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

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*

IOP Conf. Series: Materials Science and Engineering **928** (2020) 062017 doi:10.1088/1757-899X/928/6/062017

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

IOP Conf. Series: Materials Science and Engineering **928** (2020) 062017 doi:10.1088/1757-899X/928/6/062017

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 for45 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]

*hsp*70- F	5'-CGATCTGGCTGCAATCTACG-3'
hsp70- R	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

IOP Conf. Series: Materials Science and Engineering **928** (2020) 062017 doi:10.1088/1757-899X/928/6/062017

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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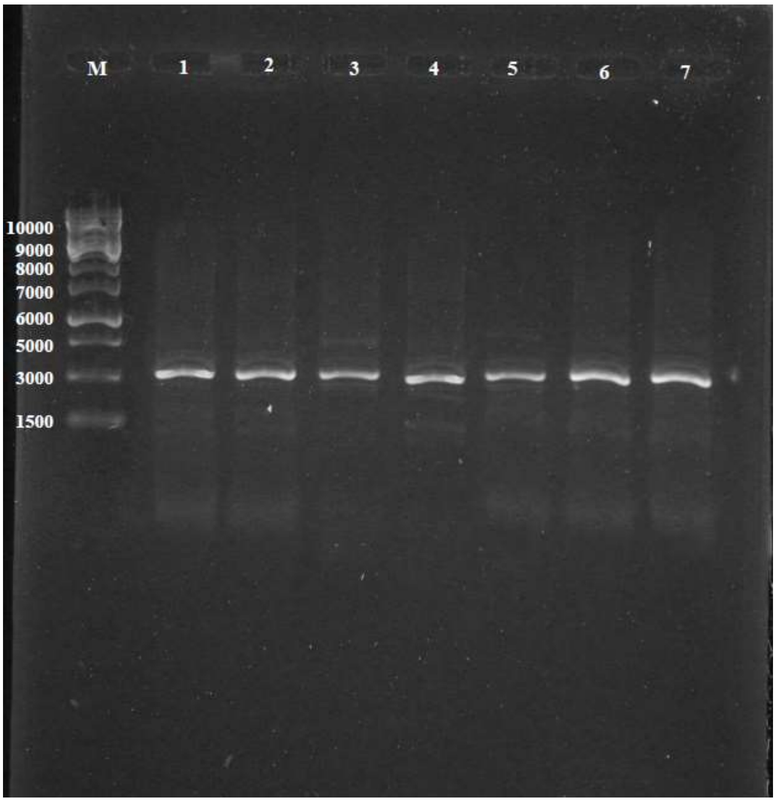

Figure 1: Gel electrophoresis of the PCR amplification to *hsp70* gene, M: 10kb DNA ladder, 1-7: DNA templates

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LC503774     TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAAAAAACAG  60
LC503773     TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAATAAACAG  60
LC503772     TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAAAAAACAG  60
J02579       TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAAAAAACAG  60
LC498496     TTGTGATTGGCTGAGGGGAGTGGCGCAGCGTAGAAAGCGAGACGGATCGAGAAAAAACAG  60
             ***************************************************** ******

LC503774     GAAGAAGCCCGATCTGGCTGCAATCTACGGGAGAGGGTTGGGCTAGAGAGTGGGCGCTAC  120
LC503773     GAAGAAGCCCGATCTGGCTGCAATCTACGGGAGAGGGTTGGGCTAGAGAGTGGGCGCTAC  120
LC503772     GAAGAAGCCCGATCTGGCTGCAATCTACGGGAGAGGGTTGGGCTAGAGAGTGGGCGCTAC  120
J02579       GAAGAAGCCCGATCTGGCTGCAATCTACGGGAGAGGGTTGGGCTAGAGAGTGGGCGCTAC  120
LC498496     GAAGAAGCCCGATCTGGCTGCAATCTACGGGAGAGGGTTGGGCTAGAGAGTGGGCGCTAC  120
             ************************************************************

LC503774     GCTTCTGATTGGGCAGGAGGCAAGGGGCGGGCGCTCTTCGGCTAGTCCGGGAGGCGGATT  180
LC503773     GCTTCTGATTGGGCAGGAGGCAAGGGGCGGGCGCTCTTCGGCTAGTCCGGGAGGCGGATT  180
LC503772     GCTTCTGATTGGGCAGGAGGCAAGGGGCCGGCGCTCTTCGGCTAGTCCGGGAGGCGGATT  180
J02579       GCTTCTGATTGGGCAGGAGGCAAGGGGCGGGCGCTCTTCGGCTAGTCCGGGAGGCGGATT  180
LC498496     GCTTCTGATTGGGCAGGAGGCAAGGGGCGGGCGCTCTTCGGCTAGTCCGGGAGGCGGATT  180
             **************************** *******************************

LC503774     CGGTCAACTGCGGCAGTCGGGTGTCTGGATTGGTCCTTCGCGTTCTGGCAGGTTCCAGAA  240
LC503773     CGGTCAACTGCGGCAGTCGGGTGTCTGGATTGGTCCTTAGCGTTCTGGCAGGTTCCAGAA  240
LC503772     CGGTCAACTGCGGCAGTCGGGTGTCTGGATTGGTCCTTAGCGTTCTGGCAGGTTCCAGAA  240
J02579       CGGTCAACTGCGGCAGTCGGGTGTCTGGATTGGTCCTTAGCGTTCTGGCAGGTTCCAGAA  240
LC498496     CGGTCAACTGCGGCAGTCGGGTGTCTGGATTGGTCCTTAGCGTTCTGGCAGGTTCCAGAA  240
             ************************************** *********************

LC503774     GAAGGCTAAGCGGACTATAAAGAGGGCGCGACGGCCGTAACGGCAGATCGCGCCGCAGAC  300
LC503773     GAAGGCTAAGCGGACTATAAAGAGGGCGCGACGGCCGTAACGGCAGATCGCGCCGCAGAC  300
LC503772     GAAGGCTAAGCGGACTATAAAGAGGGCGCGACGGCCGTAACGGCAGATCGCGCCGCAGAC  300
J02579       GAAGGCTAAGCGGACTATAAAGAGGGCGCGACGGCCGTAACGGCAGATCGCGCCGCAGAC  300
LC498496     GAAGGCTAAGCGGACTATAAAGAGGGCGCGACGGCCGTAACGGCAGATCGCGCCGCAGAC  300
             ************************************************************

LC503774     AGCAGCGAGAAGCGGGCGGAGGAGACGTGACTGCGAGCGAGCAAGTGACTGGCGGAGCGA  360
LC503773     AGCAGCGAGAAGCGGGCGGAGGAGACGTGACTGCGAGCGAGCAAGTGACTGGCGGAGCGA  360
LC503772     AGCAGCGAGAAGCGGGCGGAGGAGACGTGACTGCGAGCGAGCAAGTGACTGGCGGAGCGA  360
J02579       AGCAGCGAGAAGCGGGCGGAGGAGACGTGACTGCGAGCGAGCAAGTGACTGGCGGAGCGA  360
LC498496     AGCAGCGAGAAGCGGGCGGAGGAGACGTGACTGCGAGCGAGCAAGTGACTGGCGGAGCGA  360
             ************************************************************

LC503774     GTGGCTGACTGACCAAGAGGAATCTATCATCATGTCTGGCAAAGGGCCGGCCATCGGCAT  420
LC503773     GTGGCTGACTGACCAAGAGGAATCTATCATCATGTCTGGCAAAGGGCCGGCCATCGGCAT  420
LC503772     GTGGCTGACTGACCAAGAGGAATCTATCATCATGTCTGGCAAAGGGCCGGCCATCGGCAT  420
J02579       GTGGCTGACTGACCAAGAGGAATCTATCATCATGTCTGGCAAAGGGCCGGCCATCGGCAT  420
LC498496     GTGGCTGACTGACCAAGAGGAATCTATCATCATGTCTGGCAAAGGGCCGGCCATCGGCAT  420
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LC503774     CGATCTGGGCACCACGTATTCTTGCGTGGGTGTCTTCCAGCATGGCAAAGTGGAGATCAT  480
LC503773     CGATCTGGGCACCACGTATTCTTGCGTGGGTGTCTTCCAGCATGGCAAAGTGGAGATCAT  480
LC503772     CGATCTGGGCACCACGTATTCTTGCGTGGGTGTCTTCCAGCATGGCAAAGTGGAGATCAT  480
J02579       CGATCTGGGCACCACGTATTCTTGCGTGGGTGTCTTCCAGCATGGCAAAGTGGAGATCAT  480
LC498496     CGATCTGGGCACCACGTATTCTTGCGTGGGTGTCTTCCAGCATGGCAAAGTGGAGATCAT  480
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LC503774     TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGTGGCCTTCACCGATACAGAGCG  540
LC503773     TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGTGGCCTTCACCGATACAGAGCG  540
LC503772     TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGTGGCCTTCACCGATACAGAGCG  540
J02579       TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGTGGCCTTCACCGATACAGAGCG  540
LC498496     TGCCAACGACCAGGGGAACCGCACCACACCCAGCTATGTGGCCTTCACCGATACAGAGCG  540
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LC503774     CCTCATCGGGGATGCTGCCAAGAACCAAGTGGCAATGAACCCCACCAACACCATCTTTGA  600
LC503773     CCTCATCGGGGATGCTGCCAAGAACCAAGTGGCAATGAACCCCACCAACACCATCTTTGG  600
LC503772     CCTCATCGGGGATGCTGCCAAGAACCAAGTGGCAATGAACCCCACCAACACCATCTTTGA  600
J02579       CCTCATCGGGGATGCTGCCAAGAACCAAGTGGCAATGAACCCCACCAACACCATCTTTGA  600
LC498496     CCTCATCGGGGATGCTGCCAAGAACCAAGTGGCAATGAACCCCACCAACACCATCTTTGA  600
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LC503774     TGCCAAGCGTCTCATCGGCCGCAAGTATGATGACCCCACAGTGCAGTCAGACATGAAGCA  660
LC503773     TGCCAAGCGTCTCATCGGCCGCAAGTATGATGACCCCACAGTGCAGTCAGACATGAAGCA  660
LC503772     TGCCAAGCGTCTCATCGGCCGCAAGTATGATGACCCCACAGTGCAGTCAGACATGAAGCA  660
J02579       TGCCAAGCGTCTCATCGGCCGCAAGTATGATGACCCCACAGTGCAGTCAGACATGAAGCA  660
LC498496     TGCCAAGCGTCTCATCGGCCGCAAGTATGATGACCCCACAGTGCAGTCAGACATGAAGCA  660
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LC503774     CTGGCCCTACCGTGTGGTGAACGAGGGTGGCAAGCCCAAGGTGCAGGTGGAGTACAAGGG  720
LC503773     CTGGCCCTTCCGTGTGGTGAACGAGGGTGGCAAGCCCAAGGTGCAGGTGGAGTACAAGGG  720
LC503772     CTGGCCCTTCCGTGTGGTGAACGAGGGTGGCAAGCCCAAGGTGCAGGTGAAGTACAAGGG  720
J02579       CTGGCCCTTCCGTGTGGTGAACGAGGGTGGCAAGCCCAAGGTGCAGGTGGAGTACAAGGG  720
LC498496     CTGGCCCTTCCGTGTGGTGAACGAGGGTGGCAAGCCCAAGGTGCAGGTGAAGTACAAGGG  720
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LC503774     TGAGATGAAGACCTTCTTCCCAGAGGAGATCAGCTCTATGGTGCTCACCAAGATGAAGGA  780
LC503773     TGAGATGAAGACCTTCTTCCCAGAGGAGATCAGCTCTATGGTGCTCACCAAGATGAAGGA  780
LC503772     TGAGATGAAGACCTTCTTCCCAGAGGAGATCAGCTCTATGGTGCTCACCAAGATGAAGGA  780
J02579       TGAGATGAAGACCTTCTTCCCAGAGGAGATCAGCTCTATGGTGCTCACCAAGATGAAGGA  780
LC498496     TGAGATGAAGACCTTCTTCCCAGAGGAGATCAGCTCTATGGTGCTCACCAAGATGAAGGA  780
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LC503774         GATTGCTGAGGCCTATCTGGGAAAAAAGGTAGAGACTGCTGTTATCACAGTGCCCGCTTA  840
LC503773         GATTGCTGAGGCCTATCTGGGAAAAAAGGTAGAGACTGCTGTTATCACAGTGCCCGCTTA  840
LC503772         GATTGCTGAGGCCTATCTGGGAAGAAAGGTAGAGACTGCTTTTATCACAGTGCCCGCTTA  840
J02579           GATTGCTGAGGCCTATCTGGGAAAAAAGGTAGAGACTGCTGTTATCACAGTGCCCGCTTA  840
LC498496         GATTGCTGAGGCCTATCTGGGAAGAAAGGTAGAGACTGCTGTTATCACAGTCCCCGCTTA  840
                 *********************** **************** ********** ********

LC503774         CTTCAACGACTCCCAGCGCCAGGCCACCAAAGATGCTGGCACCATCACTGGGCTTAACGT  900
LC503773         CTTCAACGACTCCCAGCGCCAGGCCACCAAAGATGCTGGCACCATCACTGGGCTTAACGT  900
LC503772         CTTCAACGACTCCCCGCGCCAGGCCACCAAAGATGCTGGCACCATCACTGGGCTTAACGT  900
J02579           CTTCAACGACTCCCAGCGCCAGGCCACCAAAGATGCTGGCACCATCACTGGGCTTAACGT  900
LC498496         CTTCAACGACTCCCAGCGCCAGGCCACCAAAGATGCTGGCACCATCACTGGGCTTAACGT  900
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LC503774         GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGGCATGGATAAGAAAGG  960
LC503773         GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGGCATGGATAAGAAAGG  960
LC503772         GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGGCTTGGATAAGAAAGG  960
J02579           GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGGCTTGGATAAGAAAGG  960
LC498496         GATGCGTATTATCAATGAGCCCACAGCAGCTGCTATTGCCTATGGCTTGGATAAGAAAGG  960
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LC503774         TACCCGGGCTGGAGAGAAGAATGTGCTCATCTTTGACTTGGGAGGGGGCACTTTTGATGT  1020
LC503773         TACCCGGGCTGGAGAGAAGAATGTGCTCATCTTTGACTTGGGAGGGGGCACTTTTGATGT  1020
LC503772         TACCCGGGCTGGAGAGAAGAATGTGCTCATCTTTGACTTGGGAGGGGGCACTATTGATGT  1020
J02579           TACCCGGGCTGGAGAGAAGAATGTGCTCATCTTTGACTTGGGAGGGGGCACTTTTGATGT  1020
LC498496         TACCCGGGCTGGAGAGAAGAATGTGCTCATCTTTGACTTGGGAGGGGGCACTATTGATGT  1020
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LC503774         GTCCATCCTTACCATTGAGGATGGCATCTTTGAGGTGAAGTCCACAGCTGGGGACACCCA  1080
LC503773         GTCCATCCTTACCATTGAGGATGGCATCTTTGAGGTGAAGTCCACAGCTGGGGACACCCA  1080
LC503772         GTCCATCCTTACCATTGAGGATGGCATCTTTGAGGTGAAGTCCACAGCTGGGGACACCCA  1080
J02579           GTCCATCCTTACCATTGAGGATGGCATCTTTGAGGTGAAGTCCACAGCTGGGGACACCCA  1080
LC498496         GTCCATCCTTACCATTGAGGATGGCATCTTTGAGGTGAAGTCCACAGCTGGGGACACCCA  1080
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LC503774         CCTAGGTGGGGAGGACTTTGACAACCGCATGGTAAACCGTTTTGTAGAAGAGTTCAAGGG  1140
LC503773         CCTAGGTGGGGAGGACTTTGACAACCGCATGGTAAACCGTTTTGTAGAAGAGTTCAAGGG  1140
LC503772         CCTAGGTGGGGAGGACTTTGACAACCGCATGGTAAACCGTTTTGTAGAAGAGTTCAAGGG  1140
J02579           CCTAGGTGGGGAGGACTTTGACAACCGCATGGTAAACCGTTTTGTAGAAGAGTTCAAGGG  1140
LC498496         CCTAGGTGGGGAGGACTTTGACAACCGCATGGTAAACCGTTTTGTAGAAGAGTTCAAGGG  1140
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LC503774         TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCGTCTGCGTACAGCTTG  1200
LC503773         TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCGTCTGCGTACAGCTTG  1200
LC503772         TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCGTCTGCGTACAGCTTG  1200
J02579           TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCGTCTGCGTACAGCTTG  1200
LC498496         TAAGCACAAGCGTGACAATGCTGGCAATAAGCGAGCAGTGAGGCGTCTGCGTACAGCTTG  1200
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LC503774         TGAGAGGGCGAGGCGTACTCTGAGCTCTTCCACGCAAGCCAGCATTGAGATTGACTCCCT  1260
LC503773         TGAGAGGGCGAGGCGTACTCTGAGCTCTTCCACGCAAGCCAGCATTGAGATTGACTCCCT  1260
LC503772         TGAGAGGGCGAGGCGTACTCTGAGCTCTTCCACGCAAGCCAGCATTGAGATTGACTCCCT  1260
J02579           TGAGAGGGCGAGGCGTACTCTGAGCTCTTCCACGCAAGCCAGCATTGAGATTGACTCCCT  1260
LC498496         TGAGAGGGCGAGGCGTACTCTGAGCTCTTCCACGCAAGCCAGCATTGAGATTGACTCCCT  1260
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LC503774         CTTTGAGGGCATTGACTTCTACACCTCCATCACTCGTGCCCGCTTTGAGGAACTCAATGC  1320
LC503773         CTTTGAGGGCATTGACTTCTACACCTCCATCACTCGTGCCCGCTTTGAGGAACTCAATGC  1320
LC503772         CTTTGAGGGCATTGACTTCTACACCTCCATCACTCGTGCCCGCTTTGAGGAACTCAATGC  1320
J02579           CTTTGAGGGCATTGACTTCTACACCTCCATCACTCGTGCCCGCTTTGAGGAACTCAATGC  1320
LC498496         CTTTGAGGGCATTGACTTCGACACCTCCATCACTCGTGCCCGCTTTGAGGAACTCAATGC  1320
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LC503774         TGATCTTTTCCGTGGTACCCGGGAGCCAGTGGAGAAGGCCCTGCGTGATGCCAAGCTTGA  1380
LC503773         TGATCTTTTCCGTGGTACCCGGGAGCCAGTGGAGAAGGCCCTGCGTGATGCCAAGCTTGA  1380
LC503772         TGATCTTTTCCGTGGTACCCTGGAGCCAGTGGAGAAGGCCCTGCGTGATGCCAAGCTTGA  1380
J02579           TGATCTTTTCCGTGGTACCCTGGAGCCAGTGGAGAAGGCCCTGCGTGATGCCAAGCTTGA  1380
LC498496         TGATCTTTTCCGTGGTACCCTGGAGCCAGTGGAGAAGGCCCTGCGTGATGCCAAGCTTGA  1380
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LC503774         TAAGGGCCAGATCCAGGAGATTGTGCTTGTGGGGGGCTCCACTCGTATTCCTAAGATCCA  1440
LC503773         TAAGGGCCAGATCCAGGAGATTGTGCTTGTGGGGGGCTCCACTCGTATTCCTAAGATCCA  1440
LC503772         TAAGGGCCAGATCCAGGAGATTGTGCTTGTGGGGGGCTCCACTCGTATTGCTAAGATCCA  1440
J02579           TAAGGGCCAGATCCAGGAGATTGTGCTTGTGGGGGGCTCCACTCGTATTCCTAAGATCCA  1440
LC498496         TAAGGGCCAGATCCAGGAGATTGTGCTTGTGGAGGGCTCCACTCGTATTGCTAAGATCCA  1440
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LC503774         GAAGTTGCTGCAAGATTTCTTCAATGGCAAAGAGCTGAACAAGAGCATCAATCCAGATGA  1500
LC503773         GAAGTTGCTGCAAGATTTCTTCAATGGCAAAGAGCTGAACAAGAGCACCAATCCAGATGA  1500
LC503772         GAAGTTGCTGCAAGATTTCTTCAATGGCAAAGAGCTGAACAAGAGCATCAATCCAGATGA  1500
J02579           GAAGTTGCTGCAAGATTTCTTCAATGGCAAAGAGCTGAACAAGAGCATCAATCCAGATGA  1500
LC498496         GAAGTTGCTGCAAGATTTCTTCAATGGCAAAGAGCTGAACAAGAGCATCAATCCAGATGA  1500
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LC503774         AGCTGTTCCTTATGGTGCCGCTGTGCAAGCAGCTATCCTCATGGGAGACAAGTCTGAAAA  1560
LC503773         AGCTGTTCCTTATGGTGCCGCTGTGCAAGCTGCTATCCTCATGGGAGACAAGTCTGAAAA  1560
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LC503772      AGCTGTTGCTTATGGTGCCGCTGTGCAAGCAGCTATCCTCATGGGAGACAAGTCTGAAAA   1560
J02579        AGCTGTTGCTTATGGTGCCGCTGTGCAAGCAGCTATCCTCATGGGAGACAAGTCTGAAAA   1560
LC498496      AGCTGTTGCTTATGGTGCCGCTGTGCAAGCAGCTATCCTCATGGGAGACAAGTCTGAAAA   1560
              ******* ********************** *****************************

LC503774      TGTGCAAGATCTGCTCCTGCTGGATGTCACCCCCCTGTCCCTGGGCATCGAGACAGCTGG   1620
LC503773      TGTGCAAGATCTGCTCCTGTTGGATGTCACCCCCCTGTCCCTGGGCATCGAGACAGCTGG   1620
LC503772      TGTGCAAGATCTGCTCCTGTTGGATGTCACCCCCCTGTCCCTGGGCATCGAGACAGCTGG   1620
J02579        TGTGCAAGATCTGCTCCTGTTGGATGTCACCCCCCTGTCCCTGGGCATCGAGACAGCTGG   1620
LC498496      TGTGCAAGATCTGCTCCTGTTGGATGTCACCCCCCTGTCCCTGGGCATCGAGACAGCTGG   1620
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LC503774      TGGAGTGATGACTGCTCTCATCAAGCGTAACACCACCATTCCCACCAAACAAACACAGAC   1680
LC503773      TGGAGTGATGACTGCTCTCATCAAGCGTAACACCACCATTCCCACCAAACAAACACAGAC   1680
LC503772      TGGAGTGATGACTGCTCTCATCAAGCGTAACACCACCATTCCCACCAAACAAACACAGAC   1680
J02579        TGGAGTGATGACTGCTCTCATCAAGCGTAACACCACCATTCCCACCAAACAAACACAGAC   1680
LC498496      TGGAGTGATGACTGCTCTCATCAAGCGTAACACCACCATTCCCACCAAACAAACACAGAC   1680
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LC503774      CTTCACCACCTACTCGGACAACCAGAGCAGTGTCCTCGTCCAGGTGTATGAAGGTGAGAG   1740
LC503773      CTTCACCACCTACTCAGACAACCAGAGCAGTGTCCTCGTCCAGGTGTATGAAGGTGAGAG   1740
LC503772      CTTCACCACCTACTCAGACAACCAGAGCAGTGTCCTCGTCCAGGTGTATGAAGGTGAGAG   1740
J02579        CTTCACCACCTACTCAGACAACCAGAGCAGTGTCCTCGTCCAGGTGTATGAAGGTGAGAG   1740
LC498496      CTTCACCACCTACGCAGACAACCAGAGCAGTGTCCTCGTCCAGGTGTATGAAGGTGAGAG   1740
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LC503774      GGCTATGACAAAGGACAACAACTTGCTGGGCAAGTTTGACCTAACAGGCATCCCCCCGGC   1800
LC503773      GGCTATGACAAAGGACAACAACTTGCTGGGCAAGTTTGACCTAACAGGCATCCCCCCGGC   1800
LC503772      GGCTATGACAAAGGACAACAACTTGCTGGGCAAGTTTGACCTAACAGGCATCCCCCCGGC   1800
J02579        GGCTATGACAAAGGACAACAACTTGCTGGGCAAGTTTGACCTAACAGGCATCCCCCCGGC   1800
LC498496      GGCTATGACAAAGGACAACAACTTGCTGGGCAAGTTTGACCTAACAGGCATCCCCCCGGC   1800
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LC503774      ACCCCGTGGAGTTCCTCAGATCGAGGTCACTTTTGACATAGATGCTAATGGTATCCTGAA   1860
LC503773      ACCCCGTGGAGTTCCTCAGATCGAGGTCACTTTTGACATAGATGATAATGGTATCCTGAA   1860
LC503772      ACCCCGTGGAGTTCCTCAGATCGAGGTCACTTTTGACATAGATGCTAATGGTATCCTGAA   1860
J02579        ACCCCGTGGAGTTCCTCAGATCGAGGTCACTTTTGACATAGATGCTAATGGTATCCTGAA   1860
LC498496      ACCCCGTGGAGTTCCTCAGATCGAGGTCACTTTTGACATAGATGCTAATGGTATCCTGAA   1860
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LC503774      CGTCAGTGCTGTGGACAAGAGTACAGGGAAGGAGAACAAGATACCCATCACCAATGACAA   1920
LC503773      CGTCAGTGCTGTGGACAAGAGTACAGGGAAGGAGAACAAGATAACCATCACCAATGACAA   1920
LC503772      CGTCAGTGCTGTGGACAAGAGTACAGGGAAGGAGAACAAGATAACCATCACCAATGACAA   1920
J02579        CGTCAGTGCTGTGGACAAGAGTACAGGGAAGGAGAACAAGATAACCATCACCAATGACAA   1920
LC498496      CGTCAGTGCTGTGGACAAGAGTACAGGGAAGGAGAACAAGATAACCATCACCAATGACAA   1920
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LC503774      GGGTCGCCTTAGCAAAGATGATATTGACCGTATGGTACAAGAAGCAGAGAAATACAAAGC   1980
LC503773      GGGTCGCCTTAGCAAAGATGATATTGACCGTATGGTACAAGAAGCAGAGAAATACAAAGC   1980
LC503772      GGGTCGCCTTAGCAAAGATGATATTGACCGTATGGTACAAGAAGCAGAGAAATACAAAGC   1980
J02579        GGGTCGCCTTAGCAAAGATGATATTGACCGTATGGTACAAGAAGCAGAGAAATACAAAGC   1980
LC498496      GGGTCGCCTTAGCATAGATGATATTGACCGTATGGTACAAGAAGCAGAGAAATACAAAGC   1980
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LC503774      AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTTGAGTCGTATACTTA   2040
LC503773      AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTTGAGTCGTATACTTA   2040
LC503772      AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTTGAGTCGTATACTTA   2040
J02579        AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTTGAGTCGTATACTTA   2040
LC498496      AGAGGATGAAGCCAACAGAGATAGGGTGGGAGCCAAGAACTCCCTTGAGTCGTATACTTA   2040
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LC503774      CAACATGAAGCAGACAGTGGAGGATGAGAAACTGAAGGGAAAGATCAGTGACCAGGACAA   2100
LC503773      CAACATGAAGCAGACAGTGGAGGATGAGAAACTGAAGGGAAAGATCAGTGACCAGGACAA   2100
LC503772      CAACATGAAGCAGACAGTGGAGGATGAGAAACTGAAGGGAAAGATCAGTGACCAGGACAA   2100
J02579        CAACATGAAGCAGACAGTGGAGGATGAGAAACTGAAGGGAAAGATCAGTGACCAGGACAA   2100
LC498496      CAACATGAAGCAGACAGTGGAGGATGAGAAACTGAAGGGAAAGATCAGTGACCAGGACAA   2100
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LC503774      GCAGAAAGTGCTCGACAAGTGCCAGGAGGTGATCAGTTCGCTTGACCGAAACCAGATGGC   2160
LC503773      GCAGAAAGTGCTCGACAAGTGCCAGGAGGTGATCAGTTCGCTTGACCGAAACCAGATGGC   2160
LC503772      GCAGAAAGTGCTCGACAAGTGCCAGGAGGTGATCAGTTCGCTTGACCGAAACCAGATGGC   2160
J02579        GCAGAAAGTGCTCGACAAGTGCCAGGAGGTGATCAGTTCGCTTGACCGAAACCAGATGGC   2160
LC498496      GCAGAAAGTGCTCGACAAGTGCCAGGAGGTGATCAGTTCGCTTGACCGAAACCAGATGGC   2160
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LC503774      AGAGAAAGAAGAGTATGAGCACAAGCAGAAAGAGCTGGAGAAACTCTGCAACCCGATTGT   2220
LC503773      AGAGAAAGAAGAGTATGAGCACAAGCAGAAAGAGCTGGAGAAACTCTGCAACCCGATTGT   2220
LC503772      AGAGAAAGAAGAGTATGAGCACAAGCAGAAAGAGCTGGAGAAACTCTGCAACCCGATTGT   2220
J02579        AGAGAAAGAAGAGTATGAGCACAAGCAGAAAGAGCTGGAGAAACTCTGCAACCCGATTGT   2220
LC498496      AGAGAAAGAAGAGTATGAGCACAAGCAGAAAGAGCTGGAGAAACTCTGCAACCCGATTGT   2220
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LC503774      CACAAAACTGTACCAGGGAGCTGGAGGAGCTGGGGCAGGTGGCTCCGGTGGCCCAACCAT   2280
LC503773      CACAAAACTGTACCAGGGAGCTGGAGGAGCTGGGGCAGGTGGCTCCGGTGGCCCAACCAT   2280
LC503772      CACAAAACTGTACCAGGGAGCTGGAGGAGCTGGGGCAGGTGGCTCCGGTGGCCCAACCAT   2280
J02579        CACAAAACTGTACCAGGGAGCTGGAGGAGCTGGGGCAGGTGGCTCCGGTGGCCCAACCAT   2280
LC498496      CACAAAACTGTACCAGGGAGCTGGAGGAGCTGGGGCAGGTGGCTCCGGTGGCCCAACCAT   2280
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LC503774      TGAAGAAGTAGATTAAAAAGACTCTTAAACTATAGACTGGTTTATGGACAGTCACTCGAT  2340
LC503773      TGAAGAAGTAGATTAAAAAGACTCTTAAACTATAGACTGGTTTATGGACAGTCACTCCAT  2340
LC503772      TGAAGAAGTAGATTAAAAAGACTCTTAAACTATAGACTGGTTTATGGACAGTCACTCCAT  2340
J02579        TGAAGAAGTAGATTAAAAAGACTCTTAAACTATAGACTGGTTTATGGACAGTCACTCCAT  2340
LC498496      TGAAGAAGTAGATTAAAAAGACTCTTAAACTATAGACTGGTTTATGGACAGTCACTCCAT  2340
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LC503774      TCTTTGCTTTATATTTTATTCTAACGTTTAAGGAAAAACGTCATTGCCAATAACAGAGTT  2400
LC503773      TCTTTGCTTTATATAATTTTCTAACGTTTAAGGAAAAACGTCATTGCCAATAACAGAGTT  2400
LC503772      TCTTTGCTTTATATTTTTTTCTAACGTTTAAGGAAAAACGTCATTGCCAATAACAGAGTT  2400
J02579        TCTTTGCTTTATATTTTTTTCTAACGTTTAAGGAAAAACGTCATTGCCAATAACAGAGTT  2400
LC498496      TCTTTGCTTTATATTTTTTTCTAACGTTTAAGGAAAAACGTCATTGCCAATAACAGAGTT  2400
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LC503774      TATTCTGTTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTGGTTTTGATAAAAGGGAAG  2460
LC503773      TATTCTGTTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTGGTTTTGATAAAAGGGAAG  2460
LC503772      TATTCTGTTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTGGTTTTGATAAAAGGGAAG  2460
J02579        TATTCTGTTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTGGTTTTGATAAAAGGGAAG  2460
LC498496      TATTCTGTTGGGTGTGTATAAAGGCAAATCTATCAGCTTGTGGTTTTGATAAAAGGGAAG  2460
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LC503774      GCACGTCCTGCTTTATAAGGTTAGTAATAGACAAGTTTTGTTAATTCAGATACAGCTCCT  2520
LC503773      GCACGTCCTGCTTTATAAGGTTAGTAATAGACAAGTTTTGTTAATTCAGATACAGCTCCT  2520
LC503772      GCACGTCCTGCTTTATAAGGTTAGTAATAGACAAGTTTTGTTAATTCAGATACAGCTCCT  2520
J02579        GCACGTCCTGCTTTATAAGGTTAGTAATAGACAAGTTTTGTTAATTCAGATACAGCTCCT  2520
LC498496      GCACGTCCTGCTTTATAAGGTTAATAATAGACAAGTTTTGTTAATTCAGATACAGCTCCT  2520
              *********************** ************************************

LC503774      TGTATTCTGGATGTTTGTCTCTGTTTAAATGTCTCTTCTAAAGTAACCACTCGACTGTTG  2580
LC503773      TGTATTCTGGATGTTTGTCTCTGTTTAAATGTCTCTTCTAAAGTAACCACTCGACTGTTG  2580
LC503772      TGTATTCTGGATGTTTGTCTCTGTTTAAATGTCTCTTCTAAAGTAACCACTCGACTGTTG  2580
J02579        TGTATTCTGGATGTTTGTCTCTGTTTAAATGTCTCTTCTAAAGTAACCACTCGACTGTTG  2580
LC498496      TGTATTCTGGATGTTTGTCTCTGTTTAAATGTCTCTTCTAAAGTAACCACTCGACTGTTG  2580
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LC503774      CAGTTGACAAGTTTCAAGTTATGCTAGGAAAAAAAAAACTTTGTGAAAGATGAGAAATGC  2640
LC503773      CAGTTGACAAGTTTCAAGTTATGCTAGGAAAAAAAAAACTTTGTGAAAGATGAGAAATGC  2640
LC503772      CAGTTGACAAGTTTCAAGTTATGCTAGGAAAATAAAAACTTTGTGAAAGATGCGAAATGC  2640
J02579        CAGTTGACAAGTTTCAAGTTATGCTAGGAAAAAAAAAACTTTGTGAAAGATGAGAAATGC  2640
LC498496      CAGTTGACAAGTTTCAAGTTATGCTAGGAAAAAAAAAACTTTGTGAAAGATGAGAAATGC  2640
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LC503774      CAAGTGCAGCTTCTGGAAATTTTGGTAATAAATAAAATTTATTTGGGGATCC          2692
LC503773      CAAGTGCAGCTTCTGGAAATTTTGGTAATAAATAAAATTTATTTGGGGATCC          2692
LC503772      CAAGTGCAGCTTCTGGAAAATTTGGTAATAAATAAAATTTATTTGGGGATCC          2692
J02579        CAAGTGCAGCTTCTGGAAATTTTGGTAATAAATAAAATTTATTTGGGGATCC          2692
LC498496      CAAGTGCAGCTTCTGGAAATTTTGGTAATAAATAAAATTTATTTGGGGATCC          2692
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Figure 2: The multiple sequence alignment (MSA) of *hsp70* gen in Layer hens

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J02579      AMNPTNTIFDAKRLIGRKYDDPTVQSDMKHWPFRVVNEGGKPKVQVEYKGEMKTFFPEEI   120
LC503773    AMNPTNTIFGAKRLIGRKYDDPTVQSDMKHWPFRVVNEGGKPKVQVEYKGEMKTFFPEEI   120
LC503774    AMNPTNTIFDAKRLIGRKYDDPTVQSDMKHWPYRVVNEGGKPKVQVEYKGEMKTFFPEEI   120
LC498496    AMNPTNTIFDAKRLIGRKYDDPTVQSDMKHWPFRVVNEGGKPKVQVKYKGEMKTFFPEEI   120
LC503772    AMNPTNTIFDAKRLIGRKYDDPTVQSDMKHWPFRVVNEGGKPKVQVKYKGEMKTFFPEEI   120
            *********.**********************:*************:*************

J02579      SSMVLTKMKEIAEAYLGKKVETAVITVPAYFNDSQRQATKDAGTITGLNVMRIINEPTAA   180
LC503773    SSMVLTKMKEIAEAYLGKKVETAVITVPAYFNDSQRQATKDAGTITGLNVMRIINEPTAA   180
LC503774    SSMVLTKMKEIAEAYLGKKVETAVITVPAYFNDSQRQATKDAGTITGLNVMRIINEPTAA   180
LC498496    SSMVLTKMKEIAEAYLGRKVETAVITVPAYFNDSQRQATKDAGTITGLNVMRIINEPTAA   180
LC503772    SSMVLTKMKEIAEAYLGRKVETAFITVPAYFNDSPRQATKDAGTITGLNVMRIINEPTAA   180
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J02579      AIAYGLDKKGTRAGEKNVLIFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRM   240
LC503773    AIAYGMDKKGTRAGEKNVLIFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRM   240
LC503774    AIAYGMDKKGTRAGEKNVLIFDLGGGTFDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRM   240
LC498496    AIAYGLDKKGTRAGEKNVLIFDLGGGTIDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRM   240
LC503772    AIAYGLDKKGTRAGEKNVLIFDLGGGTIDVSILTIEDGIFEVKSTAGDTHLGGEDFDNRM   240
            *****:*********************.********************************

J02579      VNRFVEEFKGKHKRDNAGNKRAVRRLRTACERARRTLSSSTQASIEIDSLFEGIDFYTSI   300
LC503773    VNRFVEEFKGKHKRDNAGNKRAVRRLRTACERARRTLSSSTQASIEIDSLFEGIDFYTSI   300
LC503774    VNRFVEEFKGKHKRDNAGNKRAVRRLRTACERARRTLSSSTQASIEIDSLFEGIDFYTSI   300
LC498496    VNRFVEEFKGKHKRDNAGNKRAVRRLRTACERARRTLSSSTQASIEIDSLFEGIDFDTSI   300
LC503772    VNRFVEEFKGKHKRDNAGNKRAVRRLRTACERARRTLSSSTQASIEIDSLFEGIDFYTSI   300
            ******************************************************** ***

J02579      TRARFEELNADLFRGTLEPVEKALRDAKLDKGQIQEIVLVGGSTRIPKIQKLLQDFFNGK   360
LC503773    TRARFEELNADLFRGTREPVEKALRDAKLDKGQIQEIVLVGGSTRIPKIQKLLQDFFNGK   360
LC503774    TRARFEELNADLFRGTREPVEKALRDAKLDKGQIQEIVLVGGSTRIPKIQKLLQDFFNGK   360
LC498496    TRARFEELNADLFRGTLEPVEKALRDAKLDKGQIQEIVLVEGSTRIAKIQKLLQDFFNGK   360
LC503772    TRARFEELNADLFRGTLEPVEKALRDAKLDKGQIQEIVLVGGSTRIAKIQKLLQDFFNGK   360
            **************** *********************** ***** *************

J02579      ELNKSINPDEAVAYGAAVQAAILMGDKSENVQDLLLLDVTPLSLGIETAGGVMTALIKRN   420
LC503773    ELNKSTNPDEAVPYGAAVQAAILMGDKSENVQDLLLLDVTPLSLGIETAGGVMTALIKRN   420
LC503774    ELNKSINPDEAVPYGAAVQAAILMGDKSENVQDLLLLDVTPLSLGIETAGGVMTALIKRN   420
LC498496    ELNKSINPDEAVAYGAAVQAAILMGDKSENVQDLLLLDVTPLSLGIETAGGVMTALIKRN   420
LC503772    ELNKSINPDEAVAYGAAVQAAILMGDKSENVQDLLLLDVTPLSLGIETAGGVMTALIKRN   420
            ***** ****** ***********************************************

J02579      TTIPTKQTQTFTTYSDNQSSVLVQVYEGERAMTKDNNLLGKFDLTGIPPAPRGVPQIEVT   480
LC503773    TTIPTKQTQTFTTYSDNQSSVLVQVYEGERAMTKDNNLLGKFDLTGIPPAPRGVPQIEVT   480
LC503774    TTIPTKQTQTFTTYSDNQSSVLVQVYEGERAMTKDNNLLGKFDLTGIPPAPRGVPQIEVT   480
LC498496    TTIPTKQTQTFTTYADNQSSVLVQVYEGERAMTKDNNLLGKFDLTGIPPAPRGVPQIEVT   480
LC503772    TTIPTKQTQTFTTYSDNQSSVLVQVYEGERAMTKDNNLLGKFDLTGIPPAPRGVPQIEVT   480
            **************.*********************************************

J02579      FDIDANGILNVSAVDKSTGKENKITITNDKGRLSKDDIDRMVQEAEKYKAEDEANRDRVG   540
LC503773    FDIDDNGILNVSAVDKSTGKENKITITNDKGRLSKDDIDRMVQEAEKYKAEDEANRDRVG   540
LC503774    FDIDANGILNVSAVDKSTGKENKIPITNDKGRLSKDDIDRMVQEAEKYKAEDEANRDRVG   540
LC498496    FDIDANGILNVSAVDKSTGKENKITITNDKGRLSIDDIDRMVQEAEKYKAEDEANRDRVG   540
LC503772    FDIDANGILNVSAVDKSTGKENKITITNDKGRLSKDDIDRMVQEAEKYKAEDEANRDRVG   540
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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

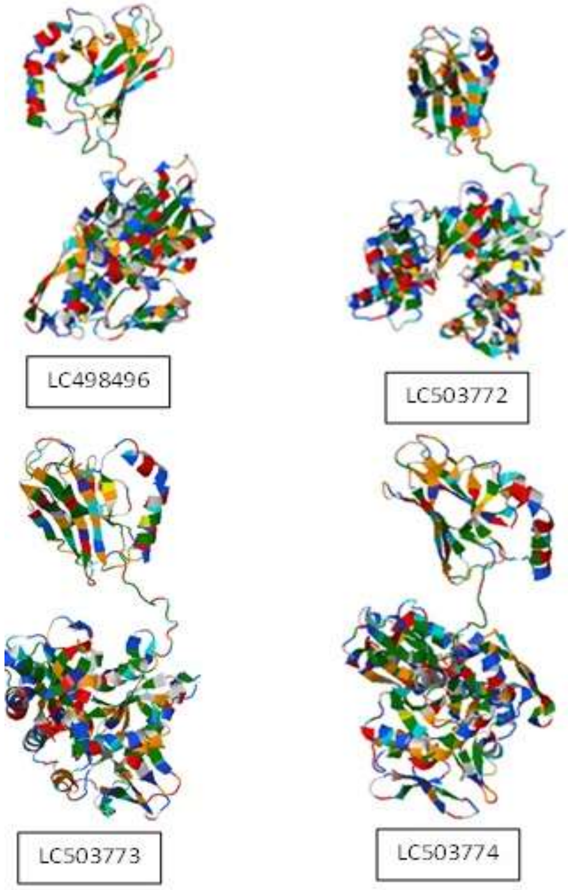

Figure 4: The expected 3D structure of the proteins of *hsp70* gene in Layer hens

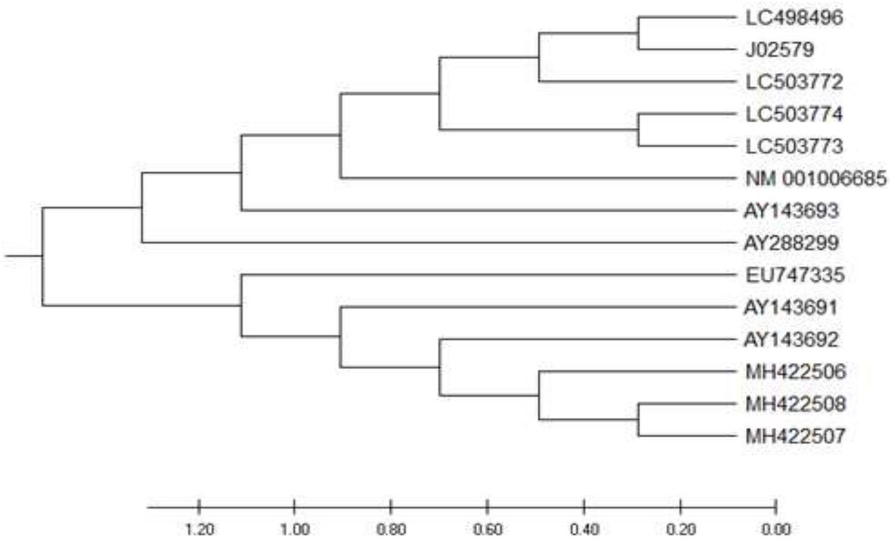

Figure 5: Phylogenetic tree of *hsp70* gene in Layer hens