

# PCR protocol

RBRC 06167 Strain name B6;129-Ece2<tm1Ywa>

Primer	primer_name	primer_seq	length	
1	Fw	CAGGGATGCAGAGTGAGACTGTC	23	RIKEN BRC
2	HK18	CTAGAATGGGCCCTACCTT	20	J. Clin Invest., 105, 1373-1382 (2000).
3		AAGAACTCGTCAAGAAGGCGATAGAAGGCG	30	

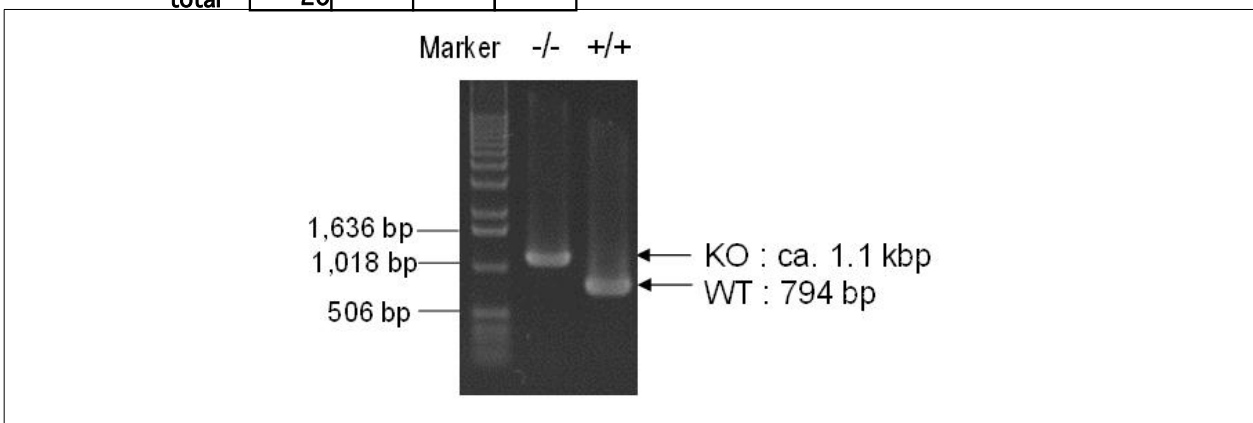
### Reaction Components

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/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>			

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



### Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

### Gel

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

### PCR products

primer_set	product_size	product
primer 1 primer 2	794 bp	WT
primer 1 primer 3	ca. 1.1 kbp	KO

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