# C57BL/6-*Actb*$^{tm3.1(Sirt1)Npa}$/J

Stock No: 013080
Protocol 28792: Standard PCR Assay - Actb<tm3.1(Sirt1)Npa>
Version 2.2

## Notes

There appears to be two misc band in the wildtype & heterozygous samples at ~ 400 bp & ~440 bp with both kapa & KLR taq. These misc bands are more than likely from the amplifying of the two other loci as mentioned by Kevin.

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 = 257 bp
Heterozygote = 257 bp and 547 bp
Wild type = 547 bp

## JAX Protocol

### Protocol Primers

| PRIMER | 5' LABEL | SEQUENCE 5' → 3' | 3' LABEL | PRIMER TYPE | REACTION | NOTE |
|---|---|---|---|---|---|---|
| 10967 | | TAT GGA ATC CTG TGG CAT CCA TGA | | | A | |
| 10968 | | CAA AGC CAT GCC AAT GTT GTC TCT | | | A | |
| 10969 | | GGC ACA TGC CAG AGT CCA AGT TTA | | | A | |

### Reaction A

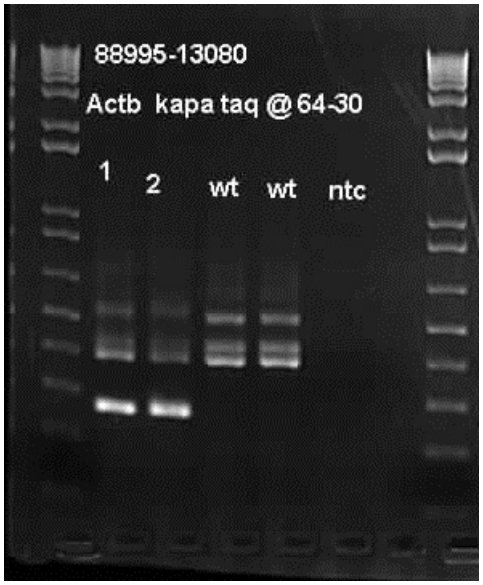
| COMPONENT | FINAL CONCENTRATION |
|---|---|
| ddH2O | |
| Kapa 2G HS buffer | 1.30 X |
| MgCl2 | 2.60 mM |
| dNTP KAPA | 0.26 mM |
| 10967 | 0.50 uM |
| 10968 | 0.50 uM |
| 10969 | 0.50 uM |
| Glycerol | 6.50 % |
| Dye | 1.00 X |
| Kapa 2G HS taq polym | 0.03 U/ul |
| DNA | |

### 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.


88995-13080
Actb kapa taq @ 64-30
1
2
wt
wt
ntc