

Kit/Enzyme: Vazyme 2 x Phanta Flash

Tarih:

04.04.24

PCR ID:

31998 Nestin-Cre

PCR Mix		
	Reagent	µl
	dH ₂ O	y
	2 x Phanta Flash Master Mix	12,5
25mM	Mg	
5M	Betaine	
	DMSO	
10mM	dNTP	
	Taq	
10µM	WT Primer_F	1
10µM	MT Primer_F	1
10µM	Primer_R	1
	Alq	15,5 + y
x ng/µl	DNA	20

Sample DNA		
Sample Name	Conc.	Vol in PCR
N1	4,44 ng/µl	4,5 µl
N2	3,54 ng/µl	5,65 µl
WT1	20 ng/µl	1 µl
WT2	20 ng/µl	1 µl

Primer	Samples
Wild type forward: CAT AAT AAA TTG TTC ACT CTC AAA GG	N1
Mutant forward: GTT CTT GAG TAG TGC GTC ATC G	N2
Common reverse: CCA GAG CTG TGA TTT GTA ATT CT	WT1
	WT2
Product Size:	Mutant= 87 bp
	WT = 93 bp

PCR Condition	Program:
initial denaturation: 98°C	30 sec
5 cycle	
denaturation: 98°C	30 sec
annealing: 63-58 °C (↓1°C/c)	5 sec
extension: 72°C	1 sec
30 cycle	
denaturation: 98°C	30 sec
annealing: 54 °C	5 sec
extension: 72°C	1 sec
Final Extension	
72°C	1 min

Notes
3.5% agarose gel / 110 V - 90 mins + 30 mins

