Sep 29, 2025

RNA isolation and qRT-PCR

DOI
https://dx.doi.org/10.17504/protocols.io.4r3l22r9pl1y/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.4r3l22r9pl1y/v1

Protocol Citation: Khaja Mohieddin Syed, Dirk Hockemeyer 2025. RNA isolation and qRT-PCR. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l22r9pl1y/v1

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

Created: January 15, 2024

Last Modified: September 29, 2025

Protocol Integer ID: 93519

Keywords: ASAPCRN, procedure for rna isolation, rna isolation, pcr primer sequence table, pcr this protocol, cdna synthesis, qrt, pcr

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000486
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-024409

Abstract

This protocol describes the procedure for RNA isolation, cDNA synthesis and qRT-PCR.

Protocol overview

A. RNA isolation and cDNA synthesis

B. qRT-PCR

qRT-PCR primer sequence table

	Gene Name	Forward Primer Sequence (5′-3′)	Reverse Primer Sequence (5′-3′)
	POU5F1	ATTCTCCAGGTTGCCTCTCA	GTGGAGGAAGCTGACAACAA
	NANOG	AGTCCCAAAGGCAAACAACCCACTTC	TGCTGGAGGCTGAGGTATTTCTGTC
	SOX2	TTCACATGTCCCCGAACTACCAGA	TCACATGTGTGAGAGGGGCAGTGTGC
	FOXA2	GGGGTAGTGCATCACCTGTT	CCGTTCTCCATCAACAACCT
	LMX1A	ACGTCCGAGAACCATCTTGAC	CACCACCGTTTGTCTGAGC
	NR4A2	GCTGGACTCCCCATTGCTTT	CGGAGCTGTATTCTCCCGAA
	KCNJ6	GCTACCGGGTCATCACAGAT	ACTGCATGGGTGGGAAAAGAC
	PITX3	CCAACCTTAGTCCGTGCCAG	TGTGTAGGGCCTAGTCCACC
	TH	CCGAGCTGTGAAGGTGTTTGA	CGGGCCGGGTCTCTAGAT
	EN2	AGGAGCTGAGCCTCAACGAGTC	CTTGGCTGTGGTGGAGTGGTTG
	AADC	GAACAGACTTAACGGGAGCCTTT	AATGCCCGGTAGTCAGTGATAAGC
	SYN1	GCAAGGACGGAAGGGATCACATCA	CCTGAGCCATCTTGTTGACCACGA
	GAPDH	TCGGAGTCAACGGATTTGGT	CCTGGAAGATGGTGATGGGA

Attachments

PDF

RNA Isolation and qR...

69KB

RNA Isolation and cDNA Synthesis

1 RNA was isolated using the RNeasy Kit (Qiagen, Cat#74014) according to manufacturer instructions.

2 Total of 2 µg RNA was used for cDNA synthesis and prepared using high capacity reverse transcriptase kit (ThermoFisher Scientific Cat#4368814) as per the manufacturer's protocol below.

	Component	Volume per reaction, µL (1X)
	100mM dNTPs	0.8
	RNAse Inhibitor	1
	Multiscribe Reverse Transcriptase	1
	10X Random Primer	2
	10x RT buffer	2
	Nuclease free water	3.2

3 2 µg of RNA was used in the reaction mix. Nuclease free water was added for a total reaction volume of 20ul. The below program was used for cDNA synthesis on the standard thermocycler.

	Temperature	Time (min)
	25°C	10
	37°C	120
	85°C	5
	4°C	Hold

4 The cDNA samples were diluted at 1:5 with nuclease free water before using them for qRT-PCR. 2 µL of the cDNA sample was used per reaction/well to check the expression of specific genes such as POU5F1, NANOG, TH, and KCNJ6.

qRT-PCR

5 Real time qRT-PCR was performed using PowerUP SYBR green (ThermoFisher Scientific, Cat#A25741) on a QuantStudio 6 flex PCR machine with 384 well plate format. Sample reactions were prepared as per reaction master mix below.

	Components	Volume per reaction, µL (1X)
	2x SYBR green	5
	Forward primer (10µM)	1
	Reverse Primer (10µM)	1
	cDNA	2
	Nuclease free water	1

6 Reaction mix was vortexed and briefly spun down before loading 10 µL of master mix per well.

7 Standard 40 cycle program was used on the QuantStudio 6 flex for the thermocycler reaction.

8 Results (ct values) were normalized to GAPDH.