

PCR protocol

RBRC 05659

Strain name B6.Cg-Ly75<tm1.1Ksat>

Primer	primer_name	primer_seq	length	
1	Cd205-exon35-FW	ATTTAGCTAATGTGAATTTTCTCTGTAAAA	30	Proc. Natl. Acad. Sci. USA, 109, 11288
2	Cd205-exon35-Rev	AAACAAAAACAAAAACAACAAAAACAAAAC	29	Proc. Natl. Acad. Sci. USA, 109, 11288
3	EAD0085Comp	GTCGCCGTCCAGCTCGACCAGG	22	

Reaction Components

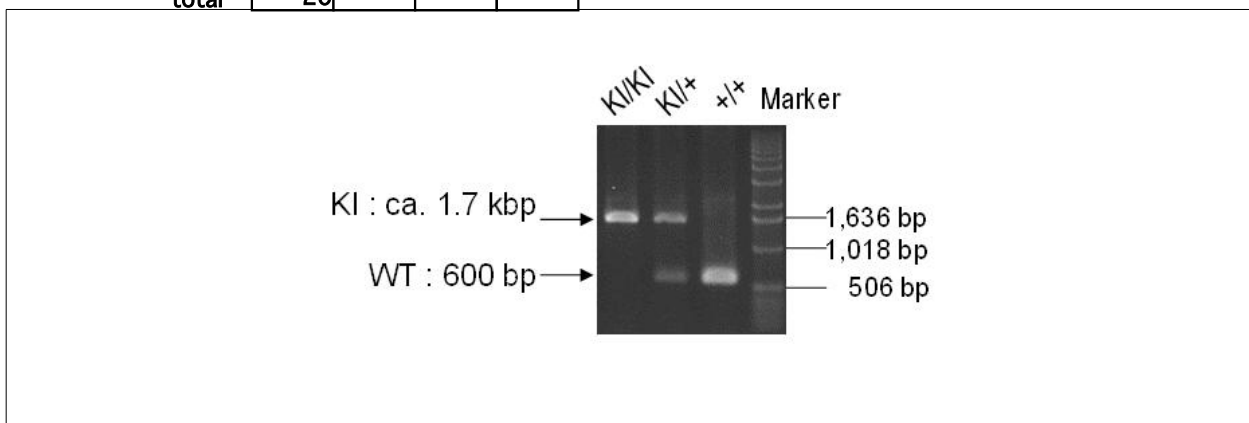
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

	Conc	Run 1	Run 2	Run 3	Run 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			

Standard PCR



Marker

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel

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

PCR products

primer_set	product_size	product
primer 1 primer 2	600 bp	WT
primer 1 primer 3	ca. 1.7 kbp	KI

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