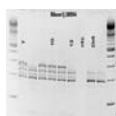




Welcome to The Genotyping Protocol System

Master Protocol**Strain Name:** C57BL/6NJ-1700001O22Rik^{em1J}J**Stock Number:** 028994**Allele:** 1700001O22Rik^{em1J}**Protocol Name:** 1700001O22Rik^{em1J}**Method:** Standard PCR**Created:** 04-April -2016 (JKELMEN)**Updated:** 22-June -2016 (ESCHAAB)**Notes**

Expected Results: Mutant = 290 bp
 Heterozygote = 188 bp and 252 bp
 Wild type = 188 bp

Attachments

View Rikem1J-28994 Gel.jpg

- View 1700001O22Rikem1jin progress 3-24-16.docx
- View 1700001O22Rik genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27490	-	GAG AGC GCA GAC CCA GAG	-	Common	A
27491	-	CTA GGG TTA TGT GGC GCT GA	-	Wild type Reverse	A
27492	-	AAC AGC TAC TAC TCA CGG ATG G	-	Mutant Reverse	A

Number Of Reactions 1

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Component	Final Concentration	Step #	Temp°C	Time	Note
ddH2O		1	94	2 min	-
Kapa 2G HS buffer	1.3 X	2	94	20sec	-
MgCl2	2.6 mM	3	65	15sec	-0.5 C per cycle decrease
dNTP KAPA	.26 mM	4	68	10sec	-
27491	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27492	.5 uM	6	94	15sec	-
27490	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
Dye	1 X	9	-	-	repeat steps 6-8 for 28 cycles
Kapa 2G HS taq polymerase	.03 U/ul	10	72	2 min	-
DNA		11	10	-	hold

Version 3.2