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Serial Dilution of Nucleofected iPSC Pools

 In 1 collection

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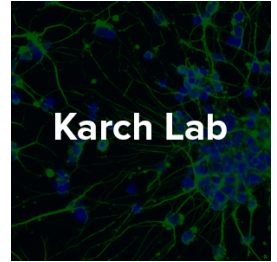
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Protocol status: Working

We use this protocol and it's working

Created: February 16, 2019

Last Modified: February 26, 2019

Protocol Integer ID: 20446



Attachments



Comprehensive

Genomi...

31KB







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 uM Rock Inhibitor.
- 10 Plate several dilutions of cells over the three wells (typically 25 µl, 50 µl and 75 µl 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.