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Original Article

Detection of myostatin gene MSTN in some goat breeds (*Capra hircus*)Y.A. Dowidar^b, M.A. El-Sayed^{a,*}, Aly M. Elrefy^b, Hytham E. Shoura^b^a Animal Genetic Resources Dept., National Gene Bank, Agricultural Research Center, Egypt^b Biotechnology Dept., Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

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ABSTRACT

Till now not information about myostatin MSTN gene in Egyptian goat breeds. Here we show more information about MSTN in some Egyptian goat breeds to enrich the database with new sequences for Egyptian goat breeds. Our conducted study focused on detection and identifying the MSTN gene as a candidate gene of the muscles growth trait in three goat breeds (Zaraibi, Baladi and Damascus). We found the similarity between the registered sequences with the accession numbers KY463684 for Zaraibi and KY463685 for Baladi and Chinese goat breeds of the MSTN gene deposited with international gene banks by up to 99% and some other species including sheep, cows and bull breeds with percentages of 95 to 97% and between 95 to 99%, respectively. There is also a correlation between the sequences of the registered pieces of Baladi with KY463686 and Damascus and Chinese breeds with KY441464 of MSTN deposited with international gene banks by up to 99% and some other species including sheep and bull breeds at a ratio of 99% for two pieces. Results demonstrated the deposited sequences of object are part of intron 1, exon 2 is fully sequenced with Zaraibi and Baladi breeds; the intron 1, exon 1 with Baladi breed; and the intron 2, part of exon 3 with Damascus breed. Therefore, the Egyptian goat breeds consider national wealth can be used to develop breeding and improvement programs which helps in more applicable scopes like biotechnology, genetic engineering and molecular biology with the help of bioinformatics tools.

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1. Introduction

Goat are the first animals that have been domesticated since about 9000 years BC [4]. According to FAO [5] the number of goats around the world about one billion and 6.8 million head are distributed worldwide at rates of 34.1% in Africa, 2.3% in Europe, 58.9% in Asia, 0.4% Oceania and 4.4% in the Americas as shown in Fig. 1. FAO [5] reported that number of goats around the world is about one billion head distributed to 1234 breed, Egypt has nearly 4,185,761 head from goat distributed in all governorates of Egypt spread over seven breeds (Abouramad, Halieb & Shalateen, Baladi, Barki, Black Sinai, Saidi, Wahati and Zaraibi Egyptian goat) according to the Domestic Animal Diversity Information System (DAD-IS). In Egypt, the goat represents the second source for the production of milk after the cattle. El-Sayed et al. [13] showed that the highest genetic distance was found between Damascus and Frafra population while the lowest value between Baladi and Zaraibi goat

breeds. Du et al. [19] constructed whole-genome dense maps of 45,953 SNP markers by using an improved RH mapping pipeline. Moreover, Ni et al. [16] showed that Cas9/gRNAs can induce precise mutations with efficiency of 9–70% in goat primary fibroblasts. A single co-transfection of pooled Cas9/gRNAs enabled isolation of cell colonies carrying simultaneous disruption of four genes with high efficiency. The Cas9/gRNA-modified fibroblasts were subjected to nuclear reprogramming by somatic cell nuclear transfer, resulting in live born goats carrying single gene mutation. Wang et al. [20] discovered ~10 million single nucleotide polymorphisms (SNPs) from eight domesticated goat breeds. They identified 22 genomic regions that may have contributed to the phenotypes in coat color patterns, body size, cashmere traits, as well as high altitude adaptation in goat populations and identified candidate functional SNPs within selected genes that may be important for each trait.

Myostatin gene (MSTN): Mcpherron et al. [3] reported the myostatin sequences of nine other vertebrate species and the identification of mutations in the coding sequence of bovine myostatin in two breeds of double muscled cattle (Belgian Blue and Piedmontese). An et al. [18] investigated polymorphisms of the MSTN gene

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Production share of Goats by region

Average 1994 - 2014

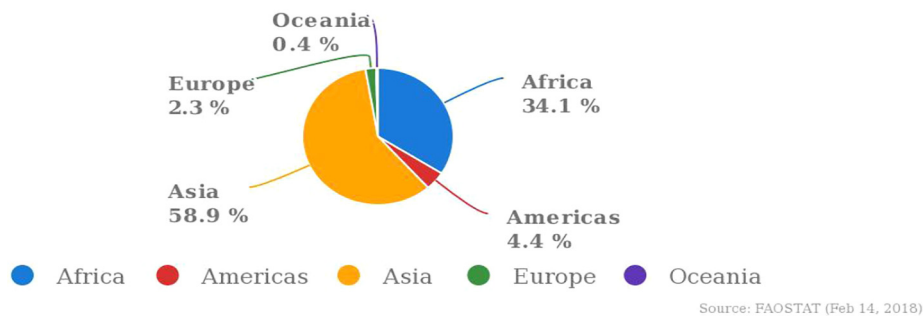


Fig. 1. The number of goats around the world according to FAO [5].

in 664 individuals from four goat populations and applied PCRSSCP and DNA sequencing analysis to reveal two single nucleotide polymorphisms (DQ167575: g.368A > C (p.Lys49Thr) and g.4911C > T. At g.368A > C locus, and suggested that the MSTN gene might play an important role in affecting the growth traits in goats. Zhang et al. [24] used gene sequencing and polymerase chain reaction-single-strand conformation polymorphism methods and identified the polymorphisms of the MSTN gene as a candidate marker for growth in Boer and Anhui white goat breeds. They showed 2 novel single nucleotide polymorphisms: DQ167575 g.197G > A and 345A > T. Singh et al. [15] reported the molecular cloning and characterization of the complete coding sequence of MSTN gene (1128 bp) in seven Indian goat breeds. The multiple sequence alignment revealed more similarity of caprine MSTN sequences to sheep and tahr (98.7–100%) than to its wild relative, *Capra ibex* (98.4–99.7%). Among the Indian goat breeds, MSTN sequence of Barbari showed the most divergence from its ancestral relatives, while Osman-Abadi showed the least. Further, Indian goat breeds were also screened for the presence of 123 T → A substitution, but polymorphism was absent. Tissue specific gene expression studies revealed the presence of MSTN in skeletal muscle. Also, they reported the characterization of the MSTN 5' upstream region and identified some putative regulatory motifs in Indian goat breeds for the first time. They identified the TTTTA deletion in 5' UTR, but they could not establish any association between genotype and growth traits because majority of goats were found homozygous for this deletion. Pothuraju et al. [11] identified the polymorphism in the coding sequence of GDF8 gene across indigenous meat type sheep breeds. A 1647 bp sequence was generated, encompassing 208 bp of the 5'UTR, 1128 bp of coding region (exon 1, 2 and 3) as well as 311 bp of 3'UTR. They observed the sheep and goat GDF8 gene sequences were to be highly conserved as compared Singh et al. [15] reported the molecular cloning and characterization of the complete coding sequence of MSTN gene (1128 bp) in seven Indian goat breeds. The sheep and goat GDF8 gene sequences were to be highly conserved as compared to cattle, buffalo, horse and pig. Several nucleotide variations were observed across coding sequence of GDF8 gene in Indian sheep. Three polymorphic sites in the 5'UTR, one in exon 1 and one in the exon 2 regions was identified. Both SNPs in the exonic region were found to be nonsynonymous. And the mutations c.539 T > G and c.821 T > A in the exon 1 and exon 2 respectively, were discovered. Ahad et al. [17] reported the first characterization of the myostatin coding regions from Bakerwal goats and compared to the ovine protein and they showed that high similarity and identity between the nucleotide and amino acid sequences from *Ovis aries* and *Capra hircus* due to the close proximity of the species which belong to the same family of Bovidae.

The objectives of this study was to investigate develop a genomic library for some of Egyptian goat breeds. All isolate one of the genes which related to growth traits in some Egyptian goat breeds and deposit them in international gene banks, including NCBI Gene Bank and to enrich the database with new sequences for Egyptian goat breeds.

2. Materials and methods

2.1. Sample collection

Blood samples were collected from three goat breeds (Zaraibi, Baladi and Damascus) by Animal Genetic Resources Department, National Gene Bank, Agricultural Research Center (ARC), Giza, Egypt, the samples were kept at 4 °C on a tube containing 0.5 ml from EDTA (0.5 M) as anticoagulant matter for DNA extraction.

2.2. DNA extraction

Total genomic DNA extraction method was according to Sambrook et al. [10]. The extraction procedures were performed as follow: Four ml of lysis buffer (20 mM Tris-HCl pH 7.6, 640 mM sucrose, 2% Triton X-100, 10 mM MgCl₂) was added to the samples. The mixture was centrifuged and the pellet suspended in 150 μl Proteinase K, 1.5 ml nuclei lysis buffer and 110 μl SDS 20%. After overnight incubation at 37 °C, the proteins were removed using of 2 ml NaCl (6M) the mixture was centrifuged. The DNA were precipitated by cold absolute ethanol. The collected DNA was dissolved by addition 500 μl TE buffer and incubated at 37 °C, the DNA samples were stored at –20 °C.

2.3. Primers design

Four primer sequences, as shown in Table 1, were designed using CLC sequence viewer (version 7.5). According to all available flat files for *capra hircus* (MSTN) genes sequences by making alignment between all available sequences for (MSTN) to identify the best primer sequences then we used the NCBI Primer-BLAST tool. To ensure the validation of primer sequences available at <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>.

2.4. PCR protocol

Emerald Amp GT Green PCR Master Mix (2X) ready-to-use (TaKaRa) was used for all PCR reactions. DNA amplification was performed in a 50 μl reaction mixture. Initial denaturation at 94 °C for 5 minutes, 35 cycles each consisted of denaturation at 94 °C for 1 minute, annealing temperature differed from one primer

Table 1

List of primer Pairs sequences used to amplify myostatin MSTN genes based on the database.

Gene	Primer Sequence (5' → 3')	Expected length
MSTN1	F: CAAGAGCCAATCACAGATCC R: GAGTATATGTTGAACTGACTGC	1493 bp
MSTN2	F: GCAGTCAGTTCAAACATATACTC R: TAATCATGTAGCACATCAATGG	1632 bp
MSTN3	F: CAGGCTAGTTAACTTGTACTACC R: TGCACAAGATGGGTATGAGG	1633 bp

to the other according to the recommended milting temperature, extension for 72 °C for 1 min per kb. A final extension time at 72 °C for 5 min was applied and then kept at 4 °C until further use.

The amplification of first, second and third fragments from MSTN gene by MSTN1, MSTN2 and MSTN3 primers showed that the optimum annealing temperature was 62 °C.

2.5. Visualization and sequencing for amplified fragments

Five µl of each PCR product was directly loaded on 1.5% agarose gel electrophoresis and visualized by Gel documentation system. The PCR products were directly purified by Gene JET PCR purification kit and sequenced on ABI3730XL DNA Analyzer apparatus throughout sigma Company. The sequenced fragments were alignment using <http://ncbi.nlm.nih.gov/BLAST/> with the published data in the NCBI databases (non-redundant nucleotide database) and submitted to the Gene Bank using Bank It tool: <http://www.ncbi.nlm.nih.gov/BankIt/>.

3. Results and discussion

3.1. Amplification of first fragment from MSTN gene

All three fragments MSTN1, MSTN2 and MSTN3 of MSTN gene were amplified by three primers to obtain 1493 bp, 1632 bp and 1633 bp represent the myostatin gene from the Egyptian goat breeds as shown in Table 2. One fragment in accordance with the expected length for three Egyptian goat breeds as shown in (Fig. 2). Stinkens et al. [2] used annealing temperature 55 °C to obtain about 6.3 kb for MSTN gene from male pigs. Furthermore, Han et al. [9] used the annealing temperatures from 54 °C to 61 °C to amplify 8003 bp of myostatin gene from New Zealand sheep breeds. Then we can say that the different between the annealing temperatures this may be due to the breed or specie and the method of primer designing.

3.2. Sequencing and submission of PCR fragments

The fragments were submitted in the NCBI using BankIt tool under the accession numbers as shown in Table 1 after modification for row sequences by Finch TV program (version 1.4.0, www.geospiza.com).

Table 2

Goat breeds, Accession numbers, primer and length of submitted fragments on the data base system.

Goat breeds	Accession No.	Primer	Length	Locus
Zaraibi	KY463684	MSTN3	984 bp	Partial of intron 1, complete exon 2
Baladi	KY463685	MSTN3	1429 bp	Partial of intron 1, complete exon 2
Baladi	KY463686	MSTN2	894 bp	Complete of exon1, partial intron 1
Damascus	KY441464	MSTN3	1405 bp	Partial of intron 2, partial of exon 3

3.2.1. Annotation of fragment KY 463684 of Zaraibi goat breed

Data was analyzed by BLASTN, Zaraibi goat breed sequenced under accession number KY463684 against nucleotide database showed that highly similarity with *Capra hircus* breeds Guizhou Black and Qianbei Ma myostatin (MSTN) gene partial cds by Feng et al. [7,8]. Also, similarity with breeds Guizhou Small Xiang myostatin (MSTN) gene complete cds which reported by Zhu et al. [6] and with *Capra hircus* breeds(Guizhou Black, Jianchang Black, Angora, Shannan White, Yichang White) myostatin MSTN gene complete cds which investigated by Liu et al. [22,23] with 99% identity and 100% query coverage under E value (0) and there were gab in one bp means that this gab may be resulted according to breed variance and there were similarity with another species like *Ovis aries* and *Bos taurus* with identity from 96 to 99% under 100% coverage and there were a variable identity percentage with a lot of mammals. The graphical alignment between these results showed that the submitted fragment consisted of partial of intron 1 and complete exon 2 as shown in Fig. 3.

3.2.2. Annotation of fragment KY463685 of Baladi goat breed

Baladi goat breed sequenced under accession number KY463685 against nucleotide database showed that highly similarity with *Capra hircus* breed Qianbei Ma and Guizhou Black myostatin (MSTN) gene partial cds by Feng et al. [7,8]. Also, Guizhou Small Xiang breed myostatin (MSTN) gene complete cds according to Zhu et al. [6] and Guizhou Black, Jianchang Black, Angora, Shannan White, Nubian and Yichang White breeds myostatin (MSTN) gene complete cds which reported by Liu et al. [22,23] with 99% identity and 100% query coverage under E value (0). There were gab in one bp means that this gab may resulted according to breed variance and there were similarity with another species like *Ovis aries* (GDF-8) gene, complete cds and *Bos taurus* myostatin (MSTN) gene, complete cds a according to Clop et al. [1] and Martinez et al. [14] with identity ranged from 95 to 99% under 99% coverage and there were a variable identity percentage with a lot of mammals, the graphical alignment between these results showed that the submitted fragment consisted of partial of intron 1 and complete exon 2 as shown in Fig. 4

3.2.3. Annotation of fragment KY463686 of Baladi goat breed

Baladi goat breed sequenced under accession number KY463686 against nucleotide database showed that highly similarity with *Capra hircus* myostatin gene complete cds, *Capra hircus* breeds Guizhou Black, Jianchang Black, Angora, Shannan White, Nubian and Yichang White) myostatin MSTN gene complete cds which investigated by Liu et al. [22,23]. Also, *Capra hircus* breeds (Guizhou Small Xiang myostatin (MSTN) gene complete cds according to Zhu et al. [6] and Guizhou Black and Qianbei Ma myostatin (MSTN) gene partial cds according to Feng et al. [7,8] and high similarity with another species like *Ovis aries* haplotypes 2, 3, 4, 5, 6, 7, 8, 9 and 10 myostatin (MSTN) gene, intron 1, promoter region, exon 1, 5' UTR and partial cds, *Ovis aries* (GDF-8) gene complete cds by Clop et al. [1], *Bos taurus* myostatin (MSTN) gene complete cds according to Martinez et al. [14] and *Bos indicus* myostatin gene complete cds which reported by Tanta et al. [12] with identity ranged from 96 to 99% and 100% query coverage under E value (0). There were gab in one bp means that this gab

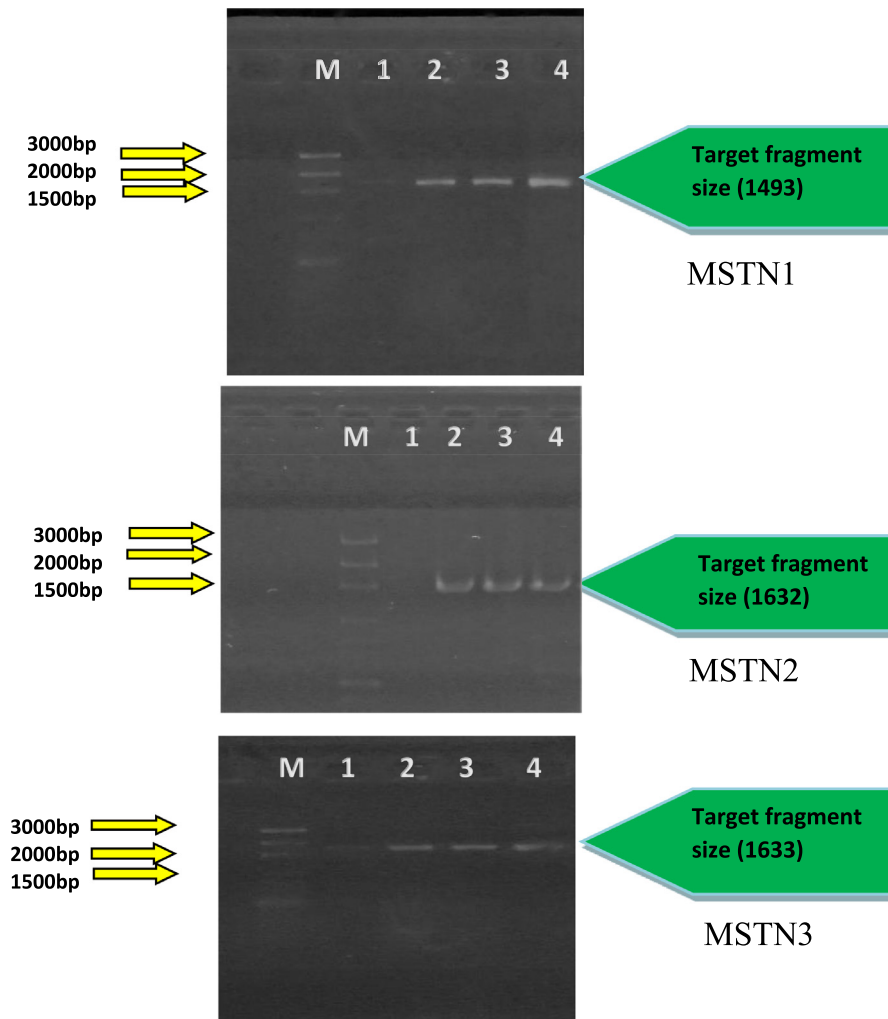


Fig. 2. The amplified fragments of gat breeds (*capra hircus*) DNA using MSTN1, MSTN2 and MSTN3 primers. M = 3 kb DNA ladder, Lane 1: negative control, Lane 2: amplified fragment for (Damascus), Lane 3: amplified fragment (Baladi) and Lane 4: amplified fragment (Zaraibi) goat breeds.

Capra hircus myostatin gene, complete cds

GenBank: EF591039.1

[GenBank](#) [FASTA](#)

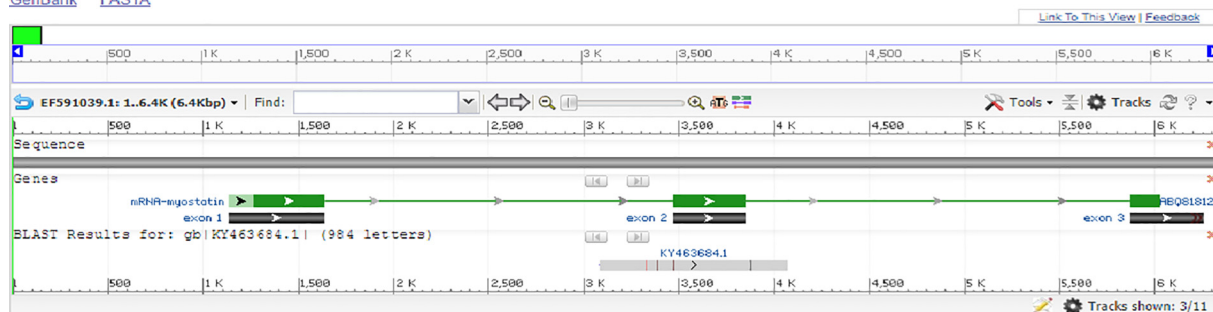


Fig. 3. Graphical alignment show the locus of the first submitted sequence on the myostatin gene.

may resulted according to breed variance, there were a variable identity percentage with a lot of mammals. The graphical alignment between the submitted fragment and *Capra hircus* myostatin gene, complete cds on database showed that the sequenced fragment loci is complete of exon1 and partial intron 1 as shown in Fig. 5.

3.2.4. Annotation of fragment KY441464 of Damascus goat breed

Damascus goat breed sequenced under accession number KY441464 against nucleotide database showed that highly similarity with *Capra hircus* breed Guizhou Small Xiang myostatin (MSTN) gene complete cds which reported by Zhu et al. [6]. Also, with *Capra hircus* breeds Jianchang Black and Yichang White myostatin

Capra hircus myostatin gene, complete cds

GenBank: EF591039.1

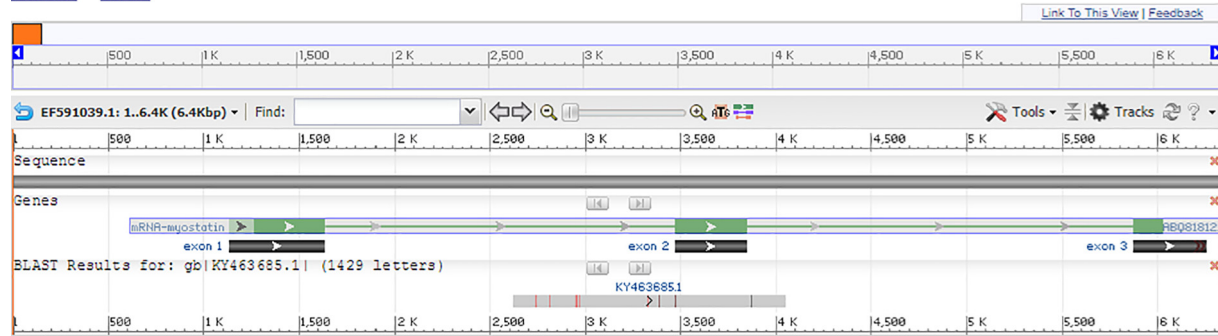
[GenBank](#) [FASTA](#)

Fig. 4. Graphical alignment show the locus of the second submitted sequence on the myostatin gene.

Capra hircus myostatin gene, complete cds

GenBank: EF591039.1

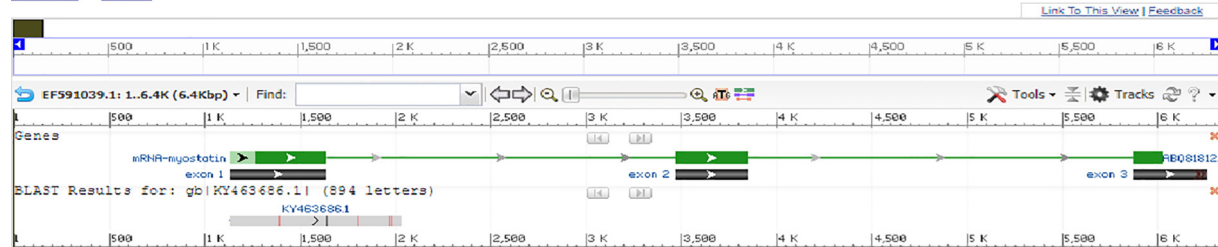
[GenBank](#) [FASTA](#)

Fig. 5. Graphical alignment show the locus of the third submitted sequence on the myostatin gene.

Capra hircus myostatin gene, complete cds

GenBank: EF591039.1

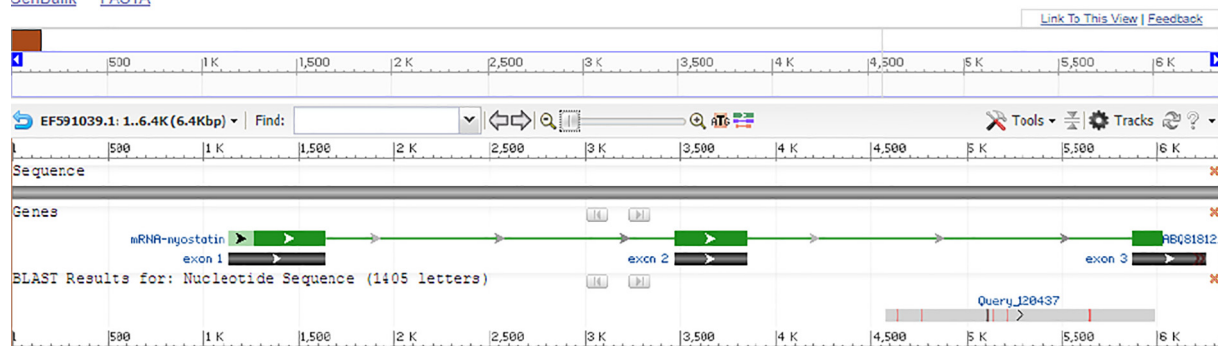
[GenBank](#) [FASTA](#)

Fig. 6. Graphical alignment show the locus of the fourth submitted sequence on the myostatin gene.

MSTN gene complete cds by Liu et al. [23], Guizhou Black, Angora, Shannan White, *Capra hircus* breeds and Nubian myostatin (MSTN) gene complete cds which investigated by Liu et al. [22,23] and *Capra hircus* breeds Qianbei Ma and Guizhou Black myostatin (MSTN) gene partial cds according to Feng et al. [7,8], *Capra hircus* myostatin (MSTN) gene exons 2, 3 and partial cds Lian et al. [21], with 99% identity and 100% query coverage under E value (0). There were gab in one bp means that this gab may resulted according to breed variance and there were similarity with another species like *Ovis aries* GDF-8 gene complete cds which reported by Clop et al. [1] and *Bos taurus* myostatin (MSTN) gene complete

cds by Martinez et al. [14] with identity from 95 to 97% under 99% coverage and there were a variable identity percentage with a lot of mammals. The graphical alignment between these results showed that the submitted fragment consisted of partial of intron 2 and partial of exon 3 as shown in Fig. 6.

4. Conclusion

There are many animal genetic resources in the Arab Republic of Egypt, which are considered national wealth, including goats.

Applications of biotechnology, genetic engineering and molecular biology with the help of bioinformatics tools can be used to isolate and define genes that are related to the productive characteristics of local goat breeds and to be registered in international gene banks. Therefore these techniques can be used to develop breeding programs and genetic improvement of Egyptian goat breeds.

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