PCR protocol

Sheet ID PS_07382
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RBRC 06292 **Strain name** B6.Cg-Ngly1<tm1Tasuz>

Primer	primer_name	primer_seq	length		Reference
1	Ngly1-82914F	GCATCACTGTCATCAGAAGCGT	22		Depositor's primer
2	Ngly1-83345R	TGACCCACATTCAAACTTCCAGG	23		Depositor's primer
3	Ngly1-85735R	AGCTGGACTTTGTAGTGG	18		Proc. Natl. Acad. Sci. USA, 112, 1398-1403 (2015).

Reaction Components

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

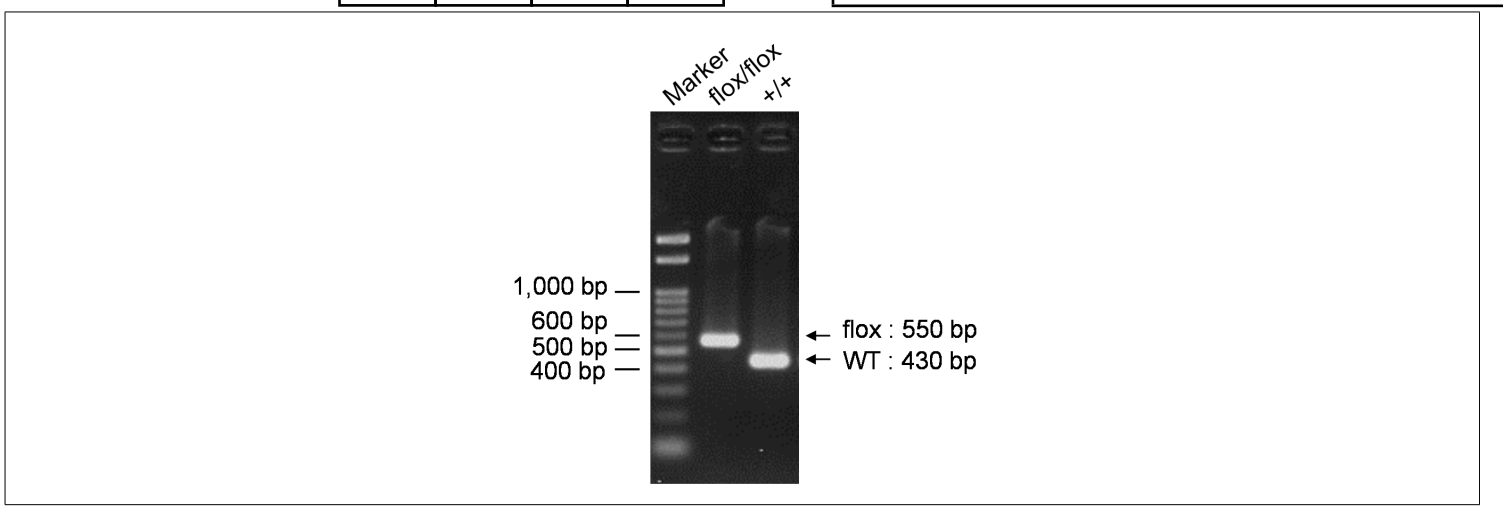
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
total		20				

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, 30 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	430 bp	WT	+/+ : 430 bp
primer 1	primer 2	550 bp	flox	flox/+ : 430 bp, 550 bp
primer 1	primer 3	800 bp	null (After cre mediated excision)	flox/flox: 550 bp

Comment Standard PCR

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