

PCR protocol

RBRC 04436 Strain name B6.129-Msi2<tm10kn>

Primer	primer_name	primer_seq	length	
1	msi2R-1	CGTGACGTCACCGCATTGGTTACA	24	Depositor's primer
2	Msi2_Ex2	CTGGTGAGGTCTGCCAGCTCAG	22	RIKEN BRC
3	msi2R-3	CCAAATTAAGGGCCAGCTCATTCT	25	Depositor's primer

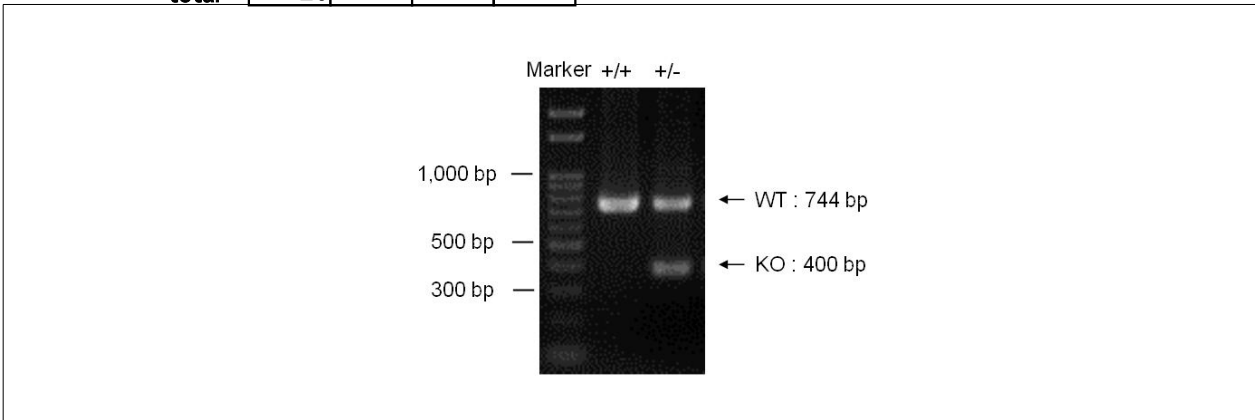
Reaction Components

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

Reaction Conditions

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

Conc	Run 1	Run 2	Run 3	Run 4
H2O	1.6			
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	0.4			
total	20			



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

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

PCR products

primer_set	product_size	product
primer 1 primer 2	744 bp	WT
primer 1 primer 3	400 bp	KO

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