PCR protocol

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RBRC 10078 **Strain name** C57BL/6N-Samd9l<em1(D764N)Ndmo>

Primer	primer_name	primer_seq	length		Reference
1	Exon2	GACACAGACATAGAGAAAGATGAATC	26		RIKEN BRC
2	Exon2	CTATAAAGGCATTGATGGCATTCTG	25		RIKEN BRC
3	WT	CTTACTTGTTCTCCAATCTCTACAAAATC	29		RIKEN BRC
4	MT4	GTGCTGTCTTGAAAAACAAAGCAACTA	27		RIKEN BRC

Reaction Components

Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

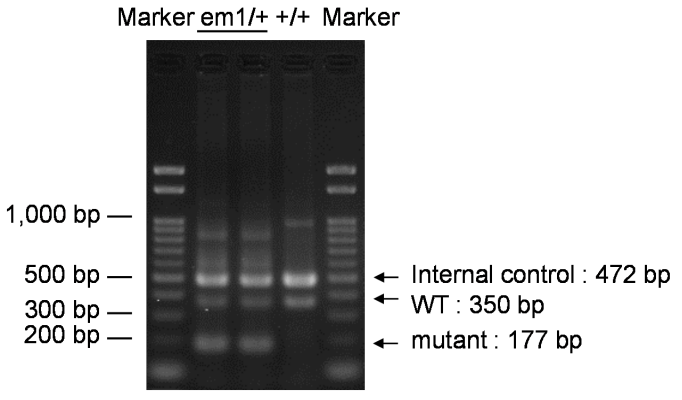
Reagent	Conc	Reaction 1	2	3	4	
primer 1	10 pmol/μl	1.0				(μL)
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Master Mix	2 X	5.0				
	total	10				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	65	90	*
4	72	60	* *Repeat for 30 cycles

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	472 bp	Internal control	+/+ (wild-type): 350 bp em1/+ (heterozygous): 350 bp and 177 bp
primer 1	primer 3	350 bp	WT	
primer 2	primer 4	177 bp	mutant	

Comment Separated PCR