PCR protocol

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RBRC 12156 **Strain name** B6;Cg-Oxtr<em4(3xHA/T2A/icre/ERT2)Yinn>/#8

Primer	primer_name	primer_seq	length		Reference
1	Oxtr Fw3	CCTGGAGAGACGAGCATTAGCAAG	24		Depositor's primer
2	Oxtr Rv1	AGGGATGCAAACCAATCAATAGGCAC	26		Depositor's primer
3	iCre_N_R2	GTCCCTGAACATGTCCATCAGGTTC	25		Depositor's primer
4	ERT2	CTTACTGACCAACCTGGCAGAC	22		RIKEN BRC

Reaction Components

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

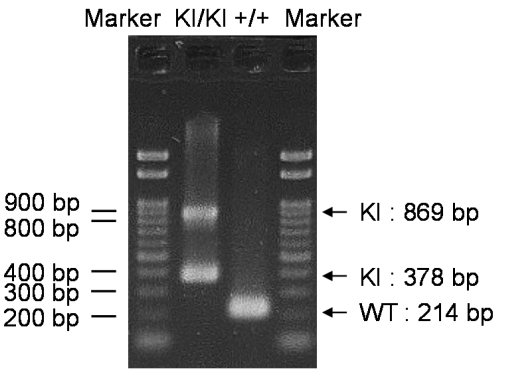
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.5				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

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	214 bp	WT	+/+ (wild-type): 214 bp KI/KI (homozygous): 378 bp and 869 bp
primer 1	primer 3	378 bp	KI (em4)	
primer 2	primer 4	869 bp	KI (em4)	

Comment Standard PCR

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