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Supporting Information for

Promoter Engineering Improves Transcription Efficiency in Biomolecular Assays

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Materials and methods

Materials

All DNA oligonucleotides (Table S1) were synthesized by Integrated DNA Technologies (Coralville, IA, USA). T7 RNA polymerase, Lambda Exonuclease (Lambda exo), Uracil-DNA Glycosylase (UDG), and DNase I were purchased from Enzynomics (Daejeon, Korea). Nt.AlwI, Exonuclease I (Exo I), RNase H, T4 polynucleotide kinase (T4 PNK), Hind III, and hOGG1 were purchased from New England Biolabs Inc. (MA, USA). SYBR green II (10000 X) was purchased from Thermo Fisher (MA, USA). To1-biotin (0.5 mg/mL in DMF) and DFHBI (20 mg/mL in DMF) were purchased from Applied Biological Materials (Richmond, BC, Canada) and Sigma Aldrich (MO, USA), respectively. Ultrapure DNase/RNase-free distilled water purchased from Bioneer (Daejeon, Korea) was used in all experiments. All other chemicals were of analytical grade and used without further purification.

Real-time monitoring of transcription reaction

The reaction sample (total volume, 20 μL) contained 50 nM template DNA, 50 nM promoter DNA, 1X T7 RNA polymerase buffer, 0.5 mM rNTP, 16 U RNase inhibitor, 20 U T7 RNA polymerase, and 1X SYBR green II. First, the reaction sample, excepting the RNase inhibitor, T7 RNA polymerase and 1X SYBR green II, was heated at 95°C for 5 min, slowly cooled to 25°C (at 0.1°C/s), and incubated at 25°C for 20 min. Next, the RNase inhibitor, T7 RNA polymerase and 1X SYBR green II were added, and the transcription reaction was initiated at 37°C in the CFX connect real-time system (Bio-Rad, CA, USA), and the fluorescence signal was measured every minute.

Transcription reaction to produce light-up RNA aptamers

The reaction sample (total volume, 50 μL) contained 50 nM template DNA, 50 nM promoter DNA, 1X T7 RNA polymerase buffer, 0.5 mM rNTP, 16 U RNase inhibitor, and 20 U T7 RNA polymerase. The reaction sample, excepting the enzyme, was heated at 95°C for 5 min, slowly cooled to 25°C (at 0.1°C/s) and incubated at 25°C for 20 min. After the Mango and Spinach aptamer transcription reactions were performed at 37°C for 5 and 30 min, respectively, To1-biotin (100 nM) and DFHBI (10 μM) were added to the Mango and Spinach samples, respectively (Since the Spinach aptamer is longer than the Mango aptamer, transcription reaction time was extended from 5 min to 30 min). Finally, the fluorescence intensities of To1-biotin and DFHBI were measured on a SpectraMax iD5 Multi-Mode Microplate Reader (CA, USA) using a black 384-well HT plate (ref: 33384, Pocheon, Korea). The excitation

wavelengths of To1-biotin and DFHBI were 507 nm and 454 nm, respectively. All experiments were performed in triplicate, and the data are displayed as the mean ± SD.

RNase H activity assay

The reaction sample (total volume, 20 µL) contained DNA probes composed of 50 nM Pro 1 and Pro 2 (Table S1), 50 nM template DNA, 1X T7 RNA polymerase buffer, and 0.5 mM rNTP. The reaction sample was heated at 90°C for 5 min, slowly cooled to 37°C (at 0.1°C/s), and incubated at 37°C for 10 min. After the addition of RNase H at different concentrations and other enzymes, the reaction sample was incubated at 37°C for 30 min, followed by the inactivation of RNase H at 65°C for 20 min. Finally, 16 U RNase inhibitor, 20 U T7 RNA polymerase and 1X SYBR green II were added to the reaction sample, and transcription was performed at 37°C for 30 min in a CFX connect real-time system (Bio-Rad, CA, USA). The fluorescence signal was measured every minute.

Polyacrylamide gel electrophoresis

The reaction products were resolved on a 15 % (w/v) polyacrylamide gel using 1X TBE as a running buffer at a constant voltage of 120 V for 50 min. After staining with EZstain™ DNA loading dye (Enzynomics, Cat. #B006) was performed, the gel image was captured using the FAS-Nano gel documentation system (Binsfelder, Germany).

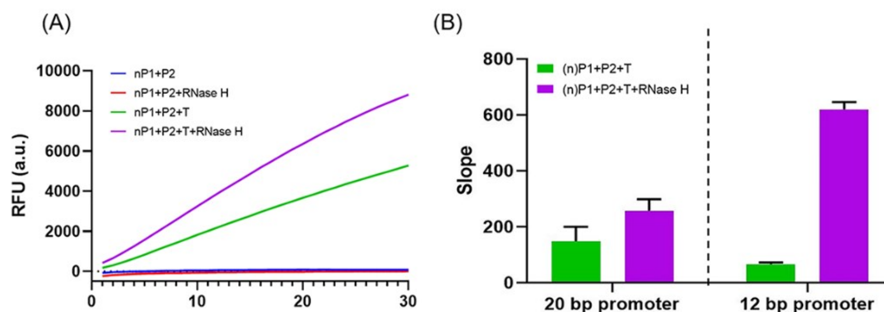

Figure S1. Detection of RNase H using the standard 20 bp promoter. (A) Time-dependent fluorescence response in the presence of RNase H. The new Pro 1 containing the standard 20 bp promoter (GCCCTAATACGACTCACTATAGGG) is hybridized to Pro 2 (TGAG*TrCrGrUrArUr*UAGGGC). The underlined sequences indicate the hybridized nucleotides between new Pro 1 and Pro 2. (B) Comparison between the 20 bp promoter and the 12 bp promoter. The slope was calculated as $(F-F_0)/5$ min, where F_0 and F are the fluorescence intensities before and after the 5 min transcription reaction, respectively. The final concentrations of new Pro 1 (nP1), Pro 1 (P1), Pro 2 (P2), template DNA (T) and RNase H were 50 nM, 50 nM, 50 nM and 1 U/mL, respectively. Error bars were estimated from triplicate measurements.

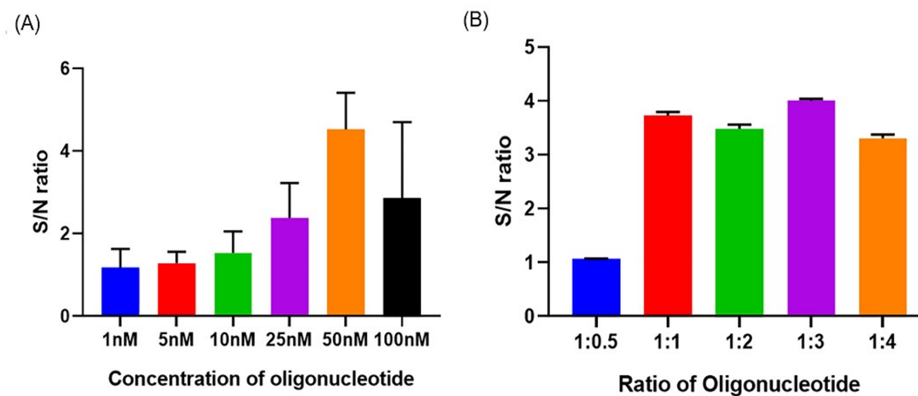

Figure S2. Optimization of RNase H activity assay conditions. (A) Optimization of DNA oligonucleotide concentrations. The ratio of Pro 1, Pro 2 and Template DNA was fixed at 1:1:1. The final concentrations of oligonucleotides were 1 nM, 5 nM, 10 nM, 25 nM, 50 nM, and 100 nM, respectively. The final concentration of RNase H was 3 U/mL. (B) Optimization of the ratio of Pro 1 and Pro 2 (Pro 1:Pro 2). The signal-to-noise (S/N) ratio was calculated as $(F_{RNase\ H} - F_0)/(F_{blank} - F_0)$, where $F_{RNase\ H}$ is the fluorescence intensity following a 30 min transcription reaction in the presence of RNase H, and F_{blank} is the fluorescence intensity following a 30 min transcription reaction in the absence of RNase H. F_0 is the fluorescence intensity following a 30 min transcription reaction in the absence of both T7 RNA polymerase and RNase H. Error bars were estimated from triplicate measurements. The final concentration of oligonucleotides was 50 nM. The final concentration of RNase H was 1 U/mL.

Table S1. Comparison of LOD with that of other RNase H activity assays

Methods	**LOD (U/mL)**	**Reference**
Tb^{3+}-induced G-quadruplex	2	[1]
CHA in combination with a G-quadruplex	0.037	[2]
Fluorescent DNA walker	0.035	[3]
Fluorescent NEAA with a G-quadruplex/thioflavin T complex	0.03	[4]
Fluorescent switching pyrene excimer probe	0.02	[5]
T7 RNA transcription	0.009	This work

Abbreviations: LOD, limit of detection; CHA, catalytic hairpin assembly; NEAA, nicking enzyme-assisted amplification.

Table S2. Oligonucleotides used in this study

Name	Oligonucleotide sequence (5' → 3')
Oligonucleotides used for the investigation of transcription efficiency	
10 bp promoter	CTTCCTAATACGACT
11 bp promoter	TTCCTAATACGACTC
12 bp promoter	TCCTAATACGACTCA
13 bp promoter	TCTAATACGACTCAC
14 bp promoter	TTAATACGACTCACT
15 bp promoter	TAATACGACTCACTA
16 bp promoter	TAATACGACTCACTAT
17 bp promoter	TAATACGACTCACTATA
18 bp promoter	TAATACGACTCACTATAG
19 bp promoter	TAATACGACTCACTATAGG
20 bp promoter	TAATACGACTCACTATAGGG
Template DNA (for Mango aptamer)	**GTACGACAACTACCCCATACCAAACCTTCCTTCGTAC**CCCTATAG TGAGTCGTATTAGGAAGGAGGG
Changed Template 1 (CT1)	**GTACGACAACTACCCCATACCAAACCTTCCTTCGTAC**CCCCGCGA TGAGTCGTATTAGGAAGGAGGG
Changed Template 2 (CT2)	**GTACGACAACTACCCCATACCAAACCTTCCTTCGTAC**CCCATATC TGAGTCGTATTAGGAAGGAGGG
Shortened Template (ST)	**GTACGACAACTACCCCATACCAAACCTTCCTTCGTAC**CCCTGAGT CGTATTAGGAAGGAGGG
Template DNA (for Spinach aptamer)	**GGGAGCTCACACTCTACTCAACAGCGCGAACGCTGGACCCGTC** **CTTCTCCCG**CCCTATAGTGAGTCGTATTAGGAAGGAGGG
Oligonucleotides used for RNase H activity assay	
Pro 1	GCCCTAATACGACTCA
Pro 2	TGAG*TrCrGrUrArUr*UAGGGC
Template DNA	**GTACGACAACTACCCCATACCAAACCTTCCTTCGTAC**CCCTATAG TGAGTCGTATTA

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