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Gap43 (MBSD; EPD0116_3_E02)

Allele: Gap43^{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	Gap43_47640_F	CAS_R1_Term x	240
Wild type PCR	Gap43_47640_F	Gap43_47640_R	369
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

Primer sequences

Primer name	Primer sequence (5' > 3')		
CAS_R1_Term	TCGTGGTATCGTTATGCGCC		
Gap43_47640_F	TGGACGCTTAGGGGAGAGAG		
Gap43_47640_R	TTCCAGGGCTCAAGAAAGG		
LacZ_2_small_F	ATCACGACGCGCTGTATC		
LacZ_2_small_R	ACATCGGGCAAATAATATCG		

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Reaction

Reagent	μΙ
DNA (~50-100 ng) 10x Buffer MgCl ₂ (50 mM) PtTaq (Platinum Taq (Invitrogen)) dNTPs (100 mM) Primer 1 (10 μ M) Primer 2 (10 μ M) H ₂ 0	1.0 2.0 0.6 0.2 0.2 0.4 0.4 15.2
Total	<u>20.0</u>

Cycling conditions

Wild type and mutant PCRs

LacZ PCR

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
4	72 °C	45 sec	4	72 °C	30 sec
5		· 34 cycles	5		- 34 cycles
6	72 °C	5 min	6	72 °C	5 min
7	12 °C	forever	7	12 °C	forever

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