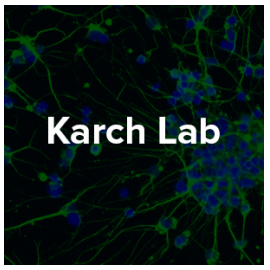
Feb 27, 2019

Characterization of iPSC



In 1 collection

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

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

Created: February 17, 2019

Last Modified: February 27, 2019

Protocol Integer ID: 20474

Attachments

Comprehensive Genomi...
31KB

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

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 01:00:00 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 µL cells .

12 gDNA pellet: 500 µL 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 µL 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 µL cells in 750 µL mTesR1. Plate 200 µL 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