Supplementary Information for

G-quadruplex-regulated exonuclease cooperative hydrolysis signal transduction strategy for processing molecular temporal information

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Supplementary Figures

Supplementary Table

Supplementary Fig. S1- Fig. S18

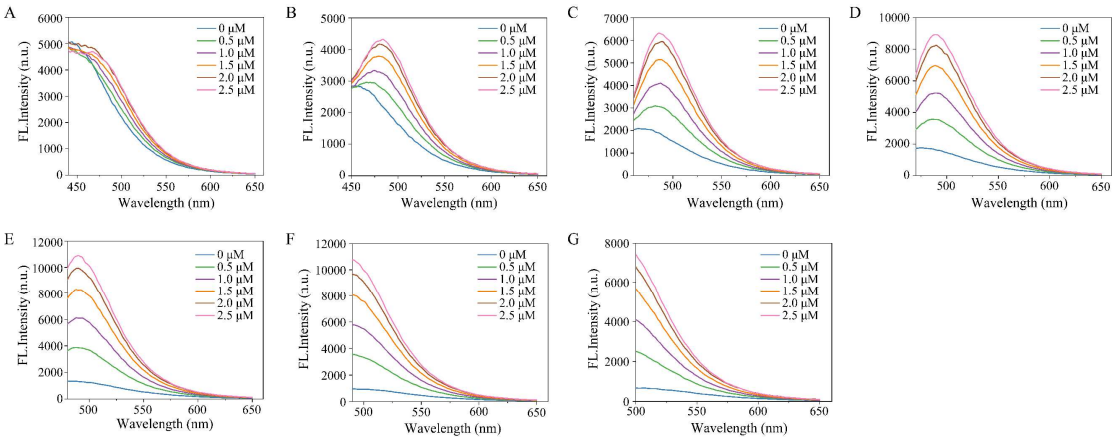

Fig. S1 Fluorescence spectra of ThT with G4 (S1/A1) at different concentrations and different excitation and emission wavelengths. (A) λ_{ex} = 400 nm; λ_{em} = 440/650 nm. (B) λ_{ex} = 410 nm; λ_{em} = 450/650 nm. (C) λ_{ex} = 420 nm; λ_{em} = 460/650 nm. (D) λ_{ex} = 430 nm; λ_{em} = 470/650 nm. (E) λ_{ex} = 440 nm; λ_{em} = 480/650 nm. (F) λ_{ex} = 450 nm; λ_{em} = 490/650 nm. (G) λ_{ex} = 460 nm; λ_{em} = 500/650 nm

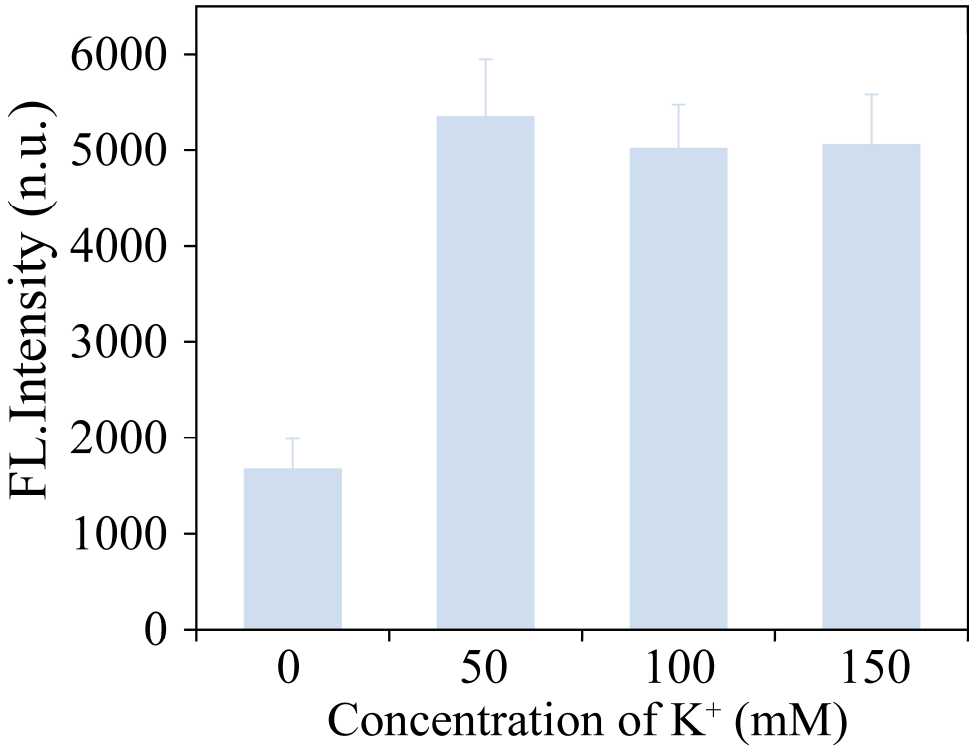

Fig. S2 Fluorescence spectra of G4 (S1/A1) and ThT at different K^+ concentrations. The concentrations of K^+ are 0, 50, 100, and 150 mM, respectively. λ_{ex} = 440 nm; λ_{em} = 490 nm. The concentration of G4 (S1/A1) is 2 μM, and the concentration of ThT is 1 μM

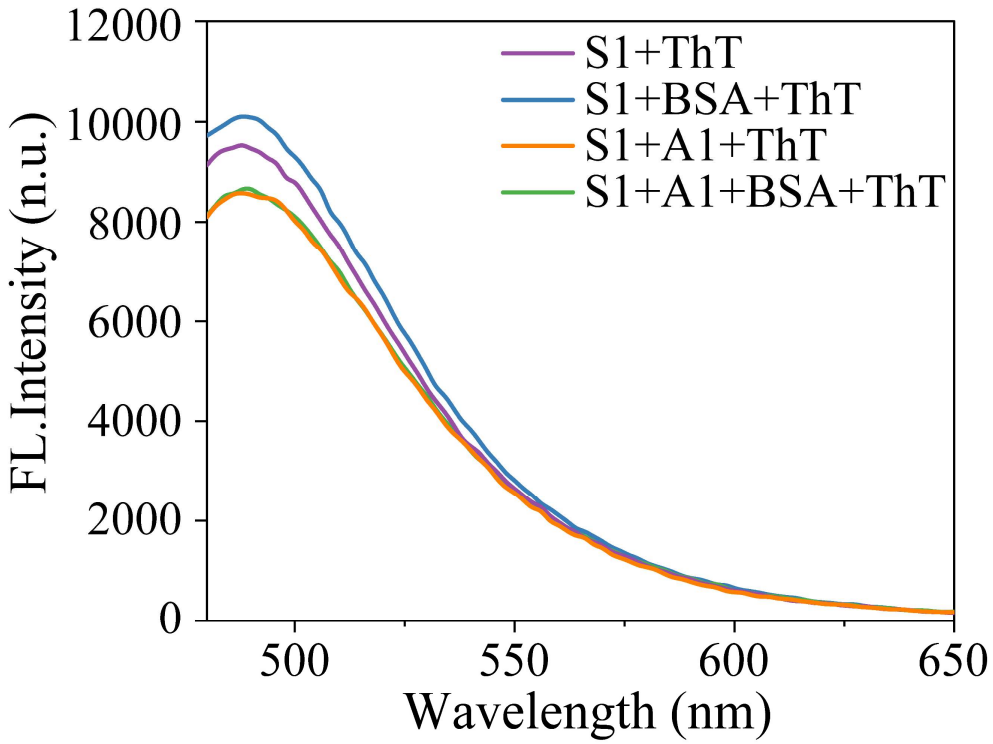

Fig. S3 The effect of A1 and BSA on G4 formation

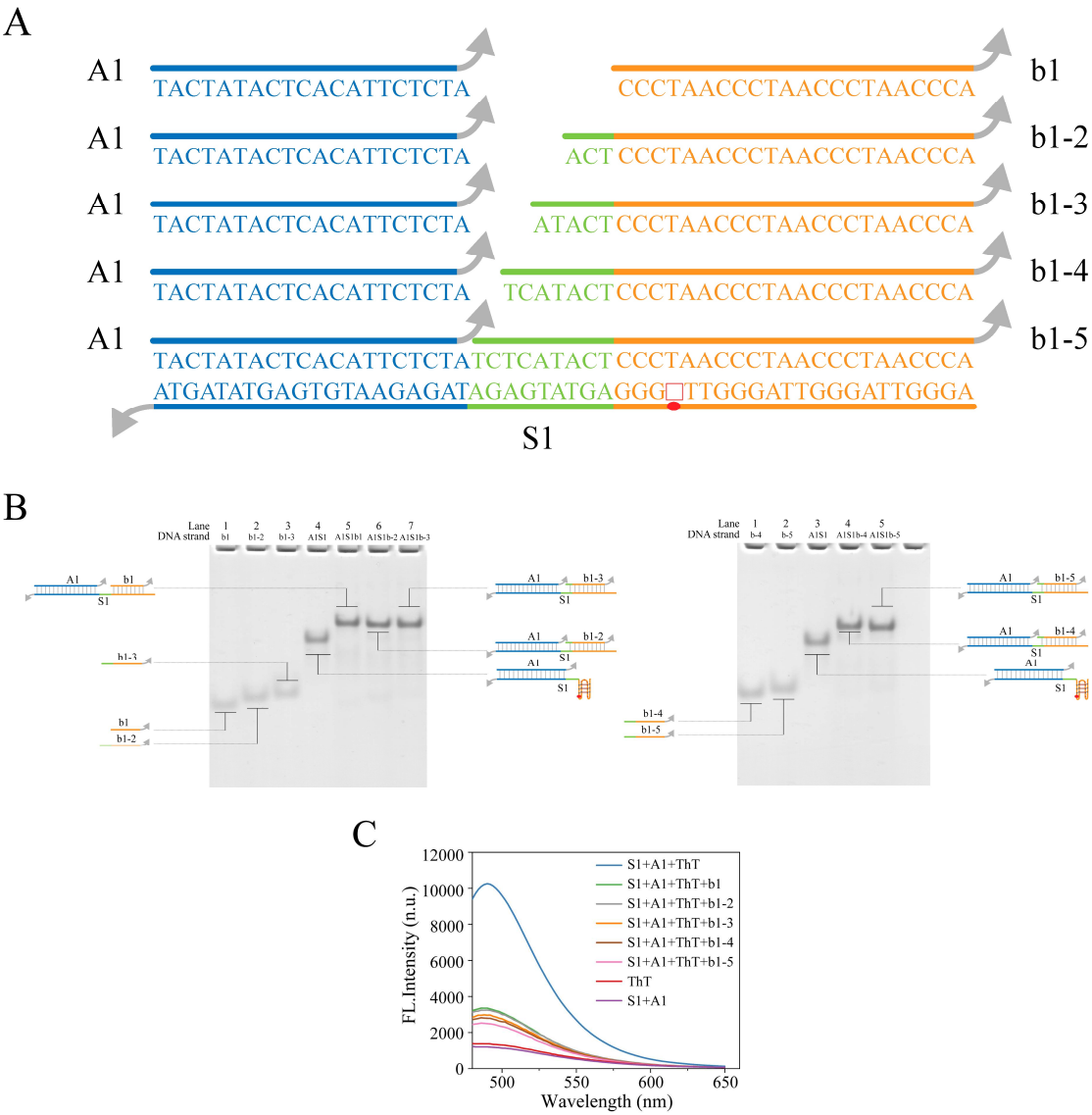

Fig. S4 The unfolding control strategy involving the G4 sequence domain (orange part of S1) fully complementary shortening of the non-G4 sequence domain (green part of S1). (A) Schematic diagram of regulating G4 unfolding using invaders of different lengths. (B) PAGE analysis of G4 structure unfolding with inputs b1, b1-2, b1-3, b1-4, and b1-5. (C) Corresponding fluorescence spectra curves

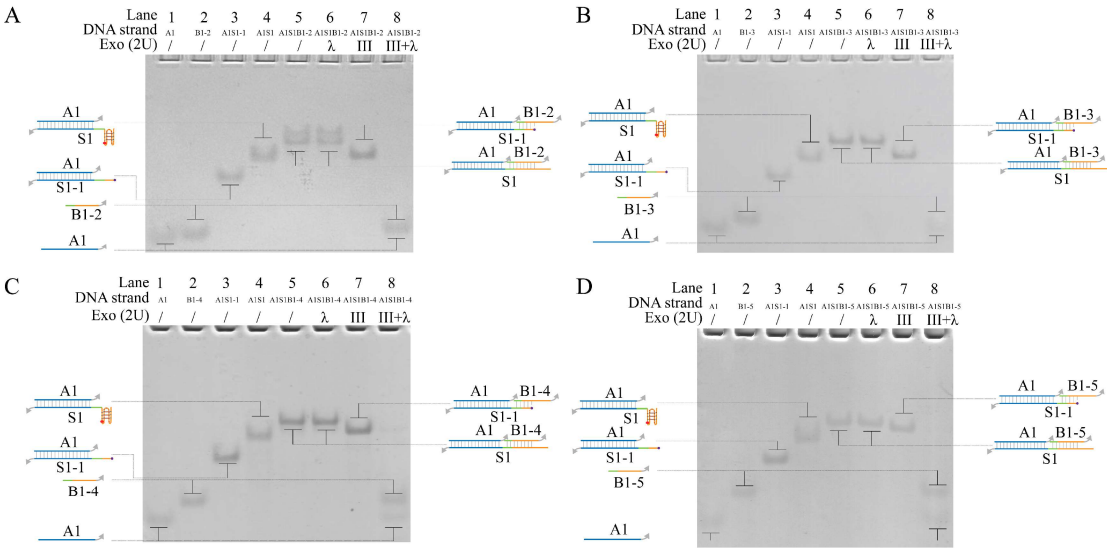

Fig. S5 PAGE analysis of signal transduction strategy. (A) Signal pathway regulated by input B1-2. Lane 1: A1; Lane 2: B1-2; Lane 3: S1-1 + A1; Lane 4: S1 + A1; Lane 5: S1 + A1 + B1-2; Lane 6: S1 + A1 + B1-2 + Exo λ; Lane 7: S1 + A1 + B1-2 + Exo III; Lane 8: S1 + A1 + B1-2 + Exo III + Exo λ. (B) Signal pathway regulated by input B1-3. Lane 1: A1; Lane 2: B1-3; Lane 3: S1-1 + A1; Lane 4: S1 + A1; Lane 5: S1 + A1 + B1-3; Lane 6: S1 + A1 + B1-3 + Exo λ; Lane 7: S1 + A1 + B1-3 + Exo III; Lane 8: S1 + A1 + B1-3 + Exo III + Exo λ. (C) Signal pathway regulated by input B1-4. Lane 1: A1; Lane 2: B1-4; Lane 3: S1-1 + A1; Lane 4: S1 + A1; Lane 5: S1 + A1 + B1-4; Lane 6: S1 + A1 + B1-4 + Exo λ; Lane 7: S1 + A1 + B1-4 + Exo III; Lane 8: S1 + A1 + B1-4 + Exo III + Exo λ. (D) Signal pathway regulated by input B1-5. Lane 1: A1; Lane 2: B1-5; Lane 3: S1-1 + A1; Lane 4: S1 + A1; Lane 5: S1 + A1 + B1-5; Lane 6: S1 + A1 + B1-5 + Exo λ; Lane 7: S1 + A1 + B1-5 + Exo III; Lane 8: S1 + A1 + B1-5 + Exo III + Exo λ

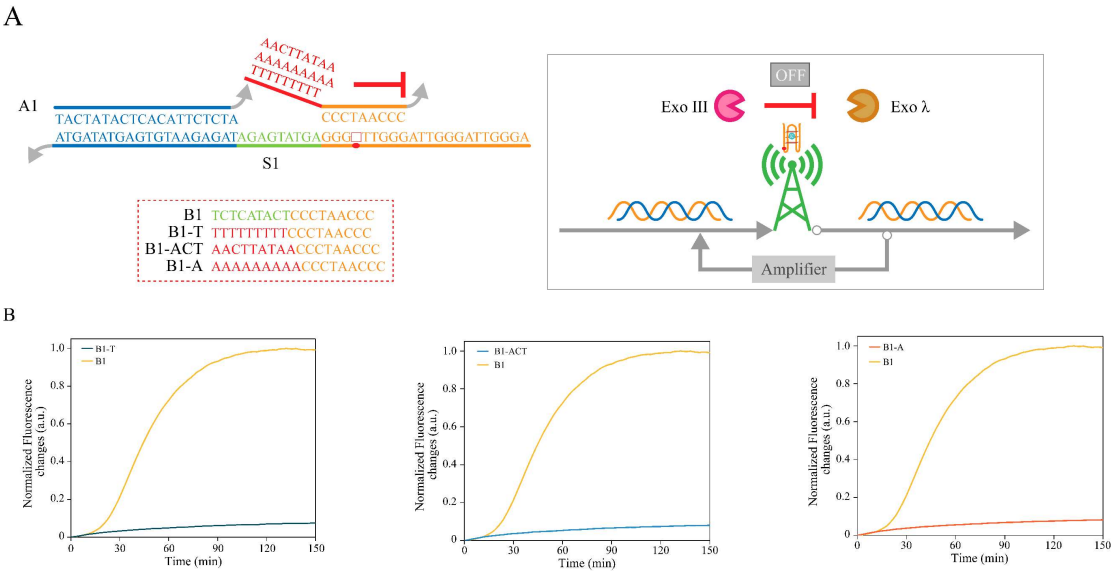

Fig. S6. Examples where G4 cannot be unfolded and therefore does not produce a signal output. (A) Invaders B1-T, B1-ACT, and B1-A have an orange part complementary to S1 and a red part non-complementary to S1, preventing the initiation of exonuclease hydrolysis. (B) Fluorescence results of ST1 triggered by invaders B1, B1-T, B1-ACT, and B1-A

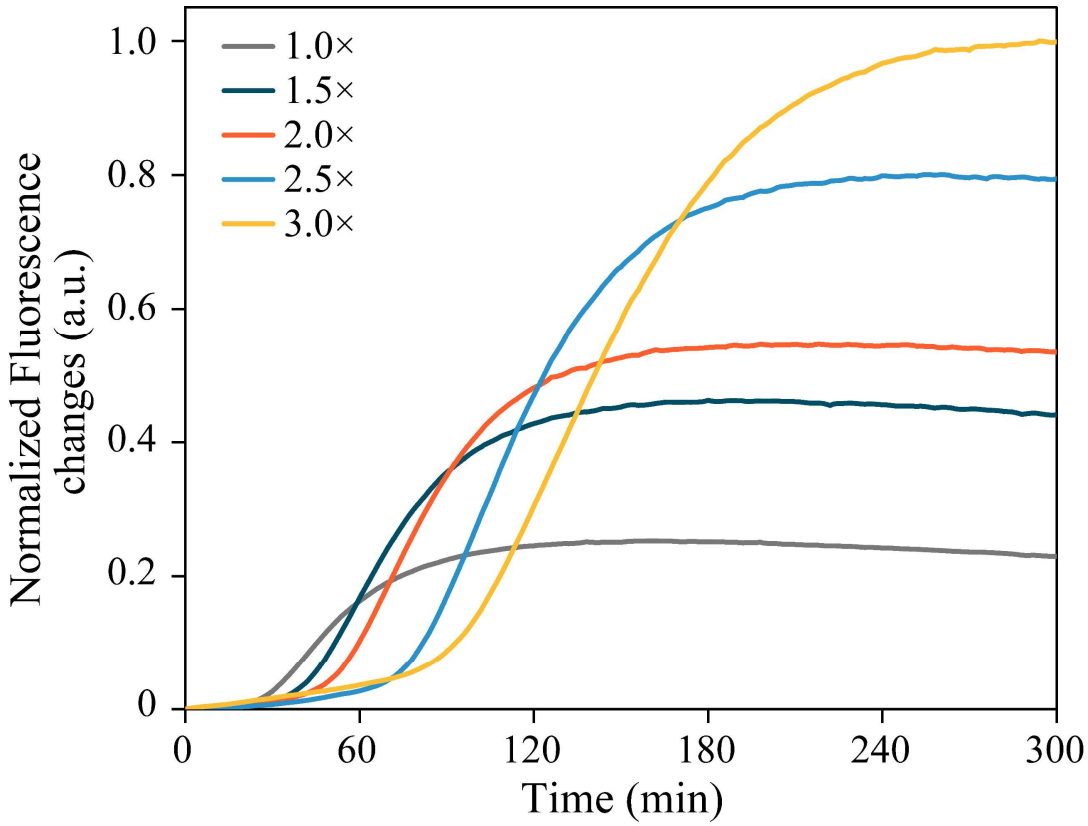

Fig. S7 Catalytic efficiency of the signal transducer at high substrate concentrations. The concentration of input B1 is 1×, and the concentration of substrate and reporter Re1 were consistent, which were 1×, 1.5×, 2×, 2.5×, and 3×, respectively

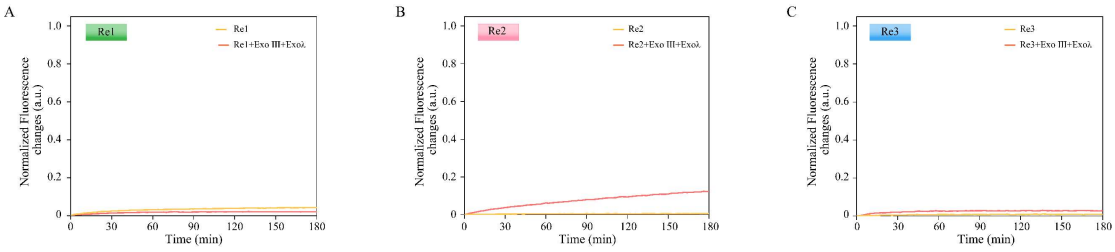

Fig. S8 Investigation of the enzyme resistance capability of the fluorescent reporters Re. (A) Fluorescence of Re1 in its silent state shows minimal change with the addition of Exo III and Exo λ. (B) Fluorescence of Re2 in its silent state shows minimal change with the addition of Exo III and Exo λ. (C) Fluorescence of Re3 in its silent state shows minimal change with the addition of Exo III and Exo λ

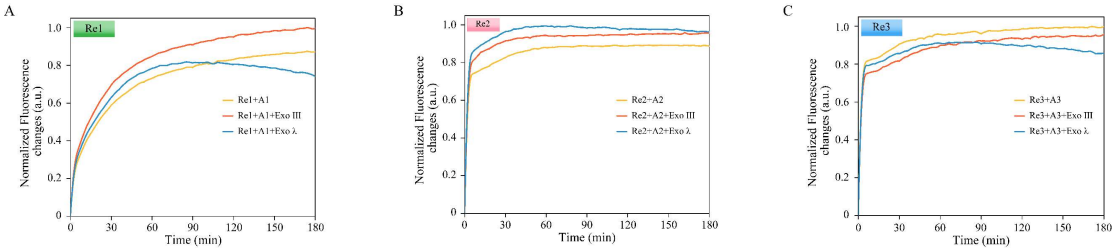

Fig. S9 Fluorescence curve of fluorescence reporter Re triggered by output**.** (A) Re1 is triggered by output B1. With the addition of Exo III, the fluorescence curve changes weakly, and with the addition of Exo λ, the fluorescence gradually attenuates. The toehold length of Re1 is 5 nt. (B) Re2 is triggered by output B2. With the addition of Exo III, the fluorescence curve changes weakly, and with the addition of Exo λ, the fluorescence gradually attenuates. The toehold length of Re2 is 6 nt. (C) Re3 is triggered by output B3. With the addition of Exo III, the fluorescence curve changes weakly, and with the addition of Exo λ, the fluorescence gradually decays. The toehold length of Re3 is 6 nt

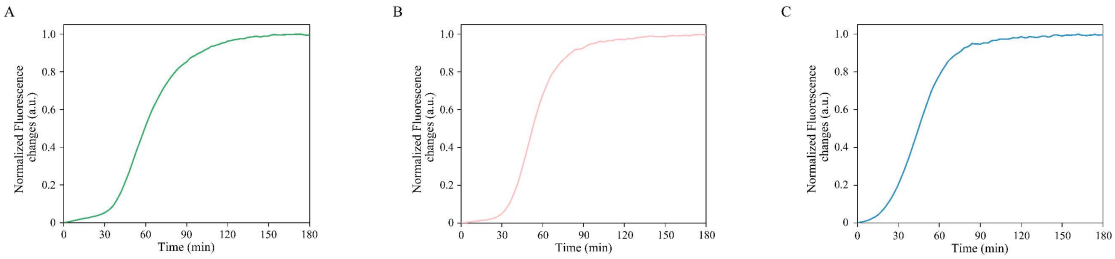

Fig. S10 Fluorescence curves of the fan-out DNA circuit at high input concentrations**.** The concentration of input B1 is 3×, and the concentrations of ST1, ST3, and ST4 are all 1×

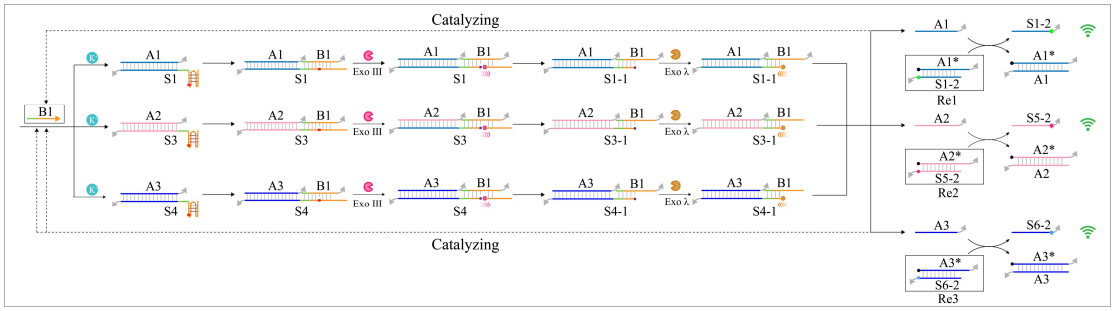

Fig. S11 Design details and reaction process of the fan-out DNA circuit controlled by B1

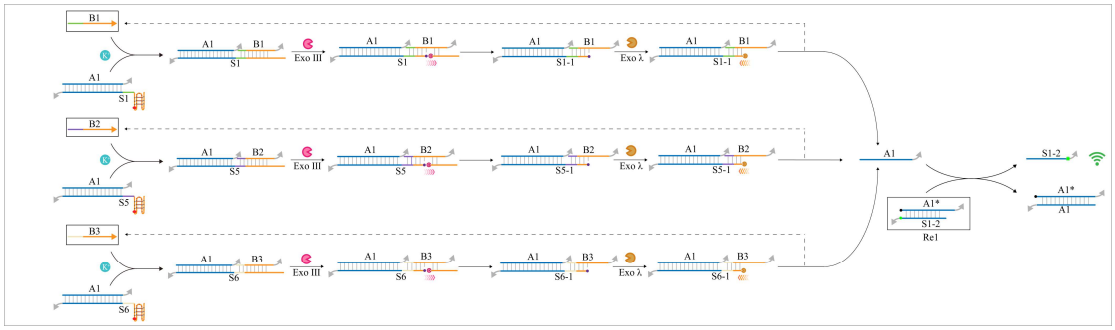

Fig. S12 Design details and reaction process of the fan-in DNA circuit controlled by B1, B2, and B3

Fig. S13 Design details and reaction process of the temporal response control achieved through dynamic control

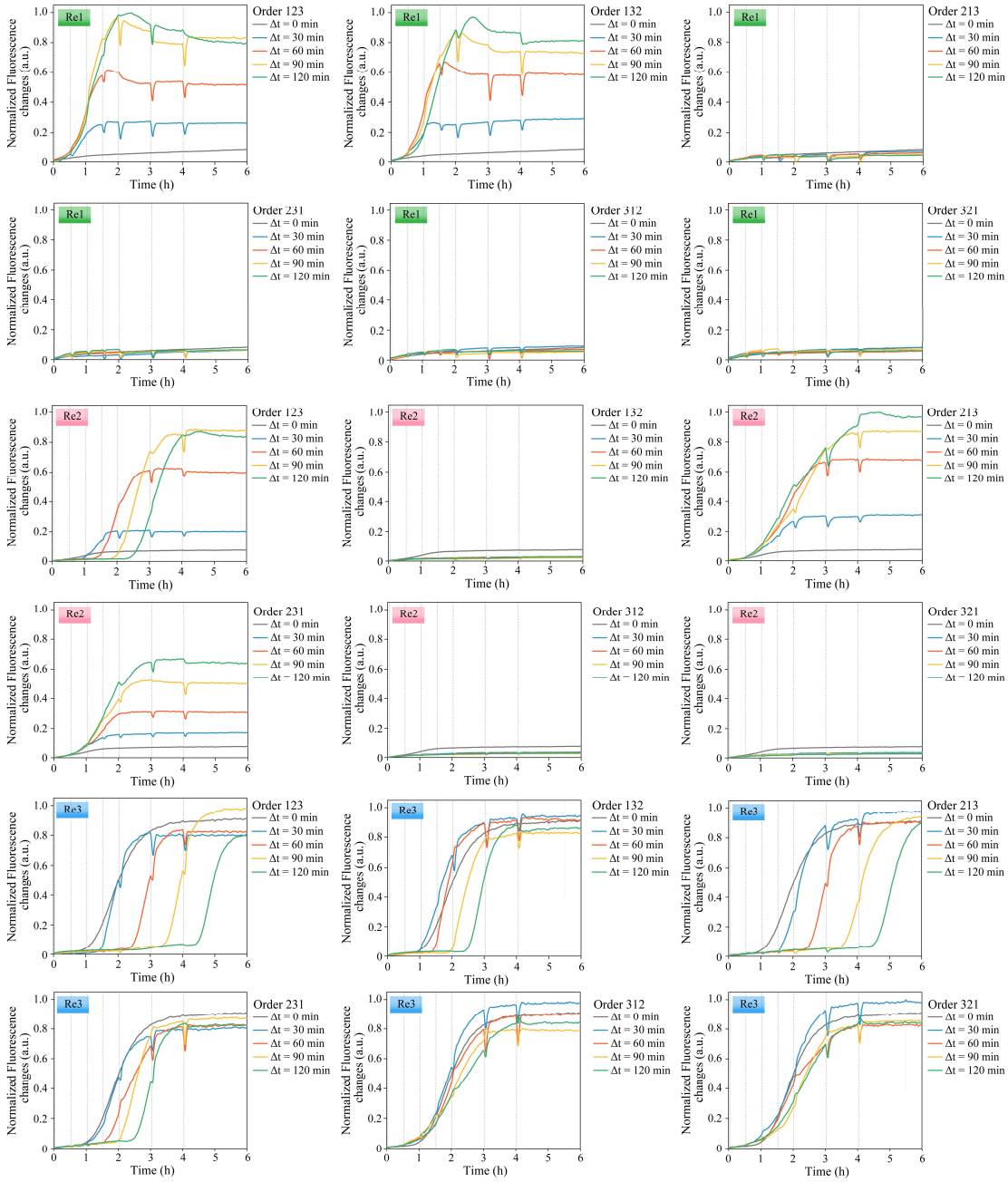

Fig. S14 Fluorescence curves for different combinations of input orders and time intervals. B1, B2 and B3 are combined into six input orders, 123, 132, 213, 231, 312 and 321, each of which represents the order of input time of B1, B2 and B3. There are 4 time intervals. Each time interval is 30 min, which are 30 min, 60 min, 90 min, and 120 min. 0 min indicates that B1, B2, and B3 are added at the same time. The concentrations of all ST and Re were 1×, and the input concentrations of B1, B2, and B3 were 3×

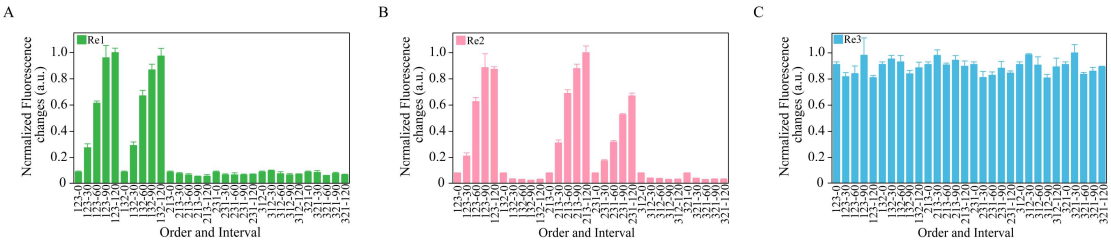

Fig. S15 Corresponding visual fluorescence output results to Fig. S14. (A) FAM fluorescence for different input orders and time interval combinations with 3× input. (B) ROX fluorescence for different input orders and time interval combinations with 3× input. (C) CY5 fluorescence for different input orders and time interval combinations with 3× input

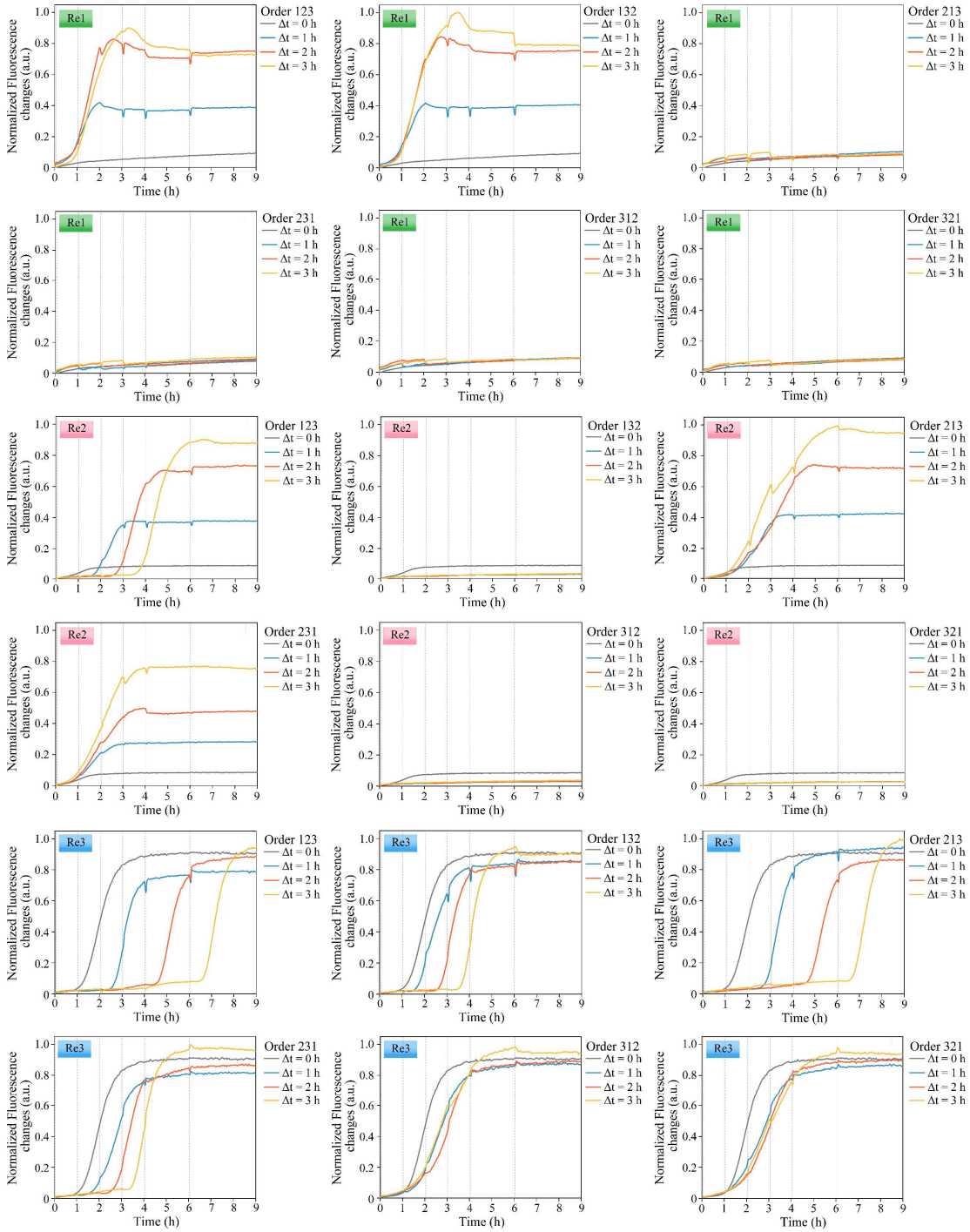

Fig. S16 Fluorescence curves for different combinations of input orders and time intervals. B1, B2 and B3 are combined into six input orders, 123, 132, 213, 231, 312 and 321, each of which represents the order of input time of B1, B2 and B3. There are 3 time intervals. The unit of each interval is 1h, which are 1 h, 3 h, and 3 h respectively. 0 h indicates that B1, B2, and B3 are added at the same time. The concentrations of all ST and Re were 1×, and the input concentrations of B1, B2, and B3 were 1×

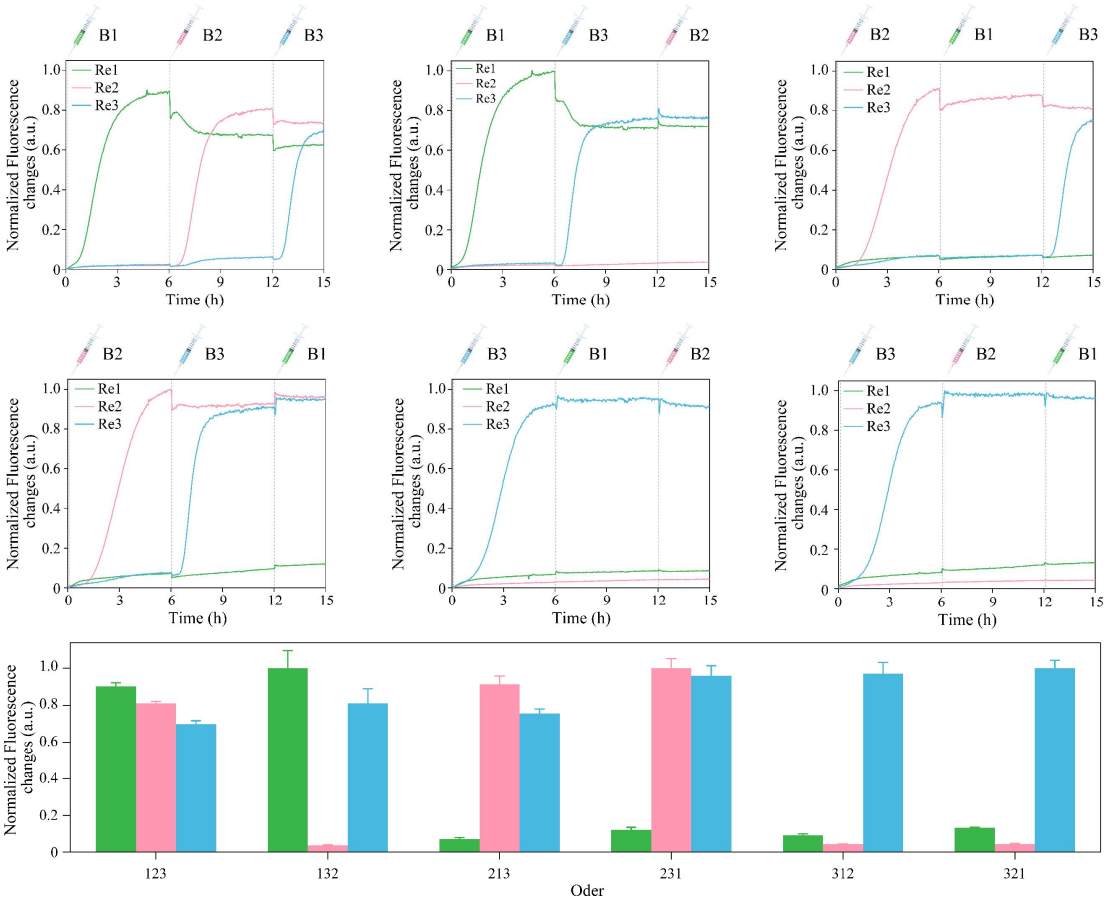

Fig. S17 Fluorescence curves and visual fluorescence output results for all input order combinations. The concentrations of all STs and the reporter Re are 1×, the input concentrations of B1, B2, and B3 are all 1×, and the time interval is 6 h

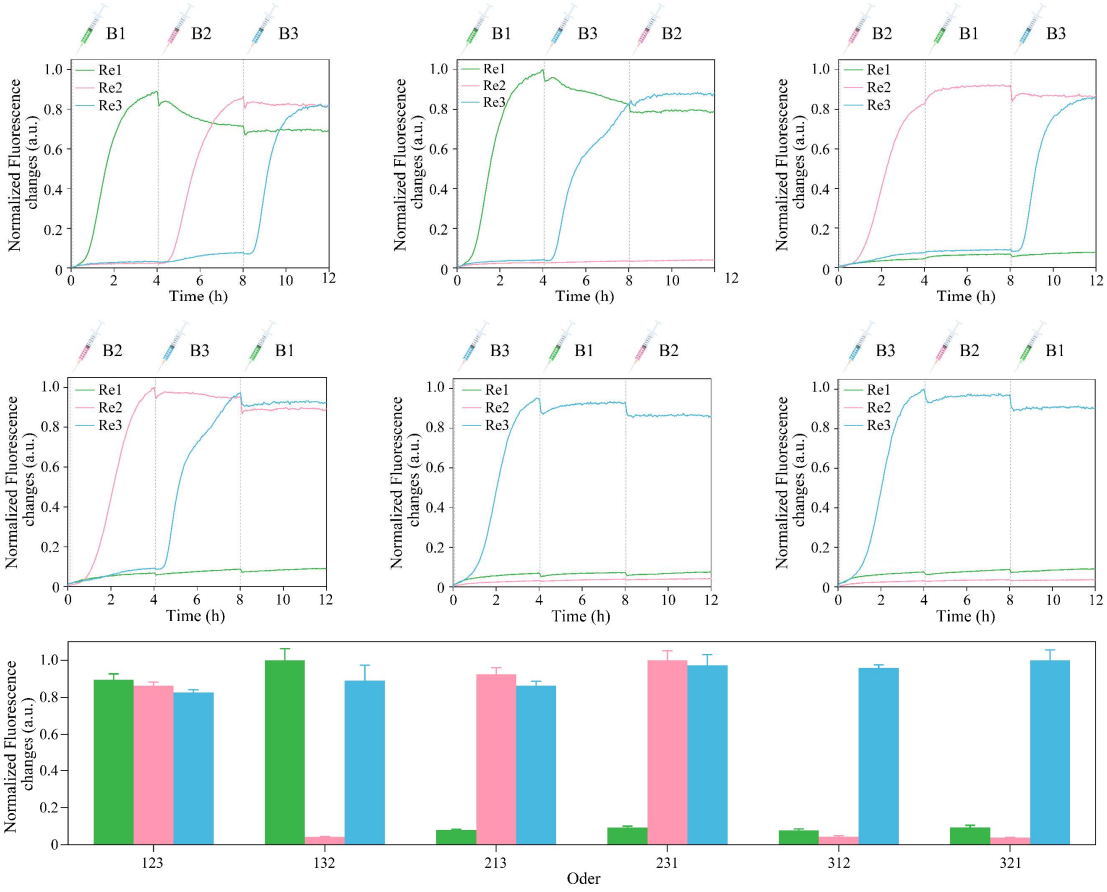

Fig. S18 Fluorescence curves and visual fluorescence output results for all input sequence combinations. The concentrations of all STs and the reporter Re are 1×, the input concentrations of B1, B2, and B3 are 3×, and the time interval is 4 h

Supplementary Table S1

Table S1 The DNA sequences used in the work

Name	Sequences from 5’ to 3’
b1	CCCTAACCCTAACCCTAACCCT***T*T*T*T**
b1-2	ACTCCCTAACCCTAACCCTAACCCT***T*T*T*T**
b1-3	ATACTCCCTAACCCTAACCCTAACCCT***T*T*T*T**
b1-4	TCATACTCCCTAACCCTAACCCTAACCCT***T*T*T*T**
b1-5	TCTCATACTCCCTAACCCTAACCCTAACCCT***T*T*T*T**
B0	TCTCATACTTAAACTATT***T*T*T*T**
B1	TCTCATACTCCCTAACCC***T*T*T*T**
B1-2	TCTCATACTCCCTAACCCTAA***T*T*T*T**
B1-3	TCTCATACTCCCTAACCCTAACCC***T*T*T*T**
B1-4	TCTCATACTCCCTAACCCTAACCCTAA***T*T*T*T**
B1-5	TCTCATACTCCCTAACCCTAACCCTAACCC***T*T*T*T**
B1-T	TTTTTTTTTCCCTAACCC***T*T*T*T**
B1-A	AAAAAAAAACCCTAACCC***T*T*T*T**
B1-ACT	AACTTATAACCCTAACCC***T*T*T*T**
A1	TACTATACTCACATTCTCTA***A*T*T*T**
A2	ACTTCAATAACCATCCAATGTAAC***T*T*T*T**
A3	CATTACACACTATTAATCACTCTA***T*T*T*T**
A7	ACTTCAATAACCATCCAATGTAAC***T*T*T*T**
A8	TAGAGAATGTGAGTATAGTA***T*T*T*T**
A9	CATTACACACTATTAATCACTCTA***T*T*T*T**
A10	TAGAGAATGTGAGTATAGTA***T*T*T*T**
A11	GTTACATTGGATGGTTATTGAAGT***T*T*T*T**
B2	ATATCAAAGCCCTAACCC***G*T*T*T**
B3	GACAGTAATCCCTAACCC***G*T*T*T**
S0*	AGTAGGATAGATGAATAGTTTAAGTATGAGATAGAGAATGTGAGTA TAGTA***T*T*T*T**

S0	AGTAGGATAGATGAATAG/idSp/TTAAGTATGAGATAGAGAATGTGAGTATAGTA***T*T*T*T**
S0-1	TTAAGTATGAGATAGAGAATGTGAGTATAGTA***T*T*T*T**
S1*	AGGGTTAGGGTTAGGGTTAGGGAGTATGAGATAGAGAATGTGAGTATAGTA***T*T*T*T**
S1	AGGGTTAGGGTTAGGGTT/idSp/GGGAGTATGAGATAGAGAATGTGAGTATAGTA***T*T*T*T**
S1-1	GGGAGTATGAGATAGAGAATGTGAGTATAGTA***T*T*T*T**
S3	AGGGTTAGGGTTAGGGTT/idSp/GGGAGTATGAGAGTTACATTGGATGGTTATTGAAGT***T*T*T*T**
S4	AGGGTTAGGGTTAGGGTT/idSp/GGGAGTATGAGATAGAGTGATTAATAGTGTGTAATG***T*T*T*T**
S5	AGGGTTAGGGTTAGGGTT/idSp/GGGCTTTGATATTAGAGAATGTGAGTATAGTA***T*T*T*T**
S6	AGGGTTAGGGTTAGGGTT/idSp/GGGAATACTGTCTAGAGAATGTGAGTATAGTA***T*T*T*T**
S7	AGGGTTAGGGTTAGGGTT/idSp/GGGCTTTGATATGTTACATTGGATGGTTATTGAAGT***T*T*T*T**
S8	AGGGTTAGGGTTAGGGTT/idSp/GGGCTTTGATATTACTATACTCACATTCTCTA***T*T*T*T**
S9	AGGGTTAGGGTTAGGGTT/idSp/GGGATTACTGTCTAGAGTGATTAATAGTGTGTAATG***T*T*T*T**
S10	AGGGTTAGGGTTAGGGTT/idSp/GGGATTACTGTCTACTATACTCACATTCTCTA***T*T*T*T**
S11	AGGGTTAGGGTTAGGGTT/idSp/GGGATTACTGTCACTTCAATAACCATCCAATGTAAC***T*T*T*T**
S1-2	TTTTTTACTCACATTCTCTA/i6FAMdT/***T*T*T*T**
A1*	TTTTT/**iBHQ1dT**/TAGAGAATGTGAGTATAGTA***T*T*T*T**
S5-2	TTTTTATAACCATCCAATGTAAC/iROXdT/***T*T*T*T**
A2*	TTTTT/**iBHQ2dT**/GTTACATTGGATGGTTATTGAAGT***T*T*T*T**
S6-2	TTTTTACACTATTAATCACTCTA/iCy5dT/***T*T*T*T**
A3*	TTTTT/**iBHQ3-NEdT**/TAGAGTGATTAATAGTGTGTAATG***T*T*T*T**