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

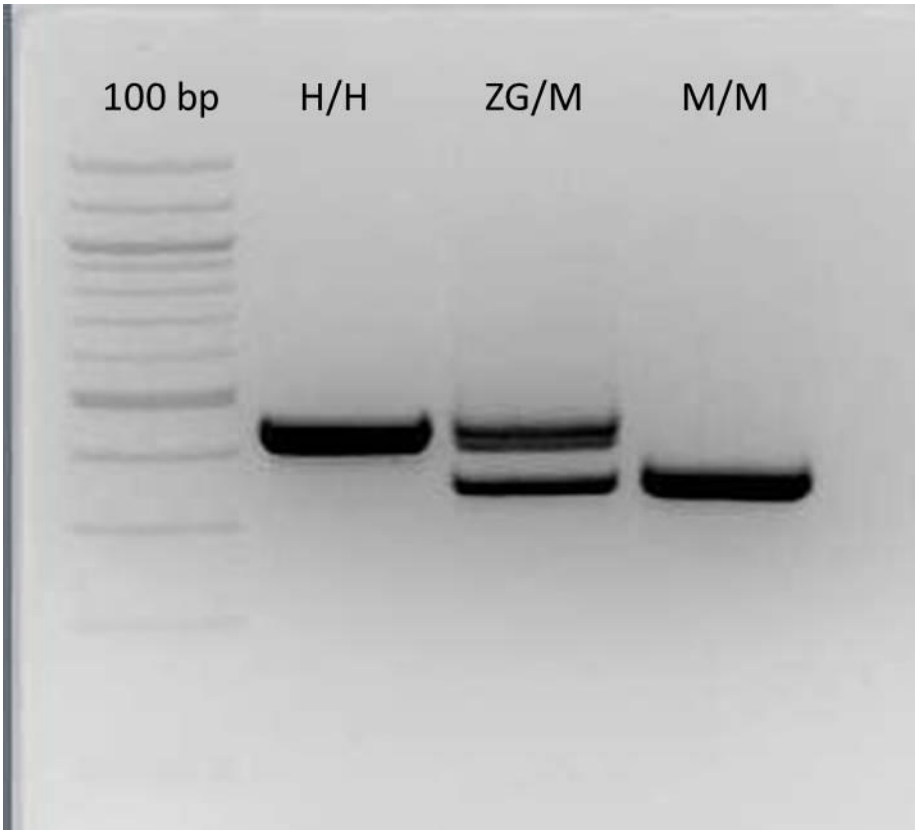
72°C – 1 minute

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. Band confirms the presence of flag tag.

The WT genomic mouse DNA which doesn't contain the flag tag.

ZG 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'