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Working

iPSC PCR: For Screening Edited Clones

In 1 collection

Celeste Karch[1], Rita Martinez[1], Jacob Marsh[1]

[1]Washington University in St Louis

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Neurodegeneration Method Development Community

Tech. support email: **ndcn-help@chanzuckerberg.com**

Celeste Karch
Washington University in St Louis

Comprehensive Genomic Editing and Screening Protocol Updated 02142019.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

This protocols is part of the Screening Edited iPSC Clones collection.

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for information about hazards, and to obtain advice on safety precautions.

BEFORE STARTING

Using the extracted gDNA, set up the following PCR and run using the following conditions.

Each cell line/mutation will require its own unique set of primers. Ensure that you have the appropriate primers for the cell line/mutation before setting up the PCR.

Note: Design primers to amplify approximately a 300-400 bp region for best results.

PCR

1 This is the set up for a 50 µL reaction.

	Volume	x# rxns
5x Green GoTaq Flexi Buffer	10 µl	
25mM MgCl2	6 µl	
25mM dNTPs	0.8 µl	
Forward Primer (10uM)	2 µl	
Reverse Primer (10uM)	2 µl	
GoTaq DNA Polymerase (5U/uL)	0.25 µl	
Milli-Q H20	26.95 µl	
Quick Extract gDNA	2 µl	
Total	50 µl	

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3 After PCR has been set up, run in thermocycler using the following conditions.

Segment	Cycles	Temperature	Time
1	1	95˚C	2 minutes
2	40	95˚C	30 seconds
		65˚C - 1˚C/cycle	30 seconds
		72˚C	1 minute
3	1	72˚C	5 minutes
4	1	4˚C	Forever

4 After PCR has been run in thermocycler, it is best to run the sample on a 2% gel to confirm the presence of PCR product.

Gel preparation

5 To make the gel, combine an appropriate amount of Agarose, TBE and Ethidium Bromide using the following guidelines.

a. The 2% gel will be cast in one of the following ways:

	15x15 cast	15x25 cast
Agarose	1.5 g	3.0 g
TBE	75 mL	150 mL
Ethidium Bromide	3.75 uL	7.5 uL

b. Combine Agarose and TBE in an appropriately sized flask and microwave until Agarose is completely dissolved. Swirl intermittently during heating.

c. Once completely dissolved add appropriate amount of Ethidium Bromide to flask and swirl until dispersed evenly.

6 Pour gel from flask into casting tray (be sure to add appropriate amount of combs to casting tray).

7 Let sit for 30-40 minutes, or until firm. 00:30:00

8 Place gel cast into the gel rig apparatus.

9 Load samples.

Be sure to include positive and negative controls when possible.

10 Load 50 bp ladder.

11 Place lid on gel rig apparatus.

12 Run gel at 150 volts for 01:30:00 (checking at 01:00:00 to ensure samples have not run too far or off the gel).

13 Turn off gel rig apparatus and remove cast.

14 Blot off excess TBE from cast.

15 Visualize gel

16 If PCR product is the approrpiate size and concentration as indicated by the gel, use the remaining PCR product to perform restriction digest