Supplementary materials

A simple fluorescence biosensing strategy for ultrasensitive detection of BCR-ABL1 fusion gene based on DNA machine and multiple primers-like rolling circle amplification

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Table S1. Synthetic oligonucleotide sequences used in the strategy

Name	**Sequence (from 5' to 3')**
BCR-ABL1	CAG ATG CTA CTG GCC GCT GAA GGG CTT TTG AAC TCT GCT TAA ATC CAG TGG CTG AGT GG
DNA1	AAT ATT ATA TAC CTC AGC GAG AAC GTT *CAG CGG CCA GTA GCA TCT*-C6
DNA2-3	*CCA CTC AGC CAC TGG ATT TAA GCA GAG TTC AAA AGC CCT* TTT **CGT**
DNA2-4	*CCA CTC AGC CAC TGG ATT TAA GCA GAG TTC AAA AGC CCT* TTT **CGT T**
DNA2-5	*CCA CTC AGC CAC TGG ATT TAA GCA AGA TTC AAA AGC CCT* TTT **CGT TC**
DNA2-6	*CCA CTC AGC CAC TGG ATT TAA GCA GAG TTC AAA AGC CCT* TTT **CGT TCT**
Amplification primer	TGA GGT ATA TAA TAT T
Splint strand	CCG GGG TGG GGC TGA GGT ATA TCC-C6
Linear RCA	GCC CCA CCC CAC CCC ACC CCA ATA TTA TAT ACC TCA GCC CCA CCC CAC CCC ACC CCA ATA TTA TAT ACC TCA
Final product	TGA GGT ATA TAA TAT TGG GGT GGG GTG GGG TGG GGC
BCR	TCC TCC CCA AAC CAG TAC TTA CTT GAA CTC TGC TTA AAT CCA GTG GCT GAG TGG
ABL	CAG ATG CTA CTG GCC GCT GAA GGG CTT CTG GAA GAG AAA GGG GGG AAC AGA AAA
NC	AGA AGC AGC AAC AAC GAT TGT TTC GCC AAT GAA GAC ATA TTC TTC TGC

The underlined italics represent the complementary portion of DNA1 and DNA2 to BCR-ABL1 fusion gene. Bold letters indicate bases where DNA2 and DNA1

hybridize to each other. C6 indicates that the C6 group has been modified at the 3' end of the oligonucleotide. Green represents the G-quadruplex functional DNA sequence. Red indicates the nicking enzyme recognition sequence.

The sequence design of this biosensing strategy mainly contained three modules: two DNA probes and a circle DNA template. Two DNA probes, named DNA1 and DNA2, were both incorporated BCR-ABL1 fusion gene recognition region and short mutual hybridization region. In addition to these complementary sequences, DNA1 also stretched out a short arm of single strand, including nicking enzyme recognition sequence and downstream primer complementary sequence. The circle DNA template for RCA was designed that contains complementary sequences of amplification primer, nicking endonuclease recognition sequence and G-quadruplex. These three complementary sequences constituted a nicking unit and there were two nicking units designed in the circle DNA template.

Table S2. Comparison between the biosensing strategy and other reported methods for DNA detection.

Signal amplification [a]	Analytical method [b]	Linear range (M)	Detection limit (M)	References
QDs/FRET	FLU	1.25×10^{-7}~10^{-9}	1.5×10^{-10}	[1]
DHMB/RCA	FLU	2×10^{-8} ~10^{-13}	1.6×10^{-14}	[2]
ExoⅢ/CHA	DPV	5×10^{-8} ~ 10^{-13}	9.2×10^{-14}	[3]
DNA machineries	ECL	10^{-7} ~ 10^{-13}	2.3×10^{-14}	[4]
3-WJ/RCA	FLU	10^{-9}~10^{-14}	5.5×10^{-15}	This work

a QDs: quantum dots, FRET: fluorescence resonance energy transfer, DHMB: double-

hairpin molecular beacon, RCA: rolling circle amplification, CHA: catalytic hairpin assembly, 3-WJ: three way junction.

b FLU: Fluorescence, ECL: Electrochemiluminescence, DPV: differential pulse voltammetry.

Table S3. The recoveries determined using the developed method via spiking synthetic DNA into 20% diluted normal serum samples.

Sample no.	Spiking value	Assayed value	Recovery	RSD
1	50.00 fM	49.63 fM	99.26%	1.80%
2	5.00 pM	4.63 pM	92.70%	2.20%
3	500.00 pM	518.86 pM	103.80%	2.70%

References

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