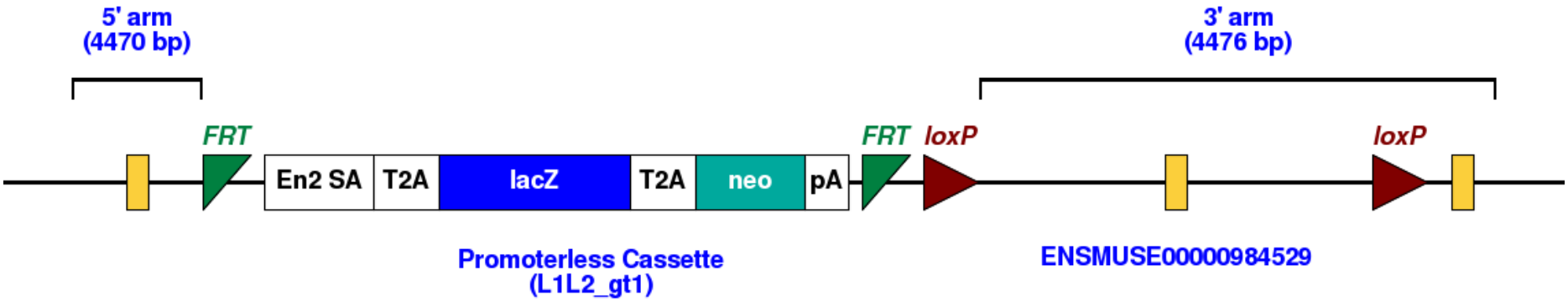
RNF7 (MYA)

Samples supplied are carrying the following allele



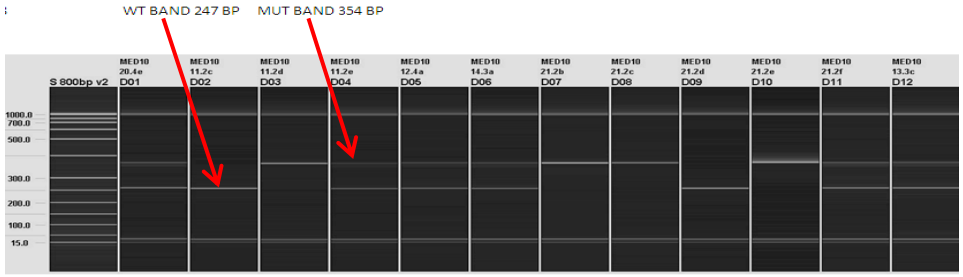
Details for the allele can be found below

http://www.knockoutmouse.org/martsearch/project/41332

RNF7 (MYA) Gel based Assay

Introduction

The gel based assays are normally run on the Qiagen QIAxcel. This is a capillary based system that provides clearer resolution and is quicker than running standard agarose gels. Different size ladders maybe loaded onto runs depending on the fragment sizes being analysed. Typically samples are run with a 50-800bp size ladder.



RNF7 primers run alongside an internal control 1260. PCR is performed using KAPA fast Taq polymerase, although alternatives may be used.

RNF7 gel based primers

RNF7_WT_for1	GGAAAGGAATTTTGCTTATGAA
RNF7_WT_for2	CACATAGCTTAAAAATGAAGGGAGT
RNF7_WT_Rev1	GCTTGCTCACACACATGGAT
MUT_Rev1	TTGGTGATATCGTGGTATCGTT
1260_1 (control)	GAGACTCTGGCTACTCATCC
1260_2 (control)	CCTTCAGCAAGAGCTGGGGAC1

Products:

WT Assay

RNF7_WT_ For1- RNF7_WT_ Rev1 292bp

MUT Assay

RNF7_WT_ For2- RNF7_MUT_Rev1 155bp

Internal Control Primer

1260_1 and 1260_2 585bp

RNF7 (MYA) EPD0057_2_C04

PCR mixes

WT Assay

Qiagen Taq PCR PCR master mix	5µl
RNF7_WT_for1 (20 µM)	0.5µl
RNF7_WT_Rev1 (20 µM)	0.5µl
1260_1 (20 µM)	0.5µl
1260_2 (20 µM)	0.5µl
H_2O	2µl
DNA	1µl

Mutant Assay

Qiagen Taq PCR PCR master mix	5µl
RNF7_WT_ For2 (20 µM)	0.5µl
RNF7_ MUT_Rev1 (20 µM)	0.5µl
1260_1 (20 µM)	0.5µl
1260_2 (20 µM)	0.5µl
H_2O	2µl
DNA	1µl

Cycling conditions

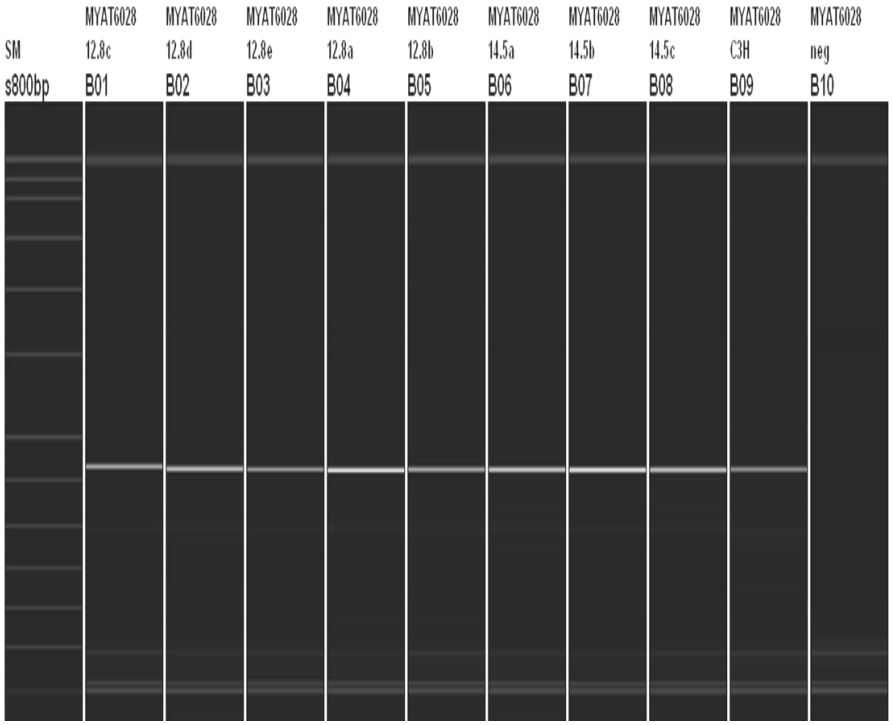
56TM30FA

1. 95ºC 1min
2. 95ºC 10s
3. 56ºC 10s
4. 72ºC 1s
5. Go to 2 for 29 cycles
6. 72ºC 30s
7. 16 ºC for ever
8. end

RNF7 (MYA) EPD0057_2_C04

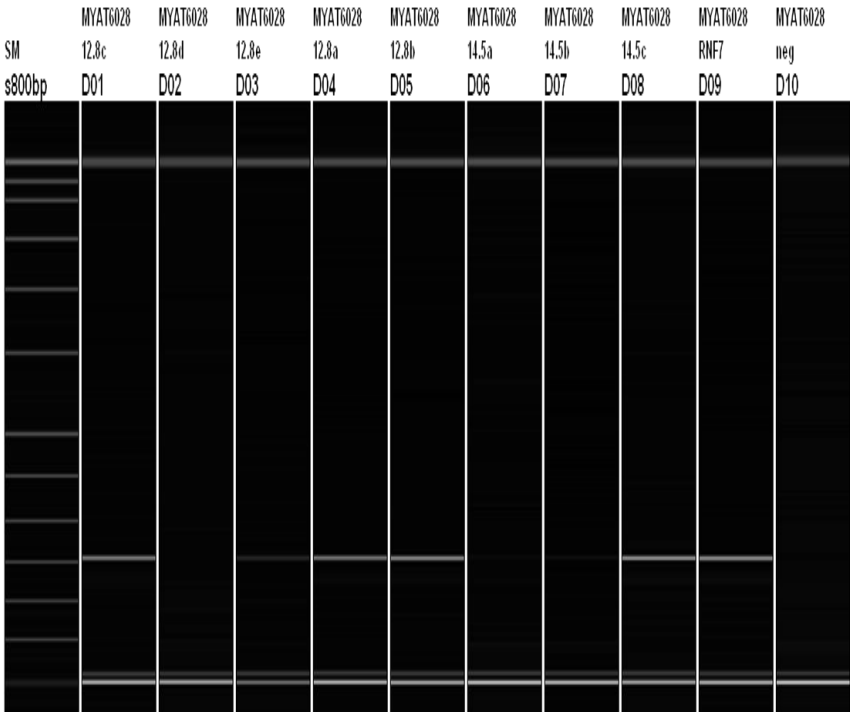
Example:
WT Assay

Expected band size (WT_ For1 and WT_ Rev1) 292bp
Samples 1-8 (controls-B6, NTC) (Internal control primer not used in this example)



Mutant Assay

Expected band size (WT_ For2 and MUT_Rev1) 155bp
Samples 1-8 (controls HET, NTC) (Internal control primer not used in this example)



qPCR Genotyping

RNF7 (MYA) sequences

5'homology arm (last 300bp) (sequence highlighted in yellow is forward qPCR primer)

TTGTTCAAGTACTTTGTCAGTGCTGAGTGACTGCATTGAGGACTAGGGGTTCGAGTCATCCTAGGCTATCTAGTTTGAGGCCAGCCTGTGCAACATGAAACCCAGTCTCAAAAACA
AAGGAAAGGAATTTTGCTTATGAAAACAACTAGAAAAAGCCTTTAACTTTCAAGTAGAGTCACATAGCTTAAAAATGAAGGGAGTATATGTGGAAACGTCATTACTGAACAGGGCT
GGGGCATGTGCCTGTGATTCCCAAACTTTGAAGAAGGTAGAGGCAGGATTTTAAGGTTGATCGTCCAA

3'homology arm (1st 300bp)

TAAACCAGAAATCAGAAAGCAGTGATTAATTATGAATATAAAATGTTAGGGGCTGGGGAGATGGCTCAGTAGGCAAAAGTTCTCGCCTCTCAAGCCTGTGAACTAAATTTGATCTC
TGGAACCCATTTAAGATAGAGAGAGAGATGGCTCCCAAAGGCCTCTGACCTGCACATGAAGCTGTGGCATGCACCTCCCCTCCCTCCCGTCCTGTACACACAATTATAAAAATAGT
TTTAAAGACAAAATCAGGATTAAAACTAAAAGGGAGTCTTCTAACAGAAGGCTCATGGTAAAACATCT

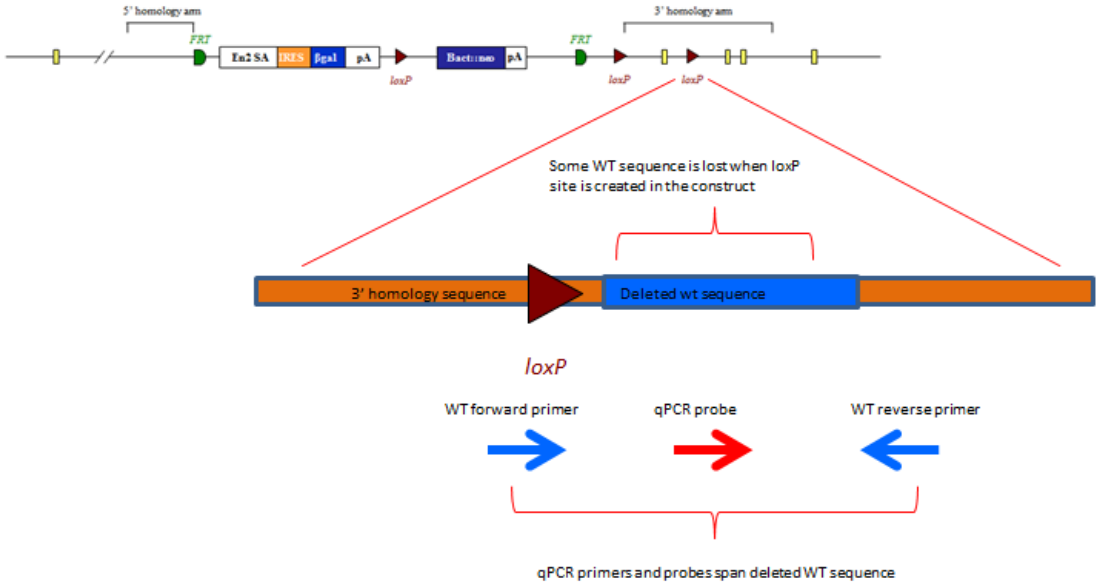
Forward qPCR primer: TCGTCCAAGTGTGGCAGTTC
Reverse qPCR primer: ACACACAGGATCTAGAAGAATACTACA
qPCR probe: AACTGGGCTATATGAGGCCCGATC

Genomic sequence (Sequences highlighted in turquoise are forward and reverse qPCR primers, sequence highlighted in red is the probe).

TTTGTTCAAGTACTTTGTCAGTGCTGAGTGACTGCATTGAGGACTAGGGGTTCGAGTCATCCTAGGCTATCTAGTTTGAGGCCAGCCTGTGCAACATGAAACCCAGTCTCAAAA
ACAAAGGAAAGGAATTTTGCTTATGAAAACAACTAGAAAAAGCCTTTAACTTTCAAGTAGAGTCACATAGCTTAAAAATGAAGGGAGTATATGTGGAAACGTCATTACTGAAC
AGGGCTGGGGCATGTGCCTGTGATTCCCAAACTTTGAAGAAGGTAGAGGCAGGATTTTAAGGTTGATCGTCCAAGTGTGGCAGTTCAGGTTAGTGGATAACTGGGCTATATG
AGGCCCGATCTCAAAAACAAGATCTTTAGTAGTTCCTGTAGTATTCTTCTAGATCCTGTGTGTATATAATCAAATAATCAATATACCGTTATGGCTTACTTATAGTGGTATCTTGA
AAGTAAATACCCCTGACTTTGATCCATGTGTGTGAGCAAGCTGTTGTCTTGCAATAACCTTTTTGGCATCTCTGTCTCGGGTGACTTCTTCTAGTGCAGTTGCCAAGCTATTTTGT
CATAAGAACTTGTAACATCATCACTGCCCTTGTAAAACCGTGGTGAGTTGTGGAATGCCATTTATTTTATTTCTCTTTAAAGAGTTGTTTTTAGATGAGAAGTTAATATTATAAG
ACTTCTCTTCATCTCTGATCCTCGGGGCTTTTGTTTGGCTTTGAGGGTCTGGGTCTTCAGTGGCCTCCTGAACTAGAGACGAGATTGGCTTTGTTTACTTTCAGATGCCTGCCTTC
GATGTCAAGCTGAAAACAAGCAAGAGGACTGTGTTGGTGCGTGGTCGTTCTTTCTCCTGCCTTTGCAACTCAAGGTTTTTCTTCTGTCTCTGTGGGAATGTTTGAATTTCCAATT
AAAATTGGCTTTATTAAACTCGCTGTGGTAACCC

qPCR assay design

Real time PCR assay for the Tm1a allele is typically designed on the deleted WT sequence around the 3' loxP breakpoint (WT break point loss of allele or BP- LOA assay). Primers or probes are designed to span the deleted WT sequence so will not be present in the EUCOMM allele. Assays may also be designed to the loxP breakpoint that is 5' to the critical region. A mutant Neo qPCR assay is also run to confirm the genotype. Both WT and Neo qPCR assays are FAM labelled and run in duplex with a VIC labelled internal control dot1l. Both sets of results are analysed together and copy counted using the ABI software CopyCaller.



Tm1a Genotyping

Example of a generic Tm1a-wt1 assay (FAM labelled probe)

Blue = Sequence in homology arm Black = breakpoint Red = critical region Example of a breakpoint region and Tm1a primers/probe

AAGTGTTGGTGAAGGGGTCACGAAGCTGAAGGCAGGTAAGGCAGGCATTTAAGCCACTCTGTGTGATGGTTTCGCCTTGTTTCTGTAGGAGCATTACAACTATTTATTTCCAACTA
TACAACTCTGTATGAGCCCATTCCTCCTCCTGGAAGAACACATATTTGGATTGATTTTAACTCGGATTTTGCAGGCTGCTTTTGCACTTGAAACTGTTTTAAATATATTAC<CCAA
ACCAATCTTGGCAAGTTAGCAAGCCTTTTTAGAGACACATCGGTGAGTCGGTGGTTTTGAAACACTC>CGTCATTCTCCATTATTTTGGGTTTTGTTTAGATCAGAAACTTTGCAA
TGAACTTTTCGTGCATGGCTGGTCAGTGTCTAAACCATGCTCTGTAGATAAAGTTTCTAAGCCTTGAGGTTGCCTTTCCAGACTTGTGTCAGCTGACGAGGGTAGAGTTCTGCTAC
GTTCACTAGAGCAGTGGTTCTCAGCCATGAAATTATTTCA

Primer 1 = GGCTGCTTTTGCACTTGAAAC Primer 2 = CCAGCCATGCACGAAAAGTTC Probe = AGACACATCGGTGAGTCGGTGGTTT

NEO assay (FAM labelled probe)

ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTC
AGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAG
CTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCT

Primer 1 = GGTGGAGAGGCTATTCGGC Primer 2 = GAACACGGCGGCATCAG Probe = TGGGCACAACAGACAATCGGCTG

Dot1l internal control (VIC labelled)

CCTAGCCATGGTGTTTGTGTGTCCAGTTCTCATGAGGCAAGCCTACAGCCTTCATCATTCTACAGTTGCCTTCATTACCCTACAGTCCACTTCTCCAGTGGAGCTGGGCCTGTGCA
AACCAGTGGGCAGTGGATGTGAAGGGCAGGAAGCTCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTCGTTTTCT
GTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGAC
TCTGTAGGATCTGCCCACACCCATCAGGTGTGCAGGGAGACAGAGCTGAGTCAGGCTCCAGCTCTGGGGAATATGTTGAGTCACCACCTCTGTAGGGTGGTTGTGCATCATAGAAC
AAGAGGACTTTGGGGTGTCACTGTGGTTGTTGGGTCCAACTGTGCATCTTTTCTCTTTCAGGACAAGCACCATGATGCTG

Primer 1 = GCCCCAGCACGACCATT Primer 2 = TAGTTGGCATCCTTATGCTTCATC Probe = CCAGCTCTCAAGTCG

qPCR Genotyping

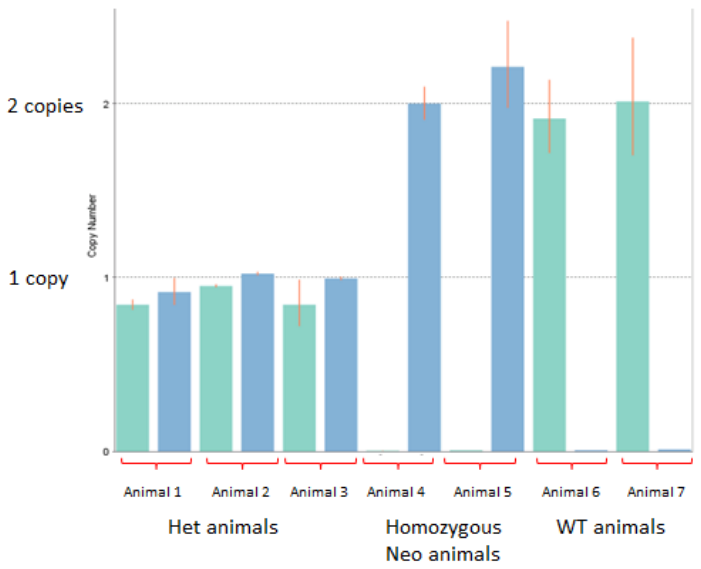
qPCR master mix

ABI GTX Taqman master mix	5µl
Primers Dot1L_2F (20uM)	0.45µl
Primers Dot1L_R (20uM)	0.45µl
Probe DotL_2M (5µM)	0.4µl
FAM Assay (probe 5µM & primers 15µM each)	0.6µl
Water	0.6µl

ALIQUOT 7.5µL

DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

Generic example of a NEO + LOA copy called result



Blue = NEO

Green = WT LOA

WT = NEO copy count of 0
LOA copy count of 2

Het = NEO copy count of 1
LOA copy count of 1

Hom = NEO copy count of 2
LOA copy count of 0