Serial Dilution of Nucleofected iPSC Pools

Celeste M M. Karch¹, Rita Martinez¹, Jacob Marsh¹

¹Washington University in St Louis

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

Celebrste M M. Karch
Washington University in St Louis

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.

Protocol Citation: Celeste M. Karch, Rita Martinez, Jacob Marsh 2019. Serial Dilution of Nucleofected iPSC Pools. protocols.io https://dx.doi.org/10.17504/protocols.io.x76frre

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: Feb 16, 2019
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.

**Note**

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 \(37 \, ^\circ \text{C}\) overnight.

13 Change mTesR1 the following day.