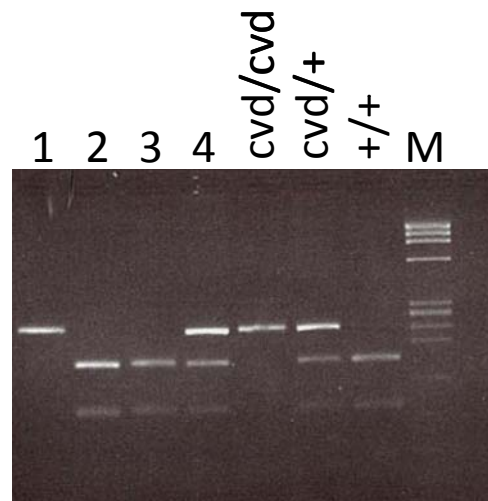


Gene symbol	<i>Unc5c^{cvd}</i>
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primer	Sequence(5'-3')	Size	target gene	accession
rUnc5h3-15 rUnc5h3-16	CAGATCAGCTCTGCTTTCCA ATTTTCAAATTGGGCAGTGG	238bp	Unc5h	NM_199407.1
comment				

PCR conditions							
Taq polymerase		BIOTAQTM HS DNA Polymerase (Bioline Reagents Ltd., UK)					
Thermal cycler		PC-808(ASTEC)					
PCR buffer		Ampdirect Plus					
		first denature	denature	anneal	extension	cycle	final extension
PCR	°C	94	94	55	72	35	72
	min	3	0.5	1	0.75		3
comments		①PCR method; Blood was applied to the FTA®card (GE Healthcare UK Ltd.,UK) and dried. 1.5mm 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 BIOTAQTM HS DNA Polymerase. ②RFLP of PCR products;Unc5c cvd mutaion causes BstEII cutting site in the Unc5c +/- genome.					

Electrocataphoresis ;4% agarose gel 300V, 30min



Strain harboring mutation	F344.CVD- <i>Unc5c^{cvd}</i> /Kyo
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