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

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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).

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Materials

- Microcentrifuge Tubes by Contributed by users
- Filter Tips by Contributed by users
- Thermocycler by Contributed by users

GoScript(TM) Reverse Transcriptase, 500 rxn A5004 by Promega

Random Primers, 20ug C1181 by Promega

Nuclease-Free Water, 1000ml P1199 by Promega

- PCR tubes by Contributed by users
- Primer by Contributed by users
- Micropipettors by Contributed by users

GoTaq® Colorless Master Mix M714 by Promega

Protocol

First-Strand cDNA Synthesis

Step 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.

First-Strand cDNA Synthesis

Step 2.

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

Component	
Experimental RNA	X µL
Random Primer (0.5µg/reaction)	X µL
Nuclease-Free Water	X µL
Final volume	5 µL

First-Strand cDNA Synthesis

Step 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 15µl 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 15µl)	Xµl
GoScript™ 5X Reaction Buffer	4.0µl
MgCl2 (final concentration 1.5–5.0mM)	1.2–4.0µ
PCR Nucleotide Mix (final concentration 0.5mM each dNTP)	1.0µ
GoScript™ Reverse Transcriptase	1.0µ

First-Strand cDNA Synthesis

Step 4.

Add 15µl aliquots of the reverse transcription reaction mix to each reaction tube on ice. Be careful to prevent cross-contamination. Add 5µl of RNA and primer mix to each reaction for a final reaction volume of 20µl per tube.

First-Strand cDNA Synthesis

Step 5.

Anneal: Place the tubes in a controlled-temperature heat block equilibrated at 25°C, and incubate for 5 minutes.

First-Strand cDNA Synthesis

Step 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.

First-Strand cDNA Synthesis

Step 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

Step 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.

cDNA Quantification Using qPCR

Step 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

cDNA Quantification Using qPCR

Step 10.

Centrifuge the reactions in a microcentrifuge for 5 seconds.

cDNA Quantification Using qPCR

Step 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