Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Highly sensitive detection of cancer-related genes based on completely fluorescence restoration of molecular beacon with functional overhang

Contents

Table 1 Oligonucleotide sequences designed in the current study [a].

Note		Sequence (5'-3')
Molecular beacon (MB)	OMB	DABCYL-*cAT**ggatcc**TGACACG**GCTGA**GG*GGTGGCGTAGGACACGA GCTCCAGGATGCATGt (FAM)TTT
Primers	Primer1	AGAGACCGGCGCACAGAGAAAACATGCATC
	Primer2	AGAGACCGGCGCACAGAGAAAACATGCA
	Primer3	AGAGACCGGCGCACAGAGAAAACATG
	Primer4	AGAGACCGGCGCACAGAGAAAACA
	Primer5	AGAGACCGGCGCACAGAGAAAA
Recognition probe (RP)	RP1	GATGCATGTTTT**AGAGACCGGCGCACAGAG**AAAACATGCATC
	RP2	ATGCATGTTTT**AGAGACCGGCGCACAGAG**AAAACATGCAT
	RP3	TGCATGTTTT**AGAGACCGGCGCACAGAG**AAAACATGCA
	RP4	GCATGTTTT**AGAGACCGGCGCACAGAG**AAAACATGC
	RP5	CATGTTTT**AGAGACCGGCGCACAGAG**AAAACATG
	RP6	ATGTTTT**AGAGACCGGCGCACAGAG**AAAACAT
	RP7	TGTTTT**AGAGACCGGCGCACAGAG**AAAACA
	RP4a	GCATGTTTT**GAGAG**ACCGGCGCACAGAGGCTCTCAAAACATGC
	RP4b	GCATGTTTT**GAG**AGACCGGCGCACAGAGGCTCAAAACATGC
	RP4c	CATGTTTT**GAG**AGACCGGCGCACAGAGGCTCAAAACATGC
	RP4d	TGTTTT**GAG**AGACCGGCGCACAGAGGCTCAAAACATGC
Nicked fragment (NF)	NF1	*TCAGCCGTGTCAGGATCCATG*
	NF2	*TCAGCCGTGTCAG*
Target DNAs	WT	TT**CCTCTGTGCGCCGGTCTCTC**CT
	MT1	TT**CCTCTGTGCGCCAGTCTCTC**CT
	MT2	TT**CCTCTGTGCGCCAGTATCTC**CT
	MT3	TT**CCTCTGTACGCCAGTATCTC**CT

[a]For OMB (full name: functional overhang-contained MB), the FAM and DABCYL were attached onto the lowercase base 't' and 'c', respectively. The strand sequence is able to fold into a stem-loop hairpin structure with a 'tTTT' overhang at the 3' end via the self-hybridization of two underlined fragments. The capital bold region is the half binding site of nicking endonuclease Nt.BbvCI, while the lowercase bold region is the half binding site of restriction endonuclease *Bam*HI. The introduction of boxed base 'G' is designated to form the mismatched-base pair in the stem region into order to inhibit the cleavage by *Bam*HI. The fragment highlighted by the gray background is designed to hybridize with the gray regions of primers and recognition probes, and the italic fragment is complementary to the nicked fragments (NF1 and NF2), which are designed to have the same base sequence as the nicked products during DNA detection. Note that recognition probes are also capable of forming the hairpin structure, in which the bold region can hybridize to the bold fragment of target DNAs. For the target DNAs, the wild target DNA is abbreviated as WT, while point mutations in mutant target DNAs (MTs) are indicated by underlines.

Table S2. Comparisons of detection capability of the present sensing system with

previous detection systems.

Reference	Sensitivity	Detection time	Linear range
polymerization-mediated strand-displacement amplification (fluorescent method)[1]	0.08 nM	~30 min	0.4 ~1000 nM
multifunction-integrated molecular beacon (fluorescent method) [2]	0.5 nM	~120 min	0.5 ~400 nM.
common MB (fluorescent method)[3]	1.1 nM	~1020 min	1.1~100 nM
common MB (electrochemical method) [4]	0.1 nM	~960 min	0.1~1000 nM
polymerization-mediated strand-displacement amplification (electrochemical method) [5]	0.2 nM	~40 min	2.6~166.7 nM
Present work	0.0082 nM	~120 min	0.01~150 nM

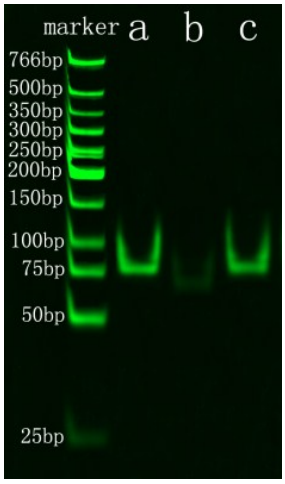

Fig. S1 Hybridization of NF1 or NF2 with OMB. Native-PAGE (12%) analysis was performed to explore mixture of OMB+ NF1 (a), OMB+ NF2 (b), OMB+ NF2+polymerase (c). The polymerization was carried out at 37 ℃ for 90 min. OMB at 300 nM was used, while the concentration of NF1 and NF2 is150 nM.

In the proposed OMB biosensing system, the extended/nicked/displaced product-mediated signal amplification is an essential issue for the improvement of the assay perform. NF1 is expected to hybridize with OMB and triggers the subsequent signal amplification procedures, while NF2, though with short base sequence, could be extended by polymerase after hybridization to OMB, generating the same hybrid as the NF1/OMB duplex. In order to affirm this assumption, the NF1 and NF2 were commercially synthesized and exposed to OMB, and the polymerase was added into the mixture of NF2/OMB. The resulting DNA products were analyzed by native PAGE, and the results are shown in Fig. S1. One can see that a clear band appears in lane a, while a dim band with faster gel mobility is observed in lane b, indicating that NF1 can easily hybridize with the OMB and NF2 almost not cannot open the OMB. However, challenge with polymerase leads to the band shift and brightness increase (seen in lane c), demonstrating the extension of NF2 and OMB opening as detected in lane a.

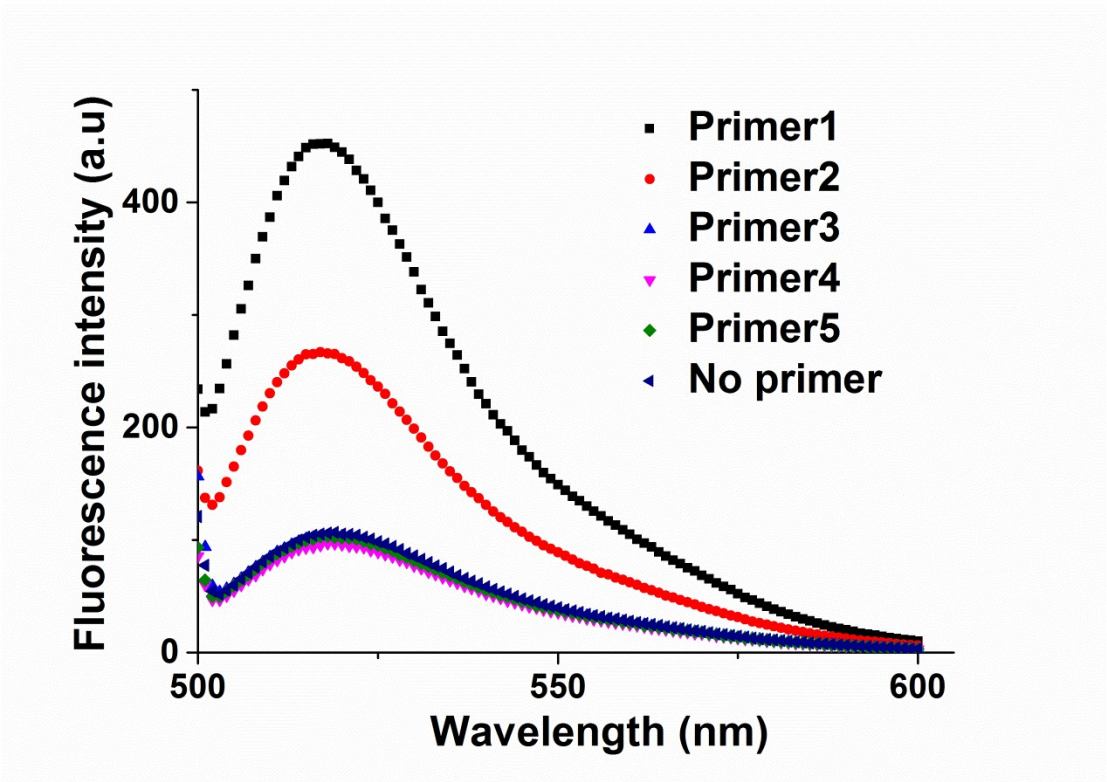

Fig. S2 The preliminary screening of primers capable of executing the polymerization in the presence of polymerase and dNTPs. The operation procedure: 46.5 μL of Tris-buffer, 1.5 μL of 10 μM OMB and 0.5 μL of 10 μM primers, 0.5 μL of KF polymerase and 1 μL of 10 mM dNTPs were successively added into an eppendorf tube. After mixing thoroughly, the resulting solution was incubated at 37 ℃ for 45 min. Then, the polymerization was terminated at 80 ℃ for 20 min, followed by slowly cooling down to room temperature and keeping on ice for 30 min. After adding 150 μL of Tris buffer, the emission spectrum was collected from 500 to 600nm. The measured data indicate that primer1 and primer2 can trigger the polymerization reaction after interaction with OMB to different degrees compared with primer3, primer4 and primer5. On the basis of these experimental results, various recognition probes (RPs), including RP1 to RP7, were designed, whose terminal fragments complementary to OMB are not longer than 12 bases.

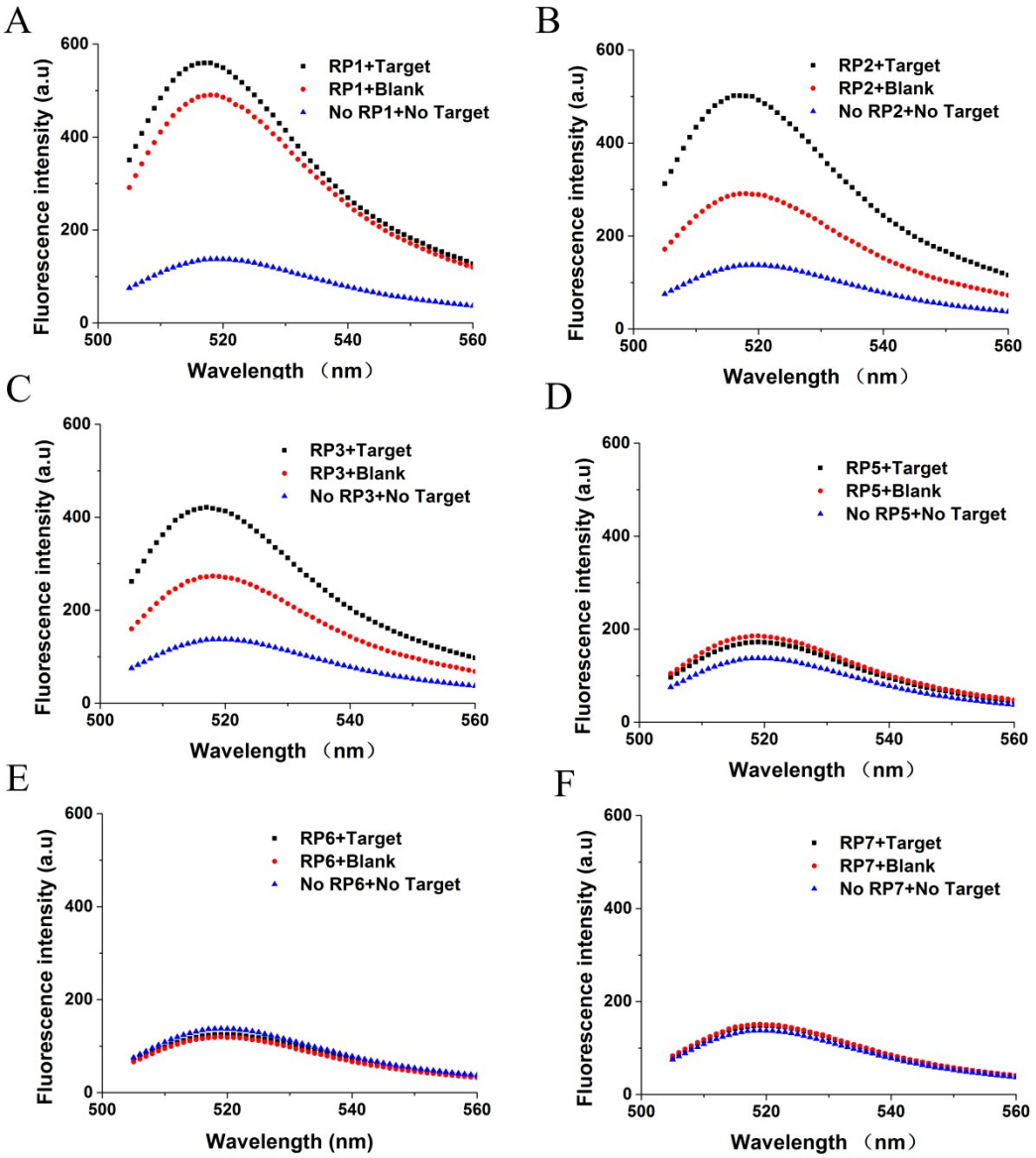

Fig. S3 The exploration of recognition probes (RPs). The fluorescence spectra wre collected from the sensing system where RP1 (A), RP2 (B), RP3 (C), RP5 (D), RP6 (E) or RP7 (F) was employed. The experiments were carried out under the identical conditions adopted in Fig. 2.

Reference

1. L.-P. Qiu, Z.-S. Wu, G.-L. Shen and R.-Q. Yu, *Analytical chemistry*, 2011, **83**, 3050-3057.
2. H. Dong, Z.-S. Wu, J. Xu, J. Ma, H. Zhang, J. Wang, W. Shen, J. Xie and L. Jia, *Biosensors and Bioelectronics*, 2015, **72**, 182-190.
3. X. Liu and W. Tan, *Analytical chemistry*, 1999, **71**, 5054-5059.

4. X. Mao, J. Jiang, X. Xu, X. Chu, Y. Luo, G. Shen and R. Yu, *Biosensors and Bioelectronics*, 2008, **23**, 1555-1561.
5. L. Qiu, Z. Shen, Z.-S. Wu, G.-L. Shen and R. Yu, *Biosensors and Bioelectronics*, 2015, **64**, 292-299.