



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Zfp329^{em1J}J
Stock Number: 028450
Allele: Zfp329^{em1J}
Protocol Name: Zfp329^{em1J}-alternate1
Method: End Point Analysis
Created: 18-January -2016 (JKELMEN) **Updated:** 19-February -2016 (ESCHAAB)

Notes

Expected Results: Mutant = -----/-----
Heterozygote = TGGAAGCCACTC/-----
Wild type = TGGAAGCCACTC/TGGAAGCCACTC

Attachments



View 28450.JPG

- View Zfp329 genomic1.gcs

Sequence

Sequence ACCAAACCTTACATAGTTGCCCTCCAAGTTATGCAGTTAAGGGAAGTCTGACGGCGATG
CTTGTGAGAAAGCCACCCAACCTTCCA[**tggaagccactc**]AACTTGTAAGAAACCAAATGAGA
GAGAAATCCTATAAATACACAGAAAGTGTTAAATCCTTAAACCACTTTA

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25536	-	GCG ATG CTT GTG AGA AAG C	-	Forward	A
25537	-	AAA GGG CAG TGG TAA AGT GG	-	Reverse	A
25538	Hex	TTC CAT GGA AGC CAC TCA ACT	Black Hole Quencher 1	WT Probe	A
26433	6-FAM	CCC AAC CTT CCA AAC TTG TAA GA	Black Hole Quencher 1	MUT Probe	A

Number Of Reactions 1

Reaction A (Endpoint 2 primer)		Cycling (TouchDown 65-60_12-12-11)			
Component	Final Concentration	Step #	Temp°C	Time	Note
Kapa Probe Fast QPCR	1 X	1	94	2 min	-
ddH2O		2	94	20sec	-
26433	.4 uM	3	65	15sec	-0.5 C per cycle decrease
25538	.4 uM	4	68	10sec	-
Wt Probe	.15 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
Mutant Probe	.15 uM	6	94	15sec	-
		7	60	15sec	-

Reaction	A	(Endpoint 2 primer)	Cycling (TouchDown 65-60_12-12-11)			
DNA			8	72	10sec	-
			9	-	-	repeat steps 6-8 for 28 cycles
			10	72	2 min	-
			11	10	-	hold

Version 3.2