PCR protocol

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RBRC 05780 **Strain name** C57BL/6N-Rufy3<tm1a(EUCOMM)Hmgu>/B08

Primer	primer_name	primer_seq	length		
1		CCTCTGTGGGTGAGTGTGTATG	22		RIKEN BRC
2		GCAACTAACTCTTGGAGGCTCTC	23		RIKEN BRC
3		GGCACATGGCTGAATATCGACGG	23		RIKEN BRC

Reaction Components

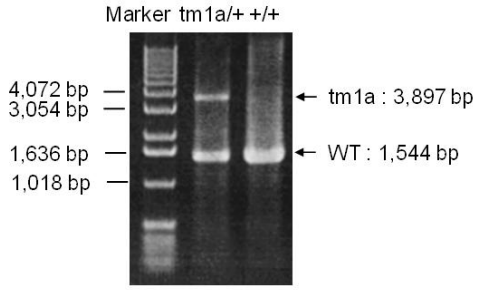
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 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			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	300	* *Repeat for 30 cycles

Standard PCR



Marker

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

Gel

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

PCR products	primer_set	product_size	product
primer 1	primer 2	1,544 bp	WT
primer 2	primer 3	3,897 bp	flox (tm1a)
primer 2	primer 3	907 bp	lacZ (tm1b)

Comment

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