Supporting Information for

A novel method to detect mutation in DNA by utilizing exponential amplification reaction triggered by the CRISPR-Cas9 system

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Table S1. Oligonucleotide sequences employed in this work.

Name	DNA sequence (5'→3')
Target mutant DNA (insertion mutation)[a]	GCA TAC GTG ATG GCT GGT GTG GGC TCC CCT **GGC TCC CC**A TAT GTC TCC CGC CTC CTG GGC ATC TGC
Target wild type DNA	GCA TAC GTG ATG GCT GGT GTG GGC TCC CCA TAT GTC TCC CGC CTC CTG GGC ATC TGC
sgRNA 1 (insertion mutation)	AUG GCU GGU GUG GGC UCC CC
sgRNA 2 (insertion mutation)	CCA GGA GGC GGG AGA CAU AU
EXPAR template 1[b]	TAT GGG GAG CCA GGG AAC A*GA TCC* ATA TGG GGA GCC AGG A
Target mutant DNA 2 (L858R mutation)[a]	TGT CCC TCA CAG CAG GGT CTT CTC TGT TTC AGG GCA TGA ACT ACT TGG AGG ACC GTC GCT TGG TGC ACC GCG ACC TGG CAG CCA GGA ACG TAC TGG TGA AAA CAC CGC AGC ATG TCA AGA TCA CAG ATT TTG GG**C GG**G CCA AAC TGC TGG GTG CGG AAG AGA AAG AAT ACC ATG CAG AAG GAG GCA AAG TAA GGA GGT GGC TTT AGG TCA GCC AGC ATT TTC CTG ACA CCA GGG ACC AGG CTG CCT TCC CAC TAG CTG TAT TGT TTA ACA
Target wild type DNA 2[a]	TGT CCC TCA CAG CAG GGT CTT CTC TGT TTC AGG GCA TGA ACT ACT TGG AGG ACC GTC GCT TGG TGC ACC GCG ACC TGG CAG CCA GGA ACG TAC TGG TGA AAA CAC CGC AGC ATG TCA AGA TCA CAG ATT TTG GGC TGG CCA AAC TGC TGG GTG CGG AAG AGA AAG AAT ACC ATG CAG AAG GAG GCA AAG TAA GGA GGT GGC TTT AGG TCA GCC AGC ATT TTC CTG ACA CCA GGG ACC AGG CTG CCT TCC CAC TAG CTG TAT TGT TTA ACA
sgRNA 1 (L858R mutation)	UCA AGA UCA CAG AUU UUG GG
sgRNA 2 (L858R mutation)	CUC UUC CGC ACC CAG CAG UU
EXPAR template 2[b] (L858R mutation)	GTT TGG CCC GCC CAAC A*GA TCC* A GTT TGG CCC GCC C
sgRNA_R-primer	AGC ACC GAC TCG GTG CCA CTT
sgRNA 1_F-primer	TAA TAC GAC TCA CTA TAG ATG GCT GGT GTG GGC TCC CCG TTT TAG AGC TAG AAA TAG C
sgRNA 2_F-primer	TAA TAC GAC TCA CTA TAG CCA GGA GGC GGG AGA CAT ATG TTT TAG AGC TAG AAA TAG C
MM 1 target[a,c]	GCA TAC GTG ATG GCT GGT GTG GGC TCC CCT **AGC TCC CC**A TAT GTC TCC CGC CTC CTG GGC ATC TGC
MM 2 target[a,c]	GCA TAC GTG ATG GCT GGT GTG GGC TCC CCT **AAC TCC CC**A TAT GTC TCC CGC CTC CTG GGC ATC TGC
MM 3 target[a,c]	GCA TAC GTG ATG GCT GGT GTG GGC TCG CCT **AAC TCC CC**A TAT GTC TCC CGC CTC CTG GGC ATC TGC
MM 4 target[a,c]	GCA TAC GTG ATG GCT GGT GTG GGC TCG CCT **AAC TCC CC**A TTT GTC TCC CGC CTC CTG GGC ATC TGC

(a) The bold characters indicate the target mutation. Red color characters represent the PAM sequence.
(b) The italic letters indicate the recognition site of Nt.AlwI.
(c) The underlined bases indicate the nonspecific bases within target insertion mutation. MM# indicates the mismatched target mutation samples containing imperfect 9 bp target insertion mutations in which a few bases (#) from one to four are replaced by different bases.

Table S2. Comparison of the CRISPR-EXPAR method with previous mutation detection methods.

Method	Linear range	Detection limit	Limitations	Reference
Rolling circle amplification	0.02 pM - 2 pM	8.6 fM	▪ Low sensitivity ▪ Prior ligation step for padlock probe	S1
Strand-displacement polymerase	0.1 pM - 50 nM	0.08 pM	▪ Low sensitivity	S2
Toehold-mediated strand displacement reaction	2 pM - 10 nM	1.8 pM	▪ Low sensitivity ▪ Multiple assay steps	S3
Helicase-dependent amplification	10 pM – 1 nM	20 pM	▪ Preparation of gold nanoparticles	S4
Strand displacement amplification	1 fM – 1 nM	0.1 fM	▪ Non-specific signal ▪ Extra step for hemin incubation	S5
CRISPR-EXPAR	1 fM - 1 nM	0.44 fM	-	This work

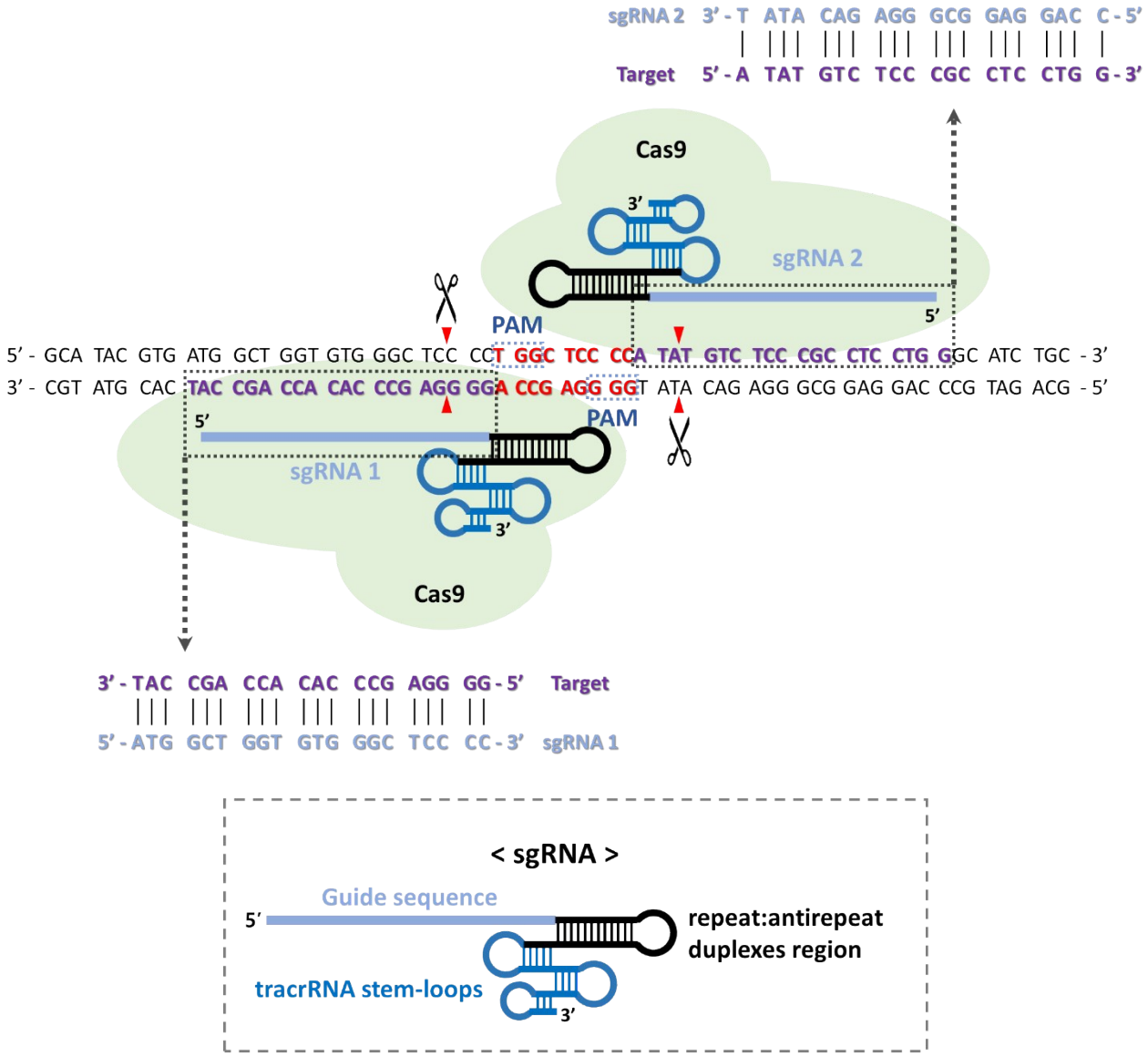


Figure S1. sgRNA sequence design. sgRNAs employed in this work consist of three segments: guide sequence, repeat:antirepeat duplexes region, and three tracrRNA stem-loops. sgRNA can bind with Cas9 to form Cas9/sgRNA complex which promotes mutation-specific cleavage of target DNA.

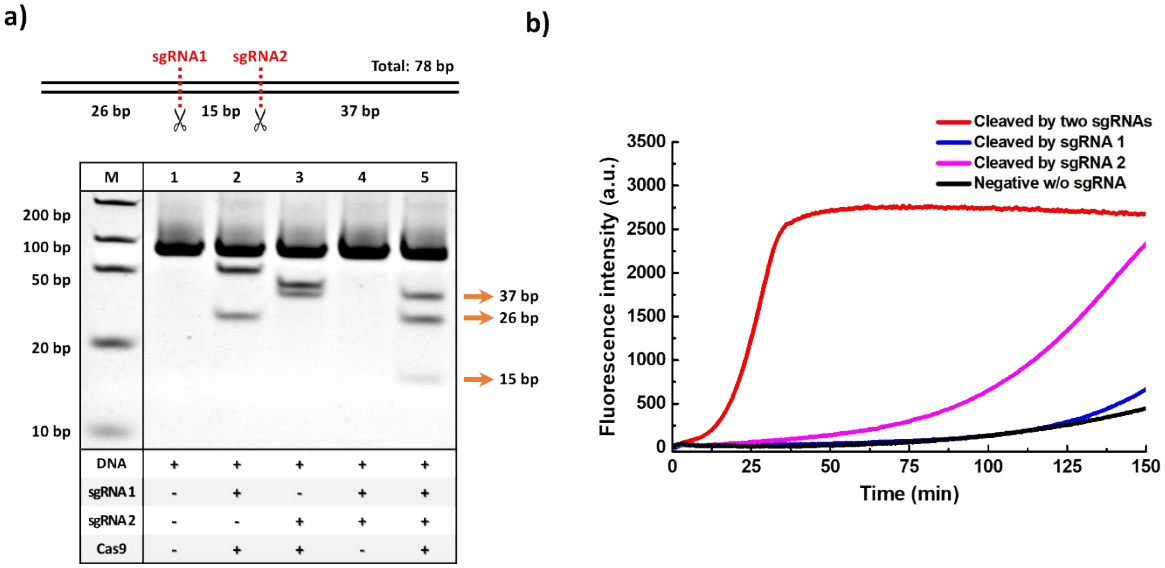


Figure S2. The requirement for two sgRNAs to promote the two Cas9-mediated cleavage reactions in the CRISPR-EXPAR reaction. a) PAGE analysis of the CRISPR-EXPAR products (1: Mutant DNA, 2: Mutant DNA + sgRNA 1 + Cas9, 3: Mutant DNA + sgRNA 2 + Cas9, 4: Mutant DNA + Two sgRNAs, 5: Mutant DNA + Two sgRNAs + Cas9). b) Real-time fluorescence curves during the CRISPR-EXPAR reaction. The final concentration of DNA, sgRNAs, and Cas9 are 200 nM, 2 μM, and 2 μM, respectively. M is marker for band analysis (Ultra low range ladder).

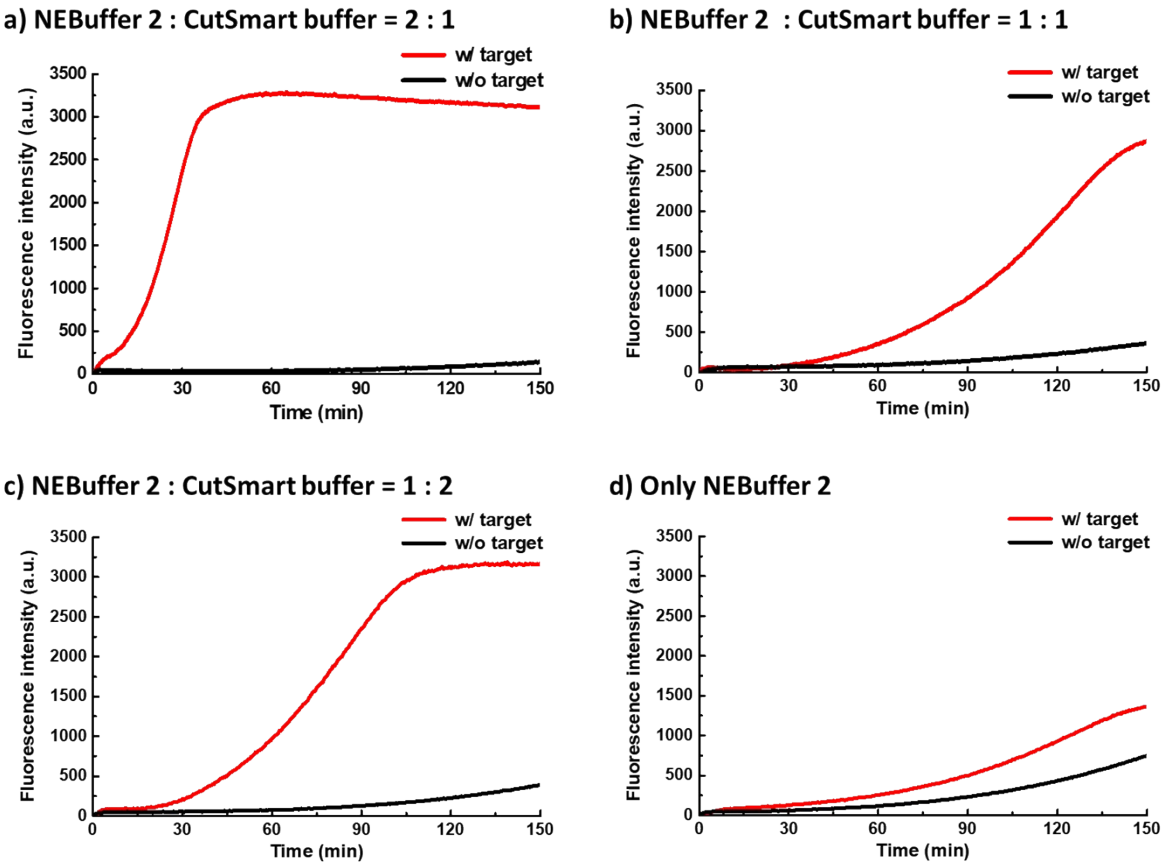


Figure S3. Optimization for reaction buffer concentration. The real-time fluorescence curves were obtained during the CRISPR-EXPAR reaction. The final concentration of DNA, sgRNAs, and Cas9 are 1 nM, 10 nM, and 10 nM, respectively.

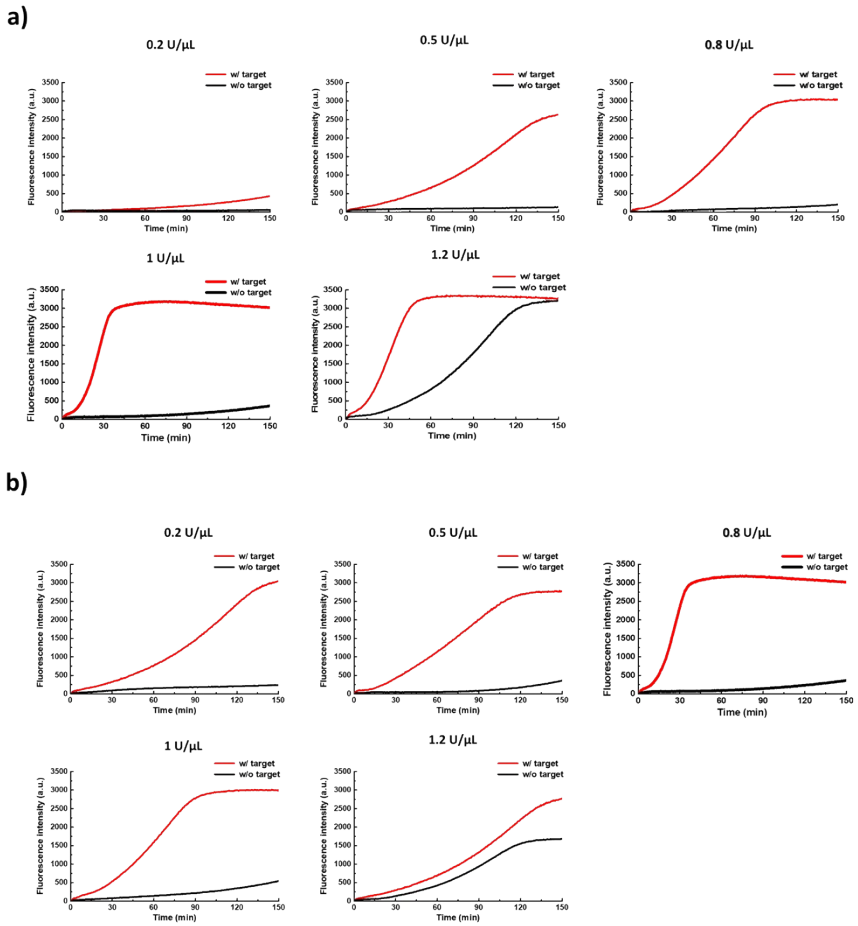


Figure S4. Optimization for the concentrations of the enzymes employed in the CRISPR-EXPAR reaction. a) Optimization of DNA polymerase (Klenow fragment) concentration. The real-time fluorescence curves were obtained during the CRISPR-EXPAR reaction with 1 nM target DNA (red line) and without target DNA (black line). The concentrations of Klenow fragment are 0.2 U/μL, 0.5 U/μL, 0.8 U/μL, 1.0 U/μL, and 1.2 U/μL. b) Optimization of nicking endonuclease (Nt.AlwI) concentration. The real-time fluorescence curves were obtained during the CRISPR-EXPAR reaction with 1 nM target DNA (red line) and without target DNA (black line). The concentrations of Nt.AlwI are 0.2 U/μL, 0.5 U/μL, 0.8 U/μL, 1.0 U/μL, and 1.2 U/μL.

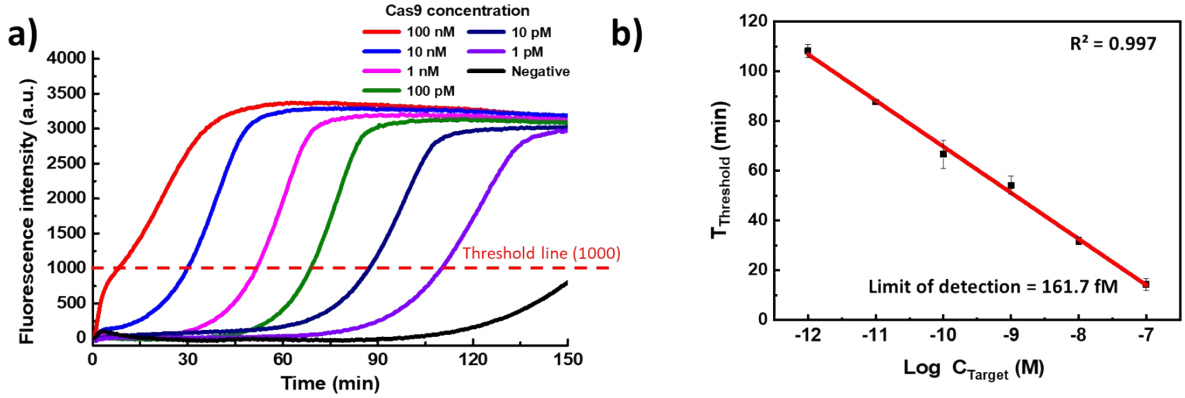
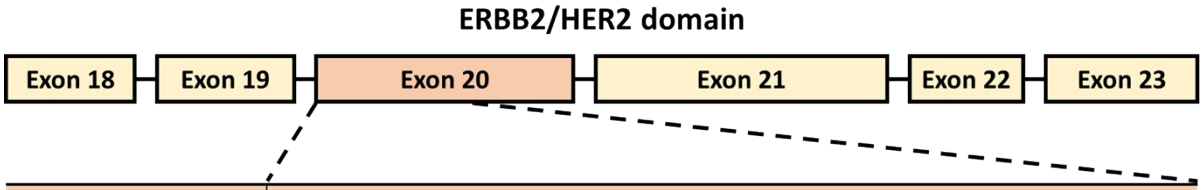


Figure S5. Assay of Cas9 activity. a) Real-time fluorescence curve for the CRISPR-EXPAR reaction by employing Cas9 at varying concentrations in the range from 1 pM to 100 nM. b) Relationship between the threshold time ($T_{Threshold}$) and the logarithm of the concentration of Cas9, where $T_{Threshold}$ is defined as the reaction time at which the fluorescence signal exceeds the threshold line (fluorescence intensity: 1000) and C_{Target} is the concentration of Cas9. The error bar is determined from triplicate measurements.



Wild type	**GAA GCA TAC GTG ATG GCT GGT GTG GGC TCC CCA TAT**
Target mutation (9 bp insertion)	GAA GCA TAC GTG ATG GCT GGT GTG GGC TCC CC**T GGC TCC CC**A TAT
12 bp insertion (1)	GAA GCA TAC GTG ATG GC**A TAC GTGA TGG C**TG GTG TGG GCT CCC CAT AT
12 bp insertion (2)	GAA GCA TAC GTG ATG GCT **TAC GTG ATG GCT** GGT GTG GGC TCC CCA TAT
6 bp insertion/ 1 bp substitution	GAA GCA TAC GTG ATG GCT **T**GT GTG **TGT GGG** GGC TCC CCA TAT

Figure S6. Insertion mutations on HER2 exon 20 studied in this work. Red color characters represent the insertion mutation.

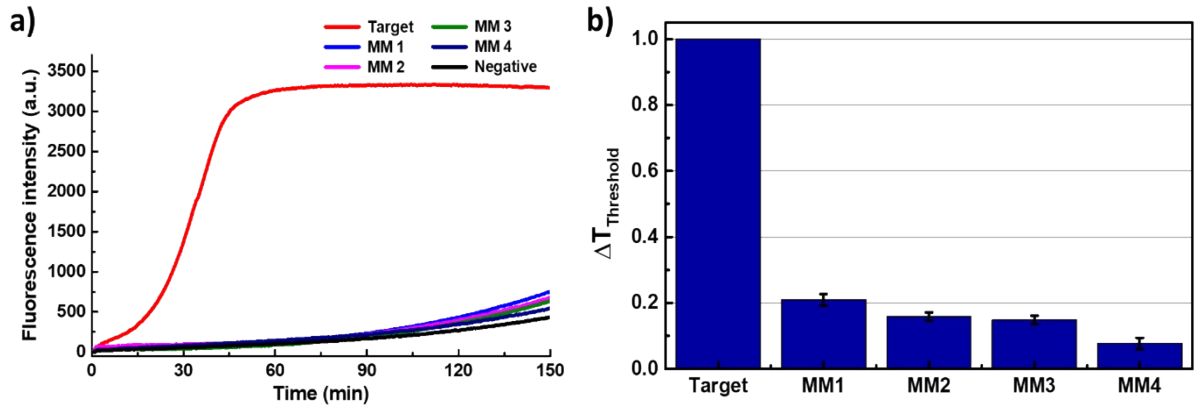


Figure S7. Selectivity of the CRISPR-EXPAR reaction system. a) Real-time fluorescence signal produced from target mutant DNA and nontarget mutant DNAs containing imperfect 9 bp target insertion mutations in which single base (MM1), two bases (MM2), three bases (MM3), and four bases (MM4) are replaced by different bases. b) The $\Delta T_{Threshold}$ is defined as $(T_{Threshold\ (Blank)} - T_{Threshold\ (MM\ target)})/(T_{Threshold\ (Blank)} - T_{Threshold\ (Target)})$, where $T_{Threshold\ (Blank)}$, $T_{Threshold\ (MM\ target)}$, and $T_{Threshold\ (Target)}$ are the threshold times from the reaction samples containing no target mutation, imperfect 9 bp target mutation, and perfect 9 bp target insertion mutation, respectively. The detailed sequences are presented in table S1. The error bar is determined from triplicate measurements.

References

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