PCR protocol

Sheet ID PS_08407
Release date : 11/18/2022
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RBRC 11753 **Strain name** C57BL/6J-Arntl<em1Yshtn>

Primer	primer_name	primer_seq	length		Reference
1	wt	GGAGCGGATTGGTCGGAACG	20		RIKEN BRC
2	RRE-Rv	AGCCATGCCGACACTCAC	18		Depositor's primer
3	RRE-Fw2	CGCCAAATGATTGGTGAAG	19		Depositor's primer
4	mut	CAGGCACCTCCGTCCCTGACTTC	23		RIKEN BRC

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

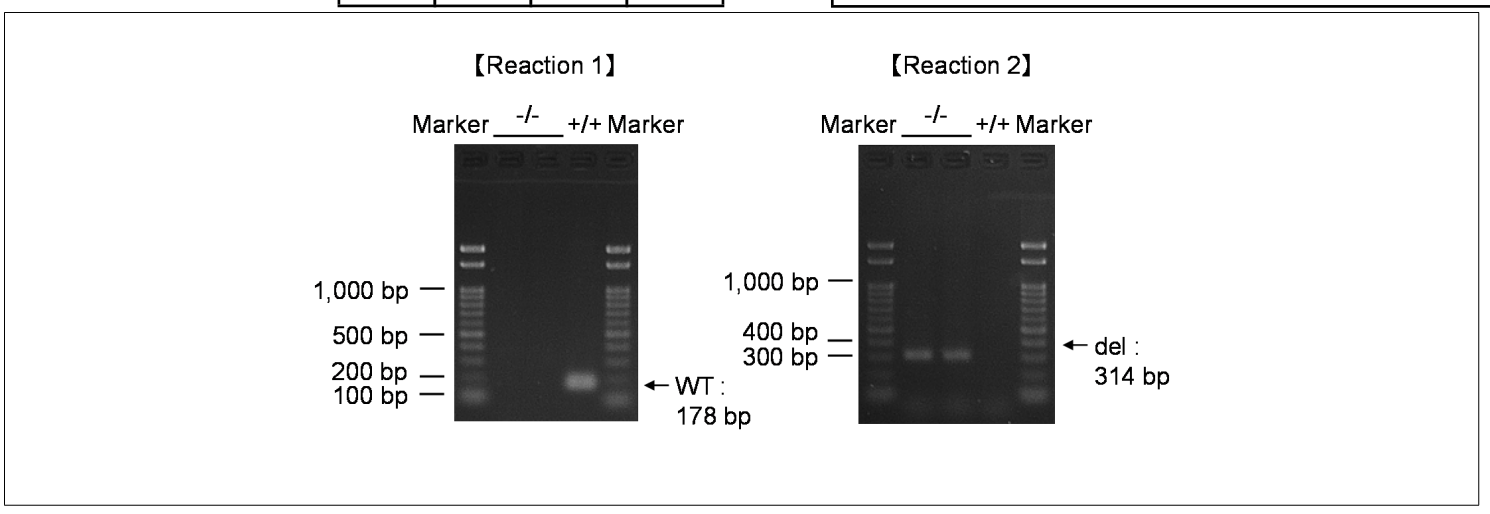
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8	2.8			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	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	178 bp	WT
primer 3	primer 4	314 bp	del

Comment

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.