



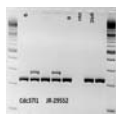
Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-Cdc3711^{em1}J/J
Stock Number: 029552
Allele: Cdc3711^{em1}J
Protocol Name: Cdc3711^{em1}J
Method: Standard PCR
Created: 12-August -2016 (LUCASM) **Updated:** 03-December -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 275 bp
 Heterozygote = 229 bp and 275 bp
 Wild type = 229 bp

Attachments

View [29552 cdc37.jpg](#)

- View [Cdc3711 genomic.gcs](#)
- View [Cdc3711em1j in progress 8-9-16.docx](#)

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
29165	-	GGA AGA AGA GGA AGA AGT TCA GC	-	Common	A
29166	-	CTC TTC ACA TAC GAG GTG TGG	-	Wild type Reverse	A
29167	-	TGG ACA ATT GAA AGC ATG AC	-	Mutant Reverse	A

Number Of Reactions

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	-
29167	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
29165	.5 uM	6	94	15sec	-
29166	.5 uM	7	60	15sec	-
Glycerol	6.5 %	8	72	10sec	-
Dye	1 X	9	-	-	repeat steps 6-8 for 28 cycles
Kapa 2G HS taq polymerase	.03 U/ul	10	72	2 min	-
DNA		11	10	-	hold

Version 3.2