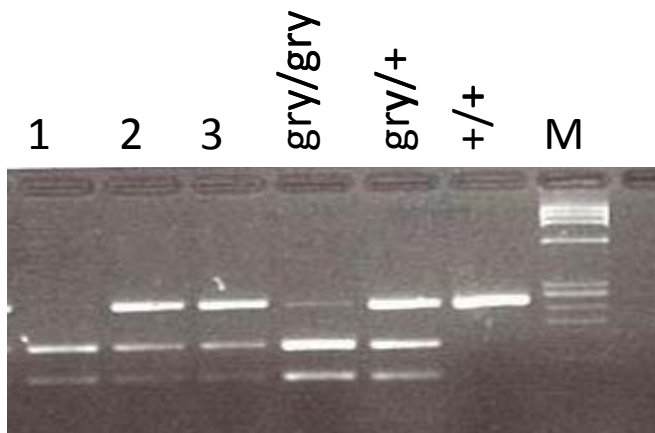


Gene symbol	<i>Cacna1a<sup>gry</sup></i>
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primer	Sequence(5'-3')	Size	target gene	accession
rCacna1a-14-F rCacna1a-14-R	CGTCCTGAAGTCAATCATGAAG AGTCTCAGCTGCTCTGTGGTT	256bp	<i>Cacna1a</i>	NM_012918.1
comment				

PCR condition							
Taq polymerase		BIOTAQ™ HS DNA Polymerase (Bioline Reagents Ltd., UK)					
Thermal cycler		PC-808(ASTEC)					
PCR buffer		Ampdirect Plus					
		<b>first denature</b>	<b>denature</b>	<b>anneal</b>	<b>extension</b>	<b>cycle</b>	<b>final extension</b>
PCR	°C	94	94	60	72	35	72
	min	3	0.5	0.5	1		3
comment		<p>①PCR method; Blood was applied to FTA®card (GE Healthcare UK Ltd.,UK) and dried. 1.5mm FTA disc was removed from the bloody-stained region on FTA®card. Untreated sample discs were placed directly in 15 μL PCR mixture containig 1 × Ampdirect®Plus, 0.2 μM each primer and 0.4 units of BIOTAQ™ HS DNA Polymerase.</p> <p>②RFLP of PCR products; <i>Cacna1a gry</i> mutaion make <i>PsiI</i> cutting site in the <i>Cacna1a gry/gry</i> genome.</p>					

Electrocataphoresis ;4% agarose gel 300V, 30min



M: (ϕX174/Hae III digest)

Strain harboring mutation	GRY/Idr, WTC.GRY- <i>Cacna1a<sup>gry</sup></i> /Kyo
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