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First-Strand cDNA Synthesis and Conventional Polymerase Chain Reaction (PCR)

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Manuscript citation:

Persistence of Yellow fever virus outside the Amazon Basin, causing epidemics in Southeast Brazil, from 2016 to 2018.

Rezende IM de*, Sacchetto L,* Mello ÉM de, Alves PA, Iani FC de M, Adelino TÉR, Duarte MM, Cury, ALF, Bernardes AFL, Santos TA, Pereira LS, Dutra MRT, Ramalho DB, Thoisy B, Kroon EG, Trindade GS, Drumond BP
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Jorge, T. A.; Mosimann, A. L. P.; Maron, L. N. A.; Santos, C. N. D. dos (2017). Isolation and characterization of a Brazilian strain of yellow fever virus from an epizootic outbreak in 2009. Acta Tropica, Volume 166, 2017, Pages 114–120, ISSN 0001-706X, <https://doi.org/10.1016/j.actatropica.2016.09.030>.

GoScript™ Reverse Transcription System (Promega, USA), more information available at:

<https://www.promega.com.br/-/media/files/resources/protocols/technical-manuals/101/goscript-reverse-transcription-system-protocol.pdf>

GoTaq® Colorless Master Mix (Promega, USA), more information available at:

<https://www.promega.com.br/-/media/files/resources/protocols/product-information-sheets/g/gotaq-colorless-master-mix.pdf>

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We use this protocol and it's working

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Abstract

For cDNA synthesis and for amplify larger fragments of YFV genome. The PCR target is of the CprM/envelope region of YFV genome described by Jorge and colleagues (2017).



Materials

MATERIALS

- ⊗ Microcentrifuge Tubes
- ⊗ Filter Tips
- ⊗ Thermocycler
- ⊗ GoScript(TM) Reverse Transcriptase, 500 rxn **Promega Catalog #A5004**
- ⊗ Random Primers, 20ug **Promega Catalog #C1181**
- ⊗ Nuclease-Free Water, 1000ml **Promega Catalog #P1199**
- ⊗ PCR tubes
- ⊗ Primer
- ⊗ Micropipettors
- ⊗ GoTaq® Colorless Master Mix **Promega Catalog #M714**

First-Strand cDNA Synthesis

- 1 Close each tube of RNA tightly. Place tubes into a preheated 70°C heat block for 5 minutes. Immediately chill in ice-water for at least 5 minutes. Centrifuge each tube for 10 seconds in a microcentrifuge to collect the condensate and maintain the original volume. Keep the tubes closed and on ice until the reverse transcription reaction mix is added.

- 2 Mix and briefly centrifuge each component before use. Combine the following:

Component	-
Experimental RNA	X μ L
Random Primer (0.5 μ g/reaction)	X μ L
Nuclease-Free Water	X μ L
Final volume	5 μ L

- 3 Prepare the reverse transcription reaction mix by combining the following components of the GoScript™ Reverse Transcription System in a sterile microcentrifuge tube on ice. Prepare sufficient mix to allow 15 μ l for each cDNA synthesis reaction to be performed. Determine the volumes needed for each component and combine them in the order listed. Vortex gently to mix, and keep on ice prior to dispensing into the reaction tubes.

Component	Amount
Nuclease-Free Water (to a final volume of 15 μ l)	X μ l
GoScript™ 5X Reaction Buffer	4.0 μ l
MgCl ₂ (final concentration 1.5–5.0mM)	1.2–4.0 μ
PCR Nucleotide Mix (final concentration 0.5mM each dNTP)	1.0 μ
GoScript™ Reverse Transcriptase	1.0 μ

- 4 Add 15 μ l aliquots of the reverse transcription reaction mix to each reaction tube on ice. Be careful to prevent cross-contamination. Add 5 μ l of RNA and primer mix to each reaction for a final reaction volume of 20 μ l per tube.
- 5 Anneal: Place the tubes in a controlled-temperature heat block equilibrated at 25°C, and incubate for 5 minutes.
- 6 Extend: Incubate the tubes in a controlled-temperature heat block at 42°C for up to one hour. The extension temperature may be optimized between 37°C and 55°C. The



reactions may be maintained frozen for long-term storage.

- 7 Inactivate Reverse Transcriptase: If the experimental goal is to proceed with PCR, the reverse transcriptase must be thermally inactivated prior to amplification. Incubate the reaction tubes in a controlled-temperature heat block at 70°C for 15 minutes.cDNA Quantification Using qPCR

cDNA Quantification Using qPCR

- 8 Thaw the GoTaq® Colorless Master Mix at room temperature. Vortex the Master Mix, then spin it briefly in a microcentrifuge to collect the material at the bottom of the tube.

- 9 Prepare one of the following reaction mixes on ice:

Component	Volume	Final Conc.
GoTaq® Colorless Master Mix, 2X	10 µL	1X
Foward primer, 10µM (Jorge et al, 2017)	0,5 µL	0.1–1.0µM
Reverse primer, 10µM (Jorge et al, 2017)	0,5 µL	0.1–1.0µM
DNA template	5 µL	<250ng
Nuclease-Free Water to	20 µL	NA

- 10 Centrifuge the reactions in a microcentrifuge for 5 seconds.

- 11 Place the reactions in a thermal cycler that has been preheated to 95°C. Perform PCR using your standard parameters.

Step	Cycles	Temperature	Time
Denaturation	1	95°C	2 minutes
Denaturation	35	95°C	30 seconds
Annealing	55°C	30 seconds	
Extension	72°C	1 minute	
Final Extension	1	72°C	10 minutes