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Tmc6 (MCVS; EPD0168_5_E05)

Allele: Tmc6^{tm1a(EUCOMM)Wtsi}

Genotyping Information

These mice may be genotyped through a combination of separate PCR reactions that detect *LacZ*, the gene-specific wild type allele, and a mutant allele-specific short range PCR. Interpretation of the consolidated results produces the genotype of the mice.

For example: LacZ positive, mutant positive, wild type positive = heterozygous.

PCRs primer pairs and expected size bands

PCR type	Forward primer	Reverse primer	Expected size band (bp)
Mutant PCR	Tmc6_47475_F	CAS_R1_Term x	185
Wild type PCR	Tmc6_47475_F	Tmc6_47475_R	269
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

Primer sequences

Primer name	Primer sequence (5' > 3')	
CAS_R1_Term	TCGTGGTATCGTTATGCGCC	
Tmc6_47475_F	CTTAACCACGGAGCCCTCAC	
Tmc6_47475_R	GCAGGAAAGTAGGTGGGCAG	
LacZ_2_small_F	ATCACGACGCGCTGTATC	
LacZ_2_small_R	ACATCGGGCAAATAATATCG	

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Last updated: 11/04/2011



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Reaction

Reagent	μΙ
DNA (~50-100 ng)	1.0
10x Buffer	2.0
MgCl ₂ (50 mM)	0.6
PtTaq (Platinum Taq (Invitrogen))	0.2
dNTPs (100 mM)	0.2
Primer 1 (10 μM)	0.4
Primer 2 (10 μM)	0.4
H_2O	<u>15.2</u>
Total	20.0

Cycling conditions

Wild type and mutant PCRs

Cycle		Cycle			
1	94 °C	5 min	1	94 °C	5 min
2	94 °C	30 sec	2	94 °C	30 sec
3	58 °C	30 sec	3	60 °C	30 sec

LacZ PCR

°C 30 sec
°C 30 sec
°C 30 sec
o to '2' + 34 cycles
.ºC 5 min
°C forever

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