PCR protocol

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RBRC 06520 **Strain name** C57BL/6N-Crh<tm2(cre)Ksak>

Primer	primer_name	primer_seq	length		Reference
1		AGCGTCACCGAAGCCTAGAGC	21		RIKEN BRC
2	Crh_Rv	CTTGCTGAGCTAACTGCTCTGC	22		RIKEN BRC
3	iCre82-R	AGGTGGCATCCACAGGGAGG	20		Endocrinology, 155, 4054-4060 (2014).

Reaction Components

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

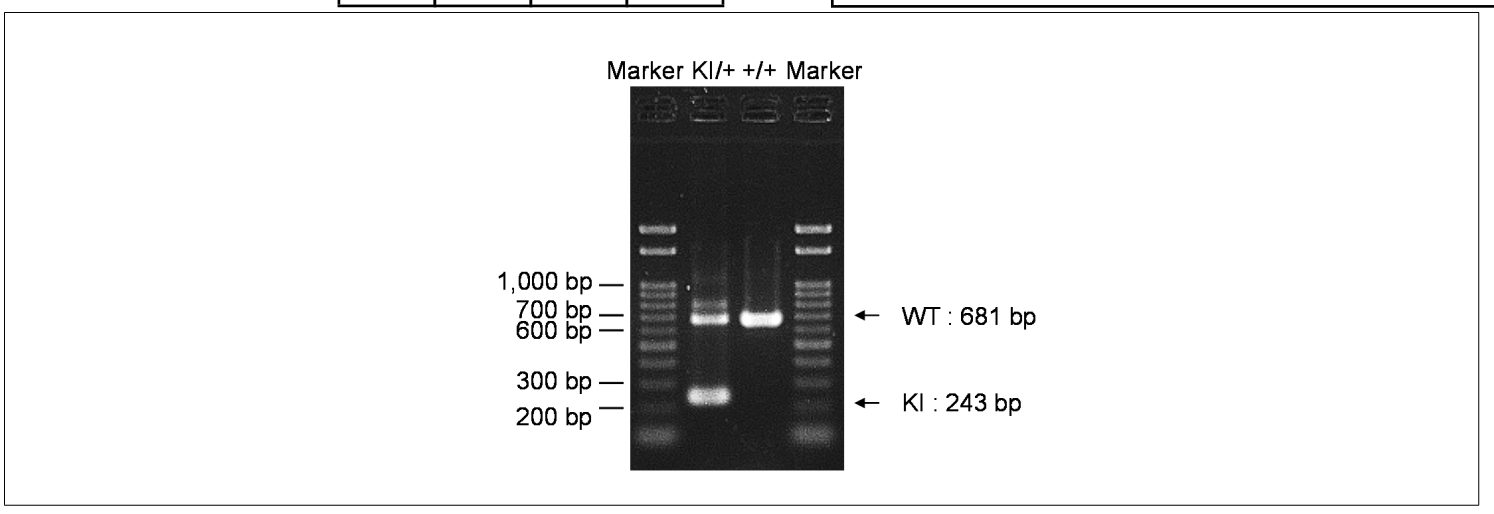
Reagent	Conc	Reaction 1	2	3	4	
H2O		3.9				(μL)
PCR buffer	2 X GC I	10.0				
dNTP	2.0 mM	2.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	5 U/μl	0.1				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	60	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	

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	681 bp	WT	+/+ (wild-type): 681 bp KI/+ (heterozygous): 681 bp and 243 bp
primer 1	primer 3	243 bp	KI	

Comment Standard PCR