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Working

Serial Dilution of Nucleofected iPSC Pools

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.

1 Coat 3 wells of a 6 well plate with **1 ml** Matrigel (supplemented with RGD fragment)

2 Aspirate media from cells in culture.

3 Wash with **1 ml** - **2 ml** PBS per well.

4 Add **1 ml** Accutase per well.

5 Incubate at **37 °C** for **00:10:00** to achieve single cells.

Individual donor lines exhibit variable sensitivity to accutase-mediated dissociation. Monitor cells regularly to identify when cells achieve single cells.

6 Collect cells in 5 mL DMEM/F12 and transfer to a 15mL conical tube.

7 Spin at 750-800 rpm for 00:03:00 .

8 Aspirate media.

9 Resuspend cells in mTesR1 supplemented with 5 uM Rock Inhibitor.

10 Plate several dilutions of cells over the three wells (typically 25 µl, 50 µl and 75 µl in 2 mL of mTesR1).

11 Freeze down the remaining cells by adding an equal volume of 2x iPSC Freezing Media (20% DMSO in FBS).

12 Incubate at 37 °C overnight.

13 Change mTesR1 the following day.