A simple colorimetric system for detecting target antigens by a 3-stage signal transformation-amplification strategy

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Supplementary Information

Table S1. The RNA sequences of ribozymes (RS)

THE78 (cm+theo3; P1=8nt):

GGGCGACCCUGAUGAGUCUGGAUACCAGCCGAAAGGCCCUUGGCAGUCUUACGAAACGGUGAAAGCCGUA^{A}GGUUGCCC

FMN72 (Class I; P1=8nt):

GGGCGACCCUGAUGAGCCUUAGGAUAUGCUUCGGCAGAAGGACGUCGAAACGGUGAAAGCCGUA^{A}GGUUGCCC

FMN103 (Same as FMN72; but P1=22nt):

GGGAACUAUACAACCUAGGGCGACCCUGAUGAGCCUUAGGAUAUGCUUCGGCAGAAGGACGUCGAAACGGUGAAAGCCGUA^{A}GGUUGCCCUAGGUUGUAUAGUU

TFH107 (THE-FMN Hybrid; P1=22nt):

GGGAACUAUACAACCUAGGGCGACCCUGAUGAGCCUUAUACCAGCCGAAAGGCCCUUGGCAGACGUCGAAACGGUGAAAGCCGUA^{A}GGUUGCCCUAGGUUGUAUAGUU

TFU76 (THE-FMN Hybrid with a U at position 11 of hRz catalytic core; P1=8nt):

GGGCGACCCUGAUGAGCCUUAUACCAGCCGAAAGGCCCUUGGCAGACGU***U***GAAACGGUGAAAGCCGUA$^{\Delta}$GGUUGCCC

TFU107 (Same as TFU76; but P1=22nt):

GGGAACUAUACAACCUAGGGCGACCCUGAUGAGCCUUAUACCAGCCGAAAGGCCCUUGGCAGACGU***U***GAAACGGUGAAAGCCGUA$^{\Delta}$GGUUGCCCUAGGUUGUAUAGUU

Underlined sequences indicate aptamer-binding domains and communication modules. Non-underlined sequences correspond to the hammerhead ribozyme (hRz) motif where $^{\Delta}$ signifies the self-cleaving site. Bolded and italicized U's are nucleotides introduced to form wobble base pairs.

Table S2. The oligonucleotide sequences for apta-beacon™ construction

Name	Sequence (5'-3')
dTHE78[a]	GGG CAA CCT ACG GCT TTC ACC GTT TCG TAA GAC TGC CAA GGG CCT TTC GGC TGG TAT CCA GAC TCA TCA GGG TCG CCC
dFMN72[b]	GGG CAA CCT ACG GCT TTC ACC GTT TCG ACG TCC TTC TGC CGA AGC ATA TCC TAA GGC TCA TCA GGG TCG CCC
dTFH76[c]	GGG CGA CCC TGA TGA GCC TTA TAC CAG CCG AAA GGC CCT TGG CAG ACG TCG AAA CGG TGA AAG CCG TAG GTT GCC C
TS41	CCG AAG CTT AAT ACG ACT CAC TAT AGG GCG ACC CTG ATG AG
RS11U-32[d]	GGG CAA CCT ACG GCT TTC TCC GTT TCA ACG TC
TS59[e]	CCG AAG CTT AAT ACG ACT CAC TAT AGG GAA CTA TAC AAC CTA GGG CGA CCC TGA TGA GC
dP1-22-32[e]	AAC TAT ACA ACC TAG GGC AAC CTA CGG CTT TC

[a] (Soukup et al. 2000)
[b] (Soukup and Breaker 1999b)
[c] (Soukup and Breaker 2000) (Frauendorf and Jaschke 2001)
[d] (Soukup and Breaker 1999a)
[e] (Nie et al. 2014)

Table S3. The oligonucleotide sequences for color development

Name	Sequence (5'-3')
GQ15[a]	GGG AGG GAG GGA GGG
GQ18[b]	CTG GGA GGG AGG GAG GGA
GQ21[c]	CAT TCG GGA GGG AGG GAG GGA

[a] (Adams et al. 2014; Gogichaishvili et al. 2014; Kankia 2014; Partskhaladze et al. 2015)
[b] (Cheng et al. 2009); also product of Nt.BstNBI from qtq template.
[c] Product of Nb.BsmI from qmq template

Table S4. The oligonucleotide sequences for EXPAR

Initiator	Sequence (5'-3')
dP1-22	GGT TGC CCT AGG TTG TAT AGT T
dP3-22	CGT TGA AAC GGT GAA AGC CGT A
P_1'-Q'	Sequence (5'-3')
p1tq51	TCC CTC CCT CCC TCC CAG $^{\Delta}$TCC AGA CTC TAA CTA TAC AAC CTA GGG CAA CCA-Biotin
P_3'-Q'	Sequence (5'-3')
p3mq41	TCC CTC CCT CCC TCC CGA ATG $^{\Delta}$CTA CGG CTT TCA CCG TTT CA-Biotin
p3tq49	TCC CTC CCT CCC TCC CAG □TCC AGA CTC TAC GGC TTT CAC CGT TTC AAC G-Biotin
Q'-Q'	Sequence (5'-3')
qtq47	TCC CTC CCT CCC TCC CAG $^{\Delta}$TCC AGA CTC TTC CCT CCC TCC CTC CCA GA-Biotin
qmq44	TCC CTC CCT CCC TCC CGA ATG $^{\Delta}$CTC CCT CCC TCC CTC CCG AAT GA-Biotin

The underlined sequences indicate recognition sites of nicking endonuclease ($^{\Delta}$ shows the relative nicking site of its complementary sequence; "t" for constructs responsive to Nt.Bst.NBI, "m" for constructs responsive to Nb.BsmI).

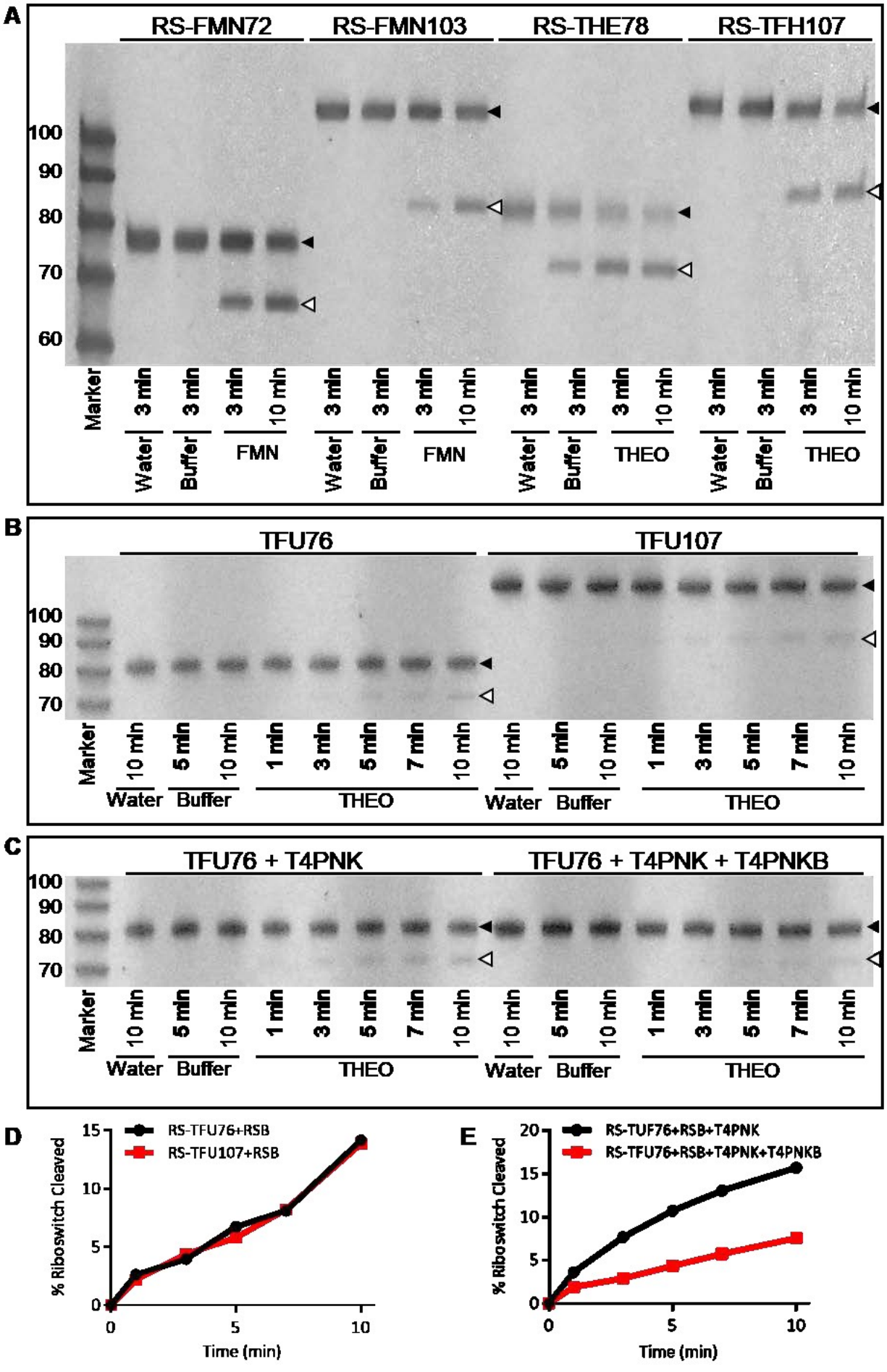
A
RS-FMN72
RS-FMN103
RS-THE78
RS-TFH107
100
90
80
70
60
Marker
3 min
10 min
Water
Buffer
FMN
THEO
B
TFU76
TFU107
1 min
5 min
7 min
C
TFU76 + T4PNK
TFU76 + T4PNK + T4PNKB
D
RS-TFU76+RSB
RS-TFU107+RSB
% Riboswitch Cleaved
Time (min)
E
RS-TUF76+RSB+T4PNK
RS-TFU76+RSB+T4PNK+T4PNKB

Figure S1. Ribozyme catalysis study by denaturing PAGE. Smaller cleavage products (22 nt for RS-FMN103, RS-TFH107, and TFU107; 8 nt for remaining constructs) were not visible by Gel-Star™ staining. Dark arrows indicate precleaved RNA. Open arrows indicate the larger cleavage products. **(A)** RS-FMN72/103 and RS-THE78/TFH107 reactions carried out in presence or absence of 800 μM corresponding targets flavin mononucleotide (FMN) and theophylline (THEO), respectively. The reactions were incubated at 23°C with 0.5 μM of ribozyme under the following conditions: DEPC-treated water only, ribozyme reaction buffer only (50 mM Tris-HCl at pH 7.5 and 20 mM $MgCl_2$), FMN, or THEO. Incubation time indicated in minutes. RS-TFH107 contains a G16-C50 communication module pairing (compare against TFU107, which contains a G16-U50 pairing). **(B)** Cleavage study of either 0.5 μM RS-TFU76 or RS-TFU107 in the presence of 10 μM THEO, ribozyme reaction buffer or DEPC-treated water only, over a 10-min time course. TFU107contains a G16-U50 communication module pairing (compare against RS-TFH107, which contains a G16-C50 pairing). **(C)** Cleavage study of either 0.5 μM RS-TFU76 with the addition of T4 Polynucleotide Kinase (PNK) or with the addition of T4 PNK and T4 PNK buffer (T4PNKB) in the presence of 10 μM THEO, ribozyme reaction buffer or DEPC-treated water only, over a 10-min time course. **(D)** Densitometry gel analysis of Figure S1B by Image J. **(E)** Densitometry gel analysis of Figure S1C by ImageJ.

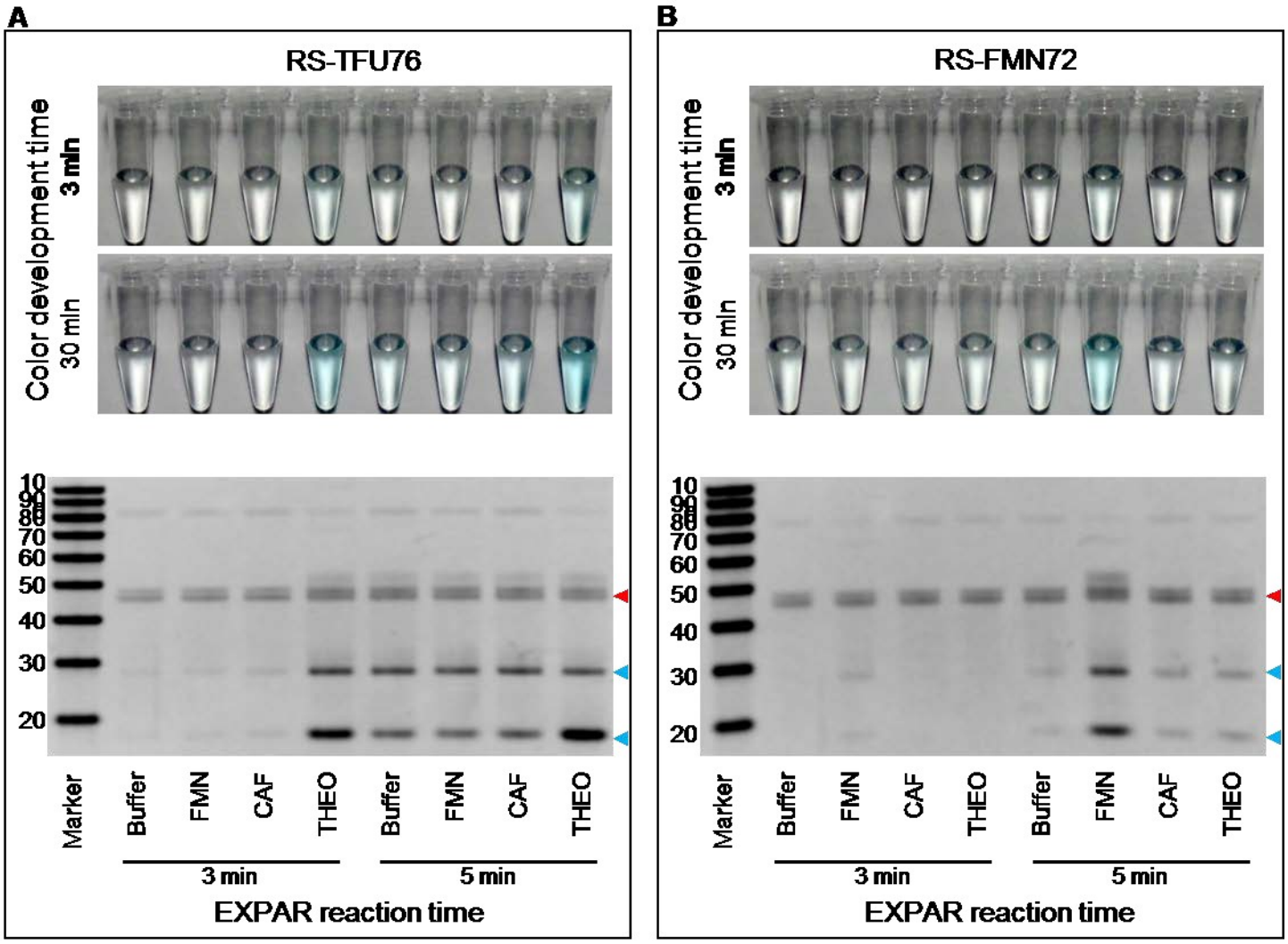

Figure S2. Specificity of RS-TFU76 and RS-FMN72. The red arrowheads indicate the pre-cleaved EXPAR product and template, and the blue arrowheads indicate the nicking products generated during isothermal amplification. The higher molecular-weight bands at the top of the gel represent full-length and incomplete extension products for templates prior to nickase cleavage. The reactions were carried out at conditions of 0.48 mM RS-TFU76 **(A)** or RS-FMN72 **(B)** combined with buffer only or 1 mM of FMN, caffeine (CAF), or theophylline (THEO). Samples were incubated at 23°C for 5 min, followed by EXPAR incubations at 55°C for 3 and 5 min. Half of the EXPAR products were used for color development of 3 min (top panel) and 30 min (middle panel); the remaining half for denaturing PAGE analysis (bottom panel).

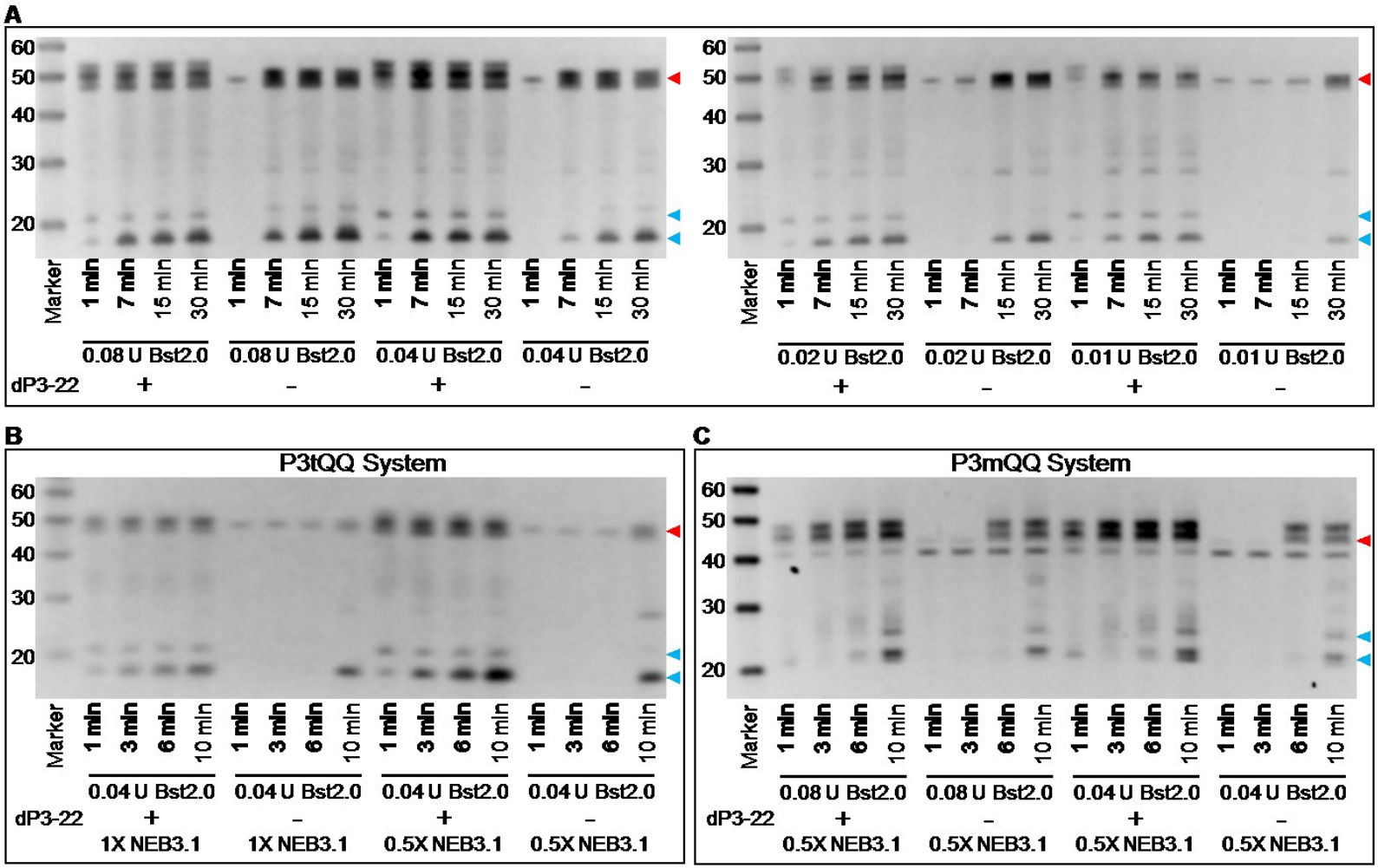

Figure S3. Optimization of EXPAR reagents. The EXPAR products were analyzed by denaturing PAGE. The red arrowheads indicate the pre-cleaved EXPAR product and template, and the blue arrowheads indicate the nicking products generated during isothermal amplification. The higher molecular weight bands at the top of the gel represent full-length and incomplete extension products for templates prior to nickase cleavage. **(A)** Determination of Bst2.0 DNA polymerase concentration. The reactions were carried out at conditions of 1X NEB3.1 buffer, 0.5 U/ml Nt.BstNBI and variable Bst2.0 DNA polymerase as indicated, 300 nM p3tq49 and 300 nM qtq47, 400 mM dNTP, in presence or absence of 300 nM dP3-22. The mixtures were incubated at 55°C for 1 to 30 min. **(B)** P3tQQ system determination of NEB3.1 buffer concentration. The reactions were carried out at 0.5X isothermal amplification buffer, 0.5 U/ml Nt.BstNBI and 0.04 U/ml Bst2.0 DNA polymerase, 300 nM p3tq49 and 300 nM qtq47, 400 mM dNTP, in presence or absence of 300 nM dP3-22, 1 to 10 min incubation at 55°C, and NEB3.1 buffer concentration as indicated. **(C)** P3mQQ system was performed with p3mq41 and qmq44 in 0.5X NEB3.1 buffer and 0.5X IAB, the mixtures were contained 0.5 U/ml Nb.BsmI and variable Bst2.0 DNA polymerase concentration as indicated, and were incubated at 65°C for 1 to 10 min.

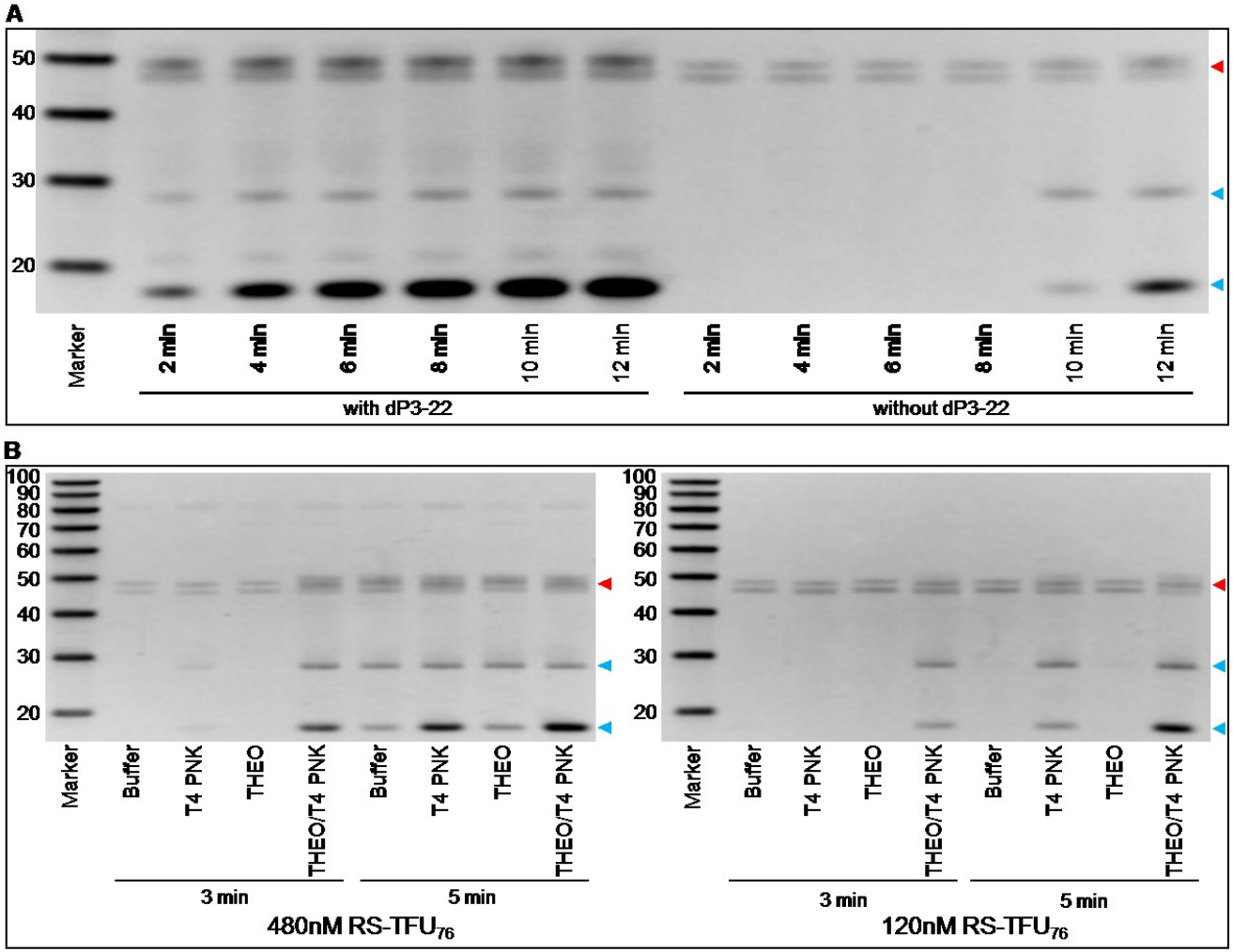

Figure S4. Determination of non-specific amplification for P3tQQ system. The red arrowheads indicate the pre-cleaved EXPAR product and template, and the blue arrowheads indicate the nicking products generated during isothermal amplification. The higher molecular-weight bands at the top of the gel represent full-length and incomplete extension products for templates prior to nickase cleavage. **(A)** Background from EXPAR templates. The reactions were carried out at 55°C with 0.5X NEBuffer 3.1, 0.5X isothermal amplification buffer (NEB), 0.8 U/ml Nt.BstNBI and 0.04 U/ml Bst2.0 DNA polymerase, 300 nM p3tq49 and 300 nM qtq47 EXPAR templates, 400 mM dNTP, in presence or absence of 300 nM dP3-22 initiating oligo, incubated for 2 to 12 min. **(B)** Background from ribozyme reaction. 480 nM or 120 nM RS-TFU76 were incubated at 23°C for 3 min in the presence of buffer only (1X ribozyme reaction buffer and 1X T4 polynucleotide kinase buffer), 1 U/mL T4 polynucleotide kinase (T4PNK), 1 mM theophylline, or 1 mM theophylline with 1 U/ml T4 polynucleotide kinase; and then followed by EXPAR under the same conditions as A, but incubated for 3 and 5 min.

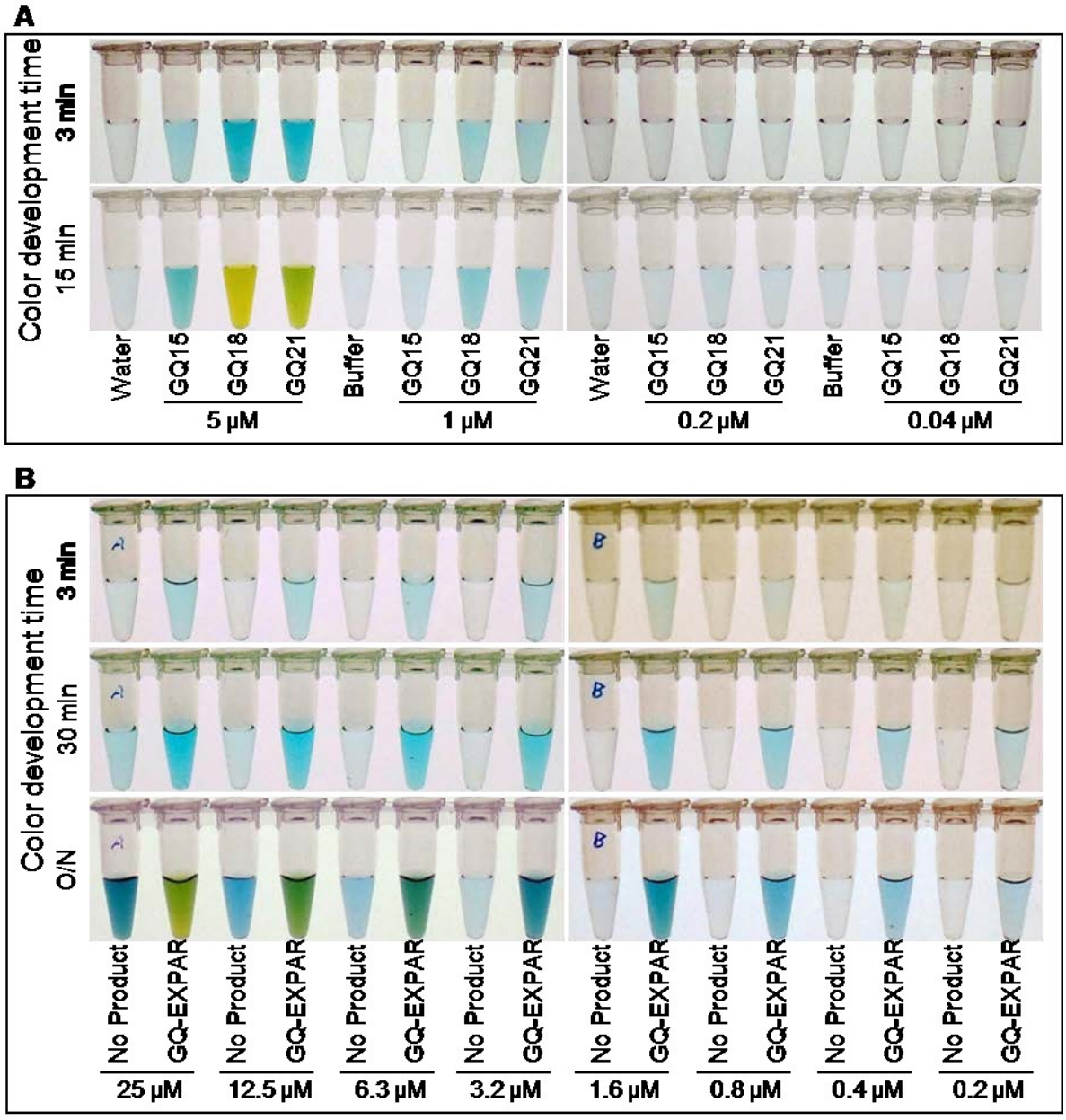

Figure S5. Determination of color development conditions. **(A)** The effects of G-quadruplex size on the redox of TMB (Table S3 for sequences). Various concentrations of G-quadruplex constructs (see Table S3) in 2.5 µl of buffer (1X isothermal amplification buffer, 0.5X 3.1 buffer) were combined with 2.5 µl of 25 µM hemin. Control reactions were also conducted using 2.5 µl of water only or buffer only, each in the presence of 2.5 µl of 25 µM hemin. Color development reactions were then started by adding 95 µl of TMB-H_2O_2 solution and allowed to incubate for 3 min (top row) or 15 min (bottom row) at room temperature. **(B)** Optimization of hemin concentration. TFU107-p3mq/qmq-EXPAR product (see Table S4 for sequences) resulting from theophylline-incubated ribozyme was divided into eight 2.5 µl samples. An equal volume of hemin at the indicated concentrations was added along with 95 µl of TMB-H_2O_2 solution. Color reaction was developed at room temperature for 3 min (top panel), 30 min (middle panel) and overnight (bottom panel) as indicated at left.

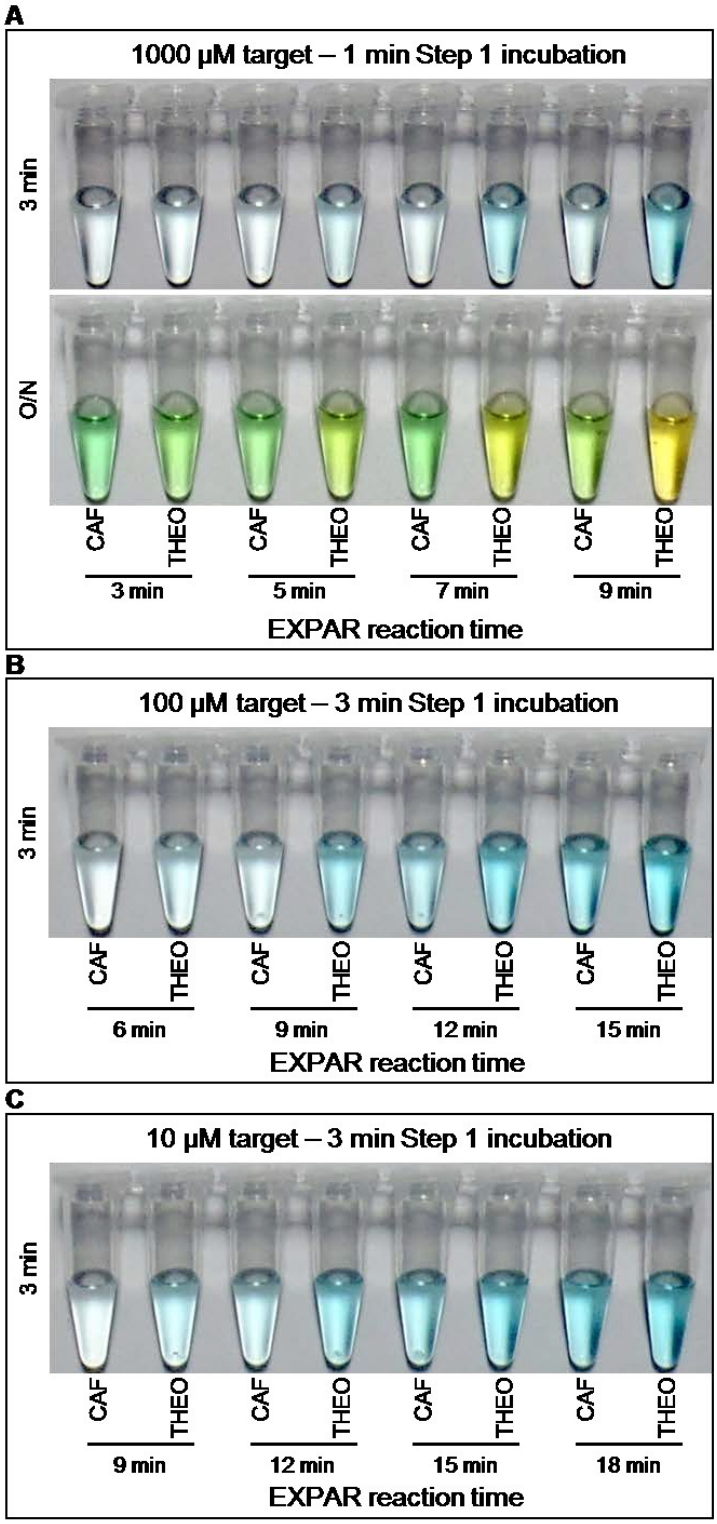

Figure S6. Detection of specific targets with non-optimized conditions for P3mQQ system using Nb.BsmI nickase. The reactions were carried out at conditions of 10 µM TFU107 combined with the indicated caffeine (CAF) or theophylline (THEO) concentrations and incubated at room temperature for 1 min **(A)** or 3 min **(B and C)**. Cleavage products were combined with 0.47X NEBuffer 3.1, 0.94X isothermal amplification buffer (NEB), 0.7 U/µl Nb.BsmI nickase, and 0.094 U/µl Bst2.0 DNA polymerase and incubated at 65°C EXPAR incubations for indicated the indicated times. Color development proceeded at room temperature for the indicated times.

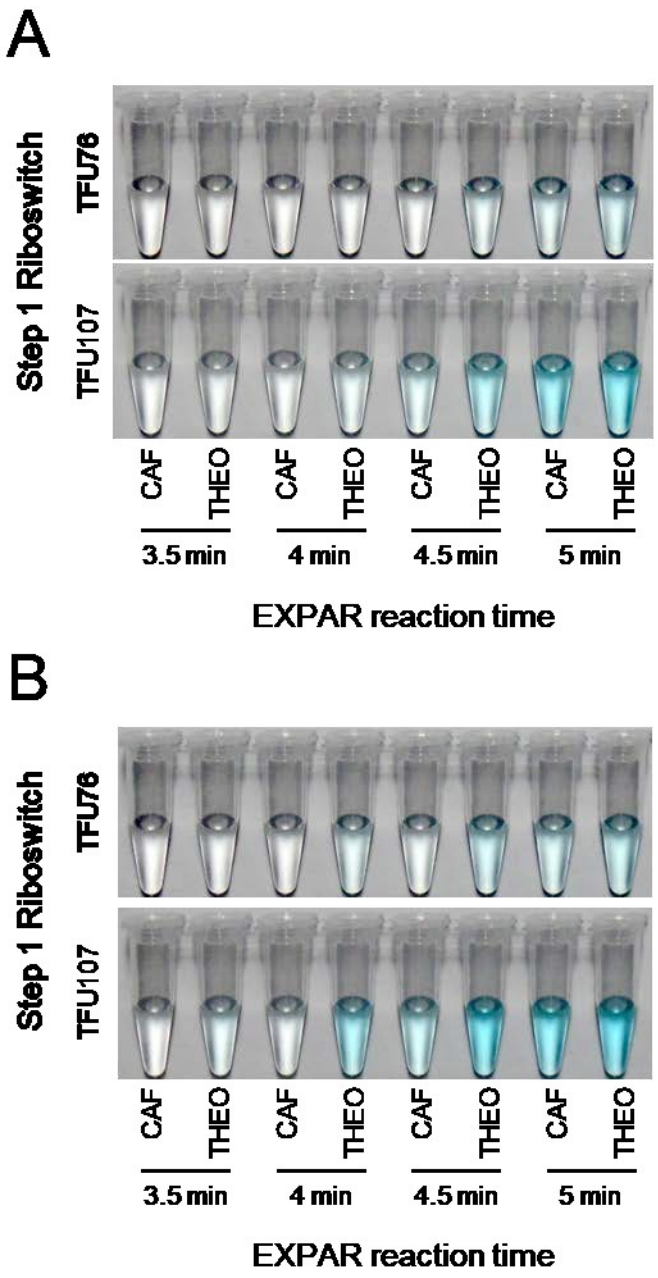

Figure S7. Color development by GQ derived from RS-TFU76-EXPAR and RS-TFU107-EXPAR. **(A)** Reactions were performed using a final concentration of 240 nM TFU76 or TFU107 combined with 10 µM caffeine or theophylline, and were incubated at 23°C for 3 min; followed by EXPAR incubations at 55°C for 3.5 to 5 min as indicated. Color development was allowed to proceed for 3 minutes before imaging. **(B)** Same experiment and samples after 30 minutes of color development.

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