

RBRC 10693

Strain name C57BL/6-Nr3c1<tm1.1Himo>

Primer	primer_name	primer_seq	length	Reference
1	GR3' LU	AAAAGAGCAGTGGAAGGTAGTGTG	24	eLife 7: e34135 (2018).
2	GR3' LL	TACTTCTGTCCCTTCCCAATGGAG	24	eLife 7: e34135 (2018).
3	intron 2	CTGGTCAGAGCAGCAGACTCG	21	RIKEN BRC

### Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/ $\mu$ l	1.0	0		
primer 2	10 pmol/ $\mu$ l	1.0	1.0		
primer 3	10 pmol/ $\mu$ l	0	1.0		
DNA		1.0	1.0		
Taq	1 U/ $\mu$ l	0.2	0.2		
<b>total</b>		<b>20</b>	<b>20</b>		

### Reaction Conditions

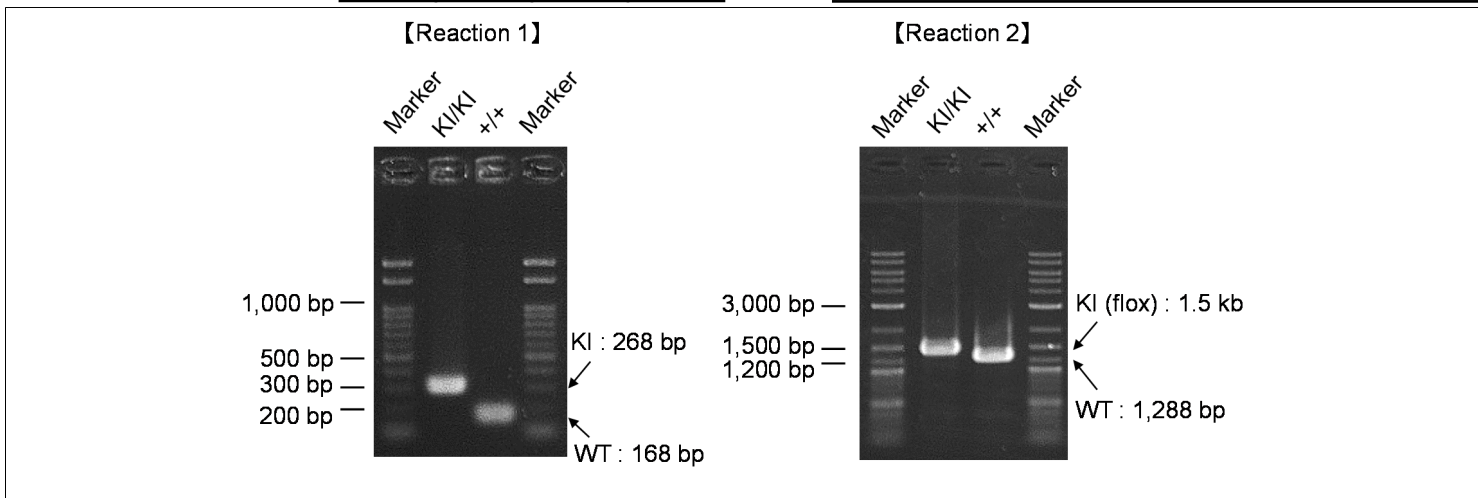
step	temp (°C)	time (sec)	note
1	94	120	[Reaction 1]
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles
1	94	120	[Reaction 2]
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

### Marker

marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 2-Log DNA Ladder (NEB, Cat. N3200)

### Gel

Run1:2% agarose in 1 X TAE (100 V, 30 min)Run2:1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1 primer 2	168 bp	WT
primer 1 primer 2	268 bp	KI
primer 2 primer 3	1,288 bp	WT
primer 2 primer 3	1.5 kb	KI (floxed)

Reaction 1    +/-: 168 bp  
                 KI/KI: 268 bp

Reaction 2    +/-: 1,288 bp  
                 KI/KI: 1.5 kb

### Comment

Standard PCR

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