Genotyping D1275N

Phire Animal Tissue Direct PCR Kit

Reaction Mix Using Dilution Method

2 x Phire Buffer	10.0 ul
F6789 Primer 10 uM	1.0 ul
R7183 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

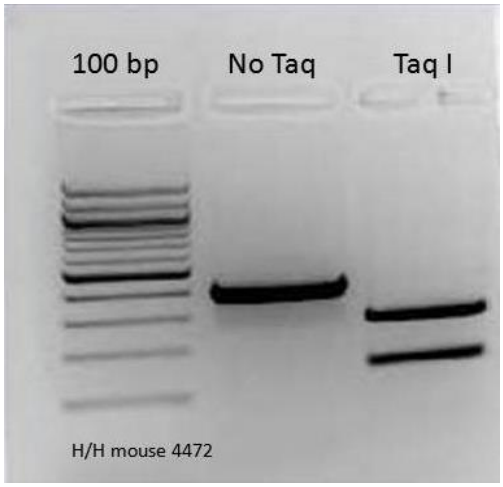
Note: both primers had a Tm of about 71°C. Two step cycling is recommended for primers with Tm between 69°C and 72°C.

Digestion with Taq alpha I

PCR purification is not necessary. Digest all of pcr product.

PCR product	20.0 ul
Cut Smart	4.0 ul
Taq I	1.0 ul
Water	15.0 ul
Total Volume	40.0 ul

Incubate at 65°C for 15 minutes. Run on 2% agarose gel with 100 bp marker.



Primer sequence:

F6789 GAGGAGCGGAAGACCATCAAGGTT

R7183 CCAAACTTCCCCGCAAAGAGGT