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

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iPSCs

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

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Keywords: i3LMN, i3Neurons, iPSC, iPSC-derived neurons, transcription factor-mediated differentiation, transfection of i3neuron, more durable expression in i3neuron, i3neuron, transient protein expression, fluorescent protein expression, transient transfection, increased protein expression, protein expression, showing fluorescent protein expression, successful dna entry into cell, refreshing neuronal medium, based transfection, transfection reagent, transfection, neuronal medium, cell, successful dna entry, using lipid

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.

Attachments

fernandopulle2018.pd...
1.7MB

Guidelines

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.

Troubleshooting

Safety warnings

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