PCR protocol

Sheet ID PS_06000
Release date : 5/6/2022
Print date : 5/6/2022

RBRC 06356 **Strain name** B6(C3)-Lgalsl<tm1(KOMP)Vlcg>

Primer	primer_name	primer_seq	length		Reference
1	5'_Fw	GCCTTCAGCCGTGGTCCAATG	21		RIKEN BRC
2	5'_Rv	GCACTCACCACCACGGCGTC	20		RIKEN BRC
3	LacZ-R5	ATGTGAGCGAGTAACAACCCGTCGGATTCT	30		Depositor's primer
4	3'_Fw	CTAGGTGGAGATTCTTTGCGAGC	23		RIKEN BRC
5	3'_Rv	CAGTGCAGATTTGATGTACCGCTG	24		RIKEN BRC
6	NeoP	CTTCTATCGCCTTCTTGACGAGTT	24		Depositor's primer

Reaction Components

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

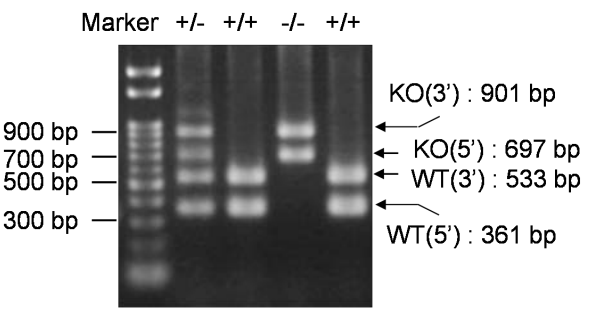
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	0.5				
primer 2	10 pmol/μl	0.5				
primer 3	10 pmol/μl	0.5				
primer 4	10 pmol/μl	0.5				
primer 5	10 pmol/μl	1.0				
primer 6	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

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

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	361 bp	WT (5')
primer 1	primer 3	697 bp	KO
primer 4	primer 5	533 bp	WT (3')
primer 5	primer 6	901 bp	KO

Comment

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