

Electronic Supplementary Information

Simultaneous multiple single nucleotide polymorphism detection based on click chemistry combining with DNA-encoded probes

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Experimental methods

Materials and apparatus

CuSO₄·5H₂O, NaH₂PO₄·2H₂O, Na₂HPO₄·12H₂O and NaCl were purchased from Xilong Chemical Co., Ltd. (Guangdong, China). Sodium ascorbate was bought from Aladdin (Shanghai, China). Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and stored at 4°C. HPLC purified oligonucleotides were obtained from Shanghai Sangon Biotech (Shanghai, China). The sequences and modifications used in this study are listed in Table S1 and S2. Deionized water was used in all experiments. All chemical reagents in the experiment were of analytical grade. Beckman coulter ssDNA 100-R Kit was acquired from Beckman (USA). The CuAAC reaction was performed on a C1000 Touch™ PCR (USA). HPLC - ESI - MS was carried out on Dionex Ultimate 3000 UHPLC and Thermo Q - Exactive mass spectrometer (Thermo Fisher, MA, USA). CuAAC products were separated and detected with a Beckman coulter PA800 plus (USA) quipped with an LIF detector (excitation at 488 nm)

The CuAAC reaction for target ssDNA detection

Probe P_M (50 nM) and probe P_N (50 nM) were mixed with different concentration of target DNA in 10 mM potassium phosphate solution (PBS, pH 7.4). The resulting solutions were heated to 85°C for 5 min and then gradually cooled down to room temperature. Then a mixture of the catalyst solutions (2 mM THPTA, 100 μM CuSO₄, 1 mM sodium ascorbate) was added to the above solutions and the CuAAC reaction was carried out at room temperature for 2 h.

The CuAAC reaction for SNP detection

A certain ratio of probe P_M and probe P_N was mixed with an appropriate amount of target DNA or mutant DNA in PBS. The resulting solutions were heated to 85°C for 5 min and then gradually cooled down to 30°C. Then a mixture of the catalyst solutions (2 mM THPTA, 100 μM CuSO₄, 1 mM sodium ascorbate) was added to the above solutions and the CuAAC reaction was carried out at 30°C for 0.5 h.

The detection of the CuAAC products

The CuAAC products were mixed with 1 μL FAM labeled internal standard chain (IS) to correct the fluorescence intensity caused by aberration of sample injection, and subsequently injected into capillaries with application of a voltage of 9.3 kV for 2 s. The separation and detection of the CuAAC products were performed on the Beckman coulter PA800 plus with an electrophoresis voltage of 9.3 kV at 30°C for an hour of running time. The positions and areas of the peaks were determined by the 32 Karat Software (Beckman). The relative peak area (RPA) which was used for the quantification of the CuAAC product was defined as the ratio of the peak area of CuAAC product

to the peak area of IS.

For HPLC-ESI-MS detection, elution was performed using 8 mM dimethylhexylamine and acetate acid (160 μ L in 500 mL solution) as mobile phase A, and acetonitrile as mobile phase B for HPLC-MS. The injection volume was 20 μ L. Scan range: 350 m/z to 4000 m/z. Mass resolution: 70 000.

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

Name	Sequence
P _M	FAM-TAGCTTATCAG-Azide
P _N	Alkyne-ACTGATGTTGA
Perfectly matched target (T)	TCAACATCAGTCTGATAAGCTA
Mismatch-1-T (N ₁ =T) target (M1)	TCAACATCAGT <u>T</u> TGATAAGCTA
Mismatch-2-C (N ₂ =C) target (M2)	TCAACATCAGT <u>C</u> GATAAGCTA
Mismatch-3-T (N ₃ =C) target (M3)	TCAACATCAGTCT <u>C</u> ATAAGCTA
Mismatch-4-A (N ₃ =T) target (M4)	TCAACATCAGTCT <u>T</u> ATAAGCTA
Mismatch-5-C (N ₃ =A) target (M5)	TCAACATCAGTCT <u>A</u> ATAAGCTA
Mismatch-6-G (N ₄ =G) target (M6)	TCAACATCAGTCTG <u>G</u> TAAGCTA
Mismatch-7-C (N ₄ =C) target (M7)	TCAACATCAGTCTG <u>C</u> TAAGCTA
IS	FAM-CTCG

Note: The underlined bases are mutant bases.

Table S2. The oligonucleotides used in the detection of STK11 rs59912467(C>G), rs184528337 (C>T) and rs587778695 (C>A) mutations (5'-3')

Name	Sequence
P _{M1} (C>G)	FAM-CGATGTCCAA-Azide
P _{N1} (C>G)	Alkyne-GAGGTCCTCG
WT ₁ (C>G)	ACGAGGACCTCTT <u>C</u> GACATCGA
MT ₁ (C>G)	ACGAGGACCTCTT <u>G</u> GACATCGA
P _{M2} (C>T)	FAM-CGAGTCTCACT-Azide
P _{N2} (C>T)	Alkyne-GTGTGCGCCGCGCCG
WT ₂ (C>T)	GGGCGACACAG <u>C</u> GAGACTC
MT ₂ (C>T)	GGGCGACACAG <u>T</u> GAGACTC
P _{M3} (C>A)	FAM-CGTCCTGATTG-Azide
P _{N3} (C>A)	Alkyne-TAGATGATGTCATCC
WT ₃ (C>A)	GATGACATCATCTACA <u>C</u> TCAAGGAC
MT ₃ (C>A)	GATGACATCATCTACA <u>A</u> TCAAGGAC

Note: The underlined bases are mutant bases.

Table S3. Effect of mismatched position for SNP detection

	Relative peak area 1 (RPA1)	Relative peak area 2 (RPA2)	Ratio of RPA1 to RPA2
$N_4=G: N_1=T$ (G-T) : (T-G)	2.329	2.997	0.777
$N_4=C: N_3=T$ (T-C) : (C-T)	0.5353	0.4366	1.226
$N_3=A: N_2=C$ (A-C) : (C-A)	0.4565	1.320	0.346

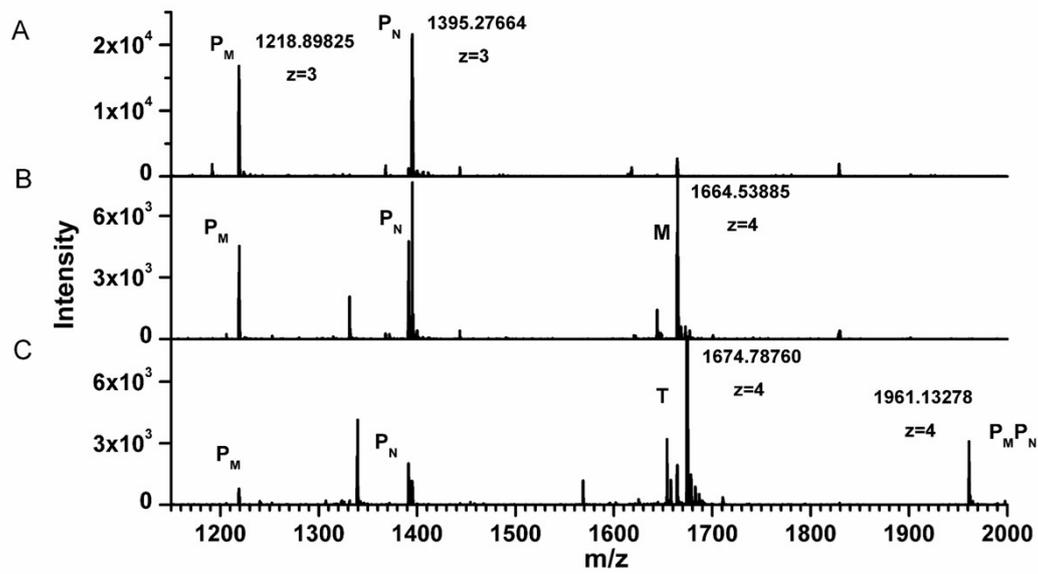


Fig. S1 Mass spectra of the template-directed CuAAC chemical reaction (A) without target (50 nM P_M +50 nM P_N), (B) with mismatched target M3 (50 nM P_M +50 nM P_N +100 nM M3), (C) with matched target T (50 nM P_M +50 nM P_N +100 nM T).

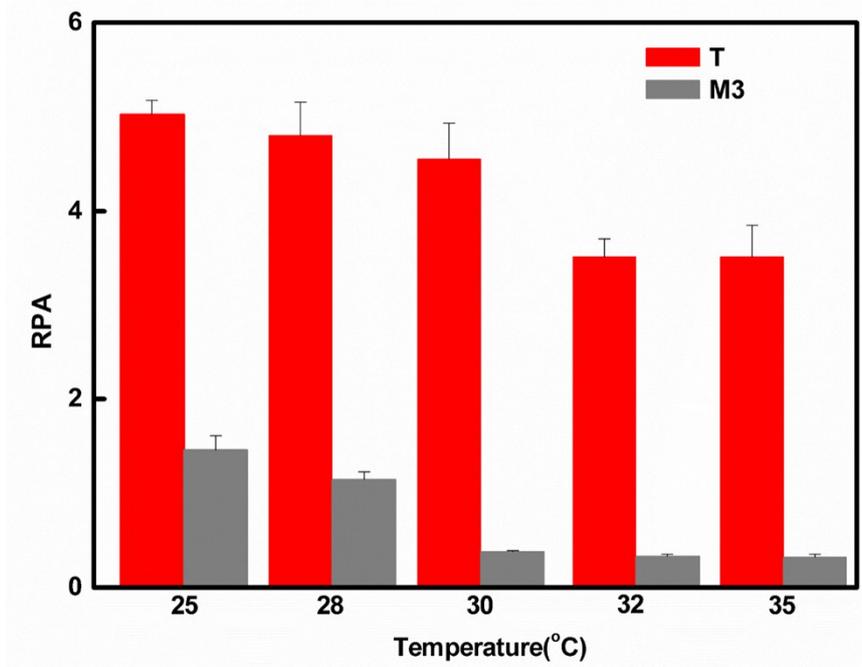


Fig. S2 The effect of temperature on SNP detection

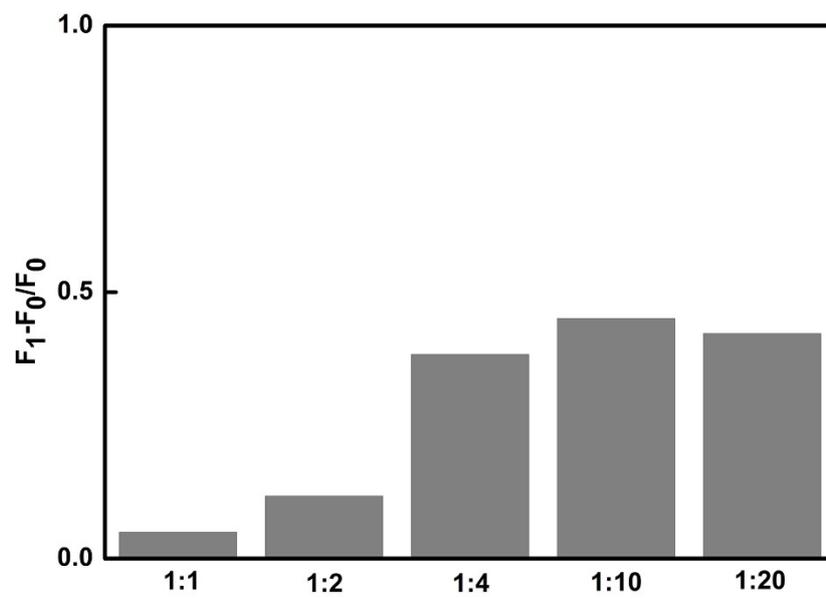


Fig. S3 The effect of ratios (P1:P2) on SNP detection. F_1 and F_0 represent the peak area produced by abundance 0.5%, and 100%, respectively.

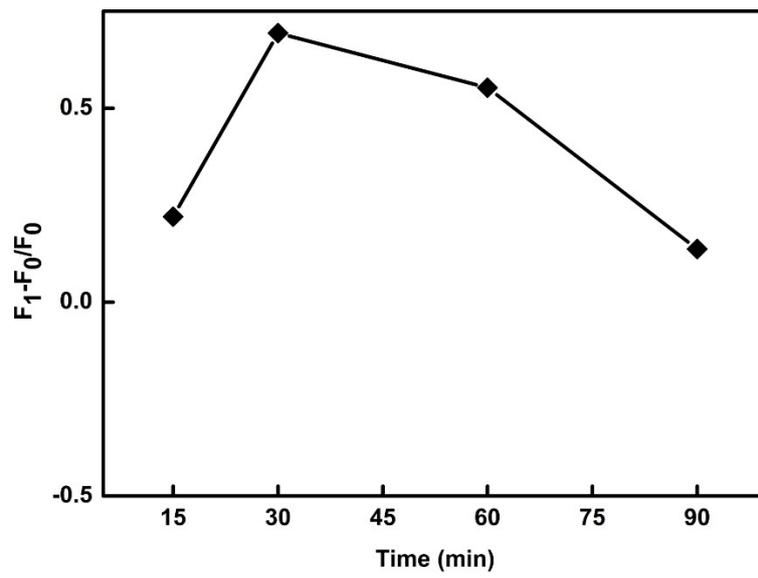


Fig. S4 The effect of reaction time on SNP detection. F_1 and F_0 represent the peak area produced by abundance 0.5%, and 100%, respectively.