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One enzyme reverse transcription qPCR using Taq DNA polymerase

Sanchita Bhadra[1], Andre Maranhao[1], Andrew Ellington[1]

[1]The University of Texas at Austin

1 Works for me This protocol is published without a DOI.

Inyup Paik

ABSTRACT

Taq DNA polymerase, one of the first thermostable DNA polymerases to be discovered, has been typecast as a DNA-dependent DNA polymerase commonly employed for PCR. However, Taq polymerase belongs to the same DNA polymerase superfamily as the Molony murine leukemia virus reverse transcriptase and has in the past been shown to possess reverse transcriptase activity. We report optimized buffer and salt compositions that promote the reverse transcriptase activity of Taq DNA polymerase, and thereby allow it to be used as the sole enzyme in TaqMan RT-qPCR reactions. We demonstrate the utility of Taq-alone RT-qPCR reactions by executing CDC SARS-CoV-2 N1, N2, and N3 TaqMan RT-qPCR assays that could detect as few as 2 copies/µL of input viral genomic RNA.

EXTERNAL LINK

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ATTACHMENTS

2020.05.27.120238v1.full.pdf

PROTOCOL CITATION

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KEYWORDS

Taq, RT-qPCR, One enzyme RT-qPCR, SARS-CoV-2

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MATERIALS TEXT

Chemicals and reagents

All chemicals were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated. All enzymes and related buffers were purchased from New England Biolabs (NEB, Ipswich, MA, USA), Thermo Fisher Scientific (Waltham, MA, USA), or Promega (Madison, WI, USA) unless otherwise indicated. All oligonucleotides and TaqMan probes (Table 1) were obtained from Integrated DNA Technologies (IDT, Coralville, IA, USA). SARS-CoV-2 N gene armored RNA was obtained from Asuragen (Austin, TX, USA). SARS-CoV2 viral genomic RNA was obtained from American Type Culture Collection (Manassas, VA, USA).

Table 1. CDC TaqMan RT-qPCR primers and probes for SARS-CoV-2[a] (adapted from https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html).

Name	Description	Sequence[b]
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5′-GACCCCAAAATCAGCGAAAT-3′
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5′-TCTGGTTACTGCCAGTTGAATCTG-3′
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5′-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3′
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5′-TTACAAACATTGGCCGCAAA-3′
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5′-GCGCGACATTCCGAAGAA-3′
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5′-FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1-3′
2019-nCoV_N3-F	2019-nCoV_N3 Forward Primer	5′-GGGAGCCTTGAATACACCAAAA-3′
2019-nCoV_N3-R	2019-nCoV_N3 Reverse Primer	5′-TGTAGCACGATTGCAGCATTG-3′
2019-nCoV_N3-P	2019-nCoV_N3 Probe	5′-FAM-AYCACATTGGCACCCGCAATCCTG-BHQ1-3′
RP-F	RNAse P Forward Primer	5′-AGATTTGGACCTGCGAGCG-3′
RP-R	RNAse P Reverse Primer	5′-GAGCGGCTGTCTCCACAAGT-3′
RP-P	RNAseP Probe	5′-FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ-1-3′

[a]According to CDC, oligonucleotide sequences are subject to future changes as the 2019-Novel Coronavirus evolves. Refer to CDC website for latest updates.

[b]FAM: 6-carboxyfluorescein; BHQ-1: Black Hole Quencher 1

Preparing RT-qPCR reaction 30m

1 RT-qPCR assays were assembled in a total volume of **25 µl** containing the indicated buffer at 1X strength (see **Generation 6A buffer** recipe in step 2)

Optimized buffer recipe

2 In this study, we report optimized buffer and salt compositions that promote the reverse transcriptase activity of Taq DNA polymerase and thereby allow it to be used as the sole enzyme in TaqMan RT-qPCR reactions.

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We recommend to use **Generation 6A** buffer for the one enzyme RT-qPCR.

10X Generation 6A buffer ([M]**600 Milimolar (mM) Tris-HCl** , pH**8.0** , [M]**20 Milimolar (mM)** $(NH_4)_2SO_4$, [M]**400 Milimolar (mM) KCl** , [M]**20 Milimolar (mM) MgCl2**)

Preparing RT-qPCR reaction 30m

15m

3 The buffer was supplemented with [M]**0.4 Milimolar (mM) deoxyribonucleotides (dNTP)** , [M]**402 Nanomolar (nM) each of forward and reverse PCR primer pairs** , [M]**102 Nanomolar (nM) of the TaqMan probe** , and **2.5 units of Taq DNA polymerase** from indicated commercial vendors.

4 Indicated copies of SARS-CoV-2 viral genomic RNA, SARS-CoV-2 N gene armored RNA, or RNaseP armored RNA prepared in **TE buffer** ([M]**10 Milimolar (mM) Tris-HCl** , pH**7.5** , [M]**0.1 Milimolar (mM) EDTA** , pH**8.0**) immediately prior to use were added to RT-qPCR reactions containing corresponding PCR primers. Negative control reactions did not receive any specific templates. 15m

Running RT-qPCR 2h

5 Amplicon accumulation was measured in real-time by incubating the reactions in a LightCycler96 qPCR machine (Roche, Basel, Switzerland) programmed to hold **60 °C** for **00:30:00** followed by **95 °C** for **00:10:00** prior to undergoing **55 cycles** of **95 °C** for **00:00:15** and **60 °C** for **00:00:30** . TaqMan probe fluorescence was measured during the amplification step (60 °C for 30 sec) of each cycle using the FAM channel. 2h