PCR protocol

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RBRC 11397

Strain name C.129P2(B6)-Il5ra<tm1Ktk>

Primer	primer_name	primer_seq	length		Reference
1	Il5ra F	CAGTATTTCCTATACTACAGGTCA	24		Immunology, 102(3):289-300 (2001).
2	Il5ra R	AATGGCAAGTGGACTGAACAGCTG	24		Immunology, 102(3):289-300 (2001).
3	Neo	CAGGATCTCCTGTCATCTCACCTT	24		Immunology, 102(3):289-300 (2001).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		1.8			
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			

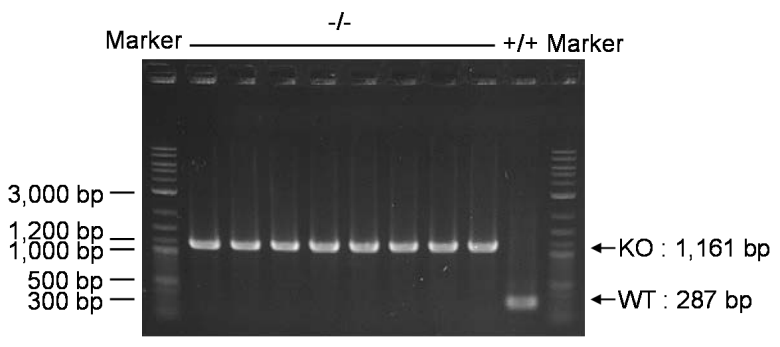
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	55	30*	
4	68	120*	*Repeat for 30 cycles

Marker: 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	287 bp	WT
primer 3	primer 2	1,161 bp	KO

+/+ (wild-type): 287 bp
-/- (homozygous): 1,161 bp

Comment Standard PCR

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