PCR protocol

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RBRC 01308 **Strain name** HB-EGF lox Knock-in mice(BL6) (HBlox#80/B6)

Primer	primer_name	primer_seq	length		
1	promoter	CAGCGGACAGTGCCTTAGTGG	21		RIKEN BRC
2	exon 1	GAGGAGGCCGCAATAAGACCC	21		RIKEN BRC
3	lacZ-R	TCTGCTTCAATCAGCGTGCC	20		Genes Cells, 1:59-72 (1996).

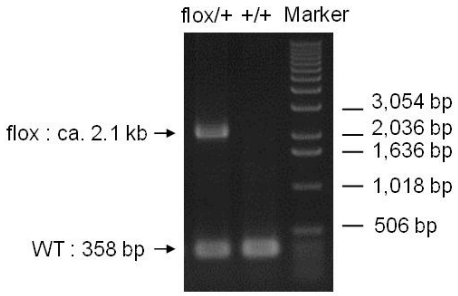
Reaction Components

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

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.6			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.4			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	68	180	* *Repeat for 30 cycles



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	358 bp	WT
primer 1	primer 3	ca. 2.1 kb	flox

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