

B6N.129X1(B6J)-*Msra*^{tm1Jmo}/RlevMmjax

Stock No: 032924

 Protocol 38098: Standard PCR Assay - *Msra*<tm1Jmo>-alternate6

Version 1.0

Notes

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Expected Results

Mutant = 225 bp

Heterozygote = 172 bp and 225 bp

Wild type = 172 bp

[>chr14:64284644-64284815](#) 172bp TTTAGCTTAGCAAACCTGGACTGG CCCCATTTCTCAGTCAGTGC

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
52979		TTT AGC TTA GCA AAC TGG ACT GG		Wild type Forward	A	Wt F
52980		CCC CAT TTC TCA GTC AGT GC		Common	A	
oIMR5316		CTA AAG CGC ATG CTC CAG AC		Mutant Forward	A	Mut F

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
Kapa 2G HS buffer	1.00
MgCl ₂	2.60 mM
MgCl ₂	2.00
dNTP KAPA	0.26 mM
dNTPS-kapa	0.20
52979	0.50 uM
52979	0.50
52980	0.50 uM
52980	0.50
10x Loading Dye	0.50
oIMR5316	0.50 uM
Glycerol	6.50 %
Kapa 2G HS taq polym	0.01
DNA	
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

32924 Msratm1Jmo Alt6

