PCR protocol

Sheet ID PS_07980
Release date : 9/21/2021
Print date : 9/21/2021

RBRC 10944 **Strain name** B6.Cg-Aicda<tm1.2Evo>

Primer	primer_name	primer_seq	length		Reference
1	AID-loxP1-F2	CATGATGATCAAAGTAGGCCTG	22		Depositor's primer
2	04892Common	CAGAGCTGAGGGATTAAAGGGCTG	24		RIKEN BRC
3	AID-loxP3-F3	ACTCAGAGTGAGTGTCAGCG	20		Depositor's primer
4		CATGCTGGACCAGAGTTCTGCC	22		RIKEN BRC

Reaction Components

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

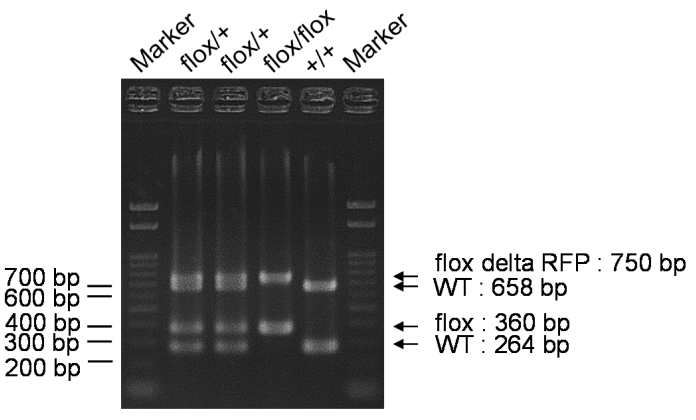
Reagent	Conc	Reaction 1	2	3	4	
H2O		0.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

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, 35 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	658 bp	WT	+/+ (wild-type): 658 bp and 264 bp flox/+ (heterozygous): 658 bp and 264 bp and 750 bp and 360 bp flox/flox (homozygous): 750 bp and 360 bp
primer 1	primer 2	750 bp	flox delta RFP	
primer 3	primer 4	264 bp	WT	
primer 3	primer 4	360 bp	flox	

Comment Standard PCR

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