PCR protocol

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RBRC 05169 **Strain name** C57BL/6-Tg(Th-FLPo)5Imayo

Primer	primer_name	primer_seq	length		Reference
1	FW_Primer	CCTGTGACAGTGGATGCAATT	21		RIKEN BRC
2	FLPo-287bp-rv	CCTCGCTGCTCTCGAACTGCAGCTG	25		Depositor's primer
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24		JAX Genotyping Protocol
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25		JAX Genotyping Protocol

Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

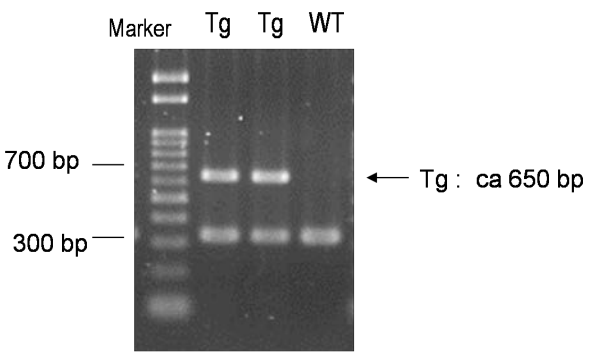
Reagent	Conc	Reaction 1	2	3	4	
H2O		11.3				(μL)
PCR buffer	10 X	2.0				
dNTP	2.5 mM	1.6				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	5 U/μl	0.1				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	60	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	

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	ca 650 bp	Tg
primer 3	primer 4	324bp	Internal control (IL-2)

Comment