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Prmt2 (MARU; EPD0197_1_C09)

Allele: *Prmt2*$^{tm1a(EUCOMM)Wtsi}$

Genotyping Information

Genotyping by short range PCR (srPCR)

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	Prmt2_40870_F	CAS_R1_Term x	274
Wild type PCR	Prmt2_40870_F	Prmt2_R3	357
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

➢ Primer sequences

Primer name	Primer sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Prmt2_40870_F	GATCGTCCAGTTCCTACGGG
Prmt2_R3	GGTCCCTTAGAAGCACAGGT
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_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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Genotyping by loss of WT allele qPCR Assay (gene-specific assay)

The wild type loss of allele (WTLoA) qPCR assay uses a hydrolysis probe assay (for example Applied Biosystems TaqMan® technology) to determine the copy number of the wild type allele in a sample. Homozygotes will show no amplification, heterozygotes one copy and wild type mice will show two copies when compared to a wild type control.

The number of copies of the Prmt2 allele can be detected using a FAM-labelled custom qPCR TaqMan® assay. These are multiplexed with a VIC® labelled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

Forward Primer Name	Forward Primer Seq.
PRMT2_WT_F	GCTCATCAATAGTCTTCCCAGTAGGT

Reverse Primer Name	Reverse Primer Seq.
PRMT2_WT_R	TGCTTCTAAGGGACCCACAGTTA

Reporter 1 Name	Reporter 1 Sequence	Reporter 1 Dye
PRMT2_WT_M	CCTCTGGTCAAGCGCA	FAM

➢ Reaction setup

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System with DNA prepared using the Sample-to-SNP™ kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems)

	Volume µl
2x GTXpress™ buffer	5
Prmt2_WT 20x assay	0.5
ddH2O	3
Tfrc 20x assay	0.5
DNA	1

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- **qPCR Cycling conditions**

Cycle		
1	95°C	20sec
2	95°C	10sec
3	60°C	30sec
4	Go to '2' + 34 cycles	

Results can be analysed using CopyCaller™ software (Applied Biosystems) or RQ Manager (Applied Biosystems). Both packages use the comparative Ct (ddCt) method to perform the analysis.
Other instrument systems will have their own analysis software – please see the manufacturer's guidelines for information about your system.

Genotyping by Neomycin copy number qPCR Assay (universal assay)

The *neo* count qPCR assay uses a hydrolysis probe assay (eg Applied Biosystems TaqMan technology) to determine the copy number of the *neo* cassette in a sample. Homozygotes will possess two copies of *neo*, heterozygotes one copy and wild type mice will show no amplification.
Please note that this assay is not gene-specific – other information should be used in conjunction with the *neo* count (for example the mutant-specific srPCR) when assigning the genotype.

Forward Primer Name	Forward Primer Seq.
NeoF	GGTGGAGAGGCTATTCGGC

Reverse Primer Name	Reverse Primer Seq.
NeoR	GAACACGGCGGCATCAG

Reporter 1 Name	Reporter 1 Sequence	Reporter 1 Dye
NeoM1	TGGGCACAACAGACAATCGGCTG	FAM

Reaction setup and amplification conditions are the same as those used for the LoA qPCR assay, with the *neo* taking the place of the Prmt2 probe and primers.

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