Electronic Supplementary Information

High-throughput ultra-selective discrimination of single nucleotide polymorphism via click chemical ligation

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Table S1. The oligonucleotides used in the exploring performance of SNP discrimination experiments (5′-3′)

Name	Sequence
P1	NH_2C_{12}-AAAAAGAGGTCCTCG-CHCH
P2	N_3-CGATGTCCAA-Biotin
Perfectly matched target (T)	TTGGACATCGCGAGGACCTC
Mismatch-1-C (M_1=C) target (M1)	TTGGACATCCCGAGGACCTC
Mismatch-2-G (M_2=G) target (M2)	TTGGACATGGCGAGGACCTC
Mismatch-3-A (M_3=A) target (M3)	TTGGACAACGCGAGGACCTC
Mismatch-4-T (M_4=T) target (M4)	TTGGACTTCGCGAGGACCTC
Mismatch-5-G (M_5=G) target (M5)	TTGGAGATCGCGAGGACCTC
Mismatch-6-T (M_6=T) target (M6)	TTGGTCATCGCGAGGACCTC
Mismatch-4-G (M_4=G) target (M7)	TTGGACGTCGCGAGGACCTC
Mismatch-4-C (M_4=C) target (M8)	TTGGACCTCGCGAGGACCTC

Note: The underlined bases are mutant bases.

Table S2. Comparison between the proposed CuAAC-LA based SNP detection strategy with other SNP detection approaches reported since 2015.

Methods	Selectivity	DNA enzyme	reference
CuAAC-LA based SNP detection	0.05-0.1%	No	This work
Dynamic sandwich assay (DSA)	0.1–0.5%	No	1
Using CCP to probe the hybridization equilibrium of short duplex	0.1%	No	2
Simulation-guided DNA probe and sink design	1%	No	3
Sequestration-assisted molecular beacon	0.5%	No	4
Combining cooperativity with sequestration	0.2%	No	5
Hairpin masking with FNP counting	0.05%-0.1%	No	6
One-step isothermal detection	1%	Taq DNA ligase	7
Isothermal ligase reaction combined with a modified cycling probe assay	1%	E.coli DNA ligase, RNase H	8
Real-time fluorescence LCR-based detection	0.1%	Ampligase	9
Rolling circle amplification (RCA)-responsive G-quadruplex and thioflavin T	0.13%	Taq DNA ligase, phi29 DNA polymerase	10
Mismatched ligation tiggered cascade strand displacement amplification	0.2%	Taq DNA ligase, Klenow fragment, Nb.BbvC	11
A genotyping–microarray by ligating a universal fluorescence-probe with SNP-encoded flaps cleaved from invasive reactions	NA	DNA Ligase, flap endonuclease	12
Mass spectrometry-based method allele-specific ligation and strand displacement amplification	NA	9°N™ DNA ligase, Vent (exo-) polymerase, and Nt. BstNBI nicking enzyme	13
Toehold strand displacement (TSD) and endonuclease IV (Endo IV)	0.5%	Endonuclease IV	14
Strand displacement and selective digestion	0.2%	lambda exonuclease	15
Nucleic Acid Self-Assembly Circuitry Aided by Exonuclease III	1%	exonuclease III	16
Ligase-based analysis with modified base-end downstream ligation fragments	NA	T4 DNA ligase	17

Lambda exonuclease and a chemically modified DNA substrate structure	0.5%	lambda exonuclease	18
Abasic site modified fluorescent probe and lambda exonuclease	0.02-0.05%	lambda exonuclease	19

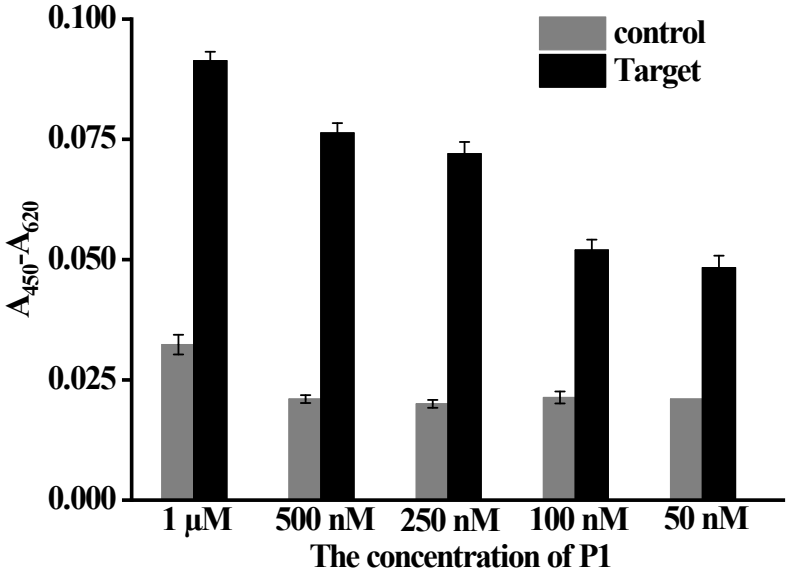

Fig. S1. The effect of the concentration of P1 for DNA detection. Experimental conditions: 50 nM P2 ,1 nM T with different concentration of P1 incubated at 25℃ for 2 h.

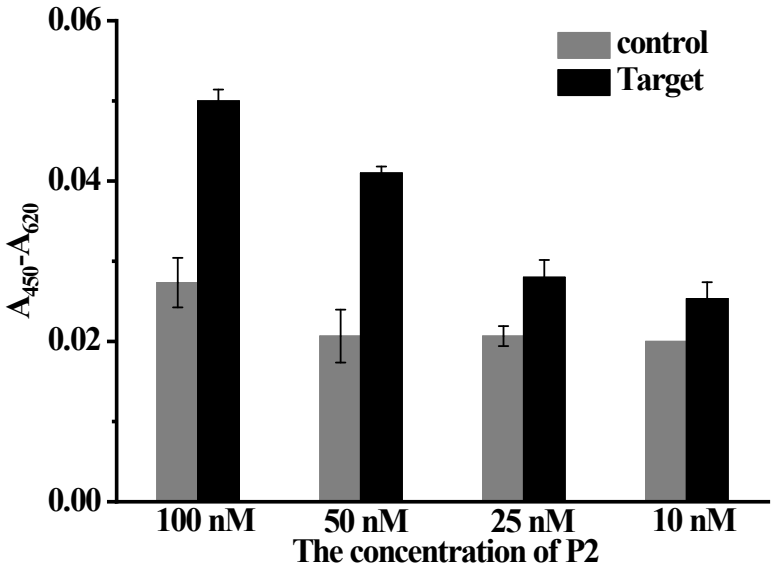

Fig S2. The effect of the concentration of P2 for DNA detection. Experimental conditions: 250 nM P1 ,1 nM T with different concentration of P2 incubated at 25℃ for 2 h.

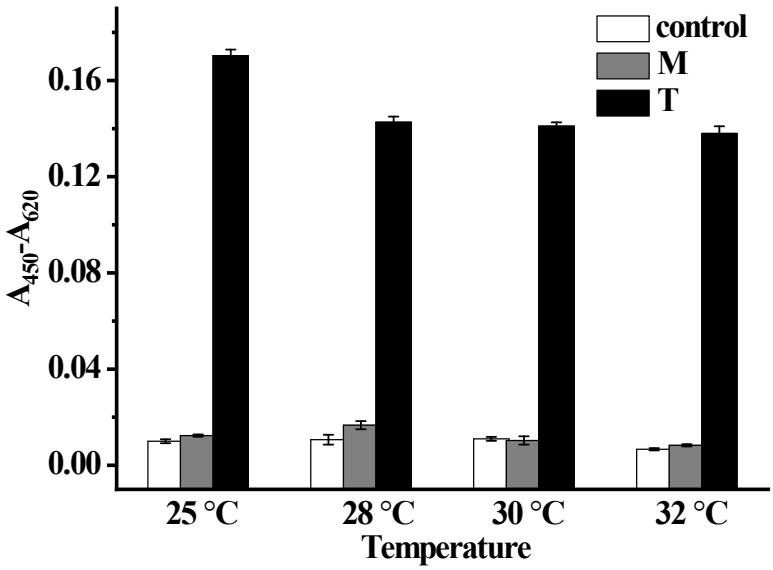

Fig S3. The effect of temperature for SNP detection. Experimental conditions: 250 nM P1, 50 nM P2 with 10 nM T or M incubated at different temperature for 2 h.

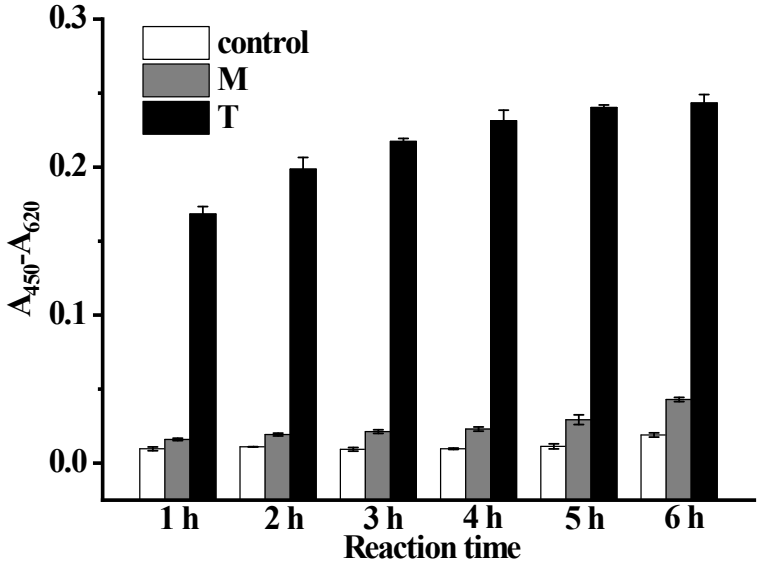

Fig S4. The effect of reaction time for DNA detection. Experimental conditions: 250 nM P1, 50 nM P2 with 10 nM T or M incubated at 25℃ for different reaction time.

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