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Rbbp7 (MBRK; EPD0126_4_F03)

Allele: *Rbbp7*$^{tm1a(EUCOMM)Wtsi}$

Genotyping Information

These mice may be genotyped through a combination of separate PCR reactions that detect *Lac*Z, 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: *Lac*Z 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	Rbbp7_41966_F	CAS_R1_Term x	134
Wild type PCR	Rbbp7_41966_F	Rbbp7_41966_R	448
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

➢ Primer sequences

Primer name	Primer sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Rbbp7_41966_F	CAGGTGAAGAGAATGAGACTGGAG
Rbbp7_41966_R	ACCAACAAAACCCTGCTTCC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

Last updated: 17/02/2010

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➢ Reaction

Reagent	**µl**
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_20	15.2
Total	**20.0**

➢ Cycling conditions

Wild type and mutant PCRs

Cycle		
1	94 °C	5 min
2	94 °C	30 sec
3	58 °C	30 sec
4	72 °C	45 sec
5	Go to '2' + 34 cycles	
6	72 °C	5 min
7	12 °C	forever

***LacZ* PCR**

Cycle		
1	94 °C	5 min
2	94 °C	30 sec
3	60 °C	30 sec
4	72 °C	30 sec
5	Go to '2' + 34 cycles	
6	72 °C	5 min
7	12 °C	forever

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