

RBRC 06415

Strain name C.Cg-Dms

Primer	primer_name	primer_seq	length	Reference
1	common	CAACTGCATGACACATGCACCAGC	24	RIKEN BRC
2	WT	CCTGATAACTGTCCAGAGAGACGG	24	RIKEN BRC
3	MT	CCTGATAACTGTCCAGAGAGACGA	24	RIKEN BRC

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

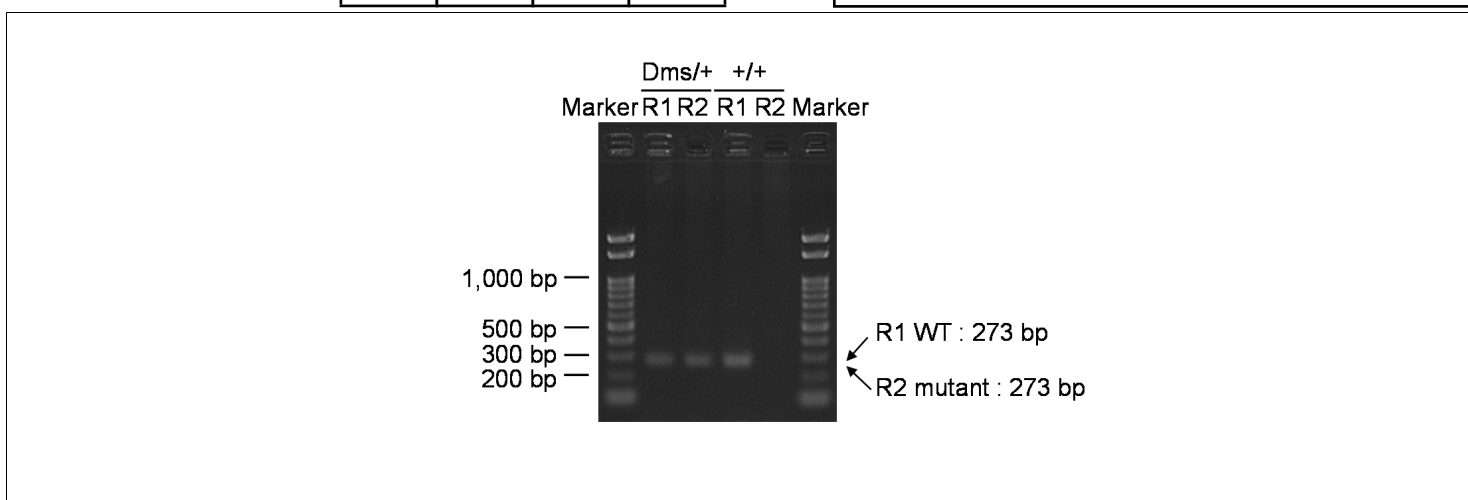
Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(μ L)
H2O		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			
Master Mix	2 X	5.0	5.0			
total		10	10			

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	273 bp	WT
primer 1	primer 3	273 bp	mutant

Reaction 1
Dms/+ (heterozygous): 273 bp
+/+ (wild-type): 273 bp

Reaction 2
Dms/+ (heterozygous): 273 bp
+/+ (wild-type): none

Comment Allele specific PCR (ASP)