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Supporting Information: Aptamer-based regulation on transcription circuits

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S1: MATERIAL AND METHODS

Materials

All DNA strands were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Unmodified DNA strands were purified by polyacrylamide gel electrophoresis (PAGE), and modified DNA strands with fluorophore and quencher were purified by high-performance liquid chromatography (HPLC). The DNA oligonucleotides were dissolved in water as the stock solution and quantified using Nanodrop 2000, and absorption intensities were recorded at λ = 260 nm. The sequences of all oligonucleotides are listed in Table S1. Thermus aquaticus DNA polymerase (Taq DNA polymerase) was purchased from Takara (Takara Biomedical Technology (Beijing) Co., Ltd.). M.SssI, S-adenosylmethionine (SAM), HpaII, T7 RNA polymerase, dNTP, and rNTP were purchased from New England Biolabs. All other chemicals were of analytical grade and used without further purification.

DNA hybridization reaction

The DNA complexes were formed by mixing corresponding single strands with equal concentrations in 0.6× RNA Pol reaction buffer. The mixture was annealed in a polymerase chain reaction (PCR) thermal cycler at the reaction condition of 85°C for 5 min, 65 °C for 30 min, 50 °C for 30min, 37 °C for 30min, 25 °C for 30min, and finally kept at 25 °C.

DNA polymerase primer extension reaction

Taq DNA polymerase (10U/mL or 25U/mL) was mixed with dNTPs (0.25 mM) and DNA template (10nM or 20nM) in 0.6×RNA Pol reaction buffer and then incubated at 25 °C for 90-160 minutes.

RNA polymerase transcription reaction

T7 RNA Polymerase (2U/uL) was mixed with rNTPs (1.5 mM), DNA template (10nM or 20nM) in 0.6×RNA Pol reaction buffer to transcribe RNA at 25°C for 90-160 minutes.

Fluorescent experiments

All experiments were performed in 0.6×RNA Pol reaction buffer using real-time fluorescence PCR (Agilent Technologies). In a typical reaction, the total volume of the solution was 25μL for detection. The FAM fluorescence was monitored at 4 min intervals. Here, fluorescence data were processed to make the initial fluorescent signal value correspond to zero. The fluorescence results were obtained by the average values from at least three times repeat experimental results. Unless specifically mentioned, all the experiments were conducted at 25°C.

PAGE experiments

The samples were mixed with 6× loading buffer (Takara) or 36% glycerin solution and

subjected to electrophoresis analysis on a 12% polyacrylamide gel. The analysis was carried out in 1×TAE buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, pH 8.0) supplemented with 12.5 mM $MgCl_2$ at 90 V for 1-2 hours at 4°C. After stains all (Sigma-Aldrich) staining, Gels were imaged using the scanner.

S2: Experimental details

(1) Experimental details of the Taq polymerase-assisted transcription: (T1/I1) was mixed with Taq polymerase, T7 RNA polymerase, dNTP, rNTP and (F/Q). The solution was subjected to fluorescence measurement after mixing. The sample was subjected to electrophoresis analysis after incubation at 25°C for 3 hours. For gel analysis, [T1/I1] =20nM, [Taq] = 25U/mL, [F/Q] = 800nM. For fluorescence assay, [T1/I1] = 10nM, [Taq] = 25U/mL, [F/Q] = 400nM.

(2) Experimental details of the aptamer-inhibited transcription: the different concentrations of A/B were mixed with DNA polymerase, dNTP and incubated for 20 minutes, followed by the addition of the mixture including T7 RNA polymerase, rNTP, (T1/I1) and (F/Q). The solution was subjected to fluorescence measurement after mixing. The sample was subjected to electrophoresis analysis after incubation at 25°C for 2 hours. For gel analysis, [T1/I1] = 20nM, [Taq] = 25U/mL, [F/Q] = 800nM. For fluorescence assay, [T1/I1] = 10nM, [F/Q] = 400nM.

(3) Experimental details in Figure 2, (A/B) was mixed with different concentrations of 'B*', DNA polymerase, dNTP and incubated for 20 minutes, followed by the addition of the mixture including T7 RNA polymerase, rNTP, the annealed (T1/I1) and (F/Q). The solution was subjected to fluorescence measurement after mixing. The sample was subjected to electrophoresis analysis after incubation at 25°C for 2 hours. For gel analysis, [T1/I1] = 20nM, [Taq] = 25U/mL, [A/B] = 50nM, [F/Q] = 800nM. For fluorescence assay, [T1/I1] = 10nM, [Taq] = 10U/mL, [F/Q] = 400nM, [A/B] = 30nM.

(4) Experimental details in two-level cascading circuits (Figure 3): (A/B) was mixed with Taq polymerase, dNTP and incubated for 20 minutes. Then the incubated sample was added into the mixture in the presence or in the absence of upstream input 'I2' containing T7 RNA

polymerase, rNTP, (T2/D2), (T1/I1) and the reporter probe (F/Q). The solution was subjected to fluorescence measurement after mixing. The sample was subjected to electrophoresis analysis after incubation at 25°C for 3 hours. For gel analysis, [A/B] = 50nM, [Taq] = 25U/mL, [T1/I1] = 20nM, [F/Q] = 800nM. For fluorescence assay, [A/B] =50nM, [Taq] = 25U/mL, [T1/I1] = 10nM, [F/Q] = 400nM. In Figure 5e, [T2/D2] =0,1,2,5, and 10 nM, [I2] = 0,1,2,5, and 10nM.

(5) Experimental details in the enzyme-controlled switch circuit in Figure 4, (A-msi/B-msi) complex incubated with HpaII (in the presence or absence of M.SssI) for 2 hours, the digested product were heated at 80°C for 20 minutes to deactivate the enzyme, followed by the addition of a mixture that contained DNA polymerase, dNTP and incubated for 20 minutes. Then the incubated sample was added into the mixture including T7 RNA polymerase, rNTP, (T1/I1) and reporter probe (F/Q). The solution was subjected to fluorescence measurement after mixing. The sample was subjected to electrophoresis analysis after incubation for 2 hours. For gel analysis, [M.SssI] = 0.5,1,3,4,5,10U/mL, [HpaII] = 10U/mL, [A-msi/B-msi] = 50nM, [Taq] = 25U/mL, [T1/I1] = 20nM, [F/Q] = 800nM. For fluorescence assay, [M.SssI] = 0.3,0.6,0.9,1.2,1.5,3U/mL, [HpaII] = 6U/mL, [A-msi/B-msi] = 30nM, [Taq] = 10U/mL, [T1/I1] =10nM and [F/Q] = 400nM.

S3: The design of the reporter probe

The 5’ end of strand ‘F’ is labeled with a FAM dye and the 3’ end of strand ‘Q’ is labeled with a BHQ quencher such that the fluorescence is low when the reporter probe is formed due to FRET while the fluorescence is high when strand ‘Q’ is free.

To avoid the undesirable elongation of the upper strand NF in the complex (NF/Q) by DNA polymerase, one base mismatch (G) was added at the 3’ end of the sequence of upper strand named as ‘F’. Because the terminal mismatching stalled action of the polymerase at the 3’ end, the extension reaction was prevented. Figure S1 shows gel electrophoresis images of the products with terminal mismatched G or without G at the 3’ end. When DNA polymerase was added into the samples, a new slower-migrating band corresponding to the extension product was observed (lane 6), whereas (F/Q) did not show extension products upon incubation with DNA polymerase (lane 7), indicating that it was suitable as the signal

reporter for the transcription circuit.

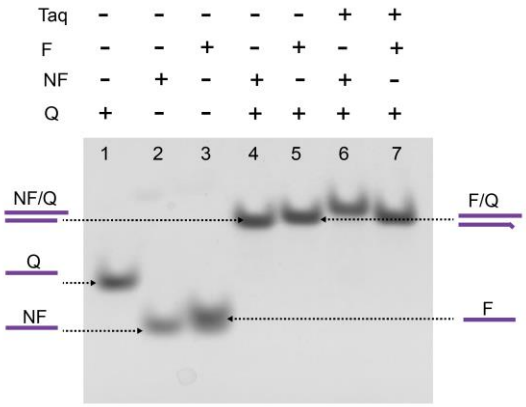

Figure S1. Page gel analysis of reporter design. '+' denotes addition of the components and '−' denotes absence of the components. Lane 1: Q, Lane 2: NF, Lane 3: F, Lane 4: (NF/Q), Lane 5: (F/Q), Lane 6: (NF/Q) upon addition of DNA polymerase, Lane 7: (F/Q) upon addition of DNA polymerase. The concentration of DNA polymerase was 0.5U/μL.

S4: Taq polymerase-assisted activator circuit

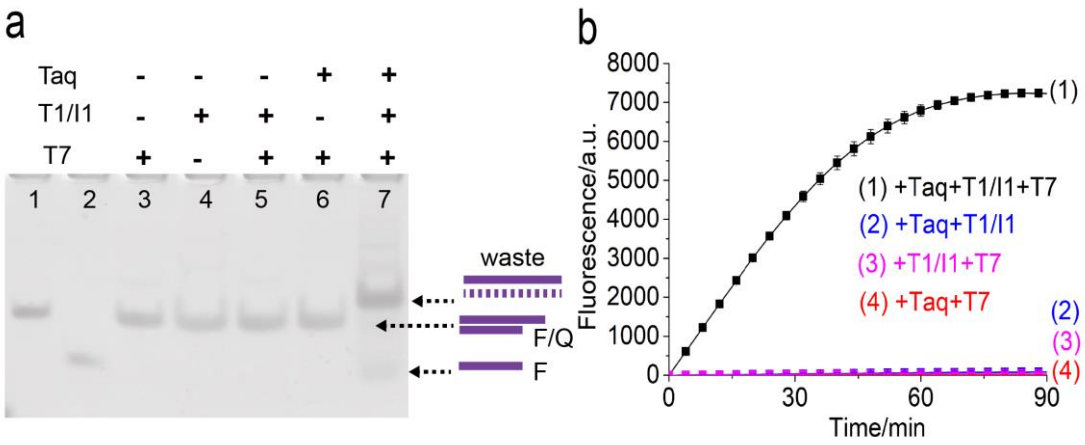

Figure S2. (a) Gel analysis of the experiment using 12% native PAGE. '+' denotes addition of the components and '−' denotes absence of the components. The components added in every lane are indicated above the image. [T1/I1] =20nM; [Taq]=25U/mL; [T7] =2U/μL; [F/Q] =800nM. (b) Time-dependent fluorescence assay of the in vitro transcription at 25°. [T1/I1] =10nM; [F/Q] =400nM; [Taq]=25U/mL; [T7] =2U/uL.

The Taq polymerase-triggered transcription reaction was confirmed by gel analysis and fluorescence assay. As shown in Figure S2a lane 3-6, only one single band corresponding to the reporter probe (F/Q) was observed upon addition of either of two polymerases or (T1/I1)

complex respectively. As expected, in the presence of both polymerase and (T1/I1) complex (lane 7), the gel band of reporter probe (F/Q) disappeared and two new bands appeared, the one that ran slower than the reporter probe was DNA/RNA waste, the other faster-migrating band corresponded to strand 'F' which was displaced from reporter probe (F/Q) by RNA. It is noted that the DNA template in the gel experiment was invisible due to low concentration (20nM). In addition, time-dependent fluorescent intensity changes were performed to verify the Taq polymerase-triggered transcription. As shown in Figure S2b, a significant increase in fluorescence signal was observed in the presence of both polymerase and incomplete DNA template (T1/I1) (black curve 1), and no distinct fluorescence increase can be obtained in the absence of DNA polymerase (purple curve 3). Meanwhile, no fluorescence signal changes can be observed in the absence of either RNA polymerase or (T1/I1) (blue curve 2 and red curve 4), thus confirming that the transcription was initiated by the DNA template completion using DNA polymerase.

S4.1: Effect of the buffer in the reaction

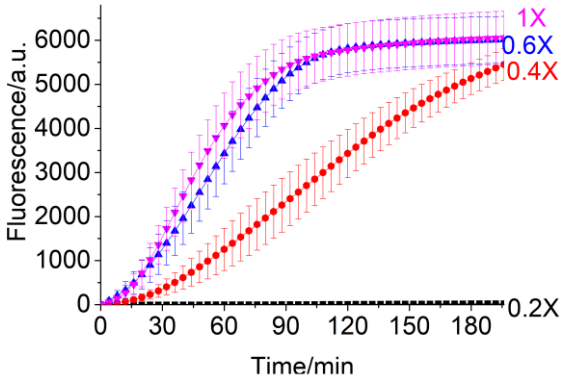

Figure S3. Fluorescence intensity analysis of the effect of the reaction buffer. [T1/I1] = 10nM, [Taq polymerase] = 25U/mL, [F/Q] = 400nM.

Considering that Taq DNA polymerase uses PCR buffer (2mM Mg^{2+}) and T7 RNA polymerase uses T7 reaction buffer (6mM Mg^{2+}) in the reaction, whereas the concentration of magnesium ions influences the fluorescence and polymerase activity. To make both polymerases work properly in this study, a series of different concentrations of T7 reaction buffer (from 0.2× to 1×) were tested by fluorescence assay. It is obvious that as the concentration of buffer increased, the fluorescence intensity grew faster correspondingly indicating the high transcription efficiency. As can be seen in Figure S3, 0.6×buffer acted

almost as 1×buffer, therefore it was chosen as the final reaction buffer in the following reaction.

S4.2: Effect of the concentration of DNA polymerase in the reaction

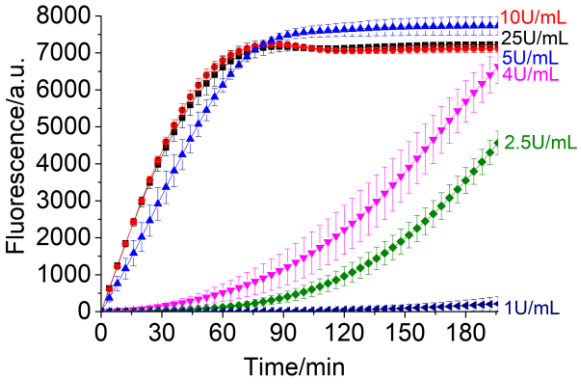

Figure S4. Fluorescence intensity analysis of the concentration of Taq polymerase. [T1/I1] = 10nM, [T7 RNA polymerase] = 2U/μL, [F/Q] =400nM.

DNA polymerase plays an important role in the primer extension reaction. In order to determine its concentration in the system, varying concentrations of Taq polymerase from 1U/mL to 25U/mL were tested by monitoring fluorescence signal. As can be seen obviously in Figure S4, as the concentration of Taq polymerase increased from 1U/mL to 5U/mL, the fluorescence grew faster correspondingly, the concentration of 10U/mL and 25U/mL almost had the same tendency. It seemed that the concentration of Taq polymerase reached the point of saturation at 10U/mL under the experimental conditions. Therefore, the concentration over 10U/mL was chosen as the final concentration of Taq polymerase.

S4.3: Effect of the concentration of T7 RNA polymerase in the reaction

In the RNA transcription reaction, the control experiments of the fluorescent assay were carried out to choose the appropriate concentration of T7 RNA polymerase (Figures S5). A series of different concentrations of T7 RNA polymerase from 0.2U/μL to 2U/μL were tested. From the experimental results, 2U/μL was selected as the final concentration of T7 RNA polymerase.

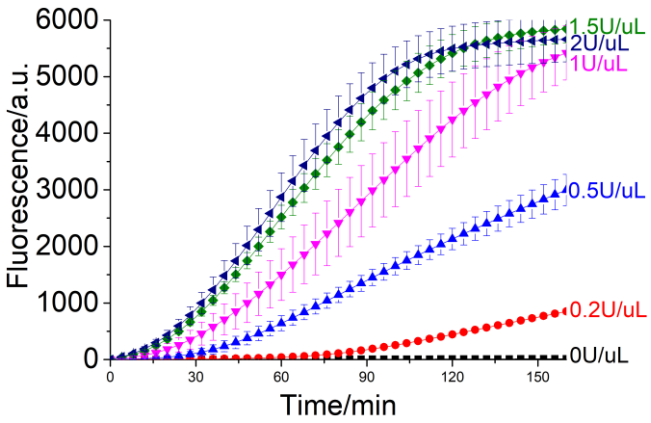

Figure S5. Fluorescent intensity analysis of the concentration of T7 RNA polymerase. [T1/I1] = 10nM, [Taq polymerase] = 25U/mL, [F/Q] = 400nM.

S4.4: Effect of the concentration of DNA template in the reaction

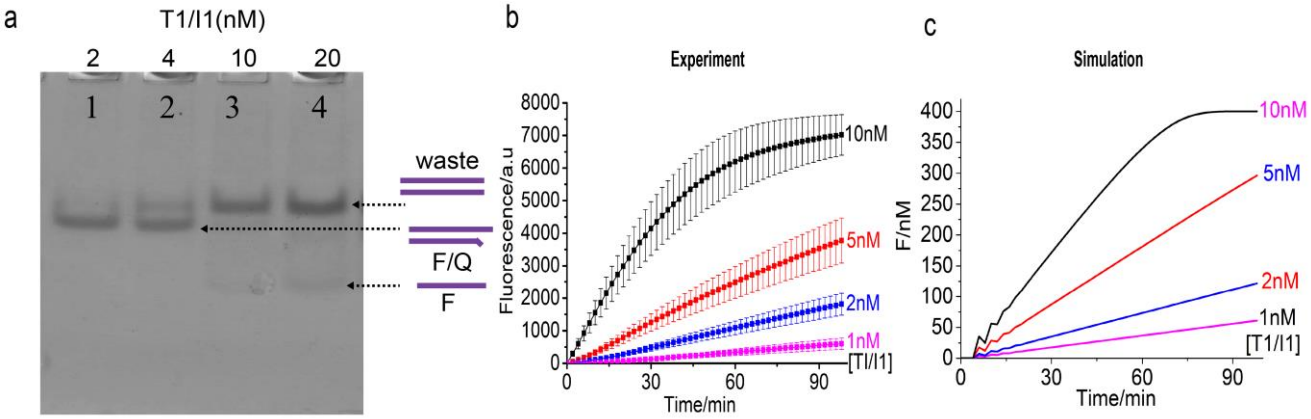

Figure S6. (a) PAGE gel analysis for different concentrations of the incomplete template (T1/I1). Lane 1 to lane 4: the reporter probe (800nM) with the addition of different concentrations of (T1/I1) (2nM, 4nM, 10nM, 20nM) respectively. [F/Q] = 400nM, [Taq polymerase] = 25U/mL, [T7 RNA polymerase] = 2U/μL. (b) Time-dependent fluorescence assay of the in vitro transcription at 25°. [T1/I1] =10nM; [F/Q] =400nM; [Taq]=25U/mL; [T7] =2U/uL. (c) The simulative characterization of the in vitro transcription. The fluorescent intensities were transformed into concentrations. The experimental details can be found in supplementary section S1. The simulation model can be found in Supplementary section S9.1.

The control experiments of PAGE analysis were implemented to determine the appropriate concentration of incomplete DNA template (T1/I1). A series of concentrations of (T1/I1) (2nM, 4nM, 10nM, 20nM) were introduced to the solution to trigger the primer extension reaction and RNA transcription reaction (Figures S6a). As the concentration of DNA template increased, the quantity of the RNA was enhanced. Notably, the reporter probe (F/Q)

remained unchanged and almost no RNA was transcribed in lane 1, due to a small amount of template (2nM). However, (F/Q) was almost completely consumed in lane 4, due to an increasing template (20nM). As the concentration of template increased, the fluorescent intensity increased correspondingly (Figure S6b). The experimental results were also confirmed via the simulation model (Figure S6c). The R1 transcribed from 10nM DNA template could react with 400nM reporter probe and rapidly release strand 'F'. To obtain the fast reaction kinetics, the concentration of over 10nM DNA template (T1/I1) was chosen as the final concentration in the reaction.

S5: Suppression of transcription using aptamer repressor

Here, DNA aptamer (A/B) can be used as the repressor to inhibit the synthetic RNA transcription. When the complete hairpin structure of aptamer repressor was formed, DNA polymerase was in the suppressed state by being captured on the hairpin loop. Therefore, the complete dsDNA promoter cannot be generated because of the lack of the primer extension reaction, thus preventing RNA polymerase binding to the DNA template.

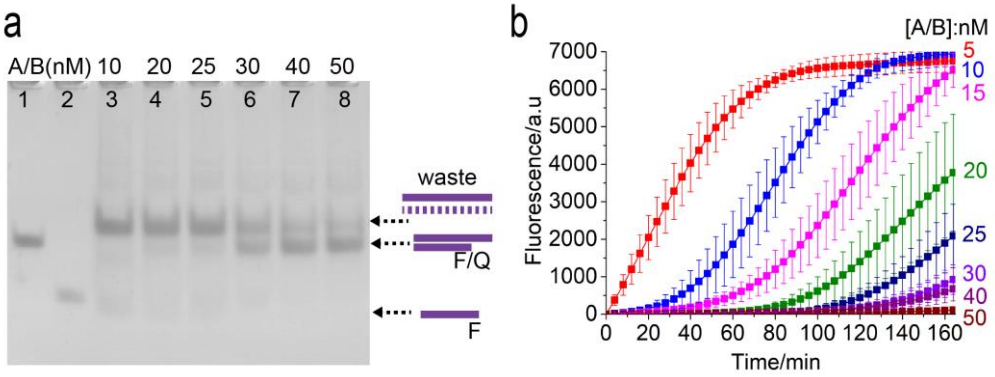

Figure S7. (a) Gel analysis of the experiment using 12% native PAGE. Lane 1: (F/Q), Lane 2: F, Lane 3 to 8: (F/Q) with the addition of different concentrations of (A/B) complex at 25°. [T1/I1] =20nM; [Taq]=25U/mL; [T7] =2U/μL; [F/Q] =800nM. (b) Time-dependent fluorescence assay of the inhibition of transcription circuit using different concentrations of (A/B) complex at 25°. [T1/I1] =10nM; [F/Q] =400nM; [Taq]=25U/mL; [T7] =2U/μL. The experimental details can be found in supplementary section S1.

The reaction of the repressive transcription was confirmed by gel analysis and fluorescence assay. As shown in Figure S7a lane 3 to 8, to test the repressing regulation, a series concentrations of aptamer repressor (hairpin A/B) (10nM, 20nM, 25nM, 30nM, 40nM,

50nM) were used at a fixed concentration of DNA polymerase (25U/mL). As the concentrations of repressor increased, the generations of the RNA decreased and the remained reporter probe increased. In gel results lanes 3 to 5, the probe (F/Q) was almost completely consumed, indicating rather poor inhibition effect with the low concentrations of aptamer repressor. Only when the repressor concentrations were more than 30 nM, the transcriptions were significantly repressed as observed (lane 6). The strong repressing effect can be obtained when repressor concentration was 50nM (lane 8). Moreover, time-dependent fluorescent intensity changes were performed to test the inhibiting effects using different repressor concentrations. As shown in Figure S7b, with the concentrations of the repressor increased, the fluorescence changing velocity decreased. The inhibiting effects had a close relationship with the concentrations of aptamer repressor. Based on the experimental results above, 50nM repressor was chosen as the final concentration to inhibit the 25U/mL Taq polymerase in transcription repressing circuit. For 10U/mL DNA polymerase, 30nM repressor was chosen to inhibit the reaction (Supplementary Figure S8).

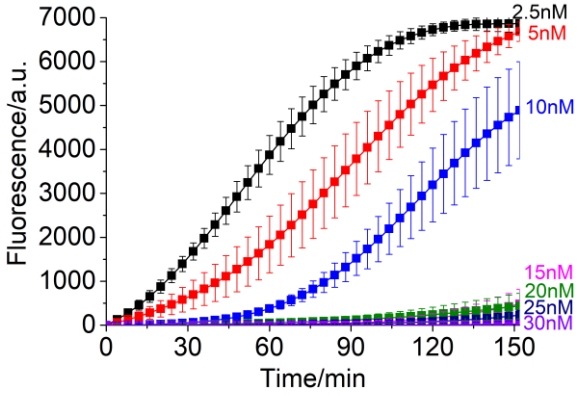

Figure S8. Time-dependent fluorescent intensity analysis of the concentration of (A/B) complex for inhibition of 10U/mL DNA Taq polymerase. [T1/I1] = 10nM, [F/Q] =400nM.

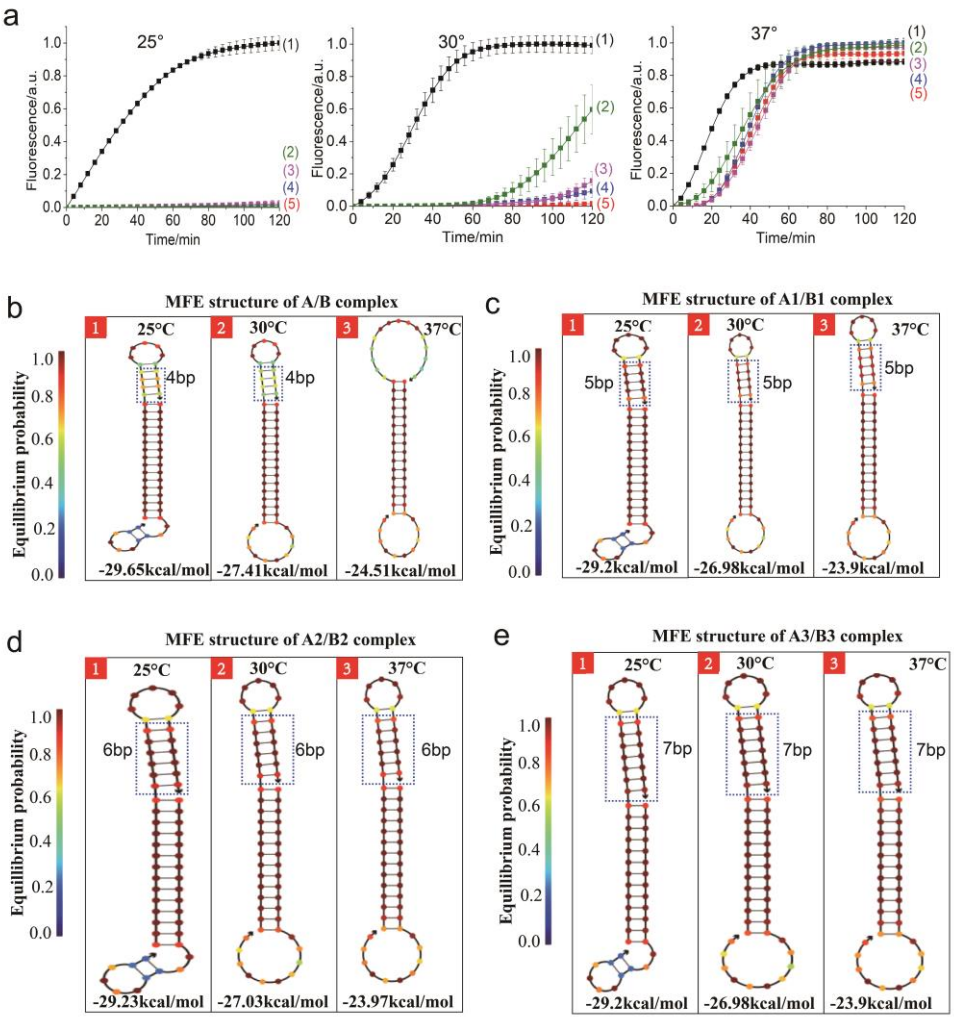

Figure S9. (a) Time-dependent normalized fluorescent analysis of the activator and repressor circuits with different aptamer complex at different temperatures. Curve 1: activator circuit 1; Curve 2: repressor circuit with (A/B); Curve 3: repressor circuit with (A1/B1); Curve 4: repressor circuit with (A2/B2); Curve 5: repressor circuit with (A3/B3). (b-e) Equilibrium probability maps and minimum free energy (MFE) structures of the aptamer complex with different stem domain predicted by NUPACK. (b) (A/B); (c) (A1/B1), (c) (A2/B2), (c) (A3/B3).

Considering the various temperature conditions in nature, different control experiments were also conducted to investigate the temperature factor influencing the above repression circuit (Supplementary Figure S9). Meanwhile, in addition to complex (A/B), another three variants of structure (A1/B1), (A2/B2) and (A3/B3), which had longer stem domain, were designed as the aptamer repressor. Specifically, (A1/B1) had a stem domain length of 5 bp, (A2/B2) had 6 and (A3/B3) had 7. In Figure S9 black curve 1, the fluorescent signal of

activator circuit grew faster along with the elevated temperatures and reached the plateau costing 120 minutes, 60 minutes and 40 minutes at 25°C, 30°C, 37°C respectively. Under 25°C, the fluorescent signal of all the four repressive circuits (curve 2-5) remained unchanged, demonstrating that they can completely inhibit the transcription. However, under 30°C, a huge reduction of the repression effect of the repressive circuit with (A/B) was observed (curve 2). When the experimental temperature was raised to 37°C, none of the repressive circuits can suppress the transcription. The experimental results demonstrated that the temperature greatly influenced the aptamer inhibiting regulation in transcription circuit.

To analyze the experiments from the perspective of thermal stability, we tested the structure of the aptamer complex by minimum free energy (MFE) method. Figure S9b displays equilibrium probability maps and secondary structure predictions of the (A/B) complex at different temperatures generated by NUPACK online. The overall free-energy of the structure quantifies the stability of the aptamer complex, where the Gibbs free energy change (ΔG) lies in the range of −29.65 to −24.51 kcal·mol−1. The MFE results suggest that the conserved sequence “CAATGTACAGTATTG” of the strand ‘A’ in the aptamer (A/B) can form a hairpin structure with a 7-base loop and 4bp stem domain at 25°C and 30°C. However, at 37°C, the hairpin structure disappeared. The Gibbs free energy was found increasing with the increase of temperature, corresponding to a less stable structure and might leading to a weaker repression effect. We speculated that the stable stem-loop conformation would be destroyed under a higher temperature, thus losing the inhibitory capability on transcription circuit. For the structure of (A1/B1), the stable stem-loop conformation remains unchanged even at 37°C (Figure S9c). The results indicated that at relatively low temperatures such as 25°C and 30°C, the hairpin DNA structure with short stem was not stable enough to inhibit transcription circuit. Nevertheless, with the length of the stem increasing, the inhibit ability increased. However, when the experimental temperature was raised to 37°C, the stable hairpin structure could not inhibit the transcription even the stem length increased to 7 bp. Therefore, in the study, all the experiments were conducted at 25° unless specifically mentioned. The MFE details of structures (A2/B2) and (A3/B3) can be found in Supplementary Figure S9d, S9e.

S6: DNA activator circuit

The repressor circuit by DNA aptamer complex could be activated upon addition of DNA strand ‘B*’, which hybridized with strand ‘B’ by strand displacement reaction through toehold and disrupted the conformation of the aptamer, thus DNA polymerase was released to participate the subsequent reaction. The control experiments of PAGE gels were implemented to verify the strand displacement reaction (Figures S10). It is obvious that as strand ‘B*’ was added to (A/B) complex in lane 5, the (A/B) complex disappeared and the band corresponding (B/B*) and strand ‘A’ appeared.

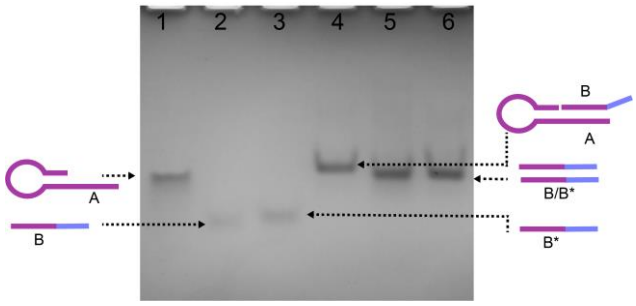

Figure S10. PAGE gel analysis for strand displacement of (A/B) complex with strand ‘B*’. Lane 1: A, Lane 2: B, Lane 3: B*, Lane 4: (A/B), Lane 5: (A/B) + B*; Lane 6: BB*; the final concentration of the sample was 0.8μM.

S7: Two-level cascading transcription circuits

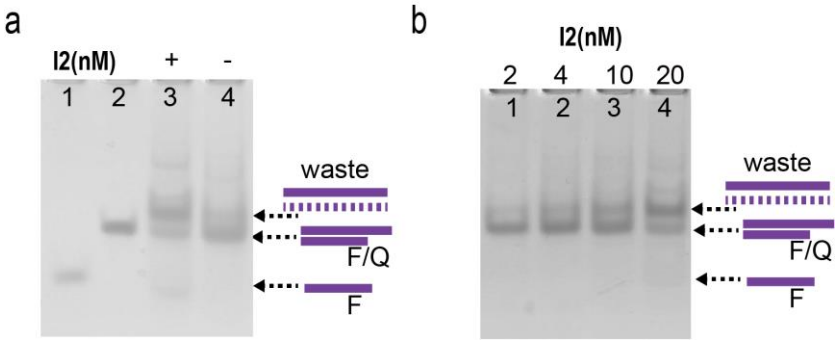

Figure S11. (a) PAGE gel analysis of the two-level cascading circuit. Lane 1: F, Lane 2: (F/Q), Lane 3: the circuit with the addition of I2; Lane 4: the circuit without I2. [T1/I1] =20nM, [T2/D2] =20nM, [I2] =20nM, [A/B] =50nM, [Taq polymerase] = 25U/mL, [F/Q] =800nM. (b) PAGE gel analysis of the circuit with different concentrations of upstream input strand ‘I2’. The concentration of each component except I2 is the same as above image.

The reaction of the two-level cascading circuit was confirmed by PAGE analysis. As shown in Figure S11a lane 3, upon addition of input strand ‘I2’, two bands corresponding to DNA/RNA waste and single strand ‘F’ appeared. In lane 4, in the absence of ‘I2’, a large amount of (F/Q) remained intact, indicating no downstream transcription occurred. With the concentration of ‘I2’ increasing from 2 to 20 nM through lane 1 to 4, the band of the reporter (F/Q) decreased and DNA/RNA waste increased, indicating that a growing number of target RNA was generated in the reactions (Figure S11b).

The control experiments were implemented as shown in Figure S12. From the results, the signal of trace 5 increased faster and reached equilibrium quickly. While in the presence of aptamer repressor, the fluorescence response gradually increased. The fluorescence intensity remained almost unchanged in three conditions: with aptamer and Taq polymerase but without the addition of input ‘I2’ (curve 1); without aptamer, Taq polymerase and ‘I2’ (curve 2); without aptamer and Taq polymerase but with the addition of input ‘I2’ (curve 3). The experiment results indicated that the downstream transcription circuit can be effectively controlled by the upstream transcription circuit through the aptamer repressor.

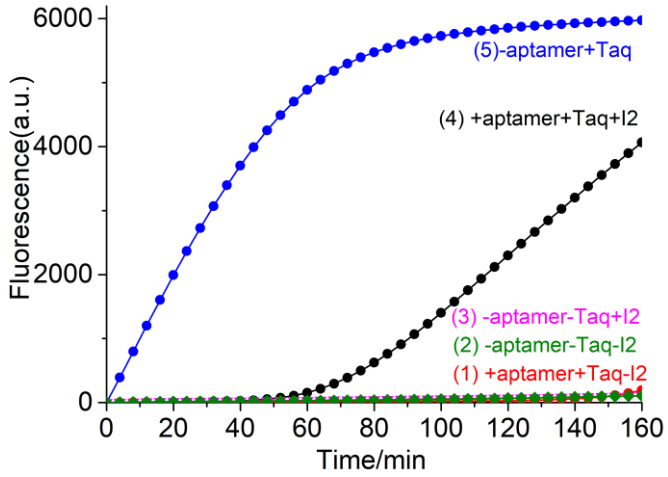

Figure S12. Time-dependent fluorescence assay of the two-level cascading circuit. [T1/I1] =10nM, [A/B] =50nM, [Taq polymerase] = 25U/mL, [F/Q] = 400nM, [T2/D2] =10nM.

S8: Regulation of transcription circuit by enzyme regulators

To block the aptamer repressor from digestion by a restriction enzyme, the control experiments of PAGE gels were performed to verify the effect of the methylase. As shown in

Figure S13, there was only one band (A-msi/B-msi) when M.SssI was present in lane 2, compared with it, when only HpaII endonuclease was added, two new bands could be observed (lane 3), suggesting that (A-msi/B-msi) complex containing the symmetrical sequences of 5'-CCGG-3′ was cleaved into two parts by HpaII endonuclease. However, when both M.SssI and HpaII endonuclease were present, there were three bands (lane 4), indicating that the methylation reaction happened and the cleavage of HpaII endonuclease was partially blocked by methylation. The reason for the residual (A-msi/B-msi) complex was a deficient amount of M.SssI (10U/mL), this could be avoided by increasing the concentration of M.SssI.

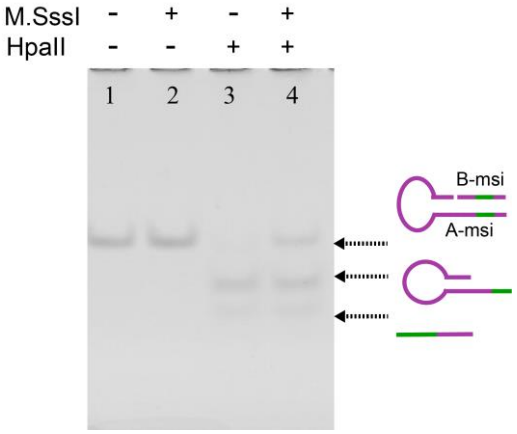

Figure S13. PAGE gel analysis of the M.SssI enzyme by the (A-msi/B-msi) complex. '+' denotes addition of the components and '−' denotes absence of the components. Lane 1: (A-msi/B-msi) without addition of M.SssI and HpaII; Lane 2: (A-msi/B-msi) with M.SssI; Lane 3: (A-msi/B-msi) with HpaII; Lane 4: (A-msi/B-msi) treated with M.SssI (10U/mL) and HpaII (100U/mL). The final concentration of aptamer complex was 1.2μM.

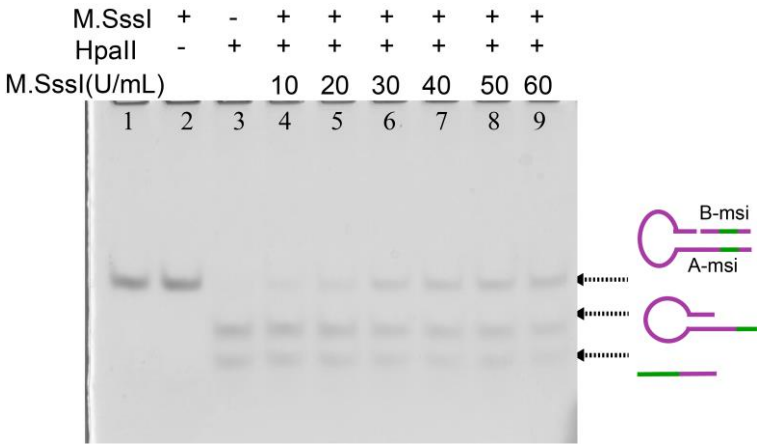

Figure S14. PAGE gel analysis of the effect of concentration of M.SssI enzyme by (A-msi/B-msi) complex.

‘+’ denotes addition of the components and ‘−’ denotes absence of the components. Lane 1: without addition of M.SssI and HpaII; Lane 2: without addition of HpaII; Lane 3: without addition of M.SssI; Lane 4 to lane 9: treated with different concentrations of M.SssI (10U/mL, 20U/mL, 30U/mL, 40U/mL, 50U/mL, 60U/mL) respectively and HpaII (100U/mL). The final concentration of (A-msi/B-msi) was 1.2μM.

A series of different concentrations of M.SssI enzyme (10U/mL, 20U/mL, 30U/mL, 40U/mL, 50U/mL, 60U/ mL) were used to verify the protection of the (A-msi/B-msi) complex from digestion (Figure S14). It is obvious that, as the concentration of M.SssI increased, the digestion of the (A-msi/B-msi) reduced.

S9: The Simulation models

S9.1: The Simulation model of polymerase-triggered transcription circuit

The circuit is triggered by the DNA polymerase on template1(T1/I1) to form the template2 which acts as the substrate for T7 RNA polymerase. After that, T7 polymerase catalyzes the transcription reaction and produce RNA, which then displace the reporter (F/Q) and lead to a significant fluorescent signal. As depicted above, the reaction can be modeled as follows:

$$\mathrm{template1} + \mathrm{Taq} \xrightarrow{K_{p1}} \mathrm{template2} + \mathrm{Taq} \quad (1)$$

$$\mathrm{template2} + \mathrm{T7} \xrightarrow{K_{p2}} \mathrm{template2} + \mathrm{T7}\text{+RNA} \quad (2)$$

$$\mathrm{RNA} + \mathrm{F/Q} \xrightarrow{K_2} \mathrm{F} + \mathrm{waste} \quad (3)$$

Equation (1) is a simplified polymerase-driven primer extension reaction with the kinetic rate K_{p1}. Equation (2) is a simplified polymerase catalysis reaction with the kinetic rate K_{p2}. Equation (3) is the strand displacement reactions and ‘F’, waste represent FAM-modified strand and DNA/RNA waste respectively. K_{p1}, K_{p2}, and K_2 are the reaction constants for equations (1) to (3), respectively.

Therefore, the rate equation of template2 can be derived from equation (1) as:

$$\mathrm{d[template2]/dt} = K_{p1}\mathrm{[template1][Taq]}$$

The rate equation of RNA can be derived from equation (2) and (3) as:

$$\mathrm{d[RNA]/dt} = \mathrm{K}_{p2}\mathrm{[template2][T7]\text{-}K_2[RNA][F/Q]}$$

The rate equation of F can be derived from equation (3) as:

$$\mathrm{d[F]/dt} = \mathrm{K_2[RNA][F/Q]}$$

When the initial condition is $[\text{template1}]_0$, $[\text{Taq}]_0$, $[\text{T7}]_0$, $[\text{F/Q}]_0$, the mass balance equations are:

$$[\text{template1}]_0 = [\text{template1}] + [\text{template2}]$$

$$[\mathrm{F/Q}]_0 = [\mathrm{F/Q}] + [\mathrm{F}]$$

In the simulation, we don't consider the delay of the fluorescent reporting reaction, because the fluorescence signal is immediately produced. This allows us to use the fluorescence signal to directly determine the concentration of F.

The differential equations can be obtained as:

$$\mathrm{template1(k+1)} = \mathrm{template1(k)} - K_{p1}\mathrm{template1(k)Taq(k)}$$

$$\mathrm{template2(k+1)} = \mathrm{template2(k)} + K_{p1}\mathrm{template1(k)Taq(k)}$$

$$\mathrm{RNA(k+1)} = \mathrm{RNA(k)} + K_{p2}\mathrm{template2(k)T7(k)} - K_2\mathrm{RNA(k)F/Q(k)}$$

$$\mathrm{F/Q(k+1)} = \mathrm{F/Q(k)} - K_2\mathrm{RNA(k)F/Q(k)}$$

$$\mathrm{F(k+1)} = \mathrm{F(k)} + K_2\mathrm{RNA(k)F/Q(k)}$$

In order to survey the kinetics of the circuit in detail, four control experiments with the varying concentrations of template1 were performed respectively. After the calculations, the simulation data have a good agreement with the experimental results (Figure S6c). Based on the fluorescence results, K_{p1}, K_{p2}, and K_2 for equations (1), (2) and (3) were fit to 7.11×10-5 L·unit-1·s-1, 5.45×10-7 L·unit-1·s-1, and 3.539×10+6 M-1·s-1, respectively. The program of simulations is listed at the end of the supplementary section S9.3.

S9.2: The Simulation model of two-level cascading transcription circuit

As shown in Figure 3, DNA polymerase was first locked by the aptamer repressor. The downstream reaction was triggered by upstream transcription circuit. The upstream incomplete template (T2/D2) hybridized with input 'I2' and constituted the substrate template3 for T7 DNA polymerase to transcribe RNA as 'R1'. The produced RNA disrupted the structure of the aptamer through strand displacement reaction, thus generated LR1 (B/R1) and strand 'A', then released the DNA polymerase which then induced downstream transcription circuit

as depicted in the basic circuit.

As described above, the reaction can be modeled as follows:

$$\text{template3} + \text{T7} \xrightarrow{K_1} \text{template3} + \text{T7} + \text{R1} \quad (1)$$

$$\text{repressor} + \text{R1} \xrightarrow{K_2} \text{Taq} + \text{LR1+A} \quad (2)$$

$$\text{template1} + \text{Taq} \xrightarrow{K_3} \text{template2} + \text{Taq} \quad (3)$$

$$\text{template2} + \text{T7} \xrightarrow{K_1} \text{template2} + \text{T7+RNA} \quad (4)$$

$$\text{RNA} + \text{F/Q} \xrightarrow{K_4} \text{F} + \text{waste} \quad (5)$$

Equation (1) and (4) are simplified RNA polymerase catalytic reactions with the kinetic rate K_1. Equation (2) is a displacement reaction with the kinetic rate K_2. Equation (3) is a DNA polymerase extension reaction with the kinetic rate K_3, and K_4 is strand displacement reaction for Equation (5). Based on the fluorescence results, K_1, K_2, K_3, and K_4 were fitted to 4.995×10+2 L·unit-1·s-1, 1.578×10-5 M-1·s-1, 9.616×10-3 L·unit-1·s-1, and 3.316×10-4 M-1·s-1, respectively.

S9.3 Python code for calculating reaction rate constant

```
import numpy as np
import matplotlib.pyplot as plt
import pandas as pd
from scipy.optimize import leastsq
'''
To load the fluroscence data
'''
df = pd.read_csv('data.csv')
data = np.asarray(df.iloc[:]).T
'''
mapping the fluroscence data# to import experimental data
'''
factor = 0.4e-6 / max_fluo
data = data * factor
'''
get value and dimension
```

```
'''
y = data[3]
l = data.shape[1]
'''
set the initial values
corresponding to the concentration: Taq, T7, template1, template2, R1, FQ, F
the unit: M/L, U/L
'''
init = [2.5e+4, 2e+6, 10e-9, 0, 0, 0.4e-6, 0]
def differ(kp1, kp2, k2, init, time):

    template1 = np.linspace(0, 0, time)
    template2 = np.linspace(0, 0, time)
    RNA = np.linspace(0, 0, time)
    FQ = np.linspace(0, 0, time)
    F = np.linspace(0, 0, time)

    p1, p2, template1[0], template2[0], RNA [0], FQ [0], F [0] = init

    for i in range(0, time-1):
        v1 = kp1 * template1[i] * p1    # taq rate
        v2 = kp2 * template2[i] * p2       #t7 rate
        v3 = k2 * RNA[i] * FQ [i]    #F/Q rate
        template1[i+1] = template1[i] - v1
        template2[i+1] = template2[i] + v1
        RNA[i+1] = RNA[i] + v2 - v3
        FQ [i+1] = FQ [i] - v3
        F[i+1] = F[i] + v3
    return F
p0 = [1e-8, 1e-6, 1e+6]
def funcerror(p, y):
     return y - differ(p[0], p[1], p[2], init, l)
p = leastsq(funcerror, p0, args=(y))[0]
print(p)
```

S10: DNA Sequences

Table S1. Sequences of the Oligonucleotides (5’ to 3’)

Name	Sequence (5′→3′)	Length (n.t.)
T1	TTTTAATACGACTCACTATAGGGAGACACTAA TGAACTACTACTAC	46
R-1	GGGAGACACUAAUGAACUACUACUAC	26
I1	GTAGTAGTAGTTCATTAGTGTCTCCC	26
NF	GGGAGACACTAATGAACTA	19
F	FAM-GGGAGACACTAATGAACTAG	20
Q	GTAGTAGTAGTTCATTAGTGTCTCCC-BHQ	26
A	CAATCAACGTTCGCGCCAATGTACAGTATTG	31
B	GCGCGAACGTTGATTGGCGGAGACCC	26
B*	GGGTCTCCGCCAATCAACGTTCGCGC	26
A1	CAATCAACGTTCGCGCCAATGTACAGTATTGG	32
B1	CGCGAACGTTGATTGGCGGAGACCC	25
A2	CAATCAACGTTCGCGCCAATGTACAGTATTGGC	33
B2	GCGAACGTTGATTGGCGGAGACCC	24
A3	CAATCAACGTTCGCGCCAATGTACAGTATTGGCG	34
B3	CGAACGTTGATTGGCGGAGACCC	23
A-msi	TCAACCTACCGGTGCACAATGTACAGTATTG	31
B-msi	TGCACCGGTAGGTTGA	16
T2	CGATCAGCAGATCTCGTAATACGACTCACTATAG GGTCTCCGCCAATCAACGTTCGCGC	59
R2	GGGUCUCCGCCAAUCAACGUUCGCGC	26

D2	GCGCGAACGTTGATTGGCGGAGACCCTATAGTGAGTCG	38
I2	TATTACGAGATCTGCTGATCG	21
T3	AAGCAAGGGTAAGATGGAATGAAATTAACCCTCACTAAAGGCGAGCGTAAGTCAATTCCACTATCATTGCTGCAAGC	77
D3	GCTTGCAGCAATGATAGTGGAATTGACTTACGCTCGCCTTTAGTGAGGG	49
I3	TTAATTTCATTCCATCTTACCCTTGCTTCAATCCGT	36