MRC Harwell Institute

Group leaders name/ contact details:
Prof. Adrian Isaacs

Users name/ contact details:

Name of Mouse model or mutation:
eGFP

Description:
Insertion of eGFP into 3110043O21Rik gene

Expected volume of samples per month:

Type of mutation:
Insertion
(define location and size)
Chromosome 4 , Exon2 of 3110043O21Rik gene

Sequence details
(please provide 100 bp of sequence either side of the SNP or deletion. If a cassette has been inserted please provide details of the cassette and the sequence of the insertion and break points)

AATAAAGGCTCATTCTTTGCTTCTATTGGTATGTGACATTTTTCTAAGTCACTTGGGGTTTGATAGATATCTTTA
AATGGCTGAACCTGATCACTGTTCTTTTGTATGTCCCTGTTTAGCTATTGCAAGCGTTCGGATAATGTGAGACC
TGGAATGCAGTGAGACCTGGGATGCAGGGATGTACCCATACGATGTTCCAGATTACGCTTACCCATACGATGT
TCCAGATTACGCTAGAAGACCACCTCGTGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCA
TCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGC
GATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGC
CCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCA
GCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGAC
GACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGA
GCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACA
ACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCC
GCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCG
ACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAA
CGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGA
CGAGCTGTACAAGCCTCCGAGACGTGGCAAACCGATTCCGAACCCGCTGCTGGGCCTGGATAGCACC
TAATTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGC
ATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATGCTAGCCTATAGGGCT
CGAGGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGCGGCTAGCTCGACTATCTGCCCCCCACCAT

CTCCTGCTGTTGCCAAGACAGAGATTGCTTTAAGTGGTGAATCACCCTTGTTGGCGGCTACCTTTGCT
TACTGGGATAATATTCTTGGTCCTAGAGTAAGGCATATTTGGGCTCCAAAGACAGACCAAGTGCTTC
TCAGTGATGGAGAAATAACTTTTCTTGCCAACCACACT

CTC= 5' Hom Arm
TAC= HA tag
GGA- eGFP
GGC= V5 tag
TTG= SV40 PolyA
GAA= FRT site
GGA= 3' Hom Arm

Current genotyping method:

Gel based assay   qPCR   Melting Analysis   Digest

Other (please expand)

Current genotyping protocol:

Current genotyping protocol:
Genomic DNA is extracted using REDExtract-N-Amp™ Tissue PCR Kit according to manufactural instructions.
The genotyping protocol involved 2 different PCR analysis. The first PCR (TG-PCR) allows to detect presence of the TG and screen positive animals. The second PCR (Repeats-PCR) allows to establish repeat length.
An additional PCR (Flp-PCR) allows to detect presence of the Flp.

TG-PCR	Volume
REDExtract-N-Amp™ PCR	5 ul
C9orf72DPRGenF4 (10 µM)	0.5 ul
C9orf72DPRR628 (10 µM)	0.5 ul
Template DNA	2 ul (from extraction)
H2O	2 ul

Temperature	Time	Cycle

94 °C	3'	
94 °C	30''	30 cycles
58 °C	30''	
72 °C	40''	
72 °C	10'	

C9orf72DPRGenFor: TAAGCACAGCAGTCATTGGA
C9orf72DPRGenRev: AAGCGTAATCTGGAACATCG

Repeats-PCR	Volume
2x Xtreme™ Buffer	12.5 ul
dNTPs (2 mM each)	5 ul
C9orf72longFor1 (10 µM)	1.25 ul
C9orf72longRev1 (10 µM)	1.25 ul
DMSO	1 ul
Betaine (5M)	5 ul
Template DNA	0.6 ul (from extraction)
KOD Xtreme™ Hot Start DNA Polymerase (1.0 U/µL)	0.5 ul

Temperature	Time	Cycle
94 °C	3'	
98 °C	15''	8 cycles
74 °C	7'	

98 °C	15''	8 cycles
72 °C	7'	
98 °C	15''	8 cycles
70 °C	7'	
98 °C	15''	35 cycles
68 °C	7'	
68 °C	10'	

C9orf72long For1: CCCATACGATGTTCCAGATTACGCTTACCC
C9orf72long Rev1: GCAATAAACAATTAGGTGCTATCCAGGCCCAG

Flip-PCR	Volume
REDExtract-N-Amp™ PCR	5 ul
Flip Forward (10 µM)	0.5 ul
Flip Reverse (10 µM)	0.5 ul
Template DNA	2 ul (from extraction)
H2O	2 ul

Temperature	Time	Cycle
94 °C	3'	
94 °C	30''	30 cycles
58 °C	30''	
72 °C	40''	
72 °C	10'	

Flip Forward: ACGGAACAGCAATCAAGAGAGCCA
Flip Reverse: TCGATCCTACCCCTTGCGCTAAA