

Aug 08, 2023

iNDI PiggyBac-TO-hNGN2 transfection protocol Version 1

DOI

<https://dx.doi.org/10.17504/protocols.io.rm7vzxb22gx1/v1>



Mark Cookson¹, michael.ward¹, Erika Lara Flores¹

¹National Institutes of Health, NIH Center for Alzheimer's and Related Dementias (CARD)

NIH Center for Alzheimer...



Erika Lara Flores

NIH/NIA

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.rm7vzxb22gx1/v1>

External link: <https://www.jax.org/jax-mice-and-services/ipsc>

Protocol Citation: Mark Cookson, michael.ward , Erika Lara Flores 2023. iNDI PiggyBac-TO-hNGN2 transfection protocol Version 1. [protocols.io https://dx.doi.org/10.17504/protocols.io.rm7vzxb22gx1/v1](https://dx.doi.org/10.17504/protocols.io.rm7vzxb22gx1/v1)



License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: August 08, 2023

Last Modified: August 08, 2023

Protocol Integer ID: 86174

Keywords: iNeurons, NGN2, Piggybac, transfection, iNDI, Jackson Laboratory, CARD, NIH, hngn2 transfection transfection protocol use of cept, hngn2 transfection transfection protocol use, hngn2 transfection protocol version, piggybac method, piggybac, protocol

Abstract

PiggyBac Method for hNGN2 transfection

- Transfection protocol
- Use of CEPT: *Nature Methods* 18, 528-541, 2021

Materials

Reagents

✕ Opti-MEM™ I Reduced Serum Medium **Gibco - Thermo Fisher Scientific Catalog #31985062**

✕ Lipofectamine™ Stem Transfection Reagent **Thermo Fisher Scientific Catalog #STEM00008**

✕ StemPro™ Accutase™ Cell Dissociation Reagent **Gibco - Thermo Fisher Scientific Catalog #A1110501**

✕ PBS 1x **Lonza Catalog #BE17-516F**

✕ • Chroman I **MedChemExpress Catalog #HY-15392**

✕ Emricasan (IDN-6556) **Selleckchem Catalog #S7775**

✕ Polyamine Supplement (1000×) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P8483**

✕ Trans-ISRIB **Tocris Catalog #5284**

✕ Essential 8™ Medium **Gibco - Thermo Fisher Scientific Catalog #A1517001**

✕ Matrigel hESC-Qualified Matrix, LDEV-free **Corning Catalog #354277**

Plasmids

PB-TO-hNGN2 (Addgene #172115)

EF1a-Transposase (sequence):

GACGGATCGGGAGATCTCCCGATCCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTA
TCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGAC
CGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATACGCGTGTG
CCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACCGGTGC
CTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTAAGTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAG
AACCGTATATAAGTGCAAGTAGTCGCCGTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCG
TGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCTTGAATTACTTCCACCTGGCTGCAGT
ACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCG
CCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTC
TCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTCTGGCAAGATAGTCTT
GTAAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGGCCCGTGCGTCCCA
GCGCACATGTTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGG
CCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCCGGTCGGCACCAAG
TTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGA
GCGGGCGGGTGAGTACCCACACAAAGGAAAAGGGCCTTTCCGTCTCAGCCGTCGCTTCATGTGACTCCACGGAGT
ACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGcGCTTTTGGAGTACGTGCTCTTTAGGTTGGGGGGAGGGGTTTT
ATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGA
ATTTGCCCTTTTTGAGTTTGGATCTTGTTTCATTgccacCATGGGCTCTAGCCTGGACgacgagcacatcctgagcgccctgctgc
agagcgacgacgaactggtgggagaggacagcgacagcgaggtcagcgaccacgtgtccgaggacgacgtgcagtcgacaccgaggaagccttca
tcgacgaggtgcacgaagtgcagcctaccagcagcggctccgagatcctggacgagcagaacgtgatcgagcagcctggcagctccctggccagcaa

cagaatcctgaccctgccccagagaacccatcagaggcaagaacaagcactgctggtccacctccaagagcaccaggcggagcagagtgtccgcccctg
aacatcgtgcggagccagagggggccccaccagaatgtgcagaaacatctacgacccccctgctgtgcttcaagctgttcttcaccgacgagatcatcagcg
agatcgtgaagtggaccaacgccgagatcagcctgaagaggcgggagagcatgaccagcgccaccttcagagacaccaacgaggacgagatctacgc
cttcttcggcatcctggtgatgaccgccgtgagaaaaggacaaccacatgagcaccgacgacctgttcgacagatccctgagcatggtgtacgtgtccgtgat
gagcagagacagattcgacttctgatcagatgcctgagaatggacgacaagagcatcagacccacccctgcgggagaacgacgtgttcacccccctgcg
gaagatctgggacctgttcatccaccagtgcacccagaactacacccctggcgcccacctgaccatcgatgagcagctgctgggcttcagaggcagatgc
cccttcagagtgtacatccccaacaagcccagcaagtacggcatcaagatcctgatgatgtgcgacagcggcaccaagtacatgatcaacggcatgccct
acctgggagaggcaccagacaaaacggcggtgcccctgggagagtactacgtgaaagaactgagcaagcctgtgcatggcagctgcaggaacatcac
ctgcgacaactggttcaccagcatccccctggccaagaacctgctgcaggaaccctacaagctgaccatcgtgggcaccgtgcggagcaacaagcggg
agatcccagaggtgctgaagaacagcagatccagacctgtgggaacaagcatgttctgcttcgacggccccctgacctggtgtcctacaagcccaagcc
cgccaagatggtgtacctgctgtccagctgcgacgaggacgccagcatcaacgagagcaccggcaagccccagatggtgatgtactacaaccagacca
agggcggcggtggacaccctggaccagatgtgcagcgtgatgacctgcagcagaaagaccaacagatggcccatggccctgctgtacggcatgatcaata
tcgctgcatcaacagcttcatcatctacagccacaacgtgtccagcaagggcgagaaggtgcagagccggaagaaattcatgcggaacctgtacatgag
cctgacctccagcttcatgagaagagactggaagccccaccctgaagagatAcctgCgggacaacatcagcaacatcctGcccaaggaagtgccag
gaacaagcgacgacagcaccgaggaacccgtgatgaagaagaggacctactgcacctactgtcccagcaagatcagaagaaaggccaacgccagct
gcaagaaatgcaaaaaagtgtatctgccgggagcacaacatcgacatGTGCCAGAGCTGTTTCTGATCTAGAGGGCCCGTTTAAACCCG
CTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCCTTGACCCCTGGA
AGGTGCCACTCCCACTGTCCTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGG
GGGTGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTC
TATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAAGCG
CGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTC
CCTTCCTTTCTCGCCACGTTCCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGT
GCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTT
TTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCT
CGGTCTATTCTTTTGATTATAAGGGATTTTGCCGATTTCCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAATAATTTA
ACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTATGCA
AAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTATGCAAGCAT
GCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTCCGCCCAGTTCCGCCCA
TTCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCTGCCTCTGAGCTATTCCAGAAG
TAGTGAGGAGGCTTTTTTTGGAGGCCTAGGCTTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTCGGATCTGATC
AAGAGACAGGATGAGGATCGTTTTGCGATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGA
GGCTATTCCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGG
CGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTG
GCTGGCCACGACGGGCGTTTCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGG
GCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGC
GGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTC
GGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACCTGTTCCGCC
AGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGT
GGAAAATGGCCGCTTTTTCTGGATTATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGC
TACCCGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCGA
TTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAA
GCGACGCCCAACCTGCCATCACGAGATTTGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTTC
CGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCACCCCAACTTGTTTATTGCA

GCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGGT
TTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGCGCTAATCATGGTCATAG
CTGTTTCCTGTGTGAAATTGTTATCCGCTCACAAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGG
GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCGCTTTCCAGTCGGGAAACCTGTCGTGCC
AGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGGCGGTTTGCCTATTGGGCGCTCTTCCGCTTCCTCGCTCACT
GACTCGCTGCGCTCGGTGCTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAA
TCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCT
GGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGA
CAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCG
GATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTA
GGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCCCGACCGCTGCGCCTTATCCGGTAACTATC
GTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGT
ATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGC
TCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTTT
TTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGAC
GCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAA
ATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGG
CACCTATCTCAGCGATCTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAG
GGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAAC
CAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGG
GAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCAGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCT
CGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAA
AGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAAGTAAAGTTGGCCGCGAGTGTTATCACTCATGGTTATGGCAGCA
CTGCATAATTCTCTTACTGTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGA
ATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAA
GTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAAC
CCACTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAA
ATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTTCAATATTATTGAAGCATT
TATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTT
CCCCGAAAAGTGCCACCTGACGTC



Transfection protocol

1h 30m

- 1 Observe **KOLF2.1** iPSCs under a phase contrast microscope to assess confluency and presence of cells debris. Dish should be dissociated at ~70% to 90% confluency.

30m

Coat **a well of 6 well plate** to be used for transfection with  1 mL of Matrigel solution, tilting to ensure coverage of entire surface area. Place in  37 °C incubator for

 00:30:00 .

- 2 Prepare E8 medium supplemented with CEPT and place in at  Room temperature to warm during dissociation.

**Note**

C.E.P.T is a cocktail that has been shown to improve the survival while transfection, however it can be used any other rock inhibitor as Y-27632 knowing that survival/transfection efficiency is not going to be as good as with CEPT.

Components of C.E.P.T cocktail

	A	B	C	D	E
	Reagent	Company	Cat. #	Final concentration	Target
	Chroman 1	MedChem Express	HY-15392	50 nM	ROCK 2 inhibitor
	Emricasan	Selleckchem	S7775	5 uM	Activated Caspase inhibitors
	Polyamine supplement (1000X)	Sigma	P8483	1x (1:1000)	Cell growth booster
	Trans-ISRIB	Tocris	5284	0.7 uM	Integrated stress response inhibitor

All the component were prepared as desired concentration following the manufacture instructions of each one.

- 3 Aspirate culture medium from well/plate that should be dissociated and wash with PBS 1X.
- 4 Aspirate PBS and add half of culture volume of Accutase.
- 5 Transfer to  37 °C incubator for  00:10:00 .

10m

**Note**

The time can vary by cell line and density (the optimal density is 70-90%) and the goal to use accutase is singularize as single cells.

- 6 Meanwhile aspirate Matrigel from well and add  2 mL culture medium E8 supplemented with CEPT.
- 7 When Incubation is ready, tilt the plate and pipet the accutase solution two to three times up and down the culture surface to break the colonies.
- 8 Quench the Accutase adding half of the culture volume of PBS.
- 9 Transfer to a new conical tube and rinse with more PBS the culture surface, combine with the cell solution in the tube.
- 10 Centrifuge  00:05:00 at 200 - 300 x g at  Room temperature . 5m
- 11 Aspirate supernatant and resuspend the cell pellet in culture medium E8 supplemented with CEPT.
- 12 Count cells and **plate $1 - 1.5 \times 10^6$ cells** into a Matrigel-coated well. Gently swirl plate to evenly distribute cells.
- 13 Return plate to  37 °C incubator.
- 14 **After ~**  01:00:00 **to**  02:00:00 **of plating cells , warm OptiMEM at**  Room temperature . 3h

Note

Do not bring Lipofectamine Stem out of refrigerator until ready to be used!

15 Prepare one tube with:

-  200 μL of OptiMEM
- Total of  3 μg of DNA mix in a 1:3 (Transposase:DNA) ratio

Note

*EF1a-transposase sequence can be found in the materials section.
Alternatively investigators can try the commercial superpiggybac transposase from SBI, but activity may be lower in iPSCs due to the use of a CMV promoter (which is rapidly silenced) rather than an EF1a promoter*

-  5 μL to  7.5 μL of Lipofectamine Stem

Note

Vortex after adding every reagent to Opti-MEM

16 Incubate transfection mix for  00:15:00 to  00:30:00 at  Room temperature

45m

Note

*Meanwhile, to remove debris and increase transfection efficiency, is recommended to wash the wells with PBS 1X and add  2 mL of fresh E8 supplemented with CEPT.
This step can be skipped when using CEPT but not when using rock inhibitor.*

17 Add transfection mix from step 15 to cells drop wise and immediately swirl the plate to evenly distribute the mix to cells.

18 Return plate to  37 $^{\circ}\text{C}$ incubator.

19 Next day, check for nuclear BFP positive signal (for PB-TO-hNGN2-BFP plasmid). If a positive transfection occurred and the well is confluent, re-plate the cells and puromycin



select 24-48 h after transfection (it all depends on the health and transfection efficiency of the cells) with [M] 8 Mass Percent for 2 to 14 days.

Note

One can start puromycin selection with low dose as [M] 1 Mass Percent and see how the cells react, then increase as convenient to reach [M] 8 Mass Percent .

- 20 Change media accordingly and expand cells when they reach 70-90% confluency.