iPSCs

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TRANSFECTION OF i^{3}NEURONS (Support Protocol 3)

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.5wug7ew

Neurodegeneration Method Development Community

ABSTRACT

Transient protein expression can easily be studied in i^{3}Neurons using lipid-based transfection. This protocol is identical to that in iPSCs (see Basic Protocol 2). i^{3}Neurons are modestly transfectable, with 5 % to 10 % of cells showing fluorescent protein expression after 24 hr. We have found that refreshing neuronal medium 1 to 2 hr after transfection both allows successful DNA entry into cells and largely prevents cytotoxicity resulting from the transfection reagent. Unlike iPSCs, i^{3}Neurons show increased protein expression/accumulation over time, with greater fluorescence 48 to 72 hr after transfection than at 24 hr. Transient transfections also show more durable expression in i^{3}Neurons than iPSCs, likely because episomes are not diluted by cell division. i^{3}Neurons can be transfected in suspension (i.e., re-plating after day 3 of differentiation) or as an adherent culture, although better results are observed in adherent cultures. They are also amenable to serial transfections (i.e., re-transfecting with the same construct 24 hr apart) if higher-percentage transfections are desired.

EXTERNAL LINK

https://doi.org/10.1002/cpcb.51

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Fernandopulle, M. S., Prestil, R., Grunseich, C., Wang, C., Gan, L., & Ward, M. E. (2018). Transcription-factor mediated differentiation of human iPSCs into neurons. Current Protocols in Cell Biology, e51. doi:https://doi.org/10.1002/cpcb.51

fernandopulle2018.pdf

GUIDELINES

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SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.