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Working

Nucleofection of iPSC

In 1 collection

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Neurodegeneration Method Development Community

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DOCX

Comprehensive Genomic Editing and Screening Protocol Updated 02142019.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

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

1

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.

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.

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 µl 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

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.

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.

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

36 Transfer **100 µl** 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 form the cuvette.

39 Transfer cells/DNA solution to appropriate pre-coated well containing 2 mL of DMEM/F12+10% FBS+10 uM Rock Inhibitor.

40 Incubate at **37 °C** overnight.

Post Nucleofection

41 Continue cultruing 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 uM Rock Inhibitor.

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

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 cultruing in mTesR1 until confluent.

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