



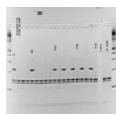
Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-H2-Ke6^{em1J/J}
Stock Number: 029001
Allele: H2-Ke6^{em1J}
Protocol Name: H2-Ke6^{em1J}
Method: Standard PCR
Created: 04-April -2016 (JKELMEN) **Updated:** 11-June -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 219 bp
 Heterozygote = 143 bp and 219 bp
 Wild type = 143 bp

Attachments

View H2-Ke6-29001 Gel.jpg

- View H2-Ke6 genomic1.gcs
- View H2-Ke6em1jin progress 3-28-16-2.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27447	-	GTC ACA GGT TCA GGG GTT CC	-	Common	A
27448	-	GAG AAG AAG GGA GGA GGT CAG	-	Wild type Reverse	A
27449	-	GGG CAC AAG TCA CAG GTT TAG	-	Mutant Reverse	A

Number Of Reactions

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	-
27448	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
27449	.5 uM	6	94	15sec	-
27447	.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