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CRISPRi Knockdown in NGN2 iPSCs

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

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Abstract

CRISPRi Knockdown in NGN2 iPSCs with dCas9 using sgRNAs

1 Doxycycline-inducible NGN2 iPSCs are maintained on Geltrex in mTeSR (STEMCELL) , and passaged using [M] 0.5 micromolar (µM)) EDTA. iPSCs should be between 50-70% confluent to increase effectiveness of transduction.

2 Prepare lentiviral stocks with sgRNAs targeting the genes for Knockdown.

3 Dilute aliquot of lentivirus preparation in enough media to cover cells in 6-well plate. We diluted our stocks 1:100 in 1mL of mTeSR.

Note

Using 1mL of media to save lentiviral stock, we transduced the cells overnight and changed the media early the next day to ensure cell survival.

4 Change media with 2 mL of mTesR per well of a 6-well plate. Leave the cells to recover for 2 days, changing the media daily.

5 Once cells look healthy after transduction step, add 0.5ug/mL of Puromycin to kill untransduced cells. Keep puromycin as long as control untransduced cells are not dead.

Note

Include antibiotic to which lentiviral construct presents the relevant resistance. Keep one well of untransduced cells to control whether the antibiotic accurately gets rid of cells that haven't taken up the plasmid. It is possible to increase the antibiotic concentration to 1ug/mL if cells sare staying alive.