PCR protocol

Sheet ID PS_08538
Release date : 10/04/2023
Print date : 10/4/2023

RBRC 09510 **Strain name** B6;Cg-Rsph4a<tm1.2Hmd>

Primer	primer_name	primer_seq	length		Reference
1	Rsph4a-flox-F	CCACAATCTTGTCCCATTGA	20		Depositor's primer
2	Rsph4a-flox-R	CCATGATGGCTCCAGTTAAA	20		Depositor's primer
3	Rsph4a-MT-F	CTCCATGGGCACTTACTTTC	20		Dev. Cell., 35(2):236-46 (2015).

Reaction Components

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

Reagent	Conc	Reaction 1	2	3	4	
H2O		2.8	2.8			(μL)
PCR buffer	2 X	10.0	10.0			
dNTP	2.0 mM	4.0	4.0			
primer 1	10 pmol/μl	1.0	0			
primer 2	10 pmol/μl	1.0	1.0			
primer 3	10 pmol/μl	0	1.0			
DNA		1.0	1.0			
Taq	1 U/μl	0.2	0.2			
	total	20	20			

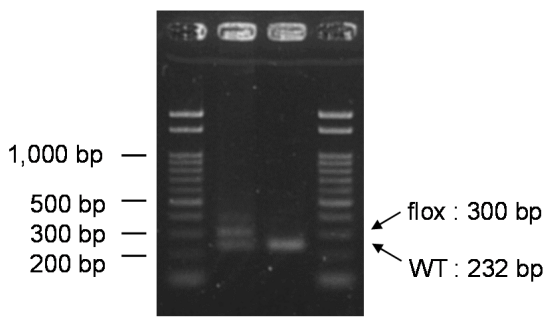
Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	[Reaction 1]
2	98	10	*
3	55	30	*
4	68	60	* *Repeat for 30 cycles
1	94	120	[Reaction 2]
2	98	10	*
3	55	30	*
4	68	240	* *Repeat for 30 cycles

Marker marker Run1: Gene Ladder 100 (Wako, Cat. 316-06951), marker Run2: 2-Log DNA Ladder (NEB, Cat. N3200)

Gel Run1:2% agarose in 1 X TAE (100 V, 30 min)Run2:1% agarose in 1 X TAE (100 V, 30 min)

【Reaction 1】



primer_set		product_size	product (allele type)
primer 1	primer 2	232 bp	WT
primer 1	primer 2	300 bp	flox
primer 2	primer 3	2,965 bp	WT
primer 2	primer 3	3 kb	flox

Comment Standard PCR

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