Feb 27, 2019

Working

Characterization of iPSC

In 1 collection

Celeste Karch[1], Rita Martinez[1], Jacob Marsh[1]

[1]Washington University in St Louis

dx.doi.org/10.17504/protocols.io.x82frye

Neurodegeneration Method Development Community

Tech. support email: **ndcn-help@chanzuckerberg.com**

Celeste Karch
Washington University in St Louis

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 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 STARTING

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).

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

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