PCR protocol

Sheet ID PS_08016
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RBRC 10980 **Strain name** B6NJcl.Cg-Mdga2<tm1Tymo>

Primer	primer_name	primer_seq	length		Reference
1	m29-2 check1 for	GTACGGTCTCGTGTGGCT	18		Neuron, 91(5):1052-1068 (2016).
2	m29-2 check1	AGACTGCAAGAGCCCATGTTTC	22		Neuron, 91(5):1052-1068 (2016).
3	Neo KO P1	ACCGCTTCCTCGTGCTTTACGGTATC	26		Neuron, 91(5):1052-1068 (2016).

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
total		20			

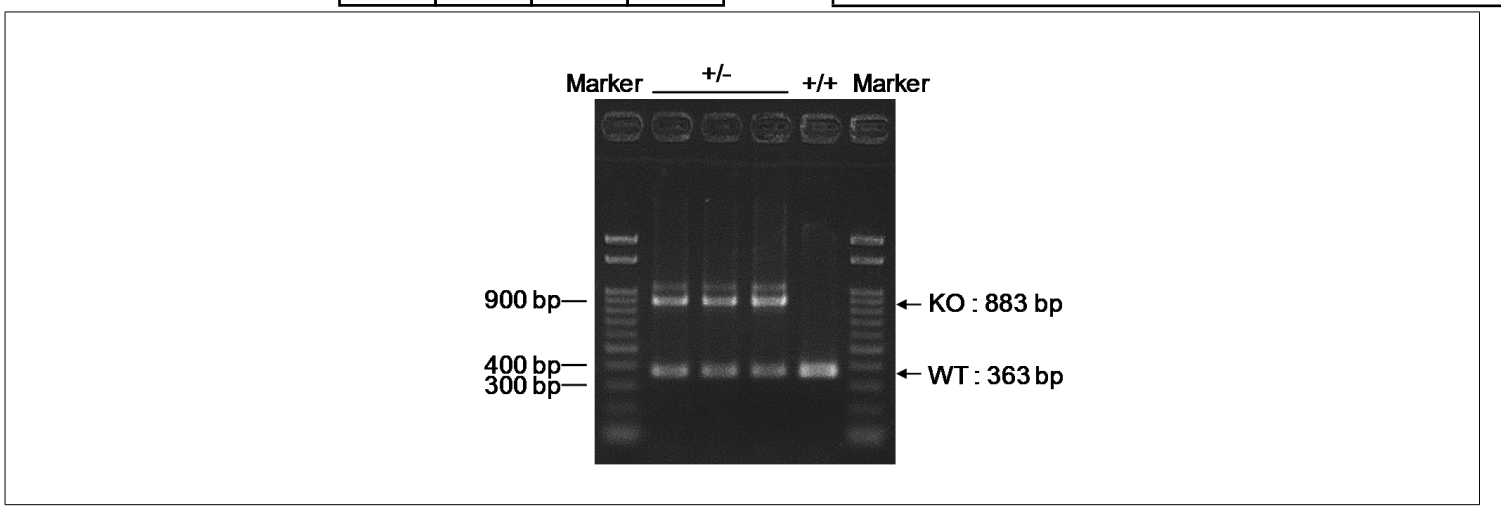
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	68	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	363 bp	WT	+/+ (wild-type): 363 bp +/- (heterozygous): 363 bp and 883 bp -/- (homozygous): 883 bp
primer 2	primer 3	883 bp	KO	

Comment Standard PCR

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.