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Working

Splitting 96 Well Plates for gDNA Extraction and Freezing Down

In 1 collection

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Neurodegeneration Method Development Community

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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

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

1 Aspirate media from original plate.

2 Wash with **200 µl** PBS and aspirate.

3 Add **25 µl** of 0.05% Trypsin.

4 Incubate at **37 °C** for **00:05:00**

5 Tap to lift cells from plate.

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

7 Add **45 µl** mTesR1 to plate and tap to mix.

8 Transfer **15 µl** to a 96 well PCR plate, while maintaining the location of each sample (this plate will be used for gDNA extraction).

9 Add **50 µl** of 2X Freezing Media (20% DMSO in FBS) to cell plate and tap to mix.

10 Wrap plate in parafilm. Add tape over parafilm. Label the plate on the outside of the tape.

11 Place plate in Styrofoam box. Fill any open areas with diapers, paper towels, or Kimwipes. Cover box and make sure that lid is closed completely.

12 Store in **-80 °C** for up to 4 months .