



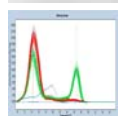
Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Acod1*^{em1J}J
Stock Number: 029340
Allele: *Acod1*^{em1J}
Protocol Name: *Acod1*^{em1J}
Method: MELT
Created: 17-June -2016 (JKELMEN) **Updated:** 06-October -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 220 bp
 Heterozygote = 157 bp and 220 bp
 Wild type = 157 bp

AttachmentsView [acod 29340.jpg](#)View [acod MCA JW.jpg](#)

- View [Acod1em1JMolDesJun2016.docx](#)
 - View [Acod1 genomic.gcs](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
26673	-	GTG GGG AGG GGA ACT ATG AG	-	Common	A
28548	-	ATT TGG AGG AAC CCC ATG AC	-	Wild type Reverse	A
28549	-	CAG CCT CTA AGC CAG ACA GC	-	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	-
28549	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28548	.5 uM	6	94	15sec	-
26673	.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