

RBRC 09617

Strain name C3H-Atp7a<Mo-ml>

Primer	primer_name	primer_seq	length	Reference
1	intron 21	GTGCATGTGTTGGAAATGTGTTGATG	26	RIKEN BRC
2	exon 22	ATGAAGCGGCCATTGCTGCGCA	22	RIKEN BRC
3	exon 22	TGGTTTTACAACCCTGGATGGGCC	24	RIKEN BRC
4	intron 22	GTTGTCCTCTGATCTCTATGTACATG	26	RIKEN BRC

Reaction Components

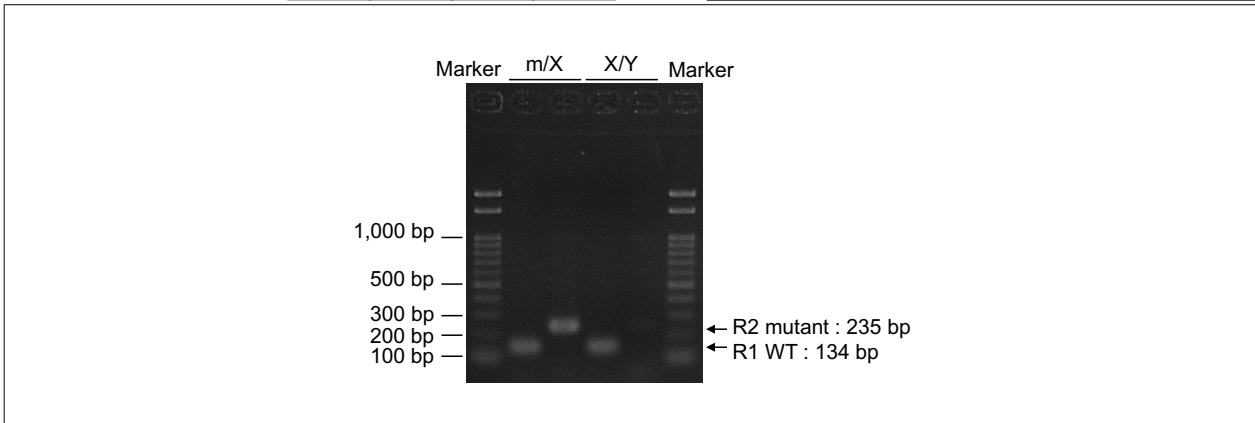
Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

Reagent	Conc	Reaction 1	2	3	4
H2O		2.0	2.0		
primer 1	10 pmol/ μ l	1.0	0		
primer 2	10 pmol/ μ l	1.0	0		
primer 3	10 pmol/ μ l	0	1.0		
primer 4	10 pmol/ μ l	0	1.0		
Master Mix	2 X	5.0	5.0		
DNA		1.0	1.0		
total		10	10		

Reaction Conditions

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	60	90*	
4	72	60*	*Repeat for 30 cycles

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	134 bp	WT
primer 3	primer 4	235 bp	mutant

X/X or X/Y (wild-type): 134 bp
m/X (Heterozygote): 134 bp and 235 bp
m/m or m/Y (Homozygote, Hemizygote): 235 bp
agouti (wild-type), agouti with white mosaic striped coat (Heterozygote),

Comment Separated PCR