Supporting information

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Instruments

ARMS-PCR were carried on LightCycler® 480 Instrument II (Rothe, Co., Ltd., Germany) and Applied Biosystems™ 7500 (Thermo Fisher Scientific, Co., Ltd., Shanghai, China). The gel electrophoresis and gel image were performed on DYY-6C electrophoresis analyzer (Liuyi Instrument Company, China) and Quantum ST4-1100, respectively. The fluorescence analysis were carried on FS5 Spectrofluorometer(Edinburgh Instruments Ltd.)

Table S1. Sequences of targets and probes used in this assay.

DNA	Sequence (5'-3')
BRAF V600E Wild target	GATGGGACCCACTCCATCGAGATTTCACTGTAGCTAGACCAAAATCACCTATTTTTACTGT
BRAF V600E Mutant target	GATGGGACCCACTCCATCGAGATTTC**T**CTGTAGCTAGACCAAAATCACCTATTTTTACTGT
K601E Mutant target	GATGGGACCCACTCCATCGAGATT**C**CACTGTAGCTAGACCAAAATCACCTATTTTTACTGT
V600R Mutant target	GATGGGACCCACTCCATCGAGATTTC**CT**TGTAGCTAGACCAAAATCACCTATTTTTACTGT
V600K Mutant target	GATGGGACCCACTCCATCGAGATTTC**TT**TGTAGCTAGACCAAAATCACCTATTTTTACTGT
Padlock probe	5po4-GACATCGATCTGGTTTTTCCTCATGCTTCTTCGGTGCCCATCATTCGATTCCTGCAGTTGCATTGAGGTAGCTCTAAAGA
Primer	GATGGGCACCGAAGAAGCAT
MB	DYBCY1-CGCACCCTAGCTACAGAGAAATCTCGAGGTGCG -FAM
FQ	DYBCY1-TTTTTTTTTTTT- -FAM
sgRNA	CUUCACUGAUAAAGUGGAGAACCGCUUCACCAAAAGCUGUCCCUUAGGGGAUUAGAACUUGAGUGAAGGUGGGCUGCUUGCAUCAGCCUAAUGUCGAGAAGUGCUUUCUUCGGAAAGUAACCCUCGAAACAAAUUCAUUUUUCCUCUCCAAUUCUGCACAAGAAAGUUGCAGAACCCGAAUAGACGAAUGAAGGAAUGCAAC**CUAGCUACAGAGAAAUCUCGA**

The mutation sites are in bold orange. The spacer sequences of crRNA are in bold red.

DNA extraction

AmoyDx@FFPE DNA Kit
180 μL Buffer DTL and 20 μL proteinase K solution was added in samples, and heated up to 56℃ for 1 hour after shaking and mixing. Next, 10 μL Buffer DES were added into the mixture and heat for 1 hour in constant temperature incubator. Then, 200 μL Buffer DTB and 200 μL absolute ethyl alcohol were mixed with the solution and transferred the solution into a DNA adsorption column followed by centrifuging (10000×g 1 min). After discarding filtrate, 600 μL Buffer DW1 was added into adsorption column to wash by centrifuging. Repeat the washing step by using 600 μL Buffer DW2. The DNA was eluted in 100μL Buffer DTE by centrifuging at 13000 g for 1 minute.

The cT value of the detected samples

Table S2. The cT value of the detected samples by ARMS-PCR

Sample code	CT value (Malignant)	Sample code	CT value (Benign
1	15.62	19	Undetermined
2	9.84	20	Undetermined
3	23.56	21	Undetermined
4	8.25	22	Undetermined
5	12.65	23	Undetermined
6	8.62		
7	13.59		
8	22.0		
9	24.15		
10	16.62		
11	11.50		
12	7.56		
13	18.69		
14	8.84		
15	19.65		
16	13.54		
17	14.83		
18	7.98		

Table S3 Comprehensive comparison of other CRISPR-based analytical performance

Signaling strategy	Detection method	Target Type	Detection limit	disease/pathogen	References
Cas9 + NASBA	Colorimetric	DNA	1 fM	Zika virus	1
dCas9 +PCR	Colorimetric	DNA	82 aM	African Swine Fever	2
Cas12a+ RPA	Fluorescence	RNA	10	SARS-CoV-2	3

			pM		
Cas12b+LAMP	Fluorescence	DNA	1pM	Several viruses	4
Cas13a +EXPAR	Electrochemiluminescence	miRNA	1 fM	Various tumor cells	5
Cas14a+RCA	Fluorescence	SNV	0.30 7 fM	BRAF V600E	This work

Table S4. The reproducibility of the developed biosensor of inter-assay and intra-assay in five parallel assays respectively (CmtDNA=10pmol)

Group	Parallel tests (a.u.)	Average (a.u.)	SD	RSD (%)
Inter-assay	169481；161415；153825；155884；165123	161145	5773.16	3.58%
Intra-assay	167452；167415；168625；163022；162565	165815	2509.95	1.51%

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