

RBRC 00857

Strain name trehalase Knockout mice

Primer	primer_name	primer_seq	length	
1	WT-S	TTCTCTTCACGCTGGCTTGTT	21	Depositor's primer
2	WT-AS	GCTCCTTGAACAAACATCCCT	21	Depositor's primer
3	oIMR0189	TGGATGTGGAATGTGTGCGAG	21	JAX Genotyping Protocol

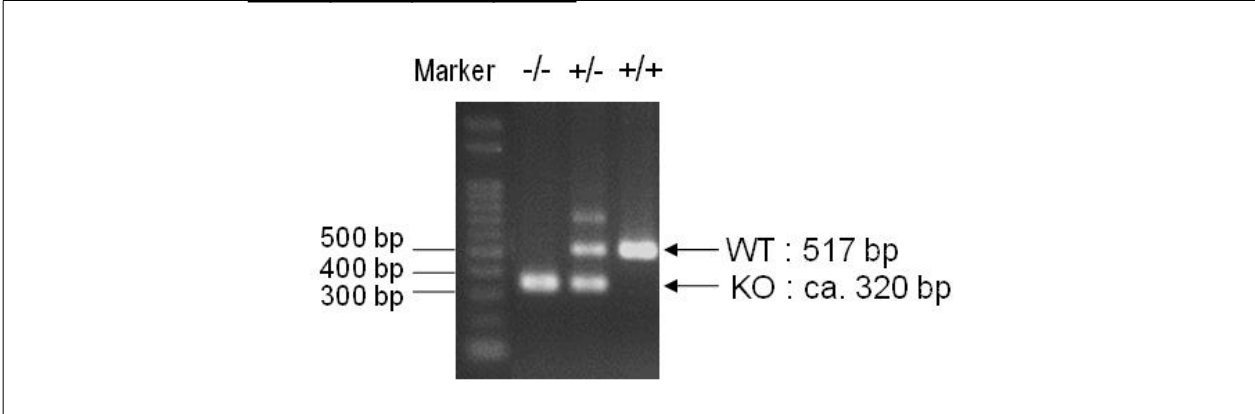
Reaction Components

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

Reaction Conditions

	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/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	1 U/ μ l	0.2			
total		20			

step	temp (°C)	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	517 bp	WT
primer 3	primer 2	ca. 320 bp	KO

Comment

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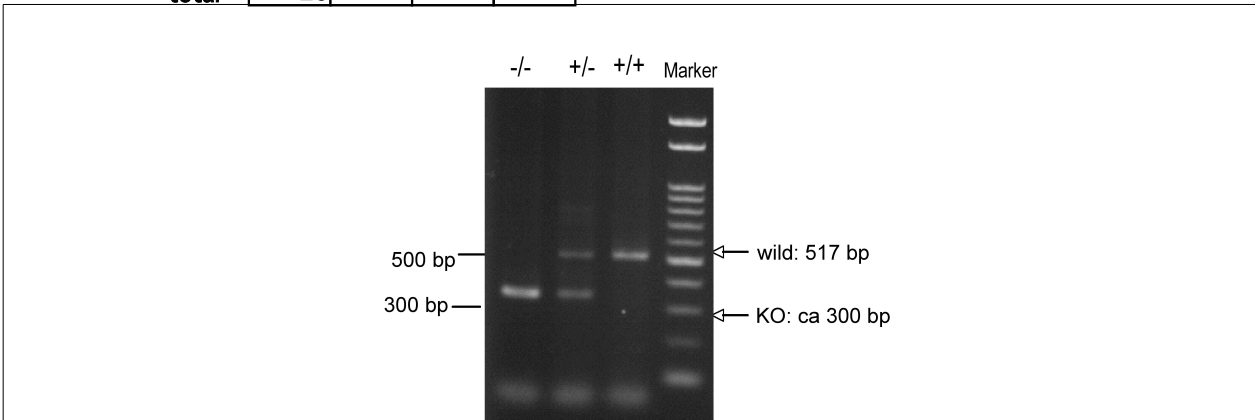
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		16.3			
PCR buffer	10 X	2.5			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ μ l	1.0			
primer 2	10 pmol/ μ l	1.0			
primer 3	10 pmol/ μ l	1.0			
DNA		1.0			
Taq	5U/ μ l	0.2			
total		25			

step	temp (°C)	time (sec)	note
1	94	300	
2	94	30*	
3	58	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	



Marker
100 bp DNA Ladder (TOYOBO)

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

PCR products

primer_set	product_size	product
primer 1	517 bp	WT
primer 2	ca 300 bp	KO

Comment