PCR protocol

Sheet ID PS_08044
Release date : 9/11/2020
Print date : 9/11/2020

RBRC 09341 **Strain name** IL-33(Acc.No.CDB0631K)

Primer	primer_name	primer_seq	length		Reference
1	ex2-F	CACTAAGACTACTCAGCCTCAG	22		Proc. Natl. Acad. Sci. USA. 107(43):18581-6 (2010).
2	WT-R	CGGTGATGCTGTGAAGTCTG	20		Proc. Natl. Acad. Sci. USA. 107(43):18581-6 (2010).
3	KO-R	GTGTTCTGCTGGTAGTGGTCG	21		Proc. Natl. Acad. Sci. USA. 107(43):18581-6 (2010).

Reaction Components

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

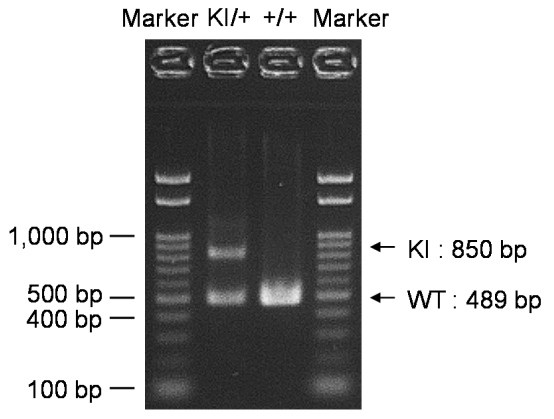
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

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	489 bp	WT	+/+ (wild-type): 489 bp KI/+ (heterozygous): 489 bp and 850 bp
primer 1	primer 3	850 bp	KI	

Comment Standard PCR

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