PCR protocol

Sheet ID PS_08574
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RBRC 06539 **Strain name** B6.Cg-Dchs2<tm1.1Hmd>

Primer	primer_name	primer_seq	length		Reference
1	Dachsous2-loxp3-01	CTTGATGTAAGGCTGCGATG	20		Development, 149(9):dev200315 (2022).
2	Dachsous2-loxp3-02	CAAACTTCAAAAGTGGACATTGAG	24		Development, 149(9):dev200315 (2022).
3	Dachsous2-null-1	GGTGGGGCACAATCTAGAAA	20		Development, 149(9):dev200315 (2022).

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 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			

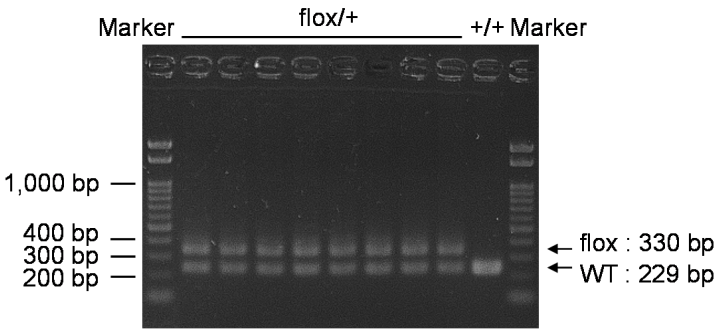
Reaction Conditions

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	360	* *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)

【Reaction 1】



primer_set		product_size	product (allele type)	
primer 1	primer 2	229 bp	WT	
primer 1	primer 2	330 bp	flox	
primer 2	primer 3	4,605 bp	WT	
primer 2	primer 3	576 bp	null (After cre mediated excision)	
primer 2	primer 3	>4,605 bp	flox delta neo	

Comment Standard PCR

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