

B6;129S-Sparc^{tm1Hwe}/J

Stock No: 003728

Protocol 26361: Standard PCR Assay - Sparc<tm1Hwe>

Version 4.2

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

Melt Curves

Wt = upper, Het = middle, Hom = lower

Mutant = ~240 bp

Heterozygote = 385 bp and ~240 bp

Wild type = 385 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 |
|----------|----------|----------------------------|----------|-------------|----------|------|
| 10760 | | TTC TTC CTT GCA ACC CTC TC | | Common | A | |
| 10761 | | TGT GGA GCT TCC TCT GTC CT | | Wild type | A | |
| oIMR8336 | | GGG GTT TGC TCG ACA TTG | | Mutant | A | |

Reaction A

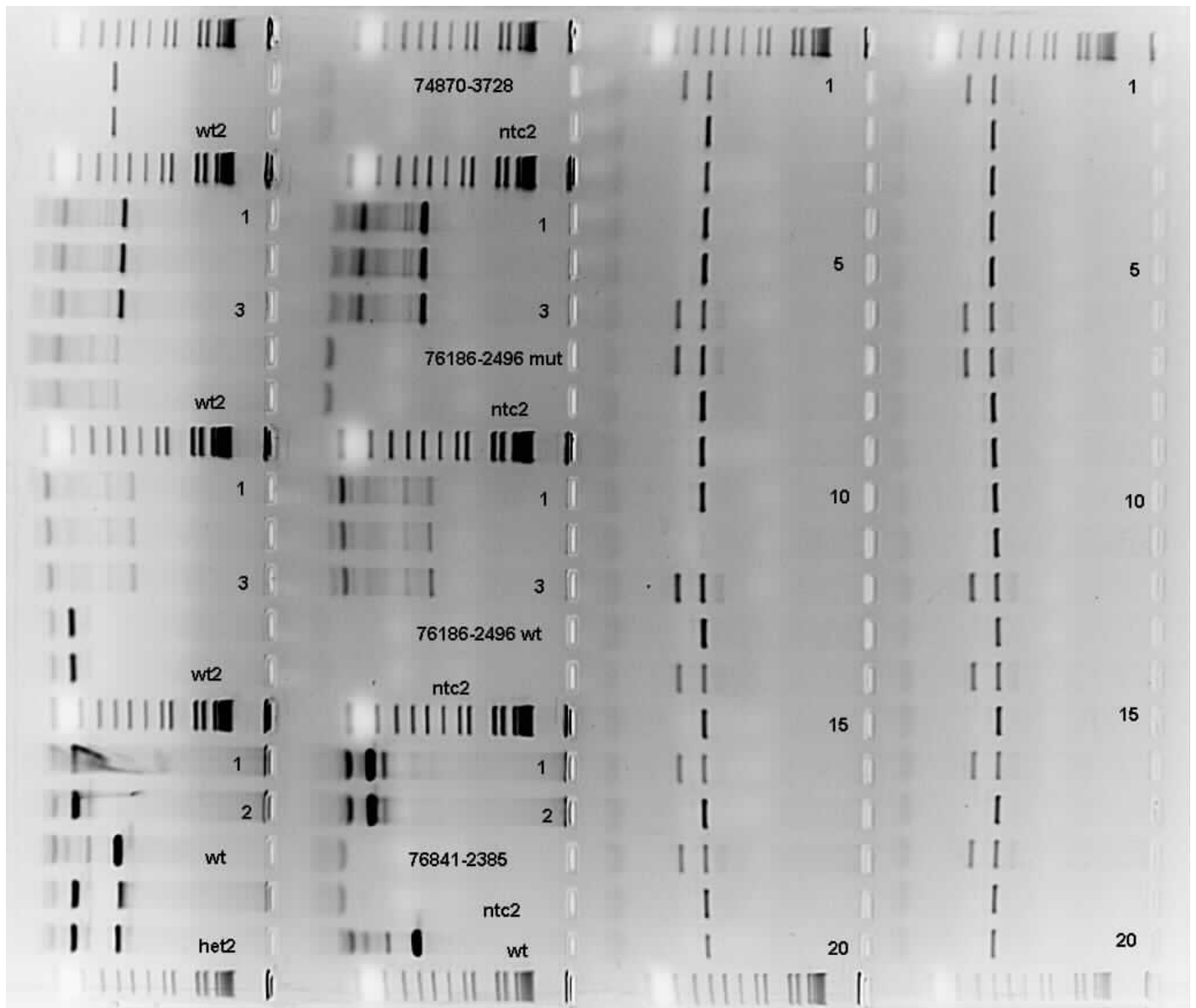
| COMPONENT | FINAL CONCENTRATION |
|----------------------|---------------------|
| ddH ₂ O | |
| Kapa 2G HS buffer | 1.30 X |
| MgCl ₂ | 2.60 mM |
| dNTP KAPA | 0.26 mM |
| 10760 | 0.50 uM |
| 10761 | 0.50 uM |
| oIMR8336 | 0.50 uM |
| Glycerol | 6.50 % |
| Dye | 1.00 X |
| Kapa 2G HS taq polym | 0.03 U/ul |
| DNA | |

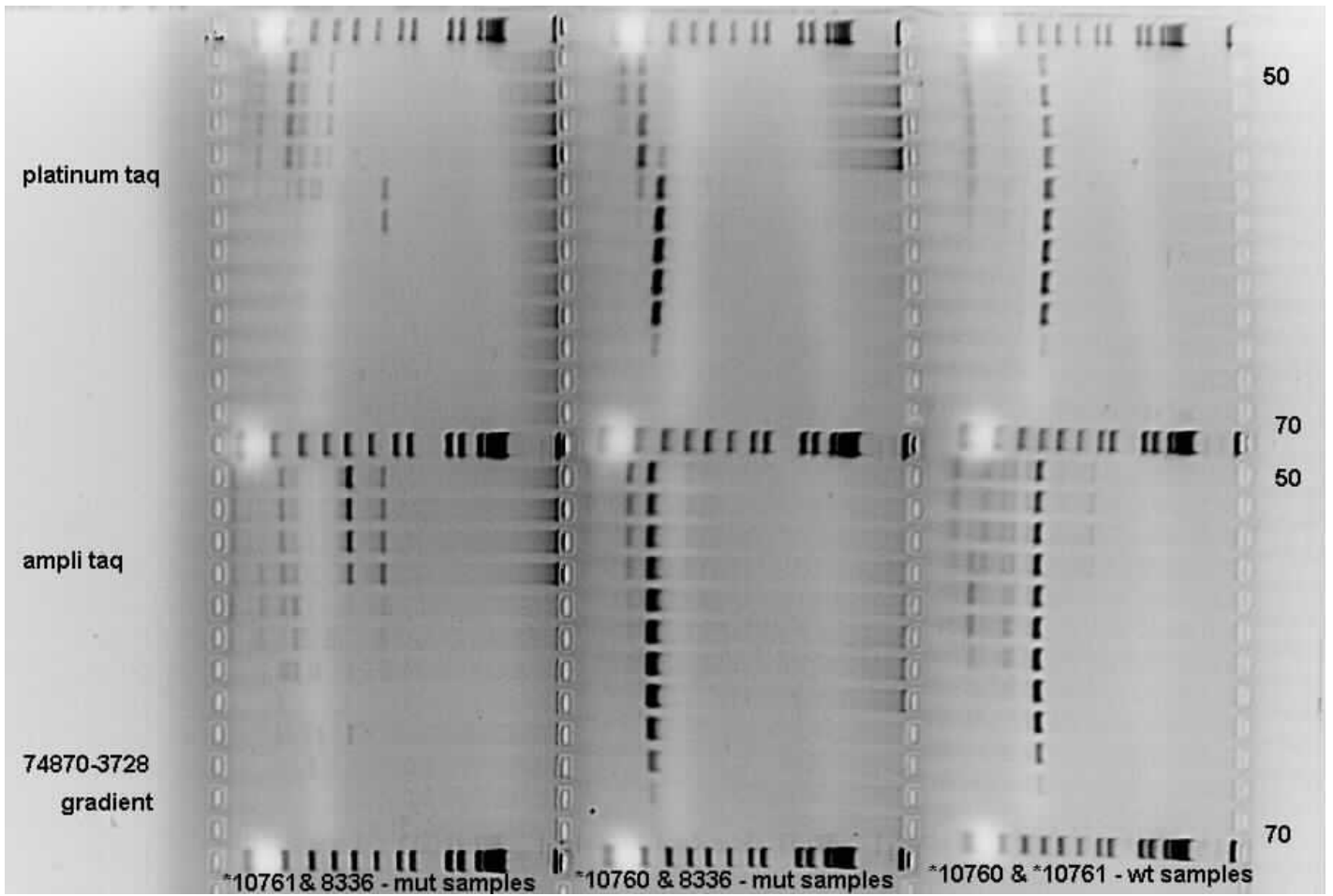
Cycling

| 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.





Normalization Graph

