**AMBN-Ires-Cre genotyping protocol**

<https://www.jax.org/protocol?stocknumber=035865&protocolid=40590>

**Protocol Primers**

| **Primer** | **5' Label** | **Sequence 5' → 3'** | **3' Label** | **Primer Type** | **Reaction** | **Note** |
| --- | --- | --- | --- | --- | --- | --- |
| 13007 |  | ACA CCG GCC TTA TTC CAA G |  | Mutant Reverse | B |  |
| 56352 |  | GGT GAT GGA GAA GCA ACC AT |  | Common | A, B |  |
| 56353 |  | TGG AAG CAA GAA GGG ACC TA |  | Wild type Reverse | A |  |

### Reaction A

| **Component** | **Final Concentration** |
| --- | --- |
| ddH2O |  |
| Kapa 2G HS buffer | 1.30 X |
| MgCl2 | 2.60 mM |
| dNTPS-kapa | 0.26 mM |
| 56352 | 0.50 uM |
| 56353 | 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 |
| 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](https://www.jax.org/news-and-insights/jax-blog/2015/september/hold-the-agarose-advanced-pcr-methods-for-genotyping-mice) and therefore has not calculated the optimal annealing temperature for each set of primers.

### Reaction B

| **Component** | **Final Concentration** |
| --- | --- |
| ddH2O |  |
| Kapa 2G HS buffer | 1.30 X |
| MgCl2 | 2.60 mM |
| dNTPS-kapa | 0.26 mM |
| 13007 | 0.50 uM |
| 56352 | 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](https://www.jax.org/news-and-insights/jax-blog/2015/september/hold-the-agarose-advanced-pcr-methods-for-genotyping-mice) and therefore has not calculated the optimal annealing temperature for each set of primers.

