



Welcome to The Genotyping Protocol System

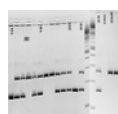
Master Protocol

Strain Name: C57BL/6NJ-*Tpd5211^{em1J}*
Stock Number: 029003
Allele: *Tpd5211^{em1J}*
Protocol Name: *Tpd5211^{em1J}*
Method: MELT
Created: 06-April -2016 (JKELMEN) **Updated:** 05-December -2016 (LUCASM)

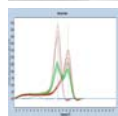
Notes

Expected Results: Mutant = 159 bp
 Heterozygote = 159 bp and 260 bp
 Wild type = 260 bp

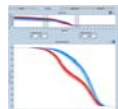
Attachments



View 29003.jpg



View 29003.JPG



View 29003 Tpd5211 hrm.JPG

- View Tpd5211em1jMolDesApr2016.docx

- View Tpd5211 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27550	-	CAC TGA GTA CCC TGG GAA TG	-	Common	A
27551	-	TTT TGC TGA CAA AAC TTG TCG T	-	Wild type Reverse	A
27552	-	CTC ATA CTT CTT TAC CTT GCT CCT	-	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	-
27551	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
		6	94	15sec	-

Reaction A (3 primer)		Cycling (TouchDown 65-60_12-12-11)			
27550	.5 uM	7	60	15sec	-
27552	.5 uM	8	72	10sec	-
Glycerol	6.5 %	9	-	-	repeat steps 6-8 for 28 cycles
Dye	1 X	10	72	2 min	-
Kapa 2G HS taq polymerase	.03 U/ul	11	10	-	hold
DNA					

Version 3.2