

# Um método rápido e não invasivo de genotipagem de camundongos mutantes deficientes de leptina por amostras de esfregaços orais e restrição enzimática.

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## Objectives:

- Lep-Ob-/- mice presents an attractive model of insulin resistance and adipose tissue inflammation, typical of T2DM
- Genotyping is necessary for maintenance of the lineage
- The DNA extraction normally requires invasive methods that can traumatize the newborn animals
- we developed a simple non-invasive method for extract DNA from oral epithelial cells for genotyping the animals

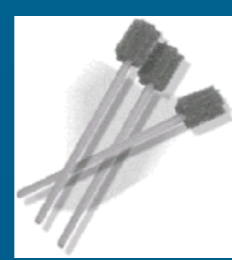
## Methods and Results :

### • DNA extraction:



18 mices (15 unknown and 3 control)

2 oral swabs collected of each



Transfer to a 600µL microtube with 200µL of TES Solution

30s vortex

TES solution: 10mM Tris-HCl pH 7.6; 1mM EDTA; 0,6% SDS

Addition of 10µL of a 10mg/mL Proteinase K solution and incubation (42°C) for 120 min

Addition of 15µL of NaCl 6M solution and centrifuge 15000g/4min

Supernatant transferred to a 1,5mL microtube and mixed with 450µL of absolute ethanol. Centrifuge 15000g/4min

Pellets resuspended in 300µL of 70% ethanol solution. Centrifuge 15000g/4min

Pellets dissolved in 15 µL of 10mM Tris-HCl pH 8.0

### • DNA quantification and purity:



Measured spectrophotometrically  
(average yield: 14.5 – 7.6µg/µL)

### • Real time PCR:



- Aliquots of 50µg DNA
- Stratagene Brilliant® II SYBR® Green qPCR Master Mix kit (50 cycles)
- FW: TGT CCA AGA TGG ACC AGA CTC;
- RV: ACT GGT CTG AGG CAG GGA GCA
- Average yield: 715 – 137µg/µL DNA

### • Restriction cut:

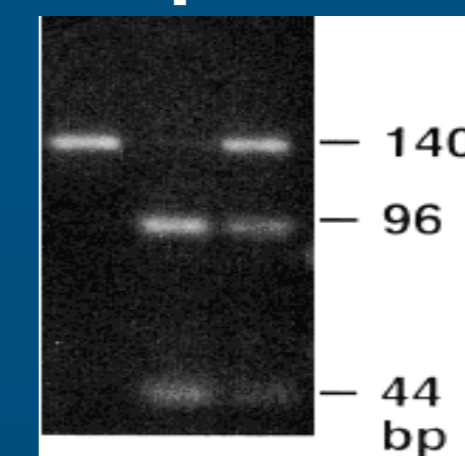
Restriction Site Sequence After Cut:  
...C TNAG...  
...GANT C...

- Aliquots of 900µg DNA
- 5 units of Ddel restriction enzyme
- incubation (37 °C) for 120min

Sequence: AAAATGTGCTGCAGATAGCCAATGACCTGGAGAATCTC(c/t)GAGACCTCCTCCATCTGCT  
Sequence: GGCTTCTCCAAGAGCTGCTCCCTGCCTCAGACCAGTGGCTGCAGAAGCCAGAGAGC

### • Eletrophoresis (4% agarose gel):

Lep +/-  
Lep +/+ ← ↑ → Lep +/-



## Conclusions:

- Venipunctures and the risk of mutilations are unnecessary
- Mouse DNA may be obtained and analyzed in less than 6h with this safe, relatively inexpensive, time-sparing and reliable method

## Acknowledgments :

I would like to thanks Prof. Silvana Almeida and Lisiane Smirdele for providing the protocol that this technique was adapted

## Apoio: