# Genotyping PCR6

## Phire Animal Tissue Direct PCR Kit

Reaction Mix Using Dilution Method

| | |
|---|---|
| 2 x Phire Buffer | 10.0 ul |
| KL176 Primer 10 uM | 1.0 ul |
| KL205 Primer 10 uM | 1.0 ul |
| Phire Polymerase | 0.4 ul |
| DNA* | 1.0 ul |
| Water | 6.6 ul |
| Total Volume | 20.0 ul |

*Protocol recommends adding DNA last.

Reaction mix can be set up at room temperature.

Use program Phire2

98°C – 5 minutes

98°C – 5 seconds

72°C - 20 seconds

Go back to step two 39 more times

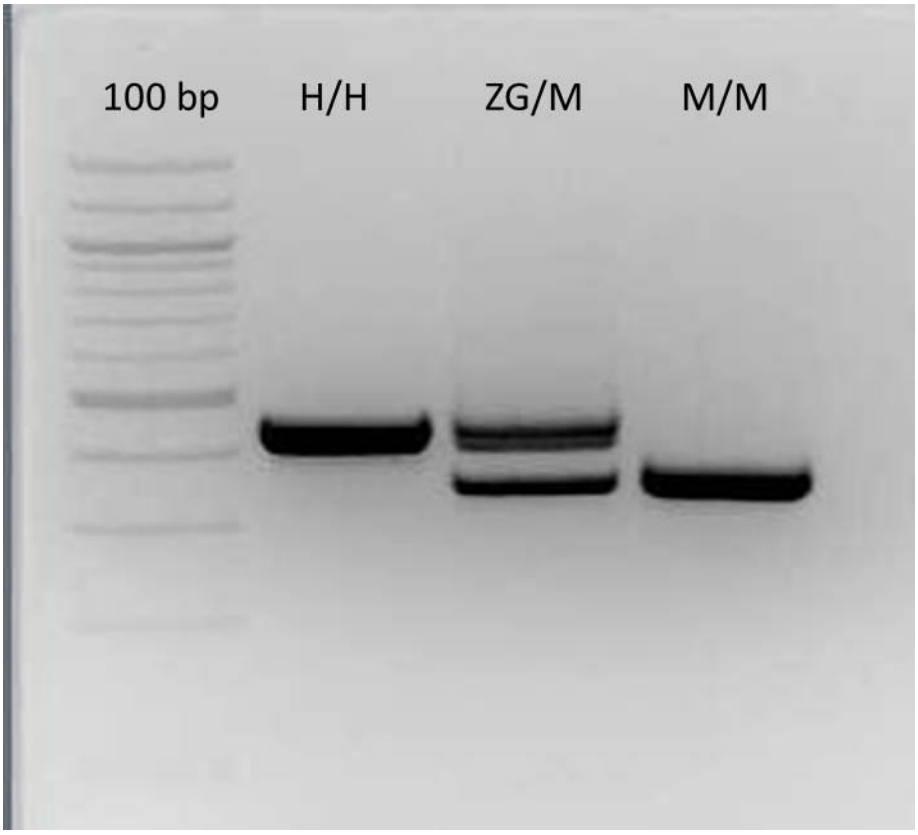
72°C – 1 minute

10°C – Hold

Use special Finnzyme Tm calculator to find proper annealing temperature.

http://www.finnzymes.com/tm_determination.html

Note: both primers had a Tm of about 76°C. Two step cycling is recommended for primers with Tm between 69°C and 72°C.




H/H can be detected with KL176 and KL205 (PCR6). Band size confirms the presence of flag tag for both alleles.

M/M band result of PCR6 when both alleles contain WT genomic mouse DNA which doesn't contain the flag tag.

ZG/M was for another mouse line.

Primer sequence

KL176A = F1 = 5' GTC GAC ATA TGG AGC AGC GAT GTG GAG 3'

KL210 = R1 = 5' TGA GCA TGT TGA AGA GCG AGT GAA CCA G 3'