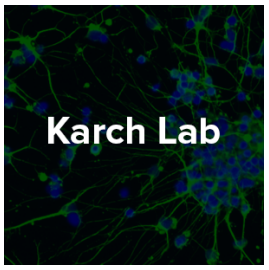
Feb 27, 2019

Serial Dilution of Nucleofected iPSC Pools



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: 20446

Keywords: serial dilution of nucleofected, ipsc pool, serial dilution, nucleofected, ipsc

Attachments

DOCX

Comprehensive Genomi...
31KB

Guidelines

This protocol is part of the **Genomic Editing: iPSC collection**.

Troubleshooting

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.