# Genotyping R222Q

## Phire Animal Tissue Direct PCR Kit

Primer F3626: 5'-GATTCTGGCTCGAGGCTTCTGC-3'

Primer R4042: 5'-GAGGTGCCGTTCTTGAGCAGGT-3'

Reaction Mix Using Dilution Method

| | |
|---|---|
| 2 x Phire Buffer | 10.0 ul |
| F3626 Primer 10 uM | 1.0 ul |
| R4042 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.

**Digestion with MlyI**

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

| | |
|---|---|
| PCR product | 20.0 ul |
| CutSmart | 4.0 ul |
| MlyI | 0.5 ul |
| Water | 15.5 ul |
| Total volume | 40.0 ul |

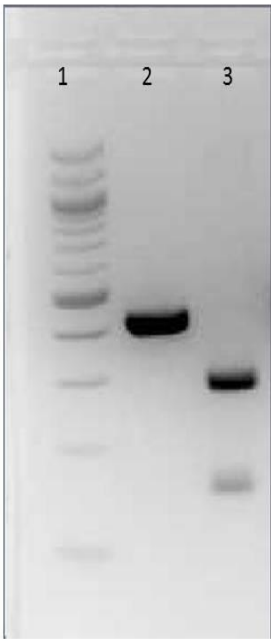
Incubate at 37C for 15 minutes. Run on 2% agarose gel with 100 bp marker.

Lane 1: 100 bp marker

Lane 2: WT R222Q pcr

Lane 3: WT R222Q pcr digested with MlyI

**Note:** R222Q mutant version will not cut with MlyI



**Roche Expand High Fidelity Method** **R222Q continued**

| | |
|---|---|
| Water | 8.5 ul |
| dNTP 10 mM | 1.0 ul |
| F3626 10 uM | 1.0 ul |
| R4042 10 uM | 1.0 ul |
| DNA* | 1.0 ul |
| Total volume | 12.5 ul |

*Used 60 ng of WT mouse genomic DNA isolated with QIAGEN Purgene kit. For pcr of mutation used plasmid containing R222Q mutation. 0.5 ng of the plasmid was used for pcr.

| | |
|---|---|
| Water | 7.1 ul |
| Buffer 2 | 2.5 ul |
| Buffer 4 (1:2) | 2.5 ul |
| Enzyme | 0.4 ul |
| Total volume | 12.5 ul |

**Cycling conditions**

94C – 2 minutes
94C – 15 seconds
62C – 30 seconds
72C – 45 seconds
Go to step two 9 more times
94C – 15 seconds
62C – 30 seconds
72C – 45 seconds adding 5 seconds for each additional cycle
Go to step five 19 more times
72C – 7 minutes
10C – Hold

Lane 1: 100 bp ladder

Lane 2: Blank

Lane 3: All 25 ul of WT pcr reaction

Lane 4: 4.5 ul of mutant plasmid pcr reaction

Lane 5: All 40 ul of WT pcr digested with MlyI

Lane 6: 7 ul of mutant plasmid digested with MlyI