PCR protocol

Sheet ID PS_08369
Release date : 8/8/2022
Print date : 8/8/2022

RBRC 11179

Strain name C57BL/6-Gt(ROSA)26Sor<tm1.1(CAG-tdTomato)Imayo>

Primer	primer_name	primer_seq	length		Reference
1	RCE-Rosa1	CCCAAAGTCGCTCTGAGTTGTTATC	25		Depositor's primer
2	RCE-Rosa2	GAAGGAGCGGGAGAAATGGATATG	24		Depositor's primer
3	oIMR0214	CCGGAGAACCTGCGTGCAATCC	22		JAX Genotyping Protocol
4		GAGGTCATCAAAGAGTTCATGCG	23		RIKEN BRC

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		0.8			
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			
primer 4	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

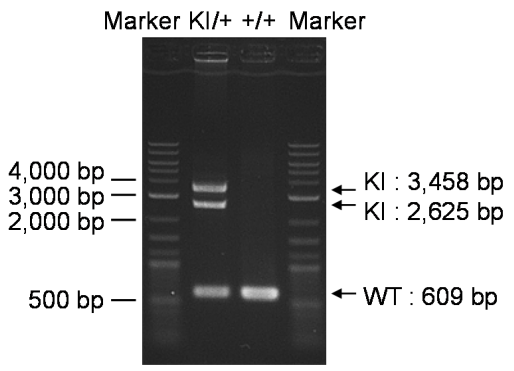
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	300*	*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	609 bp	WT
primer 1	primer 3	2,625 bp	KI
primer 2	primer 4	3,458 bp	KI

+/+ (wild-type): 609 bp
KI/+ (heterozygous): 609 bp, 2625 bp and 3458 bp

Comment

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