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

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 20446

Serial Dilution of Nucleofected iPSC Pools

In 1 collection

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ATTACHMENTS

[Comprehensive Genomic Editing and Screening Protocol Updated 02142019.docx](#)

GUIDELINES

This protocol is part of the [Genomic Editing: iPSC collection](#).

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for information about hazards, and to obtain advice on safety precautions.

1 Coat 3 wells of a 6 well plate with 1 mL Matrigel (supplemented with RGD fragment)

- 2 Aspirate media from cells in culture.
- 3 Wash with 1 mL 2 mL PBS per well.
- 4 Add 1 mL Accutase per well.
- 5 Incubate at 37 °C for 00:10:00 to achieve single cells.

Note

Individual donor lines exhibit variable sensitivity to accutase-mediated dissociation. Monitor cells regularly to identify when cells achieve single cells.

- 6 Collect cells in 5 mL DMEM/F12 and transfer to a 15mL conical tube.
- 7 Spin at 750-800 rpm for 00:03:00 .
- 8 Aspirate media.

- 9 Resuspend cells in mTesR1 supplemented with 5 μ M Rock Inhibitor.
- 10 Plate several dilutions of cells over the three wells (typically 25 μ l, 50 μ l and 75 μ l in 2 mL of mTesR1).
- 11 Freeze down the remaining cells by adding an equal volume of 2x iPSC Freezing Media (20% DMSO in FBS).
- 12 Incubate at 37 °C overnight.
- 13 Change mTesR1 the following day.