

RBRC 06210

Strain name B6.Cg-Pgap3<tm1Ymra>

Primer	primer_name	primer_seq	length	Reference
1	LOXP-shortA2	GCTGCTGCTGACAGGACACATATC	24	Depositor's primer
2	LOXP-shortS2	ACTGGATCTTCCCCTGGACTCTG	23	Depositor's primer
3	PGAP3homoS2	AGGTACTTGGGGTGGCACTATGG	23	Depositor's primer

Reaction Components

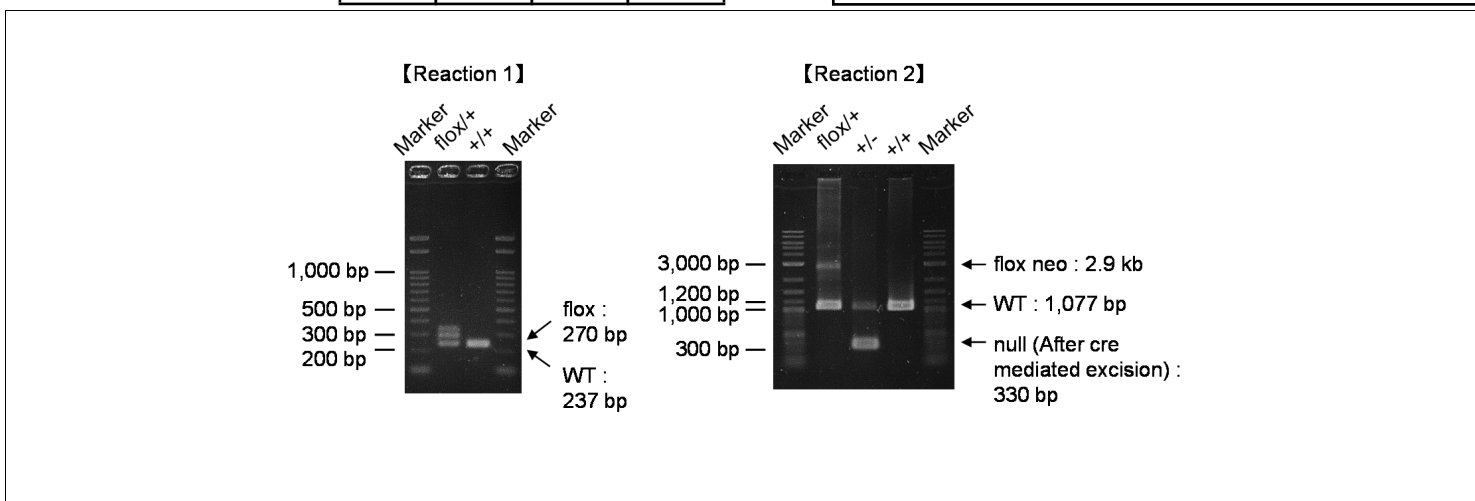
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8	2.8		
PCR buffer	2 X	10.0	10.0		
dNTP	2.0 mM	4.0	4.0		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	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 (°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	240*	*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	237 bp WT
primer 1	primer 2	270 bp flox
primer 1	primer 3	1,077 bp WT
primer 1	primer 3	2.9 kb flox neo
primer 1	primer 3	330 bp null (After cre mediated excision)

Comment Separated PCR

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