**PCR Genotyping of *Sox9 flox***

(Applied to *Sox9flox/flox etc*.)

**PCR primers**

Sox9flox-F AGACTCTGGGCAAGCTCTGG

Sox9flox-R GTCATATTCACGCCCCCATT

**Product Sizes** wt: 250bp

Mutant: 300bp

Note: in the Cre-floxed animals, the entire exon2 and exon3 are excised.

**DNA prep:** Use the proteinase K protocol for genomic DNA preparation! The Alkaline buffer protocol does not work for this specific gene.

Note: Use 2% agarose gel to separate the mutant and wt band. Run a longer gel.

**PCR Reaction Mix**

10 µl Green Master Mix (Promega M7122)

7 µl di-H2O

1 µl Forward/Reverse primer mix \*

2 µl gDNA

20 µl

\*to make primer mix, add 10 µl of forward primer (100 µM stock) and 10 µl of reversed primer (100 µM stock) to 80 µl of di-H2O.

**PCR Program (55C)**



1. 94 °C 3min
2. 94 °C 30sec
3. 55 °C 30sec
4. 72 °C 1min
5. Go to 2, repeat 34x
6. 72 °C 5min
7. Keep at 12 °C

The “Genotyping” program also works.