Supporting Information

Ratiometric fluorescence method for ctDNA analysis based on the construction of a DNA

four-way junction

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Fig. S1 Polyacrylamide gel electrophoresis analysis of (A) ctDNA, (B) template, (C) SDA without target, (D) SDA with target, (E) FWJ1, FWJ2 and FWJ3, (F) FWJ1, FWJ2, FWJ3, FWJ4. The middle ladder is 20 bp DNA Ladder.



Fig. S2 Optimization of (A) FWJ concentration, (B) Nb.BbvCI concentration, (C) reaction time in the absence and presence of target ctDNA, (D) reaction temperature.

Name	Sequence (5'-3')
Target	GTTGGAGCTAGTGGCGTAG
template	TCAAGTATCGGAAGACTCGGACTACAAGACGAGTTCATCTCTAGGCTA
	CCTCAGCTACGCCACTAGCTCCAAC
FWJ1	Cy5-CAAGACGAGTTCATCTCTAGGCTAAGAGATGAACTC
FWJ2	GAGTCTTCCGATTCAAGTATCGGAAGACTCGGACTA-Dabcyl
FWJ3	FAM- <u>TACTAGAGT</u> TCATCTCTATCGGAAG <u>ACTCTAGTA</u> -Dabcyl
FWJ4	TAGCCTAGAGATGAACTCGTCTTGTAGTCCGAGTCTTCCGATACTTGA
mismatch 1	GTTGGAGCT <u>GA</u> TGGCGTAG
mismatch 2	GTTGGAGCT <u>GC</u> TGGCGTAG
mismatch 3	GTTGGAGCT <u>GT</u> TG <u>A</u> CGTAG
mismatch 4	GTTGGAGCT <u>G</u> GTG <u>A</u> CGTAG
$^{\alpha}$ The sequences with the same colors are complementary with each other. The italic, bold, and	
underlined sequences are complementary with each other. The boxed sequence is the recognition site	
of Nb.BbvCI.	

Table S1. DNA sequences used in this work.^{α}

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