

MGPgenotyping@sanger.ac.uk www.sanger.ac.uk

Cdh19 (MELB; EPD0426_1_A11)

Allele: Cdh19^{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	Cdh19_209428_F	CAS_R1_Term	206
Wild type PCR	Cdh19_209428_F	Cdh19_209428_R	378
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

Primer sequences

Primer name	Primer sequence (5' > 3')		
CAS_R1_Term	TCGTGGTATCGTTATGCGCC		
Cdh19_209428_F	TCTTAGGAGACAACTGACTTCCACC		
Cdh19_209428_R	CCATTTCAGGCAGTGGAGATG		
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 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 ₂ 0	<u>15.2</u>
Total	20.0

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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