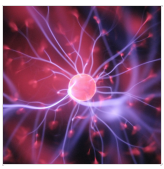

Sep 16, 2020

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

In 1 collection

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1 *Works for me* This protocol is published without a DOI.

Neurodegeneration Method Development Community
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ABSTRACT

This protocol explains Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) of lines ND1014, N1, and ND27760 from *Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons*.

EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6344911/

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons. Xue Y, Zhan X, Sun S, Karuppagounder SS, Xia S, Dawson VL, Dawson TM, Laterra J, Zhang J, Ying M. Stem Cells Transl Med. 2019 Feb;8(2):112-123. doi: 10.1002/sctm.18-0036. Epub 2018 Nov 1. PMID: 30387318

PROTOCOL CITATION

Yingchao Xue, Xiping Zhan, Shisheng Sun, Senthilkumar S. Karuppagounder, Shuli Xia, Valina L Dawson, Ted M Dawson, John Laterra, Jianmin Zhang, Mingyao Ying 2020. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). **protocols.io** https://protocols.io/view/quantitative-real-time-polymerase-chain-reaction-q-9u8h6zw

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

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EXTERNAL LINK

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6344911/

COLLECTIONS

Protocols for Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons

KEYWORDS

KEYWORDS

ND1014, N1, ND27760, ipsc, SNCA, Atoh2, Ngn2, qRT-PCR

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PARENT PROTOCOLS

Part of collection

Protocols for Synthetic mRNAs Drive Highly Efficient iPS Cell Differentiation to Dopaminergic Neurons

GUIDELINES

Primers for qRT-PCR

Primer	Sequence (5'-3')
NEUROD1	AGACACTCGTCTGTCCAGCTT; GCCCCAGGGTTATGAGACTA
FOXA2	GGAACACCACTACGCCTTCAAC; AGTGCATCACCTGTTCGTAGGC
NURR1	AAACTGCCCAGTGGACAAGCGT; GCTCTTCGGTTTCGAGGGCAAA
LMX1A	CATCGAGCAGAGTGTCTACAGC; TGTCGTCGCTATCCAGGTCATG
TH	GCTGGACAAGTGTCATCACCTG; CCTGTACTGGAAGGCGATCTCA
IFNA	ACCCACAGCCTGGATAACAG; ACTGGTTGCCATCAAACTCC
IFNB	CATTACCTGAAGGCCAAGGA; CAGCATCTGCTGGTTGAAGA
IFIT1	AAAAGCCCACATTTGAGGTG; GAAATTCCTGAAACCGACCA
OAS1	CGATCCCAGGAGGTATCAGA; TCCAGTCCTCTTCTGCCTGT
PKR	TCGCTGGTATCACTCGTCTG; GATTCTGAAGACCGCCAGAG
RIGI	GTTGTCCCCATGCTGTTCTT; GCAAGTCTTACATGGCAGCA
18s	ACCCGTTGAACCCCATTCGTGA; GCCTCACTAAACCATCCAATCGG

MATERIALS

NAME	CATALOG #	VENDOR
SYBR Green		Life Technologies
RNeasy Mini Kit	74104	Qiagen

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for safety and environmental hazards.

BEFORE STARTING

Obtain approval to work with human stem cells from an appropriate Institutional Review Board.

RNA Extraction

1 Extract total RNA using a RNeasy Mini Kit.

qRT-PCR

2 Reverse transcribe using murine leukemia virus reverse transcriptase and oligo(dT) primers.

3 Set up qRT-PCR using SYBR green PCR Master Mix in the IQ5 RT-PCR detection system. Primer sequences are listed in "Guidelines".

4 Normalize relative expression to 18S rRNA.