

PCR protocol

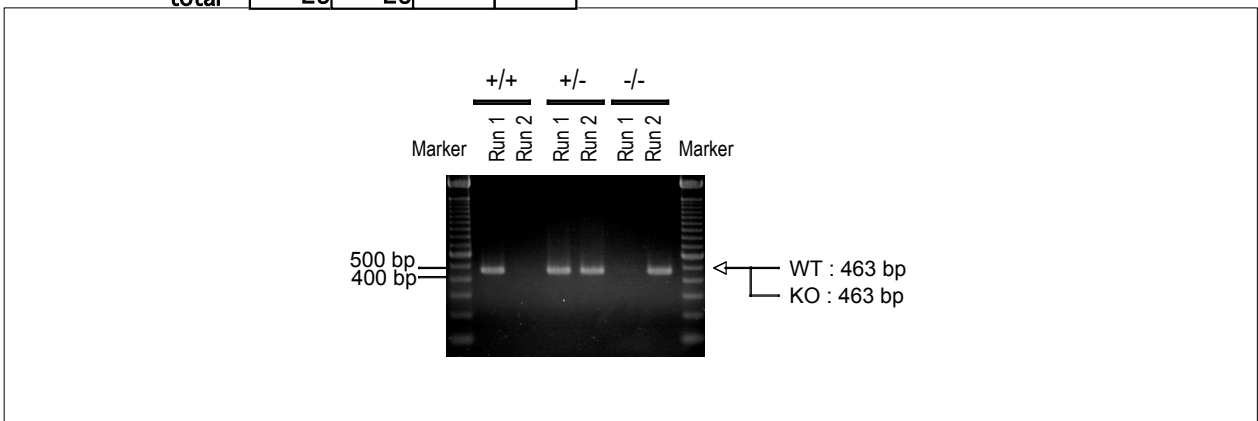
RBRC 01446
Strain name ddY/DAO<+> mice

Primer	primer_name	primer_seq	length	
1	Primer No.2	TTTGGTTAAGATGGTGATGTG	21	Depositor's primer
2	DAO_W	GAGTGGATGTGATTATCAACTGCACAG	27	RIKEN BRC
3	DAO_M	GAGTGGATGTGATTATCAACTGCACAA	27	RIKEN BRC

Reaction Components
Enzyme Roche Taq (Cat. No. BM1418432)
Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	57	30*	
4	72	60*	*Repeat for 35 cycles
5	72	300	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3	17.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/ μ l	1.0	1.0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	0	1.0		
DNA		1.0	1.0		
Taq		0.2	0.2		
total		25	25		



Marker

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

Gel

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

PCR products

primer_set	product_size	product
primer 1 primer 2	463 bp	mutant
primer 1 primer 3	463 bp	WT

Comment