RIKEN BRC

Sep. 5, 2006

BRC_No 01767 **Strain name** tob and tob2 KO(B6)

Primer

	primer_name	primer_seq	length
1	Tob-F	ACCACTCTCTTTGGATGCCTGCTCG	25
2	PGK-neo	GCTAAAGCGCATGCTCCAGACTGCCTTG	28
3	Tob-R	GTGGCTTCGCAGCTATGCAGCTTGA	25
4	Tob2mut-F	CCTTGCTTCTCTCCTCTCCTCCAGG	25
5	LacZ	TGCGCAACTGTTGGGAAGGGCGATC	25
6	Tob2WT-F	GCATTCATGCCTGTGAGTGTGTGGCTTGC	29
7	Tob2WT-R	GGGTTTCTCGGGCTTAGCCTCGTGTCA	27

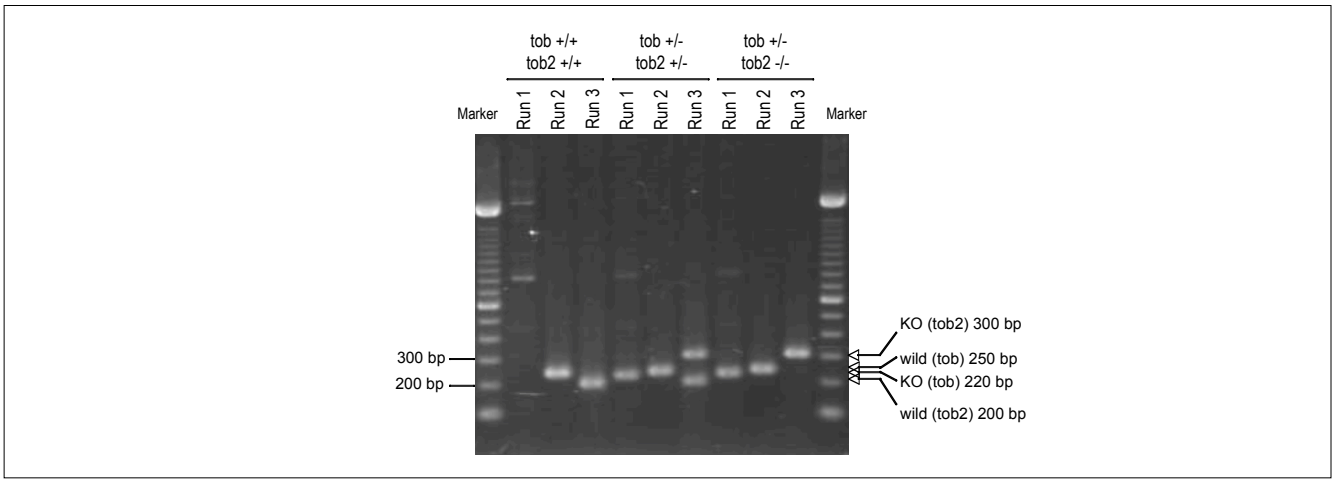
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

	Conc	Run 1	Run 2	Run 3
H2O		17.3	17.3	15.3
PCR buffer	10 X	2.5	2.5	2.5
dNTP	2.5 mM	2.0	2.0	2.0
primer 1	10 pmol/μl	1.0	1.0	0
primer 2	10 pmol/μl	1.0	0	0
primer 3	10 pmol/μl	0	1.0	0
primer 4	10 pmol/μl	0	0	1.0
primer 5	10 pmol/μl	0	0	1.0
primer 6	10 pmol/μl	0	0	1.0
primer 7	10 pmol/μl	0	0	1.0
DNA		1.0	1.0	1.0
Taq		0.2	0.2	0.2
	total	25	25	25

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	180	
2	94	30	*
3	58	30	*
4	72	60	* *Repeat for 30 cycles
5	72	300	



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

2% agarose in 1 X TAE (100 V, 35 min)

PCR products

primer_set		product_size	product
primer 1	primer 2	220 bp	KO (Tob)
primer 1	primer 3	250 bp	wild (Tob)
primer 4	primer 5	300 bp	KO (Tob2)
primer 6	primer 7	200 bp	wild (Tob2)

Comment