PCR protocol

Sheet ID PS_08263
Release date : 9/21/2021
Print date : 9/21/2021

RBRC 05269

Strain name microRNA9-2 (Acc. No. CDB0072K)

Primer	primer_name	primer_seq	length		Reference
1		GTGCAATCAAGCTGCCTGCAC	21		RIKEN BRC
2	p2	CCCTTTTCCCTGCTAGCTCTAAG	23		J. Neurosci., 31, 3407-3422 (2011).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4
H2O		2.8			
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			
DNA		1.0			
Taq	1 U/μl	0.2			
	total	20			

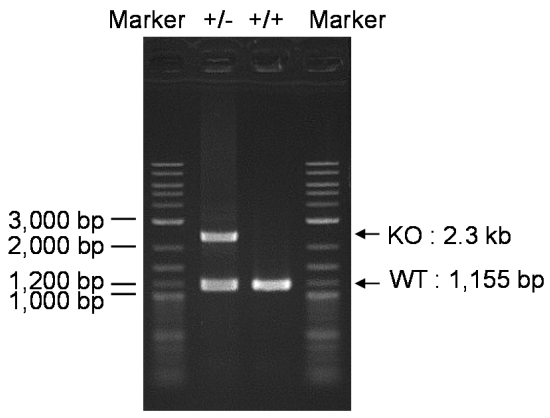
(μL)

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	63	30*	
4	68	180*	*Repeat for 30 cycles

Marker 2-Log DNA Ladder (NEB, Cat. N3200)

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



primer_set		product_size	product (allele type)
primer 1	primer 2	1,155 bp	WT
primer 1	primer 2	2.3 kb	KO(flox neo)

+/+ (wild-type): 1,155 bp
+/- (heterozygous): 1,155 bp and 2.3 kb

Comment Standard PCR

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