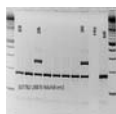




Welcome to The Genotyping Protocol System

Master Protocol**Strain Name:** C57BL/6NJ-Ndufs8^{em1J}J**Stock Number:** 028876**Allele:** Ndufs8^{em1J}**Protocol Name:** Ndufs8^{em1J}**Method:** Standard PCR**Created:** 21-September-2016 (ESCHAAB) **Updated:** 26-September-2016 (ESCHAAB)**Notes**

Expected Results: Mutant = 259 bp
 Heterozygote = 175 bp and 259 bp
 Wild type = 175 bp

Attachments

View 28876 gel.jpg

- View Ndufs8 genomic.gcs

- View Ndufs8MolDesFeb2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
26959	-	GCT CGG ATT CTG ATG TGG AC	-	Common	A
26960	-	AGT TGA TGG TGG CAG GCT CT	-	Wild type Reverse	A
26964	-	GAG CCA GGA GTG GTG GTA 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	-
26960	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
26964	.5 uM	6	94	15sec	-
26959	.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