STOCK *Esrrb*$^{tm1.1Nat}$/J

Stock No: 007674
Protocol 23022: Standard PCR Assay - Esrrb<tm1.1Nat>
Version 1.3

Notes

This assay does not work well without the use of a Hotstart (We are using Taq Start Antibody mixed 1:1 with Taq polymerase).

Use Perfect Match© (Stratagene) added before Taq to delete non-specific bands.

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 = 260 bp
Heterozygote = 180 bp and 260 bp
Wild type = 180 bp
Separated by gel electrophoresis on a 1.5% agarose gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR7535		GGG GGC CTT GGT CTA CAG GTC TAG T		Forward	A	
oIMR7536		TCT TTC TAC GGC GTT TCA GGG ACC		Reverse	A	

Reaction A

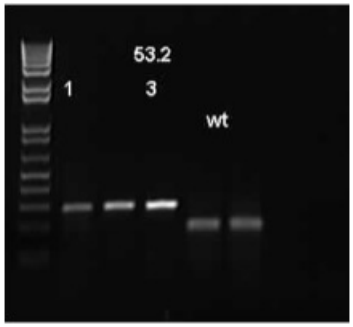
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
oIMR7535	0.50 uM
oIMR7536	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

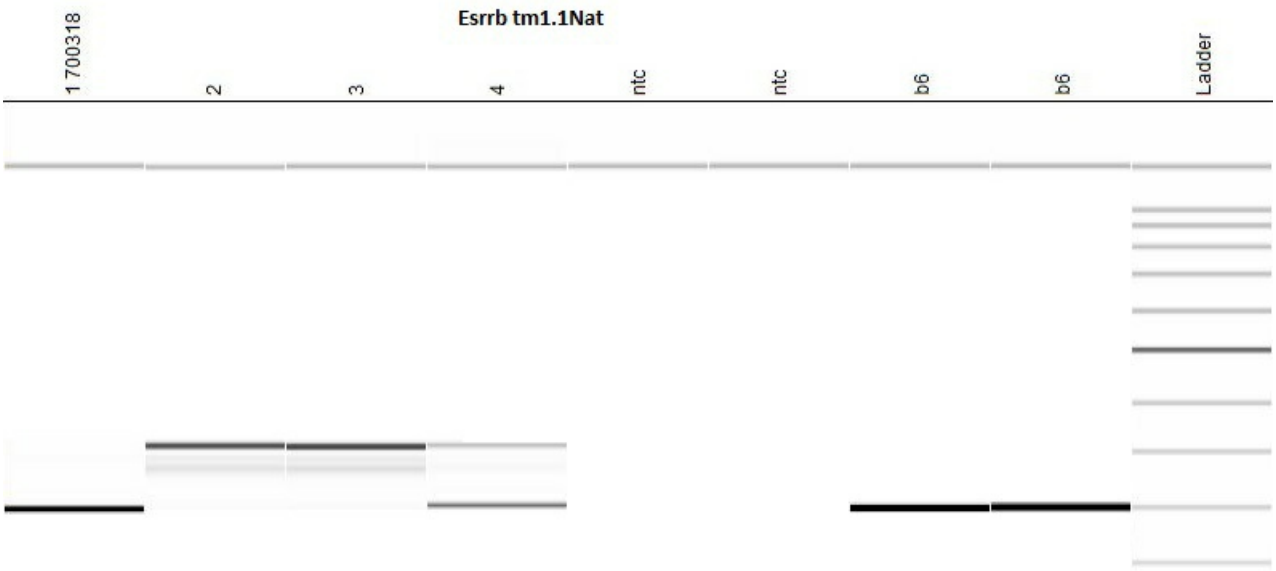
Cycling

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 (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.




Esrrb tm1.1Nat
1 700318
2
3
4
ntc
ntc
b6
b6
Ladder