Supporting Information

Innate Reverse Transcriptase Activity of DNA Polymerase for Isothermal RNA Direct Detection

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Materials and methods:

Materials

All nucleic acids listed in **Supplementary Table S1** were designed by using NUPACK software (http://www.nupack.org/) and synthesized by Shanghai Sangon Bio-Engineering Company (Shanghai, China). All enzymes used in this work were purchased from New England Biolabs. RNA pure reagent kit for rapid extraction of ultrapure RNA was obtained from Biomed (Beijing, China). 20-bp DNA ladder, and the chemicals used to prepare electrophoresis were purchased from Dalian Takara Company (China).

Methods

RNA Extraction

The total RNA of *E. coli* K-12 was extracted using RNA pure reagent kit for rapid extraction of ultrapure RNA following the manufacturer's protocol. RNA concentration was evaluated using a Cary 50 spectrophotometer (Varian Australia Pty Ltd., Melbourne, Australia). RNA integrity was verified using agarose gel electrophoresis without denaturing conditions by ethidium bromide staining (data not shown).

Reverse Transcription Reaction and Denaturing PAGE

In Figure 1, the reverse transcription reaction in 5 μL contained 2.5×10^{-6} M HCV RNA, 2.5×10^{-6} M primer, 0.7 U Vent exo$^-$, 0.7 U *Bst* LF or 1.9 U AMV, and 1.5×10^{-4} M dNTPs. RNA was first heated at 70℃ for 5 min, and cooled on ice (5 min) before reverse transcription. Then the reaction was incubated in a CFX96™ Real-Time PCR system (Bio-Rad) for 5 min at 30℃, 55 min at the optimum reaction temperature of each enzyme for the reverse transcription reaction. Subsequently, 0.2 μL RNase H was added, and incubated for 40 min at 37℃. At last, the reaction was heated at 80℃ for 20 min to inactivate enzymes for the next denaturing PAGE.

17.5% denaturing PAGE with 7 M urea was carried out using tris-acetate-EDTA (TAE) buffer (pH 8.0) at 138 V constant voltages for 55 min. After ethidium bromide staining, gels were scanned using the Champ Gel Image Analysis System (Beijing Page Creation Science Company, Beijing, China).

Real-time RT-PCR and Native PAGE

In Figure 2 and Figure 3, the reverse transcription reaction in 10 μL contained 5.0×10^{-8} M RNA including purified total RNA or synthesized RNA, 5.0×10^{-8} M primer, 0.5 U tested DNA polymerases or 1.5 U AMV,

and 1.5×10^{-4} M dNTPs. The reverse transcription reaction was carried out under the same conditions as Figure 1.

Real-time PCR

In Figure 2a and Figure 3a-c, real-time PCR was performed on a CFX96™ Real-Time PCR detection system (Bio-Rad) at 1 min intervals. The 10 μL PCR reaction system consisted of the diluted 500-fold reverse transcription product, 2.0×10^{-7} M forward primer and reverse primer, 0.25 U *Taq* DNA polymerase, 2.0×10^{-4} M dNTPs, 1×*Taq* Buffer, and 0.5×SYBR Green I. The reactions were incubated at 95℃ for 5 min, followed by 30 cycles of 95℃for 30 s, 62℃for 30 s and 72℃ for 30 s. All reactions were run in triplicate.

Native PAGE

In Figure 2b and Figure 3d, 17.5% native PAGE was carried out under the same conditions as denaturing PAGE except for 7 M urea.

Supplementary Table

Supplementary Table S1. Sequences of nucleic acids used in this work

Nucleic acids	Sequences（5′-3′）
16S rRNA of *E. coli* K-12	AAUUGAAGAGUUUGAUCAUGGCUCAGAUUGAACGCUGGC
（[a]J01859.1 [b]2-263）	GGCAGG**CCUAACACAUGCAAGUCGAA**CGGUAACAGGAA
	ACAGCUUGCUGUUUCGCUGACGAGUGGCGGACGGGUGAG
	UAAUGUCUGGGAAACUGCCUGAUGGAGGGGGAUAACUAC
	UGGAAACGGUAGCUAAUACCGCAUAACGUCGCAAGACCA
	AAGAGGGGGACCCUCGGGCCUCUUGCCAUCGGAUGUGCC
	CAGAUGGGAUUAGCUUGUUGGUGGGGUAA
Primers for 16S rRNA	
FP-16S	AATTGAAGAGTTTGATCATGGC
RP1-16S	TTCGACTTGCATGTGTTAGG
RP2-16S	CAGACATTACTCACCCGTCC
RP3-16S	TTACCCCACCAACAAGCTAA
sequence of Hepatitis C Virus	GUGGUACUGCCUGAUAGGGUGCUUGCGAGUGCCCCGGGA
(HCV)	GGUCUCGUAGA
（[a]D10749.1 [b]283-332）	
Primers for HCV	
FP-HCV	CTGTGTACTGCCTGATAG
RP-HCV	AGTGATCTACGAGACCTC

[a] GenBank accession number [b] The position of specific sequence in genomic DNA

The dotted line in sequence of *E. coli* K-12 represented the corresponding RNA sequence of FP-16S. The bold portion was the complementary sequence of RP1-16S. The boxed portion represented the complementary sequence of RP2-16S. The underlined portion was the complementary sequence of RP3-16S; In sequence of HCV, the dotted line represented the corresponding RNA sequence of FP-HCV. The underlined portion was the complementary sequence of RP-HCV. FP and RP indicated the forward primer and reverse primer in real time PCR, respectively.

Supplementary Table S2. All enzymes used in this work

Enzyme Name	Abbreviation
AMV Reverse Transcriptase	AMV
DNA polymerase I (*E. coli*)	DNA Poly I
DNA polymerase I, large (Klenow) fragment	Klenow LF
Klenow fragment (3'→5' exo$^-$)	Klenow exo$^-$
Bst DNA polymerase, large fragment	*Bst* LF
Bst 2.0 DNA polymerase	*Bst* 2.0
Bst 2.0 WarmStart DNA polymerase	WS *Bst* 2.0
Vent (exo$^-$) DNA polymerase	Vent exo$^-$
Taq DNA polymerase	*Taq*