



PCR protocol

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RBRC 06587

Strain name C57BL/6-Tg(Gt(ROSA)26Sor-ArchT/EGFP)1.2Imayo

Primer	primer_name	primer_seq	length	Reference
1	RCE-Rosa1	CCCAAAGTCGCTCTGAGTTGTTATC	25	Depositor's primer
2	RCE-Rosa2	GAAGGAGCGGGAGAAATGGATATG	24	Depositor's primer
3	RCE-Cag3	CCAGGCGGGCCATTTACCGTAAG	23	Depositor's primer
4	EGFP-336bp-fw	GTAACGGCCACAAGTTCAGCGTGTC	26	Depositor's primer
5	EGFP-336bp-rv	GTCCTCCTTGAAGTCGATGCCCTTCAG	27	Depositor's primer

Reaction Components

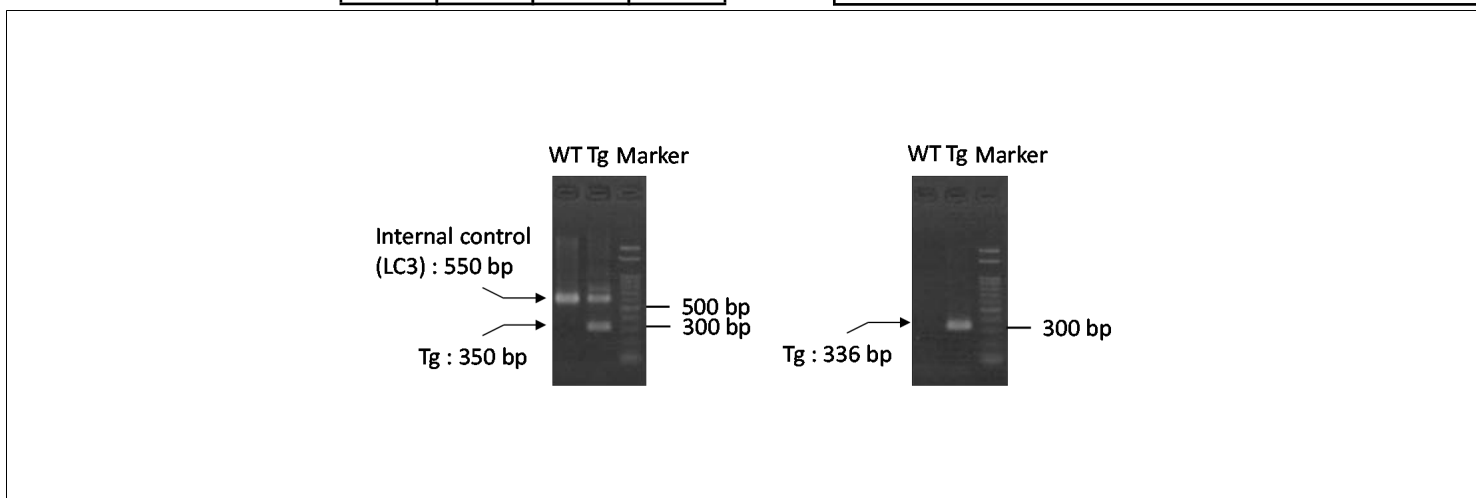
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(μ L)
H2O		1.8	2.8			
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/ μ l	1.0	0			
primer 2	10 pmol/ μ l	1.0	0			
primer 3	10 pmol/ μ l	1.0	0			
primer 4	10 pmol/ μ l	0	1.0			
primer 5	10 pmol/ μ l	0	1.0			
DNA		1.0	1.0			
Taq	1 U/ μ l	0.2	0.2			
total		20	20			

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	550 bp	Internal control
primer 1 / primer 3	350 bp	Tg
primer 4 / primer 5	336 bp	Tg

Comment

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