PCR protocol

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RBRC 05286 **Strain name** B6.Cg-Mdga1<tm1.1Tymo>/TymoRbrc

Primer	primer_name	primer_seq	length		Reference
1	m29-1 check1	CATCGGTGGCCTGTTGGATC	20		Dev. Dyn., 240(1):96-107 (2011).
2	m29-1 check2	GCACTTTGAGCAAGATCTTG	20		Dev. Dyn., 240(1):96-107 (2011).
3	LacZ+Neo-KO	GCTGGCTGCCATGAACAAAGGTTGG	25		Depositor's primer

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			

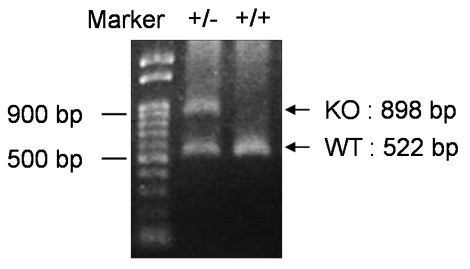
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	57	30*	
4	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	522 bp	WT
primer 3	primer 2	898 bp	KO

Comment Standard PCR

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