

# The Cytochrome b Polymorphism of Meat Lines Rabbits

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**Abstract:** The cytochrome b mtDNA was analysed from peripheral whole blood samples of meat lines rabbits. The PCR product—692 bp long fragment of *Oryctolagus cuniculus*\_cytochrome b (*OCU07566 GenBank, NCBI, USA*) was amplified. Synthetically produced oligonucleotides for the detection of cyt b were designed by own algorithm: ORYCTO-cyt b-FOR 5'- CTA TCA GCA ATC CCA TAT ATC -3' and ORYCTO-cyt b-REV 5'- CTT CAT TTG AGG ATT TTG TT -3'. Based on AluI-RFLP were described two cytochrome b haplotypes—cyt b 430 and cyt b 306. Haplotype cyt b 430 is 571A (190Threonine-T) + 877G (292Alanine-A). The new haplotype cyt b 306 is presented A571G nucleotide substitution, i.e. 571G (190Alanine-A) + 877G (292Alanine-A).

**Key words:** Rabbit, cytochrome b, SNP, new haplotype, A571G.

## 1. Introduction

MtDNA genome encodes 13 oxidative phosphorylation enzymes, two rRNA, tRNA, 22 necessary for protein synthesis and a control region (D-loop), in which begin and end of all the replication of mitochondrial DNA sequences. Mitochondrial DNA is traditionally used in population genetics as a marker of choice to characterize the phylogeny [1]. Many researchers have drawn attention to the functional role of mtDNA, and drew attention to the direct use of mitochondrial data for the needs of ecology and evolution [2-4]. Reparation mechanisms at the level of mtDNA quickly remove most of harmful mutations. However non-correction mechanism of mtDNA sequences affects the quality of production mitochondrial enzymes and therefore their effective share in the overall energy metabolism of the cell. These changes affect the male generative cells to a much greater extent in comparison with influencing somatic cells or female generative cells. Sperm have high power requirements relative to the motility, the amount of the mitochondria is only a fraction compared to the other cells [5-8]. It follows that any mutation in the mtDNA and following the relative loss of

efficiency is least in the complex OXPHOS can significantly reduce sperm function. This is also negatively affected male fertility. However, the occurrence of mtDNA mutations has no significant effect on the vitality of the eggs due to their lower energy requirements. Female fertility is therefore not adversely affected, since maternal inheritance of mtDNA, the mutant haplotype occurring in the population of the female line. This effect was called the “mother’s curse effect” [8]. The mitochondria occupy a central role in the metabolism of the eukaryotic cell [3], in Genetic disease [9], in Genetic Programming death (apoptosis) [10], RFLP identification of species [11] and aging [12]. Authors [11] used five animal species (*Mustela vison*, *Mustela putorius furo*, *Sus scrofa domesticus*, *Oryctolagus cuniculus*, *Anser anser*) for the mitochondrial cytochrome b gene analysis. Length of PCR product was 359 bp and authors used universal primers. Restriction fragment length polymorphism was analyzed by using the restriction endonuclease AluI. Results of cleavage were visualized by using electrophoresis and UV transilluminator. Every animal specie has a unique combination of restriction fragments i.e. *Mustela vison* 81 bp, 109 bp and 169 bp, *Mustela putorius furo* 169 bp and 190 bp, *Sus scrofa domesticus* 115 bp and 244 bp, *Oryctolagus cunninculus* is not cleaved by

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AluI so it has whole 359 bp fragment on agarose gel, Anser anser 130 bp and 229 bp. The results suggest that the method of PCR—RFLP is rapid and simple method for identification of species. PCR—RFLP can reliably identify chosen species.

## 2. Material and Methods

### 2.1 Animals

The experimental animals (rabbits) were bred in an approved test facility of Research Institute for Animal Production (RIAP), National Agricultural a Food Centre Nitra, Slovakia. Cytochrome b mtDNA was analysed from samples of peripheral whole blood (collected from *a. auricularis centralis* to heparinised tubes; Heparin, 25,000 IU, 4 µL/1 mL) from 18 males and 24 females breeding parental generation and their F<sub>1</sub> generation of the original meat lines (M91 and P91) rabbits bred in RIAP Nitra.

Females of the parental generation were divided into two groups: 1.—experimental group (12 animals) was subjected to strict divergent selection and selection criteria should be more stringent (selected female had to have at least three litters with 7-10 live kits). 2.—control group (12 animals) had at least three litters a large variation range of live-born kits at birth 1-15 kits. The animals were housed in individual cages, under a constant photoperiod of 14 h of light day. Temperature and humidity in the building were recorded continuously by means of a thermograph positioned at the same level as the cages (average relative humidity and temperature during the year was maintained at 60 ± 5% and 17 ± 3 °C). The rabbits were fed *ad libitum* with a commercial diet (KV; TEKRO Nitra, Ltd.) and water was provided *ad libitum* with nipple drinkers. The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic, no. SK P 28004 and Ro 2058/06-221/1c.

### 2.2 Cytochrome b mtDNA Analysis

For isolation of mtDNA from heparinized peripheral

blood, it used the Maxwell DNA Purification Kit. The concentration of DNA in the samples was measured on a UV/VIS spectrophotometer Nano Photometer (Implen). The polymerase chain reaction (PCR) technology has been used for specific segments amplification of isolated small mitochondrial (mtDNA) male and female rabbits. As a reference was the elution solution used to elute the DNA for purification. PCR conditions (PTC-200 DNA Engine, BioRad) were 95 for 2 min, 94 °C for 30 sec, 54 °C for 30 sec, 72 °C for 30 sec, 35 cycles, the last step of extension at 72 °C 10 min. The PCR reaction volume (25 µL) containing 10 mM Tris-HCl (pH 8.6 at 25 °C, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, Taq DNA polymerase, dNTPs 0.2 mM each, 5% glycerol, 0.08% Igepal CA-630, 0.05% Tween-20) (New England Biolabs), 10 pmol/µL each primer (ORYCTO-cyt b-21nt-FOR and ORYCTO-cyt b-20nt-REV), and the mtDNA samples. The amplified mtDNA was electrophoretically separated on a 2% agarose gel containing ethidium bromide at 80 mA, 120 V in 10 mM lithium borate buffer, pH 8.0 during 90 min.

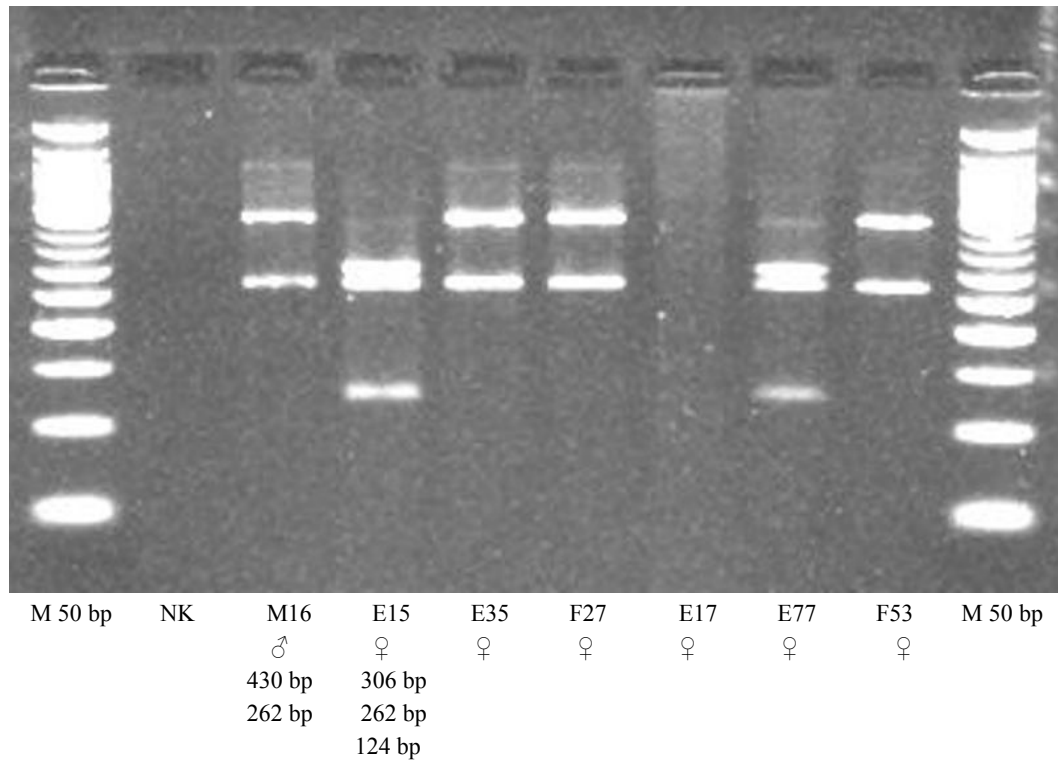
## 3. Results and Discussion

PCR products were visualized under UV light and photographed using a documentation system MiniBis Pro (Bio-Imaging Systems) (Fig. 1). It was detected 692 bp long fragment of *Oryctolagus cuniculus*\_cytochrome b (*OCU07566 GenBank, NCBI, USA*) rabbit mitochondrial DNA. To find a definition of the analyzed section of mtDNA was applied in the laboratory designed synthetically produced oligonucleotides (Table 1).

The result of AluI restriction analysis of the PCR product is the same, fragments arise which are analyzed by agarose electrophoresis and microchip MCE®-202 MultiNA (Shimadzu) (Figs. 1 and 2). On the basis of the restriction fragment was detected by two types of AluI RFLP of mtDNA b -CYT (*OCU07566 GenBank, NCBI, USA*) 306-262-124bp and 430-262bp. Based on the experimental rabbits

**Table 1** Oligonucleotides for detection of partial cytochrome b *Oryctolagus cuniculus* (OCU07566 GenBank, NCBI, USA).

Sequence (5'-3')	Anneal. temperature (°C)	Extent (bp)
ORYCTO-cyt b-FOR CTA TCA GCA ATC CCA TAT ATC	54.0 °C	692
ORYCTO-cyt b-REV CTT CAT TTG AGG ATT TTG TT		

**Fig. 1** Alu I cleavage of cytochrome b 692 bp PCR product visualised on 2% Agarose gel by MiniBis Pro (Bio-Imaging Systems). Haplotype cyt b 430 = samples M16, E35, F27, F53; haplotype cyt b 306 = samples E15 and E77; NK = negative control, M = 50 bp DNA Ladder (Jena Bioscience).

were divided into two haplotype groups cyt b 430 and cyt b 306. Haplotype cyt b 430 is 571A (Threonine-T) + 877G (Alanine-A). Haplotype cyt b 306 is presented A571G nucleotide substitution, i.e., 571G (Alanine A) + 877G (Alanine-A).

This nucleotide change is a causal consequence of the conversion and translational changes in the peptide chain, where an essential amino acid Threonine-T is replaced with Alanine amino acid -A190. Haplotype cyt b 430 is defined by one restriction site (877G) (Fig. 3). While cyt b 306 is a new haplotype defined by two restriction sites 571G and 877G (Fig. 4).

#### 4. Conclusions

The new haplotype cyt b 306 A571G polymorphism of meat lines rabbits mtDNA (cell line LEU-RAB) is registered and described as BioSample: SAMN03701526; Sample name: cyt b O.C. (Model organism or animal sample from *Oryctolagus cuniculus*), in NCBI, USA: <http://www.ncbi.nlm.nih.gov/biosample/3701526> and <http://www.ncbi.nlm.nih.gov/bioproject/284428>.

Application of these genetic and molecular methods is very useful for breeding of livestock and protection of biodiversity.

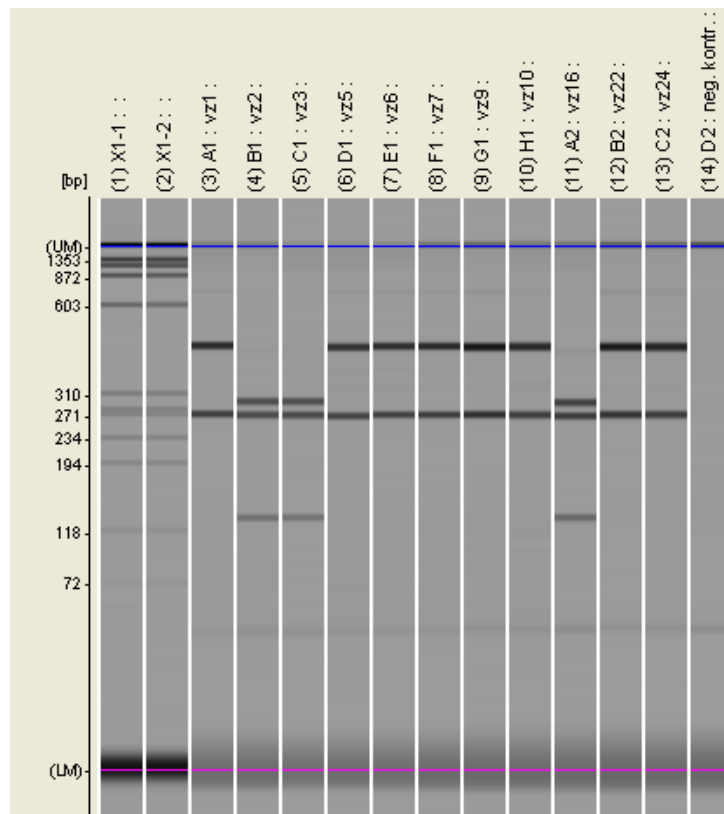


Fig. 2 The rabbit cytochrome b: PCR-RFLP (AluI) results. The Microchip electrophoresis MCE®-202 MultiNA. 1-2: X1-1 and X1-2 = phiX174 DNA/Hae III Markers (Promega), 3-13: samples, 14: negative control.

571

541 ttcacttcattcttgcattatcattgcaacttagtcttaatt  
 F H F I L P F I I A T L V L I

190

586 cacctcctctttctacatgaaactggctccaacaacccacagga  
 H L L F L H E T G S N N P T G

631 attcettcaaactcagataaaatcccttccaccctactacaca  
 I P S N S D K I P F H P Y Y T

676 atcaagacaccctaggttcttcttagccattcttctctctctt  
 I K D T L G F L V A I L L L L

721 atttagtcctattttcaccagacctattaggagaccagacaac  
 I L V L F S P D L L G D P D N

766 tacaccctgccaaccccttaataccctcccatatacaacca  
 Y T P A N P L N T P P H I K P

811 gaatgatacttctatttgctacgctatcctacgctctattcca  
 E W Y F L F A Y A I L R S I P

877

856 aataaactcggaggagtcttagctctagtcctatccatccttggt  
 N K L G G V L A L V L S I L V

292

Fig. 3 Haplotype cyt b 430: AluI Cytochrome b (*GenBank OCU07566*) = 430-262bp, 571A (190Threonine-T) + 877G (292Alanine-A).

571

541 tttcacttcattcttggcatttatcattgca gctttagtcttaatt  
 F H F I L P F I I A A L V L I

190

586 cacctcctcttttctacatgaaactggctccaacaacccacagga  
 H L L F L H E T G S N N P T G

631 attccttcaaaactcagataaaateccctttccacccctactacaca  
 I P S N S D K I P F H P Y Y T

676 atcaaagacaccctagggttcttgtagccattcttctctcctt  
 I K D T L G F L V A I L L L L

721 atttagtcctattttcaccagacctattaggagaccagacaac  
 I L V L F S P D L L G D P D N

766 tacacccctgccaaccccttaataacccctccccatataaacca  
 Y T P A N P L N T P P H I K P

811 gaatgatactttctatttgccctacgctatcctacgctctattcca  
 E W Y F L F A Y A I L R S I P

877

856 aataaactcggaggagtcctagctctagctctatccatccttgtt  
 N K L G G V L A L V L S I L V

292

**Fig. 4 Haplotype cyt b 306: AluI Cytochrome b (*GenBank OCU07566*) = 306-262-124 bp, 571G (190Alanine-A) + 877G (292Alanine-A).**

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