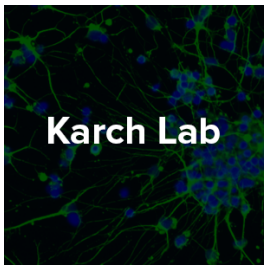
Feb 27, 2019

Splitting 96 Well Plates for gDNA Extraction and Continuing Culture



In 1 collection

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

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Protocol Integer ID: 20448

Attachments

DOCX

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

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.