



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Adgra1^{em1J/J}*
Stock Number: 027694
Allele: *Adgra1*-6965-KOMP CRISPR
Protocol Name: *Adgra1*-6965-KOMP CRISPR-alternate1
Method: Standard PCR
Created: 26-June -2015 (JKELMEN) **Updated:** 26-June -2015 (JKELMEN)

Notes

Expected Results: Mutant = X bp
 Heterozygote = X bp and Y bp
 Wild type = Y bp

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
24094	-	ACT CCA CCC TAC ACC CTT CC	-	Forward	-
24095	-	TCC TTG GGC ACA TAC ACA GA	-	Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH2O	4.55	-	-
5 X Kapa 2G HS buffer	2.40	1	-
25 mM MgCl ₂	0.96	2	-
10 mM dNTPS-kapa	0.24	.2	-
20 U Primer 1	0.30	.5	-
20 U Primer 2	0.30	.5	-
5 mM 10x Loading Dye	1.20	.5	-
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01	-
DNA	2.00	-	-

Cycling

Step #	Temp °C	Time	Note
1	94	2 min	-
2	94	20sec	-
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	-
5	-	-	repeat steps 2-4 for 10 cycles
6	94	15sec	-
7	60	15sec	-
8	72	10sec	-
9	-	-	repeat steps 6-8 for 28 cycles
10	72	2 min	-
11	10	-	hold

Number Of Reactions 1

Version 3.2