

RBRC 11278

Strain name C57BL/6N-Pkn2<tm1.1Hmuk>

Primer	primer_name	primer_seq	length	Reference
1	N2-ex19	CACAAGGGCTGTAGATTGGTGG	22	Genes Cells. Feb;22(2):220-236 (2017).
2	N2-neoR	CTGAGTCCCGTCTCATGC	18	Genes Cells. Feb;22(2):220-236 (2017).
3	intron 17	CACACACTGAGAGGTGCTGCTC	22	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		0.2	0.2		
<b>total</b>		20	20		

### Reaction Conditions

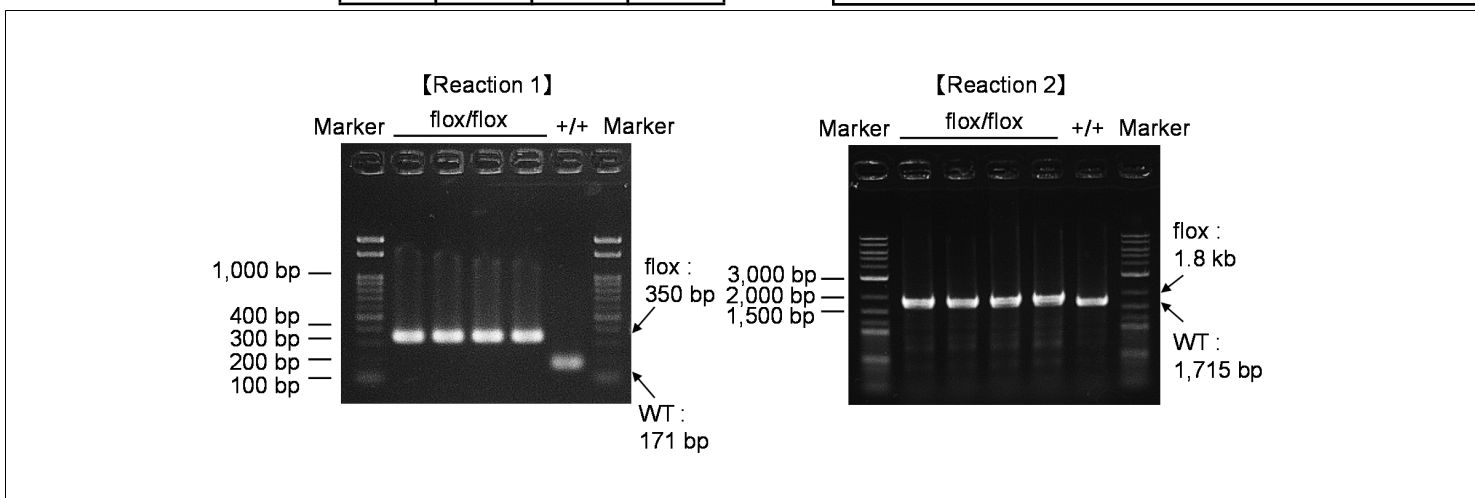
step	temp (°C)	time (sec)	note
1	94	120	[Reaction 1]
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles
1	94	120	[Reaction 2]
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

### 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	171 bp	WT
primer 1	primer 2	350 bp	flox
primer 2	primer 3	1,715 bp	WT
primer 2	primer 3	1.8 kb	flox
primer 2	primer 3		null (After cre mediated excision)
			Reaction 1
			+/+ (wild-type): 171 bp
			flox/flox (homozygous): 350 bp
			Reaction 2
			+/+ (wild-type): 1,715 bp
			flox/flox (homozygous): 1.8 kb

Comment Standard PCR

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