PCR protocol

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RBRC 02687 Strain name B6.Cg-Tnc<tm1Sia> Tnxb<tm1Kmat>

Primer	primer_name	primer_seq	length		
1	3'TN primer	AAGATGCCTGGCAGTAGCCAGGTCAC	26		Depositor's primer
2	G1-1 primer	GGTACCTGATTCCGAAGTGCATTGTCACGT	30		Depositor's primer
3	LacZ	TGCGCAACTGTTGGGAAGGGCGATC	25		Depositor's primer
4	GT-KpnI	AAGCACATGCAGCAGAGTGGGGTC	24		Depositor's primer
5	rGT-AflII	GTGCCAGCTTGGGCCGCAGTGGGAC	25		Depositor's primer

Reaction Components

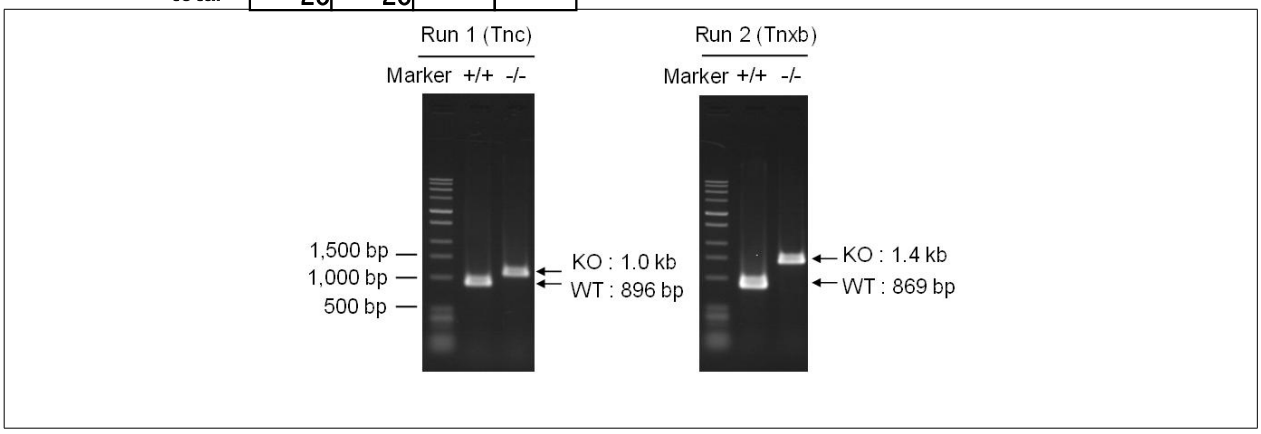
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2 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		
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	120	* *Repeat for 30 cycles

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	896 bp	WT (Tnc)
primer 2	primer 3	1.0 kb	KO (Tnc)
primer 4	primer 5	1.4 kb	KO (Tnxb)
primer 4	primer 5	869 bp	WT (Tnxb)

Comment

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