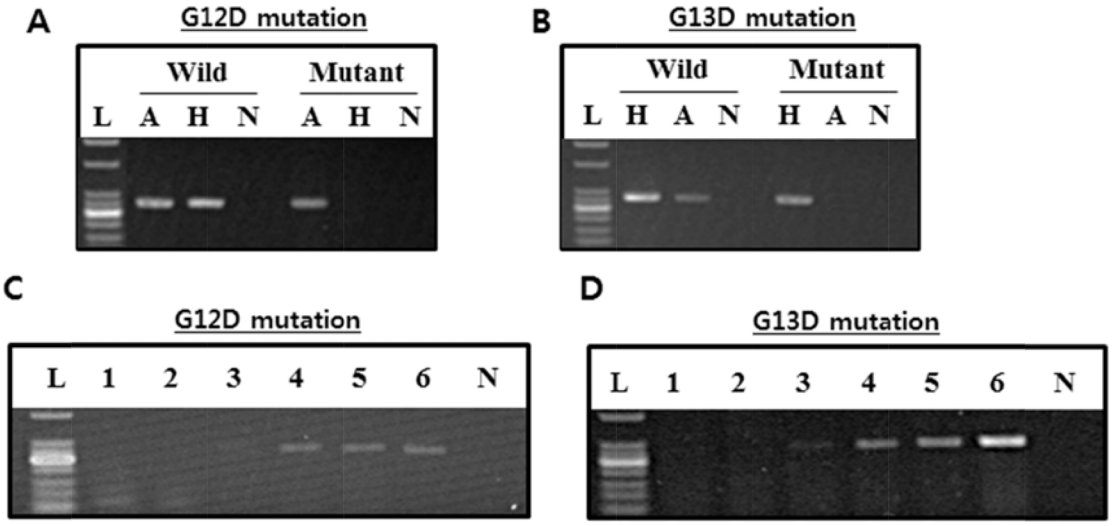
Rapid and accurate detection of *KRAS* mutations in colorectal cancers using the isothermal-based optical sensor for companion diagnostics

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Characterization of *KRAS* mutant primers using PCR. (**A**–**B**) Gel electrophoresis after PCR with the wild-type primer and G12D mutant primer (A; L: 50 bp DNA marker, A: DNA obtained from the AGS cell line, H: DNA obtained from the HCT116 cell line, N: no DNA). DNA amplification with the wild-type primer and G13D mutant primer (B; L: 50 bp DNA marker, A: DNA obtained from the AGS cell line, H: DNA obtained from the HCT116 cell line, N: no DNA). (C-D), Gel electrophoresis after PCR with the G12D mutant primer (**C**) and the G13D mutant primer (**D**) (L: 50 bp DNA marker, 1: 0%, 2: 1%, 3: 10%, 4: 30%, 5: 50%, 6: 100% of mutant cells, N: no DNA).

Supplementary Table 1: Sequences of the primers used for PCR, Sequencing and ISAD-KRAS assay

Target	Type		Sequence
G12D	PCR (Wild)	Forward	5′-TGTGGTAGTTGGAGCTGG-3′
		Reverse	5′-TCATGAAAATGGTCAGAGAAACC-3′
	PCR (Mutation)	Forward	5′-TGTGGTAGTTGGAGCTGA-3′
		Reverse	5′-TCATGAAAATGGTCAGAGAAACC-3′
	ISAD	Forward	5′-NH2-(CH2)12-CTGAATATAAACTTGTGGTAGTTGGAGCTGA-3′
		Reverse	5′-NH2-(CH2)12-CTCATGAAAATGGTCAGAGAAACCTTTATC-3′
G13D	PCR (Wild)	Forward	5′-TGTGGTAGTTGGAGCTGGTGG-3′
		Reverse	5′-TCATGAAAATGGTCAGAGAAACC-3′
	PCR (Mutation)	Forward	5′-TGTGGTAGTTGGAGCTGGTGAG-3′
		Reverse	5′-TCATGAAAATGGTCAGAGAAACC-3′
	ISAD	Forward	5′-NH_2-$(CH2)_{12}$-CTGAATATAAACTTGTGGTAGTTGGAGCTGGTGAG-3′
		Reverse	5′-NH_2-$(CH2)_{12}$-CTCATGAAAATGGTCAGAGAAACCTTTATC-3′
Exon 2	Sequencing	Forward	5′-TCATTATTTTTATTATAAGGCCTGCT-3′
		Reverse	5′-CAAGATTTACCTCTATTGTTGGATC-3′

Supplementary Table 2: Accuracy of *KRAS* mutation detection in 70 clinical samples using PCR, direct sequencing and ISAD-KRAS assay

Sample (reference)	Total tested sample	Positive sample					
		PCR result		Sequencing result		ISAD Result	
		G12D	G13D	G12D	G13D	G12D	G13D
Wild	20	0	0	0	0	4*	1*
G12D	24	22	0	21	0	24	0
G13D	26	0	25	0	22	0	26
Total	70	22/70 (31.4%)	25/70 (35.7%)	21/70 (30%)	22/70 (31.4%)	28/70 (40%)	27/70 (38.6%)

* Newly detected mutations in wild-type samples using ISAD.

Supplementary Table 3: Results of end-point PCR, direct sequencing and ISAD-KRAS assays in 70 clinical samples. See Supplementary_Table_3