Electronic Supplementary Information

Entropy-Driven Molecular Switch and Signal Amplification for Homogeneous SNPs Detection

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Chemicals. T4 DNA ligase，dNTPs, and Klenow Fragment (exo$^-$) polymerase were purchased from MBI Fermentas Crop. All oligonucleotides were supplied and HPLC purified by SBS Genetech. Co. Ltd. as seen in Table S1. The stem of molecular switch was shadowed. The region underlined was the sequence as primer. The mutation position in mutant DNA was highlighted by underline and the boldface was the complementary nucleotides to molecular switch in italic portion. The italic portion in mutant DNA was complementary with the boldface of ss-probe **2**. Ss-probe **1** and the rest 15-mer of ss-probe **2** were matched perfectly. Ss-probe **1** and **2** were labeled with the dyes, DABCYL and FAM, respectively.

Table S1. Sequences of molecular switch, target and ss- probes

	Sequence (5′to 3′)
Molecular switch	5′- PO_4 -*TGGCGTAGGCAAGAGTGCCC*TTTGTGGCGTAGATCTTGCCT ACGCCAC-3′ (48 nt)
Mutant DNA	5′-TTTT**GGG CAC TCT TGC CTA CGC CAA***CAGCTCCAACTACCA CAAG*CCCC-3′ (48 nt)
Wild-type DNA	5′-TTTTGGGCACTCTTGCCTACGCCACCAGCTCCAACTA CCACAAG CCCC-3′
Ss-probe 1	5′-GCT ACG GAC TAA ACG-DABCYL-3′ (15 nt)
Ss-probe 2	5′-FAM-CGT TTA GTC CGT AGC **CTT GTG GTA GTT GGA GCT GT**-3′ (35 nt)

Preparation of the Molecular Switch. The molecular switch had two states which were self-hybridized 14 bases and 9 bases, respectively. In absence of mutant DNA, almost all the molecular switch self-hybridized into the state of 14 bases hybridization. Whereas, in presence of mutant DNA, most of the molecular switch changed into the complex of molecule switch and mutant DNA owing to entropy-driven. The free energy change decreased in this process. The free energy difference between the molecule switch ($\triangle G = -18.21$) and the complex of molecule switch and mutant DNA which was 20 bases hybridization ($\triangle G = -30.9$) was approximated using the mFold server (http://mfold.bioinfo.rpi.edu/cgi-bin/dna-form1.cgi) using DNA parameters for

25℃, with salt conditions being 10 mM Na^+ and 12.5 mM Mg^{2+}. Such, a forward reaction automatically occurred due to $\triangle G<0$. The reaction mechanism presented here differed from the traditional view of catalysis in biological organisms in that it required no enzymes and altered no covalent bonds.

Apparatus. Fluorescence intensities were recorded on a Hitachi F-4500 spectrophotometer (Tokyo, Japan) equipped with a xenon lamp. UV-Vis measurements were performed with a Cary 50 UV-Vis-NIR spectrophotometer (Varian) for oligonucleotide quantitation.

Single-base Mutation Detection. Single-base mutation detection was performed consisting of 10^{-7} M molecular switch, 4.8×10^{-8} M ss-probe **1**, 3.0×10^{-8} M ss-probe **2**, 200 μM dNTPs, 0.25 U/μL T4 DNA ligase, 0.133 U/μL polymerase Klenow Fragment (exo^-), and 10% PEG in T4 DNA ligase reaction buffer (40 mM Tris–HCl, 10 mM magnesium chloride, 10 mM dithiothreitol and 0.5 mM ATP, pH7.8). A series of targets (mutant or wild type) at different concentrations were added to the above resulting mixture solution and the fluorescence measurements were performed.

The progress of entropy-driven of the molecule switch and mutant DNA was visualized using denatured polyacrylamide gel electrophoresis (20% separation gel, 5% spacer gel). In presence of mutant DNA, most of the molecular switch changed into the complex of molecule switch and mutant DNA. At the same time, the molecular switch transformed into self-hybridization 9 bases, and the polymerization reaction of molecular switch happened in presence of polymerase and dNTPs with 71 nt amplified products (Fig. S1).

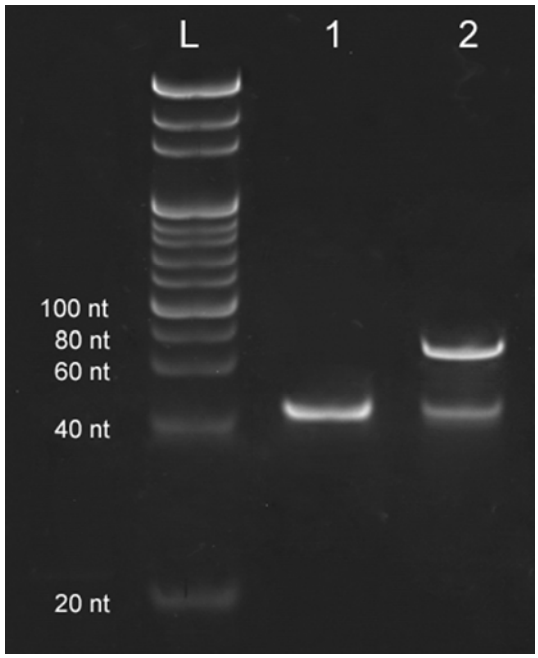

Fig. S1 Denatured polyacrylamide gel electrophoresis of the progress of entropy-driven of the molecule switch and mutant DNA. L. 20 bp DNA Ladder 1. The reaction containing only 10^{-7} M molecular switch and 10^{-7} M mutant DNA 2. The sample of lane 1 was added DNA polymerase and dNTPs.

The self-amplification process was confirmed by demonstrating formation of the DNA reaction products by gel electrophoresis. The amounts of expected reaction products (141 nt) increased with increasing the concentration of mutant DNA (Fig. S2).

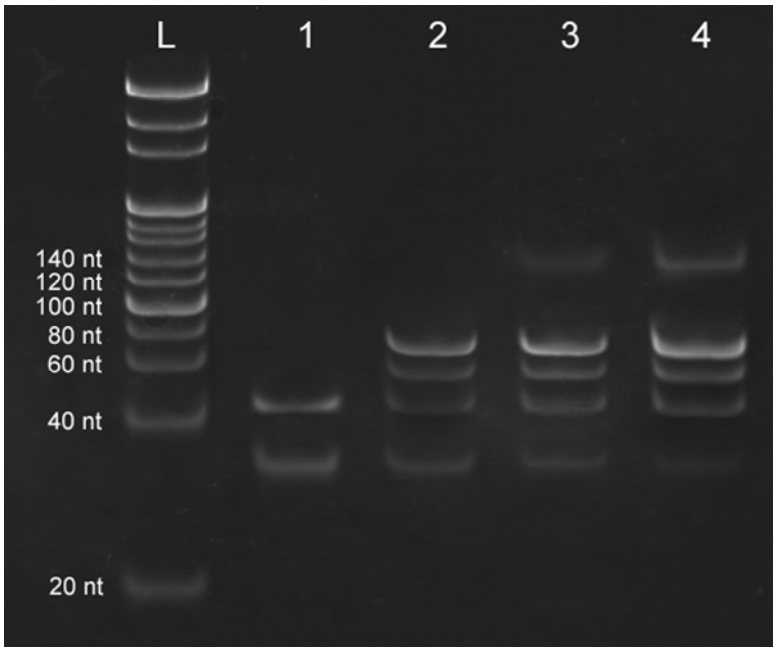

Fig. S2 Denatured polyacrylamide gel electrophoresis of different concentrations of mutant DNA L. 20 bp DNA Ladder 1-4. The amplification reactions containing 10^{-7} M molecular switch, 3.0×10^{-8} M ss-probe **2**, 200 μM dNTPs, 0.25 U/μL T4 DNA ligase, 0.133 U/μL polymerase Klenow Fragment (exo^-), which were initiated with different concentrations of mutant DNA (0 nM, 0.8 nM, 3 nM, 10 nM).

Fluorescence measurements. Before adding targets, molecular switch and ss-probes were diluted in T4 DNA ligase reaction buffer from stock solution, and incubated at room temperature for 10 min to form hairpin and heteroduplex. Following the addition of different concentrations of mutant DNA and 0.25 U/μL T4 DNA ligase were incubated at 25℃ for 30 min. Next, 0.133 U/μL DNA polymerase were added at 25℃ for 1 h. Then the fluorescence intensities were recorded. The slit widths were 5 nm. The emission spectra were obtained by exciting the samples at 480 nm and scanning the emission from 500 to 600 nm. All the experiments were performed in triplicates.