



Welcome to The Genotyping Protocol System

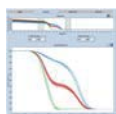
Master Protocol

Strain Name: C57BL/6NJ-*Tbc1d5*^{em1J}/*J*
Stock Number: 028894
Allele: *Tbc1d5*^{em1J}
Protocol Name: *Tbc1d5*^{em1J}
Method: MELT
Created: 29-February -2016 (JKELMEN) **Updated:** 22-April -2016 (ESCHAAB)

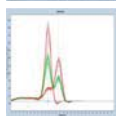
Notes

Expected Results: Mutant = 218 bp
Heterozygote = 218 bp and 280 bp
Wild type = 280 bp

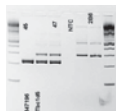
Attachments



View 28894 Tbc1d5 hrm.JPG



View 28894 Tbc1d5 mca.JPG



View 28894 Tbc1d5 gel.jpg

- View Tbc1d5MolDesFeb2016.docx
- View Tbc1d5 genomic.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
26953	-	CCA AGA AGA CAG CCA TTC GT	-	Common	A
26954	-	CCA GGC AGC TTC AAA TTG T	-	Mutant Reverse	A
26955	-	TTC TTC TTA CCT TCC AGC AAA TG	-	Wild type 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	-
26953	.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)			
26954	.5 uM	7	60	15sec	-
26955	.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