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To cite this article: Hassan Nima Habib *et al* 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* **928** 062017

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ABSTRACT DEADLINE: DECEMBER 4, 2020



May 30-June 3, 2021

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Molecular detected of heat shock protein70 gene in Layer hens (Lohmann breed)

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Abstract

The polymorphisms of the *hsp70* gene have been associated with diverse resistance of heat stress in hens. The aim of the current study was to explore the genetic variation of the *hsp70* gene in Layer hens that bred in Iraq. One hundred-fifty Lohmann breed hens aged 12 months were used in this study. Blood samples were collected during the period from 1st September to 31st December 2018 and examined for detection the polymorphism of *hsp70* gene. We have detected four main polymorphisms groups in the coding region of *hsp70* gene among these layer hens. A significant association between the silent and the missense mutations with the polymorphisms of *hsp70* gene in Layer hens was found. There was a high homology of the *hsp70* gene sequences that obtained from our local layer hens with the related sequences obtained from different hottest and coldest areas. In conclusion, this study demonstrates that the different mutations (silent and missense) in the coding region of the *hsp70* gene of these local Layer hens predict improve birds' ability to the tolerance of stress conditions, and highlights the need of further investigations.

Keywords: HSPs, *hsp70* gene, Polymorphism, Layer hens, Stress condition



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Introduction

Heat stress is one of the most environmental conditions that generally adversely influent the poultry production. Layer hens industry is particularly impacted by heat stress as it often lead to reduced feed intake, low egg weight, reduced eggshell quality, poor growth rate and could increase mortality causing significant economic losses [1-4]. Thus, it is very influential in the hottest countries including Iraq in which the temperature raises two to seven folds higher than the global rates [5].

Despite the hens has protective mechanisms against various stress conditions; heat shock proteins (HSPs), a group of proteins proliferating due to stress conditions such as high environmental temperature [6], are fully effective for protecting and repairing mechanism of cells and tissues during stress [7]. However, HSP70 is the main member of HSPs family that plays a crucial role in heat tolerance [8], which can be used as a potential biomarker for dry and heat tolerance [9-11], as a higher bioavailability of its peptides in all variants extracellular [12]. Genetically, the gene in charge of HSP (*hsp70* gene) does not contain introns in the coding region [13], therefore, the *hsp70* gene considered as a high-conservation molecular [8]. As far as possible, there are known many nucleotide polymorphisms in the coding region of the *hsp70* gene in goats [14], Japanese quail [15], duck [16], hens [17-18], cattle [19-20], sheep [21] and buffalo [22]. The polymorphisms of the *hsp70* gene have been linked with the different impedance of heat stress in hens [17,23-24]. Furthermore, *hsp70* gene polymorphisms act as genetic markers associated with heat stress tolerance, this may allow for direct selection of genes [17]. The polymorphisms of the *hsp70* gene are also linked with gene expression [18] as they can affect more than one phenotypic trait [25]. To the best of our knowledge, no single study has been attempted evaluating the polymorphism of the *hsp70* gene in Layer hens in Iraq. Therefore, the current study was aimed to detect the polymorphism of *hsp70* gene in Layer hens that bred in Iraq.

Materials and methods

Animals and experimental design

One hundred fifty Lohmann breed hens aged 50 weeks old (at close stage of egg production) were used in this study. All hens were kept in the

Poultry Field of the College of Agriculture, University of Basrah, Iraq. The study was conducted during the period from 1st September to 31st December 2018. All hens were bred under the same conditions and fed with standard recommended diet. Blood samples were collected from hens then subjected for molecular analysis of hsp70 gene as following.

Samples and DNA extraction

Ten milliliters of blood was collected from the thigh from all hens, aseptically in EDTA tubes and the samples of blood were kept frozen at -20°C till the DNA extraction process. DNA extraction was done by using the DNeasy blood and tissue kit, obtained from Qiagen®, following the methods of Aryani et al., (2019) [26].

PCR amplification

The amplification reaction was performed in 25μl, consisted of 1μl DNA template (75ng), 1μL (10μM) forward primer, 1μL (10μM) reverse primer, 9.5μL water (free nuclease) and 12.5μL of 2 X PCR master mix. The primer (Table 1) was used according to the design of Gan et al., (2015) [27], while the PCR conditions were denaturation at 94°C (3 min) followed by 32 cycles of denaturation at 94°C for 30 seconds, 62°C for 30 seconds, annealing at 72°C for 45 seconds, and a final elongation step at 72°C for 6 minutes. Then to reveal the PCR product, 1.5% ethidium bromide 0.5 μg/ml stained agarose gel was used.

Table 1: The DNA sequence of primer (forward and reverse) of hsp70 gene [27]

<i>hsp70- F</i>	5'-CGATCTGGCTGCAATCTACG-3'
<i>hsp70- R</i>	5'-AT TTCCAGAAGCTGCACTTGG-3'

The analysis of sequences

The resulting sequences were subjected to the nucleotide BLAST analysis on NCBI website, and compared with Hens 70 kd heat shock protein complete cds as a reference gene in GenBank (accession number J02579). The multiple sequence alignment (MSA) carried out on website <http://www.ebi.ac.uk/Tools/msa/clustalo/> [28]. Further analysis was done by applying “Geneious Prime 2019.0.4” software in order to determine the expected mutations in amino acids [29].

Three-dimensional structure of protein

To detect the 3D structure of the protein, the Swiss model has been used [30].

The analysis of phylogenetic tree

Mega-x version 10.0.5 [31] software was applied to conduct the analysis of the phylogenetic tree. The resulting sequences were matched with the upper 10 outcomes of the *Gallus gallus hsp70* gene in BLAST, which included each of accession numbers (J02579 USA, AY143691 Brazil, AY143692 Brazil, AY143693 Brazil, MH422506 Iran, MH422507 Iran, MH422508 Iran, EU747335 China, AY288299 China, and NM_001006685 China).

Results

The product size of PCR in the current study was 2692bp (Figure 1). However, four different polymorphism groups were obtained when compared to the reference gene (accession number J02579) in the GenBank. They were submitted to DDBJ, EMBL and GenBank, which available under accession numbers as follows: LC498496 (37 hens), LC503772 (23 hens), LC503773, (41 hens) and LC503774 (49 hens).

Multiple sequence alignment analysis (Figure 2) showed development of four groups, this was expressed in the numbers of silent mutations as well as the missense mutation that occurred due to new amino acid coding (Figure 3). Nevertheless, thirty-three different mutations occurred in all of the resulting groups, some of these mutations occurred only in one group, while some of them occurred in more than one group; the mutations were summarized as follows:

1. LC498496

The positions 710 (G>A), 832 (G>C), 1013 (T>A), 1280 (T>G), 1430 (C>G), 1694 (T>G) and 1935 (A>T) are missense mutations that happened as a result of change of amino acids from glycine to glutamic, alanine to proline, phenylalanine to tyrosine, leucine to arginine, serine to cysteine, leucine to arginine, and glutamine to histidine respectively. Whereas the positions 804 (A>G), 1413 (G>A) and 2484 (G>A) are silent mutations.

2. LC503772

The positions 149 (G>C), 710 (G>A), 821(G>T), 1013 (T>A), 1430 (C>G), 2613 (A>T), 2633 (A>C) and 2660 (A>T) are missense mutations that happened as a result of change of amino acids from arginine to proline, glycine to glutamic acid, cysteine to phenylalanine, phenylalanine to tyrosine, serine to cysteine, lysine to asparagine, glutamic acid to alanine and isoleucine to asparagine respectively. While the positions 804 (A>G) and 855 (A>C) are silent mutations.

3. LC503773

The positions 54 (A>T), 947 (T>A), 1508 (G>C), 1531 (A>T), and 2356 (T>A) are missense mutations that happened as a result of change of amino acids from lysine to asparagine, leucine to histidine, cysteine to serine, serine to cysteine, and phenylalanine to isoleucine respectively. While the position 600 (A>G) is a missense mutation that happened as an outcome of coding tryptophan, new amino acid. The positions 1341(T>G), 1488 (T>C) and 1845 (C>A) are silent mutations.

4. LC503774

The positions 219 (A>C), 947 (T>A), 1508 (G>C), 1580 (T>C), 1696 (A>G), 1904 (A>C), 2338 (C>G) and 2358 (T>A) are missense mutations that happened as a result of change of amino acids from leucine to phenylalanine, leucine to histidine, cysteine to serine, valine to alanine, arginine to glycine, asparagine to threonine, histidine to aspartic acid and phenylalanine to leucine respectively. While the positions 669 (T>A) and 1341(T>G) are silent mutations.

The 3D structure of the protein was predicted for all the resulting sequences (Figure 4). Despite the great structural similarities, there were not completely identical, taking into consideration the selection of the highest quality molds for all sequences.

The optimal tree with the sum of branch length = 9.43103611 is shown. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Consistently, phylogenetic tree analysis (Figure 5) showed that the group

LC498496 was in the same clade with the reference gene (accession number J02579), while the LC503772 group shared them origin with the same clade, the group LC503773, and LC03774 were in the same clade. Interestingly, the resulting sequences shared the same origin with all accession numbers of *hsp70* gene in Layer hens with high homology.

Discussion

The gene expression of *hsp70* gene of layer hens in the current study was higher as previously shown by Morimoto et al., (1986) [32]. Through this higher expression of *hsp70* gene, the sequences that obtained in this study did not fully coincide with any of other related sequences. However, our findings correspond well with the previously found results [27,33-34], which describe the nucleotide polymorphisms in the coding region of the *hsp70* gene in hens. Our findings suggest that the changes in the codon region of the *hsp70* gene might be explained by a mismatch that resulted from silent and missense mutations that can mainly develop this gene [27]. Furthermore, the diversity of the *hsp70* gene is directly related to the different production traits of poultry [35-36], thus, the mutations can generally enhance the function of gene [13]. Even though the silent mutations do not usually affect protein function [37], however, it may be evidence of tolerating different stress conditions [38]. However, the silent mutation impacts the related proteins by altering the transcription process and the accuracy and the efficiency of binding to mRNA [39]; therefore, its effect can reach the stability of the encoded protein [40].

As expected for missense mutations, the incidences of which were high, their influence is highly dependent on their locations [41] and on the composition and the function of resulting amino acids [42]. Therefore, so not all of these mutations cause significant changes in protein, the amino acid can be changed with another amino acid that has similar chemical properties in which the protein function remains normal and unchanged. Contrariwise, the protein can be turned into non-functional if there is a significant difference in the properties of amino acids [43]. The polymorphisms resulting from missense mutations that mentioned in previous studies are strongly linked to tolerate the stressful conditions and have better productive characteristics [44]. Another aspect is that the missense mutation may have caused a change in protein structure [45],

this change can impact the protein function [46], and thus, the *hsp70* modeling is mainly useful by giving a clear perception about its function [47].

The present data suggests that there was a potential relationship between the genetic diversity in the *hsp70* gene and the expression of gene that well corresponds with the previously reported results [24,48], where the levels of gene expression differ between polymorphisms of *hsp70* gene. By this way, the election of the most productive breeds is being fully effective, as high gene expression was positively associated with resistance to stress conditions, especially high temperature [16].

Interestingly when analyzing the sequences of *hsp70* gene in local Layer hens, all the resulting sequences were closer to the reference gene (accession number J02579) that obtained from a coldest area (USA). However, *hsp70* gene can be completely stimulated even with sharp elevation or decline of the environmental temperature [22]. Moreover, there were high homology with different *hsp70* genes that obtained from Brazil, China, and Iran. This finding is clearly indicates that the *hsp70* gene well preserved in *Gallus gallus*, these differences were also previously reported by [33], [26] and [49].

Conclusion

The present data provide the first insight into genetic variation of the *hsp70* gene of Layer hens (Lohmann breed) that bred in Iraq, and the first evidence that the different mutations (silent and missense) in the coding region of the *hsp70* gene of these local Layer hens predict improve birds'ability to the tolerance of stress conditions. Our study records four polymorphisms in the coding region of the *hsp70* gene in a limited number of local Layer hens. Further deep studies may explore the relationship between the polymorphisms of the *hsp70* gene and the productive characteristics of laying hens.

Acknowledgments

We gratefully thank the staff of the poultry field, college of agriculture, University of Basrah for their helping and supporting.

Funding

The current study was funded by the authors themselves.

Conflict of Interest

We declare that there are no conflicts of interest.

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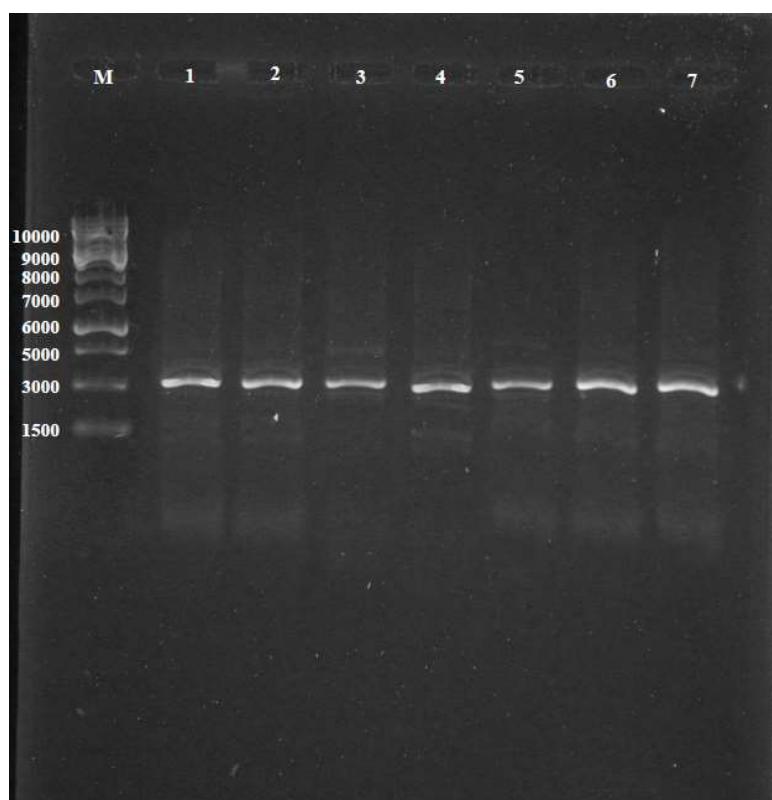


Figure 1: Gel electrophoresis of the PCR amplification to *hsp70* gene, M: 10kb DNA ladder, 1-7: DNA templates

LC503774	TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAA	AAAACAG	60	
LC503773	TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAA	AAAACAG	60	
LC503772	TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAA	AAAACAG	60	
J02579	TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAA	AAAACAG	60	
LC498496	TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAA	AAAACAG	60	

LC503774	GAAGAAAGCCCAGATCGGCTCAATCTACGGGAGAGGGTTGGCTAGAGAGTGCGCTAC	120		
LC503773	GAAGAAAGCCCAGATCGGCTCAATCTACGGGAGAGGGTTGGCTAGAGAGTGCGCTAC	120		
LC503772	GAAGAAAGCCCAGATCGGCTCAATCTACGGGAGAGGGTTGGCTAGAGAGTGCGCTAC	120		
J02579	GAAGAAAGCCCAGATCGGCTCAATCTACGGGAGAGGGTTGGCTAGAGAGTGCGCTAC	120		
LC498496	GAAGAAAGCCCAGATCGGCTCAATCTACGGGAGAGGGTTGGCTAGAGAGTGCGCTAC	120		

LC503774	GCTTCTGATTGGCAGGAGGCAAGGGGC	GGCGCTCTTCGGCTAGTCGGGAGGGCGATT	180	
LC503773	GCTTCTGATTGGCAGGAGGCAAGGGGC	GGCGCTCTTCGGCTAGTCGGGAGGGCGATT	180	
LC503772	GCTTCTGATTGGCAGGAGGCAAGGGGC	GGCGCTCTTCGGCTAGTCGGGAGGGCGATT	180	
J02579	GCTTCTGATTGGCAGGAGGCAAGGGGC	GGCGCTCTTCGGCTAGTCGGGAGGGCGATT	180	
LC498496	GCTTCTGATTGGCAGGAGGCAAGGGGC	GGCGCTCTTCGGCTAGTCGGGAGGGCGATT	180	

LC503774	CGGTCAACTGCCGAGTCGGGTGCTGGATTGGCTTC	CGCTTCTGGCAGGTTCCAGAA	240	
LC503773	CGGTCAACTGCCGAGTCGGGTGCTGGATTGGCTTC	CGCTTCTGGCAGGTTCCAGAA	240	
LC503772	CGGTCAACTGCCGAGTCGGGTGCTGGATTGGCTTC	CGCTTCTGGCAGGTTCCAGAA	240	
J02579	CGGTCAACTGCCGAGTCGGGTGCTGGATTGGCTTC	CGCTTCTGGCAGGTTCCAGAA	240	
LC498496	CGGTCAACTGCCGAGTCGGGTGCTGGATTGGCTTC	CGCTTCTGGCAGGTTCCAGAA	240	

LC503774	GAAGGCTAACGGACTATAAAGAGGGCGCAGGGCGTAACGGCAGATCGGCCCGCAGAC	300		
LC503773	GAAGGCTAACGGACTATAAAGAGGGCGCAGGGCGTAACGGCAGATCGGCCCGCAGAC	300		
LC503772	GAAGGCTAACGGACTATAAAGAGGGCGCAGGGCGTAACGGCAGATCGGCCCGCAGAC	300		
J02579	GAAGGCTAACGGACTATAAAGAGGGCGCAGGGCGTAACGGCAGATCGGCCCGCAGAC	300		
LC498496	GAAGGCTAACGGACTATAAAGAGGGCGCAGGGCGTAACGGCAGATCGGCCCGCAGAC	300		

LC503774	AGCAGCGAGAACGGCGGGAGAGCTGACTGGCAGGAGCAAGTGA	360		
LC503773	AGCAGCGAGAACGGCGGGAGAGCTGACTGGCAGGAGCAAGTGA	360		
LC503772	AGCAGCGAGAACGGCGGGAGAGCTGACTGGCAGGAGCAAGTGA	360		
J02579	AGCAGCGAGAACGGCGGGAGAGCTGACTGGCAGGAGCAAGTGA	360		
LC498496	AGCAGCGAGAACGGCGGGAGAGCTGACTGGCAGGAGCAAGTGA	360		

LC503774	GTGGCTGACTGACCAAGAGGAATCATCATCATGTCTGGCAAAGGGCGGCCATCGGCAT	420		
LC503773	GTGGCTGACTGACCAAGAGGAATCATCATCATGTCTGGCAAAGGGCGGCCATCGGCAT	420		
LC503772	GTGGCTGACTGACCAAGAGGAATCATCATCATGTCTGGCAAAGGGCGGCCATCGGCAT	420		
J02579	GTGGCTGACTGACCAAGAGGAATCATCATCATGTCTGGCAAAGGGCGGCCATCGGCAT	420		
LC498496	GTGGCTGACTGACCAAGAGGAATCATCATCATGTCTGGCAAAGGGCGGCCATCGGCAT	420		

LC503774	CGATCTGGCACACAGTATTCTGGTGGGTCTTCAGCATGGCAAAGTGGAGATCAT	480		
LC503773	CGATCTGGCACACAGTATTCTGGTGGGTCTTCAGCATGGCAAAGTGGAGATCAT	480		
LC503772	CGATCTGGCACACAGTATTCTGGTGGGTCTTCAGCATGGCAAAGTGGAGATCAT	480		
J02579	CGATCTGGCACACAGTATTCTGGTGGGTCTTCAGCATGGCAAAGTGGAGATCAT	480		
LC498496	CGATCTGGCACACAGTATTCTGGTGGGTCTTCAGCATGGCAAAGTGGAGATCAT	480		

LC503774	TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGGCCTTCACCGATAACAGAGCG	540		
LC503773	TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGGCCTTCACCGATAACAGAGCG	540		
LC503772	TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGGCCTTCACCGATAACAGAGCG	540		
J02579	TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGGCCTTCACCGATAACAGAGCG	540		
LC498496	TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGGCCTTCACCGATAACAGAGCG	540		

LC503774	CCTCATGGGATGCTCCAAGAACCAAGTGGCAATGAACCCACACCATTTGA	600		
LC503773	CCTCATGGGATGCTCCAAGAACCAAGTGGCAATGAACCCACACCATTTGA	600		
LC503772	CCTCATGGGATGCTCCAAGAACCAAGTGGCAATGAACCCACACCATTTGA	600		
J02579	CCTCATGGGATGCTCCAAGAACCAAGTGGCAATGAACCCACACCATTTGA	600		
LC498496	CCTCATGGGATGCTCCAAGAACCAAGTGGCAATGAACCCACACCATTTGA	600		

LC503774	TGCCAAGCGTCATCGGCCGCAAGTATGATGACCCCACAGTCAGTCAGACATGAAGCA	660		
LC503773	TGCCAAGCGTCATCGGCCGCAAGTATGATGACCCCACAGTCAGTCAGACATGAAGCA	660		
LC503772	TGCCAAGCGTCATCGGCCGCAAGTATGATGACCCCACAGTCAGTCAGACATGAAGCA	660		
J02579	TGCCAAGCGTCATCGGCCGCAAGTATGATGACCCCACAGTCAGTCAGACATGAAGCA	660		
LC498496	TGCCAAGCGTCATCGGCCGCAAGTATGATGACCCCACAGTCAGTCAGACATGAAGCA	660		

LC503774	CTGGCCCT	CGCTGTTGGAACGAGGGTGGCAAGGCCAAGGGCAGGTGAGTACAAGGG	720	
LC503773	CTGGCCCT	CGCTGTTGGAACGAGGGTGGCAAGGCCAAGGGCAGGTGAGTACAAGGG	720	
LC503772	CTGGCCCT	CGCTGTTGGAACGAGGGTGGCAAGGCCAAGGGCAGGTGAGTACAAGGG	720	
J02579	CTGGCCCT	CGCTGTTGGAACGAGGGTGGCAAGGCCAAGGGCAGGTGAGTACAAGGG	720	
LC498496	CTGGCCCT	CGCTGTTGGAACGAGGGTGGCAAGGCCAAGGGCAGGTGAGTACAAGGG	720	

LC503774	TGAGATGAAGACCTCTTCCCAGAGGGAGTCAGCTATGGGCTCACCAAGATGAAGGA	780		
LC503773	TGAGATGAAGACCTCTTCCCAGAGGGAGTCAGCTATGGGCTCACCAAGATGAAGGA	780		
LC503772	TGAGATGAAGACCTCTTCCCAGAGGGAGTCAGCTATGGGCTCACCAAGATGAAGGA	780		
J02579	TGAGATGAAGACCTCTTCCCAGAGGGAGTCAGCTATGGGCTCACCAAGATGAAGGA	780		
LC498496	TGAGATGAAGACCTCTTCCCAGAGGGAGTCAGCTATGGGCTCACCAAGATGAAGGA	780		

LC503774	GATTGCTGAGGCCATCTGGAA	AAAGGTAGAGACTGCTTATCACAGTC	CCCCGCTTA	840
LC503773	GATTGCTGAGGCCATCTGGAA	AAAGGTAGAGACTGCTTATCACAGTC	CCCCGCTTA	840
LC503772	GATTGCTGAGGCCATCTGGAA	AAAGGTAGAGACTGCTTATCACAGTC	CCCCGCTTA	840
J02579	GATTGCTGAGGCCATCTGGAA	AAAGGTAGAGACTGCTTATCACAGTC	CCCCGCTTA	840
LC498496	GATTGCTGAGGCCATCTGGAA	AAAGGTAGAGACTGCTTATCACAGTC	CCCCGCTTA	840

LC503774	CTTCAACGACTCCC	CGCCAGGCCACCAAAGATGCTGGCACCAC	TACTGGGCTTAACGT	900
LC503773	CTTCAACGACTCCC	CGCCAGGCCACCAAAGATGCTGGCACCAC	TACTGGGCTTAACGT	900
LC503772	CTTCAACGACTCCC	CGCCAGGCCACCAAAGATGCTGGCACCAC	TACTGGGCTTAACGT	900
J02579	CTTCAACGACTCCC	CGCCAGGCCACCAAAGATGCTGGCACCAC	TACTGGGCTTAACGT	900
LC498496	CTTCAACGACTCCC	CGCCAGGCCACCAAAGATGCTGGCACCAC	TACTGGGCTTAACGT	900

LC503774	GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGCC	ATGGATAAGAAAGG	960	
LC503773	GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGCC	ATGGATAAGAAAGG	960	
LC503772	GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGCC	ATGGATAAGAAAGG	960	
J02579	GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGCC	ATGGATAAGAAAGG	960	
LC498496	GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGCC	ATGGATAAGAAAGG	960	

LC503774	TACCCGGGCTGGAGAGAAGAATGTCATCTTGACTTTGACTTGGGAGGGGCACT	TTTGATGT	1020	
LC503773	TACCCGGGCTGGAGAGAAGAATGTCATCTTGACTTTGACTTGGGAGGGGCACT	TTTGATGT	1020	
LC503772	TACCCGGGCTGGAGAGAAGAATGTCATCTTGACTTTGACTTGGGAGGGGCACT	TTTGATGT	1020	
J02579	TACCCGGGCTGGAGAGAAGAATGTCATCTTGACTTTGACTTGGGAGGGGCACT	TTTGATGT	1020	
LC498496	TACCCGGGCTGGAGAGAAGAATGTCATCTTGACTTTGACTTGGGAGGGGCACT	TTTGATGT	1020	

LC503774	GTCATCCTTACCATTGAGGATGCGATCTTGAGGTGAAGTCCACAGCTGGGACACCCA	1080		
LC503773	GTCATCCTTACCATTGAGGATGCGATCTTGAGGTGAAGTCCACAGCTGGGACACCCA	1080		
LC503772	GTCATCCTTACCATTGAGGATGCGATCTTGAGGTGAAGTCCACAGCTGGGACACCCA	1080		
J02579	GTCATCCTTACCATTGAGGATGCGATCTTGAGGTGAAGTCCACAGCTGGGACACCCA	1080		
LC498496	GTCATCCTTACCATTGAGGATGCGATCTTGAGGTGAAGTCCACAGCTGGGACACCCA	1080		

LC503774	CCTAGGTGGGAGGACTTTGACAACCGCATGGTAACCGTTTGTAGAAGAGTTCAAGGG	1140		
LC503773	CCTAGGTGGGAGGACTTTGACAACCGCATGGTAACCGTTTGTAGAAGAGTTCAAGGG	1140		
LC503772	CCTAGGTGGGAGGACTTTGACAACCGCATGGTAACCGTTTGTAGAAGAGTTCAAGGG	1140		
J02579	CCTAGGTGGGAGGACTTTGACAACCGCATGGTAACCGTTTGTAGAAGAGTTCAAGGG	1140		
LC498496	CCTAGGTGGGAGGACTTTGACAACCGCATGGTAACCGTTTGTAGAAGAGTTCAAGGG	1140		

LC503774	TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCCCTGCGTACAGCTTG	1200		
LC503773	TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCCCTGCGTACAGCTTG	1200		
LC503772	TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCCCTGCGTACAGCTTG	1200		
J02579	TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCCCTGCGTACAGCTTG	1200		
LC498496	TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCCCTGCGTACAGCTTG	1200		

LC503774	TGAGAGGGCGAGGCCGTACTCTGAGCTTCCACCGCAAGCCAGCATGAGATTGACTCCCT	1260		
LC503773	TGAGAGGGCGAGGCCGTACTCTGAGCTTCCACCGCAAGCCAGCATGAGATTGACTCCCT	1260		
LC503772	TGAGAGGGCGAGGCCGTACTCTGAGCTTCCACCGCAAGCCAGCATGAGATTGACTCCCT	1260		
J02579	TGAGAGGGCGAGGCCGTACTCTGAGCTTCCACCGCAAGCCAGCATGAGATTGACTCCCT	1260		
LC498496	TGAGAGGGCGAGGCCGTACTCTGAGCTTCCACCGCAAGCCAGCATGAGATTGACTCCCT	1260		

LC503774	CTTGAGGGCATTGACTTACACCTCCATCACTCGTGGCCGCTTGAGGAACCTAACATGC	1320		
LC503773	CTTGAGGGCATTGACTTACACCTCCATCACTCGTGGCCGCTTGAGGAACCTAACATGC	1320		
LC503772	CTTGAGGGCATTGACTTACACCTCCATCACTCGTGGCCGCTTGAGGAACCTAACATGC	1320		
J02579	CTTGAGGGCATTGACTTACACCTCCATCACTCGTGGCCGCTTGAGGAACCTAACATGC	1320		
LC498496	CTTGAGGGCATTGACTTACACCTCCATCACTCGTGGCCGCTTGAGGAACCTAACATGC	1320		

LC503774	TGATCTTTCGGTGTACCCGGGAGCCAGTGGGAGAAGGCCCTGCGTGTGCAAGCTGA	1380		
LC503773	TGATCTTTCGGTGTACCCGGGAGCCAGTGGGAGAAGGCCCTGCGTGTGCAAGCTGA	1380		
LC503772	TGATCTTTCGGTGTACCCGGGAGCCAGTGGGAGAAGGCCCTGCGTGTGCAAGCTGA	1380		
J02579	TGATCTTTCGGTGTACCCGGGAGCCAGTGGGAGAAGGCCCTGCGTGTGCAAGCTGA	1380		
LC498496	TGATCTTTCGGTGTACCCGGGAGCCAGTGGGAGAAGGCCCTGCGTGTGCAAGCTGA	1380		

LC503774	TAAGGGCCAGATCCAGGAGATTGTGCTGTGGGGCTCCACTCGTATTCTAACAGATCCA	1440		
LC503773	TAAGGGCCAGATCCAGGAGATTGTGCTGTGGGGCTCCACTCGTATTCTAACAGATCCA	1440		
LC503772	TAAGGGCCAGATCCAGGAGATTGTGCTGTGGGGCTCCACTCGTATTCTAACAGATCCA	1440		
J02579	TAAGGGCCAGATCCAGGAGATTGTGCTGTGGGGCTCCACTCGTATTCTAACAGATCCA	1440		
LC498496	TAAGGGCCAGATCCAGGAGATTGTGCTGTGGGGCTCCACTCGTATTCTAACAGATCCA	1440		

LC503774	GAAGTTGCTGCAAGATTCTCAATGGCAAAGAGCTGAACAAGAGCACTAACATCCAGATGA	1500		
LC503773	GAAGTTGCTGCAAGATTCTCAATGGCAAAGAGCTGAACAAGAGCACTAACATCCAGATGA	1500		
LC503772	GAAGTTGCTGCAAGATTCTCAATGGCAAAGAGCTGAACAAGAGCACTAACATCCAGATGA	1500		
J02579	GAAGTTGCTGCAAGATTCTCAATGGCAAAGAGCTGAACAAGAGCACTAACATCCAGATGA	1500		
LC498496	GAAGTTGCTGCAAGATTCTCAATGGCAAAGAGCTGAACAAGAGCACTAACATCCAGATGA	1500		

LC503774	AGCTGTTCTTATGGTGCCTGTGCAAGCAGCTATCCTCATGGAGACAAGTCTGAAAAA	1560		
LC503773	AGCTGTTCTTATGGTGCCTGTGCAAGCAGCTATCCTCATGGAGACAAGTCTGAAAAA	1560		

LC503772	AGCTGTTGCTTATGGTGCCTGTGCAAGCAGCTATCCTCATGGGAGACAAGTCTGAAAA	1560
J02579	AGCTGTTGCTTATGGTGCCTGTGCAAGCAGCTATCCTCATGGGAGACAAGTCTGAAAA	1560
LC498496	AGCTGTTGCTTATGGTGCCTGTGCAAGCAGCTATCCTCATGGGAGACAAGTCTGAAAA	1560

LC503774	TGTGCAAGATCTGCTCTCGGGATGTCACCCCCCTGTCCTGGCATCGAGACAGCTGG	1620
LC503773	TGTGCAAGATCTGCTCTCGGGATGTCACCCCCCTGTCCTGGCATCGAGACAGCTGG	1620
LC503772	TGTGCAAGATCTGCTCTCGGGATGTCACCCCCCTGTCCTGGCATCGAGACAGCTGG	1620
J02579	TGTGCAAGATCTGCTCTCGGGATGTCACCCCCCTGTCCTGGCATCGAGACAGCTGG	1620
LC498496	TGTGCAAGATCTGCTCTCGGGATGTCACCCCCCTGTCCTGGCATCGAGACAGCTGG	1620

LC503774	TGGAGTGACTGCTCTCATCAAGCGTAACACCACATTCCCACCAAACAAACACAGAC	1680
LC503773	TGGAGTGACTGCTCTCATCAAGCGTAACACCACATTCCCACCAAACAAACACAGAC	1680
LC503772	TGGAGTGACTGCTCTCATCAAGCGTAACACCACATTCCCACCAAACAAACACAGAC	1680
J02579	TGGAGTGACTGCTCTCATCAAGCGTAACACCACATTCCCACCAAACAAACACAGAC	1680
LC498496	TGGAGTGACTGCTCTCATCAAGCGTAACACCACATTCCCACCAAACAAACACAGAC	1680

LC503774	CTTCACCCACTACAGACAACCCAGAGCAGTGTCTCAGTCAGGTATGAAGGTGAGAG	1740
LC503773	CTTCACCCACTACAGACAACCCAGAGCAGTGTCTCAGTCAGGTATGAAGGTGAGAG	1740
LC503772	CTTCACCCACTACAGACAACCCAGAGCAGTGTCTCAGTCAGGTATGAAGGTGAGAG	1740
J02579	CTTCACCCACTACAGACAACCCAGAGCAGTGTCTCAGTCAGGTATGAAGGTGAGAG	1740
LC498496	CTTCACCCACTACAGACAACCCAGAGCAGTGTCTCAGTCAGGTATGAAGGTGAGAG	1740

LC503774	GGCTATGACAAGGACAACAACTTGCTGGCAAGTTTGACCTAACAGGCATCCCCCGGC	1800
LC503773	GGCTATGACAAGGACAACAACTTGCTGGCAAGTTTGACCTAACAGGCATCCCCCGGC	1800
LC503772	GGCTATGACAAGGACAACAACTTGCTGGCAAGTTTGACCTAACAGGCATCCCCCGGC	1800
J02579	GGCTATGACAAGGACAACAACTTGCTGGCAAGTTTGACCTAACAGGCATCCCCCGGC	1800
LC498496	GGCTATGACAAGGACAACAACTTGCTGGCAAGTTTGACCTAACAGGCATCCCCCGGC	1800

LC503774	ACCCCGTGGAGTTCTCAGATCGAGGTCACTTTGACATAGATGTAATGGTATCCTGAA	1860
LC503773	ACCCCGTGGAGTTCTCAGATCGAGGTCACTTTGACATAGATGTAATGGTATCCTGAA	1860
LC503772	ACCCCGTGGAGTTCTCAGATCGAGGTCACTTTGACATAGATGTAATGGTATCCTGAA	1860
J02579	ACCCCGTGGAGTTCTCAGATCGAGGTCACTTTGACATAGATGTAATGGTATCCTGAA	1860
LC498496	ACCCCGTGGAGTTCTCAGATCGAGGTCACTTTGACATAGATGTAATGGTATCCTGAA	1860

LC503774	CGTCAGTGTGAGACAAGAGTACAGGGAGGAGAACAGATAACCATCACCAATGACAA	1920
LC503773	CGTCAGTGTGAGACAAGAGTACAGGGAGGAGAACAGATAACCATCACCAATGACAA	1920
LC503772	CGTCAGTGTGAGACAAGAGTACAGGGAGGAGAACAGATAACCATCACCAATGACAA	1920
J02579	CGTCAGTGTGAGACAAGAGTACAGGGAGGAGAACAGATAACCATCACCAATGACAA	1920
LC498496	CGTCAGTGTGAGACAAGAGTACAGGGAGGAGAACAGATAACCATCACCAATGACAA	1920

LC503774	GGTCGCCTTAGCAAGATGATATTGACCGTATGGTACAAGGAGAACATCAAAGC	1980
LC503773	GGTCGCCTTAGCAAGATGATATTGACCGTATGGTACAAGGAGAACATCAAAGC	1980
LC503772	GGTCGCCTTAGCAAGATGATATTGACCGTATGGTACAAGGAGAACATCAAAGC	1980
J02579	GGTCGCCTTAGCAAGATGATATTGACCGTATGGTACAAGGAGAACATCAAAGC	1980
LC498496	GGTCGCCTTAGCAAGATGATATTGACCGTATGGTACAAGGAGAACATCAAAGC	1980

LC503774	AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTGAGTCGTATACTTA	2040
LC503773	AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTGAGTCGTATACTTA	2040
LC503772	AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTGAGTCGTATACTTA	2040
J02579	AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTGAGTCGTATACTTA	2040
LC498496	AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTGAGTCGTATACTTA	2040

LC503774	CAACATGAAGCAGACAGTGGAGGTGAGAAAAGTCAGTGACCAGGACAA	2100
LC503773	CAACATGAAGCAGACAGTGGAGGTGAGAAAAGTCAGTGACCAGGACAA	2100
LC503772	CAACATGAAGCAGACAGTGGAGGTGAGAAAAGTCAGTGACCAGGACAA	2100
J02579	CAACATGAAGCAGACAGTGGAGGTGAGAAAAGTCAGTGACCAGGACAA	2100
LC498496	CAACATGAAGCAGACAGTGGAGGTGAGAAAAGTCAGTGACCAGGACAA	2100

LC503774	GCAGAAAGTGTGAGACAAGTGGAGGTGATCAGTCGCTTGACCGAAACCGAGATGGC	2160
LC503773	GCAGAAAGTGTGAGACAAGTGGAGGTGATCAGTCGCTTGACCGAAACCGAGATGGC	2160
LC503772	GCAGAAAGTGTGAGACAAGTGGAGGTGATCAGTCGCTTGACCGAAACCGAGATGGC	2160
J02579	GCAGAAAGTGTGAGACAAGTGGAGGTGATCAGTCGCTTGACCGAAACCGAGATGGC	2160
LC498496	GCAGAAAGTGTGAGACAAGTGGAGGTGATCAGTCGCTTGACCGAAACCGAGATGGC	2160

LC503774	AGAGAAAGAAGAGTATGAGCACAGCAGAAAGAGCTGGAGAAAACTCTGCAACCCGATTGT	2220
LC503773	AGAGAAAGAAGAGTATGAGCACAGCAGAAAGAGCTGGAGAAAACTCTGCAACCCGATTGT	2220
LC503772	AGAGAAAGAAGAGTATGAGCACAGCAGAAAGAGCTGGAGAAAACTCTGCAACCCGATTGT	2220
J02579	AGAGAAAGAAGAGTATGAGCACAGCAGAAAGAGCTGGAGAAAACTCTGCAACCCGATTGT	2220
LC498496	AGAGAAAGAAGAGTATGAGCACAGCAGAAAGAGCTGGAGAAAACTCTGCAACCCGATTGT	2220

LC503774	CACAAAACGTACCAAGGGAGCTGGAGGAGCTGGGGCAGGGTGGCTCCGGTGGCCCAACCAT	2280
LC503773	CACAAAACGTACCAAGGGAGCTGGAGGAGCTGGGGCAGGGTGGCTCCGGTGGCCCAACCAT	2280
LC503772	CACAAAACGTACCAAGGGAGCTGGAGGAGCTGGGGCAGGGTGGCTCCGGTGGCCCAACCAT	2280
J02579	CACAAAACGTACCAAGGGAGCTGGAGGAGCTGGGGCAGGGTGGCTCCGGTGGCCCAACCAT	2280
LC498496	CACAAAACGTACCAAGGGAGCTGGAGGAGCTGGGGCAGGGTGGCTCCGGTGGCCCAACCAT	2280

LC503774	TGAAGAAGTAGATTAAGGACTCTTAACTATAGACTGGTTATGGACAGTCACTCAT	2340
LC503773	TGAAGAAGTAGATTAAGGACTCTTAACTATAGACTGGTTATGGACAGTCACTCAT	2340
LC503772	TGAAGAAGTAGATTAAGGACTCTTAACTATAGACTGGTTATGGACAGTCACTCAT	2340
J02579	TGAAGAAGTAGATTAAGGACTCTTAACTATAGACTGGTTATGGACAGTCACTCAT	2340
LC498496	TGAAGAAGTAGATTAAGGACTCTTAACTATAGACTGGTTATGGACAGTCACTCAT	2340

LC503774	TCTTGCTTTATATTTCATTCACGTTAACGGAAAACGTCATTGCCATAACAGAGTT	2400
LC503773	TCTTGCTTTATATTTCATTCACGTTAACGGAAAACGTCATTGCCATAACAGAGTT	2400
LC503772	TCTTGCTTTATATTTCATTCACGTTAACGGAAAACGTCATTGCCATAACAGAGTT	2400
J02579	TCTTGCTTTATATTTCATTCACGTTAACGGAAAACGTCATTGCCATAACAGAGTT	2400
LC498496	TCTTGCTTTATATTTCATTCACGTTAACGGAAAACGTCATTGCCATAACAGAGTT	2400

LC503774	TATTCTGTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTTGTTGATAAAAGGGAAAG	2460
LC503773	TATTCTGTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTTGTTGATAAAAGGGAAAG	2460
LC503772	TATTCTGTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTTGTTGATAAAAGGGAAAG	2460
J02579	TATTCTGTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTTGTTGATAAAAGGGAAAG	2460
LC498496	TATTCTGTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTTGTTGATAAAAGGGAAAG	2460

LC503774	GCACGTCTGCTTATAAGGTTAATAAGACAAGTTGTTAACAGATACAGCTCCT	2520
LC503773	GCACGTCTGCTTATAAGGTTAATAAGACAAGTTGTTAACAGATACAGCTCCT	2520
LC503772	GCACGTCTGCTTATAAGGTTAATAAGACAAGTTGTTAACAGATACAGCTCCT	2520
J02579	GCACGTCTGCTTATAAGGTTAATAAGACAAGTTGTTAACAGATACAGCTCCT	2520
LC498496	GCACGTCTGCTTATAAGGTTAATAAGACAAGTTGTTAACAGATACAGCTCCT	2520

LC503774	TGTATTCTGGATGTTGTCCTGTTAAATGTCCTCTCTAAAGTAACCACTCGACTGTTG	2580
LC503773	TGTATTCTGGATGTTGTCCTGTTAAATGTCCTCTCTAAAGTAACCACTCGACTGTTG	2580
LC503772	TGTATTCTGGATGTTGTCCTGTTAAATGTCCTCTCTAAAGTAACCACTCGACTGTTG	2580
J02579	TGTATTCTGGATGTTGTCCTGTTAAATGTCCTCTCTAAAGTAACCACTCGACTGTTG	2580
LC498496	TGTATTCTGGATGTTGTCCTGTTAAATGTCCTCTCTAAAGTAACCACTCGACTGTTG	2580

LC503774	CAGTTGACAAGTTCAAGTTATGCTAGGAAAAAATTTGAAAGATGCGAAATGC	2640
LC503773	CAGTTGACAAGTTCAAGTTATGCTAGGAAAAAATTTGAAAGATGCGAAATGC	2640
LC503772	CAGTTGACAAGTTCAAGTTATGCTAGGAAAAAATTTGAAAGATGCGAAATGC	2640
J02579	CAGTTGACAAGTTCAAGTTATGCTAGGAAAAAATTTGAAAGATGCGAAATGC	2640
LC498496	CAGTTGACAAGTTCAAGTTATGCTAGGAAAAAATTTGAAAGATGCGAAATGC	2640

LC503774	CAAGTGCAGCTCTGGAAATTGGTAATAAATTTATGGGATCC	2692
LC503773	CAAGTGCAGCTCTGGAAATTGGTAATAAATTTATGGGATCC	2692
LC503772	CAAGTGCAGCTCTGGAAATTGGTAATAAATTTATGGGATCC	2692
J02579	CAAGTGCAGCTCTGGAAATTGGTAATAAATTTATGGGATCC	2692
LC498496	CAAGTGCAGCTCTGGAAATTGGTAATAAATTTATGGGATCC	2692

Figure 2: The multiple sequence alignment (MSA) of *hsp70* gen in Layer hens

J02579	AMNPTNTIFDAKRLIGRKYDOPTVQSDMKHNPFRVVNEGGKPKVQVEYKGEMKTFFPEEI	120
LC503773	AMNPTNTIFGAKRLIGRKYDOPTVQSDMKHNPFRVVNEGGKPKVQVEYKGEMKTFFPEEI	120
LC503774	AMNPTNTIFDAKRLIGRKYDOPTVQSDMKHNPYRVVNEGGKPKVQVEYKGEMKTFFPEEI	120
LC498496	AMNPTNTIFDAKRLIGRKYDOPTVQSDMKHNPFRVVNEGGKPKVQVKYKGEMKTFFPEEI	120
LC503772	AMNPTNTIFDAKRLIGRKYDOPTVQSDMKHNPFRVVNEGGKPKVQVKYKGEMKTFFPEEI	120

J02579	SSMVLTKMKEIAEAYLGKKVETAVITVPAYFNDSQRQATKDAGTITGLNWMRIINEPTAA	180
LC503773	SSMVLTKMKEIAEAYLGKKVETAVITVPAYFNDSQRQATKDAGTITGLNWMRIINEPTAA	180
LC503774	SSMVLTKMKEIAEAYLGKKVETAVITVPAYFNDSQRQATKDAGTITGLNWMRIINEPTAA	180
LC498496	SSMVLTKMKEIAEAYLGKKVETAVITVPAYFNDSQRQATKDAGTITGLNWMRIINEPTAA	180
LC503772	SSMVLTKMKEIAEAYLGKKVETAVITVPAYFNDSQRQATKDAGTITGLNWMRIINEPTAA	180

J02579	AIAYGLDKKGTRAGEKVLIFDLGGGTDFVSILTEDGIFEVKSTAGDTHLGGEDFDNRM	240
LC503773	AIAYGLDKKGTRAGEKVLIFDLGGGTDFVSILTEDGIFEVKSTAGDTHLGGEDFDNRM	240
LC503774	AIAYGMDDKGTRAGEKVLIFDLGGGTDFVSILTEDGIFEVKSTAGDTHLGGEDFDNRM	240
LC498496	AIAYGLDKKGTRAGEKVLIFDLGGGTDFVSILTEDGIFEVKSTAGDTHLGGEDFDNRM	240
LC503772	AIAYGLDKKGTRAGEKVLIFDLGGGTDFVSILTEDGIFEVKSTAGDTHLGGEDFDNRM	240

J02579	VNRFVEEFKGKHKRDNAGNKR AVRRLRTACERARRTLSSTSQTASIEIDSLFEGIDFYTSI	300
LC503773	VNRFVEEFKGKHKRDNAGNKR AVRRLRTACERARRTLSSTSQTASIEIDSLFEGIDFYTSI	300
LC503774	VNRFVEEFKGKHKRDNAGNKR AVRRLRTACERARRTLSSTSQTASIEIDSLFEGIDFYTSI	300
LC498496	VNRFVEEFKGKHKRDNAGNKR AVRRLRTACERARRTLSSTSQTASIEIDSLFEGIDFYTSI	300
LC503772	VNRFVEEFKGKHKRDNAGNKR AVRRLRTACERARRTLSSTSQTASIEIDSLFEGIDFYTSI	300

J02579	TRARFEELNADLFRGTLEPVKEKALRDAKLDKGQIQEIVLVGGSTRIPKIQKLQLQFFNGK	360
LC503773	TRARFEELNADLFRGTREPVEKALRDAKLDKGQIQEIVLVGGSTRIPKIQKLQLQFFNGK	360
LC503774	TRARFEELNADLFRGTREPVEKALRDAKLDKGQIQEIVLVGGSTRIPKIQKLQLQFFNGK	360
LC498496	TRARFEELNADLFRGTLEPVKEKALRDAKLDKGQIQEIVLVGGSTRIPKIQKLQLQFFNGK	360
LC503772	TRARFEELNADLFRGTLEPVKEKALRDAKLDKGQIQEIVLVGGSTRIPKIQKLQLQFFNGK	360

J02579	ELNIKSINPDEAVAYGAAVQAAILMGDKSENVQDLLLLDVTPSLSGIETAGGVMTALIKRN	420
LC503773	ELNIKSTNPDEAVYGAAVQAAILMGDKSENVQDLLLLDVTPSLSGIETAGGVMTALIKRN	420
LC503774	ELNIKSINPDEAVYGAAVQAAILMGDKSENVQDLLLLDVTPSLSGIETAGGVMTALIKRN	420
LC498496	ELNIKSINPDEAVYGAAVQAAILMGDKSENVQDLLLLDVTPSLSGIETAGGVMTALIKRN	420
LC503772	ELNIKSINPDEAVYGAAVQAAILMGDKSENVQDLLLLDVTPSLSGIETAGGVMTALIKRN	420

J02579	TTIPTKQTQFTTYSNDQSSVLVQVYEGERAMTKDNNLGKFDTLTGIPPAPRGPVQIEVT	480
LC503773	TTIPTKQTQFTTYSNDQSSVLVQVYEGERAMTKDNNLGKFDTLTGIPPAPRGPVQIEVT	480
LC503774	TTIPTKQTQFTTYSNDQSSVLVQVYEGERAMTKDNNLGKFDTLTGIPPAPRGPVQIEVT	480
LC498496	TTIPTKQTQFTTYSNDQSSVLVQVYEGERAMTKDNNLGKFDTLTGIPPAPRGPVQIEVT	480
LC503772	TTIPTKQTQFTTYSNDQSSVLVQVYEGERAMTKDNNLGKFDTLTGIPPAPRGPVQIEVT	480

J02579	FDIODANGILNVSAVDHSTGKENKITITNDKGRLSKDDIDRMVQEAEKYKAEDEAANRDRVG	540
LC503773	FDIODANGILNVSAVDHSTGKENKITITNDKGRLSKDDIDRMVQEAEKYKAEDEAANRDRVG	540
LC503774	FDIODANGILNVSAVDHSTGKENKITITNDKGRLSKDDIDRMVQEAEKYKAEDEAANRDRVG	540
LC498496	FDIODANGILNVSAVDHSTGKENKITITNDKGRLSKDDIDRMVQEAEKYKAEDEAANRDRVG	540
LC503772	FDIODANGILNVSAVDHSTGKENKITITNDKGRLSKDDIDRMVQEAEKYKAEDEAANRDRVG	540

Figure 3: The changes of amino acids in the *hsp70*. J02579: reference gene. LC498496, LC503772, LC503773 and LC503774 are the results sequences of *hsp70* in Layer hens

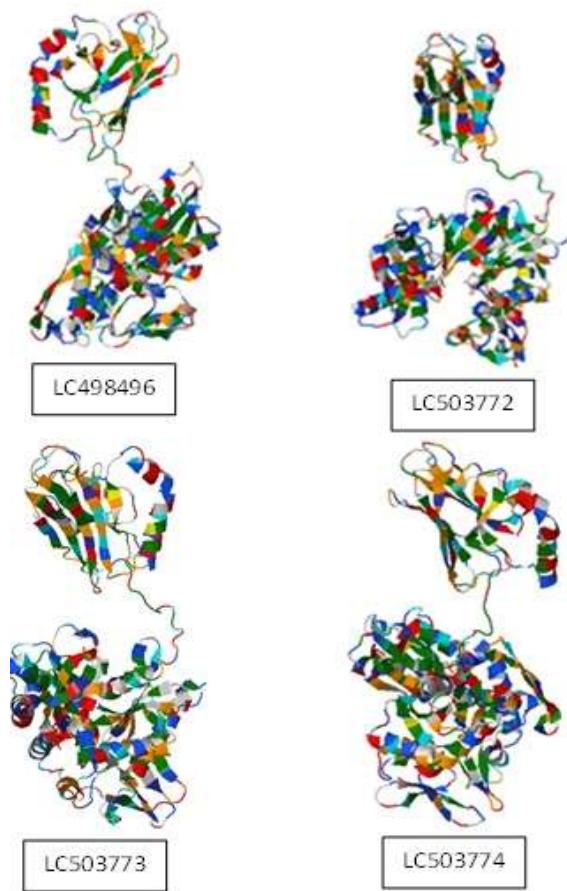


Figure 4: The expected 3D structure of the proteins of *hsp70* gene in Layer hens

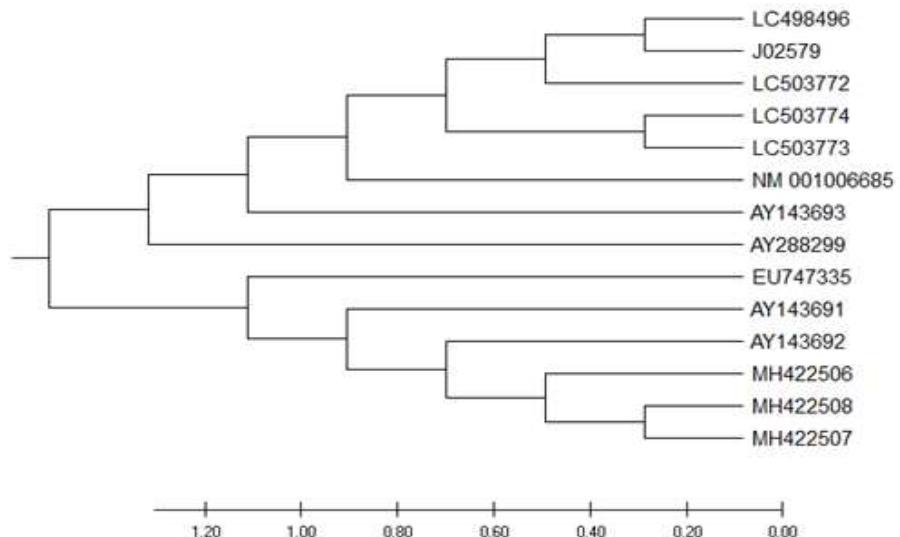


Figure 5: Phylogenetic tree of *hsp70* gene in Layer hens

