PCR protocol

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RBRC 10288 **Strain name** B6;129S4-Itpr1<tm1Tno>

Primer	primer_name	primer_seq	length		Reference
1	N1	TTGAGCTAAGCCAAATCTTAGTGAC	25		Nature, 379(6561)168-171 (1996).
2	N2	AAGGTGCTGATAAATCCATTCGTAG	25		Nature, 379(6561)168-171 (1996).
3	oIMR0214	CCGGAGAACCTGCGTGCAATCC	22		JAX Genotyping Protocol

Reaction Components

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

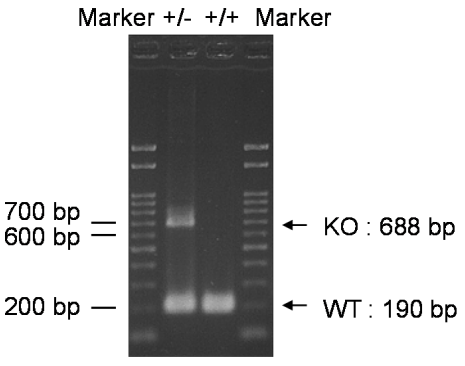
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

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	190 bp	WT
primer 1	primer 3	688 bp	KO

Comment

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