PCR protocol

RBRC 04737 **Strain name** B6.129S2-Nmu<tm1Mko> Nms<tm1Mko>

Primer	primer_name	primer_seq	length		
1	Ex8_Fw	GCAGAATACCAGAGTCCTTCCGTG	24		RIKEN BRC
2	G12	CAGGGTAGTGGAGGAAGTGAAACCACATCG	30		Depositor's primer
3	Neo	GCAATCCATCTTGTTCAATGGCC	23		Depositor's primer
4		GCGCCTGGCTGCCAAGCTCGC	21		RIKEN BRC
5	NMU2-3	TGAAAAGGAAAAATGGCCTCCCCAGGCTGG	30		Depositor's primer
6	Neo3	GGACTGGCTGCTATTGGGCGAAGTGCCGGG	30		Depositor's primer

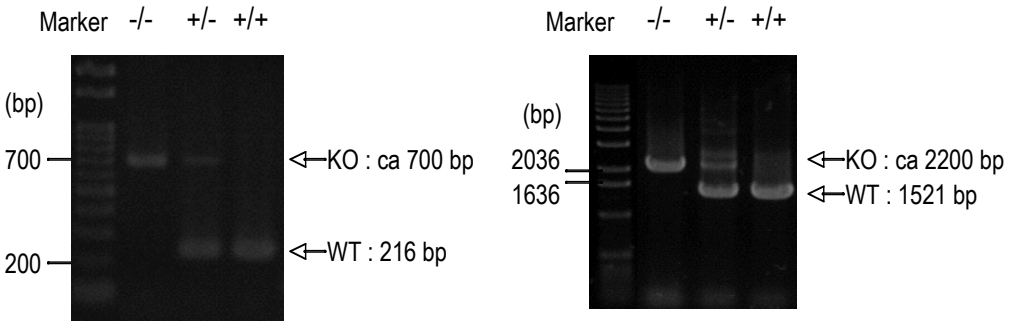
Reaction Components

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.9	3.9		
PCR buffer	2 X GC I	10.0	10.0		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	5U/μl	0.1	0.1		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	180		
2	94	30	*	
3	60	30	*	
4	72	120	*	*Repeat for 30 cycles
5	72	300		



Marker

marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	216 bp	WT (Nmu)
primer 1	primer 3	ca 700bp	KO (Nmu)
primer 4	primer 5	1521 bp	WT (Nms)
primer 4	primer 6	ca 2200 bp	KO (Nms)

Comment