

RBRC 10977

Strain name B6Jcl.Cg-Mdga1<tm1.1Tymo>

Primer	primer_name	primer_seq	length	Reference
1	MDGA1 Fwd5	GTTTCAGCACCCCTCGGTGGAC	20	Depositor's primer
2	Wild Rev5	GAGACCTTACCCCGGCATCC	20	Depositor's primer
3	Mut Rev5	CCAGGGTTTTCCCAGTCACG	20	Depositor's primer

**Reaction Components**

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

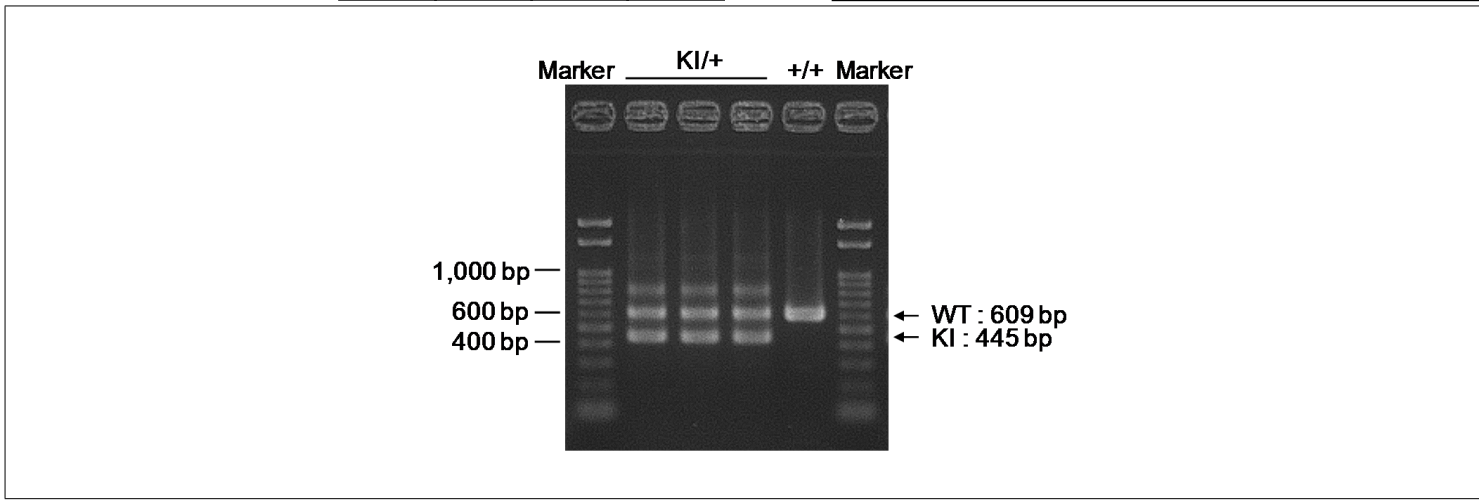
**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(μL)
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				
<b>total</b>		<b>20</b>				

**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	609 bp	WT
primer 1	primer 3	445 bp	KI

+/+ (wild-type): 609 bp  
 KI/+ (heterozygous): 609 bp and 445 bp  
 KI/KI (homozygous): 445 bp

**Comment** Standard PCR

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