

RBRC 09391

Strain name B6;129-Rapgef4<tm1Sse>

Primer	primer_name	primer_seq	length	Reference
1	Promoter	GTGGCATCGCAGCAGAACTC	20	RIKEN BRC
2	intron 1	CTGATCACATTAGCAAGCTCTTGG	24	RIKEN BRC
3	Neo2	CTTCCATCCGAGTACGTGCT	20	Cell Metab., 12(2):117-129 (2010).

Reaction Components

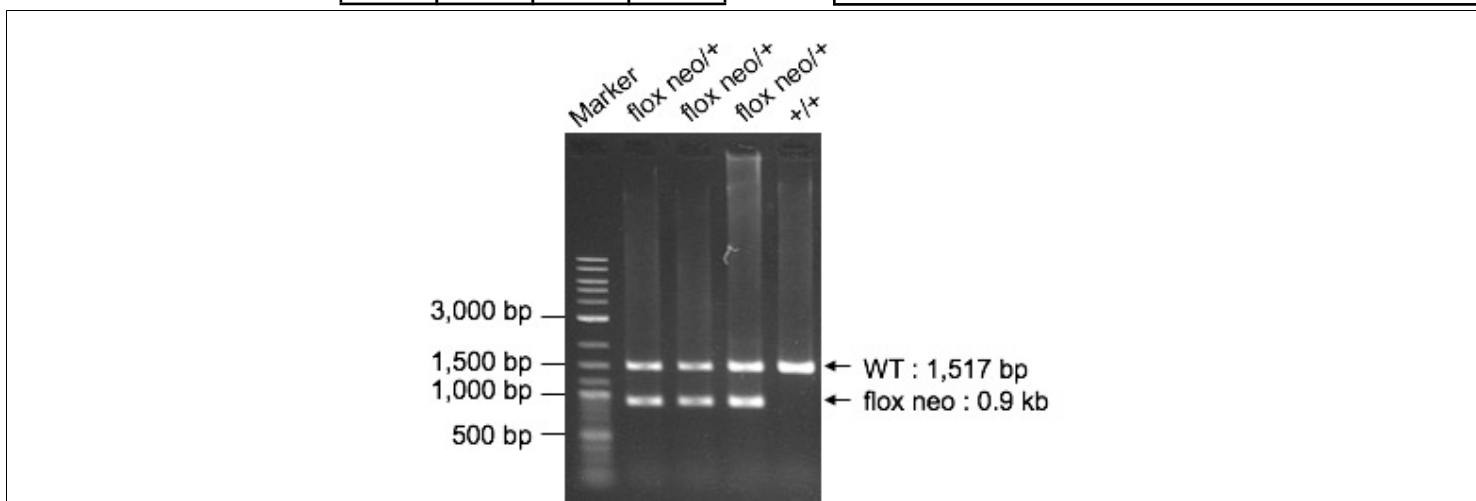
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	180*	*Repeat for 30 cycles

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		1.8				
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				

Marker 2-Log DNA Ladder (NEB, Cat. N3200)
Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)	
primer 1	primer 2	1,517 bp	WT
primer 2	primer 3	0.9 kb	flox neo

+/+ : 1517 bp
flox neo/+ : 1517 bp, 0.9 kb

Comment Standard PCR

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