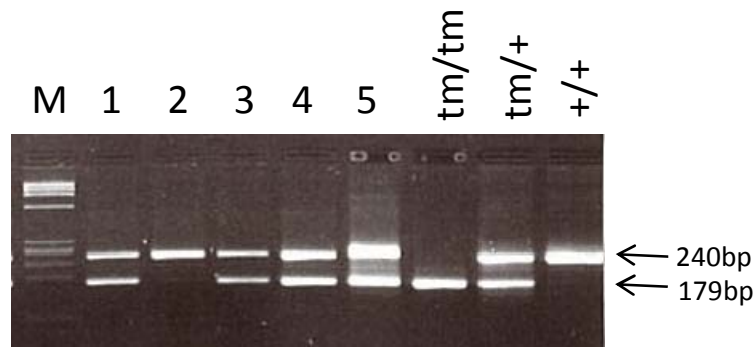


Gene symbol	<i>tm</i>
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primer	Sequence(5'-3')	Size	target gene	accession
TRM brk(+)-F TRM brk(+)-R	CAATTAGGTAAGATTCCCAGCATTT CACTCCATGCTTCCTTTTCAACAGC	240bp	Aspa	NM_024399.1
TRM brk(-)-F TRM brk(-)-R	TTCAACAGAGGAATGGATACAGAAA CACTCCATGCTTCCTTTTCAACAGC	179bp	Aspa	NM_024399.1
comment				

PCR condition							
Taq polymerase		BIOTAQ™ 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	40	72
	min	3	0.5	1	0.75		3
comment		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 μ L PCR mixture containig 1 × Ampdirect®Plus, 0.2 μ M each primer and 0.4 units of BIOTAQ™ HS DNA Polymerase					

Electrocataphoresis ;4% agarose gel 300V, 30min



M: (ϕX174/Hae III digest)

Strain harboring mutation	SER/Kyo, TRM/Kyo, TRMR/Kyo
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