Genotyping protocol

General information:

Strain name	C57BL/6-Fxr1<em2Geno>/Cnbc

Primers:

Name	Sequence	Primer type
Fxr1-Fw	5´-TCCAGAGGGCAGTGCTTTAT-3´	Gene-specific
Fxr1-Rv	5´-TGCATTTTCTTGAGCAGCAT-3´	Gene-specific
Fxr1-seq Rv	5´-TGCAATGCAAATTCTGAATGT-3´	Gene-specific

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
PCR amplification	Fxr1-Fw	Fxr1-Rv
Sequencing		Fxr1-seq Rv

Reaction mix:

ddH_2O	18,5	µl
PCR Buffer 15mM $MgCl_2$	2,5	µl
dNTPs 2,5mM	2,5	µl
Primer 100µM	0,125	µl
Primer 100µM	0,125	µl
Taq Polymerase 5U/µl	0,250	µl
DNA	1	µl
Final volume	25	µl

PCR program:

94.0	°C	5	min	
94.0	°C	30	sec	X10
62.5	°C	30	sec	
72.0	°C	45	sec	
94.0	°C	30	sec	X25
61.5	°C	30	sec	
72.0	°C	45	sec	
72.0	°C	5	min	

Expected fragment size:

PCR product length: 420 bp for wt and mutant.

Comments/Additonal information:

It´s necessary to sequence with Fxr1-seq Rv primer to differentiate the mutation

Sequencing and PCR amplification primers

Part of *Mus musculus* wild type *Fxr1* sequence (exon 15 and delACAG mutation)

GCGGTCTCGTAGACGGAGGACTGATGAAGATGCTGTTCTGATGGATGGACTGACTGAATCTGATACAGCC
TCAGTTAATGAGAATGGGCTAGGTATGTAAGCACTTAGGGAAGAGTTGTGAGTGTGTGTGGTTGCATTGT
ATTGATAATGGAGGAAGCCTTTCCTTTTTTTGTATAGAATTCACAAATGGTTTTTTTTTTTCCTTTCTTT
TCTTTTTAGTGTTTACTTTAAATGAGGGATATGGCTGCTATCTTGGGCACTCATTTTCATATCTGTCAGA
CTATCTGAACTGGGAGGATTCCTTAAGGTGCCCTTTCATACCATTAAATCAACCTAAATACTCAGTTGAT
CTGTCTACAAACAAAACTTAACTGTTTTAGTCCCTCCCCCTTTAAAAATTATAGTACATATTTATGGGGG
CTGCAAAAACCTAGCATTTATTAATAAGGGGCTTTGAGATCCAGGGTAATGTTGGAGAGATATCTTATAT
TTCTGAATGATGGTGGTTGTTTCAATTACATGGCAGTTTGCTTTATTATGAGTTATTAAAGCCCTCTTAA
TTTCCAGAGGGCAGTGCTTTATGCTGCAAAAAATGTGTATAATTCTTTGTCAGTATTGTATATGCTTTAT
CTTTGATGTTTAATTAAAAGTTTCTCTTTCCCTTCCTTAAATAAGGAAAGAAATTTTTTGTTTGTTTTTT
GTTTATTTTTTGTCAGAATAGTTGATGGTAAACTTTGCAATTACAGATGATAGTGAAAAAAAACCCCAGC
GACGCAATCGTAGCCGCAGGCGTCGTTTCAGGGGTCAGGCAGAAGATAGACAGCCAGGTAACTTGAGTGG
GCCTGTTGGTGTCAGGTCACAAGCATGAAAAAAATGTCATGTGTCTGCATTCTAAATACAGTTATATGTT
CACATGTGTGTATACATTCAGAATTTGCATTGCATAATTTATTCAACTAATAATGCTGCTCAAGAAAATG
CATTCTTCCTTCCCAGTGTAAATGTTTAGTATCTAGTCTTAAGTGAACTAATGTTGTGTCTTAATTTCTT
ACATGGACAGAATTTCAGTACCTTTACTTTTTATTCAGCTGGCAGATTCTTCAGCTTTCAGATGGGAACT
GCCATTGCAGGGACTATGGTGTGAATTGTGTTGAGGTTGATTGGCAAAACTAGGCAATTTTTTACATGGT
GCCTGCTTACTATAGATTGAGCTCTTCATTTGGTGGGAATTACAATACTCAAAATTTTAAAATAAAATGT
AGAAGTGTCTTTTTCTTTAAAAGAGGATGCATGTTAATGTTTTTGTGTTAATGTAGTGTGGTGCTTTTTC
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TGGTAACTGGAAAGAAACTTGTTTGCTATGTCTATTACTTTGAACACAATTCATTTCCTATGTACAGAGA
ATCACTGTATATTCACCTTGAAGAAATTGTATATAGTAGAAATAAACCCCTTTCAAACCAACCAATTTTT
GCATTCTTTCCAAAACATACTCTTTCATGTGTGTTCTCTATCCCCCTCCCCCCTCTCCAAATAATGTACA
TTGTAAATGTTGCACTGCAGAGGTTGTAGAGACCTGGGAATCTAGTAAAGGGTATTCTTTCCCATCATGA
AGAGTTTGTGTTTAAAAGATAGAAGGAAATGTAAAAACTGTACACTATCAAAGCTAGTGTCCAGAAAATT
TGTTTTCCCTTTATATTCCTTTTCCTTCTTAATGTTAGAGATGTATTTTAGTTGAAGAGGTCATAAATTT
CAGCTTTGAAAGTAGCACAGCAGTGAAGATTTTTGACCCTACTTTTTTTGCAAGATAGAAAATTCAGGCT
ATTAGTATGGGCTCACTAACTTGGAATGACTTTCAATCTAACTTAAGCCAGTGATCATGGTTAAATGTAC
AGACACAAAAATATCAAAACAGGATTTCTTCCAAAAGAGATGATTTTGAATGGAATTAACTAAAATGAAT
AATTTGGAGTTTTGGTAATTTTAACAGTGATGTGCTGTTGCATACTATATCTGATTTGTTTTTACTGTTC