Supporting Information for

FEN1 assisted DNA logic amplifier circuit for fast and compact DNA computing

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MATERIALS AND METHODS

DNA sequence design

All the DNA strands were synthesized by Invitrogen Corporation (Shanghai, China). All the DNA strands were dissolved in water as stock solution and quantified using Nanodrop 2000. The sequences of all strands are listed in Supplementary Table S1.

DNA logic gates preparation

The simple DNA logic gates ("AND", "OR" and "INHIBIT" logic gates) and cascade DNA logic gates ("Fan-in" and "Fan-out" and series-mode circuits) were performed on a Rotor Gene Q48 real-time fluorescent PCR reaction platform with a 20 μL reaction. The reaction system consists of 10 mM Tris-HCl (pH 8.0), 0.05% (v/v) Nonidet P40, 0.05% (v/v) Tween 20, 7.5 mM $MgCl_2$, 50 nM-250 nM fuel chain, 250 nM FRET probe, 6.7 ng/μL FEN1 (prepared in our lab according to previous study)[1] nucleic acid endonuclease and 0.05-0.5 nM input chain (detailed concentrations are listed in Table S1, S2, and S3). As reported[2], the Km of FEN1 was 10-14 μM, thus, the saturation substrate concentration of FEN1 should be more than 1 mM.

All DNA strands required for the construction of the logic gate (including input strands, fuel strands, and output signal probes), FEN1 enzyme, and the necessary buffer for the enzyme reaction are mixed in a reaction tube, and then placed into the qPCR instrument. Initially, a pre-denaturation step is performed at 95°C for 3 minutes to denature all DNA strands. Subsequently, the logic gate operation is conducted at 63°C, and the output fluorescence signals are collected using the qPCR instrument.

Supplementary Tables

Table S1. Sequences of all strands used in AND, OR and INHIBIT gates

DNA logic circuit	Name	Sequence (5'-3')	Conc. (nM)
AND	A*	GAAAGTTAAAATTCCCGTCGCTATCAT	0.5
	AB	**GCTTTCGGAGATGTCT**TGATAGCGACGGGAA TTTTAACTTTC	0.5
	C*B*	ACGGACGCGGAG**AGACATCTCCGAAAGC**	250
	Reporter	VIC-TCTT(BHQ1) AGCCGGTTTTCCGG CTAAGACTCCGCGTCCGT-C6-NH2	250
OR	AB	**GCTTTCGGAGATGTCT**TGATAGCGACGGGAA TTTTAACTTTC	0.5
	AD	*GTTGGCTTTCTGTTCCT*TGATAGCGACGGGAAT TTTAACTTTC	0.5
	A*	GAAAGTTAAAATTCCCGTCGCTATCAT	50
	C*B*	ACGGACGCGGAG**AGACATCTCCGAAAGC**	250
	C*D*	ACGGACGCGGAG*AGGAACAGAAAGCCAAC*	250
	Reporter	VIC-TCTT(BHQ1) AGCCGGTTTTCCGG CTAAGACTCCGCGTCCGT-C6-NH2	250
INHIBIT	AB	**GCTTTCGGAGATGTCT**TGATAGCGACGGGAA TTTTAACTTTC	0.05
	SAD	*GTTGCTTCTCTTAATTCCT*TGATAGCGACGGGAA TTTTAACTTTCTCACCT	0.5
	S*A*	AGGTGAGAAAGTTAAAATTCCCGTCGCTATCA T	0.05
	C*B*	ACGGACGCGGAG**AGACATCTCCGAAAGC**	250
	Reporter	VIC-TCTT(BHQ1) AGCCGGTTTTCCGG CTAAGACTCCGCGTCCGT-C6-NH2	250

The sequences of A and A*are underlined, the sequences of B and B*are bold and the sequences of D and D*are indicated in italics in the table S1.

Table S2. Sequences of all strands used in AND-OR, OR-AND, Fan-in and Fan-out circuits.

DNA logic circuit	Name	Sequence (5'-3')	Conc. (nM)
AND-OR	A*	GAAAGTTAAAATTCCCGTCGCTATCAT	0.5
	AB	**GGCTTTCGGAGATGTCT**TGATAGCGACGGGAATTTTAACTTTC	0.5
	C*	ACGGACGCGGAG	0.5
	C*B*	ACGGACGCGGAG**AGACATCTCCGAAAGCC**	250
	Reporter	VIC-TCTT(BHQ1)AGCCGGTTTTCCGG CTAAGACTCCGCGTCCGT-C6-NH2	250
OR-AND	AB	**GCTTTCGGAGATGTCT**TGATAGCGACGGGAATTTTAACTTTC	0.5
	AD	*GTTGGCTTTCTGTTCCT*TGATAGCGACGGGAATTTTAACTTTC	0.5
	CE	TGTTGCTTCTCTTAATTCCTCTCCGCGTCCGT	0.5
	A*	GAAAGTTAAAATTCCCGTCGCTATCAT	50
	C*B*	ACGGACGCGGAG**AGACATCTCCGAAAGC**	250
	C*D*	ACGGACGCGGAGAG*GAACAGAAAGCCAAC*	250
	F*E*	CGCGAGGCCGAGGAATTAAGAGAAGCAACA	250
	Reporter	VIC-TCTT(BHQ1)AGCCGGTTTTCCGG CTAAGACTCCGCGTCCGT-C6-NH2	250
Fan-in	AB	**GCTTTCGGAGATGTCT**TGATAGCGACGGGAATTTTAACTTTC	0.5
	AD	*GTTGGCTTTCTGTTCCT*TGATAGCGACGGGAATTTTAACTTTC	0.5
	AE	GTTGGCTTTCGATTCCTTGATAGCGACGGGAATTTTAACTTTC	0.5

	AF	CCAGCTTGTGGAGGTGATAGCGACGGGAATTTTAACTTTC	0.5
	A*	GAAAGTTAAAATTCCCGTCGCTATCAT	50
	C*B*	ACGGACGCGGAG**AGACATCTCCGAAAGC**	250
	C*D*	ACGGACGCGGAG*AGGAACAGAAAGCCAAC*	250
	C*E*	ACGGACGCGGAGAGGAATCGAAAGCCAAC	250
	C*F*	ACGGACGCGGAGCCTCCACAAGCTGG	250
	Reporter	VIC-TCTT(BHQ1)AGCCGGTTTTCCGGCTAAGACTCCGCGTCCGT-C6-NH2	250
Fan-out	AB	**GCTTTCGGAGATGTCT**TGATAGCGACGGGAATTTTAACTTTC	0.5
	AD	*GTTGGCTTTCTGTTCCT*TGATAGCGACGGGAATTTTAACTTTC	0.5
	A*	GAAAGTTAAAATTCCCGTCGCTATCAT	50
	C*B*	ACGGACGCGGAG**AGACATCTCCGAAAGC**	250
	C*D*	ACGGACGCGGAG*AGGAACAGAAAGCCAAC*	250
	Reporter -1	FAM-TCTT(BHQ1)AGCCGGTTTTCCGGCTAAGACTCCGCGTCCGT-C6-NH2	250
	Reporter-2	VIC-TCTT(BHQ1)AGCCGGTTTTCCGGCTAAGACTCCGCGTCCGT-C6-NH2	250

The sequences of A and A*are underlined, the sequences of B and B*are bold, the sequences of D and D*are indicated in italics, the sequences of E and E*are highlighted in gray and the sequences of F and F*are indicated by black boxes in the table S2.

Table S3. Sequences of all strands used in square-root circuits

DNA logic circuit	Name	Sequence (5'-3')	Conc. (nM)
Square-root circuit	X_1	**GCTTTCGGAGATGTCT**TGATAGCGACGGGAA TTTTAACTTTC	0.05
	X_2	*GTTGGCTTTCTGTTCCT*TGATAGCGACGGGAAT TTTAACTTTC	0.05
	X_3	TGTTGCTTCTCTTAATTCCTTGATAGCGACGGG AATTTTAACTTTCTCACCT	0.5
	X_4	TGAGGGCTGAGGTGACCCTTGTCTCTGTGTTCT TGTCCCCCAGCTTGTGGAGGGGTGAGAAAGTT AAAATTCCCGTCGCTATCAAGGAATTAAGAGA AGCAACA	0.05
	G*	GACAAGAACACAGAGACAAGGGTT	0.5
	S*A*	AGGTGAGAAAGTTAAAATTCCCGTCGCTATCA	0.05
	C*B*	ACGGACGCGGAG**AGACATCTCCGAAAGC**	250
	C*D*	ACGGACGCGGAG*AGGAACAGAAAGCCAAC*	250
	O*E*	CGCGAGGCCGAGGAATTAAGAGAAGCAACA	250
	C*F*	ACGGACGCGGAGCCTCCACAAGCTGG	250
	O*H*	CGCGAGGCCGCACCTCAGCCCTCA	250
	Reporter	FAM-TCTT-(BHQ1)-AGCCGGTTTTCCGGCTAA GACGGCCTCGCG-C6-NH2	250
	Reporter	VIC-TCTT-(BHQ1)-AGCCGGTTTTCCGGCTAA GACTCCGCGTCC GT-C6-NH2	250

The sequences of A and A*are underlined, the sequences of B and B*are bold, the sequences of D and D*are indicated in italics, the sequences of E and E*are highlighted in gray and the sequences of F and F*are indicated by black boxes and the sequences of E and E*are indicated by double-underline in the table S3. The square-root circuit contains an INHIBIT gate, in which

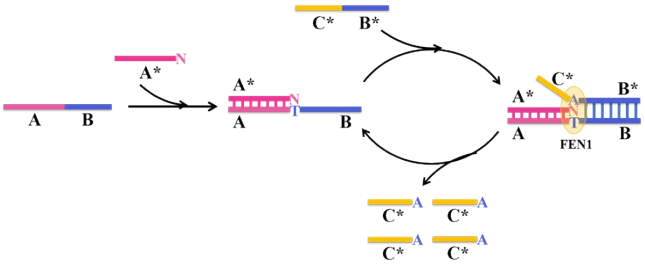
the input X_4 need to inhibit input X_3 from producing output. So, the sequences of input X_4 need contains complimentary sequences of input X_3. Besides, the input X_4 also needs to hybridize to the fuel strands of C*F*, G*, O*H* to produce output Y_1 and Y_2. Therefore, the input X_4 contains six domains respectively hybridized to SAE, F*, G*, H*.

Table S4. The response parameters of reported DNA open-root circuits

Reported work	**Completion time**	**Number of participated strands**	**Concentration of input DNA**
Qian et al.(2011)[3]	~480 min	130	100 nM
Song et al.(2019)[4]	~40 min	37	100 nM
Wang et al.(2020)[5]	~20 min	24	100 nM
Xing et al.(2022)[6]	~800 min	9	150 nM
Current work	~6 min	13	0.5 nM

Supplementary Figures

Figure S1. The principle of FEN1 catalyzed reaction



The rate of the reaction is dependent on not only the reaction temperature close to the Tm of B*B strands, but also the concentrations of the FEN1 and C*B* strands.

Figure S2. Fluorescence curves of FEN1-catalyzed reactions for logic gate under different temperatures

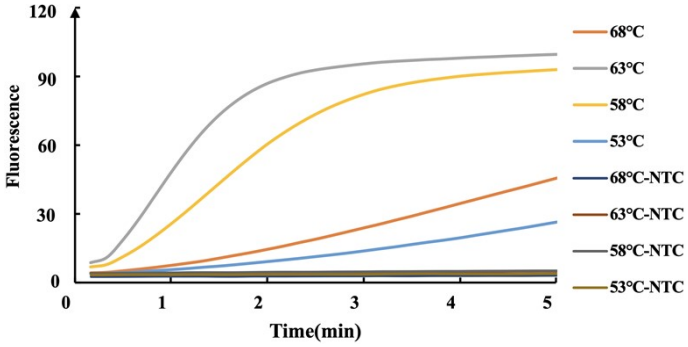


Figure S3. Fluorescence curves of FEN1-catalyzed reactions for logic gate under different concentrations of the FEN1

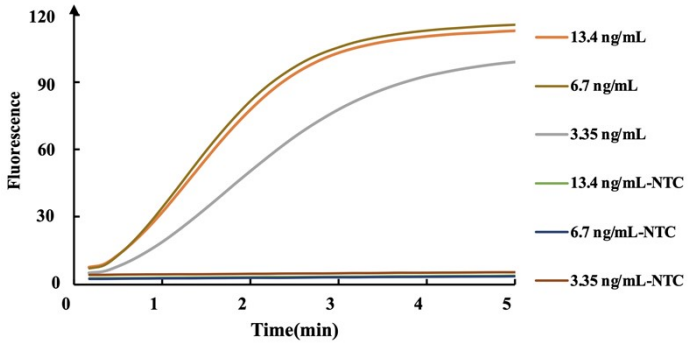


Figure S4. Fluorescence curves of FEN1-catalyzed reactions for logic gate under different concentrations of the C*B* strands

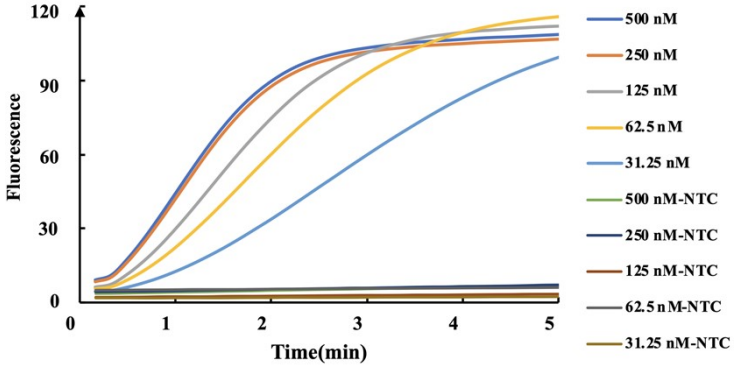
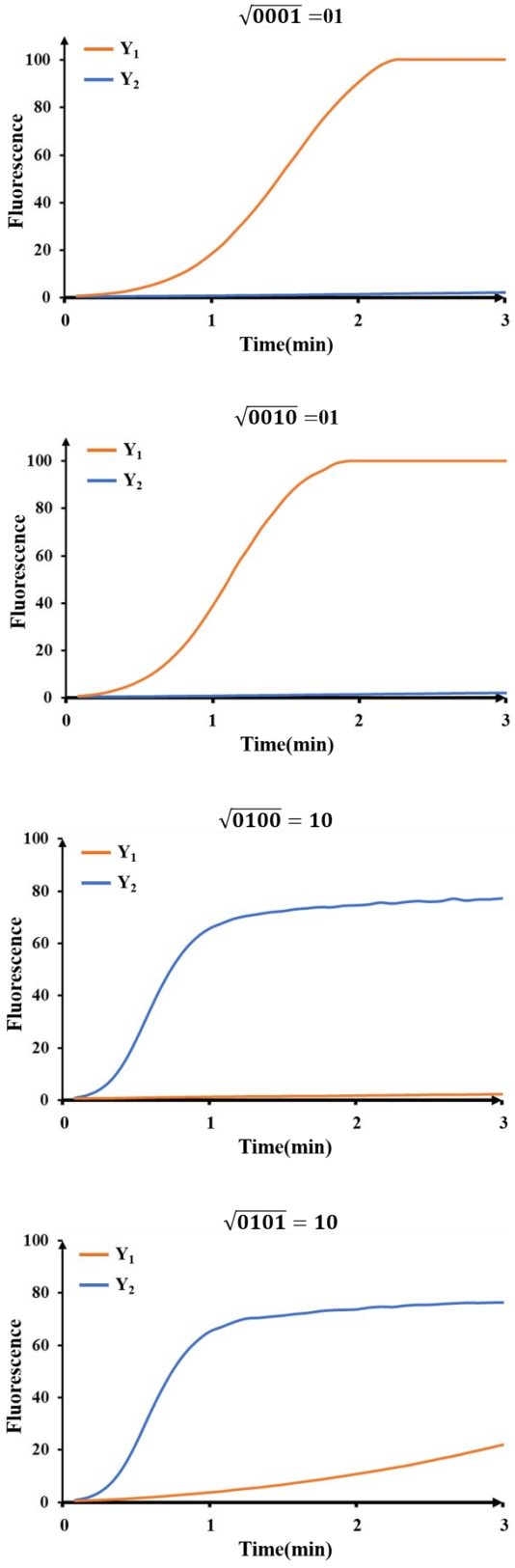
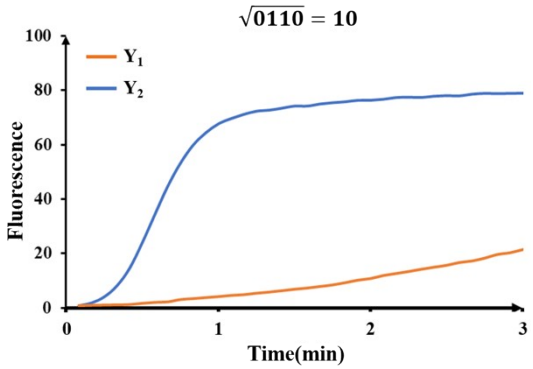


Figure S5. Experimental results for all four-bit inputs with the square-root circuit.

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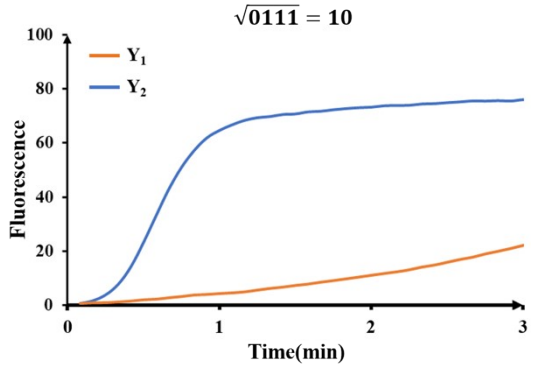
Y1
Y2
Fluorescence
Time(min)
0
20
40
60
80
100
0
1
2
3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Y2
Fluorescence
Time(min)
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80
100
0
1
2
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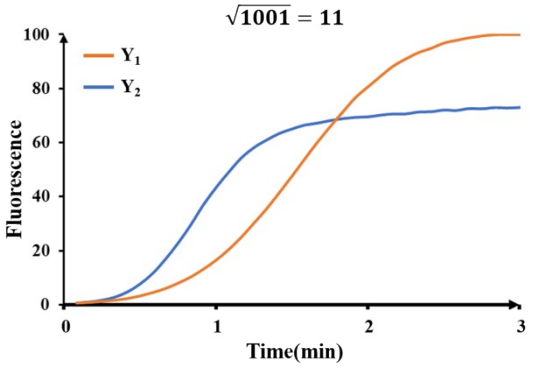
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Y1
Y2
Fluorescence
Time(min)
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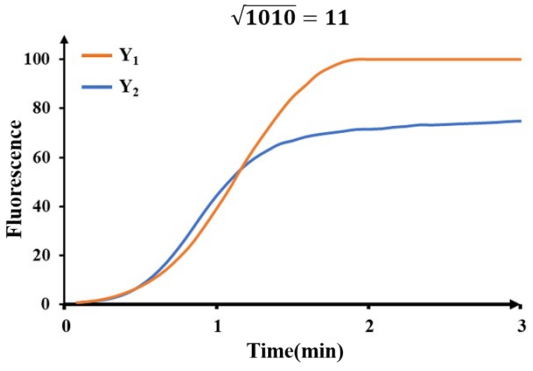
20
40
60
80
100
0
1
2
3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Y1
Y2
Fluorescence
Time(min)
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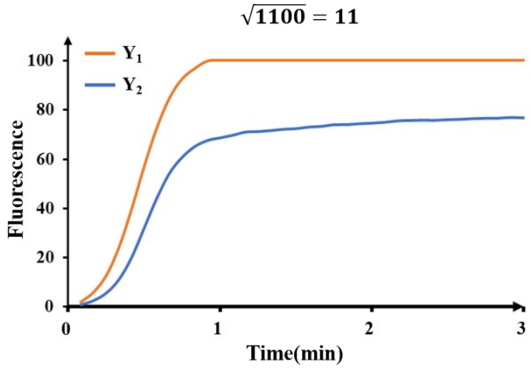
60
80
100
0
1
2
3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Y1
Y2
Fluorescence
Time(min)
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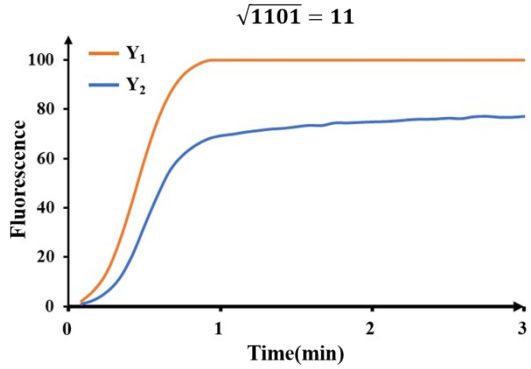
20
40
60
80
100
0
1
2
3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Y1
Y2
Fluorescence
Time(min)
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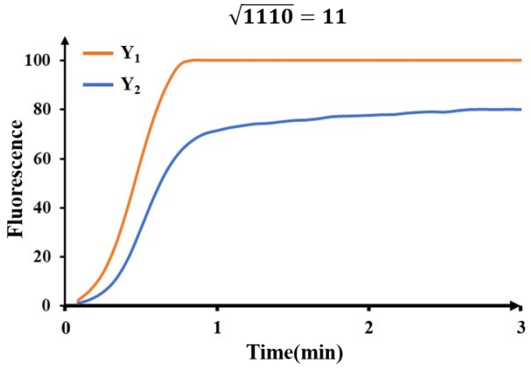
20
40
60
80
100
0
1
2
3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Y1
Y2
Fluorescence
Time(min)
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20
40
60
80
100
0
1
2
3

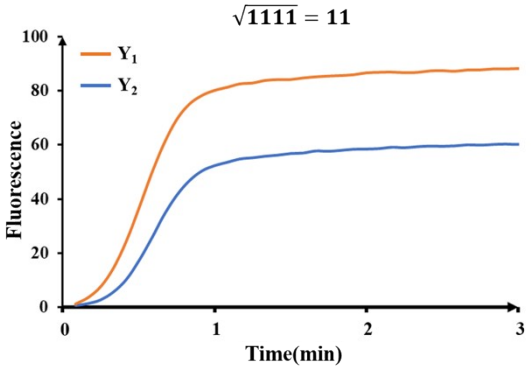
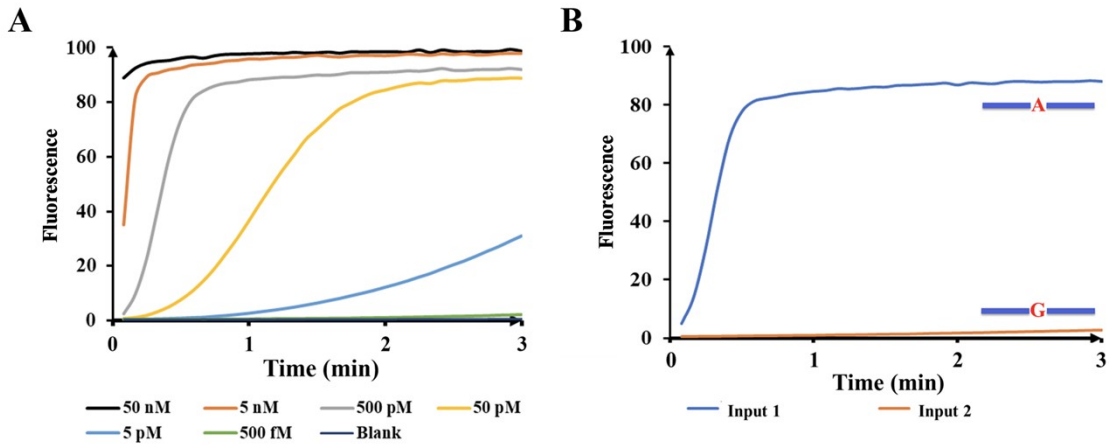

√1111 = 11
Y1
Y2
Fluorescence
Time(min)
0
20
40
60
80
100
0
1
2
3

Figure S6. Concentration and specificity of input DNA for logic gate based on FEN1-catalyzed reactions



(A) Responses of OR logic gates to different concentrations of input DNA. (B) Responses of OR logic gates to single base difference in the input DNA.

Supplementary References

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