

RBRC 05138

Strain name 129S6-Trpm1<tm1Tfur>

Primer	primer_name	primer_seq	length	Reference
1	TRPM1F1	GGAAATCCTTGGACTTGGCTACAG	24	Depositor's primer
2	TRPM1B1	GCACCCCAGTTCTAACCACTTGAG	24	Depositor's primer
3	neo3'-52	TTCGCAGCGCATCGCCTTCTATCGCCT	27	Depositor's primer

Reaction Components

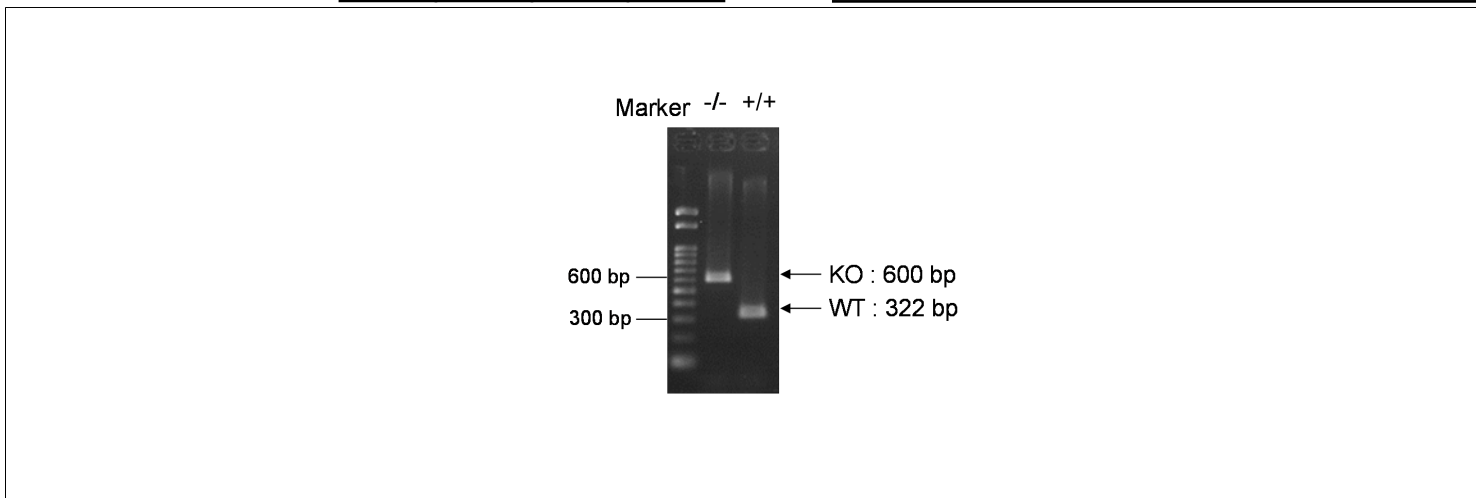
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(μ L)
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				

Marker Gene Ladder 100 (Wako, Cat. 316-06951)
Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	primer 2	322 bp WT
primer 3	primer 2	600 bp KO

Comment Standard PCR

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

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Reaction Components

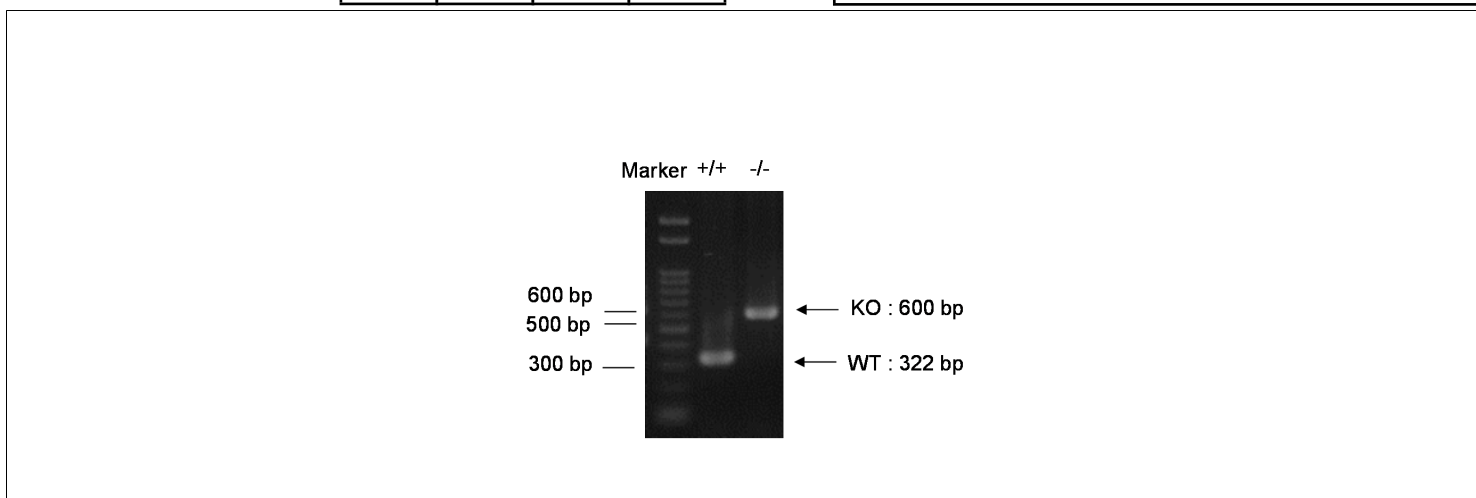
Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reagent	Conc	Reaction 1	2	3	4
H2O		12.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
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	5 U/μl	0.1			
total		20			

Reaction Conditions

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

Marker Gene Ladder 100 (Wako, Cat. 316-06951)
Gel 2% agarose in 1 X TAE (100 V, 30 min)



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primer 3	primer 2	600 bp KO

Comment Standard PCR