iPSCs

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GENOTYPING OF iPSCS WITH GENE INSERTIONS (Support Protocol 1)

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.5u9g6z6

Neurodegeneration Method Development Community

ABSTRACT

This protocol will use genomic DNA isolated from the purified iPSC clones with CLYBL or AAVS1 gene insertion to determine if integration of the transgene has occurred correctly, in a heterozygous or homozygous fashion, and if the floxed selection genes are present. Primer sequences, amplicon sizes, PCR mix composition, and thermal conditions are included in the steps.

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

MATERIALS TEXT

- Primers (see recipe in Reagents and Solutions)
- PCR reagents (e.g., Platinum SuperFi PCR Master Mix, Invitrogen, cat. no. 12358250)

Platinum™ SuperFi™ PCR Master Mix
by Thermo Fisher Scientific
Catalog #: 12358010

- Genomic DNA
- Additional reagents and equipment for PCR (Kramer & Coen, 2001) and agarose gel electrophoresis (Voytas, 2001)

SAFETY WARNINGS

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

1 Set up PCR reaction (also see Kramer & Coen, 2001).
For each 10 µl reaction, use the primer pairs recommended in Reagents and Solutions for the given safe-harbor site and donor construct chosen:

- **1 µl** [M]**10 Micromolar (µM)** Primer 1
- **1 µl** [M]**10 Micromolar (µM)** Primer 2
- **1 µl** purified genomic DNA from iPSC clone
- **2 µl** H_2O
- **5 µl** 2 × PCR Master Mix.

2 Run PCR reactions

95 °C	3min
95 °C	30 sec
64 °C for CLYBL primers, 58 °C for AAVS1 primers	30 sec
72 °C	1min
Repeat (to step 2) 34 times	
72 °C	5min
Hold at 12 °C	Indefinitely

3 Run PCR products on 1 % agarose gel (Voytas, 2001) with interpretation given above for each primer pair.

If performing the PCR on samples following Cre excision, increasing the amount of time during the extension step may be necessary to detect the larger un-excised template if a heterogeneous culture is obtained.