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Working

Splitting 96 Well Plates for gDNA Extraction and Continuing Culture

In 1 collection

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Neurodegeneration Method Development Community

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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

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%

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 µl** FBS and tap to mix.

12 Transfer **50 µl** 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 °C** .

15 After **24:00:00** , complete daily media changes with mTesR1.