PCR protocol

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RBRC 06589 **Strain name** C57BL/6-Tg(Gt(ROSA)26Sor-C1V1/EYFP)1.1Imayo

Primer	primer_name	primer_seq	length		Reference
1	RCE-Rosa1	CCCAAAGTCGCTCTGAGTTGTTATC	25		Depositor's primer
2	RCE-Rosa2	GAAGGAGCGGGAGAAATGGATATG	24		Depositor's primer
3	EGFP-336bp-rv	GTCCTCCTTGAAGTCGATGCCCTTCAG	27		Depositor's primer

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	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			

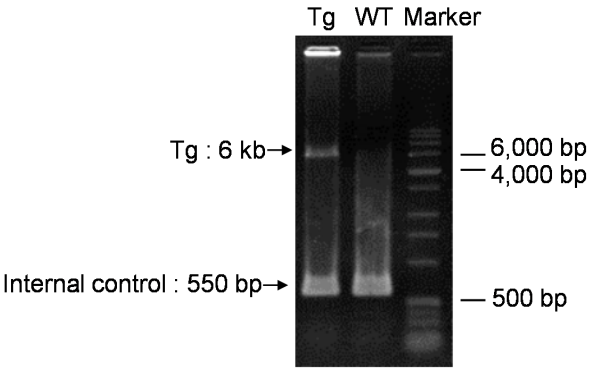
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	420*	*Repeat for 30 cycles

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

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



primer_set		product_size	product (allele type)	
primer 1	primer 2	550 bp	Internal control	
primer 1	primer 3	6 kb	Tg	

Comment Standard PCR

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