PCR protocol

Sheet ID PS_08471
Release date : 5/9/2023
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RBRC 11813 **Strain name** C57BL/6J-Fads1/Fads2<em1Tkato>

Primer	primer_name	primer_seq	length		Reference
1	intron 5	TCGGGAGGGAGGGAAGGAAC	20		RIKEN BRC
2	Fads1_Rv	ATGCTGATCACGCTGAAAGAC	21		Depositor's primer
3	Fads2_Fw	AGTGGGGATTCCTGTCTACCC	21		Depositor's primer
4	Fads2_Rv	CAGGAACCAGATGGAAGGAAC	21		Depositor's primer

Reaction Components

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

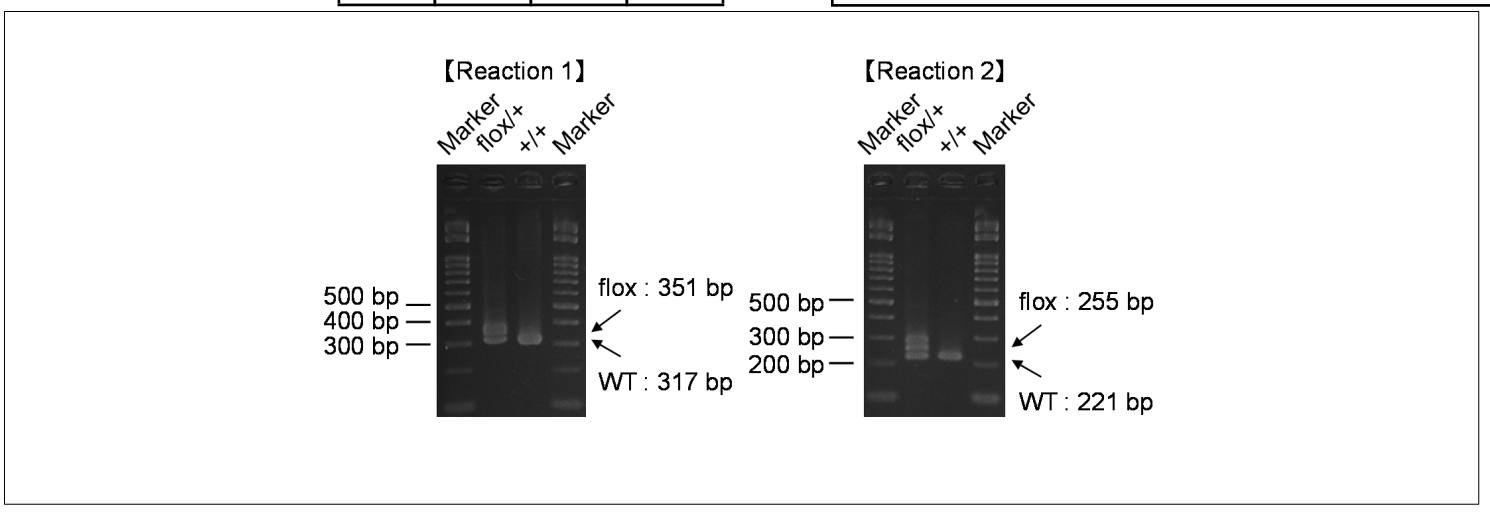
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8	2.8			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	0			
primer 3	10 pmol/μl	0	1.0			
primer 4	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

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 3% Nusieve in 0.5 X TBE (100 V, 50 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	317 bp	WT
primer 1	primer 2	351 bp	flox
primer 3	primer 4	221 bp	WT
primer 3	primer 4	255 bp	flox

Comment

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