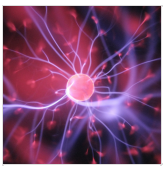

Sep 16, 2020

mRNA Synthesis and Transfection

In 1 collection

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

Neurodegeneration Method Development Community
Tech. support email: **ndcn-help@chanzuckerberg.com**

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ABSTRACT

This protocol explains the mRNA synthesis and transfection 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. mRNA Synthesis and Transfection. **protocols.io** https://protocols.io/view/mrna-synthesis-and-transfection-9u5h6y6

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

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

EXTERNAL LINK

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COLLECTIONS

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

KEYWORDS

ND1014, N1, ND27760, ipsc, SNCA, Atoh2, Ngn2, HiScribe T7 ARCA

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PARENT PROTOCOLS

Part of collection

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

GUIDELINES

Please follow the National Institutes of Health guidelines.

MATERIALS

NAME	CATALOG #	VENDOR
HiScribe T7 ARCA mRNA Kit - 20 rxns	E2065S	New England Biolabs
MEGAclear™ Transcription Clean-Up Kit	AM1908	Thermo Fisher
Lipofectamine™ MessengerMAX™ Transfection Reagent	LMRNA001	Thermo Fisher

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.

mRNA Synthesis

1 Clone coding sequence of Atoh1 into a vector containing the T7 promoter and poly(A) tail for in vitro transcription.

2 Clone coding sequence of Ngn2 into a vector containing the T7 promoter and poly(A) tail for in vitro transcription.

3

Linearize Atoh1 and Ngn2 vectors.

4 Subject linearized Atoh1 and Ngn2 vectors to mRNA synthesis using the HiScribe T7 ARCA mRNA Kit.

5 Purify mRNAs using the MEGAclear Transcription Clean-Up Kit.

Transfect mRNA

6 Transfect using a cationic lipid (e.g. Stem-In).

6.1 

For each well of a 12-well plate, incubate **0.25 µg mRNA** with **1.5 µl lipid** in **50 µl PBS** for **00:15:00** .

6.2 Add DNA:lipid complexes to cells.