Supporting Information

Novel design of DNA duplex containing programmable sensing site for nanopore-based length-resolution reading and application on Pb^{2+} and cfDNA analysis

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Table 1. All oligonucleotide sequences used in this study

Oligonucleotide sequences used when the target molecule was Pb^{2+}

Name	**Sequences（5'-3'）**
DNAzyme	AATCATCTCTGAAGTAGCGCCGCCGTAGTG
412bp-Forward primer	CTGACCCTGATGAGTTCGTGT
819bp-Forward primer	CCGAAAGAATCCGCATACCAG
15+412-Reverse primer	CTCTAGGTACTCTTASpacer18CGTTCCCGGCAGCACAAAT
20+412-Reverse primer	AGGTTCTTTGTATGCGATCASpacer18CGTTCCCGGCAGCACAAAT
25+412-Reverse primer	CTTCTTGATGATCCGACGGCCTAGTSpacer18CGT

	TCCCGGCAGCACAAAT
15+Sub+819-Reverse primer	TAAGAGTACCTAGAGCACT/rA/GGAAGAGATGATTTSpacer18ACCGGATGACGAAAACCAGAG
20+Sub+819-Reverse primer	TGATCGCATACAAAGAACCTCACT/rA/GGAAGAGATGATTSpacer18ACCGGATGACGAAAACCAGAG
25+Sub+819-Reverse primer	ACTAGGCCGTCGGATCATCAAGAAGCACT/rA/GGAAGAGATGATTSpacer18ACCGGATGACGAAAACCAGAG

Oligonucleotide sequences used when the target molecule was cfDNA

Name	**Sequences（5'-3'）**
411bp-Forward primer	GTTTTCATGTTGCCTGCCCG
411bp-Reverse primer	TTACAACCTCGTCAGAATCCSpacer18GTTCGCCAATTTTCGCCTCC
Sub+791bp-Forward primer	GGATTCTGACGAGGTTGTAAGTCCGTTCAACCTCCCATCTCAGTSpacer18CAGCGTCACAACAATCAGCC
791bp-Reverse primer	TCAGTGTCGCATTCTTCGGT

crRNA	UAAUUUCUACUAAGUGUAGAUTGGCAAACTC AGGTAGAATT
BRCA-1	GGGCAGGCACTTTATGGCAAACTCAGGTAGAA TTCTTCCTCTTCCGTCT
	AGACGGAAGAGGAAGAATTCTACCTGAGTTTG CCATAAAGTGCCTGCCC
BRCA-2	TGGCTTAGAGAAGGAATATTTGATGGTCAACC AGAAAGAATAAATACTGC
	GCAGTATTTATTCTTTCTGGTTGACCATCAAAT ATTCCTTCTCTAAGCCA
BRAF	GTTATCAGACTTGAATGTTCGTAAAGTTTATAA GCTCTGCCCTGTCTGTA
	TACAGACAGGGCAGAGCTTATAAACTTTACGA ACATTCAAGTCTGATAAC
MCRC	TTGGCTTGTGTACCGTCAATTTAGGTCAGGAAT GGCCGTGTATACATCAA
	TTGATGTATACACGGCCATTCCTGACCTAAATT GACGGTACACAAGCCAA
miR-21	ATCAATCCTGCTTGAATATCCACTTTGTACCCA TCATCGTCTGTGACATC
miR-141	ATCTTACTAGACAGTGCTGGATTTCTTGGATCT

	ATTCTAACACTGTCTGG

Sequences of special colours are random complementary sequences. The underlined sequences are substrates of GR-5 DNAzyme and CRISPR/cas12a system.

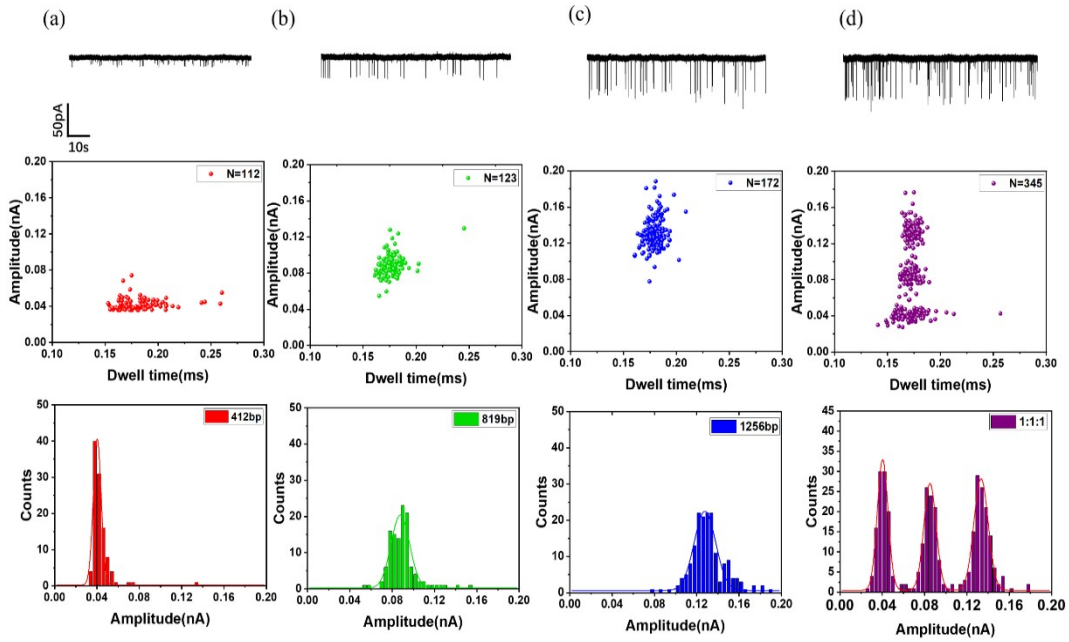

Fig. S1 Experiment results of measuring signals of dsDNA with different lengths using glass nanopores. The diagrams are the current time trace, the scatter plot of event amplitude versus dwell time, and the frequency distribution histogram of the event amplitude. (a) 412bp (b) 819bp (c) 1256bp (d) Mixed. (Concentration ratio of 412 bp: 819 bp: 1256 bp=1:1:1) All data were obtained by recording at -1000 mV for 5 mins.

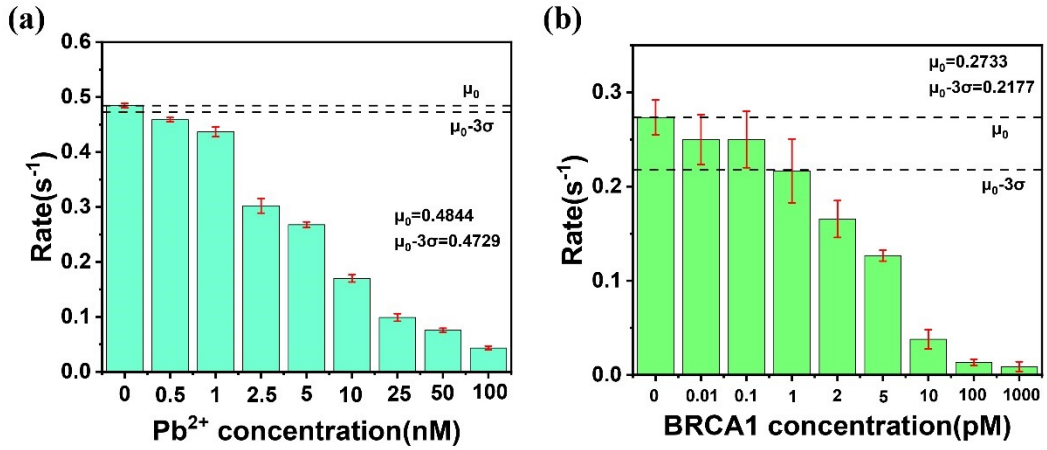

Fig. S2 Event rates of the sensor when detecting different concentration of target molecules. When the S/N =3 the detection limit was calculated as: a) 0.4 nM for detection of Pb^{2+}; b) 1 pM for detection of BRCA-1.