Supporting Information

Colorimetric detection of single-nucleotide mutation based on rolling circle amplification and G-quadruplex-based DNAzyme

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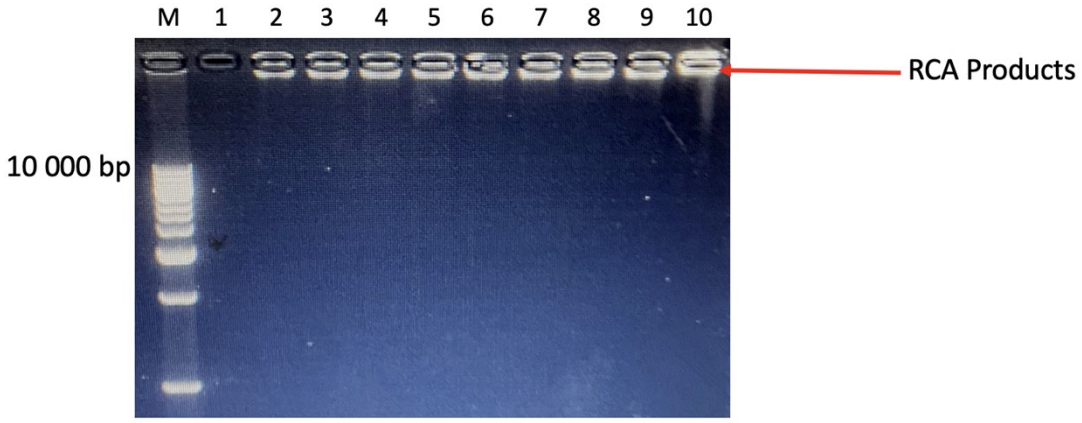

Fig. S1. Electrophoresis gel analysis of RCA products in the sensitivity assay. Lane 1 (0 nM of target DNA used), Lane 2 (0.01 nM), Lane 3 (0.1 nM), Lane 4 (0.5 nM), Lane 5 (1 nM), Lane 6 (10 nM), Lane 7 (50 nM), Lane 8 (100 nM), Lane 9 (500 nM), Lane 10 (1000 nM).

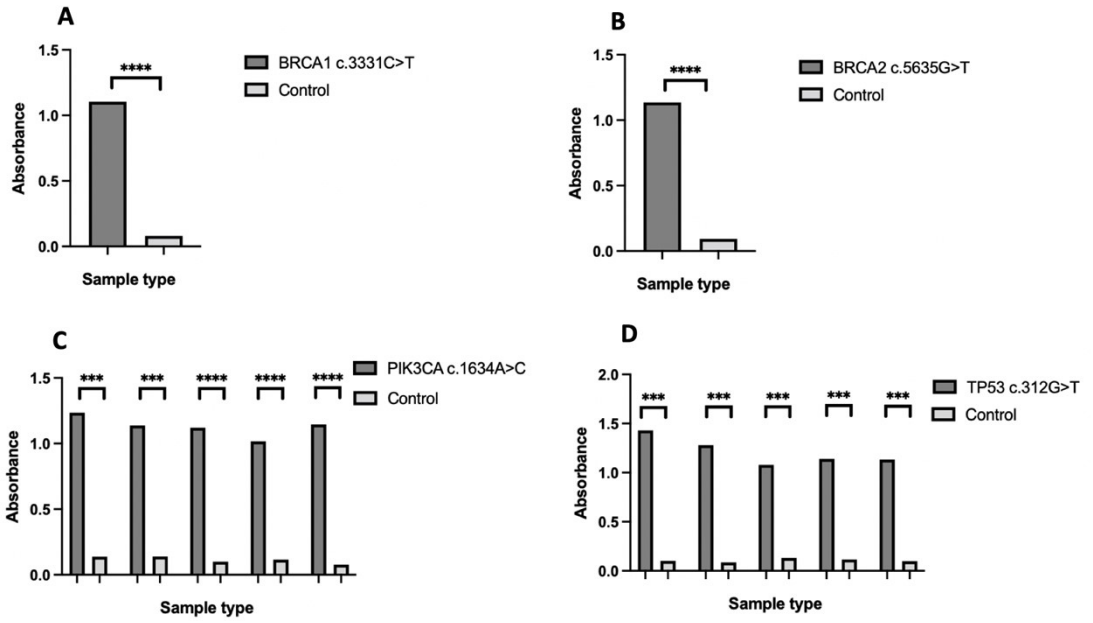

Fig.S2. Absorbance graphs for the mutation detection in breast cancer samples. (A) Graph showing the absorbance values of the mutant BRCA1 c.331C>T sample and a control WT sample. (B) Graph showing the absorbance values of the mutant BRCA2 c.5635G>T sample and a control WT sample. (C) Graph showing the absorbance values of mutant PIK3CA c.1634A>C samples and control WT samples. (D) Graph showing the absorbance values of mutant TP53 c.312G>T samples and control WT samples. All *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. The data are presented as the average and the error bars show the SD of 3 replicated experiments.

Table S1. Oligonucleotide sequences (5′ to 3′)

Targeted Mutation	Sequence* (5′ → 3′)	Size (nt)
BRCA1 (c.3331C>T)	p*TTCTTATACTTCTCCCTA*ACCCTAACCCTAACCCGACTTTATTTTTTC**A**	49
BRCA2 (c.5635G>T)	p*TCTTATTTAGTTTTCCCTA*ACCCTAACCCTAACCCATTCCTTTTGTTG**A**	49
PIK3CA (c.1634A>C)	p*CAGTGATTTCCCCTA*ACCCTAACCCTAACCCTTCTCCTGC**G**	41
TP53 (c.312G>T)	p*CCGTCGATGCCAAACCCTA*ACCCTAACCCTAACCCTCTTTTGGATGGT**A**	49
BRCA1 (c.3331C>T)	TAGGGAGAAGTATAAGAA	18
BRCA2 (c.5635G>T)	TAGGGAAAACTAAATAAGA	19
PIK3CA (c.1634A>C)	TAGGGGAAATCACTG	15
TP53 (c.312G>T)	TAGGGTTTGGCATCGACGG	19
BRCA1 (c.3331C>T)	CTGAAATAAAAAAGC/TAAGAATATGAAGA	29/29
BRCA2 (c.5635G>T)	TAAGGAAAACAACG/TAGAATAAATCAAAA	29/29
PIK3CA (c.1634A>C)	GAAATCACTGA/CGCAGGAGAA	21/21
TP53 (c.312G>T)	AGAAAACCTACCAG/TGGCAGCTACGGTTT	29/29
BRCA1 (c.3331C>T)	Forward primer: pTTAGGGGTTTTGCAACCTGAG	21
	Reverse primer: TAAGTTATCTGAAATCAGATAT	22
BRCA2 (c.5635G>T)	Forward primer: pTTTGTGTTTCACATGAAACAATT	23
	Reverse primer: ATCTAGAGAGTTATGAAGAATATC	24
PIK3CA (c.1634A>C)	Forward primer: pTGAATTAAGGGAAAATGA	18
	Reverse primer: CTGTGACTCCATAGAAAAT	19
TP53 (c.312G>T)	Forward primer: pTGCACCAGCCCCCTCCTGGC	20
	Reverse primer: GTGCAAGTCACAGACTTGGCT	21

*Red segment, C-rich region; Italic segment, RCA primer binding site; Bold segment, mutant recognition site; p, phosphorylation; nt, nucleotides; Underline, wild-type and mutant type sequence, respectively.

Table S2. Mutations and real BC samples number detected

Genes	Variants	BC samples detected
BRCA1[a]	c.3331C>T	1
BRCA2[b]	c.5635G>T	1
PIK3CA[c]	c.1634A>C	5
TP53[d]	c.312G>T	5
Control_BRCA1	-	5
Control_BRCA2	-	5
Control_PIK3CA	-	5
Control_TP53	-	5

[a]BRCA1 reference sequence NCBI RefSeq NM_007294.3 (mRNA) and NP_009225.1 (protein)

[b]BRCA2 reference sequence NCBI RefSeq NM_000059.3 (mRNA) and NP_000050.2 (protein)

[c]PIK3CA reference sequence NCBI RefSeq NM_006218.2 (mRNA) and NP_006209.2 (protein)

[d]TP53 reference sequence NCBI RefSeq NM_000546.5 (mRNA) and NP_000537.3 (protein)

Controls: Samples obtained from patients who did not harbor any of the mutations mentioned above.