Supporting information

Ultrasensitive detection of 17 β - estradiol (E2) based on multi-step isothermal amplification

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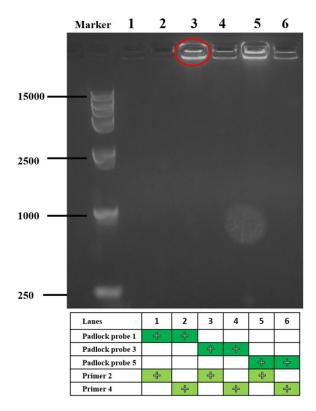


Figure S1. The Punnett method was used to selected padlock probes and primer by analyze the looping efficiency of different padlock probes and primer probes and the amount of products generated by the rolling circle amplification of the circular DNA. The sequences in each lane was shown in the table below the picture.

A crossover experiment was designed to verify the efficiency of different padlock probes to construct circ-DNA under different primer probes. Padlock and primer probe sequences were screened by the amount of product generated after RCA of circ-DNA. After the electrophoresis, the long single-stranded DNA (LssDNA) generated by RCA would be jammed in the spotting port of the polyacrylamide gel. As showed in Figure S1, the padlock probe 3 and primer probe 2 groups produced the most products. So in this experiment, the P3 and P2 were selected as the padlock probe and primer probe

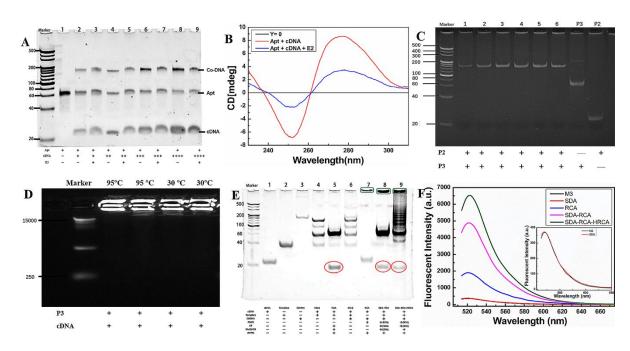


Figure S2. The result of the feasibility verification of the scheme. The E2 aptamer and cDNA were hybridized at room temperature, while E2 could bind to its aptamer and causes the release of the cDNA. (Lanes1: Apt, Lanes2: Apt(1 μ M)+cDNA (1 μ M), Lanes3: Apt(1 μ M)+cDNA (1 μ M)+cDNA (1 μ M)+cDNA (1 μ M)+cDNA (2 μ M), Lanes5: Apt(1 μ M)+cDNA (2 μ M) +E2(100ng/mL), Lanes6: Apt(1 μ M)+cDNA (3 μ M), Lanes7: Apt(1 μ M)+cDNA (3 μ M) +E2(100ng/mL), Lanes8: Apt(1 μ M)+cDNA (4 μ M), Lanes7: Apt(1 μ M)+cDNA (3 μ M) +E2(100ng/mL), Lanes8: Apt(1 μ M)+cDNA (4 μ M), Lanes7: Apt(1 μ M)+cDNA (4 μ M) +E2(100ng/mL).) This phenomenon were characterized by the electrophoretic characterization(A) and circular dichroism(B), and the looping effect of the selected padlock probe and primer probe was verified though electrophoretic (C), and compare the effect of triggering RCA reaction under different temperature (D), electrophoresis characterization of the feasibility of multi-step isothermal expansion (E), comparison of fluorescence results between single-step isothermal expansion (F).

	Sum of		Mean	F	p-value			Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F		Source	Squares	df	Square	Value	Prob > F	
Model	1.815E+007	9	2.017E+006	43.27	< 0.0001	significant	Model	1.446E+007	9	1.607E+006	212.97	< 0.0001	significa
A-ND	2.661E+006	1	2.661E+006	57.09	0.0001		A-Phi29	5.041E+006	1	5.041E+006	668.00	< 0.0001	
B-KF	1.168E+006	1	1.168E+006	25.06	0.0016		B-Nb	9.451E+005	1	9.451E+005	125.24	< 0.0001	
C-dNTPs	1.211E+006	1	1.211E+006	25.97	0.0014		C-dNTPs	8.544E+005	1	8.544E+005	113.22	< 0.0001	
AB	3.146E+005	1	3.146E+005	6.75	0.0355		AB	7.239E+005	1	7.239E+005	95.93	< 0.0001	
AC	45562.61	1	45562.61	0.98	0.3557		AC	4.972E+005	1	4.972E+005	65.88	< 0.0001	
BC	1.276E+006	1	1.276E+006	27.37	0.0012		BC	2.303E+005	1	2.303E+005	30.52	0.0009	
A ²	5.669E+006	1	5.669E+006	121.62	< 0.0001		A	4.374E+006	1	4.374E+006	579.66	< 0.0001	
B ²	2.881E+006	1	2.881E+006	61.81	0.0001		B ²	8.605E+005	1	8.605E+005	114.03	< 0.0001	
C2	1.801E+006	1	1.801E+006	38.63	0.0004		C2	4.586E+005	1	4.586E+005	60.77	0.0001	
Residual	3.263E+005	7	46610.40				Residual	52824.00	7	7546.29			
Lack of Fit	2.035E+005	3	67845.63	2.21	0.2293	not significant	Lack of Fit	22042.93	3	7347.64	0.95	0.4948	not significa
Pure Error	1.227E+005	4	30683.97				Pure Error	30781.07	4	7695.27			
Cor Total	1.848E+007	16					Cor Total	1.452E+007	16				
						г	Constraints						
Constraints						L	Constraints						
		Lower	Upper	Lower	Upper				Lower	Upper	Lower	Upper	
Name	Goal	Limit	Limit	Weight	Weight	Importance	Name	Goal	Limit	Limit	Weight	Weight	Importanc
A:Nb	is in range	0.2	2	1	1	3	A:Phi29	is in range	0.2	2	1	1	
B:KF	is in range	0.4	4	1	1	3	B:Nb	is in range	0.2	6	1	1	
C:dNTPs	is in range	0.2	2	1	1	3	C:dNTPs	is equal to 2.11	0.2	4	1	1	
fluoresent inte	maximize	786.07	10000	1	1	3	Fluorescent Inf	maximize	2667.85	10088.8	1	1	
Solutions							Solutions						
Number	Nb	KF	dNTPs flu	oresent in	Desirability		Number	Phi29	Nb	dNTPs Fl	orescentl	Desirability	
		3.04	1.57	3781.23	0.325	Selected	1	1.57	4.91	2.10	5276.95	0.352	Selecte
1	1.40												

Figure S3. The dose of different reagents in the SDA and RCA-HRCA was optimized by the response surface methodology. The fluorescence value of each group in the response surface experiment was analyzed by analysis of

variance (ANOVA) (A-B). And the recommended dosage of reagents in the experiment(C-D)

As shown in Figure S3, the amount of the dose of different reagents in the SDA and RCA-HRCA was optimized by the response surface methodology. The results of the optimization experiment were significantly different between groups by analysis of variance (ANOVA) (P<0.01), indicating that the differences between different groups were statistically significant (Figure S 3A-B). The fluorescence value of SDA (A) and RCA-HRCA(B) was analyzed by analysis of variance (ANOVA) , the P values of Both them are less than 0.01, indicating that the differences between different groups are statistically significant. According to the results of the response surface methodology, the recommended dosage of different reagents in the SDA(C) and RCA-HRCA was given(D).

strand	Sequence
Padlock probe(P3)	5'-Phosphate-ATTGAATTACACCTCAGCCCCTACCATTATTAATAGACTG
	CCTCAGCCACCATCACCTTTGCTATTTAACCTCAGCGCTTCCAGCTT-3'
Primer(P2)	5'-TGTAATTCAATAAGCTGGAAGC-3'
Aptamer	5'-GCTTCCAGCTTATTGAATTACACGCAGAGGGTAGCGGCTCTGCGCAT
	TCAATTGCTGCGCGCTGAAGCGCGGAAGC-Biotin-3'
cDNA	5'-AAAATTTAAAATGTAATTCAATAAGCTGGAAGC-3'
Template	5'-GCTTCCAGCTTATTGAATTACACCTCAGCTTCCAGCTTATTGAATTA
	CA-3'
Molecular beacon(M3)	5'-6-FAM-ATGACTACACCATCACCTTTGCTATTTAATAGTCAT-BHQ1-3'
The random ssDNA	5'-GGGTGGTCAGGTGGGATAGCGTTCCGCGTATGGCCCA-Biotin-3'
The cDNA of random ssDNA	5'-CCTGACCACCCAAAAAAAAA3'

Table S1. Oligonucleotide sequences of linear ssDNA

Table S2. Oligonucleotide sequence of padlock probe and primer probe

strand	Sequence
Padlock probe 1(P1)	ATTGAATTACACCTCAGCGCTTCCAGCTTATTGAATTACACCTCAG CGCTTCCAGCTTATTGAATTACACCTCAGCGCTTCCAGCTT
Primer 2(P2)	TGTAATTCAATAAGCTGGAAGC
Padlock probe 3(P3)	ATTGAATTACACCTCAGCCCCTACCATTATTAATAGACTGCCTC AGCCACCATCACCTTTGCTATTTAACCTCAGCGCTTCCAGCTT
Primer 4(P4)	CGAAGGTCGAATAACTTAATGT
Padlock probe 5(P5)	GAATTACACCTCAGCCCCTACCATTATTAATAGACTGCCTCAGCCA CCATCACCTTTGCTATTTAACCTCAGCGCTTCCAGCTTATT