PCR protocol

Sheet ID PS_05661
Release date : 5/11/2016
Print date : 5/11/2016

RBRC 05712 Strain name C57BL/6N-Folr4<tm1b(KOMP)Wtsi>/F07

Primer	primer_name	primer_seq	length		
1		CAGCCAAGCGACATGTCCTATCATGG	26		RIKEN BRC
2		CAAGACCAGCACGTCATGACTGTGG	25		RIKEN BRC
3		GGCACATGGCTGAATATCGACGG	23		RIKEN BRC

Reaction Components

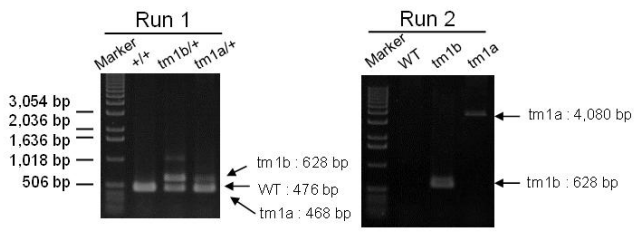
Enzyme: TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	0		
primer 2	10 pmol/μl	1.0	1.0		
primer 3	10 pmol/μl	1.0	1.0		
DNA		1.0	1.0		
Taq	1 U/μl	0.2	0.2		
	total	20	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	476 bp	WT
primer 1	primer 2	462 bp	tm1a (flox)
primer 2	primer 3	632 bp	tm1b (lacZ)
primer 2	primer 3	4,080 bp	tm1a (flox)

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