Supplementary Information

Signal amplification by rolling circle amplification on universal flaps yielded from target-specific invasive reaction

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The specificity of gap-ligation-based HRCA. Gaps (1000 fM) with an artificially mismatched base at the middle (M-mis), 3' terminal (3'-mis), and 5' terminal (5'-mis) of the gap were added to ligation reactions, respectively, followed by HRCA to investigate the specificity of gap-ligation-based HRCA. To quantify the specificity, the amount of mismatched gaps ligated with the padlock probe was measured by comparing the signal of mismatched gaps with that of the completely matched gap. As shown in Figure S1, the percentages of ligated amounts of the three mismatched-gaps relative to that of the fully matched gap are estimated as less than 0.001% (M-mis in Figure S1), about 0.01% (3'-mis in Figure S1), and 10% (5'-mis in Figure S1), respectively.

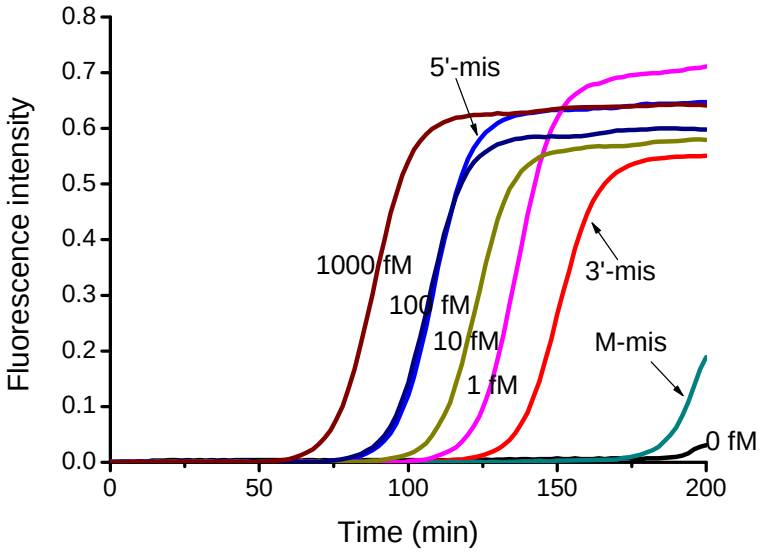

Figure S1. Time-courses of HRCA on the gap at various concentrations (1000 fM, 100 fM, 10 fM, 1 fM, and 0 fM) and gaps (1000 fM) with an artificially mismatched base at the middle (M-mis), 3' terminal (3'-mis), and 5' terminal (5'-mis).

The backgrounds of components of invasive reaction. The reagents used in invasive reaction was added to gap-ligation based HRCA reaction, and caused a high background signal as shown in Figure S2A. To look for which component is mainly responsible for the background, probes **up** and **dp**, and *Afu* flap endonulease with the invasive reaction buffer were individually added to ligation reaction followed by HRCA reaction. As shown in Figure S2B, only the probe **dp** caused the background signal, indicating the probe **dp** is the source of background. In addition, the nondenaturing polyacrylamide gel electrophoretograms (Figure S2C) of products of gap-ligation-based HRCA on **dp** and 1 pM gap had a same pattern.

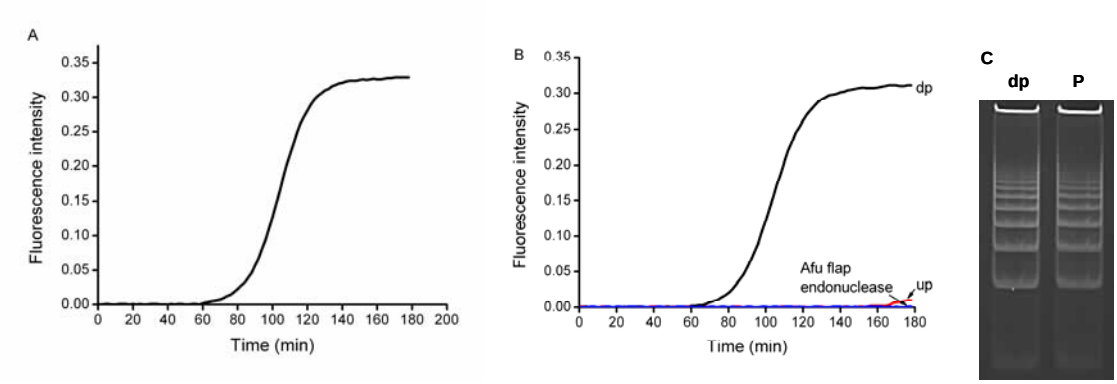

Figure S2. The background of invasive reaction coupled with HRCA. (A) Time-course of gap-ligation-based HRCA on all components in invasive reaction. (B) Time-course of gap-ligation-based HRCA on **dp**, **up**, and *Afu* flap endonuclease in invasive reaction. (C) Nondenaturing polyacrylamide gel electrophoretograms of the products of gap-ligation-based HRCA on **dp** (0.1 μM) and 1 pM gap (P).

Invasive-HRCA with different concentrations of dp. Because the high background of the probe **dp**, the sensitivity of invasive-HRCA was decreased. To lower the background, the most efficient way is to reduce the concentration of the probe **dp**. Although the background signal from negtive controls (Figure S3N) decreased as the decrease of concentrations of **dp**, the signals from positive controls (Figure S3P) also reduced in proportion to the decrease of **dp** concentrations, because the invasive reaction rate depends on **dp** concentration. To keep a high invasive reaction rate, we used the concentration of **dp** identical to that in conventional invasive reaction. [1, 2]

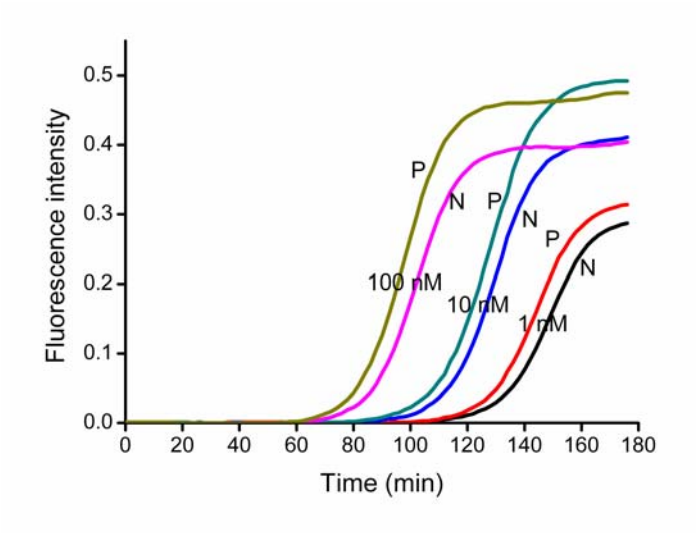

Figure S3. Time-courses of HRCA on invasive reactions using different concentrations of **dp** (100 nM, 10 nM, and 1 nM). P and N are the curves of HRCA on invasive reactions with 1 fM target DNA (**T1**) and 0 fM target DNA.

The amplification fold of invasive reaction. To investigate the amplification fold of invasive reaction, experiments of HRCA with vaious amounts of the synthesized gaps and experiments of invasive-HRCA with different amounts of targets were carried out. As shown in Figure S4, the signals from one tenth of the products of 1, 10, and 100-fM targets invasive reactions are higher than that from 1, 10, and 100 pM synthesized gaps, indicating that the amplification fold of invasive reaction is more than 10000.

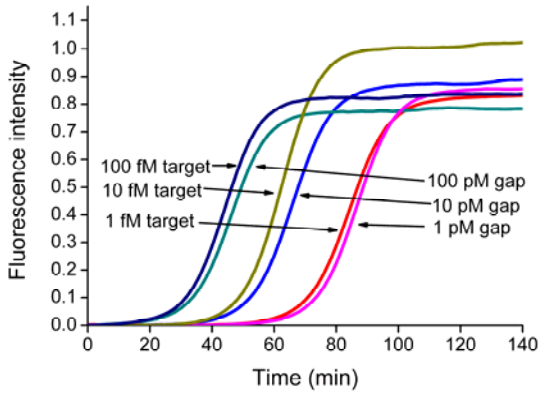

Figure S4. Comparison of HRCA signals from one tenth of 1, 10, and 100 fM-targets invasive reactions with that from 1, 10, and 100 pM synthesized gaps.

Sample detection by Invasive-HRCA. Two biological samples (**a** and **b**) from two different HBV carriers were detected by Invasive-HRCA. As shown in Figure S5, the signals from both samples were higher than that of negtive control (N). The concentrations of target HBV DNA in the two samples (**a** and **b** in Figure S5) were caculated to be 7.0 fM and 19.9 fM, respectively, according to the standard curve (data

not shown) using synthesized HBV DNA with various concentrations as templates of Invasive-HRCA.

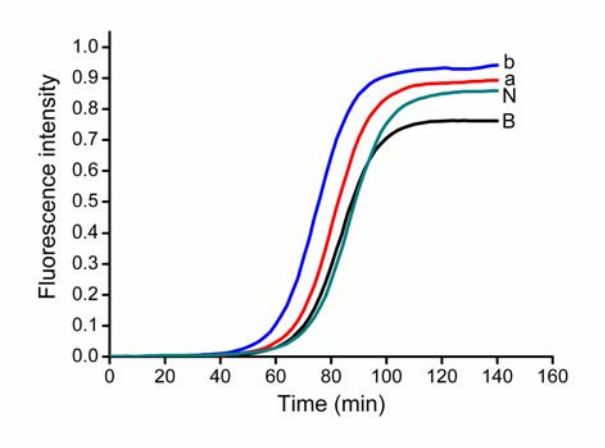

Figure S5. The time-courses of HRCA on invasive reactions for real biological samples detection. DNAs extracted from two different HBV carriers' blood (**a** and **b**) were detected. **B** is blank control (without any target), and **N** is HBV-negtive sample control.

Reference

[1] V. Lyamichev, A. L. Mast, J. G. Hall, J. R. Prudent, M. W. Kaiser, T. Takova, R. W. Kwiatkowski, T. J. Sander, M. de Arruda, D. A. Arco, B. P. Neri, M. A. Brow, Nat. Biotechnol. 1999, 17, 292-296.

[2] V. I. Lyamichev, M. W. Kaiser, N. E. Lyamicheva, A. V. Vologodskii, J. G. Hall, W. P. Ma, H. T. Allawi, B. P. Neri, Biochemistry 2000, 39, 9523-9532.