



Welcome to The Genotyping Protocol System

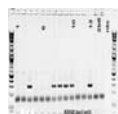
Master Protocol

Strain Name: C57BL/6NJ-Ano7^{em1J}J
Stock Number: 029341
Allele: Ano7^{em1J}
Protocol Name: Ano7^{em1J}
Method: Standard PCR
Created: 09-November -2016 (ESCHAAB) **Updated:** 09-November -2016 (ESCHAAB)

Notes

Expected Results: Mutant = 267 bp
 Heterozygote = 136 bp and 267 bp
 Wild type = 136 bp

Attachments



View JR 29341 Ano7 em1J GEL 11-7.jpg

- View Ano7em1jMolDesJun2016_edited LG.docx
- View Ano7 genomic1.gcs

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
27275	-	GGG GAC AGT TTG TGC TAA GG	-	Mutant Reverse	A
28556	-	AAG AAC GAG GGA GGA TGG AG	-	Common	A
28557	-	ATG CCA TCA GTA TCC AAC CA	-	Wild type Reverse	A

Number Of Reactions 1

Reaction A (2 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
dNTPS-kapa	.26 mM	4	68	10sec	-
27275	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
28557	.5 uM	6	94	15sec	-
Glycerol	6.5 %	7	60	15sec	-
Dye	1 X	8	72	10sec	-
Kapa 2G HS taq polymerase	.03 U/ul	9	-	-	repeat steps 6-8 for 28 cycles
DNA		10	72	2 min	-
		11	10	-	hold

Reaction B (2 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	-
MgCl ₂	2.6 mM	3	65	15sec	-0.5 C per cycle decrease
dNTPS-kapa	.26 mM	4	68	10sec	-
Primer 1	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
Primer 2	.5 uM	6	94	15sec	-
Glycerol	6.5 %	7	60	15sec	-
Dye	1 X	8	72	10sec	-
Kapa 2G HS taq polymerase	.03 U/ul	9	-	-	repeat steps 6-8 for 28 cycles
DNA		10	72	2 min	-
		11	10	-	hold

Version 3.2