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Nf1 loxP site verification

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

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Disclaimer

ETHICS DISCLAIMER

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

We have found that our *Nf1*^{flox} mouse colonies contained mice that had lost one of the two loxP sites inserted into the *Nf1* gene *(Parada laboratory, PMID:11297510,* ***JAX# 017639****)*, leading to loss of DNA recombination upon Cre-recombinase activity and loss of previously identified skeletal phenotypes. This is likely to occur in other *Nf1*^{flox} mouse colonies.
We describe here a strategy to verify conservation of the loxP sites within the *Nf1* allele and to sequence the loxP1 and loxP2 sites for detecting potential mutations or deletions. This may be done if the expression of *Nf1* (qPCR) is not reduced in Cre$^{Tg/+}$; *Nf1*$^{flox/flox}$ tissues of interest.

Attachments

PDF

Exon 31_32 and loxP ...

1.3MB

Materials

Heater (DNA denaturation/Lysis)
PCR machine
Agarose gel electrophoresis system (DNA separation)
Gel documentation system (DNA amplicon visualization and picture)

Protocol materials

Specific Primers Forward and Reverse

GoTaq® Green Master Mix **Promega Corparation Catalog #**M7123

Troubleshooting

Nf1 loxP site verification

15m

1 Specific Primers Forward and Reverse

Primers for the *Nf1* loxP1 site:
P1: 5′-CTTCAGACTGATTGTTGTACCTGA-3′
P4: 5′-TGATTCCCACTTTGTGGTTCTAAG-3′
P3: 5'-ACCTCTCTAGCCTCAGGAATGA-3'

Primers for the *Nf1* loxP2 site:
LoxP2For: 5′-GCTTTAGCTTCTGGAAATGTGAA-3′
LoxP2Rev: 5′-GCGGGCTAAAATGGCAATGTCG-3′

2 **gDNA preparation from NaOH lysate** 15m

1. Cut a 2-3mm piece of tail/ear
2. Add it to 300uL of lysis solution (NaOH 50mM)
3. Heat for 15 min at 95C
4. max rpm, Room temperature, 00:15:00 , use max speed of your bench centrifuge

3 **PCR reaction**

3.1 From a working solution of 10uM, use 1 uL of each primer (200nM each in a total volume of 20uL)
Reaction for loxP1: P1 + P2 + P3
Reaction for lopP2: LoxP2For + LoxP2Rev

3.2 Per reaction, mix 0.5uL of gDNA from the NaOH lysate (from step 2), 1 uL of each primer (from step 3.1), 10 uL 10m
GoTaq® Green Master Mix **Promega Corparation Catalog #**M7123 mix, and add water for a total volume of 20 uL

3.3 PCR cycle program 2h

	A	B	C
	Temp.	Duration	Cycles
	94C	5 min	

	A	B	C
	94C	15 sec	3 to 5, 35x
	58C	30 sec	
	72C	1 min	
	72C	10 min	

PCR temperatures and cycles

4 Run PCR reaction products on a 2% ethidium bromide agarose gel 1h

5

Expected result

Expected PCR results:

a) LoxP1 site:
480bp: WT (+) allele (P1-P3) in *Nf1* $^{+/+}$ or *Nf1* $^{f/+}$ mice, or loss of the loxP1 site in *Nf1* $^{f/f}$ mice
350bp: Presence of loxP1 (P1-P4)
in *Nf1*$^{f/f}$ mice

b) LoxP2 site:
699 bp: WT (+) allele in *Nf1* $^{+/+}$ or *Nf1* $^{f/+}$ mice, or loss of the loxP2 site in *Nf1* $^{f/f}$ mice
829 bp: Presence of loxP2 in *Nf1*
$^{f/f}$ mice

Protocol references

Ablation of NF1 function in neurons induces abnormal development of cerebral cortex and reactive gliosis in the brain, PMID:Y Zhu [1], M I Romero, P Ghosh, Z Ye, P Charnay, E J Rushing, J D Marth, L F Parada, PMID:11297510.