

Aff3 (MCND; EPD0242_2_E12)

Allele: *Aff3*^{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	Aff3_96513_F	CAS_R1_Term x	131
Wild type PCR	Aff3_96513_F	Aff3_96513_R	315
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

➤ Primer sequences

Primer name	Primer sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Aff3_96513_F	AGCAGAGATCAGGAGGAGGC
Aff3_96513_R	CTGCCCGATATGAGGGAAAG
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG

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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 ₂ O	<u>15.2</u>
Total	20.0

➤ **Cycling conditions**

Wild type and mutant PCRs

Cycle	Temp	Time
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	Temp	Time
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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