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Application of chosen *Mus musculus* mtDNA gene primers in amplification of *Chinchilla lanigera* parallel *loci*

Zastosowanie starterów wybranych genów mtDNA *Mus musculus*
do amplifikacji analogicznych *loci* w genomie *Chinchilla lanigera*

Summary. The purpose of the study was to evaluate the usefulness of primer sequences designed for amplification of mitochondrial genes of *Mus musculus* to analyze analogous *loci* in C*hinchilla lanigera's* genome. After isolation of DNA from chinchilla's hair and its quantitative and qualitative estimation, the amplification of DNA by the PCR method was carried out. Fragments of 4 genes of mitochondrial genome were under consideration. Universal primers and primers designed on the basis of *M. musculus* genome were used for amplification of analogous loci of *Ch. lanigera's* genome. PCR products of 4 examined mtDNA sequences were received. Two of the four products showed homology of flank sequences of DNA individual fragments of both studied species belonging to *Rodentia* order. PCR optimization, including changes of polymerase, the concentrations of $MgCl_2$ and primers as well as the melting temperature were determined for two of the four DNA sequences.

Key words: *Chinchilla lanigera*, primer sequences, PCR, optimization

INTRODUCTION

The Late Paleocene – a period for about 60 mln years ago is a moment when on the Earth have appeared first rodents. However, its proper and dynamic progress has started 40 mln years ago – in the Middle Miocene. Almost 2000 species in 30 families makes *Rodentia* the most numerous and the most various order of mammals. That means, *Rodentia* constitutes over 40% of all species of *Mammalia* class [Wilson and Reeder 2005].

The most known, one of the numerous and the easiest adapting to changing conditions of environment, representative of *Rodentia* is *Mus musculus* – the house mouse.

No less important and interesting in the point of view science member of this order is *Chinchilla lanigera* (Molina, 1782) – long-tailed chinchilla. Chinchillas belongs to *Rodentia* order, *Hystricomorpha* suborder, family *Chinchillidae* and genius *Chinchilla*. *Chinchilla* genius involves only 3 species: *Ch. lanigera*, *Ch. brevicaudata* and *Ch. real*. In Poland only *Ch. lanigera* have been breeding on ferms to gain fur. In contrast to mouse – which occur commonly all over the world, chinchilla is a species which comes out on the edge of extinction. Chinchilla's wild populations are on CITES lists – Convention on International Trade in Endangered Species of Fauna and Flora [CITES 1991]. In Chile chinchillas are classified as endangered species, instead in Red List of Threatened Species published by IUCN – International Union for Conservation of Nature this species is determined as critically endangered [IUCN 2008]. National Chinchilla's Reserve (*Reserva National Los Chinchilla)*, area Auco nearby Illapel and area La Higuera in Chile are sole places on the world where wild chinchillas live in nature [Jimenez 1996].

Human activity -agricultural, deforestation, cities and roads build up lead to environment 's destruction and extinction of many species animals and plants. Many scientists anticipates, that has started sixth mass extinction now [Veron 2008]. It is a moment, when knowledge from genetics and molecular biology can be used in wild life protection. Nevertheless to protect species, it is necessary to know its history, biology and genome and that is the role of molecular markers. The searches' goal was to check usefulness 2 kinds of primers: universal primers worked out on fragments mitochondrial genome of many species of animals for example house mouse and primers projected on basis of mouse's mitochondrial genome in amplification of *Ch. lanigera's* parallel loci. The second goal was optimization of conditions PCR reaction for chosen sequences *Ch. lanigera's* mitochondrial DNA (mtDNA).

MATHERIAL AND METHODS

The studied animals were 10 random chosen females of *Ch. lanigera*, bred in furbearing animal farm located in south-eastern Poland. Hair with roots were sampled for analysis. DNA was isolated using the QIAamp DNA Blood Mini KIT (Qiagen). Quantitative and qualitative DNA analysis were performed by electrophoresis in 0.8% agarose gel. DNA concentration was quantified spectrophotometric using Lightware UV/Vis diode Array (WPA). PCR (*Polymerase Chain Reaction)* was carried out for the following four gene fragments of the long-tailed chinchilla: *ND1* (mitochondrial encoded NADH dehydrogenaze 1 Gene), *ATP6* (mitochondrial encoded ATP synthase F0 subunit 6 Gene), *CYTB* (mitochondrial encoded cytochrome b Gene), *RNR1* (mitochondrial encoded 12S RNA Gene). Primers (Tab. 1) used in PCR reaction of fragments of *CYTB* and *RNR1* genes were described in literature as universal primers [Kocher *et al.* 1989, Wang *et al.* 2000]. Amplification of the other 2 fragments of genes (*ND1, ATP6)* was carried out using primers designed with the use of the Primer3 program (http://frodo.wi.mit.edu/) on the basis of mitochondrial genome of *Mus musculus*.

Table 1. Primer sequences of the analyzed *loci*
Tabela 1. Sekwencje starterowe analizowanych *loci*

Locus	Primer H Starter H	Primer L Starter L
CYTB	ACATACGAAAAACACACCCA	TTTTAGTATTTTGTCTTCGAT
RNR1	TTTCATGTTTCCTTGCGGTAC	TTTCATGTTTCCTTGCGGTAC
ATP6	TTTCATGTTTCCTTGCGGTAC	TGTTGGGGTAATGAATGAGG
ND1	TATCCTAACACTCCTCGTCCC	TGGTACTCCCGCTGTAAAAA

The reaction mixture (sample volume – 10 µl) contained: 100–20 ng DNA, 1 µl PCR buffer, 100 µM of each nucleotide, 10 pM arbitrary primer, 10 mM $MgCl_2$ and 0,5 U Taq polimerase. Samples were amplified using PCR reaction in gradient block in termocycle PTC-255 DNA Engine Tetrad. Preliminary amplification run in temperature between 55–65ºC. The amplification reaction consisted of preliminary denaturation (95ºC, 10min); 35 cycles of denaturation (95ºC, 1 min); temperature gradient 55ºC–65ºC (1 min); annealing of DNA strands (72ºC, 1 min); terminal lengthening of DNA primers (72º, 20 min) and cooling to temperature 4ºC. Quantitative and qualitative DNA analysis were performed by electrophoresis in agarose gel (2.5%). Gene Ruler 50bp DNA Ladder (Fermentas) was used as a marker of the volume of DNA fragments. The gels were analyzed in UV light (Transilluminator) and archived.

RESULTS AND DISCUSSION

In our study there was conducted evaluation of usefulness of 4 pair primer's sequences. They are normally used to amplification of gene's fragments in *M. musculus* mitochondrial genome, here they were checked in analysis analogues loci in mitochondrial genome of *Chinchilla lanigera*. Primer sequences elaborated for *CYTB* and *RNR1* genes and derived from literature [Kocher *et al.* 1989, Wang *et al.* 2000] were useful. For *ND1* and *ATP6* genes primers were designed on the basis of *Mus musculus* genome and there was no satisfactory results. DNA was visible as fluorescent in UV stripes (Fig. 1, Fig. 2). On the basis of that it was possible to determine melting temperature of primers for *CYTB* and *RNR1* and to match concentration of particular elements of reaction mixture.

Melting temperature of primers were established for two fragments: *CYTB* and *RNR1*, and were carried out adequately 58.5ºC and 64.3ºC (Tab. 2). There were quite big differences between melting temperature of primers worked out for *M. musculus's* genome and published in literature. Differences were 8.5ºC for *CYTB* and 10ºC for *RNR1*. PCR products of genes obtained in our study give evidence about homology of flank sequences of mtDNA individual fragments of both studied species of *Rodentia* because *M. musculus* and *Ch. lanigera* belong to the same order in systematic and are closely related.

In *CYTB* case it was need to change PCR reaction conditions to get specific product. Optimization concerns composition of mixture – concentration of dNTP was reduced from 100 μM to 70 μM; it was reduced quantity of primers in half; quantity of $MgCl_2$ was reduced from 10μM to 7.5 μM and concentration of polymerase from 0.5 U to 0.25 U. Firstly, besides specific amplification product, there were received extra, nonspecific products visible which after optimization did not appear.

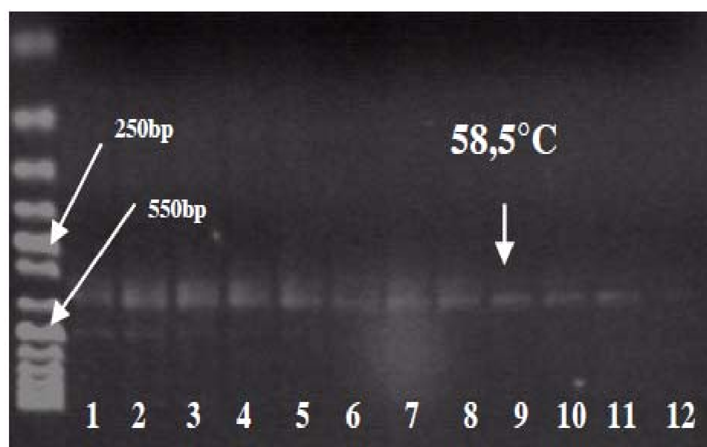

Fig. 1. Electrophoregram of *CYTB* gene in *Ch. lanigera* genome (temperature gradient 50°C – 60°C) (the first lane from the left – the size standard, lanes 1–12 amplification product)
Rys. 1. Elektroforegram fragmentu genu *CYTB* (gradient temperatury 50°C – 60°C) (pierwsza ścieżka od lewej – marker wielkości, 1–12 – produkty amplifikacji)

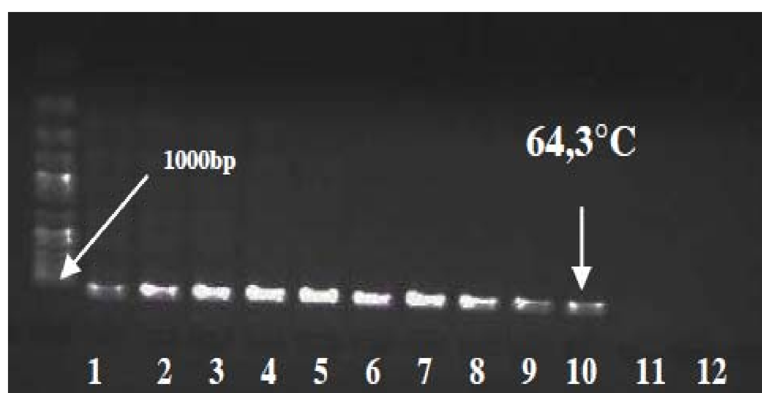

Fig. 2. Electrophoregram of *RNR1* gene in *Ch. lanigera* genome (temperature gradient 50°C – 60°C) (the first lane from the left – the size standard, lanes 1–12 amplification product)
Rys. 2. Elektroforegram fragmentu genu *RNR1* (gradient temperatury 50°C – 60°) (pierwsza ścieżka od lewej – marker wielkości, 1–12 – produkty amplifikacji)

The reason of lack of amplification products of fragments *ATP* and *ND1* genes in *Chinchilla lanigera*, which was conducted with primers designed on the basis of mitochondrial genome of *Mus musculus* was probably fact that sequences of this two genes are different in both species.

Table 2. The temperature of annealing primers
Tabela 2. Temperatura przyłączania sekwencji starterowych

Locus	The temperature of annealing primers Temperatura przyłączania starterów
CYTB	58.5°C
RNR1	64.3°C

Universal primers or primers designed on the basis of close species genome can be used in searches species which genome is not well known. Next step can be experience whole sequence those fragments or whole genes using sequencing technique. It could be use to compare species and make phylogenetic trees and also gen know about evolution of Rodents. It is worth to check usefulness of universal primers at other species of *Rodentia* order in the future, especially endangered. In Poland it could be the northern birch mouse (*Sicista betulina)*, Alpine marmot (*Marmota marmota)* or the speckled ground squirrel (*Spermophilus suslicus)*.

CONCLUSIONS

1. PCR products of *CYTB* and *RNR1* obtained in our study give evidence about the possibility of using the universal primers for the genes amplification in chinchillas.
2. During amplification of *Chinchilla lanigera's* two mitochondrial fragments of genes *ND1* and *ATP6* used primers designed on the basis of *Mus musculus* genome and there was no result.
3. Primers annealing temperature in amplification of the same DNA fragments, but of the different species can draw a distinction.
4. Changing of composition of the reaction mixture can affect on elimination non-specific products of the reaction and obtainment of specific product with very well quality.

REFERENCES

CITES, 1991. Appendices I, II, III to the Convention on International Trade in Endangered Species of wild fauna and flora. September 1, 1991. Convention on International Trade In Endangered Species, U.S. Government Printing Office, Washington D.C.

IUCN, 2008. The IUCN Red List of The Threatened Species. International Union for Conservatinon Status of Nature and Natural Resources.

Jimenez J.E., 1996. The extirpation and current status of wild chinchillas *(Chinchilla lanigera* and *Chinchilla brevicaudata)*. Biol. Cons. 77, 1–6.

Kocher T.D., Thomas W.K., Meyer A., Edwards S.V., Paabo S., Villablanca F.X., Wilson A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers, PNAS 86, 6196–6200.

Veron J.E.N., 2008. Mass extinctions and ocean acidification: biological constraints on geological dilemmas. Coral Reefs 27, 459–472.

Wang H.Y., Tsai M.P., Tu M.C., Lee S.C., 2000. Universal Primers for Amplification of the Complete Mitochondrial 12S rRNA Gene in Vertebrates. Zool. Stud. 39, 61–66.

Wilson D.E., Reeder D.M., 2005. Mammal species of the world: a taxonomic and geographic reference, 3rd ed. Smithsonian Institution Press, Washington, D.C.

Streszczenie. Przeprowadzone badania miały na celu sprawdzenie użyteczności uniwersalnych starterów i starterów opracowanych na bazie genomu mitochondrialnego *Mus musculus* w amplifikacji analogicznych *loci* genomu *Chinchilla lanigera*. Po izolacji DNA, jego ilościowej i jakościowej ocenie przeprowadzono amplifikację metodą PCR. Uzyskano produkty dwóch spośród czterech fragmentów genów. Fakt ten może świadczyć o homologii sekwencji flankujących badanych fragmentów DNA u obu badanych gatunków gryzoni. Jedną z przyczyn może być przynależność do jednego rzędu oraz bliskie pokrewieństwo *Mus musculus* i *Chinchilla lanigera*.

Słowa kluczowe: *Chinchilla lanigera*, startery, PCR, optymalizacja