

Genotyping Gba^{L444P} mice by PCR

Preparation of PCR reaction mixture

10x PCR Buffer II	5 μ l
25mM MgCl	3 μ l
2.5mM dNTP's	4 μ l
AmpliTaq Gold	0.3 μ l
Primers	1 μ l each
H ₂ O	34 μ l

Total volume 48 μ l. Mix well, add 2 μ l tail extract

PCR program: 94C 10min, 40cycles (94C 1min, 55C 1min, 72C 1min), 72C 10min

Take 9.5 μ l PCR product, add 0.5 μ l NciI 37C 2hr, 65C 20min.

There is a duplication of the Gba gene in these mice giving rise to a L444P containing gene and an inactive fragment of the normal gene. The mutant (L444P) gene contains an NciI site that can distinguish the duplicants. $Gba^{+/L444P}$ mice contain one copy of the L444P gene and two copies of the truncated normal gene (ratio 1:2). $Gba^{L444P/L444P}$ mice contain two copies of each (ratio 1 to 1).

The PCR should yield an ~400bp band corresponding to the Gba gene. If carrying the L444P mutation, the Gba PCR product will be cut in half to yield closely spaced ~200bp bands.

We use the DNA 7500 chip on the Agilent 2100 bioanalyzer for analysis of the PCR products. If the 200bp/400bp band ratio is 1 to 1, the mouse is $Gba^{L444P/L444P}$. If the 200bp /400bp band ratio is 1 to 2 the mouse is $Gba^{+/L444P}$.

Primer set:

F:5'CCC CAG ATG ACT TGA TGC TGG

R:5'CCA GGT CAG GAT CTC TGA TGG