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

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Polymerase chain reaction-based biochemical logic gates were designed for AND, OR, NOT, and AND–NOT operations, whose output signal is reported by coupled cell-free transcription–translation of green fluorescent protein.

Recently, much attention has been paid to designing a biomolecular system that responds to chemical inputs.[1] From this standpoint, polymerase chain reaction (PCR)[2] is a model reaction of a 2-input AND logic gate because it responds to the input of two kinds of primers. In the reaction, DNA-amplification responds to an output signal, which is usually reported by fluorescence enhancement based on TaqMan chemistry[3] or Molecular Beacon,[4] or by the appearance of an electrophoretic band. Since PCR features a precision molecular recognition between DNA strands and reversible duplex formation, it is intriguing to attempt to develop PCR-based logic gates representing not only an AND gate but also OR or NOT gates. In this report, designing the templates appropriately and combining two or three primers, we constructed PCR-based AND, OR, NOT, and AND–NOT logic gates. The logic gate is a PCR reaction mixture containing a linear DNA template (logic gate template) coding a green fluorescent protein (GFP)[5] and a single stranded oligodeoxyribonucleotide (ODN) that hybridizes with the logic gate template (pre-mixed primer). Fig. 1 illustrates the DNA logic gates designed and constructed in this study. The logic gate operation is executed by the addition of primer A and/or primer B as an input signal. Since the logic gate templates carry recognition sequences for T7 RNA polymerase and bacterial translational machinery, the amplified GFP-coding sequence is translated to GFP *via* a coupled bacterial cell-free transcription–translation system containing T7 RNA polymerase, wired to the logic gates as a REPORT gate, that is, the output from the PCR-based logic gate is reported by green fluorescence.

Construction of the DNA templates is described in the ESI.† The AND gate is a standard PCR mixture where both primer A and primer B are required to progress PCR; when both primers are input, the REPORT gate shows a 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 and/or primer B. In the NOT gate, 0.4 μM of primer B′ is pre-mixed; it carries a complementary sequence to B so that the PCR is undergone without any addition of primers. When primer B (0.5 μM) 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 perfectly matched, and the 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 pre-mixed. When both primer A and primer B are added, amplification of the mature GFP-coding region is inhibited by formation of truncated products between A and B and between B and C.

PCR conditions are described in the ESI.† After 50-μL scale PCR of each logic gate, 10 μL of the reaction mixture were directly subjected to a cell-free transcription–translation mixture (total volume was 50 μL, RTS 100 *E. coli* HY kit, Roche Diagnostic) and 10 μL from it were transferred into a 10 μL microwell on a polydimethylsiloxane (PDMS)-based reaction chamber. The PDMS-based reaction chamber was fabricated by putting a 2-mm thick PDMS-seat having a two-dimensional (2-D) array of numerous micro-holes (ϕ = 3 mm) onto a flat glass substrate to form the 2-D array of the microchamber ($\approx$19 μL volume). Each microchamber was filled with 10 μL of the reaction mixture, the open top was sealed with a 0.1-mm thick polycarbonate film to prevent evaporation during the reaction, and the mixture was incubated at 30 °C for 2 h. The output signal was validated on a standard UV-transilluminator (excitation wavelength was 365 nm. We used a UV-optimized variant of GFP[6]) and photographed (Fig. 2b). The 40 μL of the mixture left were also incubated in a tube at 30 °C, and the fluorescence intensity was measured on a Microtech Nition FP-3000 counter. Fig. 2c shows the normalized intensities of output signals from the REPORT gates. These results show good agreement with the theoretical truth table in Fig. 2a, showing that all the PCR-based logic gates representing AND, OR, NOT, and AND–NOT gates functioned properly.

Adopting a coupled cell-free transcription–translation of fluorescent protein as the single-input report gate is the distinctive feature of the present study. The REPORT gate cuts

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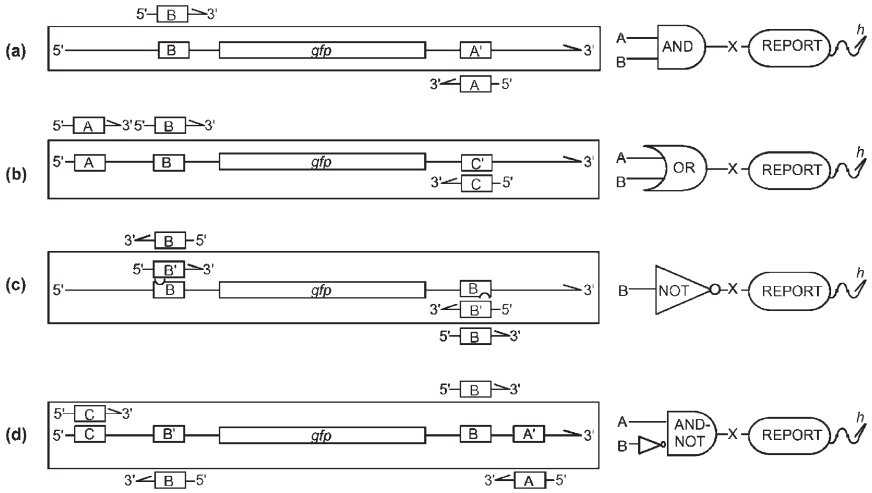
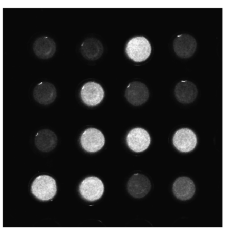


Fig. 1 PCR-based 2-input biochemical logic gates for AND, OR, NOT, and AND–NOT operations. Each logic gate is wired to a single-input REPORT gate in which cell-free synthesis of GFP is undergone. A, B, and C represent the PCR primers or the sequences in the template DNA. A′, B′, and C′ represent the complementary sequences to A, B, and C, respectively. (a) AND gate. When both primer A and primer B are input, PCR is undergone, 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 are input. (c) NOT gate. B′ is pre-mixed so that PCR progresses without any addition of primers, but is quenched when B is input due to hybridization of B and B′. There is a 3-nucleotide mismatch between the B-region of the template and primer B′, so that the pre-mixed primer B′ prefers to form 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 input, but not when A + B are input; extensions of primers A and C are interrupted by the binding of B on the template.

(a)

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

(b)



(c)

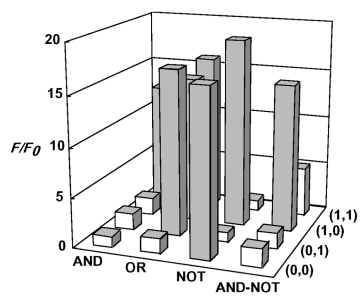


Fig. 2 Output signals from the REPORT gates wired to 2-input PCR-based logic gates. (a) Theoretical truth table of the logic gates. (b) Fluorescence of the cell-free synthesized GFP in PDMS-based microwells. (c) Normalized output signals from the logic gates reported by cell-free transcription–translation of GFP. Measured fluorescence intensities (F) are normalized to the values obtained in the absence of input primers and template DNA (F_0). A universal threshold of $F/F_0 = 10$ was chosen to define ON ($F/F_0 > 10$, dark bars) and OFF ($F/F_0 < 10$, light bars) states of the logic gates. Detailed experimental procedures are provided in the ESI.†

off any background signal from the PCR-based logic gate such as non-specific priming or primer dimer formation, which give positive signals in the TaqMan and Molecular Beacon methods. For example, in the NOT gate, addition of primer B quenches the PCR due to the formation of a B–B′ duplex, which is recognized as an output signal at the DNA-level, however, the REPORT gate shows no output signal.

It would be preferable to implement such a solution-based logic gate in a microfluidic format. Recently, Kou *et al.*[7] reported a molecular logic gate based on a microfluidic operation of fluorescent dye-containing solution where the output signal is the change in fluorescence intensity as a response to metal ions and/or pH-shift. We have reported microfluidic operation of DNA purification[8] and cell-free translation,[9–11] and based on these achievements, further research is underway in our laboratory to install the PCR-based logic gate into a microfluidic device.

In summary, PCR-based logic gates were constructed. The logic gates are a PCR mixture containing a DNA template coding GFP and recognition sites for transcriptional and translational machinery, and ODN that hybridizes with the template. Two kinds of primers represent input signals and the output from the logic gate is visualized by cell-free GFP production: the green fluorescence indicates a positive output signal from the gate. AND, OR, NOT, and AND–NOT gates were constructed and all of them functioned properly.

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