

RBRC 10449

Strain name B6.129-Hacd1 <tm1Akih>

Primer	primer_name	primer_seq	length	Reference
1	intron 5	GAAGGCGGTTGTGCACTGTGTAC	23	RIKEN BRC
2	intron 5	CCATACAAAGCATATTTAAATGCTTTTAAGAAAG	34	RIKEN BRC
3	Hacd1_P1	CATGCTCCAGACTGCCTTGGGAAAAGC	27	<i>J. Biol. Chem.</i> , 292 , 15538-15551 (2017).

Reaction Components

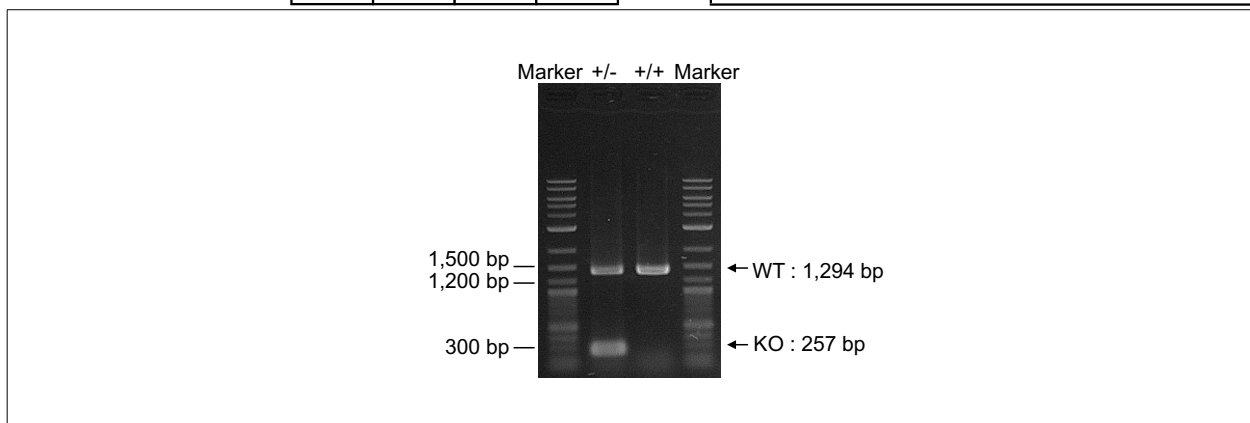
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(µL)
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				

Marker 2-Log DNA Ladder (NEB, Cat. N3200)
Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1 primer 2	1,294 bp	WT
primer 2 primer 3	257 bp	KO

+/- : 1,294 bp, 257 bp
+/: 1,294 bp

Comment Standard PCR

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