Dec 08, 2020

RNA to cDNA and RT-PCR protocol

PLOS One

Heather Robeson[1], Jing Jin[1], Mohammed S. Orloff[1]

[1]University of Arkansas for Medical Sciences

1 Works for me dx.doi.org/10.17504/protocols.io.bpatmien

Jing Jin

ABSTRACT

Used applied biosystems high-capacity RNA-to-cDNA kit and fast SYBR green master mix protocol

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243509

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Jin J, Robeson H, Fagan P, Orloff MS (2020) Association of *PARP1*-specific polymorphisms and haplotypes with non-small cell lung cancer subtypes. PLoS ONE 15(12): e0243509. doi: 10.1371/journal.pone.0243509

DOI

dx.doi.org/10.17504/protocols.io.bpatmien

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243509

PROTOCOL CITATION

Heather Robeson, Jing Jin, Mohammed S. Orloff 2020. RNA to cDNA and RT-PCR protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bpatmien

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Jin J, Robeson H, Fagan P, Orloff MS (2020) Association of *PARP1*-specific polymorphisms and haplotypes with non-small cell lung cancer subtypes. PLoS ONE 15(12): e0243509. doi: 10.1371/journal.pone.0243509

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243509

CREATED

Nov 01, 2020

LAST MODIFIED

Dec 08, 2020

PROTOCOL INTEGER ID

44083

GUIDELINES

Use up to 2 µg of total RNA per 20-µL reaction

MATERIALS TEXT

RNA-to-cDNA:
2X RT buffer
2X RT enzyme
RNA sample
384 -or 96-well plate
8-cap and tube strip

RT-PCR:
Fast SYBR Green Master Mix
cDNA samples
Primers
RNase-free water
7900HT PCR instrument

ABSTRACT

Used applied biosystems high-capacity RNA-to-cDNA kit and fast SYBR green master mix protocol

RNA to cDNA reverse transcription

1 Prepare the RT reaction mix

2X buffer mix : **10 µl**

20X RT enzyme Mix: **1 µl**

RNA sample: < **9 µl**

Nuclease-free water: quantity sufficient to **20 µl**

Total per reaction: **20 µl**

2 Prepare the reverse transcription reactions:

Aliquot **20 µl** RT reaction mix into each well

Seal the plates and centrifuge to spin down the contents and remove air bubbles
Store them on ice

3 Perform reverse transcription:
Step1:

Temp. **37 °C** , time: 60 mins

Step2:

Temp. **95 °C** , time: 5 mins

Step3:

Temp. **4 °C** , time: unlimited

Store the cDNA:
Short-term: < 24 hours before use and store at 2–8°C
Long-term: store at −25°C to −15°C

Prepare PCR reaction plate

4 For one reaction:

Citation: Heather Robeson, Jing Jin, Mohammed S. Orloff (12/08/2020). RNA to cDNA and RT-PCR protocol. https://dx.doi.org/10.17504/protocols.io.bpatmien

Fast SYBR® Green Master Mix (2×) : **10 µl**

Forward and Reverse Primers : variable

cDNA template + RNase-free water: variable

Total: **20 µl**

Mix by gentle inversion, then centrifuge the tube to spin down the contents and eliminate any air bubbles

5 Transfer the appropriate volume of each reaction to 384-well reaction plate

Run the PCR reaction plate

6 Instrument: 7900HT

AmpliTaq Fast DNA Polymerase, UP Activation :

Temp. **95 °C** , time 10 mins, Cycles HOLD

Denature :

Temp. **95 °C** , time 1second

Temp. **60 °C** , time 20 seconds

Anneal/extend:

Temp. **95 °C** , time 15 seconds

Temp. **60 °C** , time 15 seconds

Temp. **95 °C** , time 15 seconds

Reaction volume: **20 µl**

MicroAmp Optical 384-Well Reaction Plate

7 Analyze the data

Citation: Heather Robeson, Jing Jin, Mohammed S. Orloff (12/08/2020). RNA to cDNA and RT-PCR protocol. https://dx.doi.org/10.17504/protocols.io.bpatmien