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Dus2I (MFHP; EPD0536\_5\_C02)

Allele: Dus2l<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	Dus2I_50993_F	CAS_R1_Term	354
Wild type PCR	Dus2I_50993_F	Dus2l_50993_R	616
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

## Primer sequences

Primer name	Primer sequence (5' > 3')	
CAS_R1_Term	TCGTGGTATCGTTATGCGCC	
Dus2l_50993_F	CAGACACTCCAGAAGACGGC	
Dus2l_50993_R	GGCTGGAACAGTAACTCAGCAG	
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)) dNTPs (100 mM)	0.2
Primer 1 (10 μM)	0.4
Primer 2 (10 μM)	0.4
H <sub>2</sub> 0	15.2
<b>Total</b>	<b>20.0</b>

# **Cycling conditions**

Wild type and mutant PCRs		LacZ PCR			
Cycle	е		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

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