



Welcome to The Genotyping Protocol System

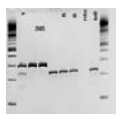
Master Protocol

Strain Name: C57BL/6NJ-Wdr45^{em1J}
Stock Number: 029405
Allele: Wdr45^{em1J}
Protocol Name: Wdr45^{em1J}
Method: MELT
Created: 21-July -2016 (LUCASM) **Updated:** 19-October -2016 (LUCASM)

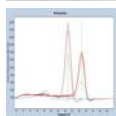
Notes

Expected Results: Mutant = 286 bp
Heterozygote = 226 bp and 286 bp
Wild type = 226 bp

Attachments



View 29405.jpg



View 29405.JPG

- View Wdr45 genomic.gcs
- View Wdr45em1jMolDescJuly2016.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
28929	-	CAC CCT AAA CTT TGG TCT TGC	-	Wild type Forward	A
28930	-	GGA GGG CGG GAG GTT AC	-	Common	A
28931	-	CCC ACC AAT ATC TGG CAA GT	-	Mutant Forward	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	-
28929	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28930	.5 uM	6	94	15sec	-
28931	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
		9	-	-	repeat steps 6-8 for 28 cycles

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2