

# PCR protocol

RBRC 05473

Strain name B6.129S6-Mad2l2<tm1Myo>

Primer	primer_name	primer_seq	length	
1	mRev7-genome-F	ACAAAGAGCTACTAAGCACCTTG	23	Depositor's primer
2		GTGGAGTGTGTCCTGGATGTACTG	24	RIKEN BRC
3	NeoS	TGCATACGCTTGATCCGGCTAC	22	

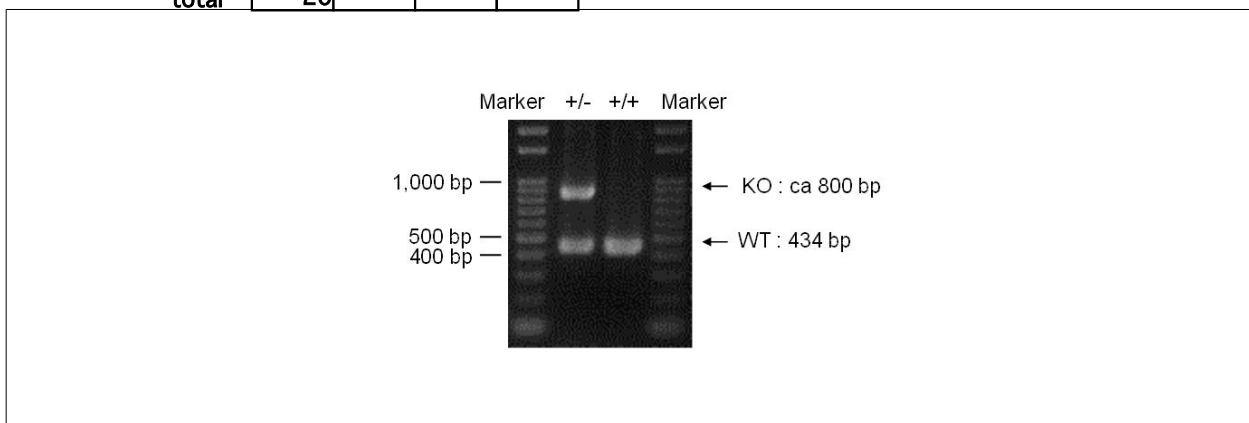
### Reaction Components

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

### Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/ $\mu$ l	1.0			
primer 2	10 pmol/ $\mu$ l	1.0			
primer 3	10 pmol/ $\mu$ l	1.0			
DNA		1.0			
Taq	1 U/ $\mu$ l	0.2			
<b>total</b>		<b>20</b>			

step	temp ( $^{\circ}$ C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	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, 35 min)

PCR products			
	primer_set	product_size	product
	primer 1	primer 2	434 bp WT
	primer 2	primer 3	ca 800 bp KO

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