PCR protocol

Sheet ID PS_08058
Release date : 3/4/2021
Print date : 3/4/2021

RBRC 11098 **Strain name** B6.Cg-Scx<tm1.1(EGFP)Skom>

Primer	primer_name	primer_seq	length		Reference
1	gsk011	CAAAATGCACTGTGGGGGTG	20		Depositor's primer
2	gsk012	ACTAGTGGCATCACCTCTTGG	21		Sci Rep., 10(1):3992 (2020).
3	EGFP-AS	CACGAACTCCAGCAGGACCATG	22		Depositor's primer

Reaction Components

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

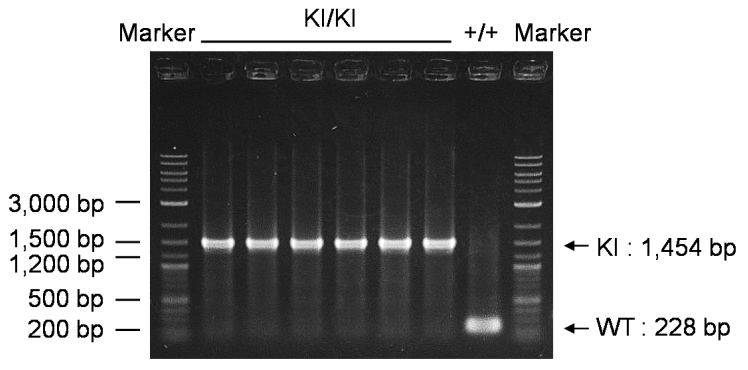
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	180	* *Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	228 bp	WT
primer 1	primer 3	1,454 bp	KI

+/+ (wild-type): 228 bp
KI/KI (homozygous): 1,454 bp

KI/+ (heterozygous): 228 bp and 1,454 bp

Comment Standard PCR

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