PCR protocol

Sheet ID PS_08361
Release date : 6/27/2022
Print date : 6/27/2022

RBRC 11203 **Strain name** B6-Rora<tm1.1Shkm>

Primer	primer_name	primer_seq	length		Reference
1	RORa-U714624	TCAGAAGAATCAAGAGCAAGG	21		Depositor's primer
2	RORa-L715049	AGAAACATTCAGTCACAGCCG	21		Depositor's primer
3	RORa-U713951	AGCCAAGGCAGATTTATGACC	21		Depositor's primer

Reaction Components

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

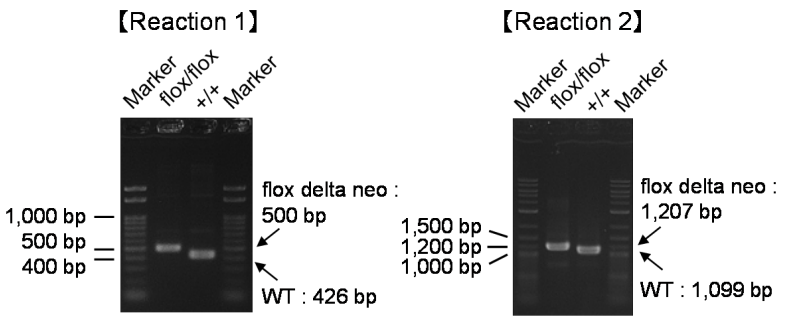
Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8	2.8			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	1.0			
primer 3	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	[Reaction 1]
2	98	10	*
3	60	30	*
4	68	60	* *Repeat for 30 cycles
1	94	120	[Reaction 2]
2	98	10	*
3	60	30	*
4	68	120	* *Repeat for 30 cycles

Marker marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 2-Log DNA Ladder (NEB, Cat. N3200)

Gel Run1:2% agarose in 1 X TAE (100 V, 30 min)Run2:1% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)
primer 1	primer 2	426 bp	WT
primer 1	primer 2	500 bp	flox delta neo
primer 2	primer 3	1,099 bp	WT
primer 2	primer 3	1,207 bp	flox delta neo
primer 2	primer 3	184 bp	null (After cre mediated excision)

Comment Standard PCR

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