

Primer	primer_name	primer_seq	length	Reference
1	wild	GGCGTTCAGACTGGGTACGGAA	22	RIKEN BRC
2	Awat1_p2	CACATTGTAGCTACTTCTCTGAAGC	25	iScience, 24, 102478 (2021).
3	Awat1_p1	GGGATCCAAACGCTAGACAGCTGAAACCATTC	32	iScience, 24, 102478 (2021).
4	mut	CTGATGTGGTTCCAGACATTCCAATC	26	RIKEN BRC
5	wild	TGGCTGAGTGCAGATACAGCACGC	24	RIKEN BRC
6	intron 6	CTGTTGGTTCAGCCTACTCTACCAG	25	RIKEN BRC
7	common	ACAGGGATCACTAAGTAACCAATGAC	26	RIKEN BRC
8	mut	AACAGGGTGGTAGAGCCTGGCTGTA	25	RIKEN BRC

Reaction Components

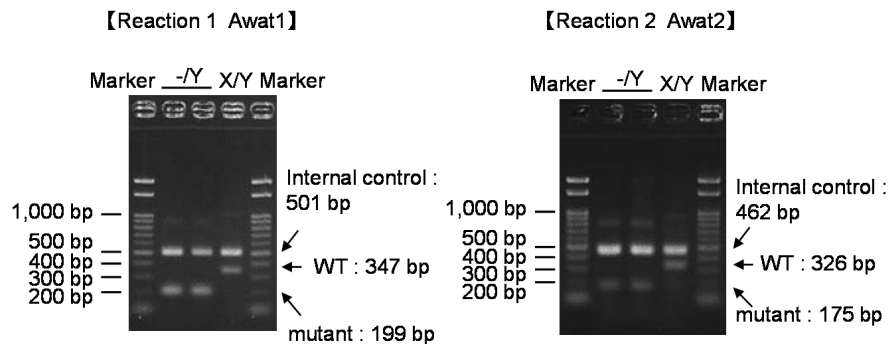
Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	95	900	
2	94	30*	
3	63	90*	
4	72	60*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(μ L)
primer 1	10 pmol/ μ l	1.0	0			
primer 2	10 pmol/ μ l	1.0	0			
primer 3	10 pmol/ μ l	1.0	0			
primer 4	10 pmol/ μ l	1.0	0			
primer 5	10 pmol/ μ l	0	1.0			
primer 6	10 pmol/ μ l	0	1.0			
primer 7	10 pmol/ μ l	0	1.0			
primer 8	10 pmol/ μ l	0	1.0			
Master Mix	2 X	5.0	5.0			
DNA		1.0	1.0			
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 2	primer 3	501 bp Internal control
primer 1	primer 2	347 bp WT
primer 3	primer 4	199 bp mutant
primer 6	primer 7	462 bp Internal control
primer 5	primer 6	326 bp WT
primer 7	primer 8	175 bp mutant

Comment Allele specific PCR (ASP)