PCR protocol

Sheet ID PS_06174
Release date : 1/13/2016
Print date : 1/13/2016

RBRC 06502 **Strain name** C.129(B6)-Aqp6<tmMysi>

Primer	primer_name	primer_seq	length		
1		TCTGTTCTGCCCTGGCCTGTG	21		RIKEN BRC
2		CTGTCTGGCCTAGGTGAATGC	21		RIKEN BRC
3	Neo-Reverse	GAGAACCTGCGTGCAATCCATC	22		

Reaction Components

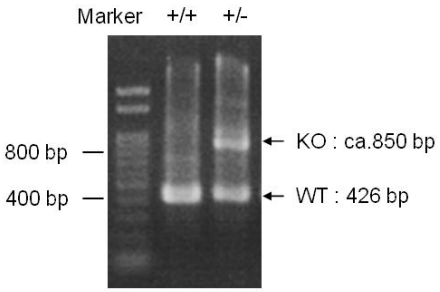
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.8			
PCR buffer	2 X	10.0			
dNTP	2.0 mM	4.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10	*
3	60	30	*
4	68	60	* *Repeat for 30 cycles

Standard PCR



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	426 bp	WT
primer 2	primer 3	ca 850 bp	KO

Comment Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

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RBRC 06502 **Strain name** C.129(B6)-Aqp6<tmMysi>

Primer	primer_name	primer_seq	length		
1	5' primer 1	GCTCCAGATCGCCATCACC	19		Depositor's primer
2	3' primer	CGGGGCAGCTCAGAATAGC	19		Depositor's primer
3	5' primer 2	CTAGGGGAGGAGTAGAAGG	19		Depositor's primer

Reaction Components

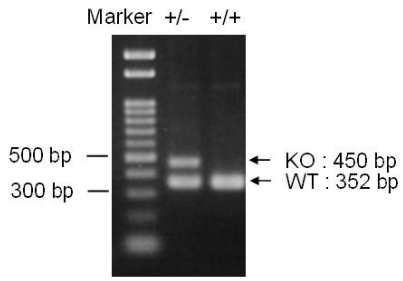
Enzyme QIAGEN Multiplex PCR Kit (Cat. No. 206143)

	Conc	Run 1	Run 2	Run 3	Run 4
H2O		1.0			
Master Mix	2 X	5.0			
primer 1	10 pmol/μl	1.0			
primer 2	10 pmol/μl	1.0			
primer 3	10 pmol/μl	1.0			
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
5	72	600	

Standard PCR



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	352 bp	WT
primer 2	primer 3	450 bp	KO

Comment