Electronic Supplementary Information

Highly specific detection of KRAS single nucleotide polymorphism by asymmetric PCR/SERS assay

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Table S1. Sequences of oligonucleotides and PCR amplicon used in this study

Oligonucleotides	5'-Sequence-3'
KRAS F-Primer (10 µM)	/5Biosg/TGACTGAATATAAACTTGTGGTAGTTG (Tm: 66.3°C)
KRAS R-Primer (2.5 µM)	GATCATATTCGTCCACAAAATGATTCTGA (Tm: 66.4°C)
KRAS G12V probe	/5ThioMC6-D/TTTTTCCTACGCCAACAG
KRAS amplicon (length: 99 nucleotides)	TGACTGAATATAAACTTGTGGTAGTTGGAGCTGTTGGCGTAGG CAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTG GACGAATATGATC

Modifications are as indicated. 5Biosg: 5'-biotin modified; 5ThioMC6-D: 5'-thiol modifier C6 S-S (disulfide). The text in blue represents position of primers; The text in red highlights complementary sequences in amplicons and probe nanotags; The underlined text refers to the single nucleotide polymorphism. The concentration-adjusted melting temperature (Tm) is calculated according to the nearest-neighbor formula,[1,2] with the concentrations of 10 mM and 3 mM for monovalent and divalent cations, respectively.

Table S2. Reaction mixture for asymmetric PCR

Reaction mixture	Volume (µL)
5x MyTaq Reaction Buffer	4
KRAS F-Primer (10 µM)	1.2
KRAS R-Primer (2.5 µM)	1.2
MyTaq HS DNA Polymerase	0.3
Input targets	2
Nuclease-free water	11.3
Total volume	**20**

Table S3. Reaction mixture for quantitative PCR

Reaction mixture	Volume (µL)
iTaq Universal SYBR Green supermix (2x)	10
KRAS F-Primer (10 µM)	1.2
KRAS R-Primer (2.5 µM)	1.2
Input targets	2
Nuclease-free water	5.6
Total volume	**20**

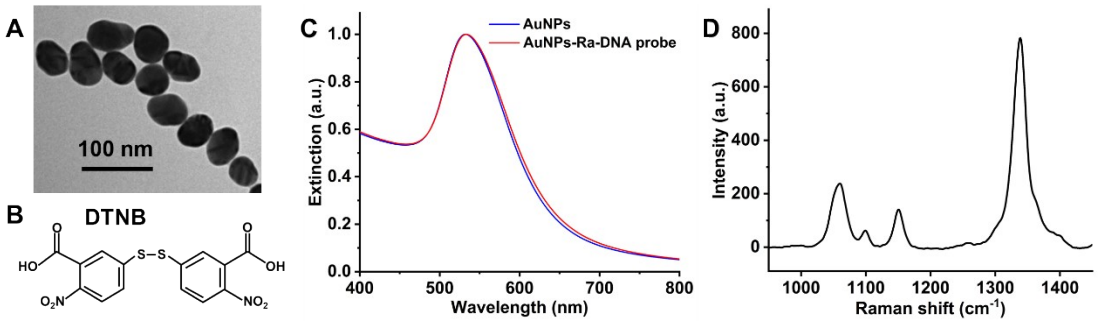

Figure S1. Transmission electron microscopy (TEM) image of AuNPs with size of about 60 nm (A), molecular structure of Raman reporter 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) (B), extinction spectra of bare AuNPs and SERS nanotags (C), and Raman spectra of SERS nanotags with the characteristic peak at 1339 cm^{-1} (D).

Figure S2. SERS nanotag functionalized with DNA probe for identifying KRAS G12V mutation.

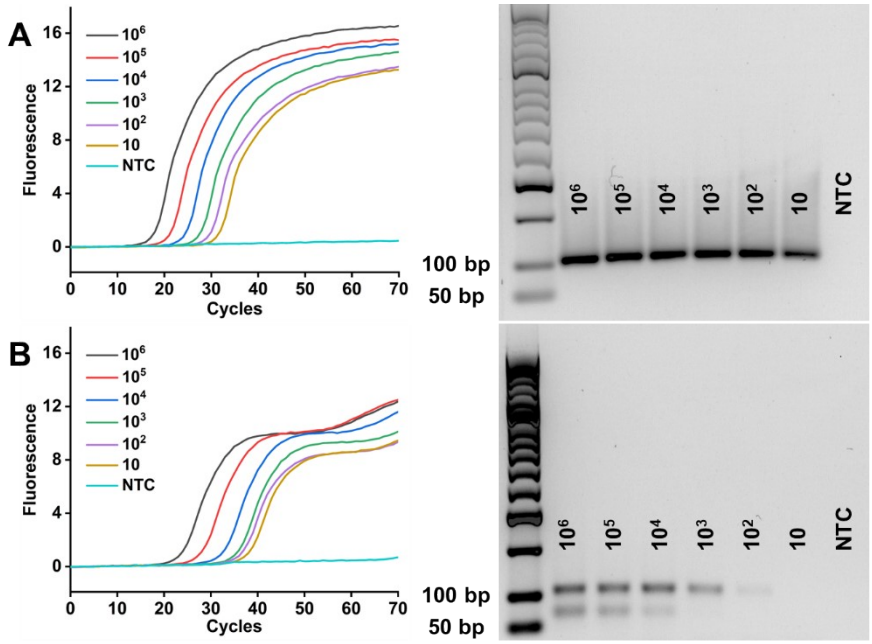

Figure S3. Quantitative PCR (left) and gel electrophoresis image (right, the amplification products are from 45 thermal cycles) of regular PCR with equal amounts of two primers (A) and Asy-PCR with excess forward primer to limiting reverse primer concentration ratio of 10:1 (B), respectively. The copies of input targets range from 10^6 to 10. NTC is the no template control.

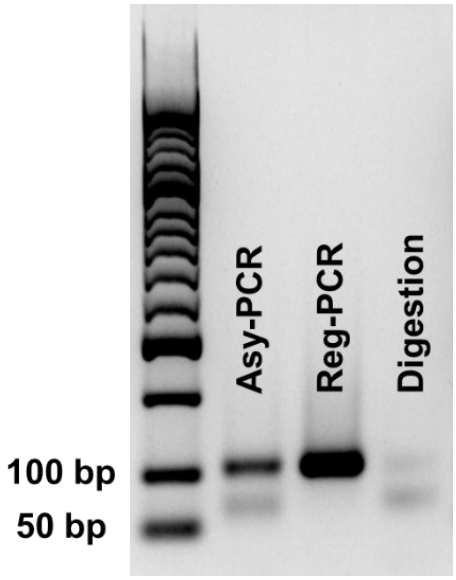

Figure S4. Gel electrophoresis image of the amplicons from asymmetric-PCR (Asy-PCR) and regular PCR (Reg-PCR) with 10^4 input synthetic targets, and the products digested from Reg-PCR with lambda exonuclease. The molecular size of ssDNA from Asy-PCR is comparable with that of ssDNA digested from dsDNA in Reg-PCR, which confirms the target amplicons from Asy-PCR.

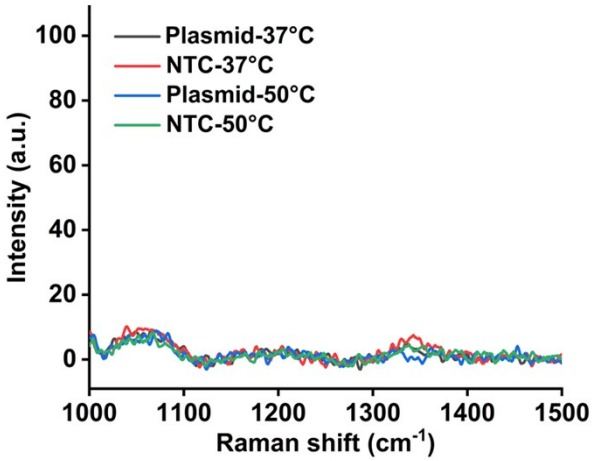

Figure S5. SERS spectra for the detection of Asy-PCR amplicons when the hybridization of ssDNA with allele-specific probe on SERS nanotags was undertaken at a constant temperature (37°C and 50°C).

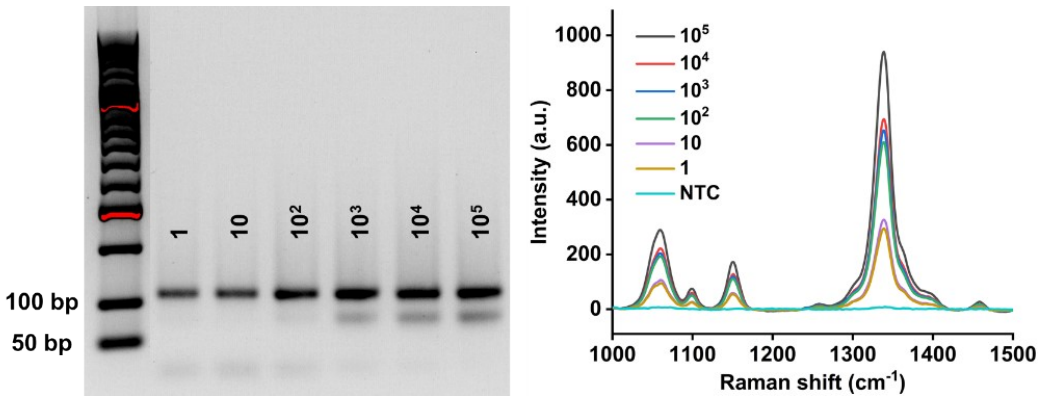

Figure S6. Amplification of KRAS G12V plasmid by Asy-PCR with excess to limiting primer concentration ratio of 4:1. Gel electrophoresis image (A) and typical raw Raman spectra (B) over a range of mutation loads from 1 to 10^5 input copies. NTC is the no template control.

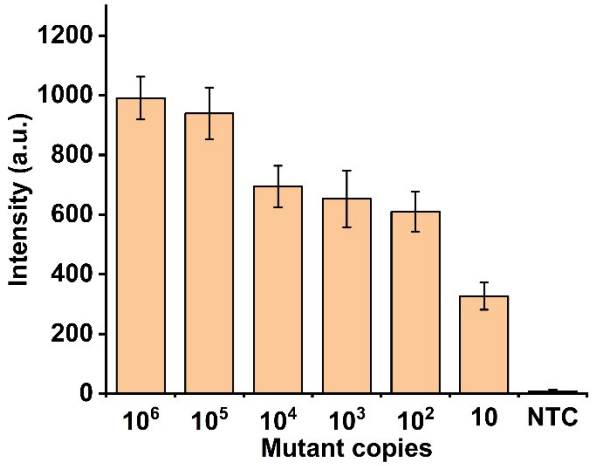

Figure S7. Bar graph of average SERS intensities at 1339 cm^{-1} over a range of mutation loads from 10^6 to 10 input copies. Error bar represents standard deviation (SD) of 3 independent experiments.

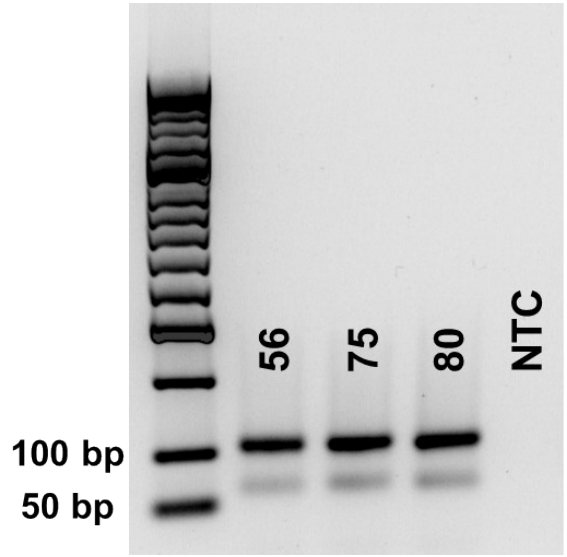

Figure S8. Gel electrophoresis image of Asy-PCR amplicons from patients' DNA samples. NTC is the no template control.

References

1 J. SantaLucia, 1998, **95**, 1460-1465.

2 N. von Ahsen, C. T. Wittwer and E. Schütz, *Clin. Chem.*, 2001, **47**, 1956-1961.