

C57BL/6J-Pm20d1^{em1Brsp}/J

Stock No: 032193

Protocol 33685: Sanger sequencing Assay - Pm20d1<em1Brsp>

Version 1.0

Notes

>[chr1:131797450+131797731](#) 282bp GGCAGCTGTGCTCCTTCTCT TACCGACGCAAGTGCTAGA

Mut = (-----)

WT= tcagcg

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

Sequence

```

GGCAGCTGTGCTCCTTCTCTTTTTCGCTACGGTCTCCGGA
TCCACTGGCCCTAGAAGCAGGGAAAATCGGGGGGCGTC
CCGGATCCCTTCCAGT(tcagcg/-----
)AGGAGGAGCGTGTCGCTATAAAAGAGGCGCTGAAAGgtg
aacctccgcccctgttctcagatctgagtcctcagtcagggcctacctcct
ctgctgagggacgcgggcgggcccagccagcgtctgcaagcttcgggacactgt
gcactggtctagcactgctcggttaa
  
```

JAX Protocol

Protocol Primers

| PRIMER | 5' LABEL | SEQUENCE 5' → 3' | 3' LABEL | PRIMER TYPE | REACTION | NOTE |
|--------|----------|----------------------------|----------|-------------|----------|------|
| 41972 | | GGC AGC TGT GCT CCT TCT CT | | Forward | A | |
| 41973 | | TTA CCG ACG CAA GTG CTA GA | | Reverse | A | |

Reaction A

| COMPONENT | FINAL CONCENTRATION |
|----------------------|---------------------|
| ddH2O | |
| Kapa 2G HS buffer | 1.30 X |
| MgCl2 | 2.60 mM |
| dNTPS-kapa | 0.26 mM |
| 41972 | 0.50 uM |
| 41973 | 0.50 uM |
| Glycerol | 6.50 % |
| 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.