



# THE USE OF MITOCHONDRIAL GENOME POLYMORPHISM TO ESTABLISH PRO-MATERNAL BREEDS IN THE FINAL HYBRIDS OF PIGS

Yelyzaveta Budakva, Konstantin Pochernyaev, Serhii Korinnyi, Mykola Povod

## ► To cite this version:

Yelyzaveta Budakva, Konstantin Pochernyaev, Serhii Korinnyi, Mykola Povod. THE USE OF MITOCHONDRIAL GENOME POLYMORPHISM TO ESTABLISH PRO-MATERNAL BREEDS IN THE FINAL HYBRIDS OF PIGS. Grail of Science, 2022, III CISP Conference "AN INTEGRATED APPROACH TO SCIENCE MODERNIZATION: METHODS, MODELS AND MULTIDISCIPLINARITY", XI (12-13), pp.198 - 204. <10.36074/grail-of-science.29.04.2022.030>. <hal-03695396>

**HAL Id: hal-03695396**

**<https://hal.science/hal-03695396v1>**

Submitted on 14 Jun 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.




Distributed under a Creative Commons Public Domain Mark: No Known Copyright - International License

DOI 10.36074/grail-of-science.29.04.2022.030

# THE USE OF MITOCHONDRIAL GENOME POLYMORPHISM TO ESTABLISH PRO-MATERNAL BREEDS IN THE FINAL HYBRIDS OF PIGS

## RESEARCH GROUP:

**Yelyzaveta Budakva** 

postgraduate student 204 «Technologies of production and processing of livestock products» specialization,

Junior researcher of the Laboratory of Genetics

*Institute of Pig Breeding and Agricultural Production NAAS, Ukraine*

**Konstantin Pochernyaev** 

Scientific Supervisor,

Deputy Director for Research, Production and Financial Activities,

Doctor of Agricultural sciences

*Institute of Pig Breeding and Agricultural Production NAAS, Ukraine*

**Serhii Korinnyi** 

Senior Researcher, Laboratory of Genetics,

Candidate of Agricultural Sciences

*Institute of Pig Breeding and Agricultural Production NAAS, Ukraine*

**Mykola Povod** 

Associate Professor, Department of Animal Feed and Feeding Technology,

Doctor of Agricultural Sciences

*Sumy National Agrarian University, Ukraine*

**Summary.** DNA markers are a convenient tool for researching the origin of pig breeds. The characteristic feature of mitochondrial DNA is that mitochondria are transmitted only maternally to all their descendants. Knowing the peculiarity of the transmission of genetic information contained in the mitochondrial DNA, it becomes possible to establish the pro-maternal breeds of pigs of the final hybrid (Large White × Landrace) × Maxgro. Comparison of the maternal-inherited set of rectal fragments of pigs' final hybrid of foreign selection (n=15) from the total sample (n=175) from RPE "Globinsky Pig Farm", Globyno, town Poltava region, Ukraine allowed getting reliable information of their origin. Laboratory research was carried out based at the Institute of Pig Breeding and Agricultural Production NAAS in the Laboratory of Genetics. As a genetic material, bristles from the auricle of pigs (Large White × Landrace) × Maxgro were used. Allocation was carried out according to Serhii Korinnyi methodology and other authors' publications in 2005

using Chelex -100 ion exchange resin. For the mitochondrial genome analysis, the method of polymorphism of the length of restriction fragments reinforced with amplified in PCR was used (Polymerase chain reaction-restriction fragment of polymorphism – PCR-RFLP). The site of the D-loop of the mitochondrial genome of a pig measuring 428 pairs of nucleotide (with *Tas* 1 recognition sites in positions 15558, 15580, 15616, 15714, 15758 p.n.) was analyzed. This method allows determining 18 haplotypes of the mitochondrial genome. With its use, mitochondrial haplotypes of pigs of the final hybrid were determined: 4 animals with haplotype C, 6 with haplotype N, and 5 with haplotype O. According to many authors pieces of research, these haplotypes characterize different breeds, namely C – Landrace, N – Large White (Asian type) and O – Landrace. The obtained data on the origin of animals of the final hybrid suggests that two-breed sows were the result of direct (Large White × Landrace) and recipient crossing (Landrace × Large White).

It was established that mitochondrial genome polymorphism is an objective marker for determining the breed belonging of animals obtained even in complex hybridization schemes. In our opinion, at the initial stage of breeding work, it is important to establish associations of mitochondrial DNA haplotypes in animals of the final hybrid. Since mitochondria are transmitted along the maternal line only, 13 genes encoding the proteins of the respiratory chain, the identified haplotypes C, N, and O will serve as stable genetic markers. Having determined the correlations of phenotype signs of animals of the final hybrid and certain mitochondrial haplotypes, it is possible to purposefully lead the selection of maternal lines.

Genetic examination in the establishment of maternal breeds of hybrid pigs with the help of markers of the mitochondrial genome and the search for a polymorphic area of the X and Y-chromosome to determine the ancestral line has become an urgent issue in continuing our research in modern pig breeding technologies of commercial lines. The work was done with the support of the National Academy of Agrarian Sciences of Ukraine 31.01.00.07. F. "Investigate the pleiotropic effect gens that the SNP use in marker-associated pig breeding". DR № 0121U109838.

**Keywords:** mitochondrial DNA, pigs, final Irish hybrid, (Large White × Landrace) × Maxgro, haplotype, haplogroup, PCR-RFLP analysis, selection, origin

**The purpose of the study was to** determine the origin of the commodity young pigs of the final hybrid (Large White×Landrace)×Maxgro using polymorphism of the lengths of the restricted fragments of mitochondrial DNA.

**Introduction.** At the present stage, molecular genetics methods have been widely used in the study of genetic biodiversity, and the origin and history of the creation of pig breeds. The study of the processes of domestication is the most popular method for the study of the analysis of the polymorphism of the mitochondrial DNA sequence – (mtDNA) (Fumihito A. et al., 1995; Niu T. et al., 2002) [1, 2]. Mitochondria are a two-membranous organelle present in most eukaryote cells. Most of the genes necessary for the formation and functioning of mitochondria are in the nucleus and only minor genetic information of these proteins is stored in the mitochondria themselves in the form of mitochondrial DNA.

In pigs, the mitochondrial genome is represented by mitochondrial DNA, which is an annular molecule measuring 16,679 pairs of nucleotides, which are part of 37 genes, of which 13 genes encoding proteins and 22 genes encoding transport RNA (16S rRNA and 12S rRNA) and a variable region - D-loops (Hao Liu, Jikun Wang, et al., 2020) [3,4,5]. The most variable part of the mitochondrial genome is a non-

coding area called the D-loop. DNA polymorphism in this area determines a specific sequence that largely characterizes the mitochondrial genome of an individual organism – haplotype. Due to the fact that recombination processes in the mitochondrial genome are absent, it is inherited along the maternal line in the form of one haplotype (Guanghui Yu, Hai Xiang, et al., 2013) [6]. In a certain population of wild pigs or breed of domestic animals, there are various haplotypes, which together form a haplogroup characteristic of the breed. Only the use of mitochondrial DNA markers allows you to determine how many maternal lines with specific haplotypes participated in the creation of the breed and the formation of its haplogroup. It is also possible to identify wild subspecies of ancestors that were the basis of domestication, and subsequently material for breeding work on improving economic qualities and fixing breed-specific features. For example, it was thus confirmed that Yorkshire pigs in Canada and the United States are direct descendants of the English great white breed (Amer P., Allain D., et al., 2015) [7], which is the most common commercial breed of pigs in the world (Lyubov Getmantseva, Siroj Bakoev, et al., 2020) [8]. Large White sows are used in the hybridization system as maternal lines due to the high growth rate, conversion of feed and less fat deposition, and most importantly the number of live birth piglets and the mass of the nest. Determine whether mitochondrial haplotypes of sows of original breeds are inherited in complex hybridization schemes, in particular, the commodity young pigs of the final hybrid (Large White × Landrace) × Maxgro became the goal of our work.

**Materials and research methods.** For the study were used pigs of the final hybrid (Large White × Landrace) × Maxgro ( $n=15$ ) from the general sample ( $n=175$ ) from RPE "Globinsky Pig Farm", Globyno, town Poltava region, Ukraine. DNA was isolated from bristle samples using Chelex-100 ion exchange resin [9]. PCR amplification of fragment *D* - loop located between positions 15558 - 15758 of the mitochondrial genome, conducted on the amplifier TERTSYK-2 (DNA-Technologies) using oligonucleotide primers: forward - *MITPRO2F* CATACAAATATGTGACCCCAAA and reverse – *MITPROR* GTGAGCATGGGCTGATTAGTC, concentrations of 258.2  $\mu\text{mol}$  and 233.6  $\mu\text{mol}$ . Alikvot of PCR product (4  $\mu\text{L}$ ) was hydrolyzed with *TasI* endonuclease (Thermo Scientific™). DNA amplification and hydrolysis products were analyzed in 8% polyacrylamide gel in an electrophoretic device in the 1×TBE buffer. As a marker of molecular weight, *pBR322* DNA/*MSP* I plasmids was used. Visualization of amplification and restriction products was carried out by painting with ethidium bromide and photographing on a transilluminator in ultraviolet light (MicroDOC Gel Documentation Digital camera with UV Transilluminator, Cleaver Scientific).

**Results of the study and their discussion.** In the position of the mitochondrial genome of the pig 15558, one monomorphic site was identified in the studied animal's endonuclease restriction *TasI* (↓AATT). Other possible Sites of endonuclease restriction *TasI* among the studied pigs were polymorphic in the positions of the mitochondrial genome of the pig 15580, 15616 and 15758 p.n. Each discrete set of discrete DNA fragments characterized a specific mitochondrial haplotype in Fig. 1, 2.

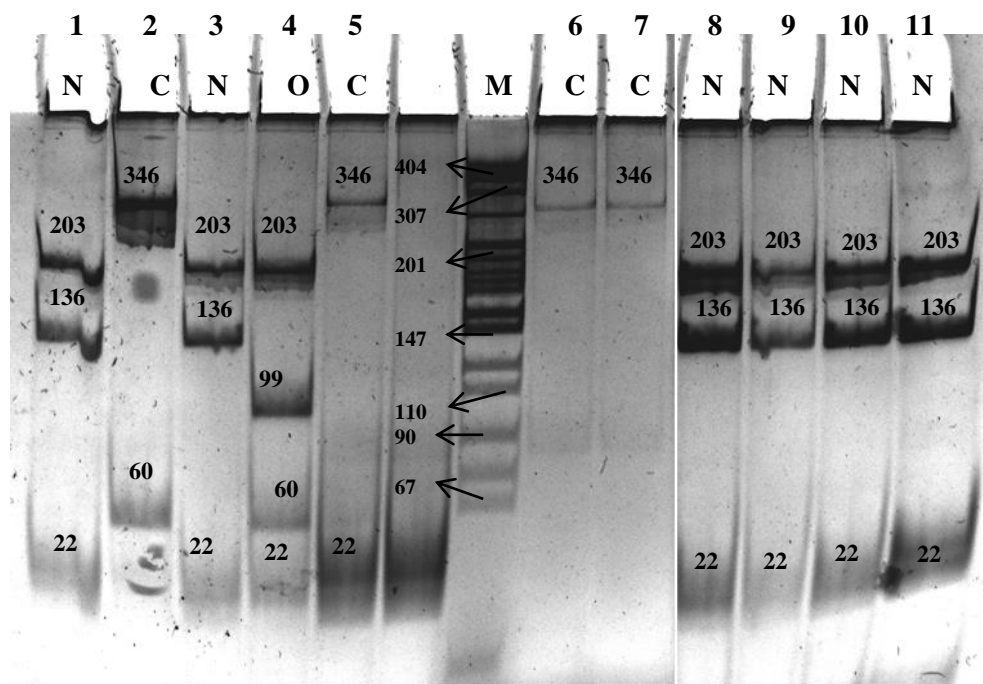


Fig. 1. Results of electrophoretic fractionation in 8% PAAG amplified in PCR and hydrolyzed using endonuclease Tas I mitochondrial DNA of pigs of the final hybrid (LW×L) × Maxgro: M - molecular weight marker pBR322 DNA/Msp I.

[author's development]

The use of this method of analysis, among the studied sample of hybrid pigs, made it possible to determine 3 haplotypes. According to the guidelines "The use of mitochondrial DNA markers to control the reliability of origin genealogical structures of sows" / K. F. Pochernyaev, M. D. Berezovsky. – Poltava: PP, 2014. – p. 27., haplotypes were given the designation in Latin capital letters (in parentheses discrete set of DNA restrict fragments): *N* - (203/136/22); *C* - (346/60/22); *O* - (203/99/60/22) (table 1).

Table 1

Mitochondrial haplotypes identified in pig sample of final hybrid (n=14) (LW×L) × Maxgro identified with the involvement of 6 single-nucleotide polymorphisms

Nucleotide positions of the mitochondrial genome by sequence Accession: AJ002189.1						Restriction fragments of mtDNA in nucleotide pairs	Mitochondrial haplotypes
15558	15580	15758	15714	15758	15917	203/136/22	<i>N</i>
15558	15580	15616	15714	15758	15917	346/60/22	<i>C</i>
15558	15580	15616	15714	15758	15917	203/99/60/22	<i>O</i>

It is believed that all descendants come from one high-performance ancestral and repeat its mitochondrial haplotype, this helps to establish the ancestral of all descendants in a number of generations (D. M. Lomako, K. F. Pochernyaev,

A. G. Bliznyuchenko, 2006) [10]. The defined mitochondrial haplotypes characteristic of the great white and landrace breeds made it possible to establish pro-maternal breeds even with complex hybridization schemes, in particular in the commodity young pigs of the final hybrid (Large White × Landrace) × Maxgro. Nucleotide replacements in mitochondrial respiratory chain gene sequences are one of the causes of the different functional states of mitochondria. Both with purebred breeding and hybridization, mitochondrial haplotypes will be persistently inherited and could potentially be a genetic marker of a specific set of mitochondrial respiratory chain genes. Thus, various mitochondrial haplotypes can potentially provide certain results of the productivity of hybrid pigs. That is, the absence of recombination processes of the mitochondrial genome allows an unchanged form to inherit breeding characteristics by descendants, which makes possible the guaranteed manifestation of phenotypic signs in the final hybrids. Therefore, in order to make an analytical decision on the selection direction in the initial specialized lines, it is important to determine which haplotypes of mitochondrial DNA in the studied population of hybrid pigs are associated with the desired signs of fattening and meat productivity.

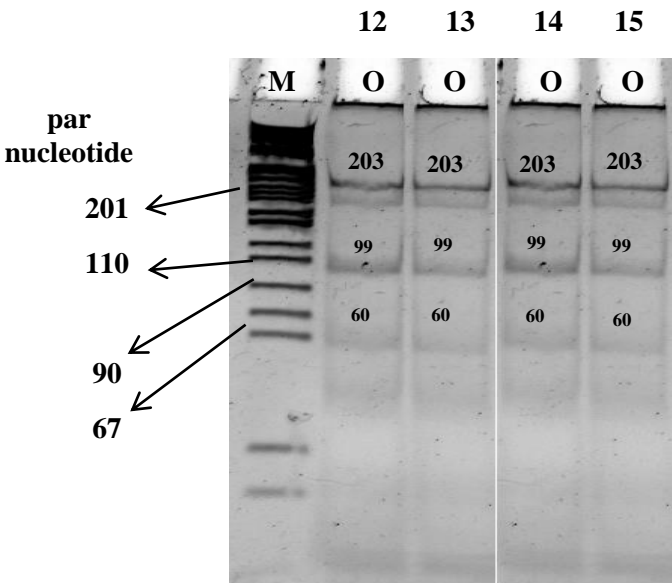


Fig. 2. Results of electrophoretic fractionation in 8% PAAG amplified in PCR and hydrolyzed using endonuclease Tas I mitochondrial DNA of pigs of the final hybrid (LW× L) × Maxgro: M - molecular weight marker pBR322 DNA/Msp I.

[author's development]

Thus, a multi-stage system for determining mitochondrial haplotypes of pigs, identified three mitochondrial haplotypes among hybrid pigs. It was confirmed that the maternal form of a large white breed with the haplotype **N** was used in the hybridization system. In our opinion, as an assumption, the presence of haplotype **O** and **C** is explained by the use of Landrace sows to obtain two-breed sows. The special value of the research performed is that the information from RPE "Globinsky Pig Farm", regarding the use of reciprocation crossbreeding Landrace × Large White was obtained only after the completion of a genetic study, that is, we are talking about

"blind tests". Thus, the identification of specific animals for the establishment of pro-maternal breeds using mitochondrial DNA markers was carried out. Identification of animals is provided for in accordance with the Law of Ukraine "On breeding in animal husbandry", this involves conducting a genetic examination of the identification of animals by laboratory methods in order to control the reliability of their origin. In the context of this article, the concept of "origin" is the attribution of animals to certain breeds along the maternal line, which was the subject of our study. For hybridization of the parent, form is created by methods of differentiated selection with an advantage on precociality, high fattening and meat qualities, and offspring resistance. As is customary, Landrace is used as a parent breed. In this regard, the results obtained require further research.

The results of our research allow us to draw the following **conclusions**:

1. With the use of mitochondrial DNA markers, maternal lines (breed) are defined, and used in the creation of the final hybrid of pigs (Large White × Landrace) × Maxgro from RPE "Globinsky Pig Farm".
2. Among the studied sample of hybrid pigs identified specific mitochondrial haplotypes **C** and **O** and haplotype **N**, allowing for the identification of the pro-maternal breeds Landrace and Large White, respectively.
3. The obtained data on the origin of animals of the final hybrid suggests, that the two-breed sows were the result of direct (Large White × Landrace) and reciprocal crossing (Landrace × Large White).
4. It is established that the polymorphism of the mitochondrial genome is an objective marker for determining the breed belonging of animals obtained even in complex hybridization schemes.

**Prospects for further research.** In our opinion, it is important at the initial stage of breeding work to determine the associations of haplotypes of mitochondrial DNA in the studied population of hybrid pigs, to study the history of the creation of commercial pigs of foreign breeding, determine what are the desired correlated signs of fattening and meat productivity in combination with the smell of boar should be improved or fix not only the maternal lines (Large White), but also the parent lines, respectively (terminal line Maxgro). After all, interspecific hybridization of subspecies of wild populations and domesticated pigs has led to the formation of modern various haplotypes of commercial pigs that cause the desired phenotypic effect.

## References:

- [1] Fumihito, A, Miyake, T, Sumi, S, Takada, M, Ohno, S, & Kondo, N. (1995). One subspecies of the red junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. *Proceedings of the National Academy of Sciences of the United States of America*, 91(26), 12505–12509. Retrieved from: <http://dx.doi.org/10.1073/pnas.91.26.12505>
- [2] Niu, T., Qin, Z. S., Xu, X., & Liu, J. S. (2002). Bayesian Haplotype Inference for Multiple Linked Single-Nucleotide Polymorphisms. *American Journal of Human Genetics* 70 (1), 157–169. Retrieved from: <https://dx.doi.org/10.1086%2F338446>
- [3] Liu, H., Wang, J., Wang, D., Kong, M., Ning, C., Zhang, X., Xiao, J., Zhang, X., Liu, J., & Zhao, X. (2020). Cybrid Model Supports Mitochondrial Genetic Effect on Pig Litter Size. *Frontiers in Genetics*, 11, 1–10. Retrieved from: <https://doi.org/10.3389/fgene.2020.579382>

- [4] Kolosova, M. A., Bakoev, N. F., Kolosov, A. Yu., Getmantseva L. V., (2029). Study of mitochondrial COX2 DNA gene of pigs of different origin. International research journal. 1 (79), 2, 10–13. Retrieved from: <https://doi.org/10.23670/IRJ.2019.79.1.030>
- [5] WANG Chen, CHEN Yao-sheng, HAN Jian-lin, MO De-lin, LI Xiu-jin, LIU Xiao-hong, (2019). Mitochondrial DNA diversity and origin of indigenous pigs in South China and their contribution to western modern pig breeds. Journal of Integrative Agriculture. 18 (10), 2338-2350. Retrieved from: [http://dx.doi.org/10.1016/S2095-3119\(19\)62731-0](http://dx.doi.org/10.1016/S2095-3119(19)62731-0)
- [6] Yu, G., Xiang, H., Wang, J., & Zhao, X. (2013). The phylogenetic status of typical Chinese native pigs: analyzed by Asian and European pig mitochondrial genome sequences. *Journal of Animal Science and Biotechnology*, 4(9). Retrieved from: <https://doi.org/10.1186/2049-1891-4-9>
- [7] Amer, P., Allain, D., Avendaño, S., Baselga, M., & Boettcher, P. (2020). Breeding strategies and programmes. FAO. The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture (4), 1-605. Retrieved from: <https://hal.inrae.fr/hal-02800594>
- [8] Getmantseva, L., Bakoev, S., Bakoev, N., Karpushkina, T., Kostyunina, O. (2020). Mitochondrial DNA Diversity in Large White Pigs in Russia. *Animals*, 10(8), Special Issue "Animal Genetics and Livestock Production: The Biodiversity Challenge", 1365. Retrieved from: <https://doi.org/10.3390/ani10081365>
- [9] Korinnyi, S. M., Pochernyaev, K. F., & Balatsky, V. M. (2005). [Animal fur as a convenient object of DNA isolation for PCR analysis]. *Veterinary biotechnology*, 7, 80–83
- [10] Lomako, D. M., Pochernyaev, K. F., & Bliznyuchenko, A. G. (2006). [Marking of a family of sows by mitochondrial DNA haplotypes]. *Bulletin of Agrarian Science of the Black Sea Region*, 3(2), 71–74. Retrieved from: <http://visnyk.mnau.edu.ua/>.