

PCR protocol

RBRC 02492

Strain name BAC-ΔP2ΔEME#2/Mesp1 Mesp2-dko

Primer	primer_name	primer_seq	length
1	Mesp-ESKO-con.F6	GGCTCTGGTATGTGAAGTAAG	21
2	ESKO-R2	ATGTCTTCCAGATGGGTGATGG	22
3	oIMR0042	CTAGGCCACAGAATTGAAAGATCT	24
4	oIMR0043	GTAGGTGGAAATTCTAGCATCATCC	25
5	P2-L3	CATCATGCCAGAGACTACAGCCTCA	25
6	P2-R3	GTCACGGCATTAGCAAGGTTGAGAA	25
7	NeoAL2	GGGGATGCGGTGGGCTCTATGGCTT	25
8	HLH-R3	GGAAGTTGAGTTCCTCATCACGATC	25

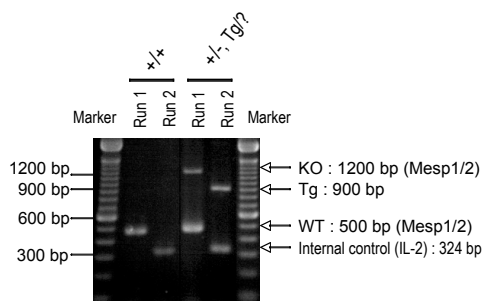
Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

Reaction Conditions

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		15.3	15.3		
PCR buffer	10 X	2.5	2.5		
dNTP	2.5 mM	2.0	2.0		
primer 1	10 pmol/μ	0	1.0		
primer 2	10 pmol/μ	0	1.0		
primer 3	10 pmol/μ	0	1.0		
primer 4	10 pmol/μ	0	1.0		
primer 5	10 pmol/μ	1.0	0		
primer 6	10 pmol/μ	1.0	0		
primer 7	10 pmol/μ	1.0	0		
primer 8	10 pmol/μ	1.0	0		
DNA		1.0	1.0		
Taq		0.2	0.2		
total		25	25		

step	temp (°C)	time (sec)	note
1	94	180	
2	94	30*	
3	65	30*	
4	72	90*	*Repeat for 30 cycles
5	72	300	



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 5	500 bp	wild (Mesp1/2)
primer 7	1200 bp	KO (Mesp1/2)
primer 1	900 bp	Tg
primer 3	324 bp	internal control (IL-2)

Comment