IJBPAS, June, 2014, 3(6): 862-871

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MOLECULAR ANALYSIS OF WEST AZERBAIJAN NATIVE CHICKEN POPULATION BASED ON HVR-I REGION OF MITOCHONDRIAL DNA

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ABSTRACT

Native chickens of importance in the economy of rural households are also important as genetic reserves that account for the reserving genetic diversity in native chicken breeds of Iran because of the little population size is necessary for breeding goals and increasing their production. The first step is determination of genetic diversity in existing populations. Among the genetic markers, mtDNA sequencing is one of the most useful and common methods employed for inferring phylogenetic relationship between close related species and population and conservation of species. The object this study was carried out for determination of the mitochondrial HVR-I sequence in west Azerbaijan native chicken in Iran. For this study blood samples were taken randomly from 20 chickens. After extracting DNA, HVR-I region of mtDNA was amplified with specific primers using PCR and after purification was sequenced. According to the results, it can be concluded that west Azerbaijan native breed has some genetic similarities with other chicken breeds.

Keywords: mtDNA, DNA Sequencing, HVR-I, West Azerbaijan Native Chicken, Phylogeny

INTRODUCTION

Chickens are classified as order: Galliformes, family: Phasianidae and genus: Gallus (jungle fowl). Domestication resulted in basic changes in the behaviour, physiology and production of the bird, but still there are some similarities between the ancestor and the current chickens **[1]**. It is widely believed that all populations of domesticated chicken

descend from a single ancestor, the red jungle fowl (*Gallus gallus gallus*), which originated in Southeast Asia **[2-3]**. Native chicken breeds are national investment and conservation of these populations is very important from biodiversity aspects **[4]**. With the burgeoning global population, techniques for the identification of highly productive food sources are needed. Many native livestock breeds, which have less productivity and are mostly found in developing countries, have diminished in number or become extinct **[5]**. In the past, attempts had been made to characterize local chickens using morphological traits (such as plumage colour, feathering pattern, etc.) which have limited utility in the study of genetic variation. The use of DNA technology by PCR-RFLP technique on D-loop mitochondrial part has also been done on Kampung chicken from some locations to base the selection program **[6]**. Studying mitochondrial genome in one breed and comparing it with other breeds can give useful information about genetic diversity in that population. Reserving genetic diversity in native chicken breeds of Iran because of little population size is necessary for breeding goals and increasing their production. The first step is determination of genetic diversity in existing populations **[4]**. The mitochondrial genome is maternally inherited and the sequences of mitochondrial DNA (mtDNA) have been extensively used in biodiversity studies of vertebrates including chickens and domestic animals **[7-12**]. Nevertheless, the genetic potential in almost all chicken breeds has not yet been much revealed. Based on previous literatures, in this study, molecular analysis of west Azerbaijan native chicken population based on HVR-I region of mitochondrial DNA were investigated to develop molecular markers for breed identification.

MATERIAL And METHODS

Animal and Blood Samples

West Azerbaijan native poultry lines which their reproductive characteristics studied for 12 generation were used in this study (Tala Tabeh, west Azerbaijan provnce, Iran). Blood samples (2ml in EDTA containing tubes) randomly collected from 20 birds via wing vain using disposable syringes in all birds and stored at -20 C° until used at hematology laboratory.

Establishment of a PCR-RFLP Assay

The PCR primers for the chicken were used in the forward between 16756-16776 bases and reverse between 845-865 bases (forward: 5'-TTGTTCTCAACTACGGGAACA-3'; reverse: 5'-CAAAGTGCATCAGTGTCAAGAT-3') using Primer premier software. DNA

amplification of each individual bird was performed according to the following conditions: the PCR was performed in a total volume of 25 μL, containing 2 μL of genomic DNA, 10 pmol of each oligonucleotide primer, 2 μL 25 mM MgCl2, 2 μL of 1 mM deoxynucleotide triphosphate mixture, and 1 U of Taq DNA polymerase; cycle parameters were 94 °C for 8 min then 35 cycles of 95 °C for 30 sec, 64 °C for 30 sec, and 72 °C for 5 min, with a final extension step for 2.07 min at 72 °C; the PCR products with length 776 bp were digested at 37 °C overnight with 10 U of Hinf I. Then the DNA primers electrophoresis on 1% agarose gel and NucleoSpin Extract II kit (Macherey-Nagel MN, Germany) used to purification DNA from agarose gel TAE/TBE.

Analysis of Primers

Obtained sequences analyzed using Chromas Lite 2.01 software. To investigate highest homology of west Azerbaijan native poultry lines, Blast procedure from NCBI was used. ClastalW software used to compare the primers.

RESULTS AND DISCUSSION

The genomic DNA fragment using PCR-RFLP method on 1% agarose gel is shown in **Figure 1**. This figure implies the accuracy of DNA fragment extraction west Azerbaijan native chicken.

Proteins are the most abundant contaminators in RNA and DNA samples which absorb 260 and 280 nm rays. Also, phenolic and alcoholic remnants in biological samples of DNA and RNA absorb 230 nm rays. Thus, by calculating DNA sample in 230, 260 and 280 nm rays and their ration on 260/280 and 260/230 the purity of the nucleoid acid will identify. Ideally, A260/A280 ratio in nucleoid acids to proteins in sample should be 1.8 to 2. In this study the quantity and quality of the DNA investigated by Nano-drop spectrophotometer (MD-1000). As seen in figure 2, our results indicated there is no phenolic and alcoholic substance in samples.

The results of electrophoresis of PCR in 776 bp on 1.5% agarose gel is presented in **Figure 3**.

In this study the sequencing applied in all 20 samples. All sequences have done by 3 different types (ab1, pdf, seq). The sequences read by Chromas Lite software using ab1 format. As seen in the **Table 4**, the sequencing was done in proper places in the sample.

All samples align using ClustalW procedure using BioEdit software. According to the data, there was a 99 percent overlap between the samples **(Figure 5)**.

Efforts to understand genetic diversity in commercial and non-commercial chickens

have also involved characterization of variation in the mitochondrial genome. Though relatively few, the mitochondrial DNA (mtDNA) based studies have also provided insight into the maternal origins of chickens **[7, 13, 14]**. As seen in **Table 1**, there was a nucleotide differences on locus in samples. There was 5 haplotype in 20 samples which 4 of them was on SNPs locus. All 4 poly morph locus was because of intra conformation purine and pyrimidine bases. Three of the poly morph locus of this study was similar to in Indonesian indigenous chickens **[6]** and pacific chickens **[15].** This result indicates that there is a common ancestor between the west Azerbaijan native chicken and other birds.

This result indicates that there is a common ancestor between the west Azerbaijan native chicken and other birds. There is little doubt that successive domestication of various wild animals contributed greatly to the sustenance and cultural development of mankind. It involves adaptation of animals to environmental condition, therefore some changes in behaviour and physiology of the animal would be expected **[16].** As reported, these behavioral physiological changes associated with domestication is a must, however, these changes vary according to type of domestication whether it is toward meat or egg production. Archaeological discoveries in China indicate that chickens had been domesticated by 5400 B.C. and chickens from Harappan culture of the Indus Valley (2500-2100 B.C) may have been the main source for diffusion through the world **[17, 18].** Birds were first domesticated for cultura and entertainment purposes, until much later birds were utilized as a source for human food **[18].** This study has proved that mtDNA and more specifically D-loop HV1 segment is a powerful molecular tool in resolving phylogenetic relationships within a species and also understanding the genetic diversity.

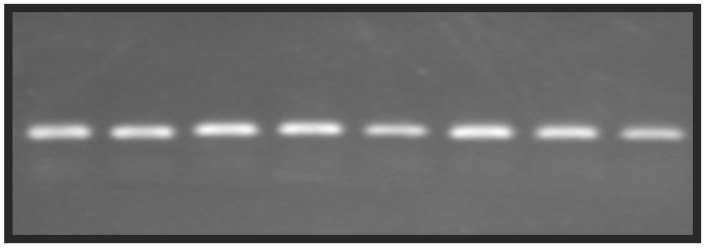


Figure 1: The Genomic DNA Fragment Amplified Using PCR-RFLP Method on 1% Agarose Gel in West Azerbaijan Native Chicken

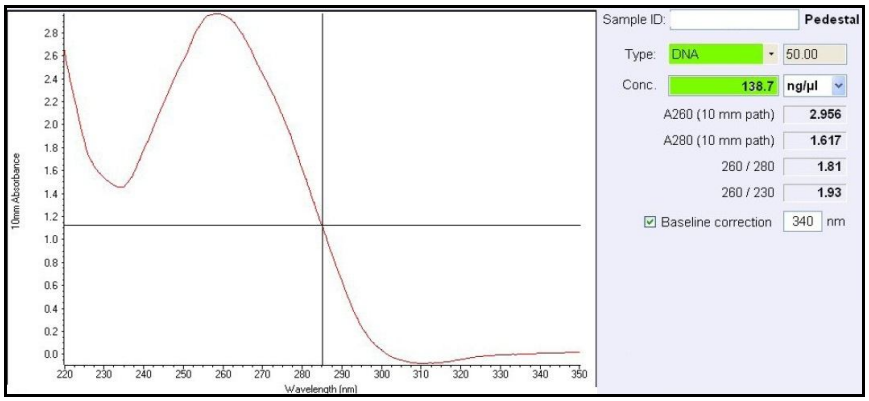


Figure 2: The Nano-Drop Output of the Spectrophotometry for Samples in West Azerbaijan Native Chicken

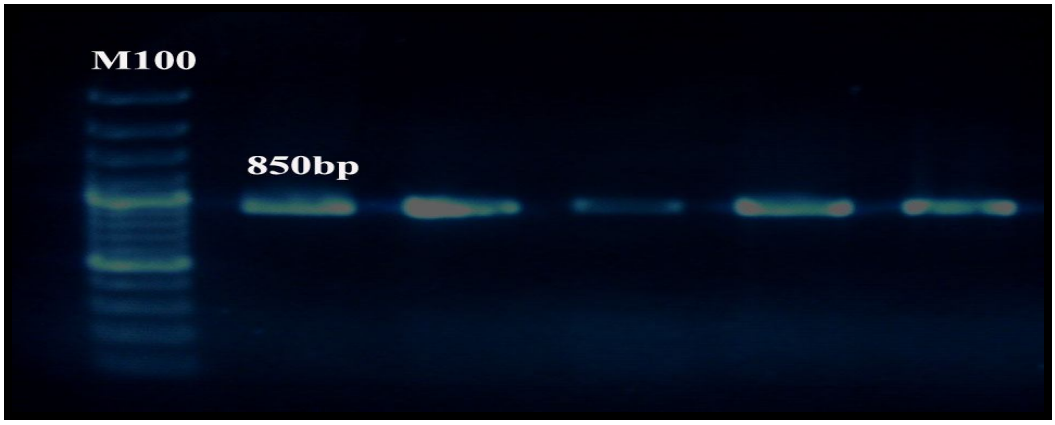


Figure 3: The Electrophoresis of PCR in 850 bp on 1.5% Agarose Gel in West Azerbaijan Native Chicken

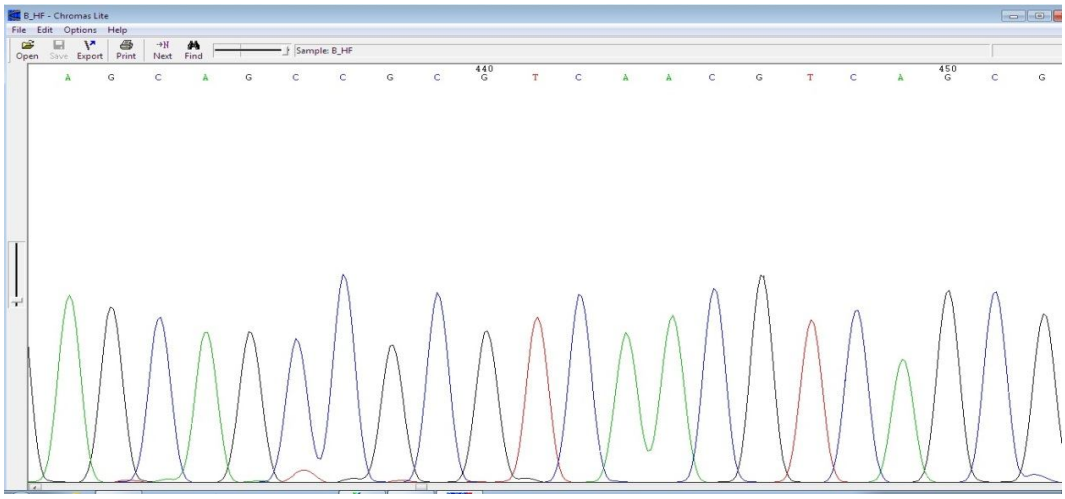
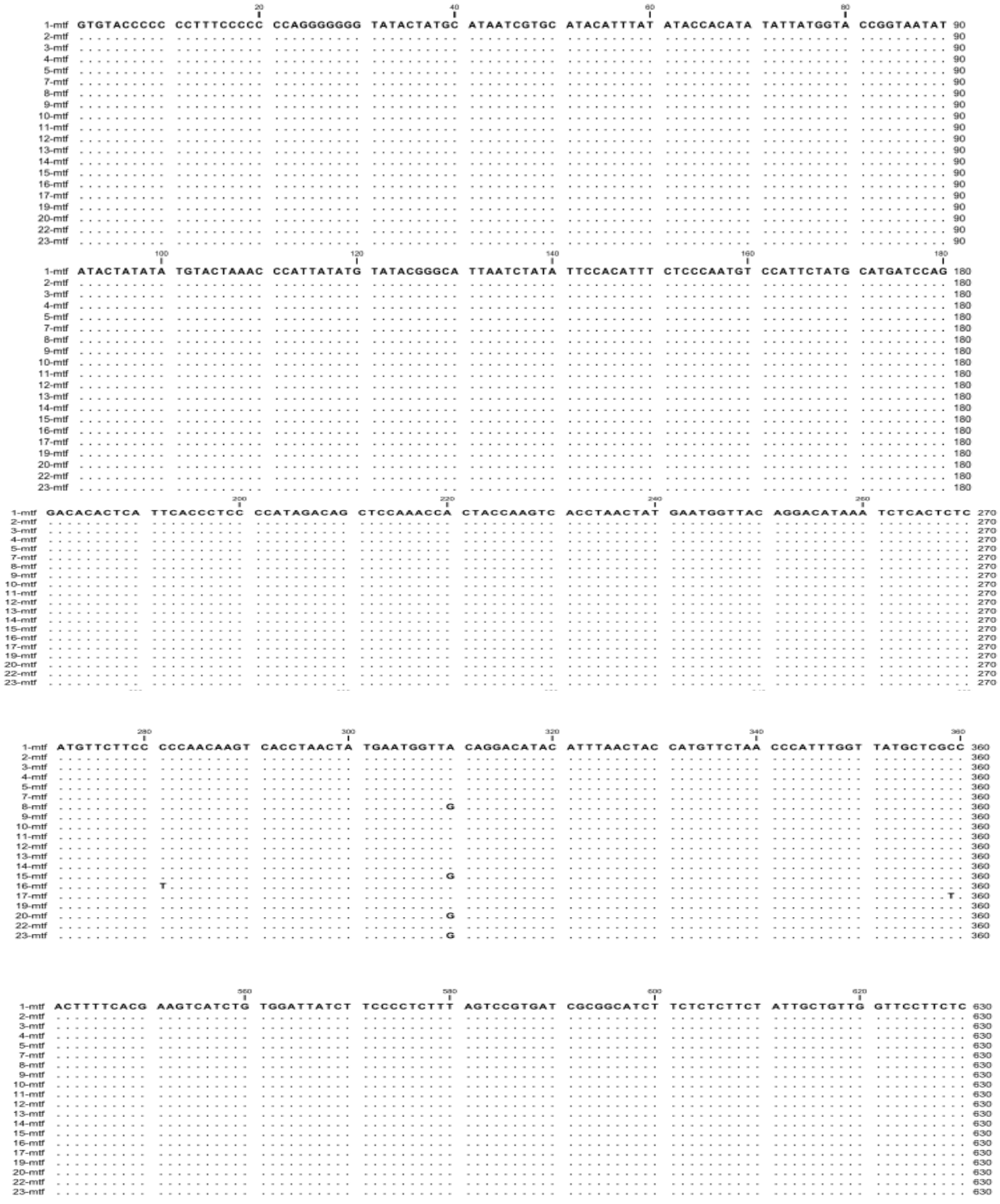


Figure 4: The accuracy of the Primers on the Samples in West Azerbaijan Native Chicken


1-mtf GTGTACCCCC CCTTTCCCCC CCAGGGGGGG TATACTATGC ATAATCGTGC ATACATTTAT ATACCACATA TATTATGGTA CCGGTAATAT 90
ATACTATATA TGTACTAAAC CCATTATATG TATACGGGCA TTAATCTATA TTCCACATTT CTCCCAATGT CCATTCTATG CATGATCCAG 180
GACACACTCA TTCACCCTCC CCATAGACAG CTCCAAACCA CTACCAAGTC ACCTAACTAT GAATGGTTAC AGGACATAAA TCTCACTCTC 270
ATGTTCTTCC CCCAACAAGT CACCTAACTA TGAATGGTTA CAGGACATAC ATTTAACTAC CATGTTCTAA CCCATTTGGT TATGCTCGCC 360
ACTTTTCACG AAGTCATCTG TGGATTATCT TCCCCTCTTT AGTCCGTGAT CGCGGCATCT TCTCTCTTCT ATTGCTGTTG GTTCCTTCTC 630

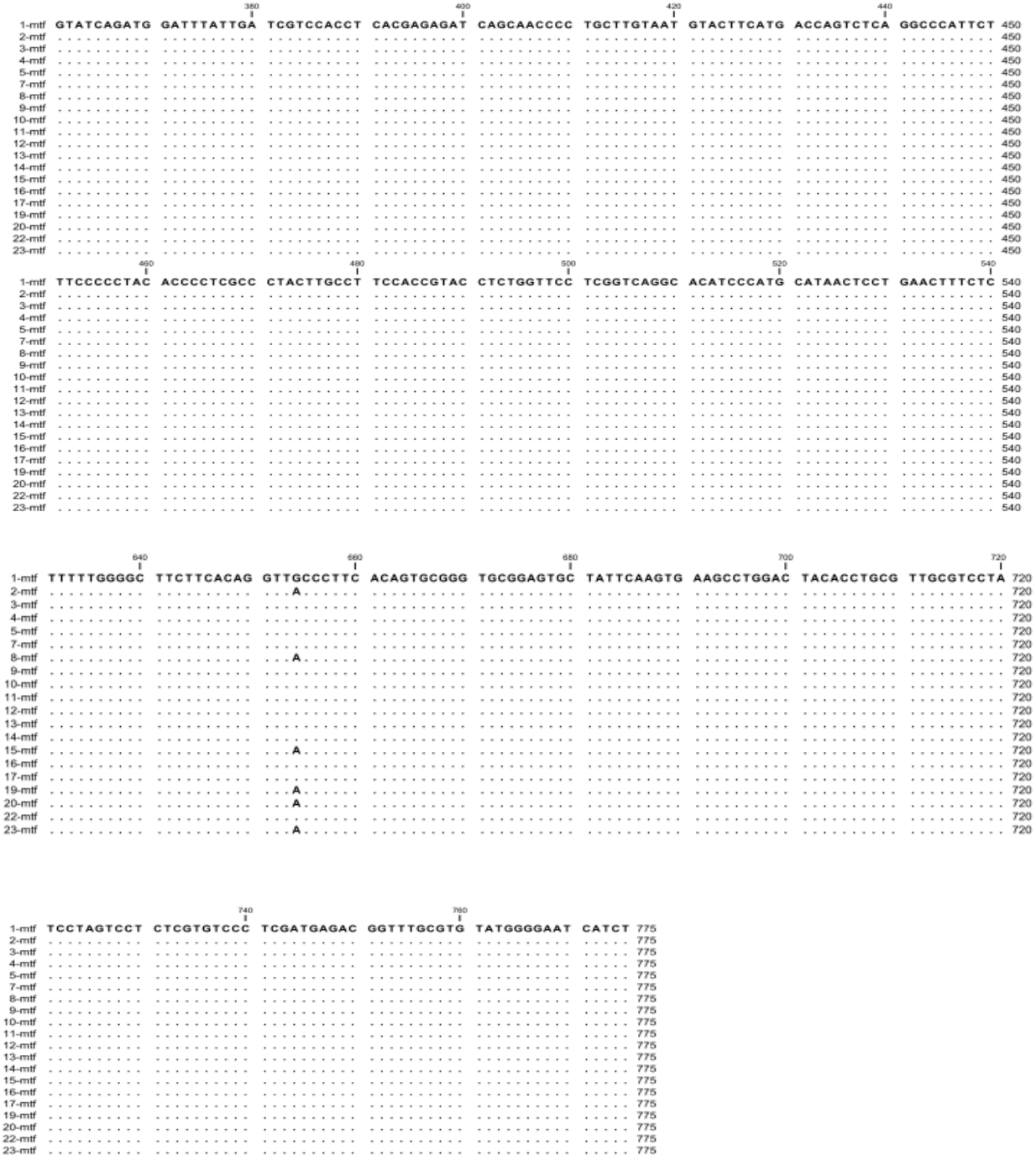


Figure 5: The BioEdit software Output Using ClustalW Procedure in West Azerbaijan Native Chicken

Table 1*:* Results of Samples Align in West Azerbaijan Native Chicken

SAMPLE	Haplotype	*281*	*310*	*359*	*654*
SAMPLE1	**A**	**C**	**A**	**C**	**G**
SAMPLE2	**D**	**C**	**A**	**C**	**A**
SAMPLE3	**A**	**C**	**A**	**C**	**G**
SAMPLE4	**A**	**C**	**A**	**C**	**G**
SAMPLE5	**A**	**C**	**A**	**C**	**G**

SAMPLE7	**A**	**C**	**A**	**C**	**G**
SAMPLE8	**E**	**C**	**G**	**C**	**A**
SAMPLE9	**A**	**C**	**A**	**C**	**G**
SAMPLE10	**A**	**C**	**A**	**C**	**G**
SAMPLE11	**A**	**C**	**A**	**C**	**G**
SAMPLE12	**A**	**C**	**A**	**C**	**G**
SAMPLE13	**A**	**C**	**A**	**C**	**G**
SAMPLE14	**A**	**C**	**A**	**C**	**G**
SAMPLE15	**E**	**C**	**G**	**C**	**A**
SAMPLE16	**B**	**T**	**A**	**C**	**G**
SAMPLE17	**C**	**C**	**A**	**C**	**G**
SAMPLE19	**D**	**C**	**A**	**T**	**G**
SAMPLE20	**E**	**C**	**G**	**C**	**A**
SAMPLE22	**A**	**C**	**A**	**C**	**G**
SAMPLE23	**E**	**C**	**G**	**C**	**A**

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