# Genetic variation analysis of cytb gene of cattle bred in Iraq

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**Abstract**

The present study was conducted at laboratory of Genetic Engineering at the University of Basrah from 01/04/2017 to 01/08/2018. The aim of study was identify genetic variations and SNPs in mtDNAcytb; among Holstein, Local Iraqi cattle and their crosses. The primer used in this study amplified 1139-bp fragments from cytb gene. The results showed the presence of 3, 12 and 2 polymorphic sites leading to the construction of 3different haplotypes for Holstein, local and crosses respectively. Haplotype and nucleotide diversity were (0.275; 0.00047), (0.417, 0.00068), and (0.733, 0.00686) respectively. Neighbor-joining trees were constructed using 34 samples showed that all studied cattle appeared into haplotype 1(H1), while Holstein also appeared in H5 and H6 (with different two or one bases respectively). Local breed included in H2 and H3 (with one different base), the crosses in H4(with 11 different bases). AMOVA showed that variation within breed (between individuals was higher (65.35%) than between breeds (34.65%). Neutrality test both Tajima's D and Fu's Fs revealed that Holstein recorded the highest negative values (-1.67053 and -0.24213 respectively). Whereas, the crosses cattle recorded positive values (1.31709 and 6.05664 respectively). The local breed showed positive and negative values near to zero (-0.93613 and 0.01635 respectively). It can be concluded from these results that Iraqi local breeds showed high genetic variability and totally differ from other breeds in the world.

Keywords: Cattle, ctyb, MtDNA, haplotype diversity, nucleotide diversity, AMOVA.

## Introduction

Mitochondrial DNA (mtDNA) is a powerful tool that can be used to determine evolutionary relationships, population composition and biology of many species due to its low molecular weight properties, simple structure, low recombination rate and rapid evolution rate (Curole and Kocher, 1999; Wong*et al*., 2004; Arif and Khan, 2009; Patwardhan et al., 2014; Hussain *et al*., 2015).

The cytochrome b (Cytb) gene is 1139 base pairs and is located between nucleotide pairs 14156 and 15295(NCBI, 2007). An encoded mitochondrial gene (Cardoso et al., 2008). This gene is involved in the transmission chain of the electron in the respiratory tract of the mitochondria (Hsieh et al., 2001; Linacre and Tobe, 2011).It is used to translate protein across membranes to synthesize ATP and various cellular processes (Chen et al., 2009).It is one of the world's

standard genes for comparing evolution rates (Avise, 2004). And in the studies of evolution and development and inheritance of breeds and the classification of different species(Johns and Avise, 1998;Li et al., 2005; Zhong et al., 2014). Comparative studies based on Ctyb lead to the recognition of new species and breeds, as well as help in understanding evolutionary relationships due to the change in Cytbsequences(Castresana, 2001). Mitochondrial geneshave been used to study genetic variation in many agricultural animals such as sheep (Othman et al., 2018; Ayied and Zqeer, 2018), goats (Amer, 2014),cattle (Tety et al., 2015) and camel (Ayied et al, 2018).

The objectives of the present study were to estimate genetic variation caused by Ctyb among different breeds of cattle in Iraq.

**Materials and Methods**

The study was conducted for the period from 01/04/2017 to 01/08/2018, at the laboratory of Genetic Engineering at the University of Basrah, following with the collection of data from the field up to 15/03/2018. The study included the use of 40 cows (20 Holstein, 10 local and 10 crosses). The blood samples (5ml/cow) from the jugular vein were collected. Milk chemical contents included fat%, protein%, lactose% and solid not fat (SNF%) were estimated by Lactoflash produced by Funke Gerber, Germany. Samples of milk (50 ml) were collected every 15 days throughout the experiment period.

The analyses were carried out on 40cows. Blood from each cow was sampled intravitally into sterile vacuum tubes containing $K_2$EDTA (dipotassium ethylene diamine tetra acetic acid) anticoagulant. A fragment (1250bp) of the mtDNACtyb in the reference cattle mitochondrial genome by using the primer F-CTCCATCAACAAGCCAGTA وR-TGTGTAGTAGGGGGATTAGAGCA(Vakalounakis and Fragkiadakis, 1999).

The PCR amplifications were conducted in a 50 µl volume containing 20 ng genomic DNA, 25 µl of Master Mix, 2 µl each primer, 15 µl free water. The amplification conditions were as follows: initial denaturation at 94 C for 2 min followed by 35 cycles of denaturation at 94 C for 0.5 min, annealing at 58 C for 0.5min, and extension at 72 C for 0.5 min, and then the final extension at 72 C for 10 min. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide to test the amplification success. The amplified products were purified with a DNA purification kit (SSufine) according to the manufacturer's instructions to remove residual primers and dNTPs. Sequencing was performed in sync ™ DNA Extraction Kit was used for DNA extraction and manufactured by the Taiwanese Geneaid company.

**Data Analysis**

ATPase8/6 sequences were aligned using the BioEdit software (Hall, 1999). Haplotype diversity (HD) and nucleotide diversity ($\pi$) were analyzed using DnaSP v5. 10 software (Librado and Rozas, 2009). Genetic distance, molecular variation (AMOVA) and neutrality test were analyzed using Arlequin 3.5.1.2 software (Excoffier and Lischer, 2010). The haplotypes network was drawn using Network 5.0.0.0 software (Bandelt et al., 1999). Neighbor-joining (NJ) tree for testing cattle breed sequences and the phylogenetic tree the three genotypes (Holstein, Local and crosses) were constructed using Megaversion 7.0 software (Kumar *et al*, 2016).

**Statistical analysis**

A complete randomized design (CRD) was used to analyze the production data using the SPSS (2016) statistical program Version 24 and compared the means using the General Linear Model within the program. The model included two factors, the first was the breed (Holstein, local and their crosses) and the second was the haplotypes within breed.

**Results and Discussion**

**Genetic diversity**

Number of Ctyb sequences were 32 (table, 1). Ten, sixteen and six of sequences belonged to local cattle, cross and Holstein respectively. Haplotypes number (H) were nine distributed equally (3 each)on local, crosses and Holstein. Local, crosses and Holstein cattle showed a polymorphisms (NH) of 2, 13 and 3 respectively. Crosses revealed the highest value of haplotype diversity (HD) (0.733), followed by the local breed (0.417) and the Holstein cattle (0.275). Cross cattlealso recorded highest nucleotide diversity ($\pi$) followed by local and Holstein cattle (0.00686, 0.00068 and 0.00047 respectively).

**Table (1) Genetic diversity of Ctyb gene among different cattle breeds**

| Breeds | Number of Sequences (N) | Haplotype number (H) | Number of Polymorphisms (NH) | Haplotype diversity (HD) | Nucleotide Diversity ($\pi$) |
|---|---|---|---|---|---|
| Local | 10 | 3 | 2 | 0.417 | 0.00068 |
| Crosses | 16 | 3 | 13 | 0.733 | 0.00686 |
| Holstein | 6 | 3 | 3 | 0.275 | 0.00047 |

**Haplotype network**

A total number of haplotypes of Ctyb gene showed by different breeds were six (fig. 1). The central circle represents Haplotype 1 ($H_1$). Five branches appeared from $H_1$, the first branch was$H_2$ which differed from $H_1$by one base (111) and represent the local cattle only. The other branch represented $H_3$ shown by local breed and differed from $H_1$ by one base (663)also. Whereas, the haplotype $H_4$showed by cross cattle which varied by 11 baes from $H_1$. H5 and $H_6$ represented the Holstein cattle and differed from $H_1$ by 2and 1 bases respectively.

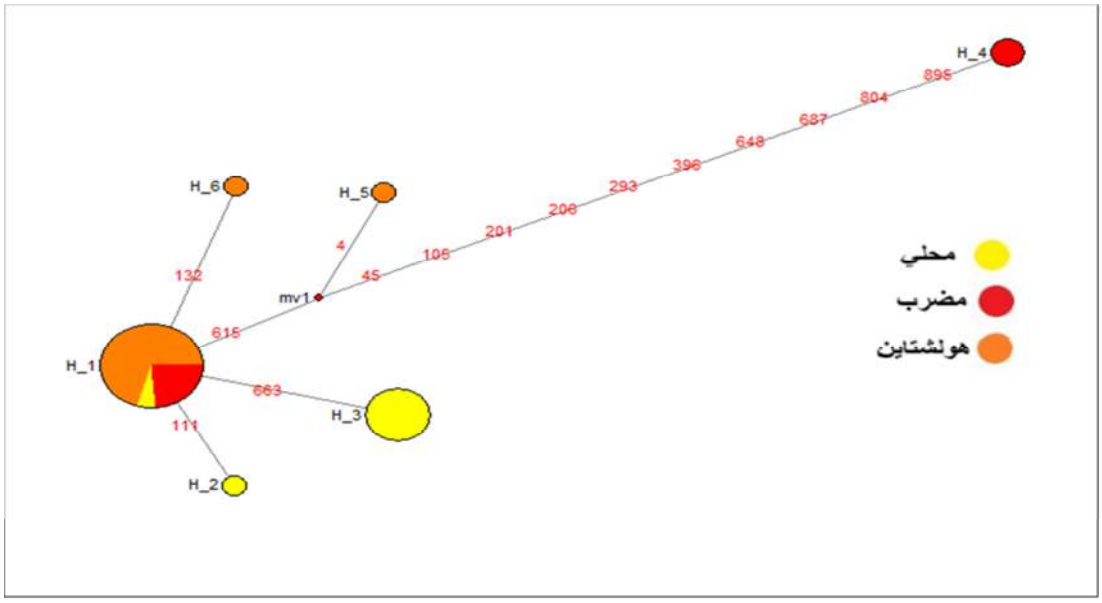


**Fig. (1) Haplotype network of Ctyb gene among studied cattle**

**Genetic Distance**

The genetic distance of the Cytb gene (Table 2) showed that the highest distance was between the local breed and the Holstein breed (0.5287) and the lowest genetic distance between the Holstein and the cross cows (0.1788). The genetic distance between the local cattle breed and the cross was 0.2890.

**Table (2) The genetic distances among studied cattle breeds in Iraq**

| Breeds | Local | Cross |
|---|---|---|
| **Local** | 0.000 | 0.2890 |
| **Holstein** | 0.5287 | 0.1788 |

**Evolutionary tree of Cytb gene between Iraqi cows and cows of the world**

The results of the evolutionary tree compared to some of the countries of Cytb and the cows breached in Iraq (Fig. 2) showed that there were two main branches. The first branch included all cattle breeds of the comparative countries, with the Iraqi bred in Iraq. Iraqi origin is different from the rest of the cows in the countries in comparison to the tree of evolution.

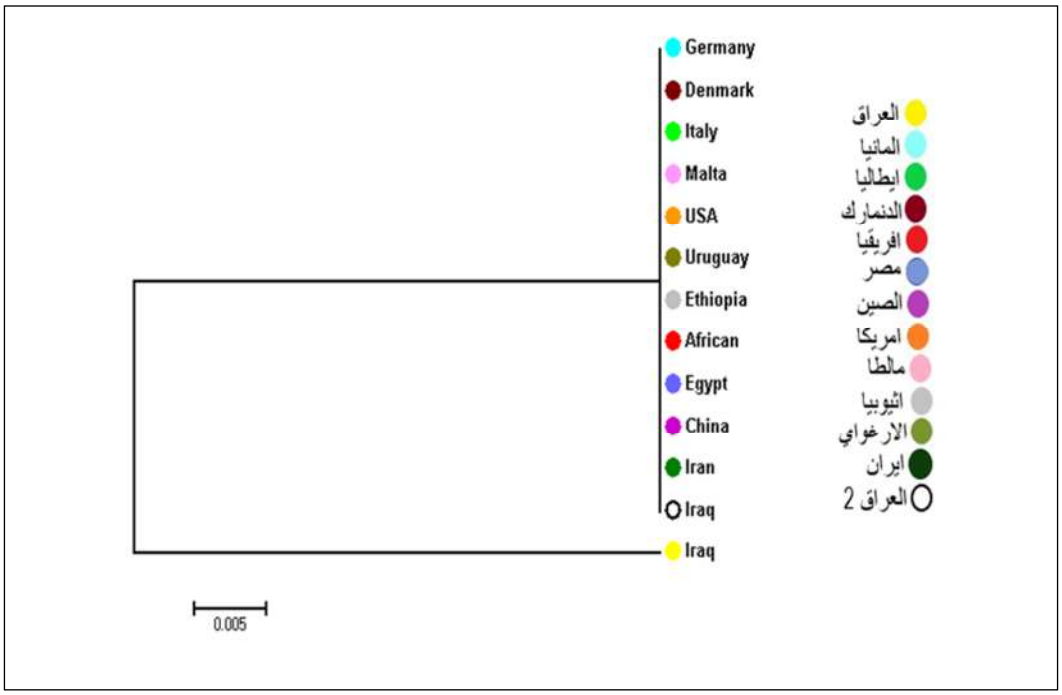


**Fig. 2 Neighboring tree of Cytb between cows in Iraq and some countries**

**Analysis of Molecular Variation (AMOVA)**

AMOVA of Ctyb gene among studied breeds of cattle resulted in between breed variation of 34.65% and within breed variation of 65.35% (table, 3).

**Table (3) Analysis of Molecular Variance (AMOVA) of Ctyb gene for studied breeds**

| Source of Variation | Degree of Freedom | Sum Squares | Variance components | Variation % |
|---|---|---|---|---|
| Between Breeds | 2 | 9.734 | 0.44289 | 34.65 |
| Within Breeds | 31 | 21.714 | 0.83516 | 65.35 |
| Total | 33 | 31.448 | 1.27806 | |

**Neutrality Test**

The results of neutrality test (Tajima's D) of the Ctyb gene (table, 3) showed negative values for local breeds (-0.92613) and Holstein (-1.67053), but it was positive (1.21709) for cross cows.

Those of Fu's Fs were 0.01635, 6.05664 and -0.24213 for local, cross and Holstein cattle respectively.

**Table (3) Neutrality test of Ctyb gene for studied cattle breeds**

| Breeds | Tajima's test (D) | Fu's Fs test |
|---|---|---|
| Local | -0.93613 | 0.01635 |
| Cross | 1.31709 | 6.05664 |
| Holstein | -1.67053 | -0.24213 |

**Discussion**

The results of the present study showed that polymorphism of cross breed reflecting that the source of genetic variation is the crossing processes or migration. These results are in agreement with those of Dadi et al (2009) in Ethiopian cross cattle. However, local breed exhibited low polymorphism which indicated that genetic content of this breed has not changed and the main factor changing the genetic variation is the genetic drift as a result of raising this breed in very small herds (not more than 3 cows/breeder). As well as this breed distributed in remote areas and kept by breeder using very old not scientific method of breeding inherited from their ancestors. These practices elevated inbreeding which increases homozygosity and genotypic fixation. Especially it showed clear reduction in nucleotide diversity of the studied gene and revealed clear genetic differences from both the Holstein and their crosses. Besides crossing local breed with Holstein is not under systematic crossing program with no clear target.

The results of the present study showed that the cross cows had the highest genetic makeup, the haplotypesdiversity and nucleotides diversity of the Cytb gene. This indicates that the source of genetic variance in this group of animals is the process of crossing and migration. These results were in agreement with those of Dadi et al. (2009) in Ethiopian cross cattle. The Iraqi local cattle breed were characterized by the lowest genetic formation of Cytb gene, which shows that the genetic content of this breed has not changed and the main factor in changing genetic variance is genetic drift. This is the result of breeding in very small herds not exceeding three cows per breeders as well as their presence in remote areas and the holder do not have the desire to conduct breeding programs and genetic improvement or modern techniques such as artificial insemination technique, which led to an increase in the inbreeding and this in turn increased the structures and showed a clear reduction in the nucleotide diversity of Cytb gene. Local breedgenetic makeup has moved away from the Holstein breed and the cross cows, especially since the cross cows in the current study may be constructed from an unprogrammedcrossing

local and the Holstein breeds, especially as some of them contained a high percentage of the genotypes of the Holstein blood. Tarekegn et al. (2018)found different genetic parameter of Ctyb diversity, that the average haplotype diversity of Abigar and Sheko breeds were 0.8333 and 0.6282 respectively and nucleotide diversity was 0.7540 and 0.0010 respectively.The local breed was characterized by the nucleotide diversity, which was comparable to the Spanish breed Lidia cattle (Cortés et al., 2008) and higher than other European cattle whose values ranged from 0.0011-0.0057 (Loftus et al., 1994).

Cytb gene is usually used to distinguish genetic variation among animals, in a study using two local Indonesianbreeds, Kebumen and Brahma, which obtained 8 and 6 haplotypes respectively and polymorphism of 36 and 7 respectively (Tarekegn et al., 2018).Cytb is a gene derived from maternal inheritance that is used to detect the origin of cattle. Romaino et al. (2014)found the possibility of distinguishing between local Malaysian cattle and wild animals using this gene as well as other studies in the world such as the Indonesian study (Sutarno and Setyawan2015), who compared the breed of Bally and Zebo cattle.

The present study showed that there were three haplotypes of the studied breeds, which was significantly lower than Chung (2013) in the Korean and Japanese black cattle (14-10 haplotypes respectively. The number of haplotypes may vary due to sample size (Tarekegn et al., 2018)Thepolymorphisms of the two breeds was 29.Cytb gene polymorphismcan be used to predict genetic diversity (Cai et al., 2007).

In conclusion the haplotypecytb network showed that local cattle were differentiated from the other studied breeds and recorded in the NCBI site, the local is of different origin from the rest of the international breeds, especially as Iraq is one of the first centers of domestication.

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