PCR protocol

Sheet ID PS_08295
Release date : 9/5/2022
Print date : 9/5/2022

RBRC 04293

Strain name B6-Pold1<exo.uchi>

Primer	primer_name	primer_seq	length		Reference
1	WT_D400	ATCACCGGCTACAACATTCAGAACTTTAA	29		RIKEN BRC
2	common	GCCGCGCTCCAAAGCATGTAC	21		RIKEN BRC
3	common	GCAAGTGCGGAGGTTGTCTTGG	22		RIKEN BRC
4	MT_A400	CCGAGAGATGAGGTATGGGAGAG	23		RIKEN BRC

Reaction Components

Enzyme: QIAGEN Multiplex PCR Kit (Cat. No. 206143)

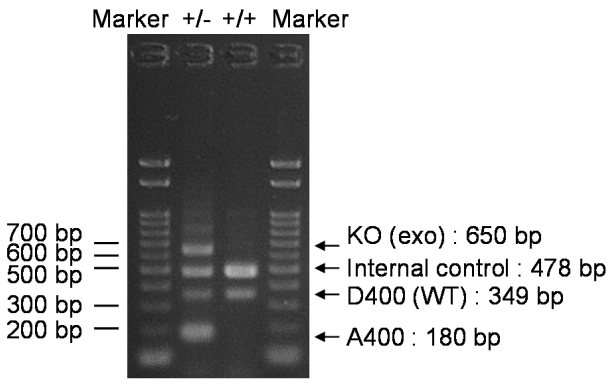
Reagent	Conc	Reaction 1	2	3	4	
primer 1	10 pmol/μl	1.0				(μL)
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
primer 4	10 pmol/μl	1.0				
Master Mix	2 X	5.0				
DNA		1.0				
	total	10				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	95	900	
2	94	30	*
3	60	90	*
4	72	60	* *Repeat for 30 cycles

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

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



primer_set		product_size	product (allele type)
primer 2	primer 3	478 bp	Internal control
primer 2	primer 3	650 bp	KO
primer 1	primer 2	349 bp	D400
primer 3	primer 4	180 bp	A400

Comment Allele specific PCR (ASP)

PCR protocol

Sheet ID PS_03684
Release date : 3/10/2014
Print date : 9/5/2022

RBRC 04293

Strain name B6-Pold1<exo.uchi>

Primer	primer_name	primer_seq	length		Reference
1	P1	GGAGTCCAGGTGTGCGTTAC	20		Depositor's primer
2	P3	CAGATTCCCCTCTGTGCATC	20		Depositor's primer

Reaction Components

Enzyme: TaKaRa Ex Taq (Cat. No. RR001A)

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

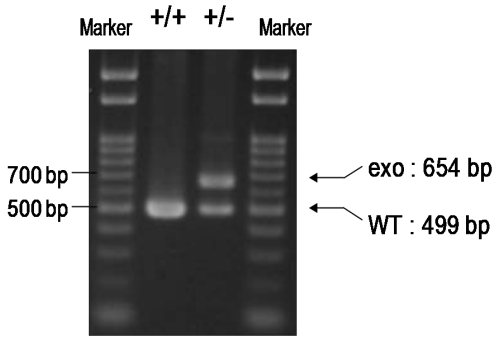
(μL)

Reaction Conditions

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

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

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



primer_set		product_size	product (allele type)
primer 1	primer 2	499 bp	WT
primer 1	primer 2	654 bp	exo

Comment Standard PCR