

RBRC 02975

Strain name B6.129S-Atg5<tm1Myok>

Primer	primer_name	primer_seq	length	
1	5L2	CAGGGAATGGTGTCTCCCAC	20	Nature, 441, 885-889(2006).
2	ATG5 check 2	ACAACGTCGAGCACAGCTGCGCAAGG	26	Nature, 441, 885-889(2006).
3	ATG5 short 2	GTA CTGCATAATGGTTAACTCTTGC	26	Nature, 441, 885-889(2006).

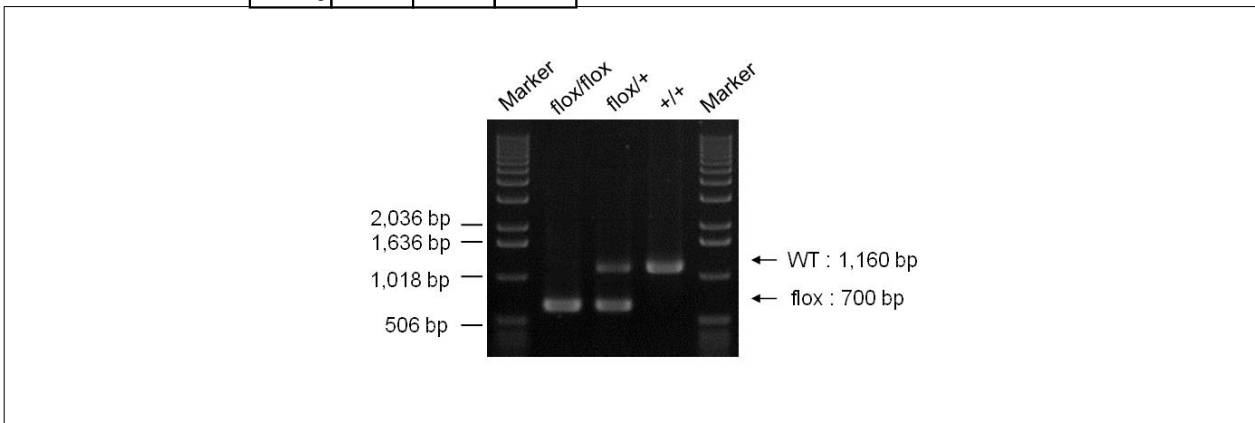
**Reaction Components**

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	90*	*Repeat for 30 cycles

	Conc	Run 1	Run 2	Run 3	Run 4
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		0.2			
<b>total</b>		<b>20</b>			



**Marker**

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

**Gel**

1% agarose in 1 X TAE (100 V, 30 min)

PCR products	primer_set	product_size	product
primer 1	primer 3	1160 bp	WT
primer 2	primer 3	700 bp	flox
primer 1	primer 3	300 bp	null (After cre mediated excision)

**Comment**

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Primer	primer_name	primer_seq	length	
1	ATG5 exon3-1	GAATATGAAGGCACACCCCTGAAATG	26	Nature, 441, 885-889(2006).
2	ATG5 check 2	ACAACGTCGAGCACAGCTGCGCAAGG	26	Nature, 441, 885-889(2006).
3	ATG5 short 2	GTAAGTGATAATGGTTAACTCTTGC	26	Nature, 441, 885-889(2006).

### Reaction Components

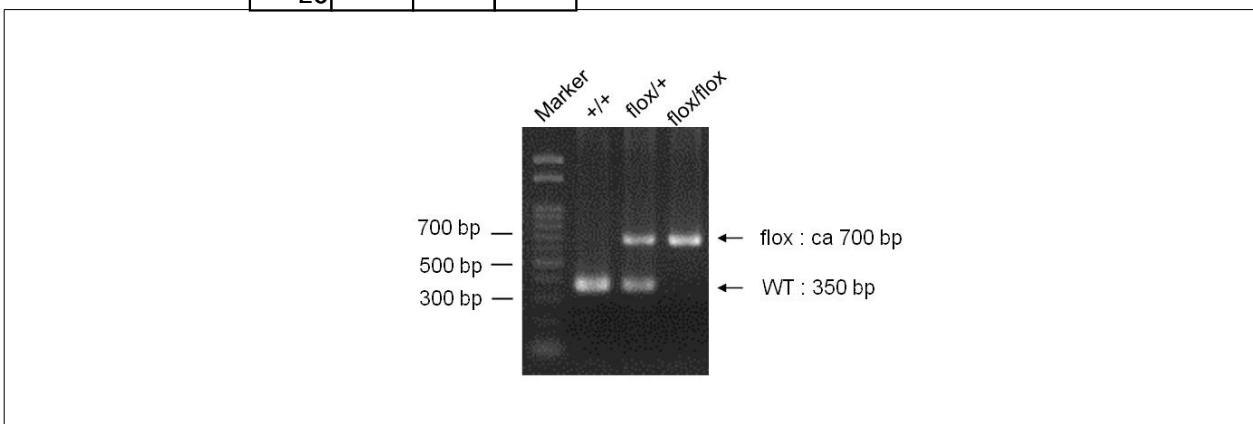
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2 mM	4.0			
primer 1	10 pmol/ $\mu$ l	1.0			
primer 2	10 pmol/ $\mu$ l	1.0			
primer 3	10 pmol/ $\mu$ l	1.0			
DNA		1.0			
Taq	1 U/ $\mu$ l	0.2			
<b>total</b>		<b>20</b>			

### Reaction Conditions

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

### Standard PCR



<b>Marker</b> Gene Ladder 100 (Wako, Cat. 316-06951)
<b>Gel</b> 2% agarose in 1 X TAE (100 V, 30 min)

PCR products	primer_set	product_size	product
primer 1	primer 3	350 bp	WT
primer 2	primer 3	700 bp	flox

### Comment

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

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2	ATG5 check 2	ACAACGTCGAGCACAGCTGCGCAAGG	26	Nature, 441, 885-889(2006).
3	ATG5 short 2	GTA CTGCATAATGGTTTAACTCTTGC	26	Nature, 441, 885-889(2006).

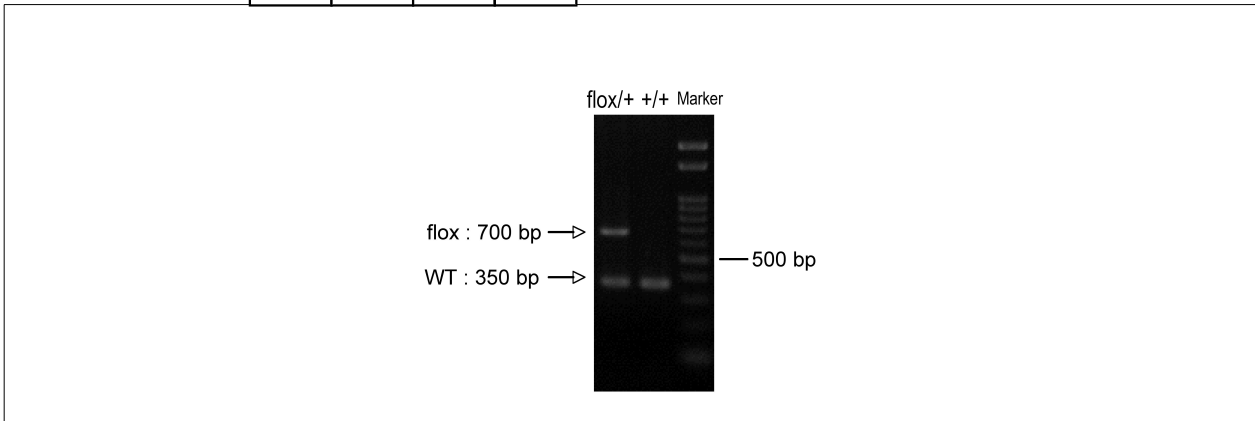
**Reaction Components**

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

**Reaction Conditions**

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		16.3			
PCR buffer	10 X	2.5			
dNTP	2.5 mM	2.0			
primer 1	10 pmol/ $\mu$ l	1.0			
primer 2	10 pmol/ $\mu$ l	1.0			
primer 3	10 pmol/ $\mu$ l	1.0			
DNA		1.0			
Taq		0.2			
<b>total</b>		<b>25</b>			

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



**Marker**  
Gene Ladder 100 (Wako, Cat. 316-06951)

**Gel**  
2% agarose in 1 X TAE (100 V, 30 min)

PCR products	primer_set	product_size	product
primer 1	primer 3	350 bp	WT
primer 2	primer 3	700 bp	flox

**Comment**