Egyptian J. Anim. Prod. 57 Suppl. Issue, January (2020):143-148

COMPARATIVE ANALYSIS OF SEQUENCE DIVERSITY DIAGRAM FOR FIVE RABBIT' BREEDS IN EGYPT USING SINGLE NUCLEOTIDE POLYMORPHISM

T.S.K.M. Rabie

Department of Animal production, Faculty of Agriculture, Suez Canal University. Ismailia, 41522. Egypt

Received: 2/9/2019 Accepted: 10/11/2019

SUMMARY

The genes coded and referred to as 18S rDNA has been selected as a universal marker. Sequence data from 18S rDNA was used to estimate the genetic variants on 128 rabbits belonging to five different breeds in Egypt, New Zealand White (NZW, n=35), California (Ca, n=35), Chinchilla (C, n=19), Flander (F, n=19) and Babion (B, n=20). Blood samples for sequencing were collected and sequence diversity diagram for multiple sequence alignments was performed. In addition, the comparative analysis according to detected SNPs was implemented. The results revealed that a 1115 bp fragment of DNA sequence was constructed using ABI5100 automated sequencer.

Random small DNA sequence mismatches (74 nucleotides; 1-29, 1071-1115) has been detected. The similarity between Ca and NZW breeds was 92%, additionally, higher similarity percentage was observed between C and both of B, and F (97.02, 97.5 respectively). Nine SNPs were detected; five single nucleotide polymorphisms were spread to (SNP: A>T, and T>A) for B and F located at 185, and 508 bp, also C>A and G>C for C located at 268, and 919 bp. In addition, A>T for B located at 267 bp, and four SNPs between Ca and NZW located at 880, 886, 899, and 900 bp were detected. The Maximum Likelihood was used to estimate the Transition/Transversion (ti/tv) Bias resulted in the estimated (ti/tv) bias (R) was 0.67. Substitution pattern and rates were estimated, the nucleotide frequencies were A = 22.12%, T/U = 21.13%, C = 26.72%, and G = 29.99%. In conclusion, the utilized five rabbit breeds possess a good reservoir for diversity which is essential for genetic improvement. Formal conservation plan is needed and must be implemented in Egypt to achieve such proper genetic improvement.

Keywords: Sequence reads; Rabbits; Diversity; Single nucleotide polymorphism

INTRODUCTION

The genetic map of rabbits is still very limited with only one partial projection (Korstanje *et al.* 2001, 2003). During the last two decates, only markers detectable by conventional biochemical, immunological, and morphological methods were in use for linkage studies in the rabbit (Korstanje 2000). Throughout the process of evolution, populations of different species preserved some common features while dropped others. This progression, referred to as natural selection, promotes survival of the strong genes which offer organisms more chances of surviving under lively habitats, reproducing vigorously and encountering longer lives. This sort of genetic change produces variations in genes over an extended duration of time, although genetic diversity happens within the momentary, neutering the characteristics between parents and offspring.

During the time of inheritance, nucleotides, but not necessarily entire genes, are rearranged and recombined to frame new mixes that are not quite the same as the parents. Also, different occasions can make new variety in the DNA sequences. Transformations or mutations are changes to at least one nucleotide in the DNA sequence. Mutations in somatic cells can cause issues, for example, growth, in a living being yet are not heritable. Transformations in regenerative cells (gametes) are acquired by the descendants. These changes in the DNA sequence can be gainful, inconvenient, or neutral. Furthermore, artificial and flavored genetic variation afterwards helps species survive with support of lowering threat of initiating defects, disorder and harmful genes. Unfortunately, the procedure of domestication tends to diminish the hereditary assorted variety of selected species, due to some degree to the hereditary bottleneck forced when just couple of animals are chosen. In addition, genetic diversity is essential since it keeps up the strength of a population, by including alleles that might be significant in resisting diseases, climate changes and other burdens.

In a small isolated population, individual might be compelled to breed with close relatives. To ensure that the breeding program practically remains on basics to screen and keep up genetic diversity, hereditary decent variety takes into account determination of prevalent animals for breeding. In the event that there is no genetic diversity, if all animals would be hereditarily the same, selection won't bring any changes or improvements in the coming generations. Recently, molecular markers have proven to be superior to the conventional methods for determining gene flow, moreover, data on genetic variation can provide crucial input to plan effective strategies for conservation and rehabilitation of natural populations. The essential

tools for advance of sequencing, eg. dense-linkage maps, genomic libraries, etc. were adapted by agricultural scientists in Europe and US (Aerts *et al*., 2003; Lee *et al*., 2003, Kersten *et al*., 2011). Nowadays, a first-generation microsatellite-based integrated genetic and cytogenetic map for the European rabbit (Oryctolagus cuniculus) was constructed by Chantry-Darmon *et al.* (2006). Although, microsatellite markers are used most widely in the analysis of genetic diversity of livestock, and population structure of rabbits (Zenger *et al*., 2003; Tian-Wen *et al*., 2010, El-Aksher *et al*. 2016), the sequence reads has been used in this study. Formerly, few efforts were existed related to Egyptian indigenous rabbits' germplasm (Rabie 2012; Emam *et al*. 2017; Abdel-Kafy *et al*., 2018; Badr *et al*. 2019). Accordingly, this study was planned to estimate the specific molecular variants between five breeds of rabbits in Egypt within the genome causing this genetic variation by using sequence reads, and single nucleotide polymorphism analysis.

MATERIALS AND METHODS

Collecting blood samples and DNA extraction:

The present study was conducted at the laboratory of biotechnology, Department of Animal Production & Fish Resources, Faculty of Agriculture, and the Biotechnology Research Institute, Suez Canal University to identify the genetic variant between five rabbit breeds to detect genetic variations between New Zealand White (NZW, n=35), California (Cal, n=35), Flander (F, n=19), Chinchilla (Ch, n=19), and Babion (B, n=20). Blood samples from the five breeds were collected according to the institutional ethical norms of the Faculty of Agriculture, Suez Canal University, Egypt. About 1ml of blood from the marginal ear vein was individually collected into coated tubes with K3-EDTA (FL medical, Italy) and stored immediately at -20°C until DNA extraction. Quick-gDNA MiniPrep (Zymo Research, USA) was used to extract the genomic DNA to provide high purity DNA yield. Furthermore, the most frequently used method to determine DNA quality is the ratio of absorbance at A260 nm /A280 nm. Ratios around 1.8 indicate good-quality DNA whereas lower values indicate protein contamination since proteins have a peak in absorption of 280 nm resulting from the aromatic amino acids. As a unique assessment of the DNA sample, the NanoDrop® ND-1000 UV-Vis spectrophotometer has been used enabling highly accurate analyses of the extremely small samples with notable reproducibility. Cuvettes and capillaries were needed to accelerate measuring sample cycling time of sample retention system. The extracted DNA was pooled for each breed and two replicates prepared and used for gene sequencing.

Gene sequencing:

The gene coded and symbolized as 18S rDNA has been used as the universal marker (5'-TTAAG CCATGCATGTCTAAG-3', 5'-GACTACGACGGT ATCTAATC-3'). The data from this gene is extensively used in molecular analysis approach due to its slow evolutionary rate which enables reconstruction of the genetic evolutionary history of organisms. Therefore, 18S marker was used for PCR amplification, each 25µl reaction contained 12 µl 2X MasterMix (Qiagen), 40ng of gDNA, 20 pmole of each primer and miliQ water. The PCR was performed at Mastercycler gradient (eppendorf) starting at 94°C for 2 min as an initial template DNA denaturation, followed by 35 amplification cycles (94°C for 30s, then 30s at 52°C, 3 min at 72 °C), then 72°C for 10 min as final extension. Furthermore, the gel advanced ver.2 software was used for the capture of gel photo, and the relative band concentration was tested by EgGel-analyzer software. Afterwards, PCR product was purified using QIA quick PCR Purification Kit (QIAGEN). The PCR product was visualized using 1.5% agarose gel documentation system, consequently analyzed by Gel Docu and performed in total volume of 20 µL using BigDye Terminator (v3.1) Cycle Sequencing Kit, each reaction contained DNA template. Template quantity was calculated according to the PCR product size, 8 µL Terminator ready reaction mix, 3.2 pmol primer, and miliQ water. The thermal profile for sequencing cycle was 60°C for one minute, followed by additional purification with CENTRI-SEP columns (Princeton Separations), Therefore, 3500 genetic analyzer (Applied Biosystems) was used for DNA sequencing starting at 96°C, followed by 25 Cycles (96°C for 10s, 50°C for 5s, and 60°C for 4 min).

Data Analysis and manipulation:

Evolutionary relationships and SNP analysis

The obtained sequences were analyzed, and the SNPs were detected according to the results of multiple sequence alignments using Mega X software (Kumar *et al*. 2018), consequently, phylogenetic trees were charted using the Neighbor-Joining method (Saitou and Nei, 1987) and the genetic distances were determined using the Composite Maximum Likelihood (CML) according to Tamura *et al*., (2004). This analysis involved 5 nucleotide sequences, and the $1^{st}+2^{nd}+3^{rd}$ codons +Noncoding were included, wherever, all ambiguous positions were removed for each sequence pair using "pairwise deletion option".

Maximum Likelihood Estimate (ML) of Transition/Transversion Bias

The initial tree generated by applying NJ was used for the ML search. In addition, BIONJ algorithms to the matrix of pairwise distances was estimated using the maximum composite likelihood approach for nucleotide sequences. To estimate Transition/Transversion (ti/tv) bias, parameters κ, κ1, and κ2 under Maximum Composite Likelihood model, thus substitution pattern and rates, were estimated under the Tamura and Nei (1993) model.

RESULTS AND DISCUSSION

In the present study, the DNA retrieved from the five rabbit breeds has been sequenced to evaluate the prospected variability among them and examine the genetic effects based on sequence reads. The results revealed that a 1115 bp fragment of DNA sequence was constructed using ABI5100 automated sequencer. Random small DNA sequence mismatches (74 nucleotides; 1-29, 1071-1115) have been detected, and may impulsively be developed in the divergent breeds. In order to facilitate the use of this information, multiple sequence alignments have been created using Mega X platform to enhance the discovery of sequence variation. In addition, the phylogenetic tree was formulated using the Neighbor-Joining method. The optimal tree with a sum of branch length 0.1029 resulted in phylogenetic diversity based on rabbit sequence reads (Figure 1) and the similarity between studied breeds is given in Table 1.

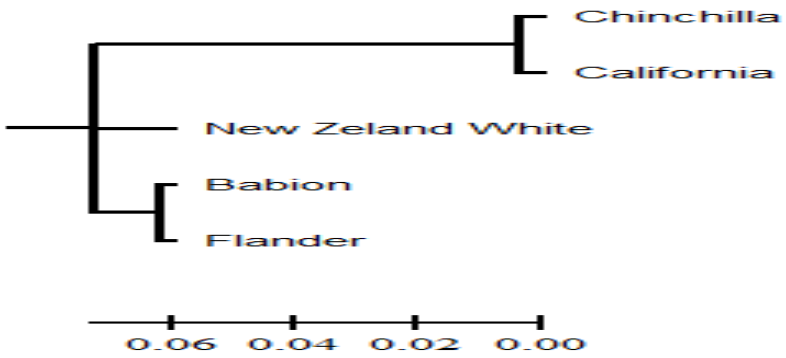

Figure 1. Phylogenetic tree developed by NJ-cluster analysis among the rabbit breeds based on gene sequence data.

Table 1. Genetic similarities (%) and distances among the studied breeds of rabbit

	Chinchilla	California	Babion	Flander
Chinchilla	100			
California	99.24	100		
Babion	89.45	90.45	100	
Flander	89.84	90.84	99.53	**100**
New Zealand White	91.03	92.02	97.02	97.5

Although C and NZW breeds have been existing in the same ecosystem for long time, they have 92% similarity to each other. The same similarity percentage (97%) was observed between C and both of B, and F which was close to that of NZW with (97.5%). However, NZW made the highest contribution to the intra-breed diversity between all analyzed breeds. These results indicated that NZW breed lowers the genetic diversity and demonstrated that quantifying the contribution to the intra-breed diversity is important for assessing conservation priorities. Identified breeds that should be given high priority for conservation purposes in terms of genetic diversity based on sequence analysis were F and B breeds.

Moreover, five single nucleotide polymorphisms have been detected (SNP: A>T, and T>A) for B and F located at 185, and 508 bp, C>A and G>C for Ch located at 268, and 919 bp, A>T for B located at 267 bp, and four SNPs between C and NZW located at 880, 886, 899, and 900 bp (Figure 2).

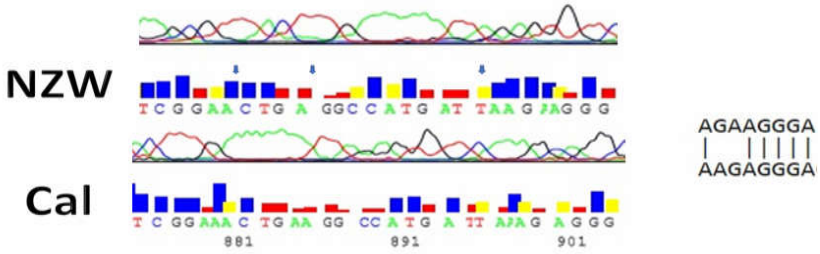

Figure 2. Single nucleotide polymorphism (SNP) detected between New Zealand white, and California breeds (the arrow shows the SNPs locations).

In spite of the fact that these SNPs represent an enormous resource for gigantic examinations, variety in recombination frequencies in the genome makes it hard to anticipate what the real hereditary separation is for firmly divided loci. The SNPs found in this study may be in a similar pattern of Carneiro *et al*.

(2014) who found that the SNPs with checked allele frequency are in contrast in wild and residential rabbits and were enhanced for conserved noncoding sites. Furthermore, the highly density segment (CCTCCCT) has been blasted against all sequence reads using BLASTN software ver. 2.3.1 (http://www.ncbi.nlm.nih.gov/blast) showing no evidence of similarity or association between them. Accurate high-density linkage maps proved to be the backbone for linkage studies (Daw *et al.,* 2000; Fingerlin *et al.,* 2006) and enhanced high-density linkage maps as of late ended up accessible in mouse (Shifman *et al.,* 2006), and human (Matise *et al.,*2007). These high-determination maps demonstrated a solid association between recombination problem areas and high density of the 7-nucleotide oligomer "CCTCCCT" either in mouse (Shifman *et al.,* 2006) or human (Myers *et al.,* 2005). Needless, to report that more advanced analysis should be done on both laboratory and bioinformatics analysis levels to get more information and add extra discovered SNPs to the rabbit's maps, also, Fabuel *et al.* (2004); and Ruane (2000) emphasized that the conservation decisions for animal genetic resources should take into account not only molecular marker-based genetic diversity but also other factors such as specific traits, productive performance, and future economic interest.

Maximum Likelihood Estimate (ML) of Transition/Transversion Bias:

The estimated Transition/Transversion (ti/tv) bias (*R*) was 0.67, estimated of substitution pattern and rates are shown in Table 2. The nucleotide frequencies were A = 22.12%, T/U = 21.13%, C = 26.72%, and G = 29.99%. For estimating ML values, the tree topology was automatically computed resulted in a maximum Log likelihood of -2111.414. Practically, the genomic DNA sequences transitions were occurred at higher frequencies than transversions (Curtis and Clegg 1984; Wakeley 1994, 1996). Thus, the analytic ratio of ti/tv is useful in a set of SNP calls, this ratio is often evaluated separately for both previously discovered and novel SNPs. Moreover, it is not clear which aspects of the evolutionary process and/or analysis that have caused the observed patterns. These results are corresponded to Yang and Yoder, (1998) who reported an expected over correction result of multiple transitions at the same site using the maximum-likelihood method at a low sequence divergence that may have significant implications. Moreover, Wakeley (1996) found a huge variance in confirmed ti/tv at lower divergence levels when examined a limited number of sites for sequences with a high transition bias. Therefore, this underlines the need for more consideration of the multiple factors that affect sequence evolution, therefore more examinations are needed using divergent breeds with a numerous sequence reads.

Table 2. Maximum Likelihood estimate of substitution matrix

	A	T/U	C	G
A	-	6.23	7.89	10.52
T/U	6.51	-	12.75	8.83
C	6.51	10.05	-	8.83
G	7.76	6.23	7.89	-

Each entry presents the probability of substitution (*r*) from one base (column) to another base (row). Tamura and Nei model (1993) was used to estimate substitution outline. Wherever, the rates of different transversionsal substitutions are shown in *italics*, and those of transitional substitutions are shown in **bold**. Relative values of instantaneous *r* should be considered when evaluating them.

CONCLUSION

In need to formal conservation plan exists in Egypt for such resources since many have been lost or now face extinction. Besides, policies to protect the local species will require more scientific contributions on the genetic structure in its local dissemination extend. The assumption is that exotic breeds are better. On the other hand, this is not generally the case.

REFERENCES

Abdel-Kafy, EM., SS.Ahmed, A .El-keredy, NI. Ali, S.Ramadan and A. Farid, 2018. Genetic and phenotypic characterization of the native rabbits in Middle Egypt, Veterinary World, 11(8): 1120-1126.

Aerts, J., S.Crooijssen, K.Hemmatian, T. Veenedaal, A. Jaader, J. van der Poel, V.Fillon, A.Vignal and M.Groenen, 2003. Intergration of chicken genomic resources to enable whole-genome sequencing. Cytogenet. Genome Res. 102,297-303.

Badr, O.A.M., I.I.S. El-Shawaf, M.H.A. Khalil, M.H. Refaat and S.I.A. (2019.) Ramadan *et al*. Molecular genetic diversity and conservation priorities of Egyptian rabbit breeds. World Rabbit Science, [S.l.], v. 27, n. 3, p. 135-141.

Carneiro, M., C-J.Rubin, F.DiPalma, Albert and J.Alföldi, 2014. Rabbit genome analysis reveals a polygenic basis for phenotypic change during domestication. Science: 1074-1079

Chantry-Darmon, C., C.Urien, H.De Rochambeau,

D.Allain, B.Pena and H.Hayes, 2006. A first-generation microsatellite-based integrated genetic and cytogenetic map for the European rabbit (Oryctolagus cuniculus) and localization of angora and albino. Animal Genetics 37:309-431.

Curtis, SE., MT. Clegg, 1984. Molecular evolution of chloroplast DNA sequences. Mol Biol Evol 1:291–301

Daw, E.W., E.A. Thompson and E.M. Wijsman, 2000. Bias in multipoint linkage analysis arising from mapmisspecification. Genet. Epidemiol.19: 366–380.

El-Aksher, S.H., H.S. Sherif, M.H.Khalil, H.A.S.El-Garhy and S.Ramadan, 2016. Comparative genetic analysis among Moshtohor line rabbits and their parental lines using microsatellite markers. 3rd International Conference on Biotechnology Applications in Agriculture (ICBAA), Benha University, Moshtohor and Sharm ElSheikh, 5-9 April 2016, Egypt. P.7-24

Emam, A.M., A.A.A.Azoz, G.M.K.Mehaisen, N.Ferrand and N.A. Ahmed, 2017. Diversity assessment among native middle egypt rabbit populations in north upper-egypt province by microsatellite polymorphism. World Rabbit Sci. 25: 9-16

Fabuel, E., C.Barragán, L.Silió, M.C.Rodríguez and M.A. Toro, 2004. Analysis of genetic diversity and conservation priorities in Iberian pigs based on microsatellite markers. Heredity 93:104– 113.

Fingerlin, T.E., G.R.Abecasis and M.Boehnke, 2006. Using sex-averaged genetic maps in multipoint linkage analysis when identity-by-descent status is incompletely known. Genet. Epidemiol. 30: 384–396.

Kersten, S., G.H.Goossens, J.Hoeks, S.van der Krieken, D.Ryu, K.E.EstherMoonen and M.K.C.Hesselink , 2011. Calorie Restriction-like Effects of 30 Days of Resveratrol Supplementation on Energy Metabolism and Metabolic Profile in Obese Humans. Cell Metabolism, Volume 14.

Korstanje, R., 2000. Development of a genetic and comparative map of the rabbit.A toolfor QTLmapping (PhD dissertation). Utrecht, The Netherlands: Utrecht University.

Korstanje, R., G.F.Gillissen, M.G.den Bieman, S.A.Versteeg, B.vanOost, R.R.Fox, H.A.van Lith and L.F. van Zutphen (2001). Mapping of rabbit chromosome 1 markers generated from a microsatellite-enriched chromosome-specific library. Animal Genetics 32, 308–12.

Korstanje, R., G.F.Gillissen, S.A.Versteeg, B.A.van Oost, A.A. Bosma, C.Rogel-Gaillard, L.F.van Zutphen and H.A.Van Lith, 2003. Mapping of rabbit microsatellite markers using chromosomespecific libraries. Journal of Heredity 94, 161-9.

Kumar, S., G.Stecher, M.Li, C.Knyaz, and K.Tamura, 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

Lee, W.L., J.R.Oberle. and J. A. Cooper, 2003. The role of the lissencephaly protein Pac1 during nuclear migration in budding yeast. J Cell Biol 160(3):355-64.

Matise, T.C., F.Chen, W.Chen, F.M.De La Vega, M.Hansen, C.He, F.C.L.Hyland, G.C. Kennedy, X.Kong and S.S.Murray, 2007. A second-generation combined linkage physical map of the human genome. Genome Res. 17: 1783–1786.

Myers,S., L.Bottolo, C.Freeman, G.McVean and P.Donnelly, 2005.A fine-scale map of recombination rates and hotspots across the human genome. Science 310: 321–324.

Rabie, T.S.K.M ,2012. Inter- and intra- Breeds Divergence in Rabbits using of SDS-PAGE for liver Protein. 3rd Medetranian poultry summit and 6th International conference for poultry sciences, Book of Proceedings, 1752-1763.

Ruane, J., 2000. A framework for prioritizing domestic animal breeds for conservation purposes at the national level: A Norwegian case study. Conserv. Biol., 14:1385–1393.

Saitou, N and M. Nei,1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

Shifman, S., J.T.Bell, R.R.Copley, M.S.Taylor, R.W.Williams, R.Mott and J.Flint, 2006. A high-resolution single nucleotide polymorphism genetic map of the mouse genome. PLoS Biol. 4: e395.

Tamura, K and M. Nei,1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

Tamura, K., M.Nei, and S.Kumar, 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

Tian-Wen, W., X.Gui-Jiang, P.Yu-Lai, X.Xi-Ping, L.Bi-Chun and W. Xin-Sheng, 2010. Study on genetic diversity of 7 rabbit populations evidenced by microsatellite makers. Journal of Animal and Veterinary Advances, 9:359-365.

Wakeley. J., 1994. Substitution rate variation among sites and the estimation of transition bias. Mol Biol Evol 11:436–442

Wakeley, J., 1996. The excess of transitions among nucleotide substitutions: New methods of estimating transition bias underscore its significance. TREE 11:158–163

Yang, Z., and A.D. Yoder, 1998. Estimation of the Transition/Transversion Rate Bias and Species Sampling. J Mol Evol (1999) 48:274–283

Zenger, K.R., B.J. Richardson and A-M. Vachot-

Griffin, 2003. A rapid population expansion retains genetic diversity within European rabbits in Australia. Molecular Ecology 12: 789-794

التحليل المقارن لمخطط التنوع التسلسلي لخمس سلالات من الأرانب في مصر باستخدام النوكليوتيدات المفردة متعددة الأشكال

طارق السعيد ربيع

قسم الانتاج الحيوانى، كلية الزراعة، جامعة قناة السويس، الإسماعيلية ٤١٥٢٢ـ جمهورية مصر العربية

تم اختيار الجينات المشفرة والمشار إليها بـ 18S rDNA كعلامة أو مميز عالميا. تم استخدام بيانات تسلسل النيوكليوتيدات الخاصة بالـ 18S rDNA لتقدير المتغيرات الوراثية على ١٢٨ أرنب ينتمون إلى خمسة سلالات مختلفة في مصر و هم كالتالي، نيوزيلندا الأبيض (NZW ، ن = ٣٥) ، كاليفورنيا (Ca ، ن = ٣٥) ، شينشيلا (C ، ن = ١٩)) ، فلاندر (F ، ن = ١٩) وبابيون (B ، ن = ٢٠). تم جمع عينات الدم من أجل إجراء تحليل النيوكليوتيدات المتسلسل وأجري الرسم البياني للتنوع لكلا من تسلسل النيوكليوتيدات. بالإضافة إلى ذلك ، تم تنفيذ التحليل المقارن وفقًا للكشف عن الـ SNP. و أشارت النتائج إلى أن ١١١٥ قاعدة نيتروجينية مزدوجة من تسلسل الحمض النووي نتجت باستخدام التسلسل الآلي عن طريق جهاز ABI5100.

تم الكشف عن عدم تطابق قطعة صغيرة عشوائية من تسلسل الحمض النووي (٧٤ النيوكليوتيدات ؛ ١-٢٩ ، ١٠٧١-١١١٥). كان التشابه بين سلالات Ca و NZW حوالي ٩٢٪ ، بالإضافة إلى ذلك ، لوحظت نسبة تشابه أعلى بين C وكلا من B ، و F (٩٧.٢ ، ٩٧.٥ على التوالي). تم الكشف عن تسعة SNPs. تم نشر خمسة أشكال متعددة النوكليوتيدات الفردية إلى (SNP: A> T ، و T> A) لـ B و F الموجودة في ١٨٥ ، و ٥٠٨ bp ، وأيضًا C> A و G> C لـ C تقع عند ٢٦٨ ، و ٩١٩ قاعدة نيتروجينية مزدوجة. تم الكشف عن A> T لـ B عند ٢٦٧ قاعدة نيتروجينية مزدوجة. تم اكتشاف أربعة SNPs بين Ca و NZW تقع في ٨٨٠ و ٨٨٦ و ٨٩٩ و ٩٠٠ bp.

تم استخدام أقصى احتمال لتقدير التحوّل الانتقالي / الانتقالي (ti / tv) الذي أدى إلى تحيز مقدّر (R) (ti / tv) كان حوالي ٠.٦٧. تم تقدير نمط الإحلال ومعدلاته ، وكانت ترددات النوكليوتيدات A=٢٢.١٢% ، T / U=٢١.١٣٪ ، C=٢٦.٧٢٪ ، و G =٢٩.٩٩٪. و عليه فقد استنتج من النتائج أن سلالات الأرانب الخمسة المستخدمة تمتلك مخزوناً جيدًا للتنوع الوراثي وهو أمر ضروري للتحسين الوراثي. هناك حاجة إلى خطة رسمية للحفظ ويجب تنفيذها في مصر لتحقيق هذا التحسن الوراثي المناسب.