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Generation of Gba L444P mutant mouse

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We use this protocol and it's working

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Abstract

This protocol was used to generate of mutant mouse carrying Gba L444P point mutation.
The protocol was also described in a previous literature (Mol Genet Metab. 2014 Feb;111(2):152-62. doi: 10.1016)

Materials

1. mouse gDNA
2. PCR kit
3. murine embryonic stem (ES) cells
4. Southern blot kit
5. Geneticin, G418 solution
6. C57BL/6 female mouse

Troubleshooting

1 The replacement targeting vector enabling positive/negative selection contained a neomycin resistance (neoR) cassette flanked by loxP sequences inserted into the intergenic regions between murine metaxin (mtx) and glucocerebrosidase (gba).

2 The L444P was introduced into a genomic clone of murine gba by PCR mutagenesis. For this, the normal murine Gba sequence in exon 9 was changed from TGACTTGGA to TGACCCGGA, resulting in the amino acid substitution of proline for leucine and introducing a Nci1 restriction site. Then, the change in sequence was confirmed by restriction digest and/or direct sequence analyses.

3 The final constructs contained a 4.0 kb 5′ gba homologous arm and a 1.4 kb 3′ mtx homologous arm.

4 A diphtheria toxin A (DTA) cassette was placed downstream as a negative selectable marker.

5 After linearization, the constructs were introduced into murine embryonic stem (ES) cells by electroporation, and the ES cells were subjected to drug selection in culture with G418 solution.

6 The correct gene targeting event in G418 resistant individual clones was identified by Southern blot and PCR analysis.

7 Cells from ES clones containing the correctly targeted mutation in one allele of the gba gene were injected into blastocysts from C57BL/6 mice and then transferred to foster mice.

8 Male offspring from these injections having more than 30% coat color chimerism were test-bred against C57BL/6 females, and progeny were screened by PCR and Southern analyses for transmission of the mutant gba allele.

9 Lines of mice containing the mutant gba allele were identified, and the DNA sequence confirmed by direct sequencing of PCR amplified DNA containing the mutation.

10 Mice heterozygous for the mutant gba gene were mated and homozygous mutant progeny were identified by Southern blot and PCR analysis.

11 In addition, heterozygous mice were mated to mice carrying a transgene for CRE DNA recombinase, resulting in the excision of the neoR marker, leaving only a 34 bp loxP sequence.

12 As expected, the targeted mutations were transmitted in a Mendelian fashion.

13 Mice homozygous for the gba mutations without the neoR cassette were used to expand the colonies.