

RBRC 10433

Strain name Runx2 (loxP) (Acc. No. CDB0832K)

Primer	primer_name	primer_seq	length	Reference
1	int4	TAAATCCAGATGCCCCTGAG	20	J. Bone Miner. Res., 28(10):2064-9 (2013).
2	int4	TTGAAACCATCCACAGGTGA	20	J. Bone Miner. Res., 28(10):2064-9 (2013).
3	int3	GCTTGCAGATTAGGCTAAGGC	21	RIKEN BRC

Reaction Components

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

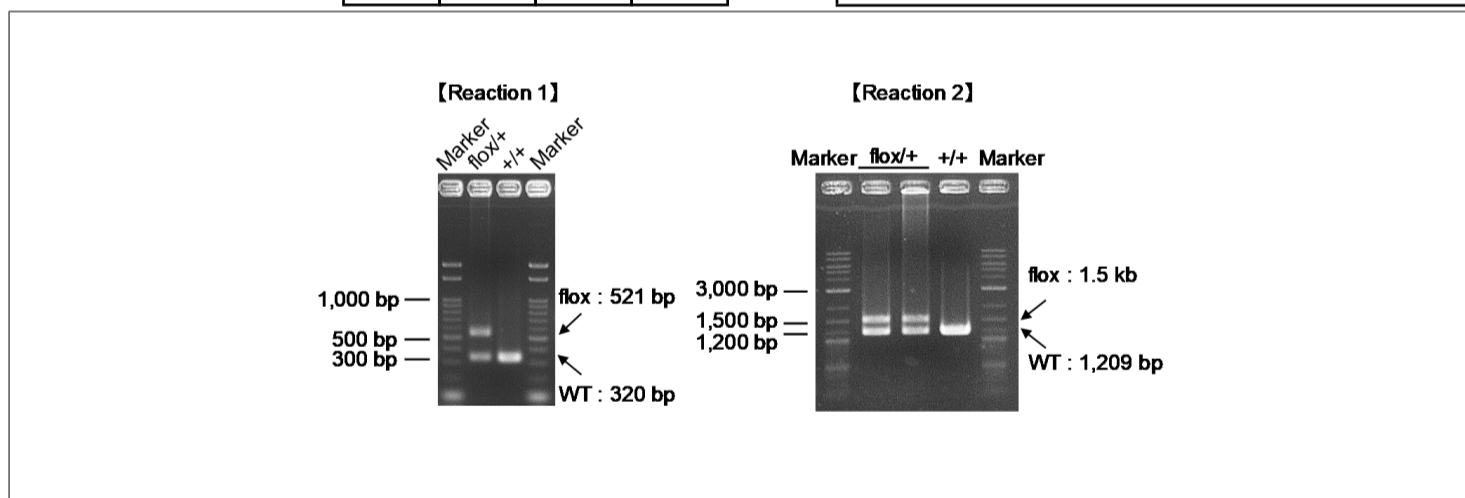
Reaction Conditions

step	temp (°C)	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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8	2.8		
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		

(μL)

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	320 bp WT
primer 1	primer 2	521 bp flox
primer 2	primer 3	1,209 bp WT
primer 2	primer 3	1.5 kb flox

[Reaction 1] +/+ : 320 bp
flox/+ : 320 bp, 521 bp
flox/flox : 521 bp
[Reaction 2] +/+ : 1,209 bp
flox/+ : 1,209 bp, 1.5 kb
flox/flox : 1.5 kb

Comment Standard PCR

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