

A novel H-shape adaptor-mediated isothermal exponential amplification reaction (HS-EXPAR) to identify target nucleic acid

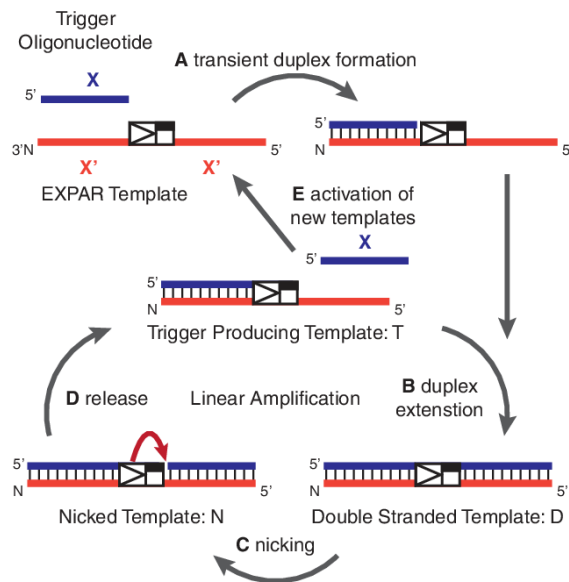


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Introduction: Exponential amplification reaction (EXPAR)



Purpose

Amplification of short nucleic acids

materials

EXPAR template, polymerase, nicking enzyme

Mechanism

Repeated extension and generation of trigger strands

Advantage

Isothermal amplification without thermal cycling

Limitation

Limited to short nucleic acids with extensible 3'-OH

➔ **Application of EXPAR technology to detect long target nucleic acids**

Qian, Jifeng et al. Nucleic Acids Research 40 (2012): e87 - e87.

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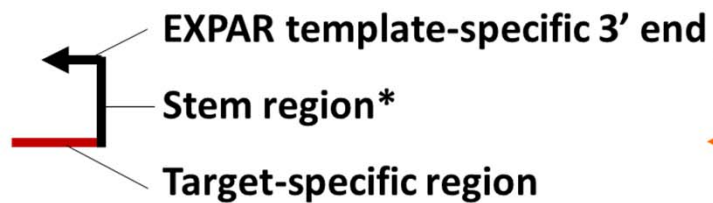


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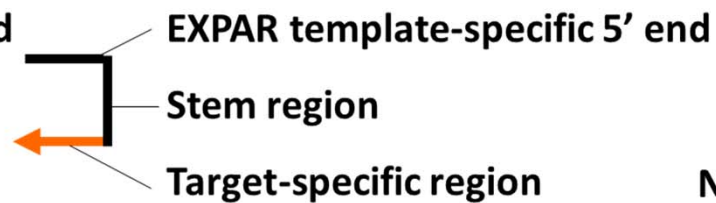
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(a) Without target DNA



AD1



AD2

Trigger-complementary sequence

Nt.BstNBI recognition sequence

EXPAR template



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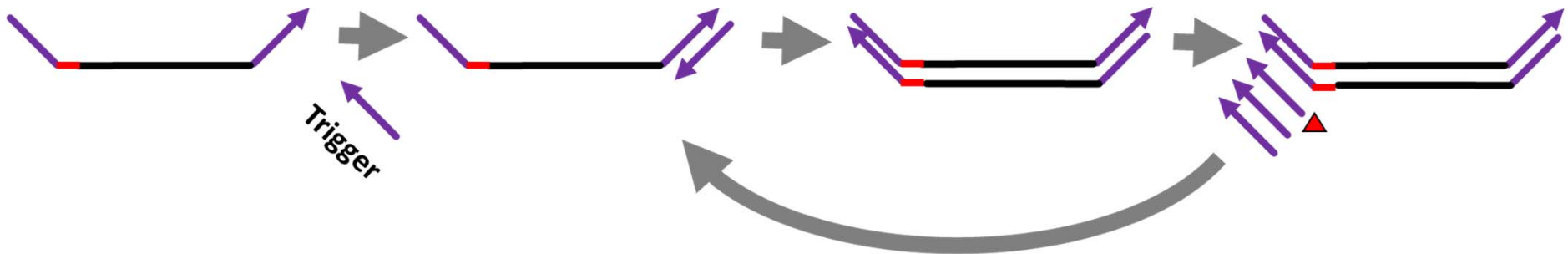


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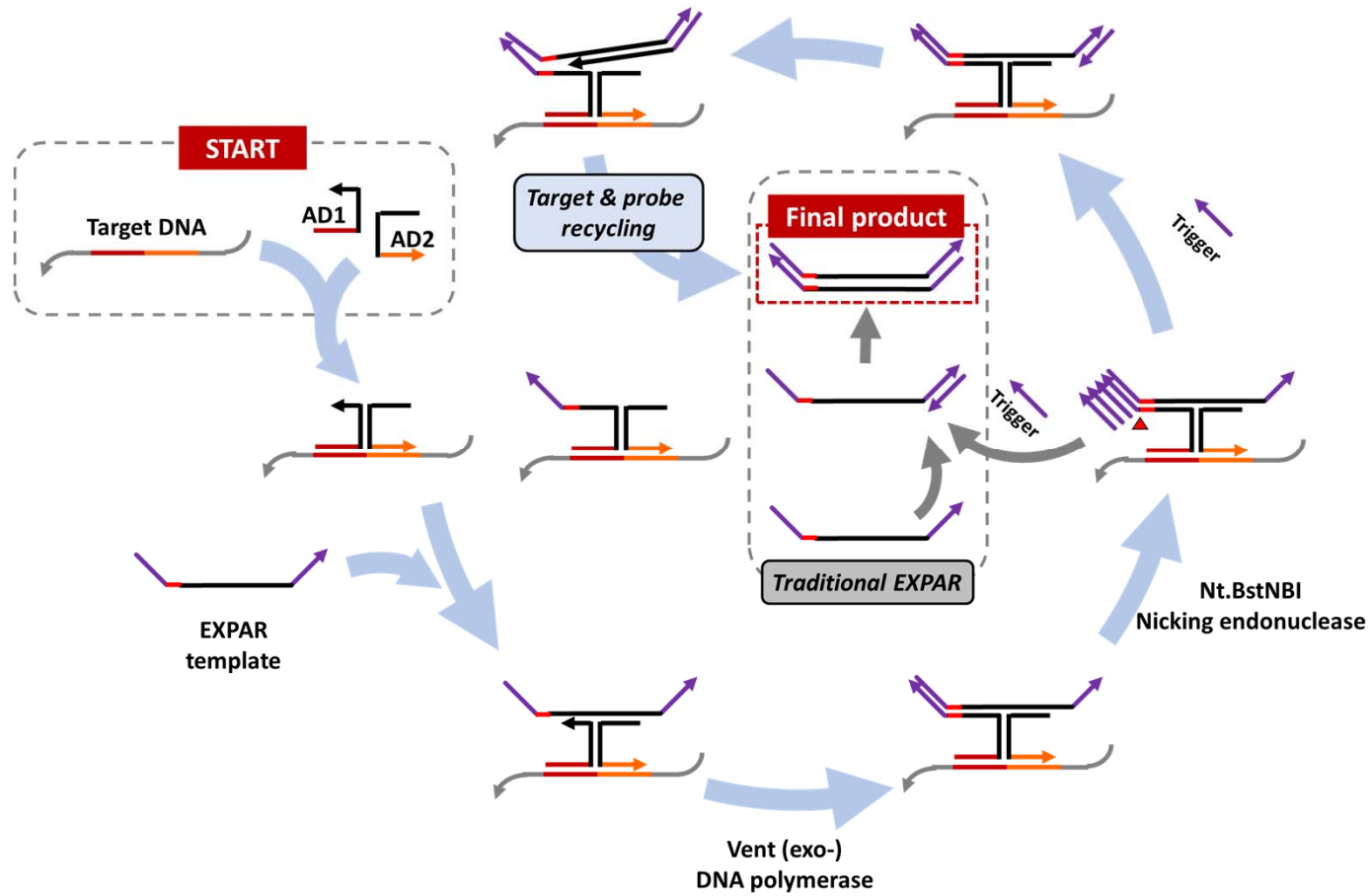
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(b) Traditional EXPAR



(c) With target DNA



➡ **Dual three way junction structure enhances target specificity**

➡ **HS-EXPAR could reliably detect long target DNA**

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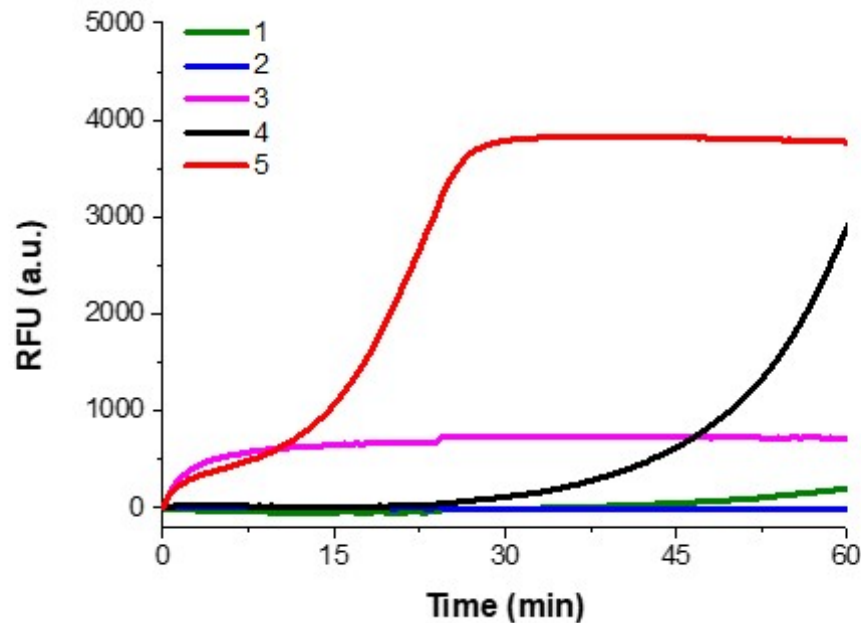


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(A) Component feasibility test



1: EXPAR template (EXT)

2: AD1+AD2

3: AD1+AD2+Target

4: AD1+AD2+EXT

5: AD1+AD2+EXT+Target

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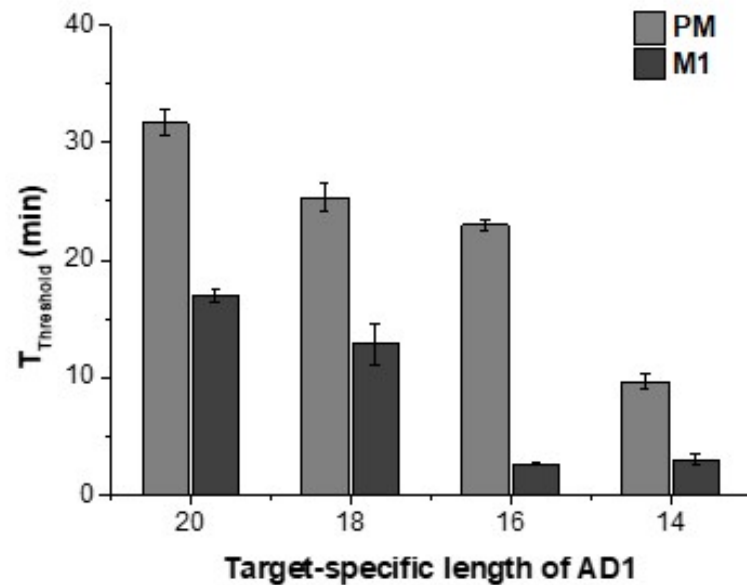


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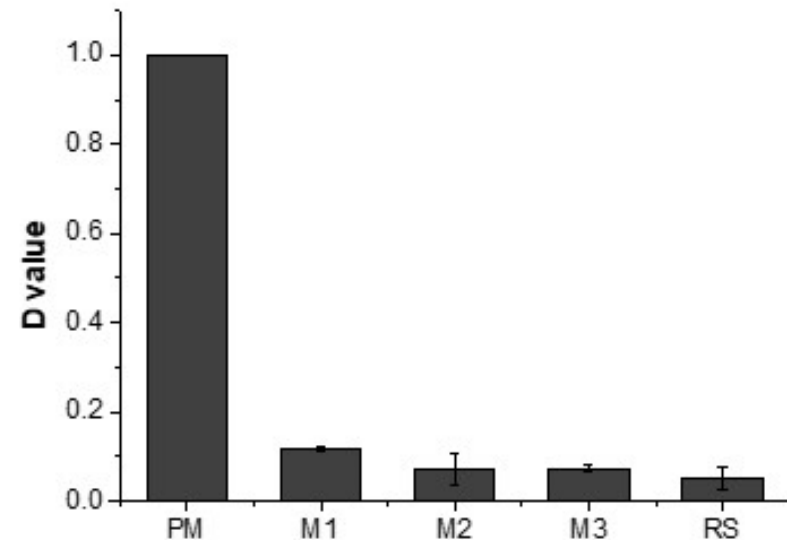
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(B) Target-specific length dependence of the reaction



(C) Discriminating capability for mismatched target sequences



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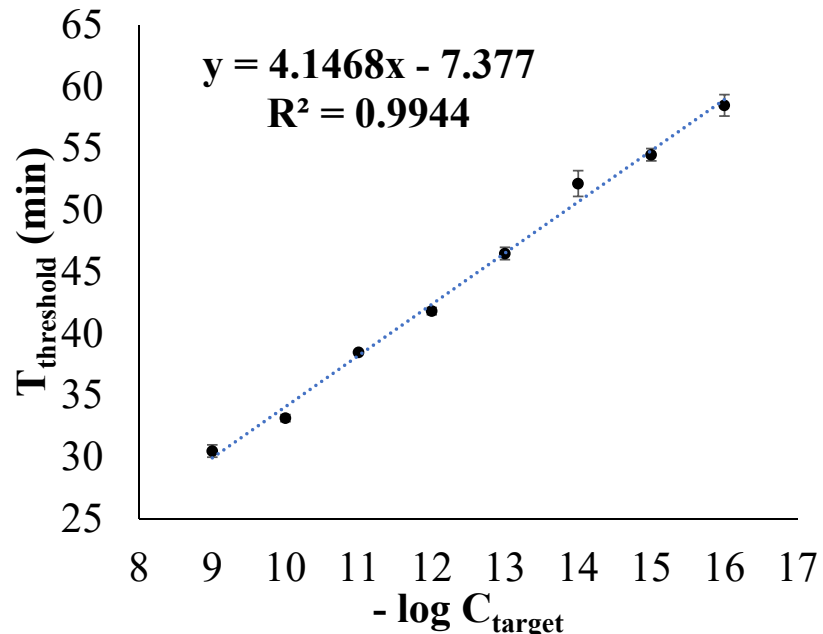


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(D) Sensitivity test



The limit of detection (LOD) ($3\sigma/\text{slope}$) was estimated to be 63 aM, which is quite comparable or even better than those from previously reported isothermal signal amplification methods.