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Protocol status: Working
 We use this protocol and it's working

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 20474

Characterization 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 protocols is part of the [Screening Edited iPSC Clones collection](#).

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

Starting Material: 1 confluent 6 well plate.

Use 2 wells to characterize the iPSC and 4 wells for freezing the iPSC.

1

Coat T25 flask, chamber slide and plates with Matrigel

prior to passaging.

- 2 Aspirate media from cell culture.
- 3 Wash with 1 mL PBS per well and aspirate.
- 4 Add 1 mL Accutase per well of 6 well plate.
- 5 Incubate in 37 °C for 3-4 minutes. 00:03:00
- 6 Collect cells from 2 wells with 3 mL DMEM/F12 per well and transfer into 15mL conical A.
- 7 Collect cells from 4 wells with 3 mL DMEM/F12 per well and transfer into 15mL conical B.
- 8 Spin cells at 750 rpm for 00:03:00 .
- 9 Aspirate media from each tube.

Tube A

- 10 To 15mL conical tube A, add 2 mL mTesR1 and distribute cells.
- 11 Karyotype: Add 2 mL of mTesR1 to T25 flask, then add 500 μ L cells .
- 12 gDNA pellet: 500 μ L cells in 1.7 mL tube, spin down at max speed for 00:00:15 , aspirate media, store in -80 °C .
- 13 RNA pellet: 900 μ L cells in 1.7 mL tube, spin down at max speed for 00:00:15 , aspirate media, store in -80 °C
- 14 ICC: Dilute 100 μ L cells in 750 μ L mTesR1. Plate 200 μ L cells per well in 4 wells of a chamber slide.

Tube B

- 15 To 15mL conical tube B, add 4 mL mTesR1 and 4 mL of 2x Freezing Media (20% DMSO, FBS).
- 16 Add 1 mL cell suspension to each vial (1 well will freeze down into approximately 2 vials).

Note

Cryovials need to be labeled with the following before freezing down:

- Cell Type
- Line Name
- Passage #
- Date
- Your Name