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Working

Sanger Sequencing

In 1 collection

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Neurodegeneration Method Development Community

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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

After identifying iPSC clones with the appropriate banding pattern, gDNA from iPSC clones of interest should be sequenced in order to confirm genotype. To do this, set up a sequencing reaction as outlined below.

Perform PCR on gDNA using the following TouchDown Protocol (50µL reaction). The PCR amplicon size should be between 300-400 bp. The primers used for the RFLP screening can also be used for Sanger Sequencing.

PCR

1 Set up PCR on ice, add reagents in desired order (however it is best to add the polymerase and gDNA last).

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

Segment	Cycles	Temperature	Time
1	1	94°C	5 minutes
2	10	94°C	30 seconds
		65°C - 1°C/ cycle	30 seconds
		72°C	1 minute
3	35	94°C	30 seconds
		55°C	30 seconds
		72°C	1 minute
4	1	72°C	10 minutes
5	1	4°C	Forever

After product has been run in the thermocycler

2 Run **15 µl** of each sample on a 2% gel to determine presence of PCR product.

3 Run product on gel at 150 V for **01:30:00** .

4 Image gel.

5 Save image.

6 After confirming the presence of PCR product, move on to sequencing reaction protocol below:

	Volume	x# rxns
PRC product	1 µl	
Primer (10µM)	1 µl	
Milli-Q H2O	10 µl	
Total	12 µl	

Send out a Forward and a Reverse for each sample.