# B6N.1297-*Ltc4s*$^{tm1Blam}$/J

Stock No: 030813
Protocol 32485: Standard PCR Assay - Ltc4s<tm1Blam>
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

>chr11:50236899-50237213 315bp CGGCATCTTCTTCCACGA TGTCCCCAGATCTCTTTCCA

Mutant = ~390 bp
Heterozygote = ~390 bp and 315 bp
Wild type = 315 bp

## JAX Protocol

### Protocol Primers

| PRIMER | 5' LABEL | SEQUENCE 5' → 3' | 3' LABEL | PRIMER TYPE | REACTION | NOTE |
|---|---|---|---|---|---|---|
| 39218 | | ATC TTG TTC AAT GGC CGA TC | | Mutant Forward | A | |
| 39219 | | CGG CAT CTT CTT CCA CGA | | Wild type Forward | A | |
| 39220 | | TGT CCC CAG ATC TCT TTC CA | | Common | A | |

### Reaction A

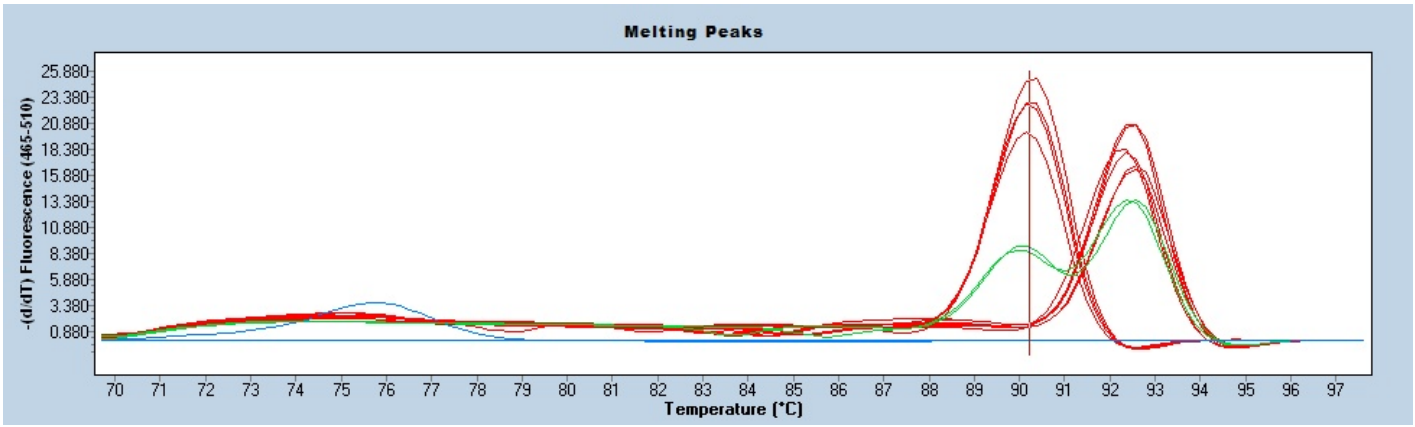
| COMPONENT | FINAL CONCENTRATION |
|---|---|
| ddH2O | |
| Kapa 2G HS buffer | 1.30 X |
| MgCl2 | 2.60 mM |
| dNTP KAPA | 0.26 mM |
| 39218 | 0.50 uM |
| 39219 | 0.50 uM |
| 39220 | 0.50 uM |
| Glycerol | 6.50 % |
| Dye | 1.00 X |
| Kapa 2G HS taq polym | 0.03 U/ul |
| DNA | |

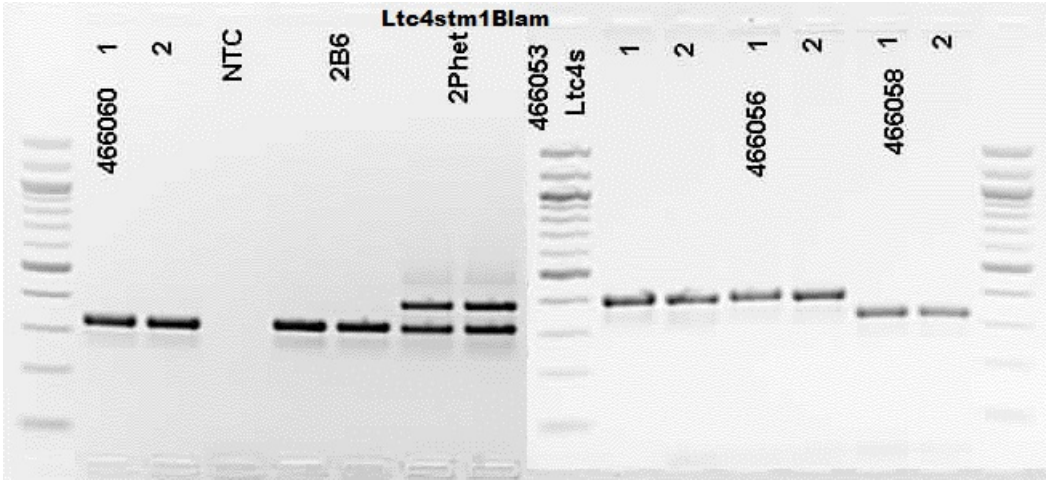
### Cycling

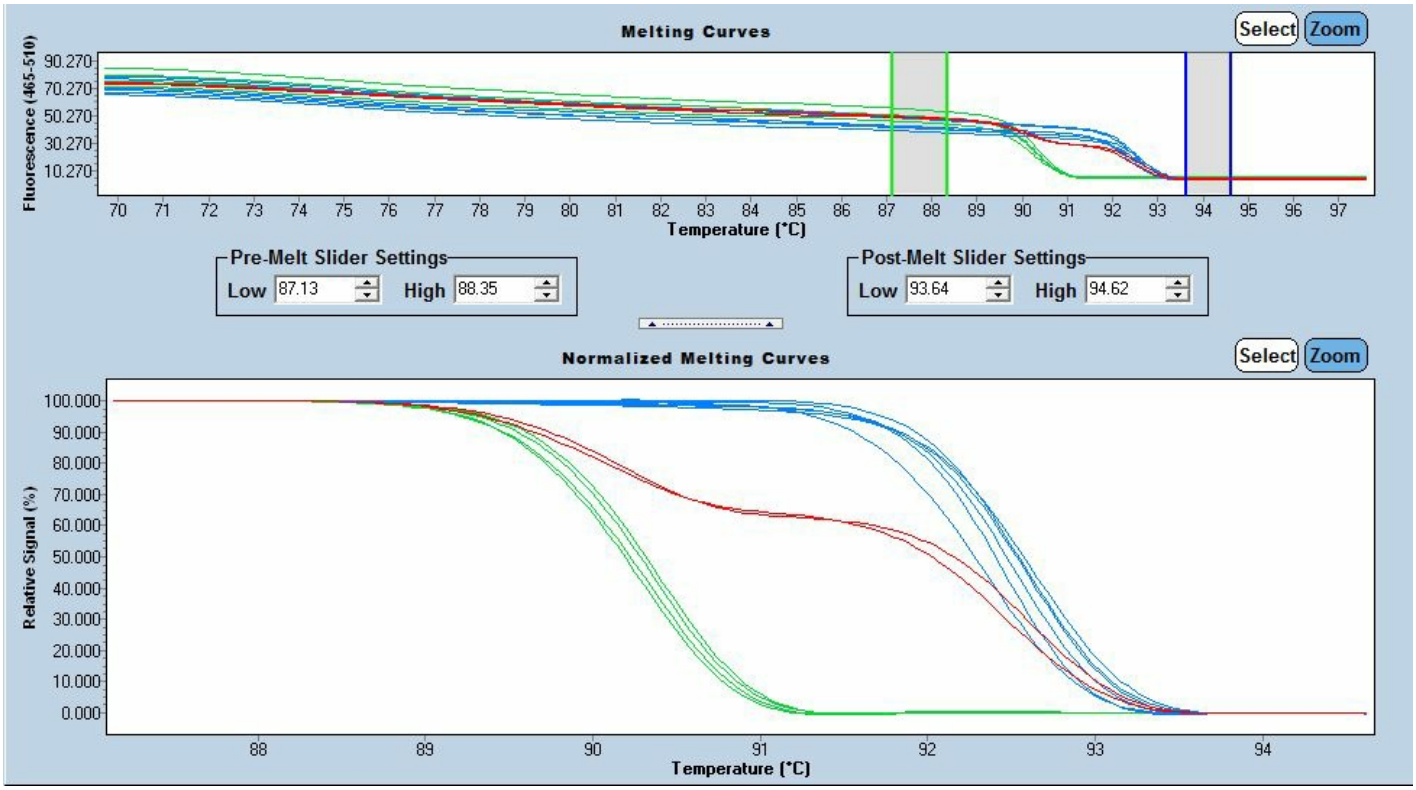
| STEP | TEMP °C | TIME | NOTE |
|---|---|---|---|
| 1 | 94.0 | -- | |
| 2 | 94.0 | -- | |
| 3 | 65.0 | -- | -0.5 C per cycle decrease |
| 4 | 68.0 | -- | |
| 5 | | -- | repeat steps 2-4 for 10 cycles (Touchdown) |
| 6 | 94.0 | -- | |
| 7 | 60.0 | -- | |
| 8 | 72.0 | -- | |
| 9 | | -- | repeat steps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


Melting Peaks
-(d/dT) Fluorescence (465-510)
25.880
23.380
20.880
18.380
15.880
13.380
10.880
8.380
5.880
3.380
0.880
70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97
Temperature (°C)


Ltc4stm1Blam
466060
1
2
NTC
2B6
2Phet
466053
Ltc4s
1
2
466056
1
2
466058
1
2


Melting Curves
Select
Zoom
Fluorescence (465-510)
90.270
70.270
50.270
30.270
10.270
70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97
Temperature (°C)
Pre-Melt Slider Settings
Low 87.13
High 88.35
Post-Melt Slider Settings
Low 93.64
High 94.62
Normalized Melting Curves
Select
Zoom
Relative Signal (%)
100.000
90.000
80.000
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0.000
88 89 90 91 92 93 94
Temperature (°C)