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Splitting 96 Well Plates for gDNA Extraction and Continuing Culture

In 1 collection

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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

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%

OPEN ACCESS

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

Created: Feb 16, 2019

Last Modified: Feb 27, 2019

PROTOCOL integer ID:
20448

1 Coat 96 well plate with 50 μ L 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 μ L mTesR1 + 10 uM Rock Inhibitor to appropriate wells.
- 5 Aspirate media from original plate.
- 6 Wash with 200 μ L PBS and aspirate.
- 7 Add 25 μ L 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.