

RBRC 09356

Strain name Sp7(Acc.No.CDB0693K)

Primer	primer_name	primer_seq	length	
1	intron 1	CCACAGGACTGAGTGGGATG	20	RIKEN BRC
2	exon 2	GAACTAGGCACTGGCAAAGG	20	J. Biol. Chem., 287(40):33179-33190 (2012).
3	olMR0214	CCGGAGAACCTGCGTGCAATCC	22	JAX Genotyping Protocol

**Reaction Components**

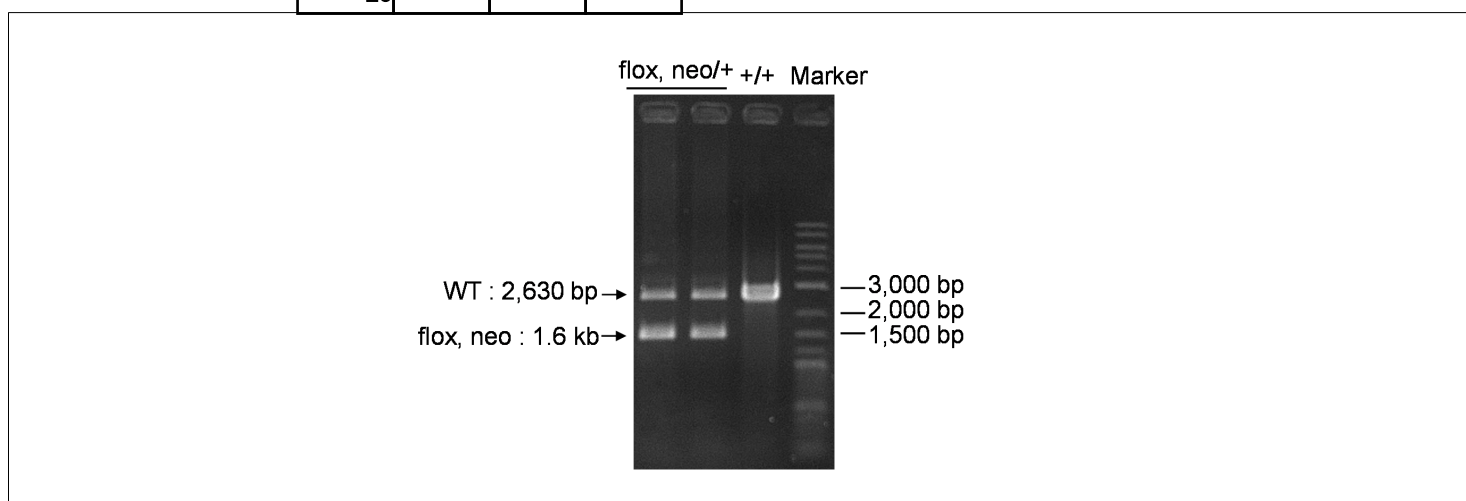
**Enzyme** TOYOBO KOD FX (Code.No : KFX-101)

**Reaction Conditions**

step	temp (°C)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	240*	*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	1 U/µl	0.2			
<b>total</b>		<b>20</b>			

Standard PCR



Marker	PCR products	primer_set	product_size	product
2-Log DNA Ladder (NEB, Cat. N3200)	primer 1	primer 2	2,630 bp	WT
	primer 1	primer 3	1.6 kb	flox, Neo

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

**Comment**

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