PCR protocol

Sheet ID PS_06755
Release date : 2/13/2017
Print date : 4/16/2019

RBRC 09538 **Strain name** B6-Tgfbi<tm1(TGFBI)Dokm>

Primer	primer_name	primer_seq	length		Reference
1	TGFBi_Wt_Fw	CACCCTTCCCACTTTTCTACGG	22		PLoS One. 10(7):e0133397(2015).
2	TGFBi_Wt_Rv	CTGTGTCCCCTTATCTCATCAACC	24		PLoS One. 10(7):e0133397(2015).
3	TGFBi_KI_Fw2	TCTGGAAGTGAGCGGTTGACC	21		Depositor's primer

Reaction Components

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

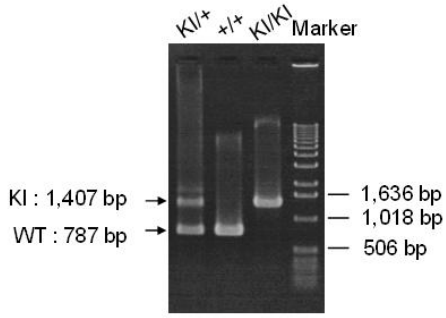
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

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

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	787 bp	WT
primer 3	primer 2	1,407 bp	KI

+/+ (wild-type): 787 bp
KI/+ (heterozygous): 787 bp and 1407 bp
KI/KI (homozygous): 1407 bp

Comment Standard PCR

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