PCR protocol

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RBRC 10866 **Strain name** B6.Cg-Hras<tm1.1(G12S)Tumg>

Primer	primer_name	primer_seq	length		Reference
1	mHras1-Fw	CGCTCAGTAAATAGTTGTAGGTTGC	25		EBioMedicine, 27:138-150 (2018).
2	mHras1-Rv	CTCAGACCAGAGAATCCACAGAAC	24		EBioMedicine, 27:138-150 (2018).

Reaction Components

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

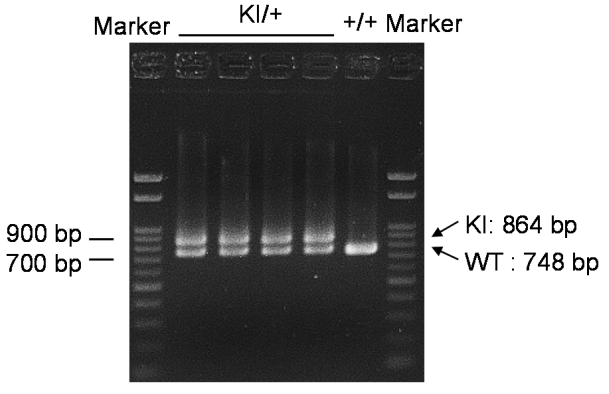
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
DNA	10 pmol/μl	1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

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

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	748 bp	WT	+/+ (wild-type): 748 bp KI/+ (heterozygous): 748 bp and 864 bp
primer 1	primer 2	864 bp	KI	

Comment Standard PCR

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