Splitting 96 Well Plates for gDNA Extraction and Continuing Culture

In 1 collection

Celeste M M. Karch¹, Rita Martinez¹, Jacob Marsh¹

¹Washington University in St Louis

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

ATTACHMENTS

Comprehensive Genomic Editing and Screening Protocol Updated 02142019.docx

GUIDELINES

This protocol 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

It takes approximately 1 week for iPSC picked into 96 well plates to be sufficiently confluent for freezing and screening. For screening purposes, a fraction of the cells picked into one well of a 96 well plate will be saved for DNA Extraction and the remaining will be kept in culture or frozen down.

Split cells upon reaching maximum 80% confluence and minimum 40%

Protocol Citation: Celeste M M. Karch, Rita Martinez, Jacob Marsh 2019. Splitting 96 Well Plates for gDNA Extraction and Continuing Culture. protocols.io https://dx.doi.org/10.17504/protocols.io.x78frw

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 96 well plate with 50 µL Matrigel per well.

2. Incubate at 37 °C for 01:00:00.

3. Prepare plate for expansion by aspirating Matrigel from plate.

4. Add 50 µL mTesR1 + 10 uM Rock Inhibitor to appropriate wells.

5. Aspirate media from original plate.

6. Wash with 200 µL PBS and aspirate.

7. Add 25 µL of 0.05% Trypsin.

8. Incubate at 37 °C for 00:05:00.
9 Tap to lift cells from plate.

10 Check under microscope to ensure that cells have detached from plate.

11 Add \( 50 \, \mu\text{L} \) FBS and tap to mix.

12 Transfer \( 50 \, \mu\text{L} \) to 96 well PCR plate, while maintaining the location of each sample (this plate will be used for gDNA extraction).

13 Transfer remaining cells (~30uL) to 96 well plate containing mTesR1.

14 Incubate at \( 37 \, ^\circ\text{C} \).

15 After \( 24:00:00 \), complete daily media changes with mTesR1.