PCR protocol

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RBRC 09893 **Strain name** C57BL/6-Crh<tm1.1(venus)Ksak>

Primer	primer_name	primer_seq	length		
1	Mcrh-ORF5'-F	GACCAATCTTACCTTTCTCC	20		Endocrinology, 155, 4054-4060 (2014).
2	Crh_Rv	CTTGCTGAGCTAACTGCTCTGC	22		RIKEN BRC
3		GCAGAAGAACGGCATCAAGGCC	22		RIKEN BRC

Reaction Components

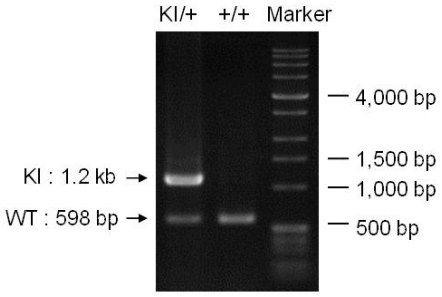
Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		3.9			
PCR buffer	2 X GC I	10.0			
dNTP	2.0 mM	2.0			
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	5U/μl	0.1			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	60*	*Repeat for 30 cycles

Standard PCR



Marker

DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL)

Gel

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

PCR products	primer_set	product_size	product
primer 1	primer 2	598 bp	WT
primer 2	primer 3	1.2 kb	KI

Comment