



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Ubn1*^{em1J/J}**Stock Number:** 027475**Allele:** *Ubn1*^{em1j}-6239-KOMP-CRISPR**Protocol Name:** *Ubn1*^{em1j}-6239-KOMP-CRISPR**Method:** Sanger sequencing**Created:** 30-April -2015 (DTG)**Updated:** 30-April -2015 (DTG)

Notes

Mut = X
Notes:
WT= Y

Sequence

Sequence SEQ

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
23475	-	AAG TTT TAG GGG CGG GAT T	-	Forward	-
23476	-	CTG CTT GTG CAA AGA GGA CA	-	Reverse	-

Reaction/Components A

Reaction Components	Volume Amt	Final Concentration	Total Volume Amt
ddH ₂ O	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	-
50 % Glycerol	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

