PCR protocol

Sheet ID PS_08277
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RBRC 11538 **Strain name** B6-Awat1<em1Akih>

Primer	primer_name	primer_seq	length		Reference
1	wt	GCAGGCGTTCAGACTGGGTACGG	23		RIKEN BRC
2	Awat1_p2	CACATTGTAGCTACTTCTCTGAAGC	25		iScience, 24, 102478 (2021).
3	Awat1_p1	GGGATCCAAACGCTAGACAGCTGAAACCATTC	32		iScience, 24, 102478 (2021).
4	mut	GTCCCTGATGTGGTTCCAGACATA	24		RIKEN BRC

Reaction Components

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

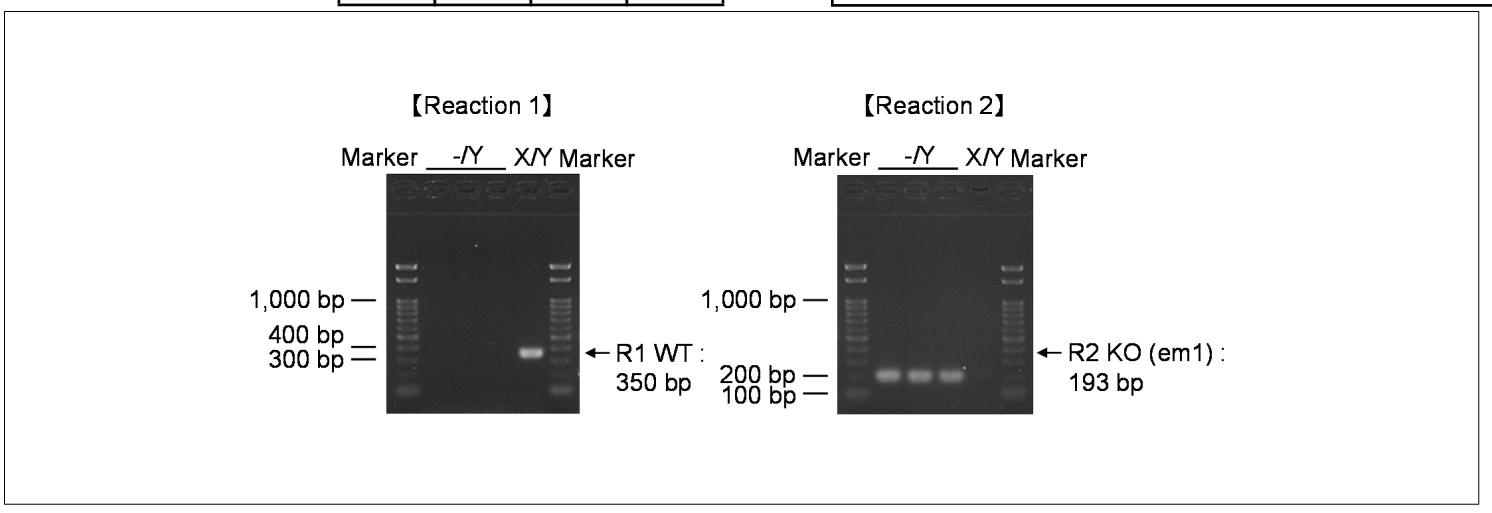
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.0	2.0			(μL)
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
Master Mix	2 X	5.0	5.0			
DNA		1.0	1.0			
	total	10	10			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	65	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	350 bp	WT
primer 3	primer 4	193 bp	KO(em1)

Reaction 1
X/X or X/Y: 350 bp
-/Y: none
Reaction 2
X/X or XY: none
-/Y: 193 bp

Comment Separated PCR