Supporting Information

Allele-Specific Isothermal Amplification Method Using Unmodified Self-Stabilizing Competitive Primers

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Supporting information includes sequences of all the genetic material utilized in the manuscript (Table S1 and S2), thermodynamic equations (Table S3), extended numerical values of the reported results (reaction speeds in Table S5, S6 and S8 and thermodynamic calculations in Table S4, S7, S9 and S11), calculations for the selection of primers (Table S10), high resolution melting analysis (Figure S1) and amplification curves of the tested alleles (Figure S2 and S3).

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Table S1
Sequences of all primers utilized in this manuscript named as SNP_name$_{length(bp)}$_specificity

Primer ID	Sequence
C580Y_F3	CTATTATACCGAATGTAGAAGCA
C580Y_B3	TGCTCCTGAACTTCTAGC
C580Y_LF	TCAAAGGTGCCACCTCTACC
C580Y_LB	GGTGGAACTAATGGTGAGAGATTAA
C580Y_sbFIP$_{17}$_WT	CACATAGCTGATGATCTTATGATCATCGTATGAAAGCATG
C580Y_sbFIP$_{19}$_WT	CACATAGCTGATGATCTAG TATGATCATCGTATGAAAGCATG
C580Y_sbFIP$_{21}$_WT	CACATAGCTGATGATCTAGGGTATGATCATCGTATGAAAGCATG
C580Y_sbFIP$_{25}$_WT	CACATAGCTGATGATCTAGGGGTATTATGATCATCGTATGAAAGCATG
C580Y_sbFIP$_{17}$_MT	TACATAGCTGATGATCTTATGATCATCGTATGAAAGCATG
C580Y_sbFIP$_{19}$_MT	TACATAGCTGATGATCTAG TATGATCATCGTATGAAAGCATG
C580Y_sbFIP$_{21}$_MT	TACATAGCTGATGATCTAGGGTATGATCATCGTATGAAAGCATG
C580Y_sbFIP$_{25}$_MT	TACATAGCTGATGATCTAGGGGTATTATGATCATCGTATGAAAGCATG
C580Y_sbBIP$_{21}$_WT	GTGTTGCTTTTGATAATAAAA GCATATGGAAATTGTTCCCAT
C580Y_sbBIP$_{25}$_WT	GTGTTGCTTTTGATAATAAAATTTA GCATATGGAAATTGTTCCCAT
C580Y_sbBIP$_{27}$_WT	GTGTTGCTTTTGATAATAAAATTTATG CTAATAAGGCATATGGAAATTG
C580Y_sbBIP$_{29}$_WT	GTGTTGCTTTTGATAATAAAATTTATGTC CTAATAAGGCATATGGAAATTG
C580Y_sbBIP$_{21}$_MT	ATGTTGCTTTTGATAATAAAA GCATATGGAAATTGTTCCCAT
C580Y_sbBIP$_{25}$_MT	ATGTTGCTTTTGATAATAAAATTTA GCATATGGAAATTGTTCCCAT
C580Y_sbBIP$_{27}$_MT	ATGTTGCTTTTGATAATAAAATTTATG CTAATAAGGCATATGGAAATTG
C580Y_sbBIP$_{29}$_MT	ATGTTGCTTTTGATAATAAAATTTATGTC CTAATAAGGCATATGGAAATTG

C580Y_FB0$_{27}$_WT	GTGTTGCTTTTGATAATAAAATTTATG
C580Y_FB0$_{29}$_WT	GTGTTGCTTTTGATAATAAAATTTATGTC
C580Y_FB0$_{31}$_WT	GTGTTGCTTTTGATAATAAAATTTATGTCAT
C580Y_FB0$_{33}$_WT	GTGTTGCTTTTGATAATAAAATTTATGTCATTG
C580Y_FB0$_{27}$_MT	ATGTTGCTTTTGATAATAAAATTTATG
C580Y_FB0$_{29}$_MT	ATGTTGCTTTTGATAATAAAATTTATGTC
C580Y_FB0$_{31}$_MT	ATGTTGCTTTTGATAATAAAATTTATGTCAT
C580Y_FB0$_{33}$_MT	ATGTTGCTTTTGATAATAAAATTTATGTCATTG
C580Y_BB0$_{17}$_WT	CACATAGCTGATGATCT
C580Y_BB0$_{19}$_WT	CACATAGCTGATGATCTAG
C580Y_BB0$_{21}$_WT	CACATAGCTGATGATCTAGGG
C580Y_BB0$_{23}$_WT	CACATAGCTGATGATCTAGGGGT
C580Y_BB0$_{17}$_MT	TACATAGCTGATGATCT
C580Y_BB0$_{19}$_MT	TACATAGCTGATGATCTAG
C580Y_BB0$_{21}$_MT	TACATAGCTGATGATCTAGGG
C580Y_BB0$_{23}$_MT	TACATAGCTGATGATCTAGGGGT

C580Y_FB1$_{33}$_MT	TATGTTGCTTTTGATAATAAAATTTATGTCATTG
C580Y_FB2$_{33}$_MT	GTATGTTGCTTTTGATAATAAAATTTATGTCATTG
C580Y_BB1$_{21}$_MT	ATACATAGCTGATGATCTAGGG
C580Y_BB2$_{21}$_MT	CATACATAGCTGATGATCTAGGG
C580Y_FB1$_{27}$_WT	TGTGTTGCTTTTGATAATAAAATTTATG
C580Y_FB2$_{27}$_WT	GTGTGTTGCTTTTGATAATAAAATTTATG
C580Y_BB1$_{21}$_WT	ACACATAGCTGATGATCTAGGG
C580Y_BB2$_{21}$_WT	CACACATAGCTGATGATCTAGGG

C580Y_FB2*$_{33}$_MT	GT**A**TGTTGCTTTTGATAATAAAATTTATGTCATTG**/3AmMo/**
C580Y_BB1*$_{21}$_MT	A**T**ACATAGCTGATGATCTAGGG**/3AmMo/**
C580Y_FB1*$_{27}$_WT	T**G**TGTTGCTTTTGATAATAAAATTTATG **/3AmMo/**
C580Y_BB2*$_{21}$_WT	CA**C**ACATAGCTGATGATCTAGGG**/3AmMo/**
C580Y_BA2*$_{33}$_MT	CAATGACATAAATTTTATTATCAAAAGCAACA**T**AC **/3AmMo/**
C580Y_FA1*$_{21}$_MT	CCCTAGATCATCAGCTATGT**A**T **/3AmMo/**
C580Y_BA1*$_{27}$_WT	CATAAATTTTATTATCAAAAGCAACA**C**A **/3AmMo/**
C580Y_FA2*$_{21}$_WT	CCCTAGATCATCAGCTATGT**G**TG **/3AmMo/**
C580Y_BA2$_{33}$_MT	CAATGACATAAATTTTATTATCAAAAGCAACA**T**AC
C580Y_FA1$_{21}$_MT	CCCTAGATCATCAGCTATGT**A**T
C580Y_BA1$_{27}$_WT	CATAAATTTTATTATCAAAAGCAACA**C**A
C580Y_FA2$_{21}$_WT	CCCTAGATCATCAGCTATGT**G**TG

Y493H_F3	GGTGTAGAATATTTAAATTCGATGG
Y493H_B3	TTGAAACATACCATACATCTCTT
Y493H_LF	TGGTAGACATAGGTGTACACATACG
Y493H_LB	ATTATAAGGCTTTATTTGA
Y493H_sbF1P$_{21}$_WT	**A**TAAGAAATTATTCAATACAGATTAGATATTAGTCAACAATGCTGG
Y493H_sbB1P$_{17}$_WT	**T**ACGTTTTTGGTGGTAA AAACGATCATACACCTCAGTT
Y493H_sbF1P$_{21}$_MT	**G**TAAGAAATTATTCAATACAGATTAGATATTAGTCAACAATGCTGG
Y493H_sbB1P$_{17}$_MT	**C**ACGTTTTTGGTGGTAA AAACGATCATACACCTCAGTT
Y493H _ FB2$_{24}$_MT	TA**C**ACGTTTTTGGTGGTAATAACTAT
Y493H _ BB1$_{26}$_MT	T**G**TAAGAAATTATTCAATACAGCACTT
Y493H _ FB1$_{24}$_WT	A**T**ACGTTTTTGGTGGTAATAACTAT
Y493H _ BB0$_{26}$_WT	**A**TAAGAAATTATTCAATACAGCACTT

Table S2

Sequences of gBlocks® utilized in this manuscript named as WT template (harboring the SNP *580C* and *493Y*) and MT template (harbpouring SNP *580Y* and *493H*). Stock concentration of 5 ng/µL with a MW of 374832.4 g/mol purchased to Integrated DNA Technologies. Location of primers for amplification of *SNP 580* are shown as example (highlighted regions in WT and MT templates). USS-sbLAMP final primer sequences for allele-specific amplification of SNP *C580Y* and SNP *Y493H* are shown below. Amplicon length (between F3 and B3) of SNP *C580Y* is 230 bp and of *SNP Y493H* is 208 bp.

WT template gBlock Gene fragment of 607 bp

CCATTCCCATTAGTATTTTGTATAGGTGGATTTGATGGTGTAGAATATTTAAATTCGATGGAATTATTAGATATTAG

TCAACAATGCTGGCGTATGTGTACACCTATGTCTACCAAAAAAGCTTATTTTGGAAGTGCTGTATTGAATAATTTC

TTA**T**ACGTTTTTGGTGGTAATAACTATGATTATAAGGCTTTATTTGAAACTGAGGTGTATGATCGTTTAAGAGATG

TATGGTATGTTTCAAGTAATTTAAATATACCTAGAAGAAATAATTGTGGTGTTACGTCAAATGGTAGAATTTATTGT

F3 F2 LF

ATTGGGGGATATGATGGCTCTTCTATTATACCGAATGTAGAAGCATATGATCATCGTATGAAAGCATGGGTAGAG

F1c B1c LB

GTGGCACCTTTGAATACCCCTAGATCATCAGCTATGTgTGTTGCTTTTGATAATAAAATTTATGTCATTGGTGGAA

BB FB B2

CTAATGGTGAGAGATTAAATTCTATTGAAGTATATGAAGAAAAAATGAATAAATGGGAACAATTTCCATATGCCTT

B3

ATTAGAAGCTAGAAGTTCAGGAGCAGCTTTTAATTACCTTAATCAAATATATGTTGTTGGAGGTATTGATAATG

MT template gBlock Gene fragment of 607 bp

CCATTCCCATTAGTATTTTGTATAGGTGGATTTGATGGTGTAGAATATTTAAATTCGATGGAATTATTAGATATTAG

TCAACAATGCTGGCGTATGTGTACACCTATGTCTACCAAAAAAGCTTATTTTGGAAGTGCTGTATTGAATAATTTC

TTA**C**ACGTTTTTGGTGGTAATAACTATGATTATAAGGCTTTATTTGAAACTGAGGTGTATGATCGTTTAAGAGATG

TATGGTATGTTTCAAGTAATTTAAATATACCTAGAAGAAATAATTGTGGTGTTACGTCAAATGGTAGAATTTATTGT

F3 F2 LF

ATTGGGGGATATGATGGCTCTTCTATTATACCGAATGTAGAAGCATATGATCATCGTATGAAAGCATGGGTAGAG

F1c B1c LB

GTGGCACCTTTGAATACCCCTAGATCATCAGCTATGTaTGTTGCTTTTGATAATAAAATTTATGTCATTGGTGGAA

BB FB B2

CTAATGGTGAGAGATTAAATTCTATTGAAGTATATGAAGAAAAAATGAATAAATGGGAACAATTTCCATATGCCTT

B3

ATTAGAAGCTAGAAGTTCAGGAGCAGCTTTTAATTACCTTAATCAAATATATGTTGTTGGAGGTATTGATAATG

Final primer sequences for SNP C580Y and SNP Y493H detection.

Primer ID	Sequence (5'-3')	Primer ID	Sequence (5'-3')
C580Y_F3	CTATTATACCGAATGTAGAAGCA	**Y493H_F3**	GGTGTAGAATATTTAAATTCGATGG
C580Y_B3	TGCTCCTGAACTTCTAGC	**Y493H_B3**	TTGAAACATACCATACATCTCTT
C580Y_LF	TCAAAGGTGCCACCTCTACC	**Y493H_LF**	TGGTAGACATAGGTGTACACATACG
C580Y_LB	GGTGGAACTAATGGTGAGAGATTAA	**Y493H_LB**	ATTATAAGGCTTTATTTGA
C580Y_sbFIP$_{17}$_WT	CACATAGCTGATGATCTTATGAT CATCGTATGAAAGCATG	**Y493H_sbF1P$_{21}$_WT**	ATAAGAAATTATTCAATACAGATT AGATATTAGTCAACAATGCTGG
C580Y_sbBIP$_{27}$_WT	GTGTTGCTTTTGATAATAAAATTTA TGCTAATAAGGCATATGGAAATTG	**Y493H_sbB1P$_{17}$_WT**	TACGTTTTTGGTGGTAAAAACG ATCATACACCTCAGTT
C580Y_sbFIP$_{17}$_MT	TACATAGCTGATGATCTTATGAT CATCGTATGAAAGCATG	**Y493H_sbF1P$_{21}$_MT**	GTAAGAAATTATTCAATACAGATT AGATATTAGTCAACAATGCTGG
C580Y_sbBIP$_{27}$_MT	ATGTTGCTTTTGATAATAAAATTTA TGCTAATAAGGCATATGGAAATTG	**Y493H_sbB1P$_{17}$_MT**	CACGTTTTTGGTGGTAAAAACG ATCATACACCTCAGTT
C580Y_FB2$_{33}$_MT	GTATGTTGCTTTTGATAATAAA ATTTATGTCATTG	**Y493H _ FB2$_{24}$_MT**	TACACGTTTTTGGTGGTAATAACTAT
C580Y_BB1$_{21}$_MT	ATACATAGCTGATGATCTAGGG	**Y493H _ BB1$_{26}$_MT**	TGTAAGAAATTATTCAATA CAGCACTT
C580Y_FB1$_{27}$_WT	TGTGTTGCTTTTGATAATAA AATTTATG	**Y493H _ FB1$_{24}$_WT**	ATACGTTTTTGGTGGTAATAACTAT
C580Y_BB2$_{21}$_WT	CACACATAGCTGATGATCTAGGG	**Y493H _ BB0$_{26}$_WT**	ATAAGAAATTATTCAATACAGCACTT

Table S3

Equations used for thermodynamic calculations of primers. The stability of the primers at the corresponding temperature (63 °C = 336.15 K) was calculated by the Gibbs free energy (ΔG°) equation (Equation 1) using the thermodynamic nearest neighbour parameters for Watson-Crick base pairs[1-3] ΔG° of the hybridization between primer and template was calculated using the software NetPrimer introducing the primer of interest, and the template as $DNA_{TEMPLATE}$ = $cDNA_{primer}$ +$1_{5'}$ + $1_{3'}$ where $1_{5'}$ and $1_{3'}$ mean the addition of one complementary nucleotide at the 3' end and at the 5' end of the $cDNA_{primer}$, respectively. Entropy (ΔS°) was adjusted to the corresponding salt conditions (Equation 2)[1] and the T_m[3] was calculated by Equation 3.

Gibbs Free Energy (ΔG°)
$\Delta G°_T = \Delta H° - T\Delta S°$ (1) where T is in Kelvin, ΔG° in kcal mol^{-1}, ΔH° in kcal mol^{-1} and ΔS° in kcal K^{-1} mol^{-1}.
Entropy (ΔS°)
$\Delta S°[Na^+] = \Delta S°[1\ M_{NaCl}] + 0.368N ln[Na^+]$ (2) where Na^+ is the concentration of monovalent cations in M and N is the number of nucleotide pairs in the primer.
Melting temperature approximation (T_m)
$T_m = \frac{\Delta H}{\Delta S° + R ln(\frac{C_T}{x})} - 273.15$ (3) where R is the gas constant 1.9872×10^{-3} kcal K^{-1} mol^{-1}, -273.15 is a conversion factor from K to °C, C_T is the total molar concentration of the strands in M, and x is considered 4 for nonself-complementary duplexes.

Table S4

Properties (ΔG, T_m, GC%, GC clamps, 3'-end nucleotide) of different length F1c and B1c primers utilized in sbLAMP experiments for the detection of SNP *C580Y*. Thermodynamic properties were calculated at DNA stock concentration of 5 ng/µL with a MW of 374832.4 g/mol, primer concentration at 2µM, free Mg^{+2} (6.6 mM), and K^+ (50 mM) using the methodology described in Experimental Section.

Primer ID		ΔG (kcal/mol)	$\Delta G_{(P\text{-}WTs)}$ (kcal/mol)	$\Delta G_{(P\text{-}MTs)}$ (kcal/mol)	T_m (◦C)	GC%	GC Clamp	3'-end
C580Y_F1c$_{bp}$_WT	**17 bp**	**-11.22**	**-17.29**	**-15.83**	**58.88**	**41.18**	**1**	**A**
	19 bp	-12.34	-18.42	-16.95	61.46	42.11	1	C
	21 bp	-16.46	-22.54	-21.07	70.31	47.62	3	C
	25 bp	-20.09	-26.16	-24.70	74.79	44.00	2	A
C580Y_B1c$_{bp}$_WT	21 bp	-14.71	-20.78	-20.10	65.37	23.81	0	A
	25 bp	-17.66	-23.73	-23.05	69.00	20.00	0	A
	27 bp	**-19.69**	**-25.76**	**-25.08**	**71.58**	**22.22**	**1**	**G**
	29 bp	-21.43	-27.51	-26.83	73.57	24.14	1	C
C580Y_F1c$_{bp}$_MT	**17 bp**	**-10.07**	**-15.83**	**-16.15**	**55.41**	**35.29**	**1**	**A**
	19 bp	-11.20	-16.95	-17.27	58.32	36.84	1	C
	21 bp	-15.31	-21.07	-21.39	67.47	42.86	3	C
	25 bp	-18.34	-24.70	-25.02	72.39	40.00	2	A
C580Y_B1c$_{bp}$_MT	21 bp	-14.59	-20.10	-20.67	64.95	19.05	0	A
	25 bp	-17.54	-23.05	-23.62	68.61	16.00	0	A
	27 bp	**-19.57**	**-25.08**	**-25.65**	**71.18**	**18.52**	**1**	**G**
	29 bp	-21.32	-26.83	-27.39	73.17	20.69	1	C

Table S5

sbLAMP amplification results of WT template (*580C*) and MT template (*580Y*) at 5x10^4 copies/µL. Mean values of two experiments with each reaction in triplicate at 5x10^4 copies/reaction loading 5µL/reaction of reaction mixture including the DNA.

sbLAMP			WT template						MT template					
B1c (bp)	F1c (bp)	Specificity	TTP mean (min)	SD (min)	ΔTTP (min)	SD (min)	p-value		TTPmean (min)	SD (min)	ΔTTP (min)	SD (min)	p-value	
21	17	WT	31.63	1.12	-16.48	5.75	2.15E-04	***	50.00	0.00	10.90	1.26	4.36E-06	****
		MT	48.11	4.63					39.11	1.26				
	19	WT	29.79	1.67	-13.99	4.82	1.58E-05	****	48.52	3.62	12.16	6.47	9.18E-05	****
		MT	43.78	3.15					36.37	2.86				
	21	WT	29.59	1.72	-12.43	5.96	3.76E-04	***	44.19	6.73	9.06	8.33	2.05E-02	*
		MT	42.02	4.24					35.13	1.60				
	25	WT	31.10	1.85	-9.56	5.15	2.85E-04	***	42.82	4.34	5.87	8.61	3.98E-02	*
		MT	40.66	3.30					36.95	4.26				
25	17	WT	27.85	1.06	-10.66	2.50	1.11E-07	****	47.68	3.87	14.15	6.02	5.83E-05	****
		MT	38.51	1.44					33.54	2.14				
	19	WT	26.30	0.99	-9.32	2.92	9.59E-06	****	41.05	5.93	9.14	7.43	1.18E-02	*
		MT	35.62	1.93					31.92	1.50				
	21	WT	26.59	1.56	-6.82	3.06	1.64E-05	****	35.44	4.44	5.14	6.40	3.64E-02	*
		MT	33.41	1.51					30.30	1.96				
	25	WT	27.83	1.09	-5.48	2.20	6.15E-06	****	36.84	4.51	4.98	6.93	4.58E-02	*
		MT	33.31	1.11					31.87	2.42				
27	**17**	**WT**	**10.28**	**0.34**	**-4.18**	**0.60**	**2.94E-09**	********	**15.15**	**1.33**	**2.29**	**1.54**	**8.03E-03**	******
		MT	**14.46**	**0.25**					**12.87**	**0.21**				
	19	WT	9.62	0.36	-3.37	0.70	1.36E-08	****	12.53	0.38	0.42	0.62	4.98E-02	*
		MT	12.99	0.35					12.11	0.24				
	21	WT	9.20	0.48	-2.42	0.83	3.38E-06	****	11.52	0.24	0.07	0.40	5.46E-01	-
		MT	11.62	0.34					11.44	0.16				
	25	WT	9.15	0.43	-2.32	0.73	1.93E-06	****	11.87	0.21	0.52	0.53	9.85E-03	**
		MT	11.47	0.30					11.35	0.32				
29	17	WT	10.44	0.31	-3.34	0.43	1.37E-07	****	13.78	0.61	1.07	0.78	6.74E-03	**
		MT	13.78	0.12					12.71	0.17				
	19	WT	9.84	0.32	-2.77	0.65	4.10E-08	****	12.29	0.67	0.38	0.91	2.37E-01	-
		MT	12.61	0.33					11.91	0.25				
	21	WT	9.27	0.36	-1.96	0.63	1.70E-06	****	11.28	0.19	0.14	0.43	2.97E-01	-
		MT	11.23	0.27					11.14	0.24				
	25	WT	9.30	0.36	-1.71	0.58	8.09E-06	****	11.24	0.06	0.08	0.15	9.83E-02	-
		MT	11.01	0.22					11.16	0.09				

Table S6

USS-sbLAMP amplification results of WT template (*580C*) and MT template (*580Y*) at 5x10^4 copies/µL utilizing sbLAMP$_{WT}$ F1c$_{17}$-B1c$_{27}$ and sbLAMP$_{MT}$ F1c$_{17}$-B1c$_{27}$. Mean values of two experiments with each reaction in triplicate at 5x10^4 copies/reaction loading 5µL/reaction of reaction mixture including the DNA.

USS$_{MT}$-sbLAMP$_{WT}$		**WT template**		**MT template**	
BB0 (bp)	**FB0 (bp)**	**TTP mean (min)**	**SD (min)**	**TTP mean (min)**	**SD (min)**
17	29	11.20	0.05	17.12	0.76
	31	11.24	0.12	17.12	0.81
	33	11.16	0.05	16.72	0.72
19	27	11.40	0.02	17.64	1.76
	29	11.43	0.05	17.51	0.99
	31	11.43	0.07	16.90	0.28
	33	11.41	0.06	17.71	0.84
21	27	12.02	0.03	18.96	1.05
	29	11.97	0.02	20.14	1.52
	31	11.95	0.02	19.09	1.48
	33	**11.73**	**0.36**	**19.92**	**1.40**
23	27	12.65	0.23	19.21	1.04
	29	12.67	0.02	19.72	0.87
	31	12.61	0.07	20.56	2.50
	33	12.57	0.08	20.04	0.75
USS$_{WT}$-sbLAMP$_{MT}$		**WT template**		**MT template**	
BB0 (bp)	**FB0 (bp)**	**TTPmean (min)**	**SD (min)**	**TTP mean (min)**	**SD (min)**
17	29	16.90	0.25	14.65	0.28
	31	17.04	0.12	14.64	0.29
	33	16.68	0.03	14.62	0.27
19	27	17.20	0.56	14.83	0.18
	29	17.56	0.27	14.83	0.15
	31	17.46	0.31	15.07	0.12
	33	17.41	0.64	14.90	0.10
21	**27**	**18.73**	**0.34**	**15.85**	**0.19**
	29	19.24	0.52	16.01	0.15
	31	19.11	0.84	15.91	0.22
	33	18.64	0.73	16.03	0.23
23	27	20.19	0.49	16.95	0.57
	29	20.50	0.61	16.93	0.33
	31	20.04	0.82	16.86	0.82
	33	20.78	0.74	16.91	0.42

Table S7

Properties (ΔG, T_m, GC%, GC clamps, 3'-end nucleotide) of different length FB and BB primers utilized in USS-sbLAMP experiments for the detection of SNP *C580Y*. Thermodynamic properties were calculated at DNA stock concentration of 5 ng/µL with a MW of 374832.4 g/mol, primer concentration at 2µM, free Mg^{+2} (6.6 mM), and K^+ (50 mM) using the methodology described in Experimental Section.

Primer ID		ΔG (kcal/mol)	$\Delta G_{(P\text{-}WTs)}$ (kcal/mol)	$\Delta G_{(P\text{-}MTs)}$ (kcal/mol)	T_m (◦C)	GC%	GC Clamp	3'-end
FB_{WT}	**27**	**-19.69**	**-25.76**	**-25.08**	**71.58**	**22.22**	**1**	**G**
	29	-21.43	-27.51	-26.83	73.57	24.14	1	C
	31	-23.46	-29.54	-28.86	75.62	22.58	1	T
	33	-25.96	-32.04	-31.35	78.12	24.24	1	G
BB_{WT}	17	-11.22	-17.29	-15.83	58.88	41.18	1	A
	19	-12.34	-18.42	-16.95	61.46	42.11	1	C
	21	**-16.46**	**-22.54**	**-21.07**	**70.31**	**47.62**	**3**	**C**
	23	-19.20	-25.28	-23.81	74.69	47.83	4	A
FB_{MT}	27	-19.57	-25.08	-25.65	71.18	18.52	1	G
	29	-21.32	-26.83	-27.39	73.17	20.69	1	C
	31	-23.35	-28.86	-29.42	75.22	19.35	1	T
	33	**-25.84**	**-31.35**	**-31.92**	**77.72**	**21.21**	**1**	**G**
BB_{MT}	17	-10.07	-15.83	-16.15	55.41	35.29	1	A
	19	-11.20	-16.95	-17.27	58.32	36.84	1	C
	21	**-15.31**	**-21.07**	**-21.39**	**67.47**	**42.86**	**3**	**C**
	23	-18.06	-23.81	-24.13	72.08	43.48	4	A

Table S8

USS-sbLAMP amplification results of WT template (*580C*) and MT template (*580Y*) at 5x10^4 copies/µL utiliz primers locating the SNP C580Y at different positions from their 5'-end in USS_{MT}-$sbLAMP_{WT}$ $F1c_{17}$-$B1c_{27}$ + FE USS_{WT}-$sbLAMP_{MT}$ $F1c_{17}$-$B1c_{27}$ + FBX_{27}/BBX_{21} . Mean values of two experiments with each reaction in trip copies/reaction loading 5µL/reaction of reaction mixture including the DNA.

USS_{MT}-$sbLAMP_{WT}$		**WT template**		**MT template**	
BB_{21}	**FB_{33}**	**TTP mean (min)**	**SD (min)**	**TTP mean (min)**	**SD (min)**
BB0	FB0	13.10	0.06	20.22	1.69
	FB1	13.40	0.04	20.54	2.48
	FB2	13.03	0.08	19.76	0.56
BB1	FB0	13.11	0.04	20.56	2.36
	FB1	13.23	0.09	19.62	1.47
	FB2	**13.06**	**0.15**	**20.64**	**1.38**
BB2	FB0	13.20	0.03	19.64	0.93
	FB1	13.15	0.08	19.77	1.29
	FB2	13.13	0.11	19.23	1.11
USS_{WT}-$sbLAMP_{MT}$		**WT template**		**MT template**	
BB_{21}	**FB_{27}**	**TTP mean (min)**	**SD (min)**	**TTP mean (min)**	**SD (min)**
BB0	FB0	20.32	0.59	16.47	0.26
	FB1	20.47	0.16	16.76	0.11
	FB2	21.53	0.93	17.00	0.19
BB1	FB0	20.29	0.29	16.63	0.51
	FB1	21.12	1.01	17.09	0.17
	FB2	22.24	1.65	17.23	0.28
BB2	FB0	20.31	0.03	16.41	0.14
	FB1	**21.86**	**0.13**	**16.49**	**0.20**
	FB2	21.68	1.02	16.85	0.15

Table S9

Properties (ΔG, T_m, GC%, GC clamps, 3'-end nucleotide) of FB and BB primers locating the SNP *C580Y* at different positions from their 5'-end utilized in USS-sbLAMP experiments for the detection of SNP *C580Y*. Thermodynamic properties were calculated at DNA stock concentration of 5 ng/µL with a MW of 374832.4 g/mol, primer concentration at 2µM, free Mg^{+2} (6.6 mM), and K^+ (50 mM) using the methodology described in Experimental Section.

Primer ID		ΔG (kcal/mol)	$\Delta G_{(P\text{-}WTs)}$ (kcal/mol)	$\Delta G_{(P\text{-}MTs)}$ (kcal/mol)	T_m (◦C)	GC%	GC Clamp	5'-end
FB_{WT}	0	-19.69	-25.76	-25.08	71.58	22.22	1	G
	1	**-21.15**	**-27.23**	**-25.08**	**73.68**	**21.43**	**1**	**T**
	2	-21.84	-27.91	-24.08	74.25	24.14	1	G
BB_{WT}	0	-16.46	-22.54	-21.07	70.31	47.62	3	G
	1	-17.14	-23.22	-21.07	71.24	45.45	3	T
	2	**-18.61**	**-24.68**	**-20.07**	**73.99**	**47.83**	**3**	**G**
FB_{MT}	0	-25.84	-31.35	-31.92	77.72	21.21	1	G
	1	-26.16	-31.35	-32.24	77.61	20.59	1	T
	2	**-26.85**	**-30.35**	**-32.92**	**78.00**	**22.86**	**1**	**G**
BB_{MT}	0	-15.31	-21.07	-21.39	67.47	42.86	3	G
	1	**-15.88**	**-21.07**	**-21.96**	**68.03**	**40.91**	**3**	**T**
	2	-17.34	-20.07	-23.42	70.81	43.48	3	G

Table S10

Calculations for the selection of the optimum concentration of USS primers in combination with the already selected sbLAMP primers. The optimum concentration was selected based on the highest ΔTTP value (difference between specific and unspecific reactions) for discrimination of both templates. ΔTTP values above 7 min are highlighted in light blue, overlapped highest ΔTTP values for both templates are highlighted in dark blue. Selected concentrations are in white lettering, in the case of USS_{WT}, 3 µM was chosen and in the case of $USS_{MT,}$ 4 µM.

WT template

USS_{WT} (µM) \ USS_{MT} (µM)	1	1.5	2	2.5	3	3.5	**4**	4.5
1	5.34	4.87	4.55	4.23	3.99	3.38	3.15	2.64
1.5	7.04	6.57	6.25	5.93	5.69	5.08	4.85	4.34
2	8.61	8.14	7.82	7.5	7.26	6.65	6.42	5.91
2.5	9.38	8.91	8.59	8.27	8.03	7.42	7.19	6.68
3	10.44	9.97	9.65	9.33	9.09	8.48	**8.25**	7.74
3.5	12.13	11.66	11.34	11.02	10.78	10.17	9.94	9.43
4	13.97	13.5	13.18	12.86	12.62	12.01	11.78	11.27
4.5	15.64	15.17	14.85	14.53	14.29	13.68	13.45	12.94

MT template

USS_{WT} (µM) \ USS_{MT} (µM)	1	1.5	2	2.5	3	3.5	**4**	4.5
1	-2.22	-3.2	-5.6	-5.88	-7.16	-8.51	-10.49	-9.74
1.5	-1.5	-2.48	-4.88	-5.16	-6.44	-7.79	-9.77	-9.02
2	-0.76	-1.74	-4.14	-4.42	-5.7	-7.05	-9.03	-8.28
2.5	-0.06	-1.04	-3.44	-3.72	-5	-6.35	-8.33	-7.58
3	0.72	-0.26	-2.66	-2.94	-4.22	-5.57	**-7.55**	-6.8
3.5	1.4	0.42	-1.98	-2.26	-3.54	-4.89	-6.87	-6.12
4	2.31	1.33	-1.07	-1.35	-2.63	-3.98	-5.96	-5.21
4.5	2.95	1.97	-0.43	-0.71	-1.99	-3.34	-5.32	-4.57

Table S11

Properties (ΔG, T_m, GC%, GC clamps, 3'-end nucleotide) of F1c, B1c,FB and BB primers utilized in sbLAMP experiments for the detection of SNP *Y493H*. Thermodynamic properties were calculated at DNA stock concentration of 5 ng/µL with a MW of 374832.4 g/mol, primer concentration at 2µM, free Mg^{+2} (6.6 mM), and K^+ (50 mM) using the methodology described in Materials and Methods.

Primer ID		ΔG (kcal/mol)	$\Delta G_{(P\text{-}WTs)}$ (kcal/mol)	$\Delta G_{(P\text{-}MTs)}$ (kcal/mol)	T_m (◦C)	GC%	GC Clamp	3'-end	5'-end
$F1c_{WT}$	21	-11.69	-17.77	-17.20	58.76	19.05	1	G	A
$B1c_{WT}$	17	-13.06	-19.14	-18.82	63.29	35.29	2	A	T
$F1c_{MT}$	21	-11.81	-17.20	-17.89	59.09	23.81	1	G	G
$B1c_{MT}$	17	-14.21	-18.82	-20.28	66.49	41.18	2	A	C
$FB1_{WT}$	24	-17.92	-24.00	-23.11	69.47	28.00	1	T	A
$BB0_{WT}$	26	-17.81	-23.88	-23.32	69.08	23.08	1	T	A
$FB2_{MT}$	24	-19.51	-22.11	-25.58	72.19	30.77	1	T	T
$BB1_{MT}$	26	-19.39	-23.32	-25.46	71.77	25.93	1	T	A

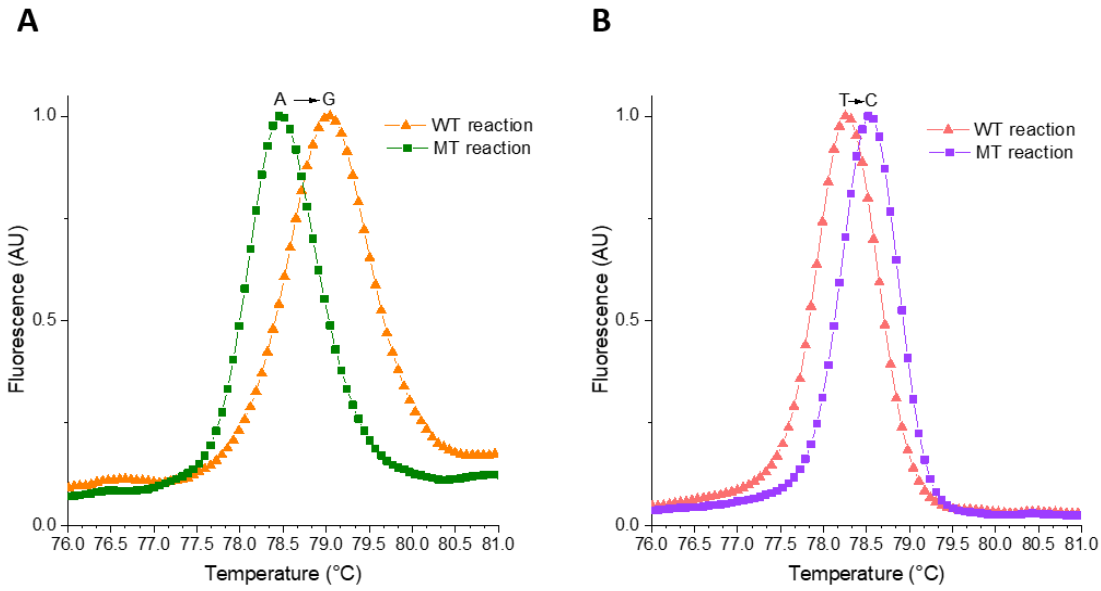


Figure S1

High Resolution Melting analysis of the products obtained after specific USS-sbLAMP amplification. **(A)** SNP *580C* ($5x10^4$ copies/reaction) using USS_{MT}-$sbLAMP_{WT}$ $F1c_{17}$-$B1c_{27}$ + $FB2_{33}$/$BB1_{21}$ (in orange) and SNP *580Y* ($5x10^4$ copies/reaction) using USS_{WT}-$sbLAMP_{MT}$ $F1c_{17}$-$B1c_{27}$ + $FB1_{27}$/$BB2_{21}$ (in green). **(B)** SNP*493Y* ($5x10^4$ copies/reaction) using USS_{MT}-$sbLAMP_{WT}$ $F1c_{21}$-$B1c_{17}$ + $FB2_{24}$/$BB1_{26}$ (in pink) and SNP *493H* ($5x10^4$ copies/reaction) using USS_{WT}-$sbLAMP_{MT}$ $F1c_{21}$-$B1c_{17}$ + $FB1_{24}$/$BB0_{26}$ (in pruple).

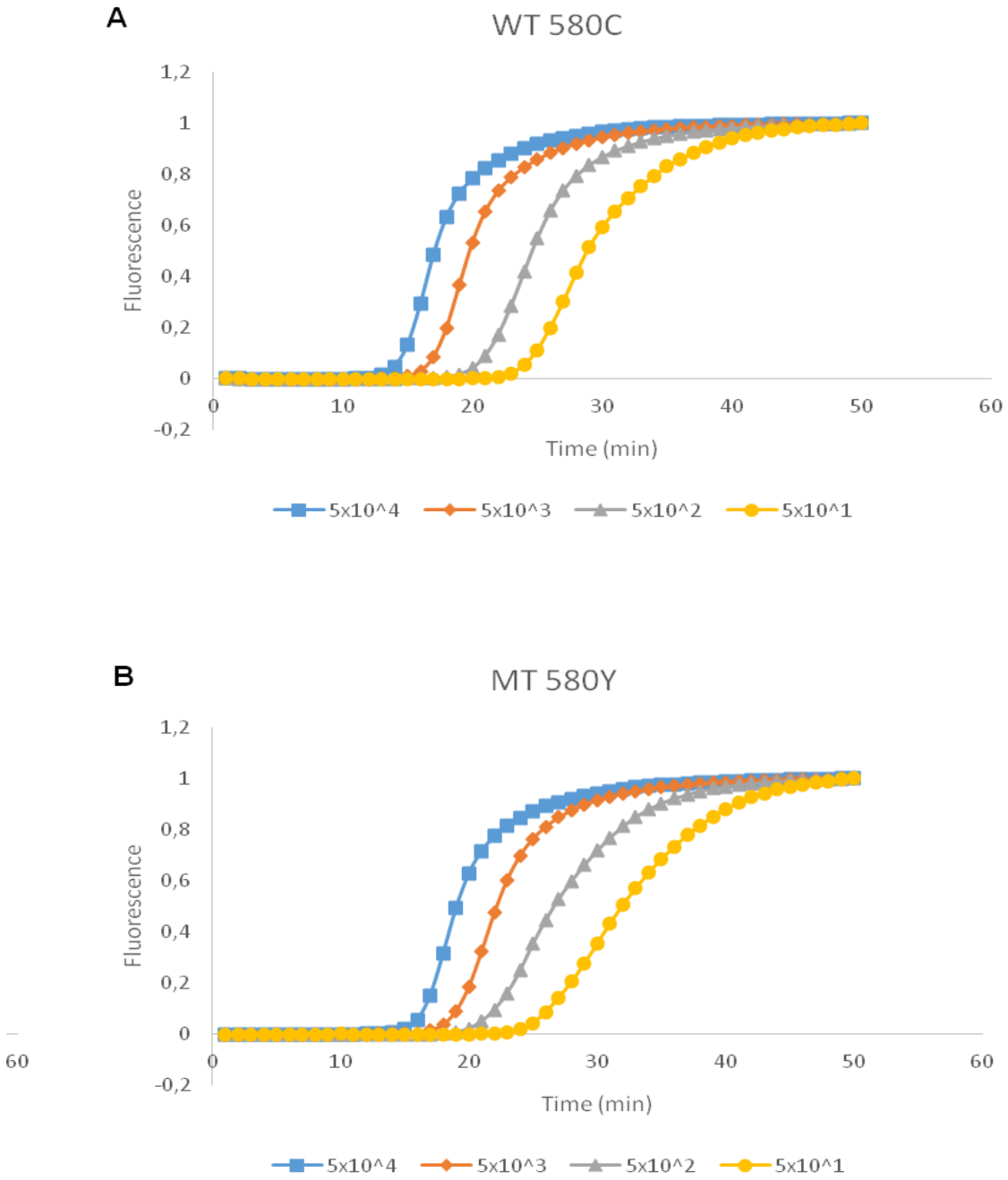
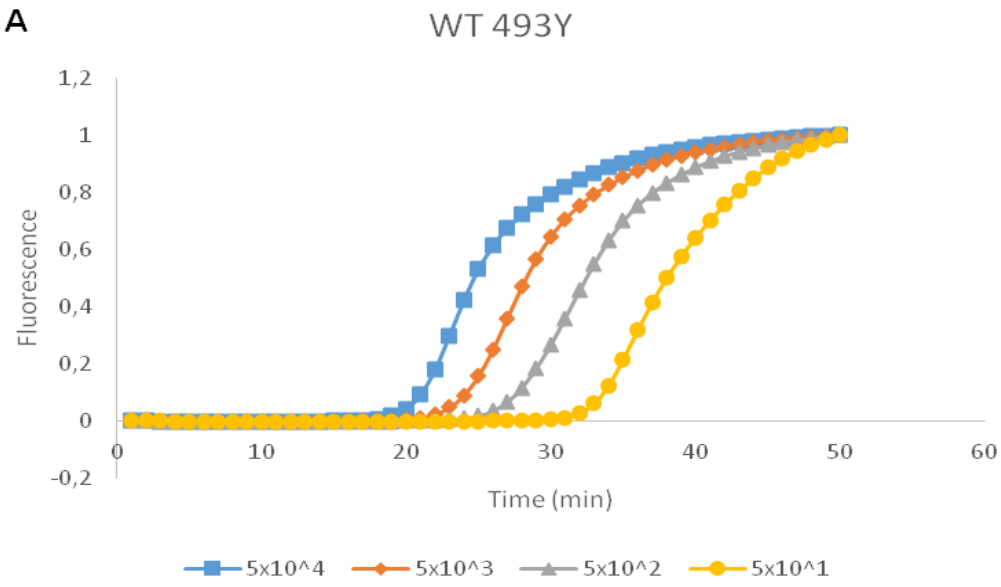


Figure S2

Amplification curves of **(A)** WT *580C* template ($5x10^4$-$5x10^1$ copies/reaction) using USS_{MT} -sbLAMP $_{W}$ $F1c_{17}$-$B1c_{27}$ + $FB2_{33}$/$BB1_{21}$ and **(B)** MT *580Y* ($5x10^4$-$5x10^1$ copies/reaction) template using USS_{WT} $sbLAMP_{MT}$ $F1c_{17}$-$B1c_{27}$ + $FB1_{27}$/$BB2_{21}$. No amplification for NTC (data not shown).



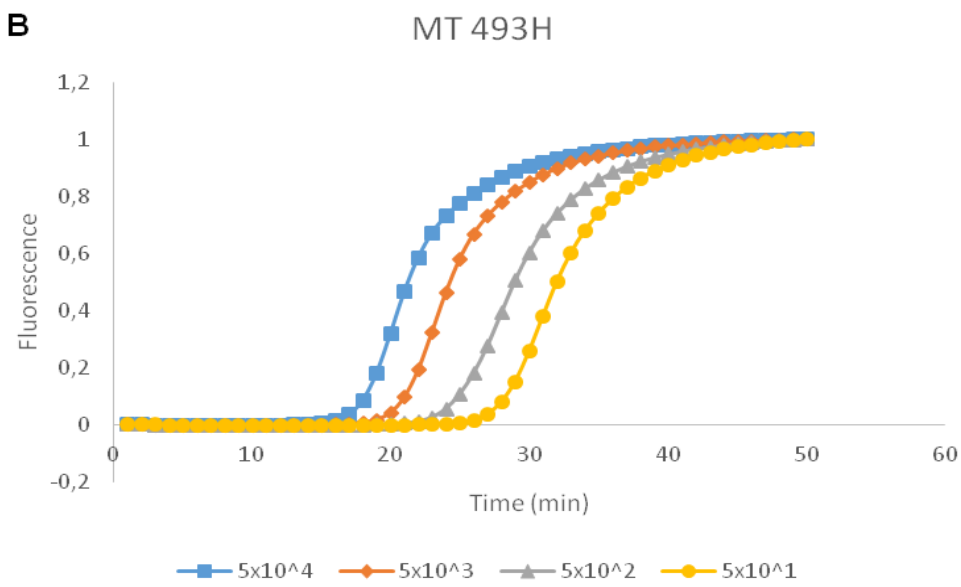


Figure S3

Amplification curves of **(A)** WT *493Y* template ($5x10^4$-$5x10^1$ copies/reaction) using USS_{MT}-$sbLAMP_{WT}$ $F1c_{21}$-$B1c_{17}$ + $FB2_{24}$/$BB1_{26}$ and **(B)** MT *493H* template ($5x10^4$-$5x10^1$ copies/reaction) using USS_{WT} -$sbLAMP_{MT}$ $F1c_{21}$-$B1c_{17}$ + $FB1_{24}$/$BB0_{26}$.