

# PCR protocol

RBRC 00989

Strain name GAD65 Knockout mice (C line)

Primer	primer_name	primer_seq	length	
1	G65S3	GGGAAGCCAGCGGAGGGCGG	20	Depositor's primer
2	G65A3	CCCATTACCTGTTGCGTGACAG	22	Depositor's primer
3	pPNT3	GGCTGCTAAAGCGCATGCTC	20	Depositor's primer

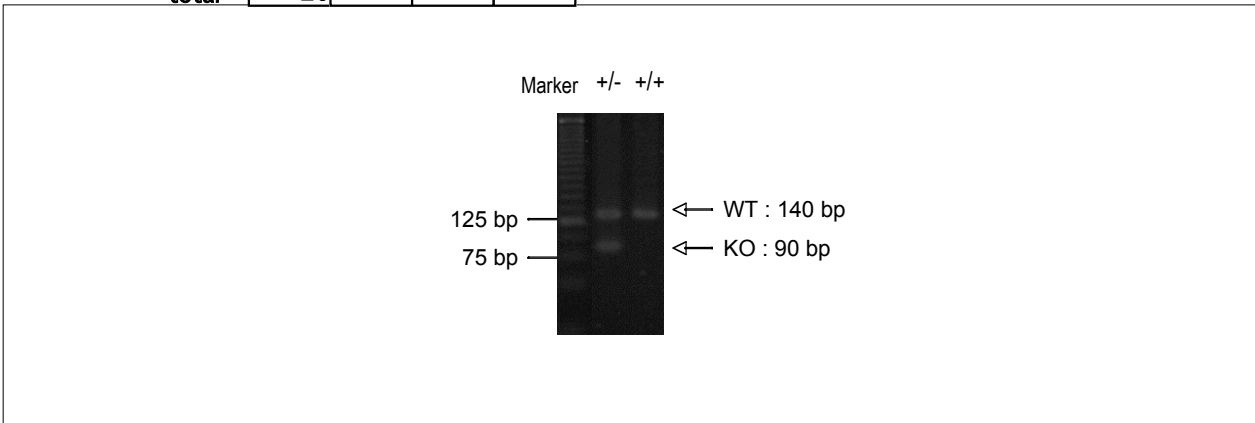
**Reaction Components**

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

**Reaction Conditions**

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		12.3			
PCR buffer	10 X	2.0			
dNTP	2.5 mM	1.6			
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		0.1			
<b>total</b>		<b>20</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**  
 25 bp DNA ladder (Invitrogen, Cat. 10597-011)

**Gel**  
 4% Nusieve in 0.5 X TBE (100 V, 45 min)

PCR products			
	primer_set	product_size	product
primer 2	primer 3	90 bp	KO
primer 1	primer 2	140 bp	WT

Comment