

RBRC 02653

Strain name B6.Cg-Tsix/Xist<tm3Sado>

Primer	primer_name	primer_seq	length	
1	R700P2	CGGGGCTTGGTGGATGGAAAT	21	Depositor's primer
2	F1063AS	GCACAACCCCGCAAATGCTA	20	Depositor's primer
3		GGTCCCTCGAAGAGGTTCACTAG	23	RIKEN BRC

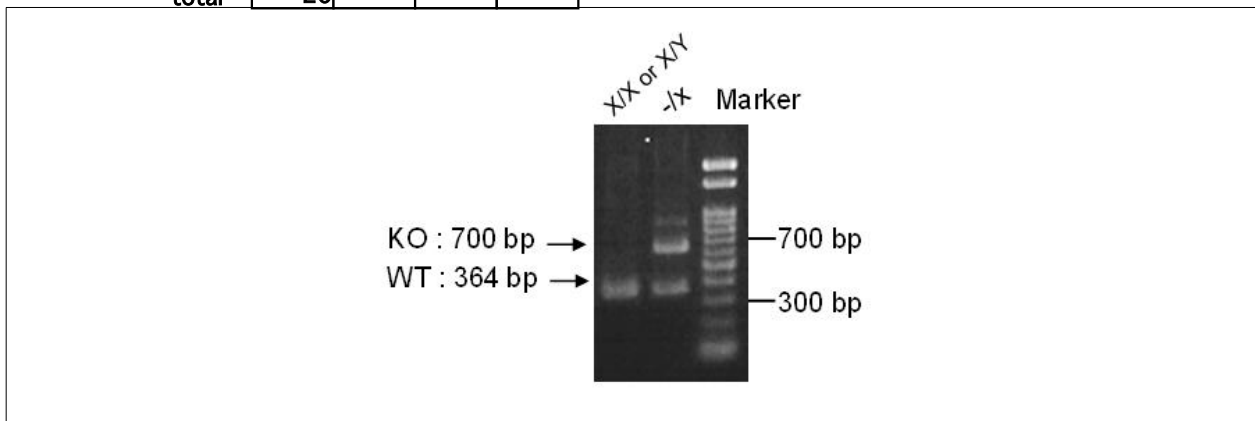
### Reaction Components

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

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



<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 2		364 bp	WT
primer 2	primer 3		700 bp	KO

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

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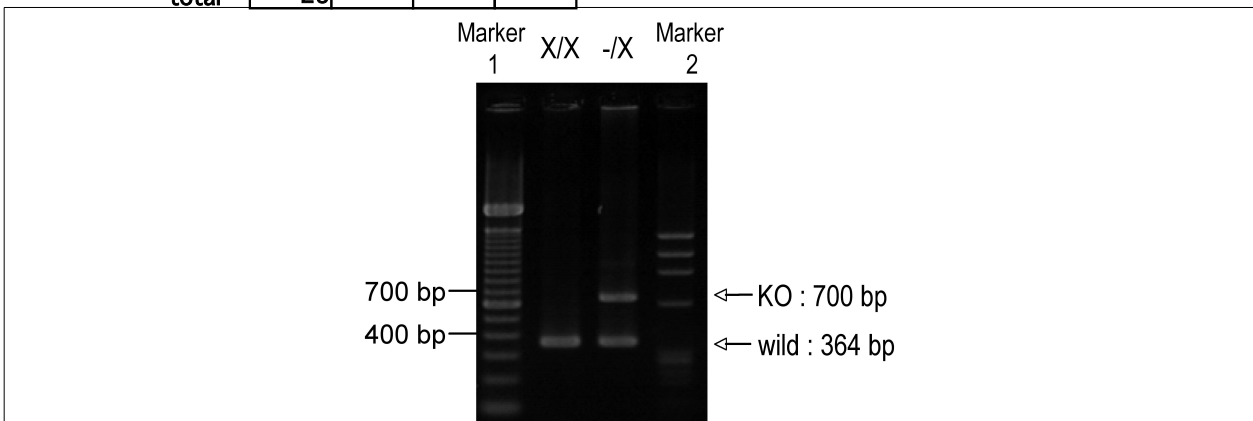
### Reaction Components

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**  
marker 1:100 bp ladder (Invitrogen, Cat. 15628-050), marker2:  $\phi$ X174 Hae III

**Gel**  
2% agarose in 1 X TAE (100 V, 35 min)

PCR products		product_size	product
primer 1	primer 2		
primer 1	primer 2	364 bp	WT
primer 3	primer 2	700 bp	KO

Comment