



Welcome to The Genotyping Protocol System

Master Protocol

Strain Name: C57BL/6NJ-*Arr3*^{em1J}/*J*
Stock Number: 028310
Allele: *Arr3*^{em1J}
Protocol Name: *Arr3*^{em1J}
Method: High Resolution Melting
Created: 30-November -2015 (EST) **Updated:** 30-November -2015 (ESCHAAB)

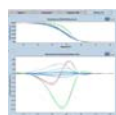
Notes

Notes: This allele from project *Arr3*-7032J M#2356 was generated at The Jackson Laboratory by injecting Cas9 RNA and 4 guide sequences: ATCCTCATTGCCCTTCCATT, CCATTCTTCAGCATCTCGGT, ATTGGCCTCATAGCTCTGCA, AGGCCAATTGAAAGGTAGAA, which resulted in a 313bp deletion beginning in intron 4 at Chromosome X positive strand position 100,606,939(GAATGGAAGGGCAATGAGGATG) and ending after (GAAAGGAAAGAATCCTTTTCAG) at 100,607,251bp (GRCm38/mm10) in intron 5. This mutation results in the deletion of exon4 and is predicted to cause a change in amino acid sequence after residue 13 and early truncation 10 amino acids later.

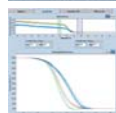
Expected Results: Mutant = 314 bp
Heterozygote = 224 bp and 314 bp
Wild type = 224 bp

X-linked

Attachments



View Arr3 HRM mplex shift-diff 11-30-15.JPG



View Arr3 HRM mplex norm-melt 11-30-15.JPG



View Mplex gel image 28310 11-30-15.jpg

- View Arr3 genomic1.gcs

- View Arr3em1j_moldesc_in progress 9-23-15.docx

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Description	Reaction
25160	-	TTC CTG TTC CCC AAA CAT TC	-	Common	A
25161	-	TTG AAG GAA TGG TCC CAA AG	-	Wild type Reverse	A
25162	-	GTG GCA TTT CAG TTG GGA CT	-	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	-
25162	.5 uM	5	-	-	repeat steps 2-4 for 10 cycles (Touchdown)
25160	.5 uM	6	94	15sec	-
25161	.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