

Feb 27, 2019

iPSC PCR: For Screening Edited Clones

 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.x8bfrsn

Celeste M M. Karch¹, Rita Martinez¹, Jacob Marsh¹

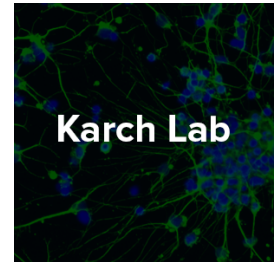
¹Washington University in St Louis

Neurodegeneration Method Development Community
Tech. support email: ndcn-help@chanzuckerberg.com



Celeste M M. Karch

Washington University in St Louis



Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.x8bfrsn>

Protocol Citation: Celeste M M. Karch, Rita Martinez, Jacob Marsh 2019. iPSC PCR: For Screening Edited Clones. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.x8bfrsn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: February 17, 2019

Last Modified: February 27, 2019

Protocol Integer ID: 20451

Keywords: screening edited clone, ipsc pcr, pcr, edited clone, ipsc

Attachments



Comprehensive

Genomi...


31KB

Guidelines

This protocols is part of the [Screening Edited iPSC Clones collection](#).

Troubleshooting

Safety warnings

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

Before start

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 μ L reaction.

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

2

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

Segm ent	Cycl es	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:

		15×15 cast	15×25 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.

Note

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 appropriate size and concentration as indicated by the gel, use the remaining PCR product to perform restriction digest