

RBRC 06248

Strain name B6.129-Brd1 &lt;tm2Hko&gt;

Primer	primer_name	primer_seq	length	Reference
1	ex2_Rv	CTGCAGAACCTCGATGGCATC	21	RIKEN BRC
2	Brd1 <sup>CKO</sup> Genotype Fw #310	GCTTGGTCTAAAGAGGTGTC	20	Depositor's prime
3	int2_Rv	CTGAAGCAGTCCCTAAACTGCAG	23	RIKEN BRC

**Reaction Components**

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

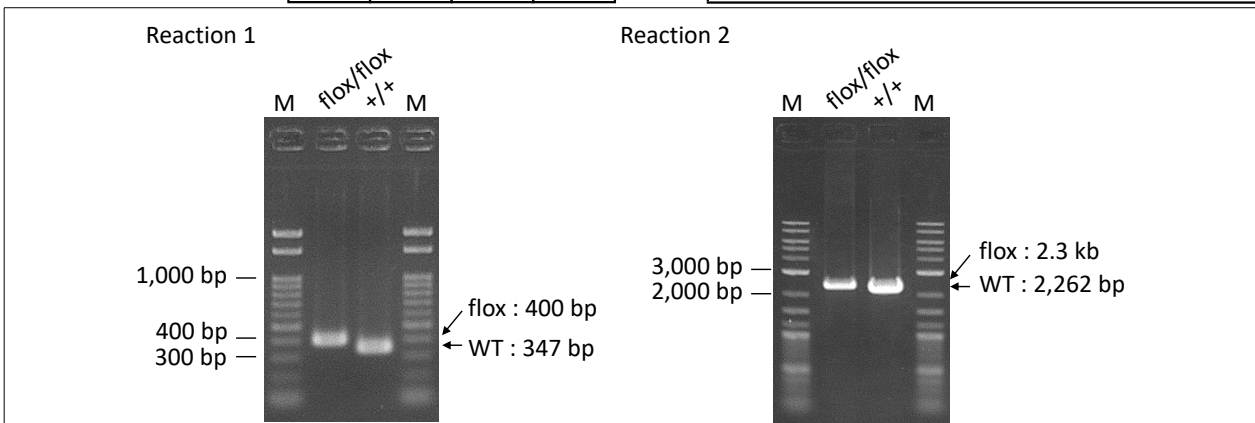
**Reaction Conditions**

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

Reagent	Conc	Reaction 1	2	3	4	( $\mu$ L)
H <sub>2</sub> O		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>			

**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	347 bp	WT
primer 1	primer 2	ca. 400 bp	flox
primer 2	primer 3	2262 bp	WT
primer 2	primer 3	2.3 kb	flox
primer 2	primer 3	predicted size 0.5 kb	null (After cre mediated excision)

Reaction 1 flox/+ : 400 bp, 347 bp  
flox/flox: 400 bp  
+/: 347 bp  
Reaction 2 flox/+ : 2.3 kb, 2262 bp  
flox/flox: 2.3 kb  
+/: 2262 bp  
+/-: 2262 bp, predicted size 0.5 kb

**Comment** [Standard PCR](#)

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