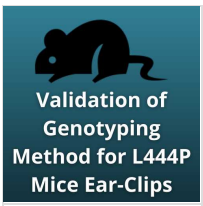

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Validation of Genotyping Method for L444P Mice Ear-Clips

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ABSTRACT

Aim: the genotyping is used to identify if mice are heterozygote (hetero) or Wild-Type (WT), and the aim of the work is to validate the digestion method, and PCR program, the PCR primers, and the interpretation of the results.

General notes: There are two sets of primers – Neo primers to distinguish between WT and hetero samples, and L444P primers to detect L444P protein. Negative and positive samples are used to verify the digestion and PCR, by gel electrophoresis. Sequencing is used to verify the interpretation of the gel electrophoresis.

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KEYWORDS

NC_000069.7, genotype, genotyping, PCR, ear tag, L444P, Neo, ASAPCRN

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45154

GUIDELINES

The sequence: (ACCESSION NC_000069) we look at GACTTGGAAACAG (about 230 to 240 on a sequencing histogram): hetero/WT are shown below, with the yellow highlighted part can either be ttg/ccg (L/P) for hetero (+/Δ), or ttg (L) for wt (+/+).

```
5221 tgggcgggaa gtgacaaggt ggcatcagag tcacagataa tccccagatg actgatgctg F
5281 gagtcctgcc acaggacagg accaaggccc agtggcacag ctgaggctca gggctaagag
                                                                    K
5341 tctggctgtg ttgcccaggg aagcctgggg tcctgatgat ttctatcttc acgttcagca
       F  I  P   E  G  S   Q  R  V  A   L  V  A   S  E  S   T  D  L  E   L444P
5401 agttcattcc cgagggatcc cagagagtgg cgttggtggc cagtgagagc actgacttgg  ccg
5461 aaacagtagc actgttacgc cctgacggct ctgcagttgt ggtcgtgtta aaccggtaag
5521 ctgggtgcaa gggcaggtct gggcctgagc cgtgtgaggt ggctggcatc agcaggttga
                            S  S   E  D  V  P   L  T  I   S  D  P
5581 gtcttgactt ttcttctctc cagatcttcg gaggatgtcc ctcttaccat cagtgatcct R
     D  L  G  F   L  E  T   V  S  P   G  Y  S  I   H  T  Y   L  W  R
5641 gacctgggct tcctggagac cgtgtcacct ggctactcca ttcacactta cctgtggcgt
     R  Q  *
5701 cgccagtgat ggaactgaca cctgagcagg cacctgggct tagcatggac attaaaggga
```


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MATERIALS TEXT

Materials

- Proteinase K (Roche, cat 10241100)
- Direct PCR (EaR) lysate buffer (Viagen, cat 402-E)
- Reaction buffer (VWR-Peqlab, cat 01-1020)
- DNA Taq polymerase (VWR-Peqlab, cat 01-1020)
- dNTP mix (40mM; VWR, cat 5100850-0500)
- RNase/DNase-free H2O (Promega, cat P1193)
- pre-cast 2% SYBR-safe E-GEL (ThermoFisher, cat G521802)
- 100bp DNA ladder (NEB, N3231)

Primers details

Neo1: 5'GATTGCACGCAGGTTCTCCG3'
Neo2: 5'CCAACGCTATGTCCTGATAG3'
L444P_F: 5'CCCCAGATGACT**T**GATGCTGG3' (marked in **bold**, an extra T, that does not appear on the sequence)
L444P_R: 5'CCAGGTCAGGATCTCTGATGG3'

Equipment

- Thermalcycler
- Gel apparatus

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Digestion 2h 30m

1

Prepare Digestion mix: **15 µL Proteinase K** (Roche, cat 10241100) per **1000 µL Direct PCR (EaR) lysate buffer** (Viagen, cat 402-E).

2 Add **50 µL digestion mix** to each ear clip sample.

3 5h

Heat for **55 °C** , **05:00:00** .

4 10m

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Heat 99 °C for 00:10:00 .

5 Cool to 10 °C .

6

Samples can be stored in -20 °C until use.

PCR 4d

7

Prepare PCR master mix (MM) as below (*per sample*):

- **2.5 µL Reaction buffer** (VWR-Peqlab, cat 01-1020)
- **0.25 µL DNA Taq polymerase** (VWR-Peqlab, cat 01-1020)
- **0.5 µL Forward primer** (10pmol/ µl)
- **0.5 µL Reverse primer** (10pmol/ µl)
- **0.5 µL dNTP mix** (40mM; VWR, cat 5100850-0500)
- **19.25 µL RNase/DNase-free H2O** (Promega, cat P1193)

Primers details:
Neo1: 5'GATTGCACGCAGGTTCTCCG3'
Neo2: 5'CCAACGCTATGTCCTGATAG3'
L444P_F: 5'CCCCAGATGACT**T**GATGCTGG3' (marked in **bold**, an extra T, that does not appear on the sequence)
L444P_R: 5'CCAGGTCAGGATCTCTGATGG3'

8 For each sample, add **23.5 µL MM** and **1.5 µL digested sample** .

9

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PCR program:

A	B	C
Temp	Time	Cycles
94°C	5 min	
94°C	30 sec	35 cycles
55°C	1 min	
72°C	1 min	
4°C	forever	

10

PCR product can be kept in **4 °C** for the short term, or at **-20 °C** .

Gel electrophoresis 30m

11 30m

Run PCR products on a pre-cast 2% SYBR-safe E-GEL (ThermoFisher, cat G521802), for **00:30:00** , along with a 100bp DNA ladder (NEB, N3231), and a negative control (no DNA, only MM).

For Neo primers, expected results are band at ~500bp for hetero samples and no band for wt (band for positive control, no band for negative control).
For L444P primers, expected results are a band for every sample (band for positive, no band for negative).

Results of validations:
Below in a gel image of L444P samples (MEFs and ear clips) that were digested and amplified as described above.
C2 and C14 are MEF samples. 1555572, 155303, 155573 are ear clips digestions, pos sample is a hetero samples that was verified by sequencing.

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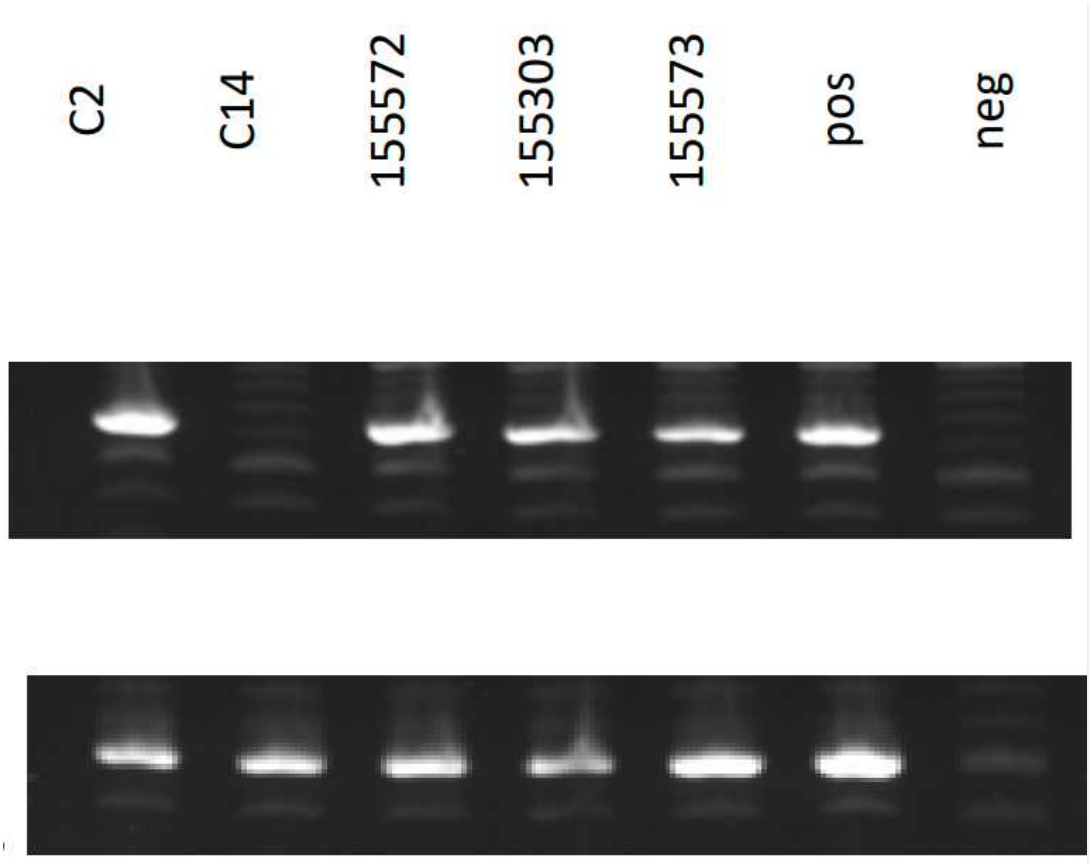

Top gel image shows samples run with **NEO primers**
Bottom gel image shows samples run with **L444P primers**

The gel show that C2 is hetero (band for both Neo and L444P), and that C14 is wt (no band for Neo, and a clear band for L444P). The sequencing results verify the identification of C2 and C14.

Sequence validations:

In order to validate the interpretation of the gel, the PCR products were sent to sequencing:

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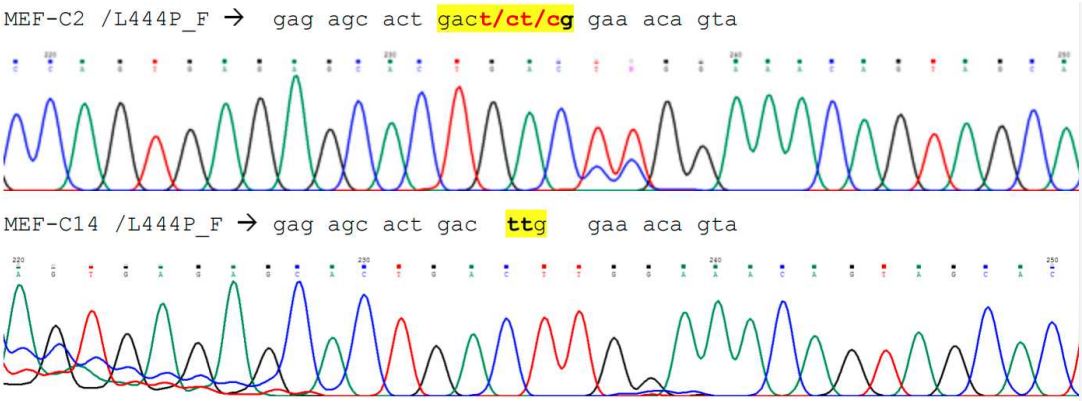

When genotyping L444P ear-clips, to identify hetero/wt, one can run PCR of the digested material with Neo primers beside L444P primers (including positive and negative control), and identify by the presence/lack of band whether it is hetero or wt. There is need to sequence the PCR product.

Routine check of ear clips:

12 Run digested ear clips with NEO primers in the first instance. Then choose one of three options:

1. If the ratio wt:hetero is 1:3, there is no need for further tests. Wt animals to be terminated.
2. If there are more wt than the 1:3 ratio, this suggested poor DNA for the samples that were not amplified, so need to amplify with L444P primers the samples that did not work, and sequence them.
3. If there are much more hetero than the 1:3 ratio, need to sequence all the samples.

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