

RBRC 02759

Strain name B6.Cg-Atg7<tm1Tchi>

Primer	primer_name	primer_seq	length	Reference
1	Hind-Fw	TGGCTGCTACTTCTGCAATGATGT	24	J. Cell Biol., 169, 425-434 (2005)
2	Pst-Rv	CAGGACAGAGACCATCAGCTCCAC	24	J. Cell Biol., 169, 425-434 (2005)
3	intron 15	CCACTGGCCCATCAGTGAGCATG	23	RIKEN BRC

Reaction Components

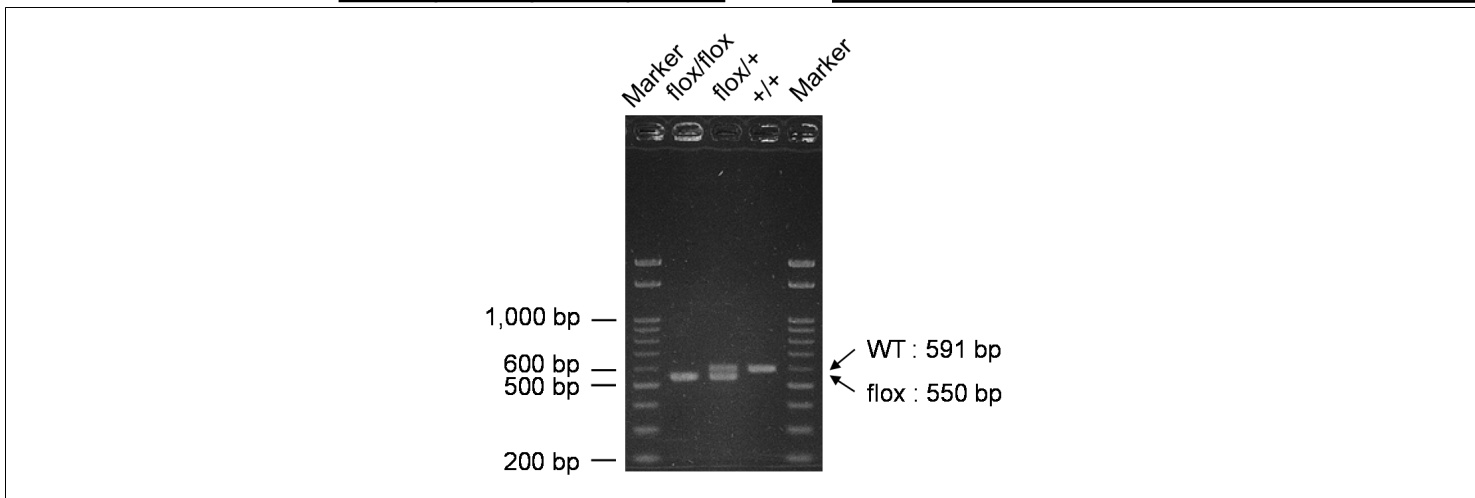
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	60*	*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 Gene Ladder 100 (Wako, Cat. 316-06951)
Gel 2% agarose in 1 X TAE (100 V, 70 min)



primer_set	product_size	product (allele type)
primer 1 / primer 3	591 bp	WT
primer 1 / primer 2	550 bp	flox

Comment Standard PCR

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

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Reaction Components

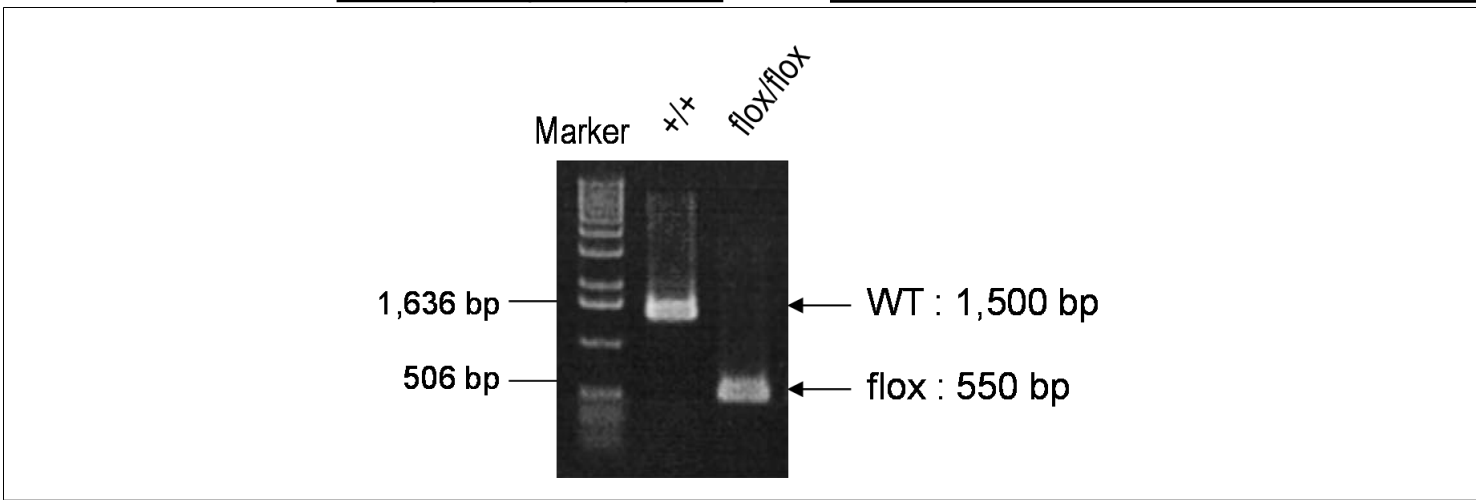
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

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

Reagent	Conc	Reaction 1	2	3	4	(μL)
H2O		2.8				
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				
DNA		1.0				
Taq	1 U/μl	0.2				
total		20				

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)
Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	550 bp	flox
primer 1	1,500 bp	WT

Comment Standard PCR

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Primer	primer_name	primer_seq	length	Reference
1	Hind-Fw	TGGCTGCTACTTCTGCAATGATGT	24	Depositor's primer
2	Pst-Rv	CAGGACAGAGACCATCAGCTCCAC	24	Depositor's primer

Reaction Components

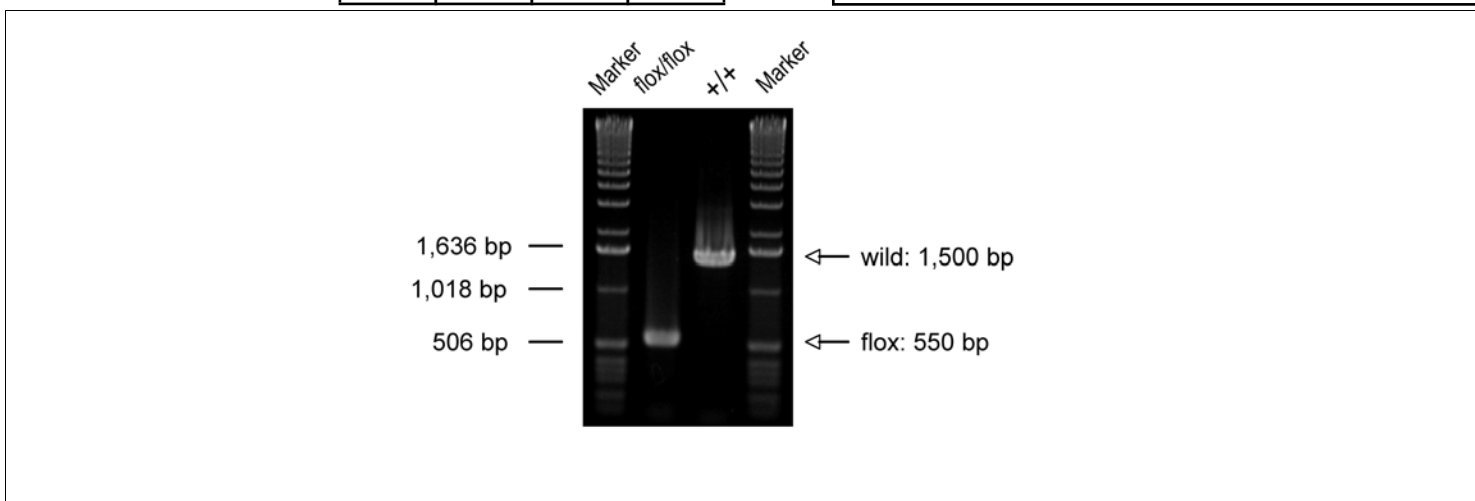
Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	60	30*	
4	72	120*	*Repeat for 30 cycles
5	72	300	

Reagent	Conc	Reaction 1	2	3	4	(µL)
H2O		4.8				
PCR buffer	2 X GC I	10.0				
dNTP	2.5 mM	2.0				
primer 1	10 pmol/µl	1.0				
primer 2	10 pmol/µl	1.0				
DNA		1.0				
Taq		0.2				
total		20				

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)
Gel 1% agarose in 1 X TAE (100 V, 30 min)



primer_set	product_size	product (allele type)
primer 1	550 bp	flox
primer 1	1500 bp	WT

Comment Standard PCR