PCR protocol

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RBRC 02467 **Strain name** B6;129-Hprt1<tm1Mok>

Primer	primer_name	primer_seq	length		
1		CTTGGCAAGATCAGCAAGTGCTA	23		RIKEN BRC
2	intron4	GGACTGTGAGGGTTAATGGTATATTC	26		RIKEN BRC
3	5Neo	TGGATTGCACGCAGGTTCTC	20		Proc. Natl. Acad. Sci. USA, 94, 13069-13074 (1997).
4		GAGAGAACTGCTACCACTGAGAG	23		RIKEN BRC
5	exon 4	GTTATTGGTGGAGATGATCTCTCAAC	26		RIKEN BRC
6	Neo10	ATCAGAGCAGCCGATTGTCTGTTG	24		

Reaction Components

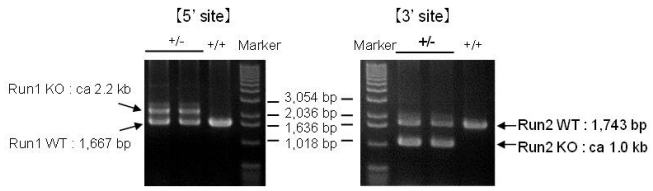
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	0.8		
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	1.0	0		
primer 4	10 pmol/μl	0	2.0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

Separated PCR



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	1,667 bp	WT(5')
primer 1	primer 3	ca 2.2 kb	KO(5')
primer 4	primer 5	1,743 bp	WT(3')
primer 4	primer 6	ca 1.0 kb	KO(3')

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