

PCR protocol

RBRC 00357

 Strain name BALB/cA-TgH(Tnc)-Foxn1^{nu}

Primer	primer_name	primer_seq	length
1	TN-G1-1	GGTACCTGATTCCGAAGTGCATTGTCACG	29 Tnc
2	TN-150R (~3'TN)	AAGCTAGAAAAGATGCCTGGC	20 Tnc
3	TN-lacZ	CTCCATGCTTGGAAACAACGAGCGCAGC	27 Tnc
4	nu_wild	ggcccagcaggcagcccaggg	21 nude
5	nu_mut	ggcccagcaggcagcccaggc	21 nude
6	common	gctgcctcctctggagctctg	21 nude

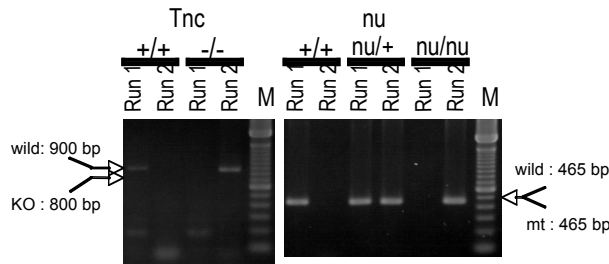
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	300	
2	94	30*	
3	55	30*	【Run 1, 2】
4	72	60*	*Repeat for 37 cycles
5	72	300	
1	94	180	
2	94	30*	
3	63	30*	【Run 3, 4】
4	72	60*	*Repeat for 30 cycles
5	72	300*	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		17.3	17.3	17.3	17.3
PCR buffer	10 X	2.5	2.5	2.5	2.5
dNTP	10 mM	2.0	2.0	2.0	2.0
primer 1	10 pmol/μl	1.0	1.0	0	0
primer 2	10 pmol/μl	1.0	0	0	0
primer 3	10 pmol/μl	0	1.0	0	0
primer 4	10 pmol/μl	0	0	1.0	0
primer 5	10 pmol/μl	0	0	0	1.0
primer 6	10 pmol/μl	0	0	1.0	1.0
DNA		1.0	1.0	1.0	1.0
Taq		0.2	0.2	0.2	0.2
total		25	25	25	25



Marker

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

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

PCR products

primer_set	product_size	product
primer 1 primer 2	900 bp	wild
primer 1 primer 3	800 bp	KO
primer 4 primer 6	465 bp	wild
primer 5 primer 6	465 bp	mutant

Comment