Genotyping protocol for Nmnat1 floxed (Nmnat1^{tm1Cole})

Import ref Q207A FESA code BSN

Method taken from the 'Application for freezing' form, submitted by the depositor in 2006.

(Michael Coleman, Babraham Institute, Cambridge).

Sequence of PCR Primers used for genotyping:

2 pairs of primers used:
a)
5'-TCGGAGTGTATCCTTGGAGT-3' (For)
5'-ACCAAGCTTTCAGCACATGG-3' (Rev)

b)
5'- CCCAGTCACTAAGACATTCAA- 3' (For)
5'-GACCCTCCTAGGCAAATATA-3' (Rev)

Reagents, conditions, thermocycling parameters for PCR:

Reagents at final concentration:

Buffer: 16mM (NH4)2SO4, 67mM TrisHCl pH8.8, 0.01% Tween-20
MgCl2: 1.5 mM
dNTP mix: 0.2 mM
Primers: 0.2 μM
Taq polymerase: 0.05U/μL

Conditions:

Use 15 μl mix plus 1-5 ng genomic DNA for genotyping

Cycling parameters:

Denature: 94 °C, 45 sec
Anneal: 56 °C, 45 sec
Elongate: 72 °C, 60 sec

Size and sequence of PCR product:

PCR product in wild type:

a. TCGGAGTGTATCCTTGGAGTCCATGTGCTGAAAGCTTGGT (size=40 bp)

b. CCCAGTCACTAAGACATTCAAATATATTTGCCTAGGAGGGTC (size = 42 bp)

PCR product in floxed:

a.
TCGGAGTGTATCCTTGGAGTGGATCCATAACTTCGTATAATGTATGCTATACGAA
GTTATCTAGAGTCGACCCATGTGCTGAAAGCTTGGT (size = 90 bp)

b.
CCCAGTCACTAAGACATTCAAGTCGACATAACTTCGTATAATGTATGCTATACGA
AGTTATAAGCTTATATATTTGCCTAGGAGGGTC (size= 88 bp)

Description of genotyping method if other than PCR.

None.

Further information

Type of construct: The targeting vector pEASYflox was used to knockin a floxed allele of Nmnat1 such that Nmnat1 exons 2 and 3 were contained between loxP sites. The NeoR gene was also floxed and was removed by Cre transient transfection. Clones were selected in which exons 2 and 3 remained in their floxed form and used to make the mice.

The stock was produced by homologous recombination. The Strain/ES cell line used to create the transgenic line was Bruce 4 (C57BL/6).

The genomic insertion site is : Distal mouse chromosome 4 (Nmnat1 locus).

The line is congenic on C57BL/6 (backcrossed for 10 generations)

There is no published reference for this line.

AR Aug 2011