PCR protocol

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RBRC 05582 **Strain name** C57BL/6N-Ikzf3<tm1(KOMP)Vlcg>/CB3

Primer	primer_name	primer_seq	length		
1	SU	TGTTTCTCAAGTAGACGTCC	20		http://www.velocigene.com/komp/detail/13584
2	TUR	GAGCCACATCTTCCATCTTCAG	23		http://www.velocigene.com/komp/detail/13584
3	lacZ-R	CGCATCGTAACCGTGCATCTGCC	23		RIKEN BRC
4	TDF	TGCGCCTCATTCACAGTTC	20		http://www.velocigene.com/komp/detail/13584
5	SD	ATTGGTGTAATGCATGTGAC	20		http://www.velocigene.com/komp/detail/13584
6	Neo30	TTCTATCGCCTTCTTGACGAGTTCTTCTGA	30		

Reaction Components

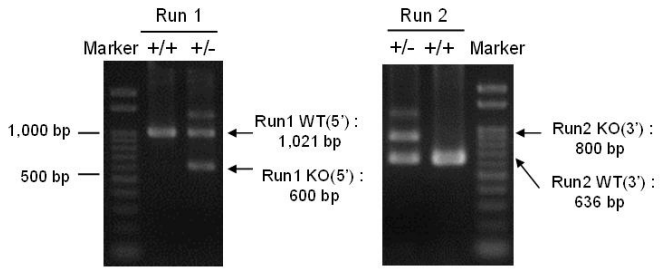
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	1.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
primer 6	10 pmol/μl	0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	20		

Reaction Conditions

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

Standard PCR



Marker
Gene Ladder 100 (Wako, Cat. 316 -06951)

Gel
2% agarose in 1 X TAE (100 V, 30 min)

PCR products	primer_set	product_size	product
primer 1	primer 2	1,021 bp	WT (5')
primer 1	primer 3	600 bp	KO (5')
primer 4	primer 5	636 bp	WT (3')
primer 5	primer 6	800 bp	KO (3')

Comment

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