Banat's University of Agricultural Sciences and Veterinary Medicine from Timisoara,
Department of Exact Sciences,
Contact: web: http://dse.usab-tm.ro, e-mail: secretariat_dse@usab-tm.ro

INVESTIGATION AND IDENTIFICATION *SALMO TRUTTA CASPIUS* BY THE GROWTH HORMONE TYPE 1 (GH1) GENE ANALOGUE

Abolhasan Rezaei[1], Sheyda Akhshabi[2], Hamid Reza Jamalzadeh[3]

[1] Department of Genetics-School of basic science, Islamic Azad University, Tonekabon Branch, Iran, a.rezaei@tonekaboniau.ac.ir
[2] Young Researchers Club, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran,
[3] Department of Fisheries Sciences and Marine Biology-School of basic science, Islamic Azad University Tonekabon Branch, Iran.

Abstract. The genetic growth hormone GH) relationships between several *salmon* and *Salmo trutta caspius* were analyzed by the sequencing technique. The GH gene is concluded by duplicated, isophorms determined as GH1 and GH2. In *salmons* GH gene 1 has 5 exons and 6 introns. At this study we have isolated and characterized GH1 gene containing 3.1 kbp. In related to, have designed one pair of primers from full length of GH gene was reported in the NCBI Network system (Accession nos. AY614010 [RYYNANEN and PRIMMER et al., 2004] and X61938 [MALE et al., 1992]), DNA genomic extracted by absorption on the glass (kit) method, we performed PCR polymorphism and the sequences of the gene were also identified, annotated and compared to each other in their coding regions and function them. We show that regions have compared between other populations of salmons by NCBI Network program. These results also were shown that high homology between Growth hormone gene in *salmo trutta caspius* and other *salmons* was recorded (approximately 97%).

Key words**:** *Salmo trutta caspius*, *Salmo salar*, Growth hormone gene type 1, DNA

Introduction

The *Salmo trutta caspius* is the most widely distributed freshwater fish native to different region [UZBEKOVA, 2000]. It naturally occurs in many different, racially distinct forms throughout Europe, the Middle East, western Asia, and parts of North Africa. From north to south, its range extends from northern Norway and north-eastern Russia, to the Atlas Mountains of North Africa. Other trout species have been also investigated. In Iran, *Salmo trutta caspius* is very important, because these fishes have been very rarely in Caspian Sea and around of Rivers it. So, we decided that research about it. Therefore we discussed about so much genes in *salmo trutta caspius*. in related to we selected Growth hormone gene, because this gene not only is necessary for regulated of growth, but also is very important for finding ancestry of salmons [AGELLON et al., 1988; RENTIER-DELRUE et al., 1989; FORBES et al. 1994].

In mammals, Growth hormone gene is encoded by a single-copy gene containing nine exons [GOUDET et al., 1996]. In fish, this gene contains an additional exon and is present as a double copy, GHR-I and -II [BILLINGTON et al., 1991; MALE et al., 1992; GROSS et al., 1995] are both actively transcribed, but their expression is not equally partitioned exhibiting some tissue-specificity, with GHR-I more actively expressed in liver and adipose tissue than GHR-II [PETER et al., 1995]. In the *Salmonid* fishes have two type GH genes (type 1 and type 2) [AGELLON et al., 1988; RENTIER DELRUE et al., 1989; FORBES et al. 1994]. These two type genes is common ancestor that double its entire genome through tetraploidy an estimated 25 – 50 million years ago. We showed about GH gene for finding polymorphism in *salmo trutta caspius*. in this project, first, we designed one pair of primer from full length of the GH1 gene's *salmo salar* (Accession nos. AY614010 [RYYNANEN and PRIMMER et al., 2004] and X61938 [MALE et al., 1992]), for the sequencing. So, we found full length polymorphism in amplification products of the GH1 gene homologue in the *salmo trutta caspius* detected polymorphism within a full length of approximately 3.1 kb on the gel electrophoresis. These results showed that there were high identity within population's *salmo trutta caspius* and other *salmons*.

Materials and Methods

Were caught adult *salmons* (The fishes has almost three years old ages) from Rivers of

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north of Iran. They were anaesthetized with 50 mg of MS222 powder.

A. DNA extraction and PCR reaction

Total DNA was extracted with phenol-chloroform and absorption on fiber glass (Roche company kit) methods. Approximately, samples of different including, bloods, fins and muscles that fins and muscles were snap frozen in liquid nitrogen and ground to a fine powder with a precooled pestle and mortar.

B. Primers: We designed one pair of primers for amplification DNA of Growth hormone gene with the BLASTn on the NCBI Network service. This pair of primer including,

Forward Primer:
5´-ACCTACACAACCGACCACCGCACTTTCAAG-3´
Reverse Primer:
5´-TCAATGCAGGGCAAGGCCTAATCTG-3´

These primers could amplify full length of almost 3.1 kb. To amplify and sequence homologous growth hormone gene from Salmo trutta caspius. Also, were used in a PCR reaction with four pair of primers, forward and reverse growth hormone gene analogue by the NCBI Network. The DNA purified from the agarose was obtained by high pure PCR product purification kit. The final elution was dried. Also fragments were sent to Chromous Geni Company-India, for sequencing by Sanger method.

C. PCR Amplification:

Designing primers: We designed one pair of primers for amplification of DNA of Growth hormone gene. These primers could amplify fragment, 3.1 kbp. **PCR reaction**: Were used in a PCR reaction, from 10X ChromTaq Assay buffer 5.0 µl, Template DNA 1.0 µl, Forward primer (100ng/ ml) 2.0 µl, Reverse primer (100ng/ ml) 2.0 µl, dNTP mix (2.5mM each) 2.0 µl, 10X ChromTaq Assay buffer (5.0 µl), ChromTaq enzyme (3U/ ml) (0.5 µl), Water (37.5 µl), Total Reaction volume (50.0 µl) . Termocycling conditions were 94ºC for 5 min, denaturation, 94ºC for 30sec, annealing 55ºC for 30sec, extension 72ºC for 1 min, final extension 72ºC for 5 min. Steps of 2, 3, 4, followed to 35 cycles. The PCR products (50 µl) were separated by electrophoresis on a 1.5% agarose gel containing ethidium bromide according to standard methods [SAMBROOK et al., 1989].

D. GEL Extraction and PCR purification by the kit SPIN-50 (RKT33): The kit is designed for rapid purification of plasmid DNA from standard or low-melt agarose in TAE or TBE. Features of the kit: High quality DNA and no phenol chloroform required. PCR products were gel eluted and sequenced using Gene specific forward and reverse primer. Finally, the PCR products were sent to the Choromous Geni Company-India for doing sequence.

Results

There are some report about Growth hormone (GH) gene in Gene Bank and there is not any report about Growth hormone(GH) gene's *Salmo trutta caspius.* Therefore we had limited for designing primers this gene. In related to we designed one pair of primer for amplify a fragment 3.1 kb *(Figure 1)*. This fragment was sent to Chromous Geni company for sequencing, hence only a 2541 bp fragment were sequenced. Nucleotide homology searches were conducted using the BLASTn on the NCBI Network service *(Figure 2)*, and were analyzed fragment DNA 2541 bp with sequences other alignment *(Figure 3 and 4)*.

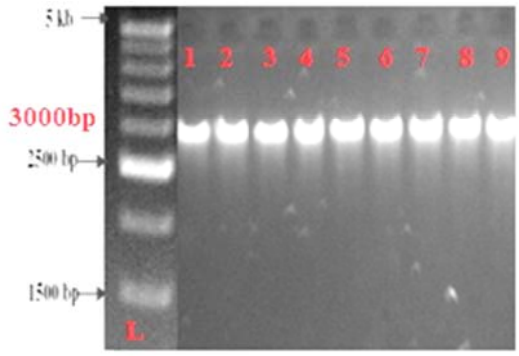

Figure 1. DNA produced from bloods of Growth hormone of gene 's *Salmo trutta caspius* 3.1 kb. Samples was electrophoresed on 1% agarose gel and stained with EtBr. **L:** Size marker: 500bp.

Sequencing: For doing sequence from PCR products, we designed gene specific forward and reverse primers for synthesis of fragments GH1 gene. In related to were sequenced four fragments and other PCR products were failed. These fragments were named: *SS1FP- SS1RP- SS2FP- SS2R* (were analysed in *Figure 2*).

Discussion

Growth hormone from a family of polypeptide [AGELLON et al., 1988]. This hormone will be regulated and involved in many other metabolic functions [reviewed in PETER and MARCHANT, 1995]. Therefore the Growth hormone gene is a potential target for studies of genetic variation in connection with studies of growth traits [RIHO GROSS and JOHN NILLSON, 1999]. For the study of Growth hormone gene, will be required full length of DNA, therefore done for this study and research. Growth hormone gene have two duplicated form (GH1 and GH2), [GROSS and NILSSON, 1995]. The GH1 has a full length of almost 4700bp in some reports in Gene Bank (Accession nos. AY614002–AY614008) also were aligned [RYYNANEN, H.J., PRIMMER, C.R., 2004 and MALE et al., 1992.]. The GH gene in *salmo trutta caspius* for first time were analyzed by us, so, it had very difficult. Therefore we had to designing four pair of primers for synthesis of full length, in related to we could getting 4 exons and introns were failed. The analysis of the genetic of GH1 in *salmo trutta caspius* with other sequences by (NCBI Network system) were found a homology with GH1 in *salmons* *(Figure 3)*. Moreover, we could find a high identity GH1 in *salmo trutta caspius* with GH2 in other *salmons* *(Figure 4)*. Also, in *Figure 2*, we were shown, there is high homology between exons of fragments, and there is high variation within introns in *salmo trutta caspius* and other *salmons.* However this research demonstrates the need to denote homology gene of GH for *salmon* species, because of the economic importance, as well as providing good evidence for the value of the approach.

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 Strand=Plus/Plus
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Sbjct  609   AATCAT-CCTTG-GCAATTAAGAGTAAAAATGGGACAAGGTAAGCCTGCTTTTTCTGTCT  666

Query  66    ATTTCttttttAGTGGGAAGTCAGTGTACCATTTAGTACAGTTTAACTTACACATTTAA  125
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Sbjct  667   ATTTCTTTTTTCAGTGGGAAGTCAGTGTACCATTTAGTACAATTTAACTTACACATTTAA  726

Query  126   TCACTGAGGCAGGGGCCAACACGGCAGAGAAAAGTGAACAAGTATTCTACTACTATGAGG  185
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Sbjct  727   TCACTGAGGCAGGGGCCAACACGGCAGAGAAAAGTGAACAAGTATTCTACTACTATGAGG  786

Query  186   TTAT-AATCTATTGACACAGAACCACCTGCTTTAACAACCTAACTATGTGATCTATAACA  244
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Sbjct  787   TTATAAATCTATTGACACAGAACCACCTGCTTTAACAACCTAACTATGTGATCTATAACA  846

Query  245   TTTACATTTGAGTCATTTAGCAGACACTCTTATCCAAAGCGACTTACAGGAGCCATTAGG  304
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Sbjct  847   TTTACATTTGAGTCGTTTAGCAGACGCTCTTATCCAGAGCGACTTACAGGAGCAATTAGG  906

Query  305   GTTAAGTGCCTTGCTCAAGGGCACATCGACAGATTTCTCACCTAGTCAGCTCAGGGATTG  364
             ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  907   GTTAAGTGCCTTGCTCAAGGGCACGTCGACAGATTTCTCACCTAGTCAGCTCAGGGATTG  966

Query  365   AAACCGGTAACCTTTCAATTACTTACCCAACGCTCTTAACCGCTGGGCTATTGGTGTACA  424
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Sbjct  967   AAACCAGTAACCTTTCAATTACTTACCCAACGCTCTTAACCGCTAGGCTATTGGTGTTCG  1026

Query  425   ATGGCTGAGAATATCTAACTAATGTATCTCACCATAATTCGACTTACTCGTTTTATACAT  484
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Sbjct  1027  ATGGCTGAGAATATCTAACTAATGTATCTCACCATAATTCGACTTACTCGTTTTATACAT  1086

Query  485   TTCTTATTTTATTTAATCTCTCTTTTAGTGTTTCTGCTGATGCCAGTCTTACTGGTCAGT  544
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Sbjct  1087  TTGTTA---T-TTT-CTCTTTCTTTTAGTGTTTCTGCTGATGCCAGTCTTACTGGTCAGT  1141

Query  545   TGTTTTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTTCAACATCGCGGTGAAC  604
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Sbjct  1142  TGTTTTCTGAGCCAAGGGGCAGCGATGGAAAACCAACGGCTCTTCAACATCGCGGTCAAC  1201

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Sbjct  1202  CGGGTGCAACATCTCCACCTAATGGCTCAGAAGATGTTCAATGACTTTGTAAGACAGCTT  1261

Query  665   TTGAATCTTCTTTTGACATATCAAATAATGTATTAATGATTGTTCTTCTTCTTGTAGACA  724
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Sbjct  1262  TTGAATCTTCTTTTGACATATCAAATAGTGTATCAATGATTGTTCTTCTTCTTGTAGACA  1321

Query  725   GTATCCTCTTTACACAACCCCTCGCGGCTaaaaaaaaaCAACAGAAAA-TCTCTCTCCCT  783
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Sbjct  1322  GTGTCCTCTTTACACAACCC-TCGTGGCAA--------CAACAAAAAAATCTCTCTCCCT  1372

Query  784   TTCTTTGTGATTTTGTGCAGGAAGGCACCCTGTTGCCTGATGAACGCAGACAGCTGAAAC  843
             | ||||||||||||||||||||||| |||||||||||||||||||||||||||||||| |
Sbjct  1373  T-CTTTGTGATTTTGTGCAGGAAGGTACCCTGTTGCCTGATGAACGCAGACAGCTGAA-C  1430

Query  844   AAGATATTCCTGCTGGAACTTTCTGTTACCTCTGACTCTATCGTGAGCCCAATCGAACA-  902
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Sbjct  1431  AAGATATTCCTGCTGGA-CTT-CTGTAAC-TCTGACTCCATCGTGAGCCCAATCGA-CAA  1486

Query  903   GCT-GAGACTCAGAGAGAGT-CAGTAGGTAACCT  934
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Sbjct  1487  GCTTGAGACTCAGA-AGAGTTCAGTAAGTAACCT  1519

 Score = 1232 bits (667),  Expect = 0.0
 Identities = 870/947 (92%), Gaps = 40/947 (4%)
 Strand=Plus/Minus

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Query  1051  AGTCTCAAGCTTGTCGATTGGGCTCACGATGGAGTCAGAGTTACAGAAGTCCAGCAGGAA  1110
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Sbjct  1496  AGTCTCAAGCTTGTCGATTGGGCTCACGATGGAGTCAGAGTTACAGAAGTCCAGCAGGAA  1437

Query  1111  TATCTTGTTCAGCTGTCTGCGTTCATCAGGCAACAGGGTGCCTTCCTGCACAAAATCACA  1170
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Query  1171  AAGAAGGGAGAGAGATTTTCT-GTTGttttttttAGCCGCGAGGGTTGTGTAAAGAGGA  1229
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Query  1230  TACTGTCTACaaaaaaaaaaCAATCATTAATACATTATTTGATATGTCaaaaaaaaaTT  1289
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Sbjct  1324  CACTGTCTACAAGAAGAAGAACAATCATTGATACACTATTTGATATGTCAAAAGAAGATT  1265

Query  1290  CAAAAGCTGTCTTACAAAGTCATTGAACATCTTCTGAGCCATTAGGTGGAGATGTTGCAC  1349
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Query  1350  CCGGTTCACCGCGATGTTGAAAAGCCGTTGGTTTTCCATCGCTGCCCCTTGGCTCAAAAA  1409
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Sbjct  1204  CCGGTTGACCGCGATGTTGAAGAGCCGTTGGTTTTCCATCGCTGCCCCTTGGCTCAGAAA  1145

Query  1410  ACAACTGACCAGTAAGACTGGCATCAGCAAAAACACTAAAAGAGAGATTAAATAAAATAA  1469
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Sbjct  1144  ACAACTGACCAGTAAGACTGGCATCAGCAGAAACACTAAAAGAAAGAG-AAA-A---TAA  1090

Query  1470  GAAATGTATAAAACGAGTAAGTCGAATTATGGTGAGATACATTAGTTAGATATTCTCAGC  1529
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Sbjct  1089  CAAATGTATAAAACGAGTAAGTCGAATTATGGTGAGATACATTAGTTAGATATTCTCAGC  1030
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Query  1530  CATTGTACACCAATAGCCCAGCGGTTAAAAGCGTTGGGTAAGTAATTGAAAGGTTACCGG  1589
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Sbjct  1029  CATCGAACACCAATAGCCTAGCGGTTAAGAGCGTTGGGTAAGTAATTGAAAGGTTACTGG  970

Query  1590  TTTCAATCCCTGAGCTGACTAGGTGAGAAATCTGTCGATGTGCCCTTGAGCAAGGCACTT  1649
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Query  1710  AAATTGTTATAGATCACATAGTTAGGTTGTTAAAGCAAGTGGTTCTGTGTCAATAGATT-  1768
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Sbjct  849   AAAT-GTTATAGATCACATAGTTAGGTTGTTAAAGCAGGTGGTTCTGTGTCAATAGATTT  791

Query  1769  ATAACCTCATAGTAGTAGAATACTTGTTCACCTTTTCCTCTGCCGTGTGGGCCC-TGCCT  1827
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Query  1828  CAGTGAATTAAATGTGTAAGTTAACT-GTACTAATTGGTTAC-CTTGAACT-CC-ACT-A  1882
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Sbjct  732   CAGTGA-TTAAATGTGTAAGTTAAATTGTACTAAATGGT-ACACT-GA-CTTCCCACTGA  677

Query  1883  AAAAAGAA-TAGGA-ACGAAAA-GC-GGCT-AC-T-GTTCCATTTTT  1922
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 Strand=Plus/Plus

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Sbjct  2267  CAATA-CAACGCAACTTCATTTTCCAATAATCTGTGGTTTCTCTACATCTACACACACAC  2325

Query  1979  ACAGGTCCTGAAGCTGCTCCATATCTCTTTCCGTCTGATTGAATCCTGGGAGTACCCTAG  2038
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Query  2159  AATGACCATTTGTGGTGCCACACTTTGTGCACTGTAAACCCCAAGGCATTTTTAACTCAA  2218
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Query  2219  ATACTTCTAGTAAGTTGAA  2237
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Sbjct  2566  ATACTTCTAGTAAGTTGAA  2584

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Sbjct  2549  TTG-GGGTTTACAGTGCACAAAGTGTGGCACCACAAATGGTCATTGTTCTCCCTCCTTTC  2491
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Query  2303  TTTACCTTGATGAGCAGGTTGATGCCCACTTTGAGGTCGCTGAGCTTCTCAGAGATCTGG  2362
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Query  2478  -A-A-A--CCACAGATTATTGGAAAATGAAGTTGCGTTGTATTGTACAGCTTGAGATTCC  2532
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Query  2533  CTATTTACA  2541
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Sbjct  2250  CTATTTACA  2242
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Figure 2. GH1 gene *salmo trutta caspius* fragments aligned with the *salmo salar* by NCBI program. Fragments including the four primers as started from first of length.

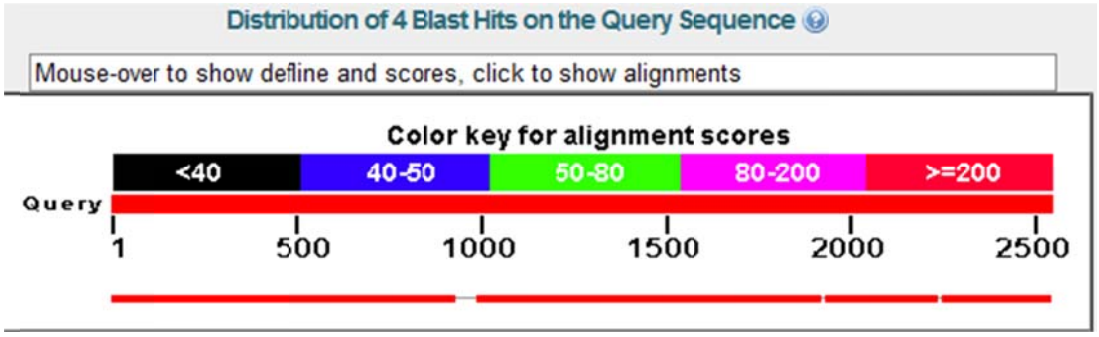

Figure 3. Sequence comparison for fragment of growth hormone (GH) gene with BLASTn programming. Similar sequences are shown. Color key denote to high homology between sequences.

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
EU621898.1	Salmo salar clone 11F04_73D15 interferon alpha 1-like gene	922	3515	90%	0.0	97%	
AY614010.1	Salmo salar isolate Ss11_GH1 growth hormone I gene, comp	922	3354	90%	0.0	97%	G
AY614009.1	Salmo salar isolate Ss10_GH1 growth hormone I gene, comp	922	3354	90%	0.0	97%	G
AY614008.1	Salmo salar isolate Ss9_GH1 growth hormone I gene, compl	922	3354	90%	0.0	97%	G
AY614007.1	Salmo salar isolate Ss8_GH1 growth hormone I gene, compl	922	3341	89%	0.0	100%	G
AY614006.1	Salmo salar isolate Ss7_GH1 growth hormone I gene, compl	922	3354	90%	0.0	97%	G
AY614005.1	Salmo salar isolate Ss6_GH1 growth hormone I gene, compl	922	3354	90%	0.0	97%	G
AY614004.1	Salmo salar isolate Ss5_GH1 growth hormone I gene, compl	922	3354	90%	0.0	97%	G
AY614003.1	Salmo salar isolate Ss3_GH1 growth hormone I gene, compl	922	3354	90%	0.0	97%	G
AY614002.1	Salmo salar isolate Ss2_GH1 growth hormone I gene, compl	922	3341	89%	0.0	100%	G
X61938.1	Salmo salar gene for growth hormone I	917	3354	90%	0.0	97%	G
EU621900.1	Oncorhynchus tshawytscha clone ChincokGH1 interferon alp	767	2676	81%	0.0	98%	
EU621899.1	Salmo salar clone 63I10 growth hormone 2 gene, complete	763	2449	73%	0.0	95%	
M21573.1	Salmon (S.salar) growth hormone gene, complete cds	760	2440	73%	0.0	95%	

Figure 4. Sequence analysis was conducted using the BLASTn on the NCBI Network service for growth hormone gene fragment. Sequence alignment strain's *Salmo trutta caspius* and other sequences in Gene Bank. Maximum identity denote conserved nucleotides. (Almost 97%).

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