Supplementary Materials

Polymerase chain reaction-based biochemical logic gate coupled with cell-free transcription-translation of green fluorescent protein as a report gate

Takahiko Nojima,* Takatoki Yamamoto, Hiroshi Kimura and Teruo Fujii
Center for International Research on MicroMechatronics, Institute of Industrial Science, The University of Tokyo, Komaba 4-6-1, Meguro-ku, Tokyo 153-8505, Japan.
E-mail: nojima@iis.u-tokyo.ac.jp

Construction of logic gate templates

All the synthetic ODN used in this report were obtained from Sigma Genosys. All the chemicals used were of the highest grade available. The plasmid coding GFPuv gene and recognition sites for transcriptional and translational machinery subjected to PCR as a starting material in preparing the logic gate templates were described in our previous paper (Nojima et al., *Chemistry Letters*, 2007, **36**, 1346). The sequences of the primers used were listed below.

AND	forward	TTT AGA TCT AAA CAG CTA TGA CTT AAT ACG ACT CAC
	reverse	TTT GGA TCC ACT GGC CGT CGT TTT ACC TCC TTT CAG
OR	forward	GTA AAA CGA CGG CCA GTA ACA GGA AAC AGC TAT GAC TTA ATA CGA CTC AC
	reverse	TTT GGA TCC GTT TTC CCA GTC ACG ACC CTC CTT TCA G
NOT	forward	GTC ATA GCT GTT TCC TGT TAA TAC GAC TCA C
	reverse	GTC ATA GCT GTT TCC TGC CGG ATA TAG TTC
AND-NOT	forward	GTA AAA CGA CGG CCA GTG TCA TAG CTG TTT CCT GCT CCT TTC AG
	reverse	GTT TCC CAG TCA CGA CGT CAT AGC TGT TTC CTG TTA ATA CGA CTC AC

PCR was performed on ASTEC PC320 thermal cycle controller with TaKaRa *ExTaq* DNA polymerase according to manufacturer's protocol in a 50-μL reaction volume containing 2 mM $MgCl_2$, 200 μM each dNTP, 0.5 μM each primer, 5 ng template DNA, and 1.25 units

polymerase. PCR buffer was supplied from the manufacturer. PCR parameters were 95°C for 60 sec, 32°C for 30 sec, and 72°C for 60 sec for 25 cycles, finishing with 72°C for 7 min. The reaction mixture were purified with QIAGEN Qiaquick PCR purification kit, and dissolved in 5 mM Tris-HCl (pH 8.5). DNA concentrations were determined by measuring absorbance at 260 nm on Hitachi Gene Spec I spectrophotometer.

PCR condition in the logic gates

The sequence and final concentration of inputted primers A and B, and pre-mixed primers B' and C are listed below.

A	GTA AAA CGA CGG CCA GT	0.5 μM
B	AAA ACA GGA AAC AGC TAT GAC	0.5 μM
B'	GTC ATA GCT GTT TCC TG	0.4 μM
C	GTT TTC CCA GTC ACG AC	0.5 μM

All the PCR were performed under the same thermal condition: 3-min dissociation at 95°C followed by 95 °C for 60 sec, 50°C for 30 sec, and 72°C for 60 sec for 30 cycles. Reaction was carried out with TaKaRa *ExTaq* DNA polymerase according to manufacturer's protocol in a 50-μL volume containing 2 mM $MgCl_2$, 200 μM each dNTP and 1.25 units polymerase. PCR buffer was supplied from the manufacturer. Since different concentrations of the logic gate templates and PCR efficiency in the logic gates may give significantly different output signals, we repeated the PCRs to find a condition in which output signals from the 4 gates are normalized. Initial template concentrations were 40, 100 and 120 ng μL^{-1} for NOT, AND, and OR and AND-NOT gates, respectively.