Supporting Information

DNA walker triggered isothermal amplification method based on freezing construction of AuNP probes and its application in ricin detection

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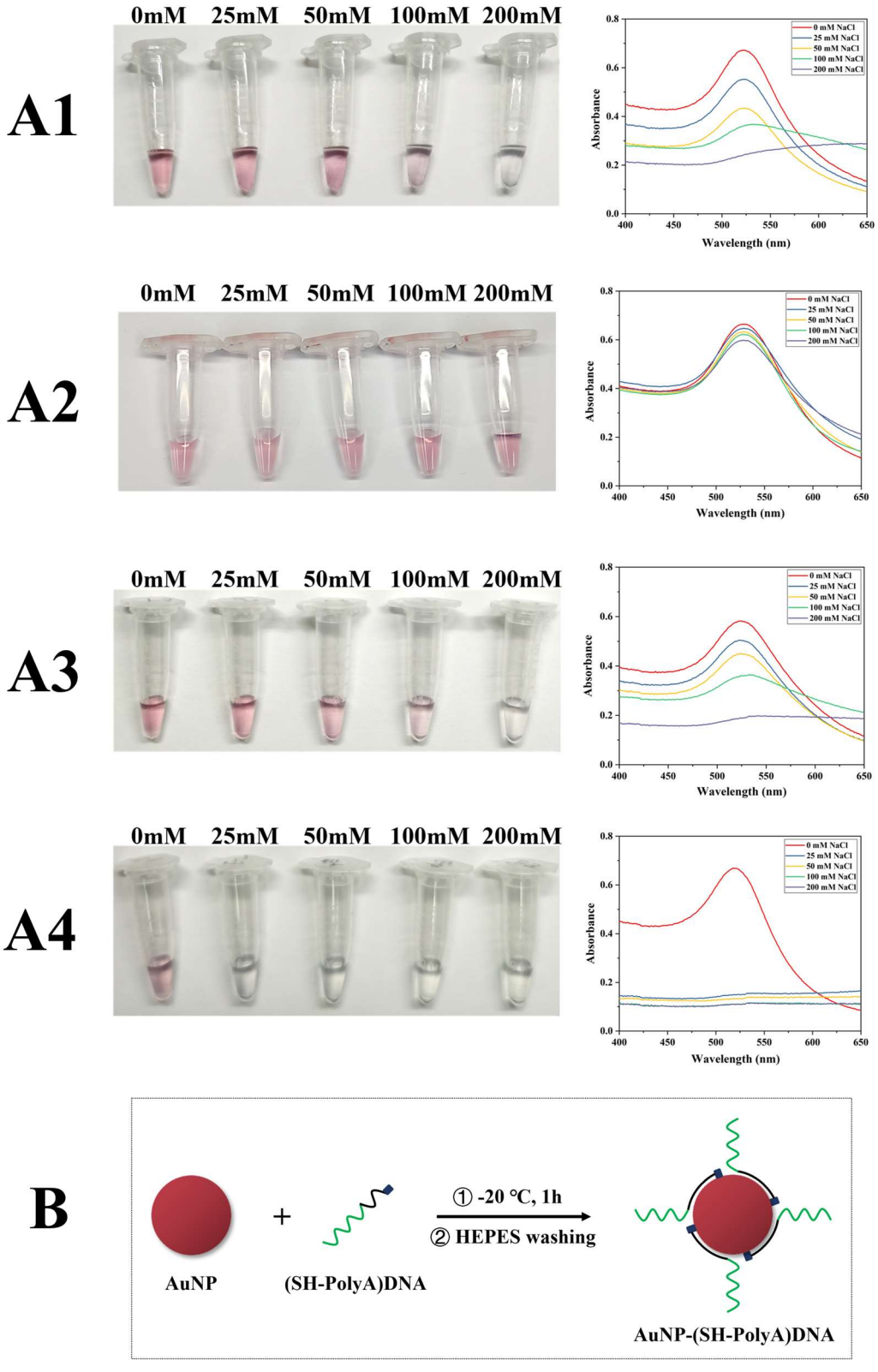

Figure S1. Stability investigation and the synthesis diagram of AuNP-DNA probes. (A1) The photograph and UV–Vis spectroscopy of the stability of AuNP-(SH)DNA after freezing construction process with the addition of different NaCl concentrations. (A2)

The photograph and UV–Vis spectroscopy of the stability of AuNP-(SH-PolyA)DNA after freezing construction process with the addition of different NaCl concentrations. (A3) The photograph and UV–Vis spectroscopy of the stability of AuNP-(PolyA)DNA after freezing construction process with the addition of different NaCl concentrations. (A4) The photograph and UV–Vis spectroscopy of the stability of AuNP-(no SH or PolyA)DNA after freezing construction process with the addition of different NaCl concentrations. The concentrations of NaCl are: 0 mM, 25 mM, 50 mM, 100 mM, 200 mM. (B) Schematic diagram of synthesizing AuNP-DNA probe with AuNP and (SH-PolyA)DNA.

The photographs at the left shows that almost no change in color only for AuNP-(SH-PolyA)DNA group can be observed with the increase of NaCl concentration (Figure S1 A2). Meanwhile, the UV–Vis spectroscopy on the right panel displays that in the presence of 0 to 200 mM NaCl, except for AuNP-(SH-PolyA)DNA group (Figure S1 A2), the absorbance peak varies greatly. Therefore, as shown in Figure S1 B, we chose (SH-PolyA) DNA and AuNP freezing construction for AuNP-DNA probe.

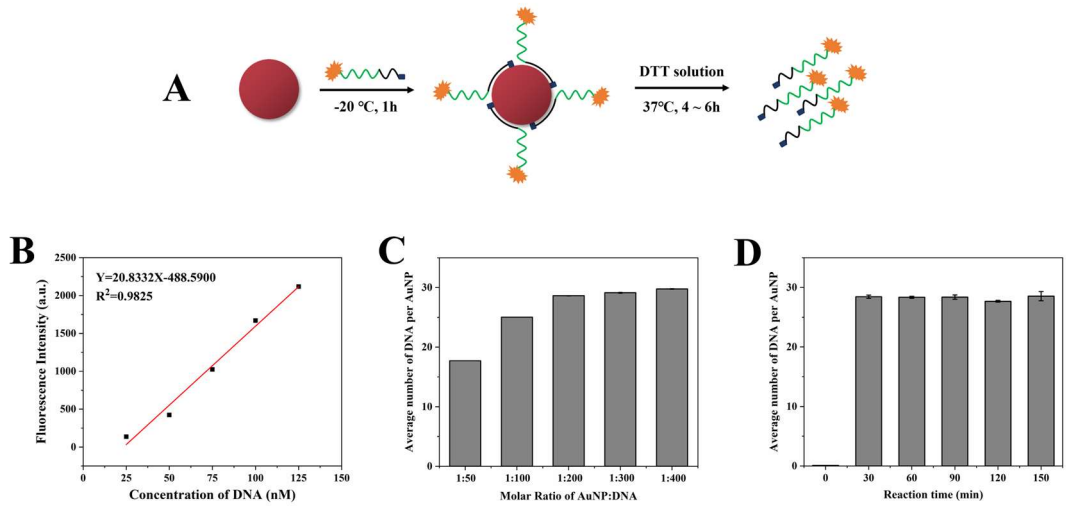

Figure S2. Optimization of the synthesis conditions of AuNP-DNA probe. (A) Schematic diagram of the acquisition of fluorescent DNA in AuNP-DNA probe. (B) Standard curve between the fluorescence intensity and the DNA concentration (25-125 nM) [Y=20.8332X-488.5900 (R^2=0.9825)]. (C) Optimization of the molar ratio of AuNP:DNA. The average number of connecting DNA per AuNP increases gradually until the ratio reaches 1:200, so the 1:200 is chosen for AuNP:DNA in the following experiment. (D) Optimization of freezing time. The average number of connecting DNA per AuNP reaches the maximum at 30 min. But the freezing effect (-20°C) is related to the performance of the refrigerator, sample size, and sample placement position, we choose the freezing time of 60 min to ensure that the sample is completely frozen.

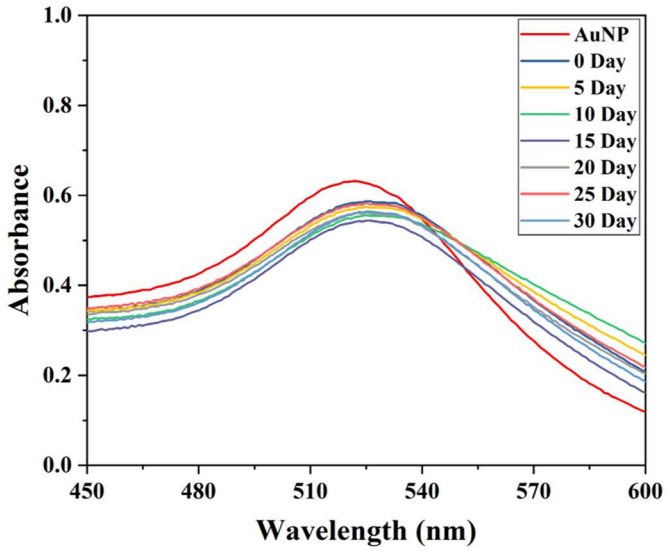

Figure S3. The UV–Vis spectroscopy of the stability of AuNP-DNA probe.

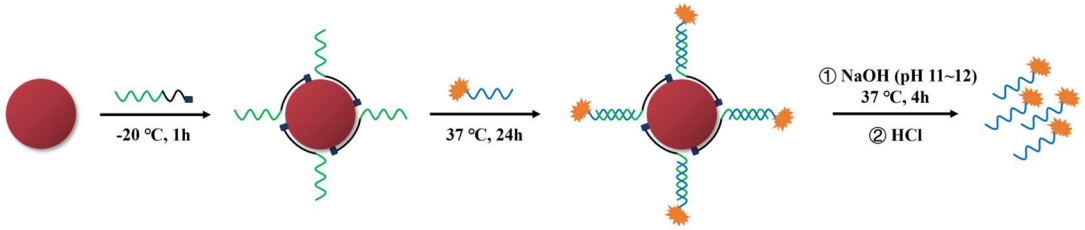

Figure S4. Schematic diagram of the acquisition of complementary fluorescent DNA in AuNP-DNA probe.

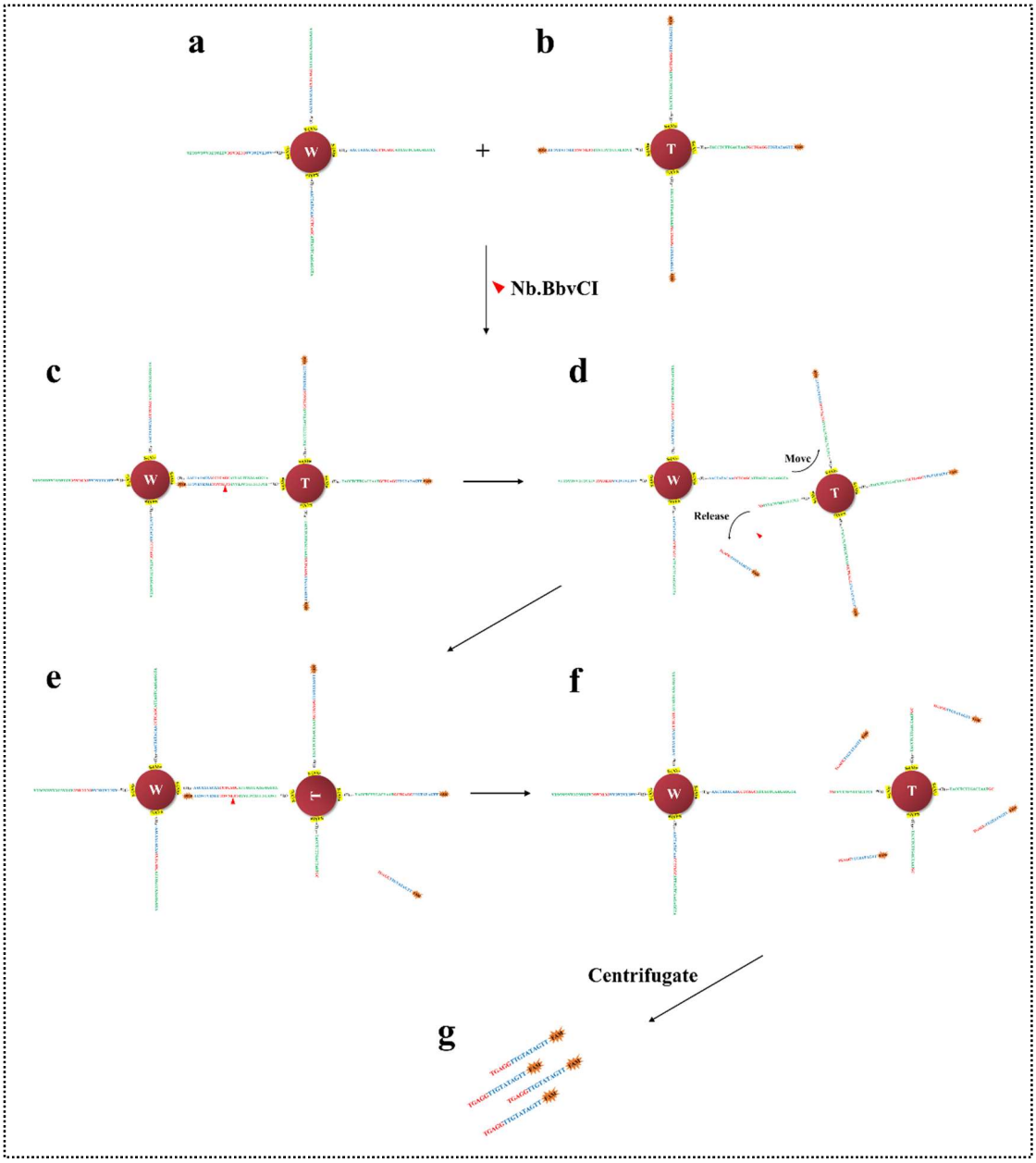

Figure S5. Schematic diagram of the DNA Walker reaction. AW (AuNP-Walker probe) recognizes and cleaves the DNA (FAM) on the AT (AuNP-Track probe). After the relative motion of AT, the remaining DNA track strands are also cleaved by the AW in turn, producing a large amount of ssDNA (FAM).

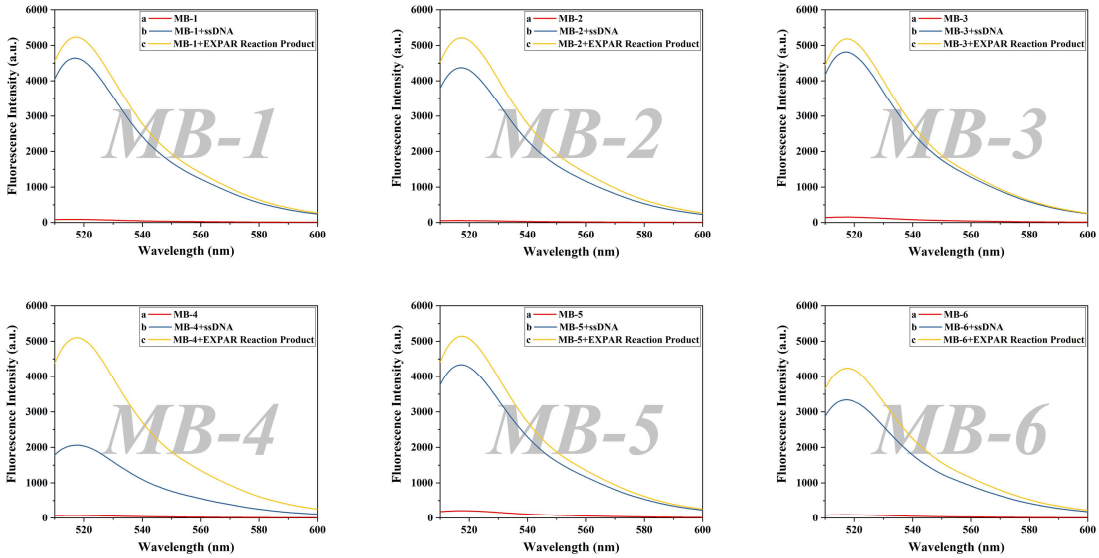

Figure S6. Fluorescence curve of EXPAR reaction with 6 MBs. Group a, MB; Group b, MB is mixed with ssDNA (1:1); Group c, MB is mixed with the product of EXPAR.

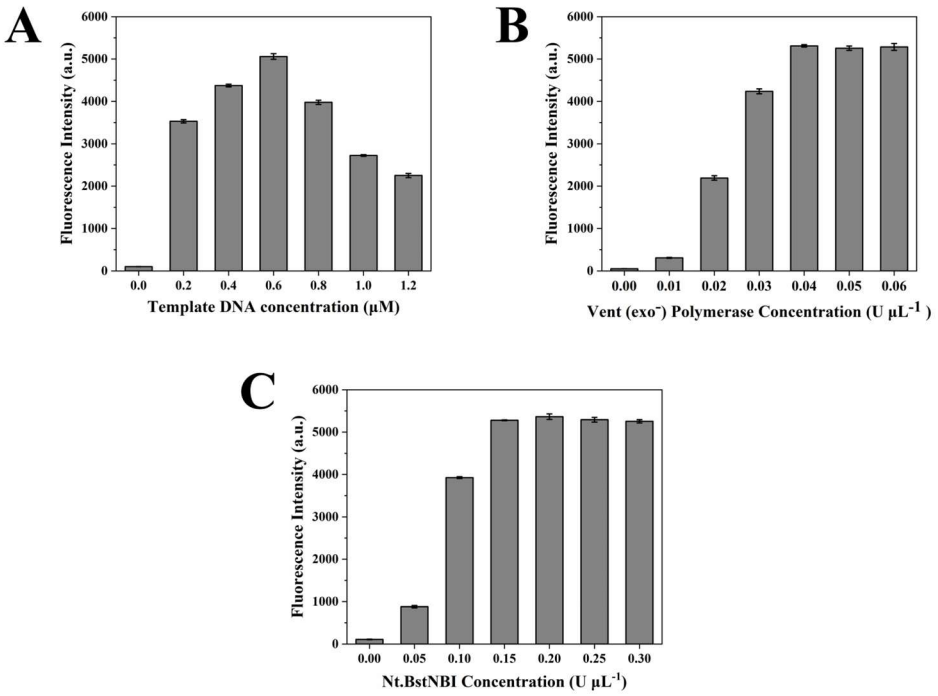

Figure S7. Condition optimization of EXPAR-MB method. (A) Optimization of the Template DNA concentration. The fluorescence intensity reaches a maximum at 0.6 μM, so the 0.6 μM Template DNA is selected in the following experiment. (B) Optimization of the Vent (exo$^-$) DNA polymerase concentration. As the concentration of vent (exo$^-$) DNA polymerase increases, the fluorescence intensity increases gradually to 0.04 U μL^{-1}. Thus, 0.04 U μL^{-1} Vent (exo$^-$) DNA polymerase is chosen for this experiment. (C) Optimization of the Nt.BstNBI concentration. The produced fluorescence signals increase with increasing concentration of Nt.BstNBI until the concentration reaches 0.15 U μL^{-1}. Hence, we select 0.15 U μL^{-1} Nt.BstNBI in the subsequent research.

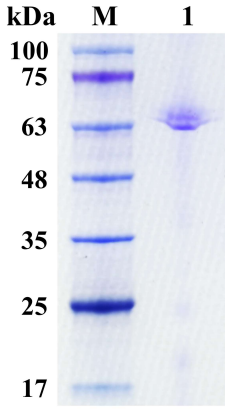

Figure S8. Native-PAGE analysis of the ricin. Lane M, protein marker; lane 1, ricin (~64kDa).

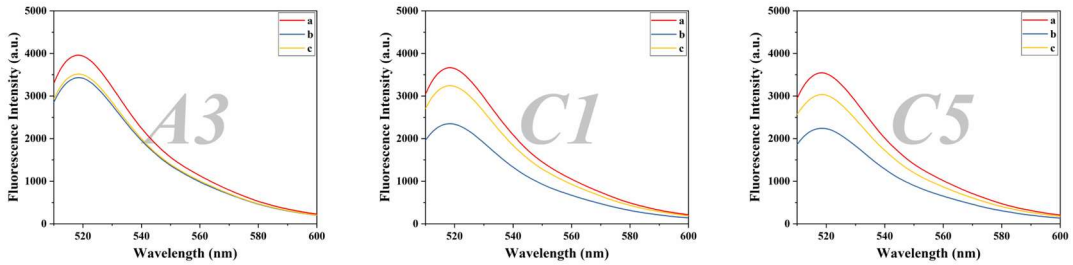

Figure S9. Fluorescence spectra of 3 aptamers (A3, C1, and C5) under different conditions. Curve a, only aptamer(FAM); Curve b, the supernatant after mixing and centrifuging solutions aptamer(FAM) and AW; Curve c, the supernatant after mixing and centrifuging solutions aptamer(FAM), AW, and ricin.

Table S1 Hybridization efficiency of different type of AuNP-DNA probes

Type	Connecting DNA		Complementary DNA		Hybridization efficiency (%) [c]
	Concentration (nM)	Average number [a]	Concentration (nM)	Average number [b]	
AuNP-(SH)DNA	107.37	27	58.52	15	55.50
AuNP-(SH-PolyA)DNA	116.40	**30**	91.56	**23**	**78.66**
AuNP-(PolyA)DNA	82.74	21	56.94	14	68.82

[a] The average number of connecting DNA per AuNP can be calculated by dividing the concentration of connecting DNA by the concentration of AuNP.

[b] The average number of complementary DNA per AuNP can be calculated by dividing the concentration of complementary DNA by the concentration of AuNP.

[c] The hybridization efficiency (%) was calculated by dividing the concentration of complementary DNA by the concentration of connecting DNA.

Table S2. Application of the method for ricin detection in practical samples

Sample	Original Value (pM)	Add (pM)	Found (pM)	Recovery (%)	RSD (%)
Spring Water	Not found	10^4	0.9887×10^4	98.87	2.87
	Not found	10^3	1.0296×10^3	102.96	1.20
	Not found	10^2	1.0339×10^2	103.39	3.19
Skim Milk	Not found	10^4	0.9684×10^4	96.84	0.68
	Not found	10^3	1.0136×10^3	101.36	4.21
	Not found	10^2	0.9821×10^2	98.21	2.93
Apple Juice	Not found	10^4	0.9962×10^4	99.62	1.18
	Not found	10^3	1.0423×10^3	104.23	3.76
	Not found	10^2	0.9759×10^2	97.59	2.38

Table S3. Sequences of synthesized DNA

DNA name	Sequence (5' to 3') description
(SH)DNA	(SH) AACTATACAACCTCAGCATTAGTCAAGAGGTA
(SH-PolyA)DNA/ Walker DNA	(SH)A_{20}AACTATACAACCTCAGCATTAGTCAAGAGGTA
(PolyA)DNA	A_{20}AACTATACAACCTCAGCATTAGTCAAGAGGTA
DNA	AACTATACAACCTCAGCATTAGTCAAGAGGTA
Track DNA	(SH)A_{20}TACCTCTTGACTAATGCTGAGGTTGTATAGTT
Template	AACTATACAACCTCAAACAGACTCAAACTATACAACCTCAA
ssDNA	TGAGGTTGTATAGTT