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 We use this protocol and it's working

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 20410

Nucleofection of iPSC

In 1 collection

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Neurodegeneration Method Development Community

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ATTACHMENTS

[Comprehensive Genomic Editing and Screening Protocol Updated 02142019.docx](#)

GUIDELINES

This protocol is part of the [Genomic Editing: iPSC collection](#).

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for information about hazards, and to obtain advice on safety precautions.

Nucleofection - Expand iPSCs

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Note

Cells should be passaged as single cells prior to nucleofection. Split cells into 3 wells of a 6 well plate 48 hours prior to nucleofection (plan for cells to be confluent in 48 hours). You will need 3 million cells per nucleofection, so 3-6 confluent wells should be sufficient

Split 1 well from a 6 well plate into 3 wells in a 6 well plate.

- 2 Coat 6 well plate with 1 mL Matrigel (supplemented with RGD fragment) for 01:00:00
- 3 Aspirate media from cell culture.
- 4 Wash with 1 mL - 2 mL of PBS, per well.
- 5 Incubate at 37 °C for 00:05:00 to 00:10:00 to achieve single cells.

Note

Individual donor cell lines exhibit variable sensitivity to accutase-mediated dissociation. Thus, monitor cells closely to determine when single cell dissociation is achieved.

- 6 Collect cells in a 5 mL DMEM/F12 and transfer to a 15 mL conical tube.
- 7 Spin at 750-800 rpm for 00:03:00 .

- 8 Aspirate media.
- 9 Resuspend cells in mTesR1 (2 mL per well) supplemented with 1 uM Rock Inhibitor.
- 10 Plate 2 mL of cells per well on a Matrigel (supplemented with RGD fragment) coated plate.
- 11 Change media daily with mTesR1
- 12 Coat plate for nucleofection (2-3 wells) with matrigel supplemented with RGD for 01:00:00
- 13 Just prior to splitting cells for nucleofection, equilibrate Matrigel/RGD coated plate with 3-5 mL/well DMEM/F12+10% FBS supplemented with 10 uM Rock Inhibitor.
- 14 Aliquot DNA into 1.7 mL tubes using the table below.

DNA	Concentration	Volume (uL)	Final Concentration
pMaxGFP	1 ug/ul	1 ul	1 ug
gRNA	ng/ul	ul	1 ug
Cas9 SM168	ug/ul	ul	3 ug
Donor Oligo	100uM	3 ul	300uM

Nucleofection - Split cells for nucleofection

- 15 Aspirate media.
- 16 Wash with 1 mL - 2 mL PBS per well.
- 17 Add 1 mL of accutase per well.
- 18 Incubate at 37 °C for 00:05:00 to 00:10:00 minutes (checking at 5 minutes) to achieve single cells.

Note

Individual donor cell lines exhibit variable sensitivity to accutase-mediated dissociation. Thus, monitor cells closely to determine when single cell dissociation is achieved.

- 19 Collect cells in 5 mL PBS and transfer to a 15 mL conical tube.
- 20 Spin at 750-800 rpm for 00:03:00 .

Nucleofection - Count cells

- 21 Resuspend cell pellet in 3 mL PBS in 15 mL conical tube.
- 22 Pipette 10 μ L into cell counter.
- 23 Using all four corners of the countess slide, calculate the average number of cells.
- 24 Multiply the average by 10,000 (10^4).
- 25 Multiply product from step 24 by 3 to get the total number of cells.
- _____ average number of cells x 10,000 x 3 = total number of cells.

Nucleofection - Calculate 3 million cells

- 26 Take total number of cells calculated in step 25 and divide by 3 million.
- 27 Take answer from previous step and divide by 3 to get the volume of cells necessary for nucleofection.

Centrifugation

- 28 Transfer the desired volume of cells to microcentrifuge tube.

29 Centrifuge cells at 90 xg for 00:05:00 .

30 Aspirate PBS.

Make reaction mix from Lonza Kit

31 Make reaction mix from Lonza Kit: P3 Primary Cell 4D (V4XP-3024)- total of 100 μL per nucleofection.

Note

If performing multiple nucleofections, make a master mix.

32 82 μL P3 solution

33 18 μL of Supplement

Mixing

34 Combine Reaction Mix from step 31 (100 μL) with previously aliquoted DNA from
go to step #14

- 35 Mix Reaction Mix and DNA with cell pellet by pipetting up and down with p200 pipette.

Note

Try to pipette as little as possible. Pipette only until mixed.

- 36 Transfer 100 μ L to a cuvette.

Nucleofection

- 37 Nucleofect with Lonza program CA-137 in P3 solution.
- 38 Add a small amount of media to cuvette via dropper in order to obtain all the cells from the cuvette.
- 39 Transfer cells/DNA solution to appropriate pre-coated well containing 2 mL of DMEM/F12+10% FBS+10 μ M Rock Inhibitor.
- 40 Incubate at 37 $^{\circ}$ C overnight.

Post Nucleofection

- 41 Continue culturing the iPSC in 1 well of a 6 well plate for 5-7 days post nucleofection, changing mTesR1 daily.
- a. 24:00:00 post nucleofection--add mTesR1 with 5 μ M Rock Inhibitor.

- b. 48:00:00 post nucleofection-- add mTesR1 with 2.5 uM Rock Inhibitor.

Note

24 hours after nucleofection, cells exhibit extended processes - this is expected. Over the following 24-72 hours, cells recover and return to typical rounded iPSC morphology. The exact timeline for the morphological recovery is dependent on donor lines.

- c. 72:00:00 post nucleofection-- add mTesR1 with 1uM Rock Inhibitor.

42 Continue culturing in mTesR1 until confluent.

Note

Cells must be maintained for 5 days post-nucleofection prior to screening to minimize chimeric clones.