

PCR protocol

RBRC 00238

Strain name C57BL/6-TgH(AchR-gamma/epsilon)

Primer	primer_name	primer_seq	length
1	LCB668 (Neo1007)	CTTCCTCGTGCTTTACGGTA	20 ^{neo}
2	LCB665 (g436r)	GTTCTCCAGGACGATACTG	19
3	LCB665 (g301)	CCTCACAACCTAACGTCTGGA	20
4	LCB667 (e179)	AGGTTGGTTAGGGTGACCTT	20
5	LCB666 (e48)	CAGAAGCCAGGGAAAGAATG	20

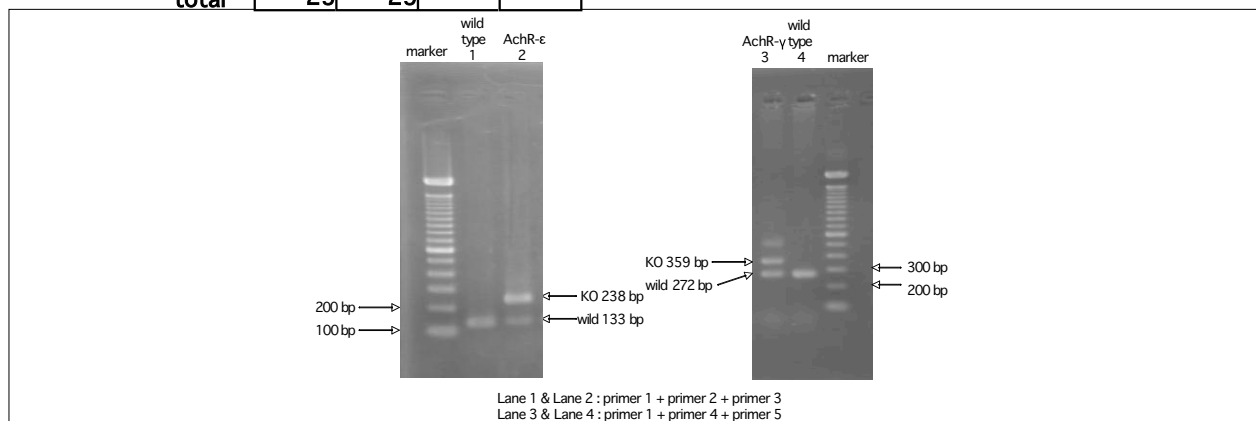
Reaction Components

Enzyme Run1: Roche Taq (Cat. No. BM1418432) Run2:
TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

step	temp (°C)	time (sec)	note
1	94	300	
2	94	30*	
3	55	60*	
4	72	60*	*Repeat for 32 cycles
5	72	300	

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		16.75	5.75		
PCR buffer	10 X	2.5	0		
PCR buffer	2 X GC I	0	12.5		
dNTP	10 mM	0.5	1.5		
primer 1	10 pmol/μl	1.0	1.0		
primer 2	10 pmol/μl	1.0	0		
primer 3	10 pmol/μl	1.0	0		
primer 4	10 pmol/μl	0	1.0		
primer 5	10 pmol/μl	0	1.0		
DNA		2	2		
Taq		0.25	0.25		
total		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 2	primer 3	272 bp	WT (gamma)
primer 1	primer 2 + primer	359 bp	KO (gamma)
primer 4	primer 5	133 bp	WT (epsilon)
primer 1	primer 4 + primer	238 bp	KO (epsilon)

Comment