

Supplementary Information

Molecular Beacon-Based Half Adder and Half Subtractor

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1. Materials and Methods

Half-Adder

Eight separate microtubes corresponding to the four possible states of the half-adder (0,0; 1,0; 0,1; 1,1) were prepared by adding the AND gate (8 μ L, 0.6 μ M) or the XOR gate (8 μ L, 0.6 μ M) to each tube, together with I_A (4 μ L, 0.6 μ M) and I_B (4 μ L, 0.6 μ M) in annealing buffer (100mM K-acetate, 30 mM HEPES-KOH, pH 7.4, 2mM Mg-acetate to give a 50 μ L total volume). The DNA annealing was performed in a thermal cycler (polymerase chain reaction machine, BioRed), that the annealing condition was programmed as following: incubation at 95°C for 4 minutes followed by incubation at 70°C for 10 minutes and gradually decreasing the temperature 1°C per minute until 4°C. The DNA hybridization reaction was completed after the temperature kept in 4°C for 30 minutes and the reaction content for each reaction was subjected for fluorescence measurement. The contents of each tube were transferred in a 96-well plate and diluted by adding 200 μ L annealing buffer. The 96-well plate was subjected to fluorescence intensity measurement using the Microplate Fluorometer (Fluoroskan Ascent, Thermo). For detecting the FAM fluorescence, the excitation wavelength was set at 485nm and the emission wavelength was set at 538nm. For CAL Fluor orange detection, the excitation wavelength was set at 538nm and the emission wavelength was set at 590nm. Three individual experiments were performed independently and the fluorescence intensity was analyzed.

Half-Subtractor

Eight separate microtubes corresponding to the four possible states of the half-subtractor (0,0; 1,0; 0,1; 1,1) were prepared by adding the AND gate (8μL, 0.6μM) to four tubes, together with I_A' (4μL, 0.6μM) and I_B' (4μL, 0.6μM) in annealing buffer (100mM K-acetate, 30 mM HEPES-KOH, pH 7.4, 2mM Mg-acetate to give a 50μL total volume).

The DNA annealing condition and fluorescence intensity measurement were the same as that described in the Half-adder above.

2. Sequence Assignment

strand name	sequence
AND	5' <u>CCGGCGAGTCCTTCCACGACTCCAGCCGG</u> --Q 3'
XOR	5' <u>F</u> -- <u>CCGGCGAGTCCTTCCACGACTCCAGCCGG</u> --Q 3'
I_A	3' ATGATCGTGCAGGCGTCACGGTGACCA <u>GAAGGTGCTGAGGT</u> <u>CGGCC</u> -- <u>F</u> 5'
I_B	3' <u>GGCCGCTCAGGAAGGTGCT</u> TGGTCACCGTGACGCCTGCACGATCAT 5'
I_A'	3' Q -- ATGATCGTGCAGGCGTCAG <u>GAAGGTGCTGAGGT</u> <u>CGGCC</u> 5'
I_B'	3' <u>GGCCGCTCAGGAAGGTGCT</u> TGGTCACCGTGACGCCTGCACGATCAT -- <u>F</u> 5'

Fig. S Sequences used in AND and XOR logic gates and two input sets for the half-adder (I_A and I_B) and for the half-subtractor (I_A' and I_B'). In AND and XOR gate molecules, the underlined regions represent the stem sequences of the hairpins. In the four input sequences, the regions highlighted in yellow background are complementary to the AND and XOR gate molecules. Q stands for the quencher, BHQ-1; F stands for the red fluorophore, CAL Fluoro Orange 560; F stands for the green fluorophore, FAM. Sequences are typed in colour to match Figure 1 and Figure 2 in the article.