, that is, the fluorescence reports positive output signal. CFTT of GFP was also adopted to evaluate on-chip capillary electrophoresis (CE)-based DNA fractionation, which was developed to isolate single DNA species from reaction mixture of DNA ligase-catalyzed DNA-assembly. As a model system, GFP gene was inserted in the target DNA fragment. The collected fraction was amplified with PCR and subjected to a CFTT system, and green fluorescence was observed showing that the fractionation was successful. These results showed that CFTT of GFP is a useful tool to verify, estimate, and monitor microfluidic chip-related technologies in which cell-free protein synthesis is involved.

## 2. INTRODUCTION

Production of proteins by biological synthesis based on recombinant DNA technology has been a basic technique in modern biosciences and biotechnologies (1). A variety of proteins are synthesized based on this technology today. Host cell incorporates recombinant DNA carrying genetic information and transcribes it to messenger RNA (mRNA), which is then served as a template for the protein biosynthesis. Because recombinant DNA is introduced into living host cells, the protein synthesis should be carried out in a biohazard facility. This fact has prevented us from applying the recombinant DNA technology into other technologies including electronics, informatics, or nanotechnology. Cell-free translation (CFT), otherwise known as *in vitro* translation (IVT), is therefore attractive approach because it requires no living cells for protein production and thereby no usage of biohazard facilities. There are two options in the way to input the genetic information into CFT reaction according to the substances: DNA or mRNA. DNA-dependent mRNA synthesis can be carried out together with CFT by adding RNA polymerase and ribonucleoside triphosphates. It is coupled cell-free transcription-translation (CFTT). Thanks to the improvement of CFT systems (2-5), various kinds of recombinant proteins are prepared in CFT today. Most of them are CFTT system.

Recently, much attention has been paid for CFT from the viewpoint of not only protein production but also protein screening (6-8). Especially, after the completion of human genome sequencing project (9,10), demand has been raised for conversion system that converts genetic information to protein one by one. One solution for this demand is miniaturized reactor for CFT. We therefore developed a microstructure, having 0.2 µL volume, fabricated in a silicon wafer, and installed cell-free translation system prepared from *E. coli* on it (11,12). We demonstrated two kinds of mRNA-dependent polypeptide synthesis: polyphenylalanine [poly(Phe)] synthesis depending on polyuridylic acid [poly(U)], where $^{14}$C-labeled phenylalanine was incorporated into acid-insoluble fraction, and polymerization of $^{14}$C-labeled amino acids depending on bacteriophage MS2 mRNA It was the world's first achievement of CFT in a microfabricated structure. To confirm as if the protein synthesized in a microfabricated structure has enzymatic activity, the synthesized material should be analyzed with biochemical assay. Baba and his group compared the biochemical specificity of the proteins synthesized in a microstructure, a plastic tubes, and living cells, and showed no differences among them (13).

We introduced GFP (14,15) as a model protein synthesized in a microfabricated structure because of its convenience for detection that it shows green emission without any substances or chemical energy sources. For the observation of cell-free GFP expression, we constructed microreactor array on a transparent plastic plate, embedded on a temperature control unit (16). The reaction volume was 125 nL. We adopted two kinds of commercially available GFP variant as the first step toward multi-parallel protein expression. The variants were an ultraviolet-optimized GFPuv (F99S, M153T, V163A) (17) and a blue-shifted BFP (F64L, S65T, T145F) (18). They have same excitation wavelength, around 380 nm, so that the setting of optical equipments was compatible with standard system optimized for fluorescein detection (19). The emission wavelengths are 440 nm and 509 nm for BFP and GFPuv, respectively. The GFP synthesis by CFTT was monitored with charge coupled device (CCD) camera. We demonstrated that GFP variants were successfully synthesized in the microreactor. Parameter-tuning and system-optimization for protein synthesis in this microreactor system were carried out based on GFP-expression, and our system could translate not only GFP variants but also other proteins (20).

After that, CFTT of GFP has become a standard reaction as a reporter in the field of micro- and nano-scale biotechnology involving CFT. The reaction has been adopted in picoliter chamber array (21), nanowell chip (22), and picoliter droplets (23).

In this review, we introduce our approach to apply CFT of GFP as (i) a report gate in PCR-based biochemical logic system, and (ii) verification tool for DNA collection with on-chip capillary electrophoresis system.

The first topic in this review is PCR-based biochemical logic gate where CFT of GFP is utilized as a report gate to monitor the amplification (24). Much attention has been paid to designing a biomolecular system that responds to chemical inputs and remarkable progress has been achieved in the development of logic gates based on both proteins and nucleic acids. For example, Katz and his co-worker have reported enzyme-based logic systems for information processing, regarding enzyme's redox activity as signal processing (25). Willner and his group have developed DNA-based logic gate systems, focusing on DNA's abilities as signal processing, such as structure formation, molecular recognition, and amplification, (26). Our approach has started from the viewpoint that standard PCR (27) is 2-input AND gate. The reaction responds to the input of two kinds of primers, and DNA amplification responds to output signal. Usually, the product is analyzed with TaqMan chemistry (28), Molecular Beacon (29), or electrophoresis, however, we adopted CFTT of GFP instead of them. The positive signal is reported as green emission.

The second topic is DNA fractionation with on-chip CE, where CFTT of GFP is utilized to verify as if the target DNA fragment is collected properly (30). Although various kinds of recombinant proteins are synthesized with CFTT system, the template DNA subjected to the CFT reaction is still prepared with living cell-based method because the template is constructed with standard recombinant DNA technology, in which DNA ligase-catalyzed DNA assembly and living cell-dependent screening of the construction are utilized. Because DNA ligase assembles DNA fragments randomly to generate complex mixture (31), it is required to separate the target DNA species from the mixture, where living cell-based selection has been widely utilized, which should be carried out in a biohazard facility. In order to construct the template DNA without using living cells, we performed direct PCR from the ligation mixture, followed by collection with on-chip fractionation with CE. We adopted GFP-coding gene as a model DNA and after the fractionation, the DNA was subjected to CFTT system. Green fluorescence showed the total process was carried out properly.

## 3. APPLICATION OF CELL-FREE GFP-EXPRESSION AS A REPORT GATE IN BIOMOLECULAR LOGIC GATE SYSTEM

Figure 1 shows PCR-based biomolecular logic gate system. This system is consisted of two reactions: one was PCR and another was CFTT. In the PCR, input signal is addition of primers, and output signal is the amplification of the target DNA. Normal PCR is 2-input AND logic gate because it undergoes responding to input of two kinds of primers. In this study, designing the templates appropriately and combining two primers or three, we constructed PCR-based AND, OR, NOT and AND-NOT logic gates. Our logic gate was a reaction mixture of PCR

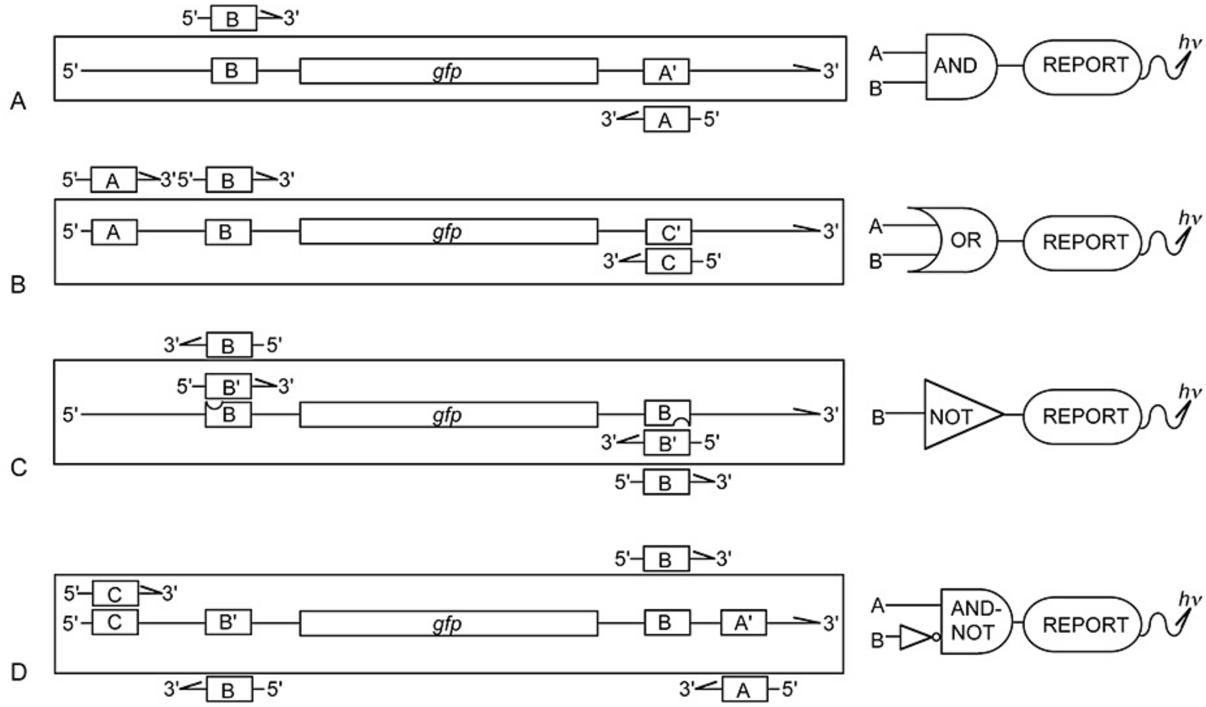


**Figure 1.** PCR-based 2-input biochemical logic gates for AND, OR, NOT and AND-NOT operations. Each logic gate is wired to a single-input REPORT gate in which CFTT of GFP undergoes. A, B and C represent the PCR primers or the sequences in the template DNA. A', B' and C' represent the complement sequences to A, B and C, respectively. (A) AND gate. When both primer A and primer B are inputted, PCR undergoes, the logic gate outputs a positive signal, and the REPORT gate shows green fluorescence. (B) OR gate. Primer C is pre-mixed so that PCR progresses when A, B, or both is inputted. (C) NOT gate. B' is pre-mixed so that PCR progresses without any addition of primers, but quenched when B is inputted due to hybridization of B and B'. There are 3-nucleotides mismatch between the B-region of the template and primer B', so that the pre-mixed primer B' prefers to from a duplex with primer B when it is added. (D) AND-NOT gate. Primer C is premixed. PCR of the GFP-coding region progresses when A is inputted, but not when A+B are inputted; extensions of primers A and C are interrupted by the binding of B on the template.

containing linear DNA template (logic gate template) coding GFPuv and a single stranded oligodeoxyribonucleotide (ODN) that hybridizes with logic gate template (pre-mixed primer). The logic operation is executed by an addition of primer A and/or primer B as an input signal. Since the logic gate templates carried recognition sequences for transcriptional and translational machinery, the amplified GFPuv-coding sequence is translated to GFPuv throughout CFTT, wired to the logic gates as a REPORT gate, that is, the output from the PCR-based logic gate is reported by green fluorescence.

The AND gate is a standard PCR mixture where both primer A and primer B are required to progress PCR; when both primers are inputted, the REPORT gate shows positive output signal. In the OR gate, primer C is pre-mixed in the initial condition and the logic gate operation is executed by inputting primer A, primer B, or both. In the NOT gate, primer B' is pre-mixed that carries a complement sequence to B so that the PCR undergoes without any addition of primers. When primer B is added to the reaction, the PCR is strongly inhibited due to the hybridization of B with B'. There is a 3-nucleotide mismatch between primer B' and the B-region of the template, whereas primers B and B' are perfect matched, and the molar ratio of B/B' was 5/4. Addition of B to the NOT gate therefore perfectly blocks the PCR. In the AND-NOT gate, inputting primer A progresses the PCR because C is already premixed. When both primer A and primer B are added, amplification of mature GFPuv-coding region is inhibited by formation of truncated products between A and B and between B and C.

After 50-μL scale PCR of each logic gate, 10 μL of the reaction was directly subjected to a CFTT (total volume was 50 μL) and 10 μL from it was transferred into a 10-μL microwell on a polydimethylsiloxane (PDMS)-based reaction chamber (32). The chamber was fabricated by putting a 2-mm thick PDMS-seat having two-dimensional (2-D) array of numerous micro-holes (the diameter was 3 mm) onto a flat glass substrate to form the 2-D array of microchamber (the volume was 19 μL). Each chamber was filled with the 10 μL of reaction mixture, sealed the open top with a thick polycarbonate film (thickness was 0.1 mm) to prevent evaporation during the reaction, and incubated at 30 ºC for 120 min. The output signal was validated on a standard UV-visible transilluminator and photographed (Figure 2). Fourty μL

**Table 1**. Theoretical truth table of the logic gates

| **Input** | | **Output** | | | |
|---|---|---|---|---|---|
| A | B | AND | OR | NOT | AND-NOT |
| 0 | 0 | 0 | 0 | 1 | 0 |
| 0 | 1 | 0 | 1 | 0 | 0 |
| 1 | 0 | 0 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 0 | 0 |

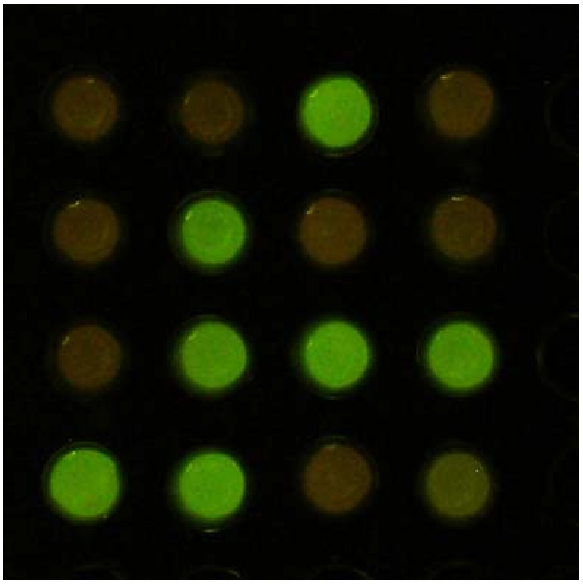

**Figure 2**. Fluorescence of the cell-free synthesized GFP in PDMS-based microwells. The positions of each well correspond to Table 1.

mixture left was also incubated in a tube at 30 ° C, and the fluorescence intensities were quantified. Figure 3 shows the normalized intensities of output signals from the REPORT gates. These results show good agreement with theoretical truth table (Table 1) showing that all the PCR-based logic gates representing AND, OR, NOT, and AND-NOT gates properly functioned.

Adopting CFT of GFP as the single-input report gate is the characteristics in this study. The REPORT gate cuts off a background signal from the PCR-based logic gate such as non-specific priming or primer dimer formation, which gives positive signal in TaqMan or Molecular Beacon methods. For example, in NOT gate, addition of primer B quenches the PCR due to the formation of B-B' duplex, which is recognized as an output signal in DNA-level, however, the REPORT gate shows no output signal.

We are trying to expand the variation of logic operation: NAND, NOR and NOR. The construction is underway, and CFT of GFP will be adopted as the REPORT gate again.

## 4. APPLICSTION OF CELL-FREE GFP-EXPRESSION AS A REPORTER IN ON-CHIP CAPILLARY ELECTROPHORESIS FRACTIONATION OF DNA

To realize a method for DNA construction without living cell-based screening, we introduced on-chip CE fractionation of DNA construct and enzymatic reactions. The model system we employed was introduction of GFPuv gene into an expression vector carrying T7 RNA polymerase-specific promoter and terminator (33), and bacterial translational machinery. When the construction is succeeded, transcription of the target gene will be under the control of T7 RNA polymerase, and green fluorescence will observed after CFT of GFP.

The backbone of expression vector coding recognition sites for T7 RNA polymerase and bacterial translational machinery, and the insert carrying GFPuv gene were isolated from commercial vectors with a standard PCR using primer set carrying restriction sites. After the digestion with restriction enzymes, the PCR-amplified fragments were assembled with T4 DNA ligase with a standard condition (34). From the reaction mixture, the complete region that T7 RNA polymerase transcribes was amplified with PCR (1st PCR).

The amplified DNA was then collected with an on-chip CE-based DNA collection using a microfluidic device (35,36). Figure 5 illustrates design of the device for the on-chip CE-based DNA collection. Electrodes were patterned on a glass substrate, and covered with PDMS-based fluidic chip in which six ports for sample injection and collection, and electrophoretic channels were fabricated and filled with 1.2 % hydroxyethyl cellulose (37) in 1 x TBE buffer. Aliquot of PCR mixture was loaded from port A to port E, and the loaded sample was separated by an electrophoresis to port C, then the target band was separated at BD-FC intersection by switching the direction of the electrophoresis. The operation was monitored with CCD camera coupled with a video recorder.

Figure 7 shows captured images of the fluidic operation. This operation was repeated twice and total 8 µL of sample was recovered. Without any purification, the recovered material was directly subjected to a PCR (2nd PCR) as a starting material. After the reaction, 1 µg of the amplified DNA was subjected to CFTT containing T7 RNA polymerase, and after 4 h incubation for maturing at 30 °C (38), the product eliminated green fluorescence (Figure 7). It shows that the construction of the recombinant template DNA for CFTT was successful in a cell-free manner.

## 5. SUMMARY AND PERSPECTIVE

In order to estimate the performance of protein synthesis reaction, it is required to monitor the activity of the protein synthesized in the system. GFP is an ideal protein for this purpose, especially for the evaluation of protein synthesis carried out in a microstructure, in which supplying substrates or sampling is difficult. In this review, we introduced our approach to apply CFTT of GFP into (i) PCR-based biomolecular logic gate system as a report gate, and (ii) on-chip CE-based DNA fractionation as a confirmation reaction. In both study, due to the feature of GFP, execution in logic gate and fractionation in on-chip CE were simply monitored.

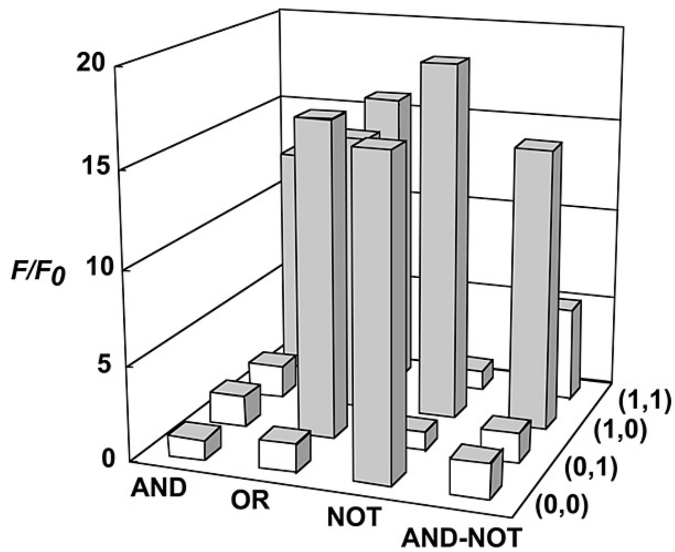


**Figure 3.** Normalized output signals from the logic gates reported by CFTT of GFP. Measured fluorescence intensities (F) are normalized to the values obtained in the absence of input primers and template DNA (F0). A universal threshold of F/F0 = 10 was chosen to define ON (F/F0 > 10, dark bars) and OFF (F/F0 < 10, light bars) states of the logic gates. X-axis represents types of logic operation; AND, OR, NOT, and AND-NOT operations. Y-axis represents the combination of input primer; "0" and "1" mean "no input" and "input", respectively.

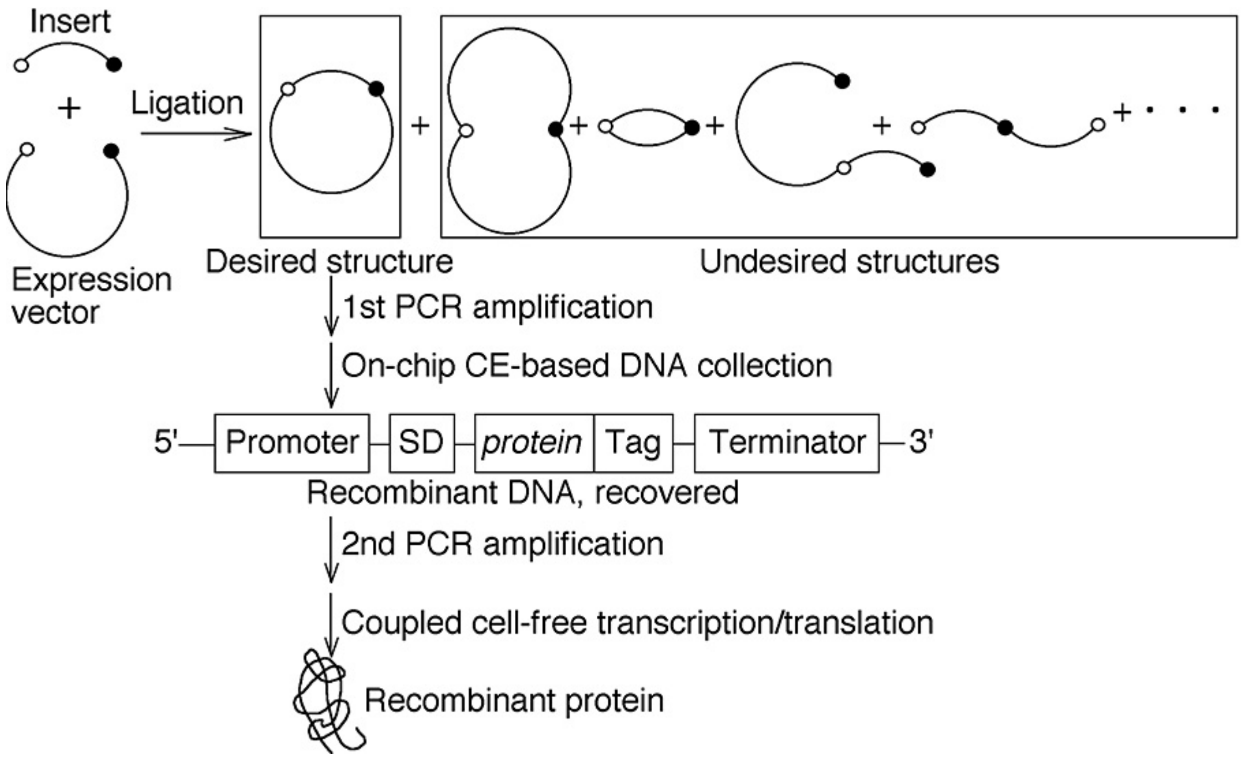


**Figure 4**. Construction of a template DNA for CFTT by using on-chip CE-based DNA collection.

DNA-based computing and DNA-based logic operation have been attracting interest in many field, including nanotechnology, electronics, materials, and informatics. PCR-based logic gate system is advantageous because it realizes various logic operations by designing the construction and sequences of template DNA and pre-mixed primers, without changing input primer set. We are now trying to expand the variation of the logic operation.

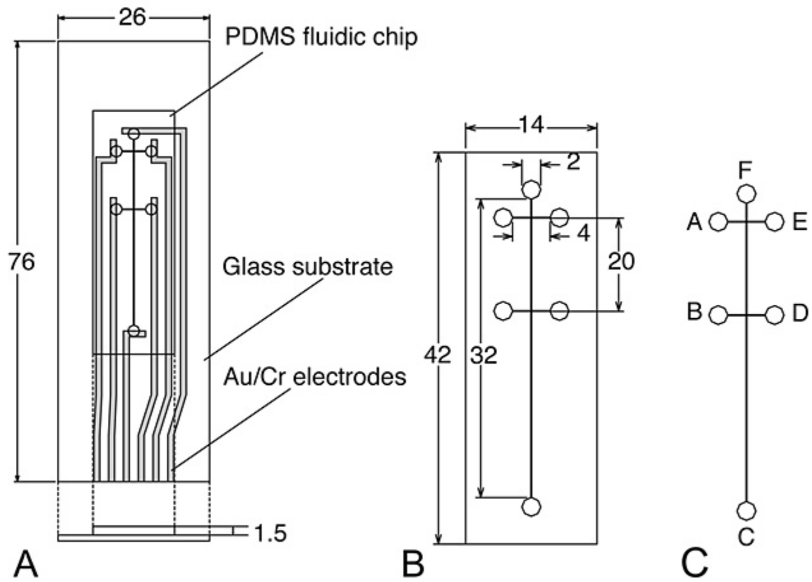


**Figure 5**. (A) Design of the microfluidic device for the on-chip CE-based DNA collection. The size is described in mm scale. (B) Structure of the PDMS fluidic chip. (C) Layout of ports and channels. The volume of each port is less than 5 µL. The channel width is 90 µm and the channel depth is 30 µm. The channel was filled with 1.2 % hydroxyethyl cellulose in 1xTBE buffer. The glass substrate patterned with electrodes (A) and PDMS chip (B) were brought into contact. Sample is loaded from port A to port E, and the loaded sample is separated by an electrophoresis to port C, then the target band was separated at BD-FC intersection by switching the direction of the electrophoresis. The band is collected from port B.

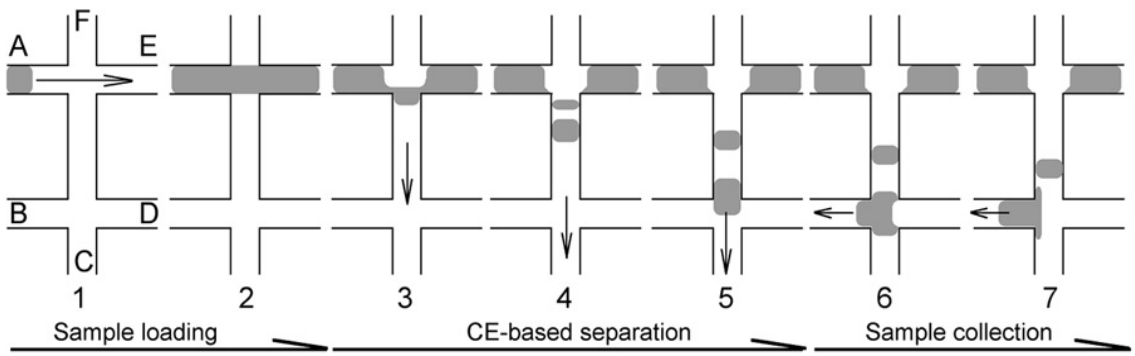


**Figure 6.** Separation and collection of the target DNA with on-chip CE-based DNA collection. (1, 2) Sample DNA mixture is loaded from Port A to Port E. (3-5) The loaded sample is separated by an electrophoresis to Port C. (6, 7) The target band is separated at the BD-FC intersection by switching the direction of electrophoresis.

Because DNA is water-soluble biomolecule, and usually handled as an aqueous solution, it is of immediate interest to apply microfluidic technology to DNA-based computing and DNA-based logic operation. To build up the PCR-based logic gate system for a fluidic computing system, integration of PCR and CFTT on one chip is necessary.

Development of a platform in which total operation of gene expression including PCR and CFTT is also desired in the field of genetic engineering. Although we achieved total operation of genetic engineering in cell-free format by applying on-chip CE fractionation of DNA instead of living cell-based screening, each step is still separated. Integration and systematization of each step on one device, a useful tool would be realized for protein preparation under cell-free condition. It would open the door for "cell-free genetic engineering".

Both logic gate system and cell-free genetic engineering, we have to estimate the performance of the protein expression reaction. CFT of GFP is capable as an ideal reporter system.

## 6. ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan

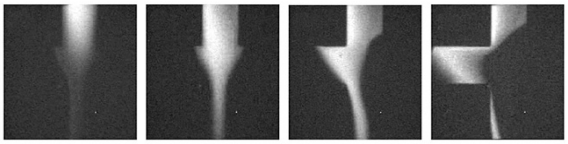


**Figure 7.** Captured video images of the target DNA collection at the BD-FC intersection of Figure 5. The reaction mixture was stained with SYBR Green I and a 4 µL portion (containing 100 ng DNA mixture) was subjected for the separation. (1, 2) The target fraction came down to the intersection in vertical direction. (3, 4) The direction of electrophoresis was switched to horizontal and the target fraction goes to the recovery port. Electrophoretic operations were implemented by a computer-controlled high voltage supply. The operation was monitored with an inverted fluorescence microscope equipped with a CCD camera and recorded by a video recorder.

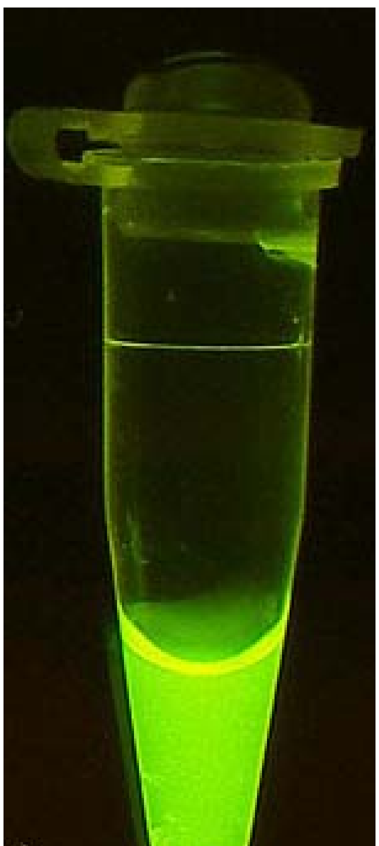

**Figure 8.** Fluorescence of GFPuv synthesized in CFTT. DNA collected from the on-chip CE was subjected to a PCR and 1 µg of the amplified DNA was subjected to a 100 µL scale of a CFTT system as a template. The reaction was carried out at 30 °C for 4 h. Picture was taken on a standard UV-visible transilluminator.

(20550148 and 23550197) and Chuo University Joint Research Grant 2009.

## 7. REFERENCES

1. Joseph Sambrook and David W Russell. In: Molecular Cloning A Laboratory Manual Third Edition, Cold Harbor Laboratory Press, New York (2001)

2. Vladimir I Baranova, Igor Yu. Morozova, Stephen A Ortlepp and Alexander S Spirin: Gene expression in a cell-free system on the preparative scale. *Gene* 84, 463-466 (1989)

3. Takanori Kigawa, Takashi Yabuki Yasuhiko Yoshida, Michio Tsutsui, Yutaka Ito, Takehiko Shibata and Shigeyuki Yokoyama: Cell-free production and stable-isotope labeling of milligram quantities of proteins. *FEBS Lett* 442, 15-19 (1999)

4. Kairat Madin, Tatsuya Sawasaki, Tomia Ogasawara and Yaeta Endo: A highly efficient and robust cell-free protein synthesis system prepared from wheat embryos: plants apparently contain a suicide system directed at ribosomes. *Proc Natl Acad Sci USA* 97, 559-564 (2000)

5. Yoshihiro Shimizu, Akio Inoue, Yukihide Tomari, Tsutomu Suzuki, Takashi Yokogawa, Kazuya Nishikawa and Takuya Ueda: Cell-free translation reconstituted with purified components. *Nat Biotechnol* 19, 751-755 (2001)

6. Kim A. Woodrow, Isoken O. Airen and James R. Swartz: Rapid expression of functional genomic libraries. *J Proteome Res* 5, 3288-3300 (2006)

7. Qian Mei, Carl K Frederickson, Andrew Simon, Ruba Khnouf and Z Hugh Fan: Cell-free protein synthesis in microfluidic array devices. *Biotechnol Prog* 23, 1305-1311 (2007)

8. Ruba Khnouf, Daniel Olivero, Shouguang Jin, Matthew A Coleman and Z Hugh Fan: Cell-free expression of soluble and membrane proteins in array device for drug screening. *Anal Chem* 82, 7021-7026 (2010)

9. J. Craig Venter, Mark D. Adams, Eugene W. Myers, Peter W. Li, Richard J. Mural, Granger G. Sutton, Hamilton O. Smith, Mark Yandell, Cheryl A. Evans, Robert A. Holt, Jeannine D. Gocayne, Peter Amanatides, Richard M. Ballew, Daniel H. Huson, Jennifer Russo Wortman, Qing Zhang, Chinnappa D. Kodira, Xiangqun H. Zheng, Lin Chen, Marian Skupski, Gangadharan Subramanian, Paul D. Thomas, Jinghui Zhang, George L. Gabor Miklos, Catherine Nelson, Samuel Broder, Andrew G. Clark, Joe Nadeau, Victor A. McKusick, Norton Zinder, Arnold J. Levine, Richard J. Roberts, Mel Simon, Carolyn Slayman 0, Michael Hunkapiller, Randall Bolanos, Arthur Delcher, Ian Dew, Daniel Fasulo, Michael Flanigan, Liliana Florea, Aaron Halpern, Sridhar Hannenhalli, Saul Kravitz, Samuel Levy, Clark Mobarry, Knut Reinert, Karin Remington, Jane Abu-Threideh, Ellen Beasley, Kendra Biddick, Vivien Bonazzi, Rhonda Brandon, Michele

Cargill, Ishwar Chandramouliswaran, Rosane Charlab, Kabir Chaturvedi, Zuoming Deng, Valentina Di Francesco, Patrick Dunn, Karen Eilbeck, Carlos Evangelista, Andrei E. Gabrielian, Weiniu Gan, Wangmao Ge, Fangcheng Gong, Zhiping Gu, Ping Guan, Thomas J. Heiman, Maureen E. Higgins, Rui-Ru Ji, Zhaoxi Ke, Karen A. Ketchum, Zhongwu Lai, Yiding Lei, Zhenya Li, Jiayin Li, Yong Liang, Xiaoying Lin, Fu Lu, Gennady V. Merkulov, Natalia Milshina, Helen M. Moore, Ashwinikumar K Naik, Vaibhav A. Narayan, Beena Neelam, Deborah Nusskern, Douglas B. Rusch, Steven Salzberg , Wei Shao, Bixiong Shue, Jingtao Sun, Zhen Yuan Wang, Aihui Wang, Xin Wang, Jian Wang, Ming-Hui Wei, Ron Wides , Chunlin Xiao, Chunhua Yan, Alison Yao, Jane Ye, Ming Zhan, Weiqing Zhang, Hongyu Zhang, Qi Zhao, Liansheng Zheng, Fei Zhong, Wenyan Zhong, Shiaoping C. Zhu, Shaying Zhao , Dennis Gilbert, Suzanna Baumhueter, Gene Spier, Christine Carter, Anibal Cravchik, Trevor Woodage, Feroze Ali, Huijin An, Aderonke Awe, Danita Baldwin, Holly Baden, Mary Barnstead, Ian Barrow, Karen Beeson, Dana Busam, Amy Carver, Angela Center, Ming Lai Cheng, Liz Curry, Steve Danaher, Lionel Davenport, Raymond Desilets, Susanne Dietz, Kristina Dodson, Lisa Doup, Steven Ferriera, Neha Garg, Andres Gluecksmann, Brit Hart, Jason Haynes, Charles Haynes, Cheryl Heiner, Suzanne Hladun, Damon Hostin, Jarrett Houck, Timothy Howland, Chinyere Ibegwam, Jeffery Johnson, Francis Kalush, Lesley Kline, Shashi Koduru, Amy Love, Felecia Mann, David May, Steven McCawley, Tina McIntosh, Ivy McMullen, Mee Moy, Linda Moy, Brian Murphy, Keith Nelson, Cynthia Pfannkoch, Eric Pratts, Vinita Puri, Hina Qureshi, Matthew Reardon, Robert Rodriguez, Yu-Hui Rogers, Deanna Romblad, Bob Ruhfel, Richard Scott, Cynthia Sitter, Michelle Smallwood, Erin Stewart, Renee Strong, Ellen Suh, Reginald Thomas, Ni Ni Tint, Sukyee Tse, Claire Vech, Gary Wang, Jeremy Wetter, Sherita Williams, Monica Williams, Sandra Windsor, Emily Winn-Deen, Keriellen Wolfe, Jayshree Zaveri, Karena Zaveri, Josep F. Abril , Roderic Guig , Michael J. Campbell, Kimmen V. Sjolander, Brian Karlak, Anish Kejariwal, Huaiyu Mi, Betty Lazareva, Thomas Hatton, Apurva Narechania, Karen Diemer, Anushya Muruganujan, Nan Guo, Shinji Sato, Vineet Bafna, Sorin Istrail, Ross Lippert, Russell Schwartz, Brian Walenz, Shibu Yooseph, David Allen, Anand Basu, James Baxendale, Louis Blick, Marcelo Caminha, John Carnes-Stine, Parris Caulk, Yen-Hui Chiang, My Coyne, Carl Dahlke, Anne Deslattes Mays, Maria Dombroski, Michael Donnelly, Dale Ely, Shiva Esparham, Carl Fosler, Harold Gire, Stephen Glanowski, Kenneth Glasser, Anna Glodek, Mark Gorokhov, Ken Graham, Barry Gropman, Michael Harris, Jeremy Heil, Scott Henderson, Jeffrey Hoover, Donald Jennings, Catherine Jordan, James Jordan, John Kasha, Leonid Kagan, Cheryl Kraft, Alexander Levitsky, Mark Lewis, Xiangjun Liu, John Lopez, Daniel Ma, William Majoros, Joe McDaniel, Sean Murphy, Matthew Newman, Trung Nguyen, Ngoc Nguyen, Marc Nodell, Sue Pan, Jim Peck, Marshall Peterson, William Rowe, Robert Sanders, John Scott, Michael Simpson, Thomas Smith, Arlan Sprague, Timothy Stockwell, Russell Turner, Eli Venter, Mei Wang, Meiyuan Wen, David Wu, Mitchell Wu, Ashley Xia, Ali Zandieh, Xiaohong Zhu: The sequence of the human genome. *Science* 291, 1304-1351 (2001)

10. International Human Genome Sequencing Consortium: Initial sequencing and analysis of the human genome. *Nature* 409, 860-921 (2001)

11. Teruo Fujii, Kazuo Hosokawa, Takahiko Nojima, Shuichi Shoji, Akira Yotsumoto and Isao Endo: *In vitro* protein synthesis on a silicon chip. *Proc Int Conf New Frontiers Biomechanic Eng*, 103-104 (1997)

12. Takahiko Nojima, Teruo Fujii, Kazuo Hosokawa, Akira Yotsumoto, Shuichi Shoji and Isao Endo: Cell-free protein synthesis in a microfabricated reactor. *Bioprocess Eng* 22, 13-17 (2000)

13. Mari Tabuchi, Mami Hino, Yasuo Shinohara and Yoshinobu Baba: Cell-free protein synthesis on a microchip. *Proteomics* 2, 430-435 (2002)

14. Osamu Shimomura, Frank Johnson and Yo Saiga: Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. *J Cell Comp Physiol* 59, 223-239 (1962)

15. Roger Tsien: The green fluorescent protein. *Annu Rev Biochem* 67, 509-544 (1998).

16. Takatoki Yamamoto, Teruo Fujii and Takahiko Nojima: PDMS-glass hybrid microreactor array with embedded temperature control device. Application to cell-free protein synthesis. *Lab Chip* 2, 197-202 (2002)

17. Andreas Crameri, Erik Whitehorn, Emily Tate and Willem Stemmer: Improved green fluorescent protein by molecular evolution using DNA shuffling. *Nat Biotechnol* 14, 315-319 (1996)

18. Roses Heim, Douglas Prasher and Roger Tsien: Wavelength mutations and posttranslational autoxidation of green fluorescent protein. *Proc Natl Acad Sci USA* 91, 12501-12504 (1994)

19. Takahiko Nojima, Angela C. Lin, Teruo Fujii, and Isao Endo: Determination of the termination efficiency of the transcription terminator using different fluorescent profile in green fluorescent protein mutants. *Anal Sci* 21, 1479-1481 (2005)

20. Takatoki Yamamoto, Mami Hino, Rei Kakuhata, Takahiko Nojima, Yasuo Shinohara, Yoshinobu Baba and Teruo Fujii: Evaluation of cell-free protein synthesis using PDMS-based microreactor arrays. *Anal Sci* 24, 243-246 (2008)

21. Takeshi Kinpara, Ryuta Mizuno, Yuji Murakami, Masaaki Kobayashi, Shouhei Yamaura, Quamrul Hasan, Yasutaka Morita, Hideo Nakano, Tsuneo Yamane and Eiichi Tamiya1: A picoliter chamber array for cell-free protein synthesis. *J Biochem* 136, 149-154 (2004)

22. Philip Angenendt, Lajos Nyarsik, Witold Szaflarski, Jorn Glokler, Knud H Nierhaus, Hans Lehrach, Dolores J Cahill and Angelika Lueking: Cell-free protein expression and functional assay in nanowell chip format. *Anal Chem* 76, 1844-1849 (2004)

23. Fabienne Courtois, Luis F Olguin, Graeme Whyte, Daniel Bratton, Wilhelm T S Huck, Chris Abell and Florian Hollfelder: An integrated device for monitoring time-dependent *in vitro* expression from single genes in picolitre droplets. *ChemBioChem* 9, 439-446 (2008)

24. Takahiko Nojima, Takatoki Yamamoto, Hiroshi Kimura and Teruo Fujii: Polymerase chain reaction-based molecular logic gate coupled with cell-free transcription-translation as a reporter. *Chem Comm* 32, 3771-3773 (2008)

25. Evgeny Katz and Vladimir Privman: Enzyme-based logic systems for information processing. *Chem Soc Rev* 39, 1835-1857 (2010)

26. Itamar Willner, Bella Shlyahovsky, Maya Zayats and Bilha Willner: DNAzymes for sensing, nanobiotechnology and logic gate applications. *Chem Soc Rev* 37, 1153-1165 (2008)

27. Randall Saiki, Stephan Scharf, Fred Faloona, Kary Mullis, Glenn Horn, Henry Erlich and Norman Arnheim: Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230, 1350-1354 (1985)

28. T Morris, B Robertson and M Gallagher: Rapid reverse transcription-PCR detection of hepatitis C virus RNA in serum by using the TaqMan fluorogenic detection system. *J Clin Microbiol* 34, 2933-2936 (1996)

29. Amy Piatek, Sanjay Tyagi, Armo Pol, Amalio Telenti, Lincoln Miller, Fred Kramer and David Alland: Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis. *Nat Biotechnol* 16, 359-363 (1998)

30. Takahiko Nojima, Shohei Kaneda and Teruo Fujii: On-chip capillary electrophoresis fractionation of DNA construct for cell-free protein expression. *Chem Lett* 36, 1346-1347 (2007)

31. Randy Legerski and Donald Robberson: Analysis and optimization of recombinant DNA joining reactions. *J Mol Biol* 181, 297-312, (1985)

32. David Duffy, J Cooper McDonald, Oliver Schueller, George Whitesides: Rapid prototyping of microfluidic systems in poly(dimethylsiloxane), *Anal Chem* 70, 4974-4978 (1998)

33. William Studier and Barbara Moffatt: Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J Mol Biol* 189, 113-130 (1986)

34. B Weiss, A Jacquemin-Sablon, T. Live, G Fareed and C. Richardson: Enzymatic breakage and joining of deoxyribonucleic acid. VI. Further purification and properties of polynucleotide ligase from Escherichia coli infected with bacteriophage T4. *J Biol Chem* 243, 4543-4555 (1968)

35. John Hong, Teruo Fujii, Minoru Seki, Takatoki Yamamoto and Isao Endo: Integration of gene amplification and capillary gel electrophoresis on a polydimethylsiloxane-glass hybrid microchip. *Electrophoresis* 22, 328-333 (2001)

36. Shohei Kaneda and Teruo Fujii: Combining droplet-based liquid handling and on-chip capillary electrophoresis with a new sample injection method. *Proc µTAS 2003,* 1279-1282 (2001)

37. Adam T Woolley and Richard A Mathies: Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips. *Proc Natl Acad Sci USA* 91, 11348-11352 (1994)

38. Angela Coxona and Timothy H. Bestor: Proteins that glow in green and blue. *Chem Biol* 2, 119-121 (1995)

**Abbreviations:** CCD: charge coupled device, CE: capillary electrophoresis, CFTT: coupled cell-free transcription-translation, GFP: green fluorescent protein, mRNA: messenger ribonucleic acid, ODN: oligodeoxyribonucleotide, PCR: polymerase chain reaction, PDMS: polydimethylsiloxane

**Key Words:** Capillary electrophoresis, DNA, GFP, Logic gate, Microreactor, Transcription, Translation, Review

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