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We use this protocol and it's working

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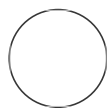
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🌐 Generation of Gba L444P mutant mouse

Tae-Un Han¹, Edward Ginns²

¹National Institute of Health; ²University of Massachusetts Medical School



Tae-Un Han

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

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- 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.