PCR protocol

RBRC 00494 **Strain name** B10.MOL-TEN1

Primer	primer_name	primer_seq	length		
1	common	GAGGACAGCTTCAAGGGCTCTG	22		RIKEN BRC
2	B6	GGGACTCTCCGAACTCAGTTTG	22		RIKEN BRC
3	B10	GGGACTCTCCGAACTCAGTTTT	22		RIKEN BRC
4	common	GCCCTCCAAGTAGACCCTCCAG	22		RIKEN BRC
5	b, k	CAGAAGTACTGGGATGACCAGTC	23		RIKEN BRC
6	f	CAGAAGTACTGGGATGACCAGTA	23		RIKEN BRC

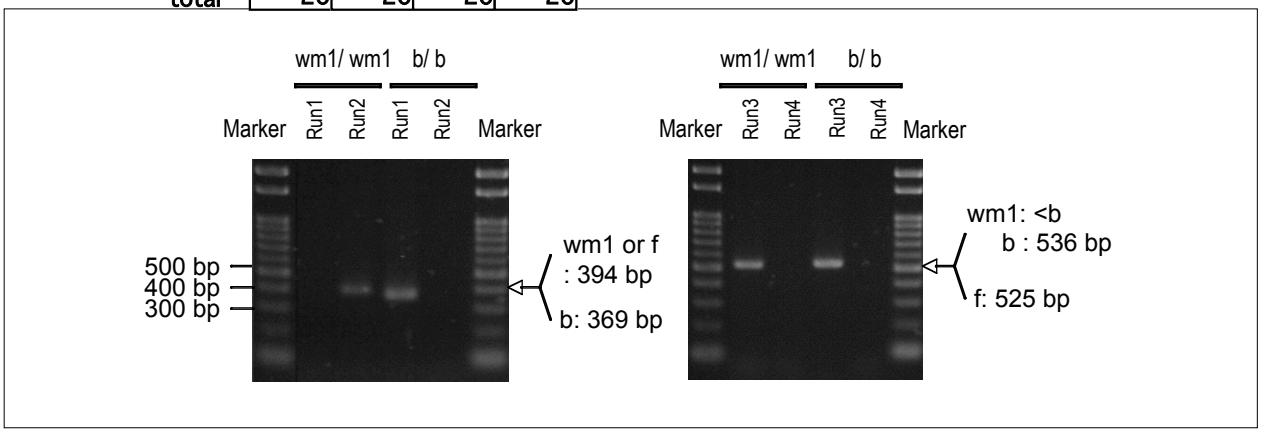
Reaction Components

Enzyme Roche Taq (Cat. No. BM1418432)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		13.3	13.3	13.3	13.3
PCR buffer	10 X	2.0	2.0	2.0	2.0
dNTP	2.5 mM	1.6	1.6	1.6	1.6
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	1.0
primer 5	10 pmol/μl	0	0	1.0	0
primer 6	10 pmol/μl	0	0	0	1.0
DNA		1.0	1.0	1.0	1.0
Taq	5U/μl	0.1	0.1	0.1	0.1
	total	20	20	20	20

Reaction Conditions

step	temp (℃)	time (sec)		note
1	94	180		
2	94	30	*	
3	60	30	*	
4	72	60	*	*Repeat for 35 cycles
5	72	300		



Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

Gel

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

PCR products

primer_set		product_size	product
primer 1	primer 2	369 bp	B6 (Type : b)
primer 1	primer 3	394 bp	B10.M(Type : f), B10.MOL-TEN1
primer 4	primer 5	536 bp	Type : b
primer 4	primer 5	<Type : b	Type : wm1
primer 4	primer 6	525 bp	Type : f

Comment