

# *Cre Genotyping*

---

## **Primer Sequences:**

5'' – AGCGATGGATTCCGTCTCT (Ad For)

5'' – CACCAGCTTGCATGATCTCC (Ad Rev)

## **Ear Lysis:**

NaOH extraction of ear sample

- a. Dissolve 1 pellet (0.1g) of NaOH in 45 ml of distilled water (approx. 0.06M)
- b. Add 300  $\mu$ l of NaOH solution in each tube
- c. Boil at 100<sup>o</sup>C for 10 min (in heating block)
- d. Leave for 10 min at room temperature
- e. Add 50  $\mu$ l of 1M Tris pH 7.5 - 8

## **PCR Master Mix:**

Reddy Mix	9.0 $\mu$ l
Ad Forward Primer (5uM)	0.5 $\mu$ l
Ad Reverse Primer (5uM)	0.5 $\mu$ l
DNA	1.0 $\mu$ l

## **PCR Conditions:**

- 1) 95<sup>o</sup>C x 03:00
- 2) 95<sup>o</sup>C x 00:35
- 3) 55<sup>o</sup>C x 01:00
- 4) 72<sup>o</sup>C x 01:00
- 5) Repeat 2-4 x 29 cycles
- 6) 72<sup>o</sup>C x 02:00
- 7) 16<sup>o</sup>C x 10:00

## **Bands Present Represent:**

Positive band is approximately 200 base pairs.