Supporting Information for

CRISPR/Cas12a collateral cleavage activity for an ultrasensitive assay of RNase H

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Contents

Materials and Methods

1. Materials

All the oligonucleotides employed in this study were synthesized and purified with polyacrylamide gel electrophoresis (PAGE) by Bioneer® (Daejeon, Korea), except for blocker RNA (purified by high-performance liquid chromatography (HPLC)). The sequences of the oligonucleotides used in this study are listed in Table S1. Alt-R® A.s. Cas12a (Cpf1) Ultra and Alt-R® A.s. Cas12a crRNA were purchased from IDT (Coralville, IA, USA). Escherichia coli (E. coli) RNase H, DNase I, exonuclease I (Exo I), exonuclease III (Exo III), and FEN1 were purchased from Enzynomics (Daejeon, South Korea). T4 polynucleotide kinase (PNK), uracil DNA glycosylase (UDG), lambda exonuclease (λ exo), HIND III, Dpn I, Nt.BstI, and APE1 were purchased from New England Biolabs Inc. (Beverly, MA, USA). Ribonuclease A (RNase A) was purchased from Takara (Japan). Duplex-specific nuclease (DSN) was purchased from Evrogen (Konkov, Moscow, Russia). Dulbecco's modified Eagle's medium (DMEM) and Fetalgro Bovine Growth Serum (FBS) were purchased from Welgene Inc. (Gyeongsan-si, Korea) and RMBIO® (Missoula, MT, USA), respectively. Tumor cell lines (A549 and HT-29) were purchased from Korean Cell Line Bank (Seoul, South Korea). Ultrapure DNase/RNase-free distilled water (DW) was purchased from Bioneer Co. and used throughout all the experiments. All other chemicals were of analytical grade and used without further purification.

2. Procedures of proposed assay

6 μL of DW, 1 μL of 10X RNase H buffer (500 mM Tris-HCl, 750 mM KCl, 30 mM MgCl2, 100 mM DTT, pH 8.3), 1 μL of activator DNA (AD) (100 nM), 1 μL of blocker RNA (BR) (100 nM), and 1 μL of RNase H at varying concentrations were mixed to make total 10 μL of RNase H reaction solution. The RNase H reaction solution was incubated at 37 ℃ for 30 min. Next, the detection solution consisting of 4 μL of DW, 2 μL of 10X NEBuffer 2.1 (500 mM NaCl, 100 mM Tris-HCl, 100 mM MgCl2, 1 mg mL-1 BSA, pH 7.9), 2 μL of FAM- and BHQ1-labeled reporter probe (F-Q reporter) (1 μM), 1 μL of crRNA (1 μM), and 1 μL of Cas12a (1 μM) was added to the reaction mixture, which was then incubated at 25 ℃ for 15 min. The fluorescence signal from the reaction solution was measured from 500 nm and 600 nm at an excitation wavelength of 460 nm by using a Tecan Infinite M200 pro microplate reader (Männedorf, Switzerland) and 384-well Greiner Bio-One microplates (Ref. 781077, Courtaboeuf, France). In the experiments for the evaluation of selectivity and the assay for the tumor lysates, enzyme inactivation step was additionally conducted at 65 ℃ for 15 min after the RNase H reaction to eliminate the possible effect by other potentially interfering enzyme activities.

3. Biological sample test

Two tumor cells (A549 and HT-29) were cultured in DMEM supplemented with 10 % FBS under humidified atmosphere containing 5 % CO_2 at 37 ℃ and collected during the exponential growth phase, which were estimated to be 1×10^7 cells based on the cell counter, LUNA-II™ (Logos Biosystems Inc., Gyeonggi-do, Korea). Collected cells were washed three times with 1X PBS (10 mM phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.4). The cells were lysed and cellular extracts were obtained by using PRO-PREP™ protein extraction solution kit (Intron Biotechnology, South Korea). The cellular extracts were quantified by Pierce™ bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) and resuspended in nuclease-free water at a concentration of 2 μg/ μL. The RNase H activity was then assayed by following the same procedure as described above.

4. Gel electrophoresis analysis

For PAGE analysis, 10 μL reaction solution was mixed with 2 μL loading buffer (6X) purchased from Bioneer®, which was resolved on 15 % polyacrylamide gel at a constant voltage of 120 V for 120 min using 1X TBE as the running buffer. After GelRed staining, the gel image was taken with a ChemiDoc™ Imaging System (Bio-Rad, Hercules, USA). In the experiments to employ FAM-labeled reporter probe, the staining step was omitted.

Table S1. Oligonucleotide sequences used in this work.

Oligonucleotide	**Sequence (5' → 3')**
Blocker RNA (BR)	rArCrC rUrUrC rCrUrC rCrGrC rArArU rArCrU rCrCrC rCrCrA rGrGrU
Activator DNA (AD)[(a)]	ACC TGG GGG AGT ATT GCG GAG GAA GGT
F-Q reporter	[FAM]-TCG TAT CCA GTG CGA ATC ACT C-[BHQ1]
F reporter	[FAM]-AGA ACC GAA TTT GTG TAG CTT ATC AGA CTG
crRNA[(a)]	rUrArA rUrUrU rCrUrA rCrUrC rUrUrG rUrArG rArUrU rUrCrC rGrCrA rArUrA rCrUrC rCrCrC rCrArG rGrU
Blocker DNA (BD)	ACC TTC CTC CGC AAT ACT CCC CCA GGT

[(a)] Sequences written in red represent complementary sequences between AD and crRNA.

Table S2. Comparison of the proposed assay with alternative methods for RNase H assay.

Key components/Methods	**Limit of detection ($U\ mL^{-1}$)**	**Detection range ($U\ mL^{-1}$)**	**Assay time**	**Limitations**	**Reference**
Rolling circle amplification	0.019	0-1	20 min	• Expensive synthesis of chimeric RNA-DNA oligonucleotides	[S1]
DNA templated fluorescent copper nanoclusters	0.00055	0.0006-30	1 h		[S2]
Graphene oxide nanosheets	0.0007	0.001-5	3 h 15 min	• Long reaction time	[S3]
G-quadruplex / NMM complex	0.037	0-0.7	2 h 50 min	• Long reaction time • Low sensitivity	[S4]
Target-activated DNA polymerase activity	0.016	0-10	> 3 h		[S5]
G-quadruplex / thioflavin T	0.03	0.03-1	1 h 5 min	• Low sensitivity	[S6]
Tb3+-induced G-quadruplex	2	0-20	40 min		[S7]
Collateral cleavage of Cas12a	0.00024	0.00025-50	1 h	-	This work

Figure S1. Optimization of the Cas12a reaction temperature in the sensing system. The signal to background ratio (F/F_0, where F and F_0 are the fluorescence intensities at 520 nm from the samples with and without RNase H, respectively) was obtained by comparing the fluorescence intensity of the sample with or without RNase H. The concentration of RNase H, RNP, chimeric duplex, and Cas12a reaction time were 0.25 U mL^{-1}, 50 nM, 5 nM, and 30 min, respectively. Error bars represent the standard deviation from three independent experiments.

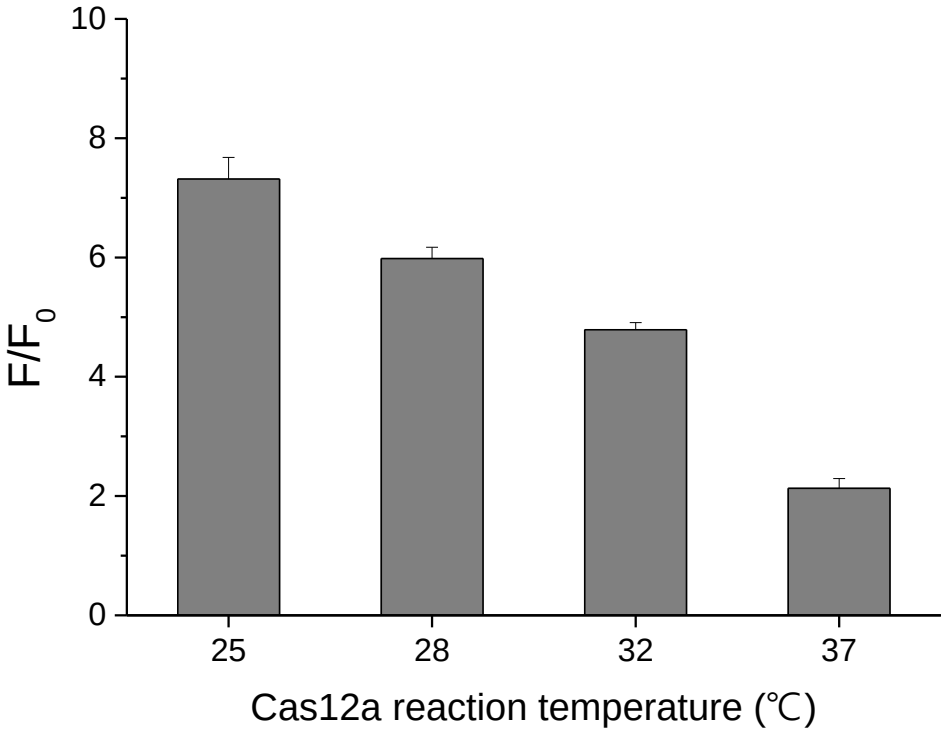


Figure S2. Optimization of the ratio of RB to AD in the sensing system. The signal to background ratio (F/F_0, where F and F_0 are the fluorescence intensities at 520 nm from the samples with and without RNase H, respectively) was obtained by comparing the fluorescence intensity of the sample with or without RNase H. The concentration of RNase H, AD, RNP, Cas12a reaction temperature, and Cas12a reaction time were 0.25 U mL^{-1}, 5 nM, 50 nM, 25 ℃, and 30 min, respectively. Error bars represent the standard deviation from three independent experiments.

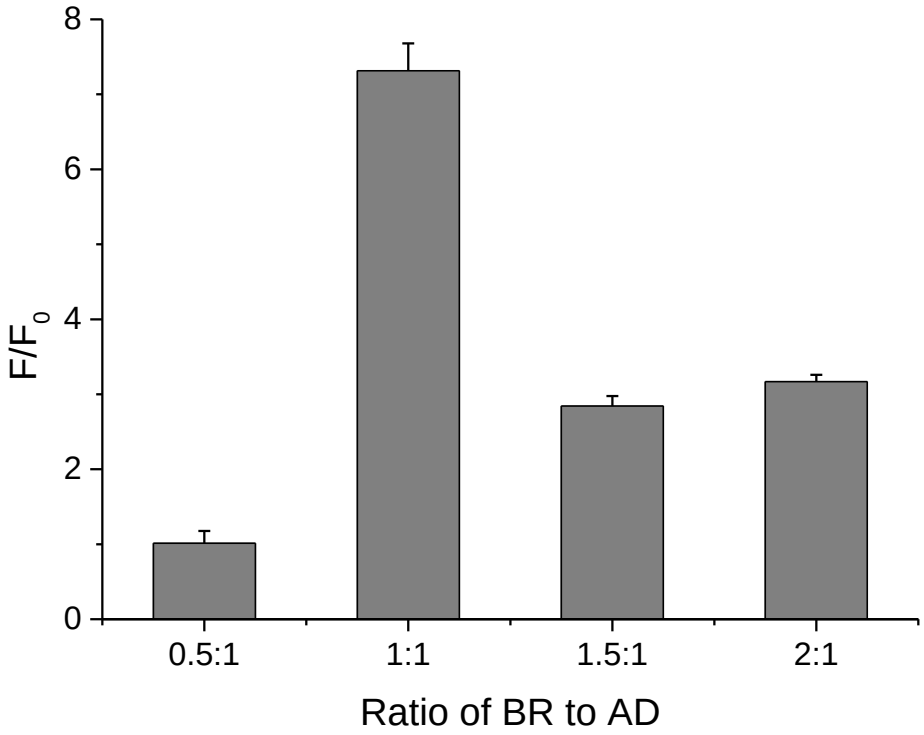


Figure S3. Optimization of the Cas12a reaction time in the sensing system. The signal to background ratio (F/F_0, where F and F_0 are the fluorescence intensities at 520 nm from the samples with and without RNase H, respectively) was obtained by comparing the fluorescence intensity of the sample with or without RNase H. The concentration of RNase H, RNP, chimeric duplex and Cas12a reaction temperature were 0.25 U mL^{-1}, 50 nM, 5 nM, and 25 ℃, respectively. Error bars represent the standard deviation from three independent experiments.

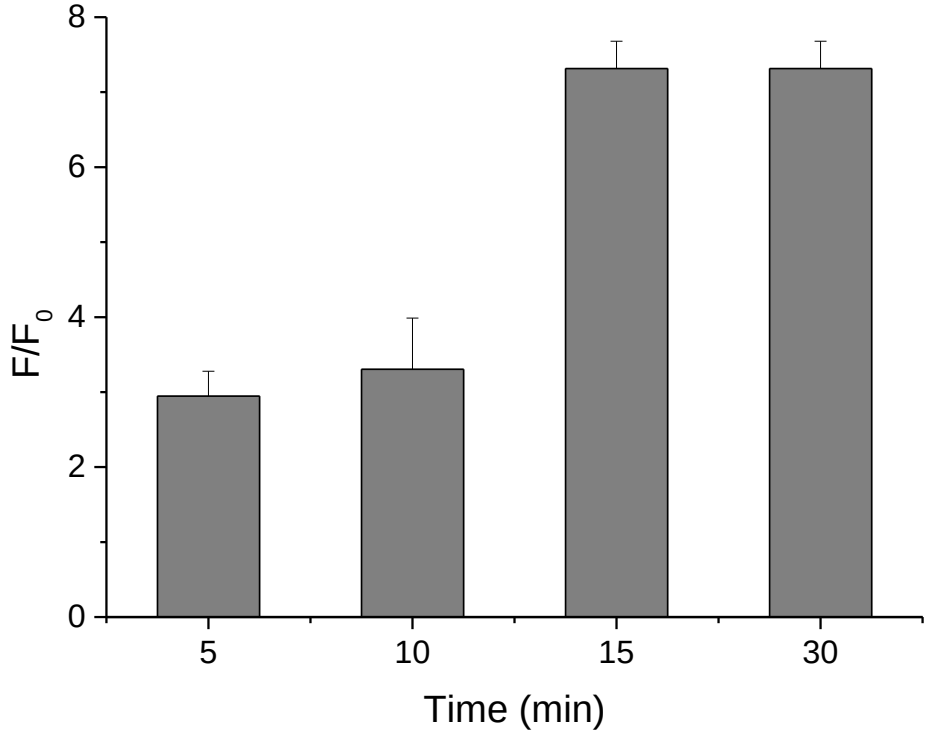
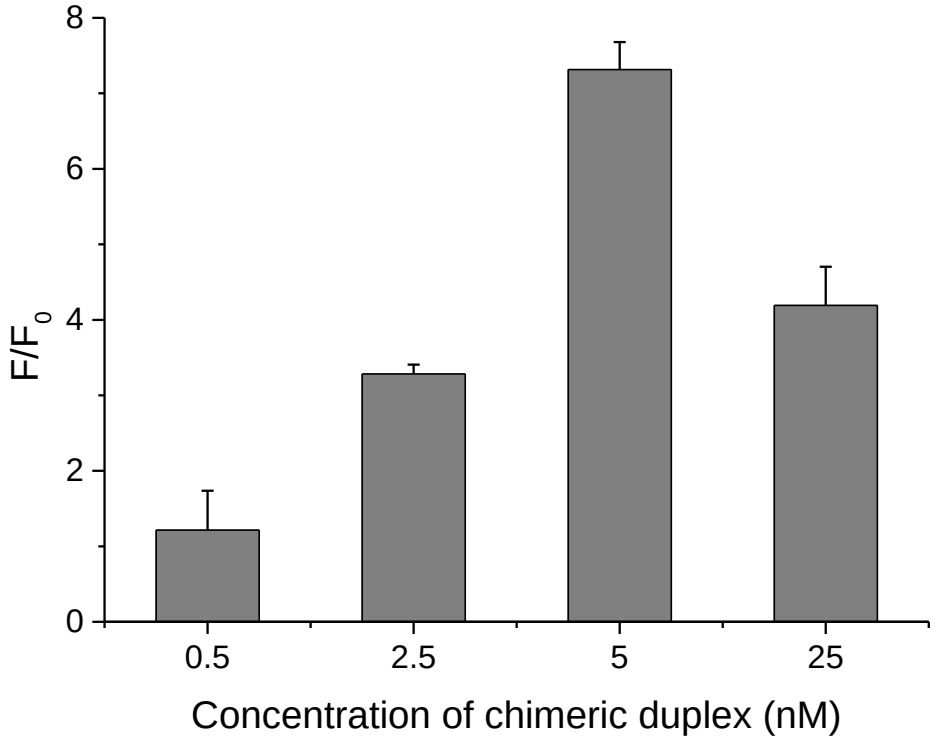


Figure S4. Optimization of the chimeric duplex concentration in the sensing system. The signal to background ratio (F/F_0, where F and F_0 are the fluorescence intensities at 520 nm from the samples with and without RNase H, respectively) was obtained by comparing the fluorescence intensity of the sample with or without RNase H. The concentration of RNase H, RNP, Cas12a reaction temperature, and Cas12a reaction time were 0.25 U mL^{-1}, 50 nM, 25 ℃, and 15 min, respectively. Error bars represent the standard deviation from three independent experiments.



Reference (Supporting)

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