PCR protocol

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RBRC 00193 **Strain name** GRS.Cg-Tnc<tm1Sia>/Rbrc

Primer	primer_name	primer_seq	length		
1	TN-G1-1	GGTACCTGATTCCGAAGTGCATTGTCACG	29		Depositor's primer
2	TN-150R (~3'TN)	AAGCTAGAAAGATGCCTGGC	20		Depositor's primer
3	ZA-6	ACGGCAAACGACTGTCCTGGCCGTAACCGACC	32		Depositor's primer

Reaction Components

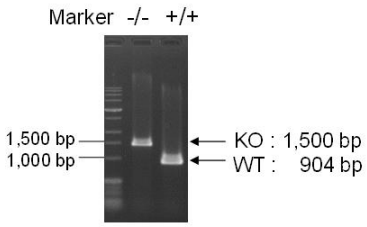
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
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	120*	*Repeat for 30 cycles

Standard PCR



Marker

DNA Ladder,1 kb Plus (Affymetrix, Cat. 76714 500 UL)

Gel

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

PCR products	primer_set	product_size	product
primer 1	primer 2	904 bp	WT
primer 1	primer 3	1,500 bp	KO

Comment

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