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Rab7l1 (MCSP; EPD0418\_1\_B05)

Allele: Rab7l1<sup>tm1a(EUCOMM)Wtsi</sup>

## **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	Rab7l1_39964_F	CAS_R1_Term x	219
Wild type PCR	Rab7l1_39964_F	Rab7l1_39964_R	485
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

# Primer sequences

Primer name	Primer sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Rab7l1_39964_F	CCAGGCAGTAACAAGGGGAG
Rab7l1_39964_R	GATGTCCAAAACCCTGTCTGC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

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#### Reaction

Reagent	μΙ
DNA (~50-100 ng)	1.0
10x Buffer	2.0
MgCl <sub>2</sub> (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
$H_20$	<u>15.2</u>
Total	20.0

# Cycling conditions

Wild type and mutant PCRs

Cycle				Cycle		
A *	04.00			A *	04.00	

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	Go to '2' -	+ 34 cycles	5	Go to '2' +	34 cycles
6	72 °C	5 min	6	72 °C	5 min
7	12 °C	forever	7	12 °C	forever

LacZ PCR

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