PCR protocol

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RBRC 05463

Strain name C57BL/6N-Trpc3<tm1a(EUCOMM)Wtsi>/D08

Primer	primer_name	primer_seq	length		Reference
1		GAGACTTCTATCCTGATAGTTGACC	25		RIKEN BRC
2		CTCACAGAGGGTTCTGTGACTC	22		RIKEN BRC
3		GGCACATGGCTGAATATCGACGG	23		RIKEN BRC

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			

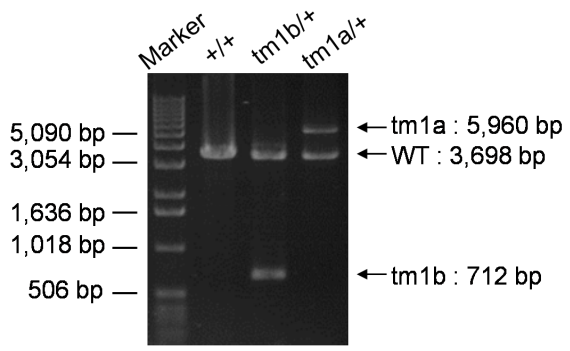
(μL)

Reaction Conditions

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

Marker: 1 kb DNA ladder (Invitrogen, Cat. 15615-024)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	3,698 bp	WT
primer 2	primer 3	5,960 bp	flox (tm1a)
primer 2	primer 3	712 bp	lacZ(tm1b)

Comment Standard PCR

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