PCR protocol

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RBRC 09796 **Strain name** B6.Cg-Ano6<tm1.1Naga>

Primer	primer_name	primer_seq	length		
1	16F-WT-F1	CTCCAGAGTTTGTAAGTAACACAT	24		J. Biol. Chem., 288, 13305-13316 (2013).
2	16F-R1	AGACTGATTTCCAAGGTTATCGAA	24		J. Biol. Chem., 288, 13305-13316 (2013).
3	16F-KO-F1	CAGTCATCGATGAATTCATAACTT	24		J. Biol. Chem., 288, 13305-13316 (2013).

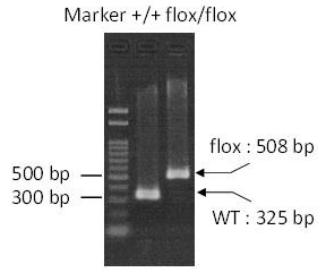
Reaction Components

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	60	* *Repeat for 30 cycles



Marker
Gene Ladder 100 (Wako, Cat. 316 -06951)

Gel
2% agarose in 1 X TAE (100 V, 30 min)

PCR products	primer_set	product_size	product
primer 1	primer 2	325 bp	WT
primer 1	primer 2	508 bp	flox
primer 1	primer 3	199 bp	null (After cre mediated excision)

Comment

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