PCR protocol

RBRC 01862 **Strain name** Mesp2-GFP

Primer

	primer_name	primer_seq	length	
1	Mesp2-R1	ggcacttccaaggcagcccaatc	23	
2	PGK-R	CTAAAGCGCATGCTCCAGACT	21	
3	P2-L3	CATCATGCCAGAGACTACAGCCTCA	25	

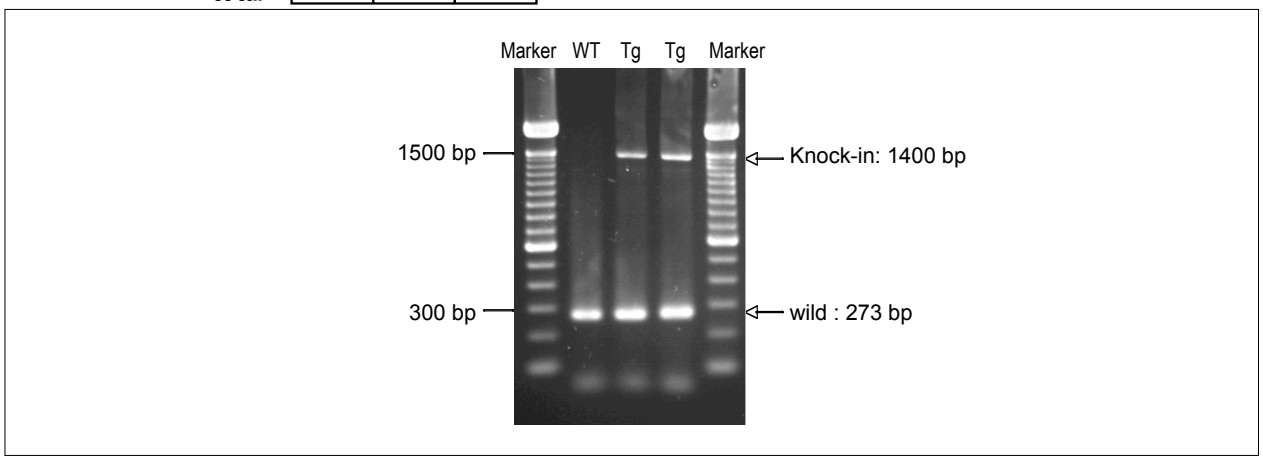
Reaction Components

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

	Conc	Run 1	Run 2	Run 3
H2O		3.8		
PCR buffer	2 X	10.0		
dNTP	2.5 mM	2.0		
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		
	total	20		

Reaction Conditions

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



Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	1400 bp	knock-in
primer 1	primer 3	273 bp	wild

Comment