Electronic Supplementary Information:

A label-free amplified fluorescence DNA detection based on isothermal circular strand-displacement polymerization reaction and graphene oxide

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Experiment Section

Materials and reagents

Klenow fragment (3'→5' exo$^-$, KF), deoxyribonucleoside triphosphates (dNTPs) were purchased from New England Biolabs Ltd (Beijing, China). SYBR Green I (SG, 10000×concentrate) was obtained from Dingguo Biochemical Reagents (Beijing, China). GO was synthesized by a modified Hummers' method.[1] DNA oligonucleotides used in this work were carefully designed by our team, and synthesized by Sangon Biotechnology Co. Ltd (Shanghai, China). The sequences of oligonucleotides are given in Table 1. All other purchased materials were used as received without further purification. All the reagents were prepared with ultrapure water (resistance > 18.2 MΩ.cm, prepared from Millipore Milli-Q water purification system) unless otherwise stated and deionized and sterilized ultrapure water was used throughout experiment. The buffer solutions used in this study were as following. Reaction buffer was 1×NEB buffer 2 (10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, PH 7.9), and detection buffer contains 10 mM Tris-HCl, 100 mM NaCl, and 5 mM $MgCl_2$ (pH 8.0).

Assay procedures

HP was firstly heated at 95°C for 5 min then being transferred onto the ice surface and kept for 10 min, the solution was diluted to 1 μM and kept at 4 °C for later use. In a typical assay, 3 μL HP (1 μM), 3 μL primer 1 (1 μM) and different concentrations of target DNA (3 μL) were firstly incubated in reaction buffer, followed by addition of 2.5 units KF and 3 μL dNTPs (1mM), leading to a final reaction volume of 30 μL.

Subsequently, the above reaction mixture was kept at 37°C for 1 h. After that, SG (2 μL, 100×) was added to stain the SDA products at room temperature for 10 min. Finally, 6 μL GO (200 μg/mL) and detection buffer were injected to give a final volume of 100 μL. After 10 min reaction time, the fluorescence spectra were recorded.

Instruments

All fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrometer (Hitachi Ltd., Japan). The optical path length of a quartz fluorescence cell was 1.0 cm. Excitation and emission wavelengths were set at 497 and 525 nm, respectively, and the fluorescence spectra were recorded from 508-600 nm. Both the excitation and emission slits were set for 5 nm and PMT detector voltage was 800 V. All fluorescence detections were carried out under room temperature unless otherwise indicated.

Table 1 Synthesized oligonucleotide sequences used in the experiments.

Oligonucleotides	Sequence (5'→3')
HP	TAGAGAGAGAGAGAGAGTCTTGGACACAGTAAAGAGAG GTGCGCCCATTGTGTCCAAGA-p
Target DNA	ATGGGCGCACCTCTCTTTACTGTGTCTTT
Primer 1	TCTTGGAC
Primer 2	TCTTGGACA
Primer 3	TCTTGGA
T1-m	ATGGGCGCACCTATCTTTACTGTGTCTTT
T4-m	ATGGGCTCACCTATCTTTAATGTTTCTTT
T-n	TTCGTTCTTCAATGAAGTGGGACGACA

The mutation site is highlighted in red in target sequence. Letter "p" at the 3' end of HP sequence represents the phosphate.

Table 2 Analytical performance comparisons of different assay methods for DNA.

Method	Linear range	LOD	Ref
Immobilization-free electrochemical DNA sensor	1-20 nM	0.1 nM	13
Amplified electrochemiluminescence detection	~	10 pM	14
Label-free fluorescent enzymatic amplification	0.6-3 nM	36 pM	26
GO-based chemiluminescence biosensor	0.1-3 nM	34 pM	22
Multicolor fluorescent DNA analysis	0-25 nM	100 pM	16
GO-based molecular beacon	5-500 nM	2 nM	15
LrRET strategy	0.5-100 nM	0.31 nM	17
DNA–CdTe Quantum Dots	50-1600 nM	10.4 nM	23
Bifunctional oligonucleotide probe based fluorescence	0.4-1000 nM	80 pM	24
Label-free optical bifunctional oligonucleotide probe	2-2000 nM	2 nM	25
label-free amplified fluorescence detection	0.01-10 nM	4 pM	This work

LrRET: long-range resonance energy transfer; LOD: limits of detection.

References

1 Y. G. Li and Y. Y. Wu, *J. Am. Chem. Soc.*, 2009, **131**, 5851-5857.