

RBRC 11605

Strain name B6.Cg-Keap1<em1Iha>//Ahosh

Primer	primer_name	primer_seq	length	Reference
1	Left flox forward	CAAGCTGTAAACTGCCAGTCA	21	Depositor's primer
2	left flox reverse	GGACTGGTCTTCTGGCTTGG	20	Depositor's primer
3	right flox forward	GAGACGGTACCTGTTCTTGTCC	22	Depositor's primer
4	right flox reverse	CCAGAGCACTACCACTGAACT	21	Depositor's primer

**Reaction Components**

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

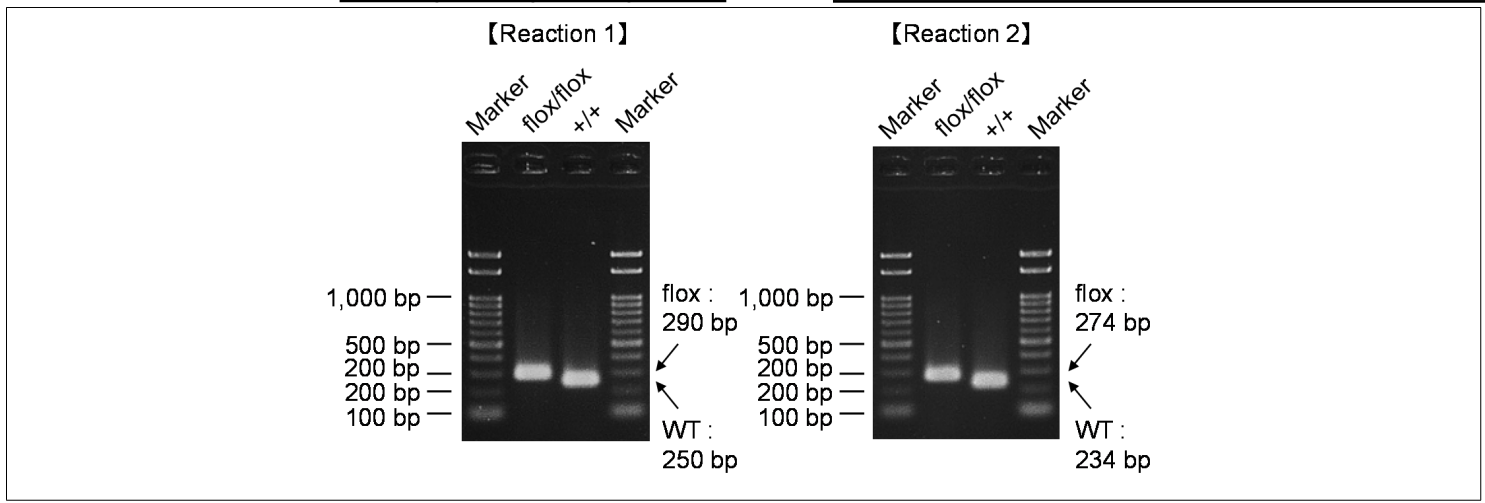
**Reaction Conditions**

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

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

**Marker** Gene Ladder 100 (Wako, Cat. 316-06951)

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



primer_set	product_size	product (allele type)	
primer 1	primer 2	250 bp	WT
primer 1	primer 2	290 bp	flox
primer 3	primer 4	234 bp	WT
primer 3	primer 4	274 bp	flox
			Reaction 1
			+/+ (wild-type): 250 bp
			flox/flox (homozygous): 290 bp
			Reaction 2
			+/+ (wild-type): 234 bp
			flox/flox (homozygous): 274 bp

**Comment** Standard PCR

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