

RBRC 01390

Strain name Nrf2 knockout mouse/C57BL6J

Primer	primer_name	primer_seq	length	
1	NRF2-5'	TGGACGGGACTATTGAAGGCTG	22	Depositor's primer
2	lac Z	GCGGATTGACCGTAATGGGATAGG	24	Depositor's primer
3	Nrf2-AS	GCCGCCTTTTCAGTAGATGGAGG	23	Depositor's primer

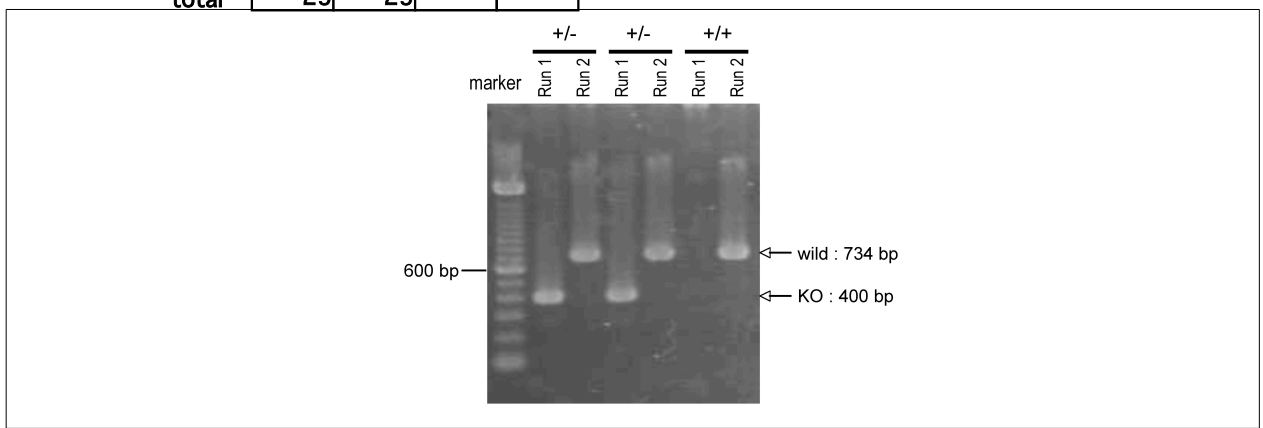
### Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

### Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/ $\mu$ l	1.0	1.0		
primer 2	10 pmol/ $\mu$ l	1.0	0		
primer 3	10 pmol/ $\mu$ l	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
<b>total</b>		<b>25</b>	<b>25</b>		

step	temp (°C)	time (sec)	note
1	94	300	
2	94	30*	
3	62	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 2	400 bp	KO
primer 1	primer 3	734 bp	WT

Comment

# PCR protocol

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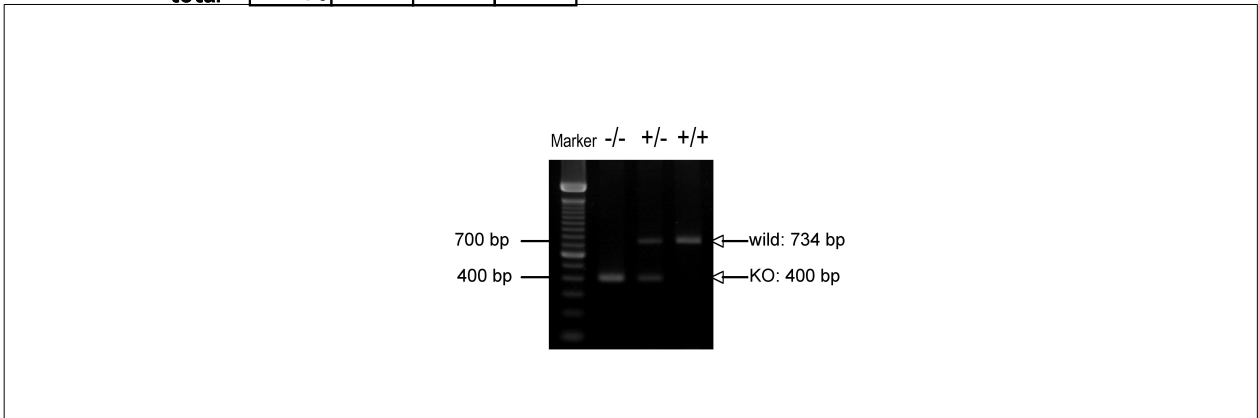
### Reaction Components

Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

### Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.0			
primer 1	10 pmol/ $\mu$ l	1.0			
primer 2	10 pmol/ $\mu$ l	1.0			
primer 3	10 pmol/ $\mu$ l	1.0			
Master Mix	2 X	5.0			
DNA		1.0			
total		10			

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	90*	*Repeat for 25 cycles
5	72	600	



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

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

PCR products				
primer_set	primer_1	primer_2	product_size	product
primer 1	primer 2		400 bp	KO
primer 1	primer 3		734 bp	WT

Comment

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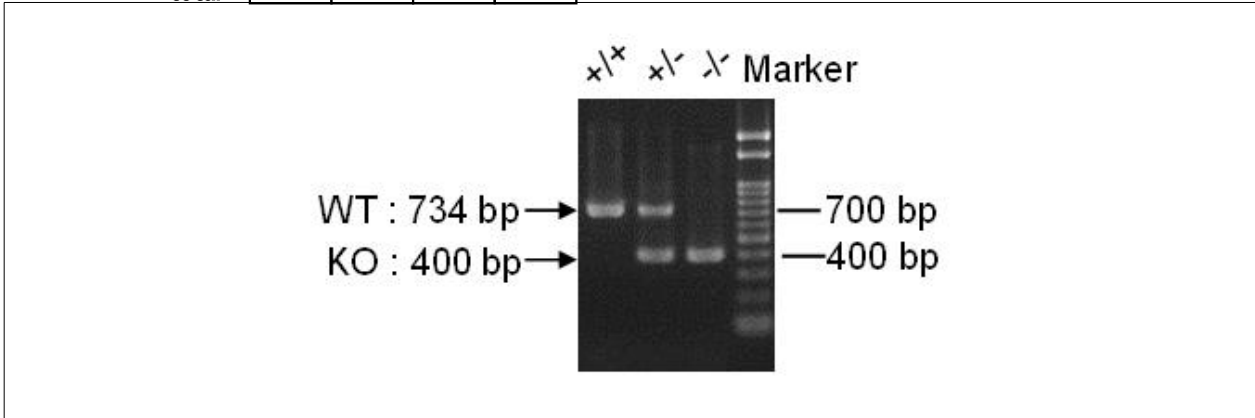
### Reaction Components

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

	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>			



**Marker**  
 Gene Ladder 100 (Wako, Cat. 316-06951)  
**Gel**  
 2% agarose in 1 X TAE (100 V, 30 min)

PCR products		product_size	product
primer 1	primer 2	400 bp	KO
primer 1	primer 3	734 bp	WT

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