

# PCR protocol

RBRC 02097

Strain name STOCK Izumo1 &lt;tmOsb&gt;

Primer	primer_name	primer_seq	length
1	Pr-B	GGGTTCACTCTCCAGCTACCCCAAACCTCAC	30
2	Pr-C	GCTTGCCGAATATCATGGTGGAAAATGGCC	30
3	02097_1	gtgaagcattagcgcttgagggcg	24

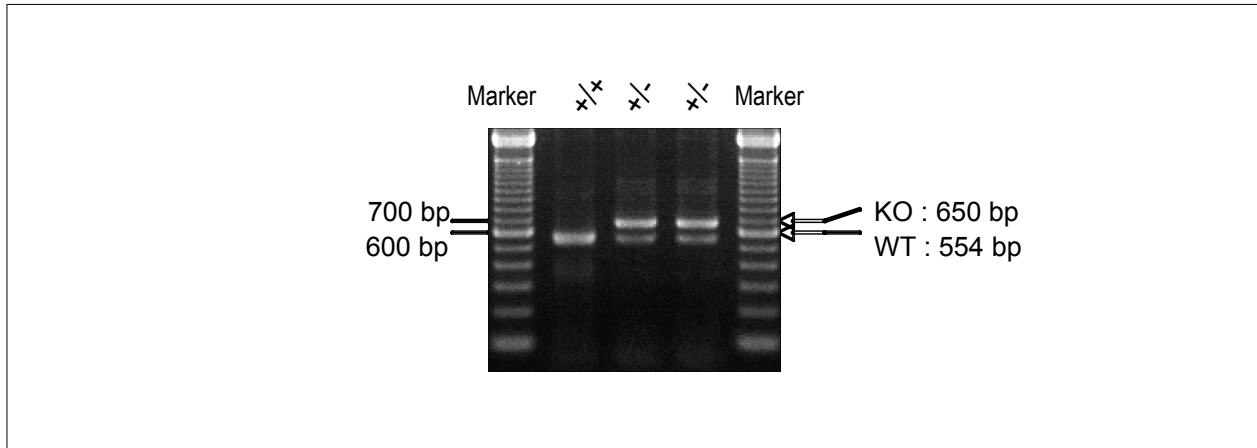
**Reaction Components**

Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

**Reaction Conditions**

	Conc	Run 1	Run 2	Run 3
H2O		3.8		
PCR buffer	2 X GC I	10.0		
dNTP	2.5 mM	2.0		
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		0.2		
<b>total</b>		<b>20</b>		

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*repeat for 30 cycles
5	72	300	


**Marker**

100 bp ladder (Invitrogen, Cat. 15628-050)

**Gel**

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

**PCR products**

primer_set	product_size	product
primer 1 primer 3	554 bp	wild
primer 2 primer 3	650 bp	KO

**Comment**