Jun 07, 2018

Real-time Reverse Transcription Polymerase Chain Reaction (RT- qPCR)

DOI
dx.doi.org/10.17504/protocols.io.pw8dphw

Izabela M Rezende[1], Lívia Sacchetto

[1]Stanford University School of Medicine

MRCA

Izabela M Rezende
Stanford University School of Medicine

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.pw8dphw

Protocol Citation: Izabela M Rezende, Lívia Sacchetto 2018. Real-time Reverse Transcription Polymerase Chain Reaction (RT-qPCR). **protocols.io https://dx.doi.org/10.17504/protocols.io.pw8dphw**

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
PLoS Neglected Tropical Disease 2018;12: e0006538. doi:10.1371/journal.pntd.0006538
*These authors contribully equally to this work

Domingo, C., Patel, P., Yillah, J., Weidmann, M., Méndez, J. A., Nakouné, E. R., &Niedrig, M. (2012). Advanced Yellow Fever Virus Genome Detection in Point-of-Care Facilities and Reference Laboratories. Journal of Clinical Microbiology, 50(12), 4054–4060. http://doi.org/10.1128/JCM.01799-12

Protocol status: Working
We use this protocol and it's working

Created: May 05, 2018

Last Modified: June 07, 2018

Protocol Integer ID: 11968

Abstract

For investigate the presence of YFV genome by quantitative PCR assays in the hydrolysis probe detection format. The target is the 5′-noncoding region (5′-NC) of the YFV genome (Domingo et al., 2012).

Materials

MATERIALS

- Microcentrifuge Tubes
- Filter Tips
- Nuclease-Free Water, 1000ml **Promega Catalog #**P1199
- GoTaq® Probe 1-StepRT-qPCR System **Promega Catalog #**A6120
- Primer
- Probe
- Micropipettors
- Real time PCR instrument
- PCR tubes (for qPCR)
- PCR plate (for qPCR)
- adhesive plate seal

1 Each sample is tested at least in duplicate, or triplicate.

2 The final reaction volume in this protocol is 20µl.

3 Estimate the appropriate amount of each reagent for each test/sample.

4 Determine the number of reactions to be set up, including negative control from the RNA extraction, for the non-template control and for the positive control reactions.

5 Add 1 or 2 reactions to this number to compensate for pipetting error.

6 Prepare the reaction mix (minus the RNA template) by combining the GoTaq® Probe qPCR Master Mix with dUTP, GoScript™ RT Mix for 1-Step RT-qPCR, primers, hydrolysis probe and Nuclease-Free Water as described below. Vortex briefly to mix.

	Component	Volume	Concentration
	GoTaq® Probe qPCR Master Mix with dUTP*	10 µL	1X
	GoScript™ RT Mix for 1-Step RT-qPCR	0,4 µL	1X
	Forward primer 20X (Domingos et al, 2012)	1,0 µL	200 nM-1µM
	Reverse primer 20X (Domingos et al, 2012)	1,0 µL	200 nM-1µM
	Hydrolysis probe (Domingos et al, 2012)	0,5 µL	100-300nM
	RNA temlate	5,0 µL	10pg-1µg
	Nuclease-Free Water	To final volume of 20 µL	

*The GoTaq® Probe qPCR Master Mix included in the GoTaq® Probe 1-Step RT-qPCR System is formulated with dUTP. When dUTP is incorporated into the amplification products, the amplicons are susceptible to degradation by uracilDNA glycosylase (UNG); this allows you to incorporate UNG into subsequent reactions to control possible carryover contamination.

7 Add the appropriate volume of reaction mix (without the RNA template) to each PCR tube or well of an optical grade PCR plate.

8 Add the RNA template (or water for the no-template control reactions) to the appropriate wells of the reaction plate.

9 Seal the tubes or optical plate; centrifuge briefly to collect the contents of the wells at the bottom. Protect from extended light exposure or elevated temperatures before cycling. The samples are ready for thermal cycling.

10 Run the cycling parameters below:

	Step	Cycles	Temperature	Time
	Reverse transcription	1	45°C	15 minutes
	Reverse transcriptase inactivation and GoTaq® DNA Polymerase activation	1	95°C	2 minutes
	Denaturation	40	95°C	15 seconds
	Annealing and extension	60°C	1 minutes	

11 When the cycling ends, analyze the results according to the manufacturer's instructions.

12 RNA extraction control and non-template controls must be negative and positive control must be positive.

13 To be considered positive a sample must present at least two replicates with amplification equal of bellow the cycle threshold of 37.