Selection for higher fertility reflects in the seminal fluid proteome of modern domestic chicken

Mohammad Atikuzzaman, Libia Sanz, Davinia Pla, Manuel Alvarez-Rodriguez, Marie Rubér, Dominic Wright, Juan J. Calvete and Heriberto Rodriguez-Martinez

Journal Article

N.B.: When citing this work, cite the original article.

Original Publication:

Mohammad Atikuzzaman, Libia Sanz, Davinia Pla, Manuel Alvarez-Rodriguez, Marie Rubér, Dominic Wright, Juan J. Calvete and Heriberto Rodriguez-Martinez, Selection for higher fertility reflects in the seminal fluid proteome of modern domestic chicken, Comparative Biochemistry and Physiology - Part D, 2017. 21, pp.27-40.

<http://dx.doi.org/10.1016/j.cbd.2016.10.006>

Copyright: Elsevier

<http://www.elsevier.com/>

Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-132624>

Selection for higher fertility reflects in the seminal fluid proteome of modern domestic chicken

Mohammad ATIKUZZAMAN^{1,*}, Libia SANZ^{2,*}, Davinia PLA², Manuel ALVAREZ-RODRIGUEZ¹, Marie RUBÉ¹, Dominic WRIGHT³, Juan J. CALVETE^{2,‡}, Heriberto RODRIGUEZ-MARTINEZ^{1,‡}

¹ Department of Clinical and Experimental Medicine, ³ Department of Physics, Chemistry and Biology, University of Linköping, Linköping, Sweden

² Instituto de Biomedicina de Valencia, CSIC, Valencia, Spain

* These authors have made equal contribution to the work, and should both be considered "first authors".

Running title: Comparative seminal fluid proteomics of high and low egg-laying chicken

‡Corresponding authors: For questions concerning biological aspects of this work, please contact Heriberto Rodriguez-Martinez (heriberto.rodriguez-martinez@liu.se), Linköping University, Department of Clinical and Experimental Medicine, Faculty of Medicine and Health Sciences; Lasarettsgatan 64/65, Lanken, Floor 12, SE-581 85 Linköping, Sweden; For issues regarding proteomic analysis, please contact Juan J. Calvete (jcalvete@ibv.csic.es), Laboratorio de Venómica Estructural y Funcional, Instituto de Biomedicina de Valencia, C.S.I.C., Jaime Roig 11, 46010 Valencia, Spain. Phone: +34 96 339 1778, Fax: 34 96 369 0800

Abbreviations

WL	White Leghorn
RJF	Red Junglefowl
AIL	Advanced intercross line
SF	seminal fluid
TGF-β2	transforming growth factor-beta 2
CXCL10	C-X-C motif chemokine 10

Key words: Rooster seminal fluid proteome; cytokines; egg-laying capacity; Red Junglefowl; White Leghorn; Advanced Intercross Line; chicken

Abstract

The high egg-laying capacity of the modern domestic chicken (i.e. White Leghorn, WL) has arisen from the low egg-laying ancestor Red Junglefowl (RJF) via continuous trait selection and breeding. To investigate whether this long-term selection impacted the seminal fluid (SF)-proteome, 2DE electrophoresis-based proteomic analyses and immunoassays were conducted to map SF-proteins/cytokines in RJF, WL and a 9th generation Advanced Intercross Line (AIL) of RJF/WL-L13, including individual SF (n= 4, from each RJF, WL and AIL groups) and pools of the SF from 15 males of each group, analyzed by 2DE to determine their degree of intra-group (AIL, WL, and RJF) variability using Principal Component Analysis (PCA); respectively an inter-breed comparative analysis of intergroup fold change of specific SF protein spots intensity between breeds. The PCA clearly highlighted a clear intra-group similarity among individual roosters as well as a clear inter-group variability (e.g. between RJF, WL and AIL) validating the use of pools to minimize confounding individual variation. Protein expression varied considerably for processes related to sperm motility, nutrition, transport and survival in the female, including signaling towards immunomodulation. The major conserved SF-proteins were serum albumin and ovotransferrin. Aspartate aminotransferase, annexin A5, arginosuccinate synthase, glutathione S-transferase 2 and L-lactate dehydrogenase-A were RJF-specific. Glyceraldehyde-3-phosphate dehydrogenase appeared specific to the WL-SF while angiotensin-converting enzyme, γ -enolase, coagulation factor IX, fibrinogen α -chain, hemoglobin subunit α -D, lysozyme C, phosphoglycerate kinase, Src-substrate protein p85, tubulins and thioredoxin were AIL-specific. The RJF-SF contained fewer immune system process proteins and lower amounts of the anti-inflammatory/immunomodulatory TGF- β 2 compared to WL and AIL, which had low levels- or lacked pro-inflammatory CXCL10 compared to RJF. The seminal fluid proteome differs between ancestor and modern chicken, with a clear enrichment of proteins and peptides related to immune-modulation for sperm survival in the female and fertility.

Highlights

- Seminal fluid proteomes of high- (WL) and low (RJF) egg-laying-capacity roosters were analyzed
- Seminal fluid proteome of 9th generation RJF x WL-L13 interbreed line was analyzed
- Cytokine/chemokine profiles of WL, RJF, and RJF x WL-L13 seminal fluids were determined
- Considerable variation in expression of seminal fluid proteins/cytokines was observed
- Selection for higher fertility has an impact in the composition of the seminal fluid proteome
- Ancestor and modern chicken seminal fluids varied in immune modulatory proteins/cytokines which might impact production performance

1. Introduction

Semen is composed of a complex mixture of organic and inorganic components built by secretions of the male reproductive organs, which the ejaculated spermatozoa bathe in (Mann et al., 1982; Blesbois and Hermier, 1990). In mammals, seminal plasma contains a huge bulk of proteins and peptides (25-60 g/L) per ejaculate, mostly derived from the accessory sex glands (Batruch et al., 2011; Rodríguez-Martínez et al., 2011). In the chicken, due to a lack of accessory sex glands, with the exception of the vascular bodies in the cloaca which resemble the mammalian bulbo-urethral glands, the seminal fluid derives from the testis, the rudimentary epididymis and the ductus deferens (Etches, 1996; Fujihara, 1992) and contains a 10-fold lower protein load than mammals (2.0-2.4 g/dL) (Harris and Sweeney, 1971; Thurston et al., 1982). In mammals, seminal plasma proteins have been ascribed a range of diverse functions, including germicidal effects, promotion of sperm survival, assistance in sperm interactions with different microenvironments in the female genital tract, and the signaling to the immune system of the female so that a state of maternal tolerance to foreign spermatozoa (and embryos and placentae) is established (Rodríguez-Martínez et al., 1998, 2008, 2011; Novak et al., 2010; Kareskoski et al., 2011; Caballero et al., 2012; Druart et al., 2013; Milardi et al., 2013; Rodrigues et al., 2013; Sharma et al., 2013; Bromfield et al., 2014). Whether such roles can also be ascribed to avian seminal fluid (SF) proteins remains to be explored.

Proteomic research in Reproductive Biology, accelerated by methodological developments in mass spectrometry (Druart et al., 2013; Rodrigues et al., 2013; Sharma et al., 2013; Calvete et al., 1994; Pilch and Mann, 2006; Kelly et al., 2006), has increased our knowledge of the composition of seminal plasma in mammals, revealing relevant roles for sperm function (Soggiu et al., 2013; Soler et al., 2016) and immune modulation, including the role of specific cytokines and chemokines (Schjenken and Robertson, 2014; Crawford et al., 2015) with relevance for fertility. Proteomic studies investigating avian sperm and ejaculates are comparatively scarce, yet those that do exist assess the potential correlations with sperm motility (Froman et al., 2011), attempt to identify and classify proteins (Marzoni et al., 2013), or correlate the proteome to the semen phenotype (Labas et al., 2015). Few studies (Marzoni et al., 2013; Labas et al., 2015) describe seminal plasma proteins (e.g. beta-defensin as gallinacins, ovotransferrin, serum albumin and peroxiredoxin-6) and their role in antimicrobial activity and sperm survival (Das et al., 2011). Comparative cytokine/chemokine studies are also restricted to cytokine expression in avian testis (Ocón-Grove et al., 2010; Michailidis et al., 2014) and the oviduct. In the latter, pro-inflammatory cytokines (tumor necrosis factor (TNF) and interleukin (IL)-1 β) as well as immunosuppressive cytokines (as transforming growth factor (TGF)- β) and their receptors are expressed in the vagina and utero-vaginal-junction respectively, after seminal deposition (Das et al., 2006, 2008, 2009). The lack of cytokine mappings in the chicken SF, which might be an important factor for sperm function, comparative to what is known in mammals (Barranco et al., 2015), is thus required.

Fertility varies among animals, irrespective of classes. Red Junglefowl (RJF), the wild progenitor of modern domestic chicken breeds, has a low fertility, expressed in terms of fertile oviposition rates per season, while domestic modern laying chicken breeds such as the White Leghorn (WL)

display the opposite situation, with high laying/fertility rates. Sperm quality and fertility varies among males, particularly when considering the pressure of selection applied. Interestingly, when a chicken breed is selected for a particular trait- as egg production, a decrease in semen quality has been recorded in the male line (Murugesan et al., 2013). Comparative studies have shown differences in sperm concentration and forward sperm motility between wild RJF and domestic chicken with an advantage for the RJF, without consideration of eventual roles for the seminal fluid (Malik et al., 2013). The composition of the seminal fluid/plasma also varies with varying fertility among males, even if definite links with reproductive outcome are yet to be fully established. Of even greater relevance, semen deposition elicits gene expression shifts in the female genital tract (Fazeli et al., 2004; Atikuzzaman et al., 2015), calling for the identification of the pertinent signals involved, including components of the SF-proteome that could be related to sperm survival and immunomodulation by peptides as cytokines, an as yet undiscovered chapter in the rooster SF.

The present study aims to map the proteome, including cytokines, of the seminal fluid of the ancestor RJF (n= 31 birds), of the highly-selected modern WL (n= 20 birds), and of an AIL (n= 23 birds) - intercross between wild and domestic chicken (RJF x WL-L13), with clear differences in egg-laying/fertility, evolved during selection. The SF was analyzed for protein profiles as ejaculate pools per breed (15 males/breed) to minimize individual variation or as individual ejaculates (39-79 ejaculates/breed) or pool of ejaculates of a male (10 males/breed) for quantitation of cytokines.

2. Material and methods

2.1 Animals and seminal fluid source

Sexually mature, proven fertile roosters from a RJF-pure line, a pure WL line and from a 9th generation AIL- intercross between RJF and WL-L13 lines have been used (see Johnsson et al., 2012 for details of the cross and breeds used as well as for details on rearing and breeding routines). Food and water were available *ad libitum* and the chicken were held under controlled temperature and light regimes (12h light: 12h darkness cycle, 5 lux) in 1-2 m² pens depending on age for their first seven weeks, in compliance with European Community (Directive 2010/63/EU) and Swedish (SJVFS 2012:26) current legislation. Throughout all experiments, animals were handled carefully to avoid any unnecessary stress. The experiments were approved by the 'Regional Committee for Ethical Approval of Animal Experiments' (Linköpings Djurförsöksetiska nämnd) in Linköping, Sweden (permit no. 75-12).

Semen was collected from pre-trained roosters by manual abdominal massage. The success of ejaculation was confirmed by extending 2 µl of semen in Dulbecco's medium (1:10 v/v) for examination on a Carl Zeiss microscope equipped with a thermal plate (41⁰ C) and positive phase contrast optics (10x objective). Ejaculates mixed with faeces or blood or lacking live sperm were excluded from the study. Semen from 31 RJF (45 ejaculates), 23 RJF/WL-L13 (43 ejaculates) and 20 WL (79 ejaculates) roosters were finally selected for the study. The selected ejaculates were centrifuged at +5°C, 21,000xg for 10 minutes immediately after collection to harvest the

extra-cellular SF-supernatant, which was thereafter transferred to liquid nitrogen (LN₂) before storage at -80⁰ C prior to analysis.

2.2 Identification of proteins by 2DE followed by mass spectrometry

Seminal fluid samples collected from four animals per group (breed) were individually analyzed by 2DE to determine their degree of individual variability. As well, pools of SF from 15 males of each group (breed) were built a group/breed sample. The rationale behind this pooling was our interest in unveiling SF proteome differences that may underline a breed population fitness trait, rather than individual differences, and thus to minimize confounding individual intra- and inter-male variation. Each breed pool was analyzed in triplicate by 2DE and only spots consistently found in all three experiments were considered in the subsequent inter-breed comparative analysis.

Sample preparation: 150 µg of SF from each breed pool (RJF, WL and AIL) was subjected to cleansing using a 2D clean-Up kit (GE Healthcare). The cleansed SF of each breed was suspended in 90 µl of rehydration solution containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 0.5% (v/v) IPG buffer, 40 mM DTT plus 1 µl of bromophenol blue. The solution, after centrifugation at 13,000xg for 5 minutes, was loaded into a prior overnight rehydrated (rehydration solution containing 7 M urea, 2 M thiourea, 2% w/v CHAPS, 0.5% v/v IPG buffer, 0.002% bromophenol blue and 40 mM DTT) seven cm long IPG strip (pH range 3-10 L).

2DE (two-dimensional gel electrophoresis): The isoelectric focusing was carried out following Calvete et al. (2009) at 20⁰ C and 50 µA using Ettan IPGphor 3 (GE Healthcare) with a modification of running schedule: First step and hold, 500 V (125 Vh); Second gradient, 1000 V (500 Vh); and Third gradient, 5000 V (6666 Vh). Prior to perform second dimension sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the IPG strip was equilibrated for 7 minutes twice in SDS equilibration buffer solution containing 6 M urea, 75 mM Tris-HCl pH 8.8, 29.3% glycerol (v/v), 2% SDS (w/v) and 0.002% bromophenol blue (w/v) with a gentle shaking at 15 rotations per min.

The IPG strips were then placed on a SDS polyacrylamide 4% stacking gel (0.75 mm thick) with a SDS polyacrylamide 15% separation gel (1.5 mm thick), according to Laemmli (1970), into a vertical SDS-PAGE gel slab filled with electrode buffer. A 200 kDa molecular weight marker (Mark12TM, Invitrogen Corporation) was loaded on to the stacking gel before running the electrophoresis. The electrophoresis was performed at 100V until the dye reached to the bottom of the separating gels (Calvete et al., 2009).

Protein spot visualization: 2DE gels of individual and pooled SF samples were stained with Coomassie Brilliant Blue G-colloidal at room temperature on a plate shaker at 15 rpm until the spots were visible. The stained gels were then washed with deionized water until the backgrounds became clear. Gels were stored for further analysis immediate after scanning with a high resolution scanner, LabScan (Amersham Pharmacia Biotech, Sweden). The scanned 2D-gel images were analyzed for intragroup (AIL, WL, and RJF) variability using MarkerViewTM 1.2.1

Software (AB Sciex, Ontario, Ca), and for intergroup fold change of specific protein spots intensity comparing AIL and WL with RJF, using SameSpots (TotalLab Ltd. Newcastle, UK).

Protein digestion and mass spectrometry: Immediately after scanning, the visible protein spots on 2D gels were cut and subjected to automated reduction with DTT and alkylation with iodoacetamide and digestion with sequencing grade modified porcine trypsin (Promega) using a ProGest™ digester (Genomic Solutions) following the manufacturer guidelines. The dried digests were re-suspended with 8 µl of 0.1% formic acid, and 6 µl aliquots injected and analyzed by Liquid Chromatography-Electrospray Ionization-Quadropole-Time-of-Flight-Mass Spectrometry (LC-ESI-Q-TOF-MS/MS) using a Synapt G2 instrument (Waters, Manchester, UK) hyphenated to a liquid nano-chromatographic separation system (NanoACQUITY-UPLC, Waters) equipped with a C18 column (100 µm x 100 mm, 1.7 µm particles (BEH130 C18, Waters) operated at a flow rate of 0.6 µl/min. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The optimized ultra-performance liquid chromatography (UPLC) elution conditions were 0-1 min 1-12% B; 1-16 min, 12-40% B; 16-18 min, 40-85% B; 18-20 min, 85-1% B; 20-30 min, 1% B. The autosampler was maintained at +10 °C. All the analyses were performed in data dependent analysis (DDA) mode that automatically triggers the MS/MS experiments. The scan range was from 100 to 2,000 m/z. For positive nanospray mode, the capillary and cone voltage were set at 3kV and 28V respectively. The deviation gas was set at 100 L/h, the cone gas at 10L/h and the source temperature at +100° C. The mass spectrometer was operated in V-optics mode with 20,000 resolution using dynamic range extension. The data acquisition rate was set to one scan time. All analyses were acquired using the LockSpray to ensure accuracy and reproducibility. Leucine-enkephalin was used as the lockmass at a concentration of 200 ng/ml and a flow rate of 0.5 µl/min. Data were collected in continuum mode and the spray frequency was set at 30 s. The data acquisition and spectra were analyzed by MassLynx4.1 (Waters).

The *Gallus gallus* SF-proteins were identified from their peptide sequences using MASCOT MS/MS Ions Search Engine version 2.5 (Matrix Science, Boston, MA) along with the latest updated version of Swiss-Prot protein database (UniProtKB/Swiss-Prot). Carbamidomethyl cysteine and oxidized methionine residues were selected as fixed and variable modifications, respectively. The peptide tolerance and the MS/MS tolerance were set at 1.2 Da and 0.6 Da, respectively and peptide charges were set at 2⁺, 3⁺ and 4⁺. Trypsin digestion (allowing two possible missed cleavages) and taxonomy of bony vertebrate were set. The data format and instrument information was set as Micromass (PKL) and ESI-QUAD-TOF, respectively along with decoy. The ion scores have been reported as $-10 \times \log_{10}(P)$ where P is the probability that the observed match is a random even. Individual MS/MS peptide ion scores >39 indicated identity or extensive homology ($P < 0.05$) for the MS/MS ion search have been taken into consideration for protein identification. Identified proteins underwent bioinformatic analysis using the PANTHER classification system (Version 10.0) (Mi et al., 2016).

2.3 Measurement of the concentrations of cytokines and chemokines

Presence and relative concentration of a battery of cytokines and chemokines including IL-6, CXCL8 (IL-8), CCL2 (monocyte chemo-attractant protein-1, MCP-1) or the growth factor

granulocyte-macrophage colony-stimulating factor (GM-CSF, associated with a pro-inflammatory immune response); the anti-inflammatory IL-10 and TGF- β 1, TGF- β 2 and TGF- β 3; the Th1-associated IFN- γ and CXCL10 (interferon gamma-induced protein, IP-10); the Th2-associated CCL22 (macrophage-derived chemokine, MDC); and the Th17-associated IL-17 and CXCL1 (growth-regulated oncogene, GRO), as well as the inducer of NK cell proliferation IL-15 were first examined in the SF of individual ejaculates of RJF, WL and AIL roosters ($n > 15$ birds per breed, range: 15-79 ejaculates) using a multiplexed microsphere-based flow cytometric assay (Luminex's xMAP®) following the protocol of Barranco et al. (2015). In brief, pre-coated magnetic beads (Cat#HCYTOMAG-60K-11 for human reactivity, Merck Millipore, Billerica, MA, USA) were used for the determination of cytokines and chemokines except TGF- β s, while a 3-plex kit (Cat#TGFB-64K-03 for pig, human, mouse, rat, non-human primate, canine, feline reactivity, Merck Millipore) were used for TGF- β s following the methods described by manufacturers in 96-well multiscreen plates, albeit samples were ran as singlets. A 25 μ l SF (acidified in case of TGF- β s) was used per sample well to measure the concentration.

Concentrations of TGF- β 2, TGF- β 3 and CXCL10 were further measured in pooled SF of each male (10 birds per breed) using a chicken-specific ELISA kit for TGF- β s (Nori™ Chicken TGF- β 2 and TGF- β 3 kit, Genorise Scientific, Inc., Glen Mills, PA, USA) and for CXCL10 (CXCL10 ELISA kit, Cat#MBS2505839, MyBiosource, Inc., San Diego, CA, USA), after preparation of a standard curve, following the manufacturer protocol. The 96-well microplate loaded with duplicate-samples was incubated, washed using an automatic microplate washer (Ref# 30022011, Hydroflex, Tecan Austria GmbH, Grödig, Austria) and the optical density of each well was determined using a microplate reader (Ref# 16039400, Sunrise, Tecan Austria GmbH) at 450 nm.

An independent t-test analysis was employed to compare the mean concentration values (expressed as pg/ml, mean $\pm$ SEM) of cytokines and chemokines in the SF of the different breeds. $P < 0.05$ was considered significant.

3. Results

A Principal Component Analysis (PCA) of the 2DE images of 12 individual SF samples (4 per group RJF, WL and AIL) (**Fig. 1**) was performed in order to visualize the 2DE data in terms of sample grouping and gain insights into their intra-group and inter-group variability. Figure 1 (panel D) highlighted a clear intra-group similarity among individual roosters as well as a clear inter-group variability (e.g. between RJF, WL and AIL). Under such circumstances group/breed pools built by equal SF-amounts of 15 roosters per group were subjected to 2DE separation and compared using SameSpots, to analyze inter-group fold change of specific SF protein spots intensity between breeds.

3.1 The major conserved SF-proteins were serum albumin and ovotransferrin

Out of 107 (RJF), 52 (WL) and 98 (AIL) spots on the 2D gels analyzed (**Fig. 2**), a total of 28 (26.16 %), 21 (40.38%) and 38 (38.78%) proteins were detected in SF (**Table 1 and Supplementary Table S1**). Around 50% of the detected SF-proteins (from the total number of

spots analyzed per breed) were serum albumin (11.21%, 21.15%, and 13.27%) and ovotransferrin (15.89%, 17.31%, and 12.24%) in RJF, WL, and AIL, respectively (**Table 1**). Among the identified proteins, aspartate aminotransferase, annexin A5, arginosuccinate synthase, glutathione S-transferase 2 and L-lactate dehydrogenase-A were RJF-specific, while glyceraldehyde-3-phosphate dehydrogenase were found to be specific to the WL-SF. On the other hand, angiotensin-converting enzyme, γ -enolase, coagulation factor IX, fibrinogen α -chain, hemoglobin subunit α -D, lysozyme C, phosphoglycerate kinase, Src substrate protein p85, tubulins and thioredoxin were AIL specific (**Table 1**).

3.2 Eleven SF-proteins were down-expressed while eight proteins were over-expressed in RJF compared with WL and AIL

Compared to the WL, the down-regulated proteins (shown subsequently as fold changes, and spot number on the 2D gels) in the SF of the RJF were serum albumin (4.3, 126), ovotransferrin (2.1, 123), ovoinhibitor (1.9, 129), creatinine kinase B-type (2.6, 131), α -enolase (5.0, 132), L-lactate dehydrogenase B chain (5.3, 140), triosephosphate isomerase (9.1, 145), retinol-binding protein (3.4, 146) and apolipoprotein A-I (3.8, 147) and over-expressed proteins in the SF of RJF were malate dehydrogenase (1.7, 139), glyceraldehyde-3-phosphate dehydrogenase (3.0, 141), cystatin (2.7, 152) and gallinacin-9 (1.9, 157) (**Fig. 3A-B and Supplementary Table S2A**). A similar comparison revealed down-regulated proteins in RJF, including serum albumin (3.2, 214), ovotransferrin (4.0, 192), phosphoglycerate mutase 1 (2.1, 230) and tubulin β -1 chain (2.0, 172) while, the over-expressed proteins were transthyretin (2.6, 85), Ig λ -chain C (2.0, 243), protein NEL (2.3, 162) and arginosuccinate synthase (3.1, 62) as compared to the AIL (**Fig. 3C-D and Supplementary Table S2B**).

3.3 The SF of RJF contained fewer immune system process proteins compared to WL and AIL

The identified proteins were classified according to the 12 biological process categories for each breed of chicken (**Fig. 4A and Table 2A**). The highest number of proteins in the SF of RJF, WL and AIL were found in the metabolic process category (10, 8 and 13 respectively) while in the 'immune system process' category 3, 5 and 6 proteins were identified, respectively. The protein class analysis revealed 11, 15, and 18 types of proteins, respectively, in the seminal fluid of RJF, WL and AIL (**Fig. 4B and Table 2B**). Proteins were also screened in the EMBL-EBI Quick GO database to ascertain if any one of them were from sperm regions of *Gallus gallus*. We found that the trypsin inhibitor CITI-1 (ISK1L_CHICK) is an acrosome membrane protein (GO: 0002080) and was detected in the SF of each breed.

3.4 The seminal fluid of RJF, WL and AIL contain TGF- β 2 and CXCL10

The Luminex screening solely detected TGF- β 2 (RJF, 14810.47 $\pm$ 1492.56; WL, 17111.14 $\pm$ 1028.78; AIL, 13706.36 $\pm$ 1318.50, pg/ml, mean $\pm$ SEM, **Fig. 5A**) and CXCL10 (RJF, 48.74 $\pm$ 5.75; WL, 0.00; AIL, 56.65 $\pm$ 14.93, pg/ml, mean $\pm$ SEM, **Fig. 5B**). The other cytokines or chemokines (IFN γ , CCL22, CXCL1, IL-17, GM-CSF, CCL2, IL-6, CXCL8, IL-15, IL-10, TGF- β 1 and TGF- β 3) were not in the detectable range when measured by Luminex. The following use of a chicken specific ELISA could not confirm the presence of TGF- β 2 in RJF but could on WL

and AIL samples (RJF: 0.00; WL: 2467.52 ± 731.93 ; AIL: 2844.30 ± 664.36 , pg/ml, mean $\pm$ SEM, **Fig. 5C**). Regarding CXCL10, the ELISA confirmed its presence in the SF of all breeds in contrast to the Luminex (RJF: 107.65 ± 16.40 ; WL: 71.54 ± 5.97 ; AIL: 86.46 ± 7.36 , pg/ml, mean $\pm$ SEM, **Fig. 5D**). ELISA measurements depicted further differences between breeds; thus, while TGF- β 2 levels did not differ between WL and AIL, the cytokine was absent in RJF. The concentrations of CXCL10 differed significantly between RJF and WL ($P < 0.05$) with the SF of AIL having intermediate concentrations (n.s.).

4. Discussion

As it is the case in mammals, rooster seminal fluid has been considered to be a modulator of sperm functions, including fertilization (Douard et al., 2005; Froman et al., 2011; Marzoni et al., 2013). While even seminal plasma in mammals has been ascribed relevant roles in fertility including that of livestock selected for fertility, as pigs (Robertson, 2005; Song et al., 2016) such counterstudies are not available for chicken. Selection for fertility, marking differences in egg-laying capacity seemed to have affected sperm function. Murugesan et al. (2013) found high egg laying WL-lines had poorer sperm concentration and forward sperm motility than low-egg laying WL-lines. Uncertainty prevails, since other studies did not found any such differences (Frankham and Doornenbal, 1972; Niranjana et al., 2001). Progressive sperm motility appeared significantly higher in RJF compared to the domestic bantam chicken (Malik et al., 2013). Whether comparative changes have occurred in SF-components and whether eventual changes affect the endowment that SF provides the ejaculated spermatozoa with, as it is proven for mammals (Rodriguez-Martinez et al., 2011; Perez-Patiño et al., 2016) is yet to be explored. Chicken spermatozoa are stored for days to weeks in the sperm-storage oviduct area (UVJ). Since spermatozoa (and the SF) are foreign to the hen, some sort of negotiation with the female immune system must be achieved to allow such long survival, with maintenance of potential fertilizing capacity. In mammals, the seminal plasma induces a state of immune tolerance (Robertson et al., 2005; Rodriguez-Martinez et al., 2011). Mating is capable of inducing changes in the expression of genes at the sperm storage region in the chicken oviduct, including genes involved in immune functions (Atikuzzaman et al., 2015), indicating that similar mechanisms might be present in either animal classes. Ongoing studies replacing mating for infusion of seminal fluid in RJF and WL and exploring the utero-vaginal junction (UVJ) containing sperm-storage tubuli (SST) by oligonucleotide microarray has shown changes in the expression of immune-responsive genes (7 genes in RJF and 9 genes in WL) in this specific area of the oviduct (Atikuzzaman et al., unpublished results). The capacity of the seminal fluid to modulate immune responsive genes would therefore imply that a differential protein abundance in the seminal fluid might have a regulating capacity of the oviductal sperm storage area by modulating the local immune responsive genes. If so, selection might have changed this capacity, not affecting the spermatozoa but the SF proteome. Further studies are of course needed to explore this possibility. Differences in SF-proteins between the low egg-laying progenitor RJF, the high egg-laying modern domestic WL and an AIL- intercross between the two, are hereby made evident.

4.1 The SF of RJF lacks astacin-like metalloendopeptidase which is commonly found in most biological process and protein class categories

The present study showed that the numbers of identified SF-proteins from RJF, in the biological process categories and protein classes, were closer to WL, yet less than in the AIL (**Fig. 4 and Table 2**). Among the biological process categories, the RJF-SF contained stimulus responsive proteins (gallinacin-9 and apolipoprotein A-I) maintained in WL and AIL (Table 2A). However, the RJF-SF lacked several stimulus responsive proteins that were only detected in the SF of WL (i.e. astacin-like metalloendopeptidase) or AIL (i.e. astacin-like metalloendopeptidase, coagulation factor IX and thioredoxin). Astacin-like metalloendopeptidase, relevant in most of the biological processes and protein class categories (**Table 2**), is present in the seminal fluid of *D. melanogaster*, and is responsible for processing other reproductive proteins and for inducing a variety of post-mating responses in the female fly (Ram et al., 2006; Sirot et al., 2009; Ayroles et al., 2011; LaFlamme et al., 2012). Our results suggest this protein is also important in the chicken, reinforcing results previously reported (Labas et al., 2015). Interestingly, this protein was apparently absent in the SF of the ancestor RJF, calling for further studies to determine whether evolution has elevated the levels of this particular protein in modern chicken. Coagulation factor IX is an anti-hemophilic B single chain polypeptide with M_r 57,000 Da in human and bovine which circulates in blood plasma (DiScipio et al., 1978; Katayama et al., 1979). In humans, the coagulation factor IX has been detected in the seminal plasma of both fertile and infertile donors (Lwaleed et al., 2005). However, further research is necessary to disclose whether coagulation factor IX could be a biomarker for rooster fertility. Thioredoxin has been reported in the *Gallus gallus* seminal fluid being classified as defense- or immunity-protein (Marzoni et al., 2013) while in the present study it was classified as a stimulus responsive protein (**Table 2A**). Cytoskeletal proteins especially tubulins are strongly expressed in human normal sperm compared to sperm from infertile man (Salvolini et al., 2013). In the present study, cytoskeletal proteins were only detected in the AIL-SF, crossbred individuals without fertility deficiencies. Further analyses are needed to explore this exception.

4.2 The rooster seminal fluid conserves serum albumin and ovotransferrin, but they are over-expressed in modern WL and AIL, suggesting a role for production performance

There were a number of proteins conserved in modern chicken. Around fifty percent of the identified proteins in the seminal fluid of all breeds from the 2D gels were ovotransferrin and serum albumin (**Table 1**), confirming recent reports (Castillo et al., 2011; Marzoni et al., 2013). Serum albumin (spots 126 and 214) was differentially expressed (with lesser expression in the RJF than in WL and AIL) (**Fig. 3 and Supplementary Table 2**). Serum albumin reported in both chicken (Marzoni et al., 2013) and sheep (Soleilhavoup et al., 2014), originates from the epididymis and prostate gland in humans (Elzanaty et al., 2007) but its origin is not determined as yet in chicken. Serum albumin has been classified as a defense or immunity protein (Marzoni et al., 2013) and it is used as an additive in animal semen handling industry (Matsuoka et al., 2006; Fukui et al., 2007; Hossain et al., 2007; Xia and Ren, 2009; Nang et al., 2012), since it appears to increase the percentage of motile spermatozoa and sperm velocity (Bakst and Cecil, 1992). The overall consideration is that the different expression levels between ancestors and modern breeds might relate to their documented differences in fertility.

Ovotransferrin (Spots 123 and 192), a member of iron-binding transferrin and metalloproteinases, has antimicrobial activity (Valenti et al., 1983) and is found in all roosters

yet in lesser quantities (2 to 4 fold) in the SF of RJF than in that of WL and the AIL (**Fig. 3 and Supplementary Table 2**). In mammalian seminal plasma, transferrin originates in the Sertoli cells (Holmes et al., 1982; Gilmont et al., 1990) and thus been shown to be an important biomarker for spermatogenesis (Holmes et al., 1982; Bharshankar and Bharshankar, 2000). Ovotransferrin as a defensive protein (Marzoni et al., 2013) is also present in egg albumin, depicting a strong bactericidal effect (Baron et al., 2014). It is therefore possible that during chicken mating, the ovotransferrin contained in the SF acts as an antimicrobial during semen deposition in the female cloaca, thus decreasing pathogen overload during sperm colonization and storage in the oviduct reservoir (sperm-storage tubules). A lower amount of the protein in RJF-SF as compared to WL- and AIL- might therefore be related to production performance.

Another conserved protein is the cytoskeletal protein-gelsolin family, present in all roosters albeit in different types (Gelsolin, Src substrate protein p85, tubulin α -5 chain, tubulin β -1 chain and tubulin β -5 chain) (**Table 2B**). Among these cytoskeletal proteins, Gelsolin is reported as influencing the acrosome reaction during human fertilization (Finkelstein et al., 2010). Although it is unclear what role this protein plays in chicken fertilization, it is interesting that it is conserved in such a primitive phenomenon as internal fertilization.

4.3 Other differentially expressed proteins in the seminal fluid might mirror egg-laying capacity evolved through selection pressure

In addition to serum albumin and ovotransferrin, ovoinhibitor (spot 129), creatinine kinase B-type (spot 131), α -enolase (spot 132), L-lactate dehydrogenase B chain (spot 140), triosephosphate isomerase (spot 145), retinol-binding protein (spot 146), apolipoprotein A-I (spot 147), malate dehydrogenase (spot 139), glyceraldehyde-3-phosphate dehydrogenase (spot 141), cystatin (spot 152) and gallinacin-9 (spot 157) were differentially expressed in the SF of RJF compared to WL, whereas, phosphoglycerate mutase 1 (spot 230), tubulin beta-1 chain (spot 172), transthyretin (spot 85), Ig λ -chain C (spot 243), protein NEL (spot 162) and arginosuccinate synthase (spot 62) were differentially expressed in the SF of RJF while compared with AIL (**Fig. 3 and Supplementary Table 2**).

Among these differentially expressed SF proteins between RJF and WL, ovoinhibitor, creatinine kinase B-type, α -enolase, L-lactate dehydrogenase B-chain, triosephosphate isomerase, retinol binding protein and apolipoprotein A-I were down-expressed in RJF compared to WL, whereas, glyceraldehyde-3-phosphate dehydrogenase, cystatin and gallinacin-9 were over-expressed in RJF compared to WL. Ovoinhibitor was reported as having antibacterial activity in turkey seminal fluid (Slowinska et al., 2014). Creatinine kinase B-type, α -enolase, triosephosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase are reported chicken seminal fluid proteins, classified as energy metabolism proteins (Marzoni et al., 2013). This author also reported apolipoprotein A-I as a transport or binding protein and gallinacin-9 as a defense or immunity protein in chicken-SF. Malate dehydrogenase was reported to be 2.23 fold increased in the SF of infertile chicken (Labas et al., 2015). Our findings of down-expression of proteins with bactericidal (ovoinhibitor), sperm motility-enhancing (creatinine kinase B-type), energy metabolism (α -enolase and triosephosphate isomerase) and binding (apolipoprotein A-I) capacities; as well as the over-expression of defense or immunity protein (gallinacin-9) in the SF

of low-laying RJF compared to high-laying WL (**Fig. 3A and Supplementary Table S2A**) suggest these proteins relate to their differential fertility.

Phosphoglycerate mutase 1 and tubulin β -1 chain are down-expressed in the SF of RJF compared to AIL (**Fig. 2C and Supplementary Table S2B**). Phosphoglycerate mutase 1 has been categorized into energy metabolism group (Marzoni et al., 2013) has also been identified in rooster sperm (Labas et al., 2015). In ruminant seminal plasma (bull, buck and ram) however, only phosphoglycerate mutase 2 has been identified (Druart et al., 2013). In a more recent report (Labas et al., 2015), predicted tubulin alpha-3 and tubulin β -3 chains were detected in rooster sperm while tubulin β -2C, isoform CRA-b was detected in the seminal plasma of rams (Soleilhavoup et al., 2014), indicating that differences are present between these classes. Four proteins (transthyretin, Ig λ -chain C, protein NEL and arginosuccinate synthase) were found over-expressed in the seminal fluid of RJF compared with AIL (**Fig. 3C and Supplementary Table S2B**). Transthyretin was found overexpressed in the yellow seminal fluid of sub-fertile turkey compared to fertile males (Slowinska et al., 2015). The Ig λ -chain C-a defense/immunity protein (**Table 2**), previously reported in rooster-SF (Labas et al., 2015), and protein NEL- is a developmental process and calcium ion-binding protein (**Table 2A**) found in bovine seminal plasma (Kelly et al., 2006)- were over-expressed in RJF-SF compared to AIL-SF, in the current study, albeit reported before as down-expressed in infertile roosters (Labas et al., 2015). Argininosuccinate synthase seems to be involved in disposing excess ammonium in semen (Dietz and Flipse, 1969), and related to nitric oxide metabolism. However, it is not known whether the increment of this protein, as found in the present study, relates the enzyme to sperm survival in neither ancestor nor modern chicken. Its character as biomarker for sperm quality warrants further studies of this enzyme.

4.4 The seminal fluid of RJF lacks TGF- β 2 but is rich in CXCL10: a relation to lower sperm survival?

Cytokines and chemokines are important modulators of the immune system process. They are necessary to help the female eliminate pathogens entering with the semen but at the same time allow spermatozoa to survive in the sperm reservoir at the utero-vaginal junction (UVJ), as it happens in the mammalian tubal sperm reservoirs (Robertson et al., 2002). A bead-based immune-assay screening of a battery of cytokines/chemokines reported in mammals (Barranco et al., 2015) was initially done, which resulted in only few cytokines being detectable in rooster SF, i.e. the immune-suppressive TGF- β and the immune cell-chemoattractant CXCL10, those related to the above mentioned dual effects (Agostini et al., 2001; Dufour et al., 2002; Das et al., 2006). Since this initial detection in SF was not present in all breeds, a new attempt was done to confirm their presence by using a chicken-specific ELISA, where we could find that TGF- β 2 levels did not differ between WL and AIL but that the cytokine was absent in the SF of RJF (**Fig. 5C**). The concentrations of CXCL10 differed significantly between RJF and WL ($P < 0.05$) with AIL values in between (**Fig. 5D**). The cytokine TGF- β 2 is present in the seminal plasma of normal fertile men (Nocera and Chu, 1995; Srivastava et al., 1996). The presence of TGF β in the seminal fluid is usually in latent form therefore, has to be activated in the female reproductive tract post-insemination (Robertson, 2005). In chicken, insemination increases the expression of TGF- β 2 in the UVJ, which is apparently responsible for the immunosuppression as well as it helps sperm

survival (Das et al., 2006). In contrast, the chemokine CXCL10 is a chemoattractant that recruits activated T-cells (Agostini et al., 2001). CXCL10^{-/-} KO-mice show impaired T-cell responses (Dufour et al., 2002). Interestingly, the present study shows that immune-modulating cytokine/chemokine concentrations in the SF differ between low-laying and high-laying chicken breed, the RJF depicting high amounts of immune reacting CXCL10 and lack of the immunosuppressive cytokine-TGF- β 2. The modern domesticated chicken has evolved from this common ancestor RJF, towards a higher egg-laying capacity. Such selection has, apparently, contributed to differential SF-cytokines, which may be key determinants of sperm survival in the UVJ.

Conclusion

We are aware that the lack of orthogonal quantitative validation of the comparative 2DE data represents a potential weakness of the study. However, the current lack of commercial antibodies with proven specificity, and the absence of SILAC chicken model for quantitative proteomics, relegate orthogonal validation beyond the scope of this study. Consequently, our work should be seen as preliminary, and the quantitative validation of relevant proteins should be pursued in future studies. Given this limitation, our findings suggest that several proteins (especially gallinacin-9 and Ig λ -chain C) and specific cytokines (TGF- β 2 and CXCL10) are differentially expressed in the SF between ancestor-RJF and modern- (WL and AIL) chicken, presumably due to the selection for production traits during chicken breeding.

Conflict of interest statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

The project has been financed by the Research Council FORMAS, Stockholm, Sweden (Project number: 221-2011-512), and by grant BFU2013-42833-P from the Ministerio de Ciencia e Innovación (Madrid, Spain),

Acknowledgements

Prof. Jordi Altimiras and Per Jensen are acknowledged for putting at our disposal the chicken included in this study. We thank Prof. Maria Jenmalm for her suggestions regarding cytokine analyses.

References

Agostini C, Calabrese F, Rea F, Facco M, Tosoni A, Loy M, Binotto G, Valente M, Trentin L, and Semenzato G. CXCR3 and its ligand CXCL10 are expressed by inflammatory cells

- infiltrating lung allografts and mediate chemotaxis of T cells at site of rejection. *Am J Pathol* 158 (2001) 1703.
- Atikuzzaman M, Bhai RM, Fogelholm J, Wright D, and Rodriguez-Martinez H. Mating induces the expression of immune- and pH-regulatory genes in the utero-vaginal junction containing mucosal sperm-storage tubuli of hens. *Reproduction* 150 (6) (2015) 473-83.
- Ayroles JF, LaFlamme BA, Stone EA, Wolfner MF, and Mackay TF. Functional genome annotation of *Drosophila* seminal fluid proteins using transcriptional genetic networks. *Genet Res* 93 (2011) 387-395.
- Bakst MR, and Cecil HC. Effect of bovine serum albumin on motility and fecundity of turkey spermatozoa before and after storage. *J Reprod Fertil* 94 (1992) 287-293.
- Baron F, Jan S, Gonnet F, Pasco M, Jardin J, Giudici B, Gautier M, Guérin-Dubiard C, and Nau F. Ovotransferrin plays a major role in the strong bactericidal effect of egg white against the *Bacillus cereus* group. *J Food Prot* 77(6) (2014) 955-62.
- Barranco I, Rubér M, Perez-Patiño C, Atikuzzaman M, Martinez EA, Roca J, Rodriguez-Martinez H. The seminal plasma of the boar is rich in cytokines, with significant individual and intra-ejaculate variation. *Am J Reprod Immunol* 74 (2015) 523-532.
- Batruch I, Lecker I, Kagedan D, Smith CR, Mullen BJ, Grober E, Lo KC, Diamandis EP, and Jarvi KA. Proteomic analysis of seminal plasma from normal volunteers and post-vasectomy patients identifies over 2000 proteins and candidate biomarkers of the urogenital system. *J Proteome Res* 10 (2011) 941-953.
- Bharshankar RN, Bharshankar JR. Relationship of seminal plasma transferrin with seminal parameters in male infertility. *Indian J Physiol Pharmacol* 44(4) (2000) 456-60.
- Blesbois E and Hermier D. Effects of high-density lipoproteins on storage at 4°C of fowl spermatozoa. *J Reprod Fertil* 90 (1990) 473-482.
- Bromfield JJ, Schjenken JE, Chin PY, Care AS, Jasper MJ, and Robertson SA. Maternal factors contribute to paternal seminal fluid impact on metabolic phenotype in offspring. *Proc Natl Acad Sci* 111 (2014) 2200-2205.
- Caballero I, Parrilla I, Almiñana C, del Olmo D, Roca J, Martínez EA, and Vázquez JM. Seminal plasma proteins as modulators of the sperm function and their application in sperm biotechnologies. *Reprod Domest Anim* 47 (Suppl.3) (2012) 12-21.
- Calvete JJ, Fasoli E, Sanz L, Boschetti E, and Righetti PG. Exploring the venom proteome of the Western Diamondback Rattlesnake, *Crotalus atrox*, via snake venomomics and combinatorial peptide ligand library approaches. *J Proteome Res* 8 (2009) 3055-3067.
- Calvete JJ, Nessau S, Mann K, Sanz L, Sieme H, Klug E, and Töpfer-Petersen E. Isolation and biochemical characterization of stallion seminal-plasma proteins. *Reprod Domest Anim* 29 (1994) 411-426.
- Castillo A, Marzoni M, Sagona S, Citti L, Rocchiccioli S, Romboli I, and Felicioli A. Ovotransferrin and gallinacin-9 found in seminal plasma rooster. 6th ITPA Annual National Conference, Torino (2011).
- Crawford G, Ray A, Gudi A, Shah A, and Homburg R. The role of seminal plasma for improved outcomes during *in vitro* fertilization treatment: review of the literature and meta-analysis. *Hum Reprod Update* 21 (2015) 275-284.

- Das SC, Isobe N, and Yoshimura Y. Changes in the expression of interleukin-1 β and lipopolysaccharide-induced TNF factor in the oviduct of laying hens in response to artificial insemination. *Reproduction* 137 (2009) 527-536.
- Das SC, Isobe N, and Yoshimura Y. Expression of Toll-like receptors and avian b-defensins and their changes in response to bacterial components in chicken sperm. *Poult Sci* 90 (2011) 417-425.
- Das SC, Isobe N, and Yoshimura Y. Mechanisms of prolonged sperm storage and sperm survivality in hen oviduct: A review. *Reprod Immunol* 60 (2008) 477-481.
- Das SC, Isobe N, Nishibori M, and Yoshimura Y. Expression of TGF β -isoforms and their receptors in utero-vaginal junction of hen oviduct in presence or absence of resident sperm with reference to sperm storage. *Reproduction* 132 (2006) 781-790.
- Dietz RW, and Flipse RJ. Metabolism of bovine semen. XX. Role of ammonia in interactions between the citric acid and urea cycles. *Biol Reprod* 1(1969) 200-206.
- DiScipio RG, Kurachi K, and Davie EW. Activation of human factor IX (Christmas factor). *J Clin Invest* 61 (1978) 1528-1538.
- Douard V, Hermier D, Labbe C, Magistrini M, and Blesbois E. Role of seminal plasma in damage to turkey spermatozoa during in vitro storage. *Theriogenology* 63 (2005) 126-137.
- Druart X, Rickard JP, Mactier S, Kohnke PL, Kershaw-Young CM, Bathgate R, Gibb Z, Crossett B, Tsikis G, Labas V, Harichaux G, Grupen CG, and de Graaf SP. Proteomic characterization and cross species comparison of mammalian seminal plasma. *J Proteomics* 91 (2013) 13-22.
- Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, and Luster AD. IFN- γ -inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. *J Immunol* 168 (2002) 3195-3204.
- Elzanaty S, Erenpreiss J, and Becker C. Seminal plasma albumin: origin and relation to the male reproductive parameters. *Andrologia* 39 (2007) 60-65.
- Etches RJ. The male. In: Etches, RJ (Ed.), *Reproduction in poultry*. CAB International, Oxford (1996) 208-233.
- Fazeli A, Affara NA, Hubank M, and Holt WV. Sperm-induced modification of the oviductal gene expression profile after natural insemination in mice. *Biol Reprod* 71 (2004) 60-65.
- Finkelstein M, Etkovitz N, and Breitbart H. Role and regulation of sperm gelsolin prior to fertilization. *J Biol Chem* 285 (2010) 39702-39709.
- Frankham F, Doornebal H. Semen characteristics of lines selected for increased part-record egg production. *Poult Sci* 51 (1972) 1468-1469.
- Froman DP, Feltmann AJ, Pendarvis K, Cooksey AM, Burgess SC, and Rhoads DD. A proteome-based model for sperm mobility phenotype. *J Anim Sci* 89 (2011) 1330-1337.
- Fujihara N. Accessory reproductive fluids and organs in male domestic birds. *Worlds Poult Sci J* 48 (1992) 39-56.
- Fukui Y, Kohno H, Togari T, and Hiwasa M. Fertility of ewes inseminated intrauterinally with frozen semen using extender containing bovine serum albumin. *J reprod Dev* 53 (2007) 959-962.
- Gilmont RR, Senger PL, Sylvester SR, and Griswold MD. Seminal transferrin and spermatogenic capability in the bull. *Biol Reprod* 43 (1990) 151-157.

- Harris GC Jr and Sweeney MJ. Changes in the protein concentration of chicken seminal plasma after rapid freeze-thaw. *Cryobiology* 7 (1971) 209-215.
- Holmes SD, Lipshultz LI, and Smith RG. Transferrin and gonadal dysfunction in man. *Fertil Steril* 38 (1982) 600-604.
- Hossain MS, Hyeong LJ, Miah AG, and Tsujii H. Effect of fatty acids bound to bovine serum albumin-V on acrosome reaction and utilization of glucose in boar spermatozoa. *Reprod Med Biol* 6 (2007) 109-115.
- Johnsson M, Gustafson I, Rubin C-J, Sahlqvist A-S, Johnsson KB, Kerje S, Ekwall O, Käämpe O, Andersson L, Jensen P & Wright D. A sexual ornament in chickens is affected by pleiotropic alleles at *HAOI* and *BMP2*, selected during domestication. *PLoS Genet* 8 (2012) e1002914.
- Kareskoski AM, Rivera del Alamo MM, Güvenc K, Reilas T, Calvete JJ, Rodriguez-Martinez H, Andersson M, and Katila T. Protein composition of seminal plasma in fractionated stallion ejaculates. *Reprod Domest Anim* 46 (2011) e79-e84.
- Katayama K, Ericsson LH, Enfield DL, Walsh KA, Neurath H, Davie EW, and Titani K. Comparison of amino acid sequence of bovine coagulation factor IX (Christmas factor) with that of other vitamin K-dependant plasma proteins. *Proc Natl Acad Sci* 76 (1979) 4990-4994.
- Kelly VC, Kuy S, Palmer DJ, Xu Z, Davis SR, and Cooper GJ. Characterization of bovine seminal plasma by proteomics. *Proteomics* 6 (2006) 5826-5833.
- Labas V, Grasseu I, Cahier K, Gargaros A, Harichaux G, Teixeira-Gomes A-P, Alves S, Bourin M, Gérard N, and Blesbois E. Qualitative and quantitative peptidomic and proteomic approaches to phenotyping chicken semen. *J Proteomics* 112 (2015) 313-335.
- Laemmli, UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227 (1970) 680-685.
- LaFlamme BA, Ram KR, and Wolfner MF. The *Drosophila melanogaster* seminal fluid protease “seminase” regulates proteolytic and post-mating reproductive process. *PLoS Genet* 8 (2012) e1002435.
- Lwaleed BA, Greenfield RS, Hicks J, Birch BR, and Cooper AJ. Quantitation of seminal factor IX and factor IXa in fertile, nonfertile, and vasectomy subjects: a step closer toward identifying a functional clotting system in human semen. *J Androl* 26 (2005) 146-152.
- Malik A, Haron AW, Yosoff R, Nesa M, Bukar M, Kasim A. Evaluation of the ejaculate quality of the red jungle fowl, domestic chicken, and bantam chicken in Malaysia. *Turkish J Vet Anim Sci* 37 (2013) 564-568.
- Mann T, Mann CL, and Dixon RL. Passage of chemicals into human and animal semen: mechanisms and significance. *Crit Rev Toxicol* 11(1) (1982) 1-14.
- Marzoni M, Castillo A, Sagona S, Citti L, Rocchiccioli S, Romboli I, and Felicioli A. A proteomic approach to identify seminal plasma proteins in roosters (*Gallus gallus domesticus*). *Anim Reprod Sci* 140 (2013) 216-223.
- Matsuoka T, Imai H, Kohno H, and fukui Y. Effects of bovine serum albumin and trehalose in semen diluents for improvement of frozen-thawed ram spermatozoa. *J Reprod Dev* 52 (2006) 675-683.

- Mi H, Poudel S, Casagrande AMJT, and Thomas PD. PANTHER version10: expanded protein families and functions, and analysis tools. *Nucleic Acids Res* 44 (D1) (2016) D336-42 doi:10.1093/nar/gkv1194.
- Michailidis G, Anastasiadou M, Guibert E, and Froment P. Activation of innate immune system in response to lipopolysaccharide in chicken sertoli cells. *Reproduction* 148 (2014) 259-270.
- Milardi D, Grande G, Vincenzoni F, Castagnola M, and Marana R. Proteomics of human seminal plasma: identification of biomarkers candidates for fertility and infertility and the evolution of technology. *Mol Reprod Dev* 80 (2013) 350-357.
- Murugesan S, Matam N, Kulkarni R, Bhattacharya TK, Chatterjee R. Semen quality in white leghorn chicken selected for egg production traits. *Turk J Vet Anim Sci* 37 (2013) 747-749.
- Nang CF, Osman K, Budin SB, Ismail MI, Jaffar FHF, Mohamad SFS, and Ibrahim SF. Bovine serum albumin: survival and osmolarity effect in bovine spermatozoa stored above freezing point. *Andrologia* 44 (2012) 447-453.
- Niranjan M, Datt C, Das A. Semen quality studies in two White Leghorn lines maintained at Tripura. *Indian J Poult Sci* 36 (2001) 287-289.
- Nocera M, Chu TM. Characterization of latent transforming growth factor-beta from human seminal plasma. *Am J Reprod Immunol* 33 (1995) 282-291.
- Novak S, Ruiz-Sánchez A, Dixon WT, Foxcroft GR, and Dyck MK. Seminal plasma proteins as potential markers of relative fertility in boars. *J Androl* 31 (2010) 188-200.
- Ocón-Grove OM, Krzysik-Walker SM, Maddineni SR, Hendricks II GL, and Ramachandran R. NAMPT (visfatin) in the chicken testis: influence of sexual maturation on cellular localization, plasma levels and gene and protein expression. *Reproduction* 139 (2010) 217-226.
- Perez-Patiño C, Barranco I, Parrilla I, Martinez EA, Rodriguez-Martinez H, and Roca J. Characterization of the porcine seminal plasma proteome comparing ejaculate portions. *J Proteomics* 142 (2016) 15-23.
- Pilch B and Mann M. large-scale and high-confidence proteomic analysis of human seminal plasma. *Genome Biol* 7 (2006) R40.
- Ram KR, Sirot LK, and Wolfner MF. Predicted seminal astacin-like protease is required for processing of reproductive proteins in *Drosophila melanogaster*. *Proc Natl Acad Sci* 103 (2006) 18674-18679.
- Robertson SA, Ingman WV, O'Leary S, Sharkey DJ, Tremellen KP. Transforming growth factor β -a mediator of immune deviation in seminal plasma. *J Reprod Immunol* 57 (2002) 109-128.
- Robertson SA. Seminal plasma and male factor signaling in the female reproductive tract. *Cell Tissue Res* 322 (2005) 43-52.
- Rodrigues MAM, Souza CEA, Martins JAM, Rego JPA, Oliveira JTA, Domont G, Nogueira FCS, and Moura AA. Seminal plasma proteins and their relationship with sperm motility in Santa Ines rams. *Small Ruminant Res* 109 (2013) 94-100.
- Rodríguez-Martínez H, Iborra I, Martínez P, and Calvete JJ. Immunoelectroscopic imaging of speradhesin AWN epitopes on boar spermatozoa bound *in vivo* to the zona pellucida. *Reprod Fertil Dev* 10 (1998) 491-497.

- Rodríguez-Martínez H, Kvist U, Ernerudh J, Sanz L, and Calvete JJ. Seminal plasma proteins: what role do they play? *Am J Reprod Immunol* 66 (Suppl. 1) (2011) 11-22.
- Rodríguez-Martínez H, Saravia F, Wallgren M, Roca J, and Pena FJ. Influence of seminal plasma on kinematics of boar spermatozoa during freezing. *Theriogenology* 70 (2008) 1242-1250.
- Salvolini E, Buldreggini E, Lucarini G, Vignini A, Lenzi A, Di Primio R, and Balercia G. Involvement of sperm plasma membrane and cytoskeletal proteins in human male infertility. *Fertil Steril* 99 (2013) 697-704.
- Schjenken JE, and Robertson SA. Seminal fluid and immune adaptation for pregnancy-comparative biology in maternal species. *Reprod Domest Anim* 49 (Suppl. 3) (2014) 27-36.
- Sharma R, Agarwal A, Mohanty G, Jesudasan R, Gopalan B, Willard B, Yadav SP, and Sabanegh E. Functional proteomic analysis of seminal plasma proteins in men with various semen parameters. *Reprod Biol Endocrinol* 11 (2013) 38.
- Sirot LK, LaFlamme BA, Sitnik JL, Rubinstein CD, Avila FW, Chow CY, and Wolfner MF. Molecular social interactions: *Drosophila melanogaster* seminal fluid proteins as a case study. *Adv Genet* 68 (2009) 23-56.
- Slowin'ska M, Liszewska E, Nynca J, Bukowska J, Hejmej A, Bilin'ska B, Szubstarski J, Kozlowski K, Jankowski J, and Ciereszko A. Isolation of an ovinhibitor, a multidomain kazal-like inhibitor from turkey (*Meleagris gallopavo*) seminal plasma. *Biol Reprod* 91(5) (2014) 108.
- Slowinska M, Kozlowski K, Jankowski J, and Ciereszko A. Proteomic analysis of white and yellow seminal plasma in turkeys. *J Anim Sci* 93(6) (2015) 2785-95.
- Soggiu A, Piras C, Hussein HA, De Canio M, Gaviraghi A, Galli A, Urbani A, Bonizzi L, and Roncada P. Unravelling the bull fertility proteome. *Mol Biosyst* 9 (2013) 1188-1195.
- Soler L, Labas V, Thélie A, Grasseau I, Teixeira-Gomes A-P, and Blesbois E. Intact cell MALDI-TOF MS on sperm: a molecular test for male fertility. *Mol Cell Proteomics* 15 (2016) 1998-2010.
- Soleilhavoup C, Tsikis G, Labas V, Harichaux G, Kohnke P, Dacheux J-L, Guérin Y, Gatti J-L, De Graaf SP, Druart X. Ram seminal plasma proteome and its impact on liquid preservation of spermatozoa. *J Proteomics* 109 (2014) 245-260.
- Song Z-H, Li Z-Y, Li D-D, Fang W-N, Liu H-Y, Yang D-D, Meng C-Y, Yang Y, and Peng J-P. Seminal plasma induces inflammation in the uterus through the $\gamma\delta$ T/IL-17 pathway. *Sci Rep* 6 (2016) 25118.
- Srivastava MD, Lippes J, Srivastava BL. Cytokines of the human reproductive tract. *Am J Reprod Immunol* 36 (1996) 157-166.
- Thurston RJ, Hess RA, Froman DP, Biellier HV. Elevated seminal plasma protein: a characteristic of yellow turkey semen. *Poult Sci* 61(9) (1982) 1905-11.
- Valenti P, Antonini G, Von Hunolstein C, Visca P, Orsi N, and Antonini E. Studies of the antimicrobial activity of ovotransferrin. *Int J Tissue React* 5 (1) (1983) 97-105.
- Xia J and Ren D. The BSA-induced Ca(2+) influx during sperm capacitation is CATSPERM channel-dependent. *Reprod Biol Endocrinol* 7 (2009) 119.

Table 1. Comparative identified proteins in chicken seminal fluid detected by 2D SDS-PAGE followed by mass spectrometry. Total protein spots on 2D gels -107 of RJF, 52 of WL and 98 of AIL- were analysed using LC-ESI-Q-TOF-MS/MS. Percent calculated from total number of spots analyzed in each breed.

Proteins identified in the chicken seminal fluid	Proteins detected in the seminal fluid % (total number of spots identified for a protein)			Spot number(s) on acrylamide gels
	RJF	WL	AIL	
Aspartate aminotransferase, cytoplasmic	1.87 (2)	0 (0)	0 (0)	71, 72
Angiotensin-converting enzyme (Fragment)	0 (0)	0 (0)	1.02 (1)	161
Serum albumin	11.21 (12)	21.15 (11)	13.27 (13)	2, 4, 7, 9, 10, 12, 14, 16, 42, 51, 52, 53, 108, 109, 111, 112, 113, 114, 116, 117, 124, 125, 126, 169, 170, 171, 182, 188, 195, 196, 199, 212, 213, 214, 217, 246
Apolipoprotein A-I	1.87 (2)	5.78 (3)	4.08 (4)	80, 90, 142, 143, 147, 237, 241, 242, 244
Annexin A5	0.93 (1)	0 (0)	0 (0)	86
Argininosuccinate synthase	0.93 (1)	0 (0)	0 (0)	62
Astacin-like metalloendopeptidase	0 (0)	1.92 (1)	1.02 (1)	141, 252
Complement factor B-like protease (Fragment)	0 (0)	1.92 (1)	1.02 (1)	120, 184
Cystatin	0.93 (1)	1.92 (1)	3.06 (3)	100, 152, 248, 250, 251
Alpha-enolase	1.87 (2)	1.92 (1)	1.02 (1)	57, 66, 132, 222
Beta-enolase	0.93 (1)	0 (0)	1.02 (1)	66, 222
Gamma-enolase	0 (0)	0 (0)	1.02 (1)	222
Coagulation factor IX	0 (0)	0 (0)	1.02 (1)	169
Fatty acid-binding protein, brain	1.87 (2)	1.92 (1)	3.06 (3)	97, 98, 149, 247, 248, 249
Fibrinogen alpha chain	0 (0)	0 (0)	1.02 (1)	213
Fibrinogen beta chain (Fragment)	1.87 (2)	0 (0)	4.08 (4)	50, 56, 210, 211, 212, 213
Gelsolin	2.80 (3)	3.85 (2)	2.04 (2)	30, 32, 34, 118, 128, 180, 182
Gallinacin-9	1.87 (2)	1.92 (1)	1.02 (1)	103, 105, 157, 256
Gallinacin-10	4.67 (5)	0 (0)	1.02 (1)	63, 103, 104, 105, 106, 256
Glutathione S-transferase 2	0.93 (1)	0 (0)	0 (0)	79
Glyceraldehyde-3-phosphate dehydrogenase	0 (0)	1.92 (1)	0 (0)	141
Hemoglobin subunit alpha-D	0 (0)	0 (0)	1.02 (1)	251
Ig mu chain C region	1.87 (2)	5.77 (3)	2.04 (2)	6, 31, 114, 116, 117, 182, 188
Ovoinhibitor	2.80 (3)	1.92 (1)	4.08 (4)	45, 46, 47, 129, 206, 207, 208, 209
Trypsin inhibitor CITI-1	1.87 (2)	3.85 (2)	2.04 (2)	101, 102, 154, 155, 253, 254

Creatine kinase B-type	0.93 (1)	1.92 (1)	2.04 (2)	61, 131, 218, 221
Pyruvate kinase PKM	0.93 (1)	0 (0)	1.02 (1)	54, 204
Ig lambda chain C region	3.74 (4)	1.92 (1)	4.08 (4)	87, 88, 89, 90, 142, 239, 241, 242, 243
L-lactate dehydrogenase A chain	0.93 (1)	0 (0)	0 (0)	75
L-lactate dehydrogenase B chain	0.93 (1)	1.92 (1)	2.04 (2)	74, 140, 228, 229
Lysozyme C	0 (0)	0 (0)	1.02 (1)	252
Malate dehydrogenase, cytoplasmic	0.93 (1)	1.92 (1)	1.02 (1)	73, 139, 227
Protein NEL	0.93 (1)	0 (0)	3.06 (3)	3, 161, 162, 170
Phosphoglycerate mutase 1	0 (0)	0 (0)	1.02 (1)	230
Phosphoglycerate kinase	0 (0)	0 (0)	1.02 (1)	225
Retinol-binding protein 4	0.93 (1)	1.92 (1)	1.02 (1)	94, 146, 244
Src substrate protein p85	0 (0)	0 (0)	1.02 (1)	141
Tubulin alpha-5 chain	0 (0)	0 (0)	2.04 (2)	172, 173
Tubulin beta-1 chain	0 (0)	0 (0)	1.02 (1)	172
Tubulin beta-5 chain	0 (0)	0 (0)	2.04 (2)	172, 173
Thioredoxin	0 (0)	0 (0)	1.02 (1)	246
Triosephosphate isomerase	1.87 (2)	1.92 (1)	1.02 (1)	79, 81, 145, 232
Ovotransferrin	15.89(17)	17.31 (9)	12.24 (12)	16, 17, 18, 26, 27, 28, 31, 32, 34, 35, 36, 37, 38, 39, 41, 52, 102, 114, 116, 117, 118, 119, 120, 121, 122, 123, 181, 185, 188, 189, 191, 192, 193, 194, 201, 203, 235, 252
Transthyretin	3.74 (4)	3.85 (2)	4.08 (4)	84, 85, 96, 97, 134, 148, 211, 237, 238, 245

Table 2A. Biological process analysis of identified protein in the seminal fluid of Red Junglefowl (RJF), White Leghorn (WL) and Advanced Intercross Line (AIL) male chicken.

Category name (Accession)		(Number of protein identified) Identified protein symbols		
		RJF	WL	AIL
1	Cellular component organization or biogenesis (GO:0071840)	(2) Gelsolin Apolipoprotein A-I	(2) Gelsolin Apolipoprotein A-I	(3) Gelsolin Src substrate protein p85 Apolipoprotein A-I
2	Cellular process (GO:0009987)	(5) Gelsolin Protein NEL Argininosuccinate synthase Fibrinogen beta chain Apolipoprotein A-I	(3) Gelsolin Astacin-like metalloendopeptidase Apolipoprotein A-I	(8) Gelsolin Protein NEL Astacin-like metalloendopeptidase Fibrinogen alpha chain Thioredoxin Fibrinogen beta chain Src substrate protein p85 Apolipoprotein A-I
3	Localization (GO:0051179)	(4) Serum albumin Retinol-binding protein 4 Transthyretin Apolipoprotein A-I	(5) Serum albumin Astacin-like metalloendopeptidase Retinol-binding protein 4 Transthyretin Apolipoprotein A-I	(7) Hemoglobin subunit alpha-D Serum albumin Astacin-like metalloendopeptidase Retinol-binding protein 4 Coagulation factor IX Transthyretin Apolipoprotein A-I
4	Apoptotic process (GO:0006915)	(1) Trypsin inhibitor CITI-1	(1) Trypsin inhibitor CITI-1	(2) Coagulation factor IX Trypsin inhibitor CITI-1
5	Biological regulation (GO:0065007)	(1) Apolipoprotein A-I	(2) Astacin-like metalloendopeptidase Apolipoprotein A-I	(4) Astacin-like metalloendopeptidase Coagulation factor IX Thioredoxin Apolipoprotein A-I
6	Response to stimulus (GO:0050896)	(2) Gallinacin-9 Apolipoprotein A-I	(3) Astacin-like metalloendopeptidase Gallinacin-9 Apolipoprotein A-I	(5) Astacin-like metalloendopeptidase Coagulation factor IX Thioredoxin Gallinacin-9 Apolipoprotein A-I
7	Developmental process (GO:0032502)	(4) Fatty acid-binding protein, brain Gelsolin	(4) Fatty acid-binding protein, brain Gelsolin	(7) Fatty acid-binding protein, brain Gelsolin Protein NEL

		Protein NEL Apolipoprotein A-I	Astacin-like metalloendopeptidase Apolipoprotein A-I	Astacin-like metalloendopeptidase Coagulation factor IX Src substrate protein p85 Apolipoprotein A-I
8	Multicellular organismal process (GO:0032501)	(1) Apolipoprotein A-I	(2) Astacin-like metalloendopeptidase Apolipoprotein A-I	(5) Hemoglobin subunit alpha-D Astacin-like metalloendopeptidase Fibrinogen alpha chain Coagulation factor IX Apolipoprotein A-I
9	Biological adhesion (GO:0022610)	(2) Apolipoprotein A-I Ovotransferrin	(2) Astacin-like metalloendopeptidase Ovotransferrin	(4) Astacin-like metalloendopeptidase Fibrinogen alpha chain Fibrinogen beta chain Ovotransferrin
10	Metabolic process (GO:0008152)	(10) Annexin A5 L-lactate dehydrogenase A chain Beta-enolase Malate dehydrogenase, cytoplasmic L-lactate dehydrogenase B chain Triosephosphate isomerase Argininosuccinate synthase Gallinacin-9 Alpha-enolase Apolipoprotein A-I	(8) Malate dehydrogenase, cytoplasmic L-lactate dehydrogenase B chain Triosephosphate isomerase Astacin-like metalloendopeptidase Gallinacin-9 Glyceraldehyde-3-phosphate dehydrogenase Alpha-enolase Apolipoprotein A-I	(13) Beta-enolase Malate dehydrogenase, cytoplasmic Phosphoglycerate kinase L-lactate dehydrogenase B chain Triosephosphate isomerase Astacin-like metalloendopeptidase Gamma-enolase Coagulation factor IX Thioredoxin Gallinacin-9 Src substrate protein p85 Alpha-enolase Apolipoprotein A-I
11	Immune system process (GO:0002376)	(3) Gallinacin-9 Ig mu chain C region Ig lambda chain C region	(5) Astacin-like metalloendopeptidase Gallinacin-9 Complement factor B-like protease Ig mu chain C region Ig lambda chain C region	(6) Astacin-like metalloendopeptidase Coagulation factor IX Gallinacin-9 Complement factor B-like protease Ig mu chain C region Ig lambda chain C region
12	Fertilization (GO:0009566)	(1) Trypsin inhibitor CITI-1	(1) Trypsin inhibitor CITI-1	(1) Trypsin inhibitor CITI-1

Table 2B. Protein class analysis of identified proteins in the seminal fluid Red Junglefowl (RJF), White Leghorn (WL) and Advanced Intercross Line (AIL) male chicken.

Category name (Accession)		(Number of protein identified) Identified protein symbols		
		RJF	WL	AIL
1	Extracellular matrix protein (PC00102)	-	(1) Astacin-like metalloendopeptidase	(1) Astacin-like metalloendopeptidase
2	Protease (PC00190)	-	(2) Astacin-like metalloendopeptidase Complement factor B-like protease	(3) Astacin-like metalloendopeptidase Coagulation factor IX Complement factor B-like protease
3	Cytoskeletal protein (PC00085)	(1) Gelsolin	(1) Gelsolin	(5) Gelsolin Src substrate protein p85 Tubulin alpha-5 chain Tubulin beta-1 chain Tubulin beta-5 chain
4	Transporter (PC00227)	(2) Transthyretin Ovotransferrin	(3) Astacin-like metalloendopeptidase Transthyretin Ovotransferrin	(3) Astacin-like metalloendopeptidase Transthyretin Ovotransferrin
5	Transferase (PC00220)	-	-	(1) Phosphoglycerate kinase
6	oxidoreductase (PC00176)	(3) L-lactate dehydrogenase A chain Malate dehydrogenase, cytoplasmic L-lactate dehydrogenase B chain	(4) Malate dehydrogenase, cytoplasmic L-lactate dehydrogenase B chain Astacin-like metalloendopeptidase Glyceraldehyde-3-phosphate dehydrogenase	(4) Malate dehydrogenase, cytoplasmic L-lactate dehydrogenase B chain Astacin-like metalloendopeptidase Thioredoxin
7	Lyase (PC00144)	(2) Beta-enolase Alpha-enolase	(1) Alpha-enolase	(3) Beta-enolase Gamma-enolase Alpha-enolase
8	Cell adhesion molecule (PC00069)	-	(1) Astacin-like metalloendopeptidase	(1) Astacin-like metalloendopeptidase
9	Signaling molecule (PC00207)	(1) Fibrinogen beta chain	(1) Astacin-like metalloendopeptidase	(4) Astacin-like metalloendopeptidase Fibrinogen alpha chain Coagulation factor IX Fibrinogen beta chain
10	Enzyme modulator	(3)	(4)	(5)

	(PC00095)	Ovoinhibitor Cystatin Trypsin inhibitor CITI-1	Astacin-like metalloendopeptidase Ovoinhibitor Cystatin Trypsin inhibitor CITI-1	Astacin-like metalloendopeptidase Coagulation factor IX Ovoinhibitor Cystatin Trypsin inhibitor CITI-1
11	Calcium-binding protein (PC00060)	(2) Gelsolin Protein NEL	(1) Gelsolin	(3) Gelsolin Protein NEL Coagulation factor IX
12	Defense/immunity protein (PC00090)	(3) Gallinacin-9 Ig mu chain C region Ig lambda chain C region	(4) Gallinacin-9 Complement factor B-like protease Ig mu chain C region Ig lambda chain C region	(4) Gallinacin-9 Complement factor B-like protease Ig mu chain C region Ig lambda chain C region
13	Hydrolase (PC00121)	-	(2) Astacin-like metalloendopeptidase Complement factor B-like protease	(3) Astacin-like metalloendopeptidase Coagulation factor IX Complement factor B-like protease
14	Transfer/carrier protein (PC00219)	(3) Serum albumin Retinol-binding protein 4 Transthyretin	(4) Serum albumin Astacin-like metalloendopeptidase Retinol-binding protein 4 Transthyretin	(5) Hemoglobin subunit alpha-D Serum albumin Astacin-like metalloendopeptidase Retinol-binding protein 4 Transthyretin
15	Transcription factor (PC00218)	-	-	(1) Src substrate protein p85
16	Kinase (PC00137)	-	-	(1) Phosphoglycerate kinase
17	Isomerase (PC00135)	(1) Triosephosphate isomerase	(1) Triosephosphate isomerase	(1) Triosephosphate isomerase
18	Receptor (PC00197)	-	(1) Astacin-like metalloendopeptidase	(2) Astacin-like metalloendopeptidase Coagulation factor IX
19	Ligase (PC00142)	(1) Argininosuccinate synthase	-	-

Figure 1. Coomassie Brilliant Blue stained 2D gels of the seminal fluid of individual males (n= 4) from each the Advanced Intercross Line (AIL, panel A), White Leghorn (WL, panel B), and Red Junglefowl (RJF, panel C) groups. Panel D presents a PCA plot showing the overall degree of intra- and intergroup variability.

Figure 2. Coomassie Brilliant Blue stained 2D gels of pooled seminal fluid of Red Junglefowl (A, n= 15), White Leghorn (B, n= 15) and Advanced Intercross Line (C, n= 15) roosters. The gel spots (see the numerical numbers for each spot) were examined by LC-ESI-Q-TOF-MS/MS followed by Mascot ions search using SwissProt high quality, curated protein database.

Figure 3. Differentially expressed proteins (based on staining intensities of protein spots on 2D gels) in the seminal fluid (SF) of Red Junglefowl (RJF), White Leghorn (WL) and Advanced Intercross Line (AIL) roosters. A, fold changes of differentially expressed proteins in the SF between RJF (red column) and WL (black column); B, the protein spots on the 2D gels that had different staining intensities as shown in figure A; C, fold changes of differentially expressed proteins in the SF between RJF (red column) and AIL (light blue column); D, the protein spots on the 2D gels that had different staining intensities as shown in figure C.

Figure 4. Biological process (A) and protein class (B) analysis of identified proteins in pooled seminal fluid of roosters from the Red Junglefowl (n= 15), White Leghorn (n= 15) and Advanced Intercross Line (n= 15) breeds using PANTHER gene ontology classification following the protocol, described by Mi et al. 2016.

Figure 5. Transforming growth factor β -2 and CXCL10 concentrations in the seminal fluids of RJF, WL and AIL roosters. In Luminex, individual ejaculates were used to measure the concentrations of TGF- β 2 (A): RJF (ejaculates, n=40), WL (ejaculates, n=79), AIL (ejaculates, n=39) and CXCL10 (B): RJF (ejaculates, n=45), WL (ejaculates, n=79), AIL (ejaculates, n=43). In ELISA, ejaculates of individual birds were pooled and 10 pools (birds, n=10) per breed were used to measure the concentrations of TGF- β 2 (C) and CXCL10 (D). Different letters on the column represents values are significantly different ($P < 0.05$).

Identification by LC-MS/MS of the seminal fluid prot

Spot ID	Breed	Appr. MW (kDa)	Gene Name	Protein Name (Short)
1	RJF	200		NS
2	RJF	200	ALB	ALBU_CHICK
3	RJF	96	NEL	NEL_CHICK
4	RJF	156	ALB	ALBU_CHICK
5	RJF	77		NS
6	RJF	80	N/A	IGHM_CHICK
			N/A	TRFE_CHICK
7	RJF	80	ALB	ALBU_CHICK
8	RJF	80		NS
9	RJF	80	ALB	ALBU_CHICK
10	RJF	66.3	ALB	ALBU_CHICK

11	RJF	66.3		NS
12	RJF	66.3	ALB	ALBU_CHICK
13	RJF	60		NS
14	RJF	66.3	ALB	ALBU_CHICK
15	RJF	50	MIB2 DDA1 ALB	MIB2_CHICK DDA1_CHICK ALBU_CHICK
16	RJF	200	N/A	TRFE_CHICK
17	RJF	200	ALB N/A	ALBU_CHICK TRFE_CHICK
18	RJF	200	N/A	TRFE_CHICK
19	RJF	100		NS
20	RJF	100		NS
21	RJF	156		NS
22	RJF	156		NS

23	RJF	156		NS
24	RJF	156		NS
25	RJF	156	CNTN2	CNTN2_CHICK
26	RJF	156	N/A	TRFE_CHICK
27	RJF	156	N/A	TRFE_CHICK
			CNTN2	CNTN2_CHICK
28	RJF	156	N/A	TRFE_CHICK
29	RJF	97.4		NS
30	RJF	80	GSN	GELS_CHICK

31	RJF	66.3	GSN	GELS_CHICK
----	-----	------	-----	------------

	N/A	TRFE_CHICK
--	-----	------------

	N/A	IGHM_CHICK
--	-----	------------

32	RJF	80	GSN	GELS_CHICK
----	-----	----	-----	------------

	N/A	TRFE_CHICK
--	-----	------------

33	RJF	97.4		NS
34	RJF	80	N/A	TRFE_CHICK

			GSN	GELS_CHICK
--	--	--	-----	------------

35	RJF	66.3	N/A	TRFE_CHICK
----	-----	------	-----	------------

36	RJF	66.3	N/A	TRFE_CHICK
----	-----	------	-----	------------

37	RJF	66.3	N/A	TRFE_CHICK
----	-----	------	-----	------------

38	RJF	66.3	N/A	TRFE_CHICK
----	-----	------	-----	------------

39	RJF	66.3	N/A	TRFE_CHICK
----	-----	------	-----	------------

40	RJF	66.3		NS
41	RJF	66.3	N/A	TRFE_CHICK
42	RJF	60	ALB	ALBU_CHICK
43	RJF	60	ALB	ALBU_CHICK
44	RJF	60	ALB	ALBU_CHICK
45	RJF	55.4	OIH	IOV7_CHICK
46	RJF	55.4	OIH	IOV7_CHICK
47	RJF	55.4	OIH	IOV7_CHICK
48	RJF	55.4	OIH	IOV7_CHICK
49	RJF	52		NS
50	RJF	55.4	FGB	FIBB_CHICK
51	RJF	60	ALB	ALBU_CHICK

52	RJF	60	N/A	TRFE_CHICK
----	-----	----	-----	------------

			ALB	ALBU_CHICK
--	--	--	-----	------------

53	RJF	60	N/A	TRFE_CHICK
----	-----	----	-----	------------

			ALB	ALBU_CHICK
--	--	--	-----	------------

54	RJF	58	PKM	KPYM_CHICK
----	-----	----	-----	------------

55	RJF	60	ALB	ALBU_CHICK
----	-----	----	-----	------------

56	RJF	55.4	PKM FGB	KPYM_CHICK FIBB_CHICK
----	-----	------	------------	--------------------------

57	RJF	49	PKM ALB	KPYM_CHICK ALBU_CHICK
----	-----	----	------------	--------------------------

58	RJF	49	ENO1 ALB	ENOA_CHICK ALBU_CHICK
59	RJF	49	ALB	ALBU_CHICK
			CKB	KCRB_CHICK
60	RJF	48		NS
61	RJF	45	CKB	KCRB_CHICK
62	RJF	48	ASS1	ASSY_CHICK
63	RJF	50	GAL10	GLL10_CHICK
64	RJF	45		NS
65	RJF	36.5	CDCA8	BORE1_CHICK
66	RJF	50	ENO1	ENOA_CHICK

			ENO3	ENOB_CHICK
67	RJF	41		NS
68	RJF	55.4		NS
69	RJF	50		NS
70	RJF	45	PGK	PGK_CHICK
71	RJF	45	PGK	PGK_CHICK
			GOT1	AATC_CHICK
72	RJF	45	GOT1	AATC_CHICK
73	RJF	40	MDH1	MDHC_CHICK
74	RJF	36.5	LDHB	LDHB_CHICK
75	RJF	36.5	LDHA	LDHA_CHICK
76	RJF	40	MYOD1	MYOD1_CHICK
77	RJF	31		NS
78	RJF	31		NS
79	RJF	29	TPI1	TPIS_CHICK

			GSTM2	GSTM2_CHICK
80	RJF	28	APOA1	APOA1_CHICK
81	RJF	28	TPI1	TPIS_CHICK
82	RJF	23		NS
83	RJF	36.5		NS
84	RJF	36.5	TTR	TTHY_CHICK
			APOA1	APOA1_CHICK
85	RJF	36.5	ALB	ALBU_CHICK
86	RJF	28	TTR	TTHY_CHICK
			ANXA5	ANXA5_CHICK
87	RJF	29	APOA1	APOA1_CHICK
88	RJF	29	N/A APOA1	LAC_CHICK APOA1_CHICK

89	RJF	29	N/A ALB	LAC_CHICK ALBU_CHICK
90	RJF	28	N/A N/A	LAC_CHICK LAC_CHICK
			APOA1	APOA1_CHICK
91	RJF	23	VMO1	VMO1_CHICK
92	RJF	26	ALB	ALBU_CHICK
93	RJF	26	ALB	ALBU_CHICK
94	RJF	25	RBP4	RET4_CHICK
95	RJF	23	APOA1	APOA1_CHICK NS
96	RJF	15	TTR	TTHY_CHICK
97	RJF	14.5	FABP7	FABP7_CHICK

98	RJF	14	TTR FABP7	TTHY_CHICK FABP7_CHICK
99	RJF	14		NS
100	RJF	10	N/A	CYT_CHICK
101	RJF	6	N/A	ISK1L_CHICK
102	RJF	6	N/A	ISK1L_CHICK
103	RJF	5	N/A GAL9 GAL10	TRFE_CHICK GLL9_CHICK GLL10_CHICK
104	RJF	5	GAL10	GLL10_CHICK
105	RJF	4	GAL10 GAL9	GLL10_CHICK GLL9_CHICK
106	RJF	4	GAL10	GLL10_CHICK
107	RJF	4		NS
108	PWL	66.3	ALB	ALBU_CHICK
109	PWL	66.3	ALB	ALBU_CHICK
110	PWL	66		NS
111	PWL	66	ALB	ALBU_CHICK

112	PWL	97	ALB	ALBU_CHICK
-----	-----	----	-----	------------

113	PWL	90	N/A	TRFE_CHICK
			N/A	IGHM_CHICK
			ALB	ALBU_CHICK

114	PWL	100	ALB	ALBU_CHICK
			N/A	TRFE_CHICK
115	PWL	98	N/A	IGHM_CHICK NS
116	PWL	70	N/A	TRFE_CHICK
			N/A	IGHM_CHICK
117	PWL	66.3	ALB N/A	ALBU_CHICK TRFE_CHICK
			N/A ALB	IGHM_CHICK ALBU_CHICK

118	PWL	66.3	N/A	TRFE_CHICK
			GSN	GELS_CHICK
119	PWL	97	N/A	TRFE_CHICK
120	PWL	95	N/A	TRFE_CHICK
121	PWL	79	N/A	CFBL_CHICK
			N/A	TRFE_CHICK
122	PWL	79	N/A	TRFE_CHICK
123	PWL	66.3	N/A	TRFE_CHICK

124	PWL	66.3	ALB	ALBU_CHICK
			ALB	ALBU_CHICK
125	PWL	66.3	ALB	ALBU_CHICK

126	PWL	66.3	ALB	ALBU_CHICK
-----	-----	------	-----	------------

127	PWL	52		NS
128	PWL	50	GSN	GELS_CHICK
			ALB	ALBU_CHICK
129	PWL	55	OIH	IOV7_CHICK
130	PWL	55		NS
131	PWL	50	CKB	KCRB_CHICK

132	PWL	50	ASS1	ASSY_CHICK
			ENO1	ENOA_CHICK

			ENO3	ENOB_CHICK
--	--	--	------	------------

133	PWL	36.5		NS
134	PWL	36.5	TTR	TTHY_CHICK
135	PWL	36		NS
136	PWL	38		NS
137	PWL	55.4		NS
138	PWL	35		NS
139	PWL	37	MDH1	MDHC_CHICK
			LDHB	LDHB_CHICK
140	PWL	36.5	LDHB	LDHB_CHICK
141	PWL	40	ASTL	ASTL_CHICK
142	PWL	30	GAPDH APOA1	G3P_CHICK APOA1_CHICK
143	PWL	30	N/A APOA1	LAC_CHICK APOA1_CHICK
144	PWL	31		NS
145	PWL	28	TPI1	TPIS_CHICK
146	PWL	24	RBP4	RET4_CHICK

147	PWL	25	APOA1	APOA1_CHICK
148	PWL	15	TTR	TTHY_CHICK
149	PWL	14.4	FABP7	FABP7_CHICK
150	PWL	14.4		NS
151	PWL	14.4		NS
152	PWL	14.4	N/A	CYT_CHICK
153	PWL	10		NS
154	PWL	6	N/A	ISK1L_CHICK
155	PWL	6	N/A	ISK1L_CHICK
156	PWL	10		NR
157	PWL	6	GAL9	GLL9_CHICK
158	PWL	10		NS
159	PWL	4		NS
160	AIL	200		NS
161	AIL	200	ACE	ACE_CHICK
			NEL	NEL_CHICK
162	AIL	115	NEL	NEL_CHICK
163	AIL	136		NS
164	AIL	90		NS
165	AIL	90		NS
166	AIL	90		NS
167	AIL	90		NS
168	AIL	90	UQCRFS1	UCRI_CHICK
169	AIL	66.3	ALB	ALBU_CHICK
			F9	FA9_CHICK

170	AIL	66.3	ALB	ALBU_CHICK
-----	-----	------	-----	------------

			NEL	NEL_CHICK
			SERPINC1	ANT3_CHICK
171	AIL	66.3	ALB	ALBU_CHICK

172	AIL	55.4	N/A	TBB1_CHICK
-----	-----	------	-----	------------

			N/A	TBB5_CHICK
--	--	--	-----	------------

			N/A	TBA5_CHICK
173	AIL	55.4	N/A	TBA5_CHICK
			N/A	TBB5_CHICK
174	AIL	48		NS
175	AIL	117		NS
176	AIL	117		NS
177	AIL	117		NS
178	AIL	117		NS
179	AIL	97.4		NS
180	AIL	90	GSN	GELS_CHICK
181	AIL	150	N/A	TRFE_CHICK
			ALB	ALBU_CHICK
182	AIL	80	GSN	GELS_CHICK

			ALB	ALBU_CHICK
			N/A	IGHM_CHICK
183	AIL	117		NS
184	AIL	150	N/A	CFBL_CHICK
185	AIL	150	N/A	TRFE_CHICK
186	AIL	150		NS
187	AIL	117		NS
188	AIL	70	N/A	TRFE_CHICK

			ALB	ALBU_CHICK
			N/A	IGHM_CHICK
189	AIL	117	N/A	TRFE_CHICK
190	AIL	120		NS
191	AIL	70	N/A	TRFE_CHICK

192	AIL	95	N/A	TRFE_CHICK
------------	------------	----	-----	------------

193	AIL	95	GAL9 N/A	GLL9_CHICK TRFE_CHICK
------------	------------	----	-------------	--------------------------

194	AIL	68	N/A	TRFE_CHICK
------------	------------	----	-----	------------

195	AIL	66.3	ALB	ALBU_CHICK
------------	------------	------	-----	------------

196	AIL	66.3	ALB	ALBU_CHICK
-----	-----	------	-----	------------

197	AIL	66.3	ALB	ALBU_CHICK
-----	-----	------	-----	------------

198	AIL	66.3		NS
199	AIL	64	ALB	ALBU_CHICK

200	AIL	67		NS
201	AIL	65	N/A	TRFE_CHICK

			ALB	ALBU_CHICK
--	--	--	-----	------------

202	AIL	65	ALB	ALBU_CHICK
-----	-----	----	-----	------------

203	AIL	70	ALB	ALBU_CHICK
-----	-----	----	-----	------------

			N/A	TRFE_CHICK
--	--	--	-----	------------

204	AIL	66.3	PKM	KPYM_CHICK
-----	-----	------	-----	------------

205	AIL	66.3		NS
206	AIL	60	OIH	IOV7_CHICK
207	AIL	60	OIH	IOV7_CHICK
208	AIL	60	OIH	IOV7_CHICK
209	AIL	60	OIH	IOV7_CHICK
210	AIL	60	FGB	FIBB_CHICK
			OIH	IOV7_CHICK
211	AIL	52	FGB	FIBB_CHICK
			OIH	IOV7_CHICK
			TTR	TTHY_CHICK
212	AIL	55	FGB	FIBB_CHICK
			ALB	ALBU_CHICK
213	AIL	55	FGB	FIBB_CHICK
			ALB	ALBU_CHICK
			FGA	FIBA_CHICK
214	AIL	49	ALB	ALBU_CHICK
215	AIL	49		NS
216	AIL	48		NS
217	AIL	50	ALB	ALBU_CHICK
			CKB	KCRB_CHICK
218	AIL	48	CKB	KCRB_CHICK

219	AIL	36		NS
220	AIL	48		NS
221	AIL	48	CKB	KCRB_CHICK

222	AIL	50	ENO1	ENOA_CHICK
-----	-----	----	------	------------

ENO2	ENOG_CHICK
------	------------

ENO3	ENOB_CHICK
------	------------

223	AIL	47		NS
224	AIL	50		NS
225	AIL	50	PGK	PGK_CHICK

226	AIL	48		NS
227	AIL	37	MDH1	MDHC_CHICK

228	AIL	35	LDHB	LDHB_CHICK
-----	-----	----	------	------------

229	AIL	31.5	LDHB	LDHB_CHICK
-----	-----	------	------	------------

230	AIL	27	PGAM1	PGAM1_CHICK
-----	-----	----	-------	-------------

231	AIL	25.5	TPI1	NS
232	AIL	25.5		TPIS_CHICK

233	AIL	24	N/A	NS
234	AIL	24		NS
235	AIL	25		TRFE_CHICK

236	AIL	39	GAL9	GLL9_CHICK
			MRPL50	RM50_CHICK
237	AIL	35	TTR	NS
				TTHY_CHICK

			APOA1	APOA1_CHICK
--	--	--	-------	-------------

238	AIL	35	TTR	TTHY_CHICK
239	AIL	32	N/A	LAC_CHICK
240	AIL	24		NS
241	AIL	28	APOA1	APOA1_CHICK
			N/A	LAC_CHICK
242	AIL	28	CTTN1 APOA1	SRC8_CHICK APOA1_CHICK
			N/A	LAC_CHICK
243	AIL	28	N/A	LAC_CHICK
244	AIL	23	RBP4	RET4_CHICK
			APOA1	APOA1_CHICK
245	AIL	17	TTR	TTHY_CHICK

246	AIL	13	ALB	ALBU_CHICK
			TXN	THIO_CHICK
247	AIL	15	FABP7	FABP7_CHICK
248	AIL	15	FABP7	FABP7_CHICK
			N/A	CYT_CHICK
249	AIL	15	FABP7	FABP7_CHICK
250	AIL	18	N/A	CYT_CHICK
251	AIL	10	N/A	CYT_CHICK
			HBAD	HBAD_CHICK
252	AIL	15	N/A	TRFE_CHICK

			LYZ	LYSC_CHICK
253	AIL	6	ASTL N/A	ASTL_CHICK ISK1L_CHICK
254	AIL	6	SUPT5H N/A N/A	SPT5H_CHICK ISK1L_CHICK TRFE_CHICK
255	AIL	6		NS
256	AIL	6	GAL9	GLL9_CHICK
			GAL10	GLL10_CHICK
257	AIL	4		NS

Supplementary Table S1

eins of Red Junglefowl (RJF), White Leghorn (WL) and 9th-generation Advance

Protein name detail

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Protein NEL OS=Gallus gallus GN=NEL PE=2 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ig mu chain C region OS=Gallus gallus PE=2 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

E3 ubiquitin-protein ligase MIB2 OS=Gallus gallus GN=MIB2 PE=2 SV=1

DET1- and DDB1-associated protein 1 OS=Gallus gallus GN=DDA1 PE=3 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Contactin-2 OS=Gallus gallus GN=CNTN2 PE=1 SV=1
Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2
Contactin-2 OS=Gallus gallus GN=CNTN2 PE=1 SV=1
Ovotransferrin OS=Gallus gallus PE=1 SV=2

Gelsolin OS=Gallus gallus GN=GSN PE=2 SV=1

Gelsolin OS=Gallus gallus GN=GSN PE=2 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ig mu chain C region OS=Gallus gallus PE=2 SV=2

Gelsolin OS=Gallus gallus GN=GSN PE=2 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Gelsolin OS=Gallus gallus GN=GSN PE=2 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2
Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Fibrinogen beta chain (Fragment) OS=Gallus gallus GN=FGB PE=1 SV=1
Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Pyruvate kinase PKM OS=Gallus gallus GN=PKM PE=2 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Pyruvate kinase PKM OS=Gallus gallus GN=PKM PE=2 SV=2

Fibrinogen beta chain (Fragment) OS=Gallus gallus GN=FGB PE=1 SV=1

Pyruvate kinase PKM OS=Gallus gallus GN=PKM PE=2 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Alpha-enolase OS=Gallus gallus GN=ENO1 PE=2 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Creatine kinase B-type OS=Gallus gallus GN=CKB PE=1 SV=1

Creatine kinase B-type OS=Gallus gallus GN=CKB PE=1 SV=1

Argininosuccinate synthase OS=Gallus gallus GN=ASS1 PE=2 SV=1

Gallinacin-10 OS=Gallus gallus GN=GAL10 PE=2 SV=1

Borealin OS=Gallus gallus GN=CDCA8 PE=2 SV=1

Alpha-enolase OS=Gallus gallus GN=ENO1 PE=2 SV=2

Beta-enolase OS=Gallus gallus GN=ENO3 PE=1 SV=3

Phosphoglycerate kinase OS=Gallus gallus GN=PGK PE=2 SV=2

Phosphoglycerate kinase OS=Gallus gallus GN=PGK PE=2 SV=2

Aspartate aminotransferase, cytoplasmic OS=Gallus gallus GN=GOT1 PE=1 SV=3

Aspartate aminotransferase, cytoplasmic OS=Gallus gallus GN=GOT1 PE=1 SV=3

Malate dehydrogenase, cytoplasmic OS=Gallus gallus GN=MDH1 PE=2 SV=1

L-lactate dehydrogenase B chain OS=Gallus gallus GN=LDHB PE=1 SV=3

L-lactate dehydrogenase A chain OS=Gallus gallus GN=LDHA PE=1 SV=3

Myoblast determination protein 1 homolog OS=Gallus gallus GN=MYOD1 PE=2 SV=2

Triosephosphate isomerase OS=Gallus gallus GN=TPI1 PE=1 SV=2

Glutathione S-transferase 2 OS=Gallus gallus GN=GSTM2 PE=1 SV=4

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Triosephosphate isomerase OS=Gallus gallus GN=TPI1 PE=1 SV=2

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Annexin A5 OS=Gallus gallus GN=ANXA5 PE=1 SV=2

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Ig lambda chain C region OS=Gallus gallus PE=4 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Ig lambda chain C region OS=Gallus gallus PE=4 SV=1
Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ig lambda chain C region OS=Gallus gallus PE=4 SV=1
Ig lambda chain C region OS=Gallus gallus PE=4 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Vitelline membrane outer layer protein 1 OS=Gallus gallus GN=VMO1 PE=1 SV=1
Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Retinol-binding protein 4 OS=Gallus gallus GN=RBP4 PE=1 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Fatty acid-binding protein, brain OS=Gallus gallus GN=FABP7 PE=2 SV=2

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Fatty acid-binding protein, brain OS=Gallus gallus GN=FABP7 PE=2 SV=2

Cystatin OS=Gallus gallus PE=1 SV=2

Trypsin inhibitor CITI-1 OS=Gallus gallus PE=1 SV=1

Trypsin inhibitor CITI-1 OS=Gallus gallus PE=1 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Gallinacin-9 OS=Gallus gallus GN=GAL9 PE=2 SV=1

Gallinacin-10 OS=Gallus gallus GN=GAL10 PE=2 SV=1

Gallinacin-10 OS=Gallus gallus GN=GAL10 PE=2 SV=1

Gallinacin-10 OS=Gallus gallus GN=GAL10 PE=2 SV=1

Gallinacin-9 OS=Gallus gallus GN=GAL9 PE=2 SV=1

Gallinacin-10 OS=Gallus gallus GN=GAL10 PE=2 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ig mu chain C region OS=Gallus gallus PE=2 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ig mu chain C region OS=Gallus gallus PE=2 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ig mu chain C region OS=Gallus gallus PE=2 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ig mu chain C region OS=Gallus gallus PE=2 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Gelsolin OS=Gallus gallus GN=GSN PE=2 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Complement factor B-like protease (Fragment) OS=Gallus gallus PE=1 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Gelsolin OS=Gallus gallus GN=GSN PE=2 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Creatine kinase B-type OS=Gallus gallus GN=CKB PE=1 SV=1

Argininosuccinate synthase OS=Gallus gallus GN=ASS1 PE=2 SV=1

Alpha-enolase OS=Gallus gallus GN=ENO1 PE=2 SV=2

Beta-enolase OS=Gallus gallus GN=ENO3 PE=1 SV=3

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Malate dehydrogenase, cytoplasmic OS=Gallus gallus GN=MDH1 PE=2 SV=1

L-lactate dehydrogenase B chain OS=Gallus gallus GN=LDHB PE=1 SV=3

L-lactate dehydrogenase B chain OS=Gallus gallus GN=LDHB PE=1 SV=3

Astacin-like metalloendopeptidase OS=Gallus gallus GN=ASTL PE=2 SV=1

Glyceraldehyde-3-phosphate dehydrogenase OS=Gallus gallus GN=GAPDH PE=2 SV=3

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Ig lambda chain C region OS=Gallus gallus PE=4 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Triosephosphate isomerase OS=Gallus gallus GN=TPI1 PE=1 SV=2

Retinol-binding protein 4 OS=Gallus gallus GN=RBP4 PE=1 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Fatty acid-binding protein, brain OS=Gallus gallus GN=FABP7 PE=2 SV=2

Cystatin OS=Gallus gallus PE=1 SV=2

Trypsin inhibitor CITI-1 OS=Gallus gallus PE=1 SV=1

Trypsin inhibitor CITI-1 OS=Gallus gallus PE=1 SV=1

Gallinacin-9 OS=Gallus gallus GN=GAL9 PE=2 SV=1

Angiotensin-converting enzyme (Fragment) OS=Gallus gallus GN=ACE PE=2 SV=1

Protein NEL OS=Gallus gallus GN=NEL PE=2 SV=1

Protein NEL OS=Gallus gallus GN=NEL PE=2 SV=1

Cytochrome b-c1 complex subunit Rieske, mitochondrial OS=Gallus gallus GN=UQCRFS1 PE=1 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Coagulation factor IX OS=Gallus gallus GN=F9 PE=2 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Protein NEL OS=Gallus gallus GN=NEL PE=2 SV=1

Antithrombin-III (Fragment) OS=Gallus gallus GN=SERPINC1 PE=1 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Tubulin beta-1 chain OS=Gallus gallus PE=2 SV=1

Tubulin beta-5 chain OS=Gallus gallus PE=3 SV=1

Tubulin alpha-5 chain OS=Gallus gallus PE=3 SV=1

Tubulin alpha-5 chain OS=Gallus gallus PE=3 SV=1

Tubulin beta-5 chain OS=Gallus gallus PE=3 SV=1

Gelsolin OS=Gallus gallus GN=GSN PE=2 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Gelsolin OS=Gallus gallus GN=GSN PE=2 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ig mu chain C region OS=Gallus gallus PE=2 SV=2

Complement factor B-like protease (Fragment) OS=Gallus gallus PE=1 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ig mu chain C region OS=Gallus gallus PE=2 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Gallinacin-9 OS=Gallus gallus GN=GAL9 PE=2 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Pyruvate kinase PKM OS=Gallus gallus GN=PKM PE=2 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Fibrinogen beta chain (Fragment) OS=Gallus gallus GN=FGB PE=1 SV=1

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Fibrinogen beta chain (Fragment) OS=Gallus gallus GN=FGB PE=1 SV=1

Ovoinhibitor OS=Gallus gallus GN=OIH PE=1 SV=2

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Fibrinogen beta chain (Fragment) OS=Gallus gallus GN=FGB PE=1 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Fibrinogen beta chain (Fragment) OS=Gallus gallus GN=FGB PE=1 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Fibrinogen alpha chain OS=Gallus gallus GN=FGA PE=1 SV=4

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Creatine kinase B-type OS=Gallus gallus GN=CKB PE=1 SV=1

Creatine kinase B-type OS=Gallus gallus GN=CKB PE=1 SV=1

Creatine kinase B-type OS=Gallus gallus GN=CKB PE=1 SV=1

Alpha-enolase OS=Gallus gallus GN=ENO1 PE=2 SV=2

Gamma-enolase OS=Gallus gallus GN=ENO2 PE=2 SV=1

Beta-enolase OS=Gallus gallus GN=ENO3 PE=1 SV=3

Phosphoglycerate kinase OS=Gallus gallus GN=PGK PE=2 SV=2

Malate dehydrogenase, cytoplasmic OS=Gallus gallus GN=MDH1 PE=2 SV=1

L-lactate dehydrogenase B chain OS=Gallus gallus GN=LDHB PE=1 SV=3

L-lactate dehydrogenase B chain OS=Gallus gallus GN=LDHB PE=1 SV=3

Phosphoglycerate mutase 1 OS=Gallus gallus GN=PGAM1 PE=1 SV=3

Triosephosphate isomerase OS=Gallus gallus GN=TPI1 PE=1 SV=2

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Gallinacin-9 OS=Gallus gallus GN=GAL9 PE=2 SV=1

39S ribosomal protein L50, mitochondrial OS=Gallus gallus GN=MRPL50 PE=2 SV=1

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Ig lambda chain C region OS=Gallus gallus PE=4 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Ig lambda chain C region OS=Gallus gallus PE=4 SV=1

Src substrate protein p85 OS=Gallus gallus GN=CTTN1 PE=1 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Ig lambda chain C region OS=Gallus gallus PE=4 SV=1

Ig lambda chain C region OS=Gallus gallus PE=4 SV=1

Retinol-binding protein 4 OS=Gallus gallus GN=RBP4 PE=1 SV=1

Apolipoprotein A-I OS=Gallus gallus GN=APOA1 PE=1 SV=2

Transthyretin OS=Gallus gallus GN=TTR PE=1 SV=1

Serum albumin OS=Gallus gallus GN=ALB PE=1 SV=2

Thioredoxin OS=Gallus gallus GN=TXN PE=3 SV=2

Fatty acid-binding protein, brain OS=Gallus gallus GN=FABP7 PE=2 SV=2

Fatty acid-binding protein, brain OS=Gallus gallus GN=FABP7 PE=2 SV=2

Cystatin OS=Gallus gallus PE=1 SV=2

Fatty acid-binding protein, brain OS=Gallus gallus GN=FABP7 PE=2 SV=2

Cystatin OS=Gallus gallus PE=1 SV=2

Cystatin OS=Gallus gallus PE=1 SV=2

Hemoglobin subunit alpha-D OS=Gallus gallus GN=HBAD PE=1 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Lysozyme C OS=Gallus gallus GN=LYZ PE=1 SV=1

Astacin-like metalloendopeptidase OS=Gallus gallus GN=ASTL PE=2 SV=1

Trypsin inhibitor CITI-1 OS=Gallus gallus PE=1 SV=1

Transcription elongation factor SPT5 OS=Gallus gallus GN=SUPT5H PE=2 SV=1

Trypsin inhibitor CITI-1 OS=Gallus gallus PE=1 SV=1

Ovotransferrin OS=Gallus gallus PE=1 SV=2

Gallinacin-9 OS=Gallus gallus GN=GAL9 PE=2 SV=1

Gallinacin-10 OS=Gallus gallus GN=GAL10 PE=2 SV=1

oid Interbreed Line (RJFxWL) (AIL) separated by 2DE as in panels A-C of Figure 1

PI (Gels)	Protein PI (M)	Accession Number	Nominal Mass (Mr)	Protein Score
4.3				
5	5.51	P19121	71868	424
4.3	5.23	Q90827	96096	335
5	5.51	P19121	71868	233
3.9				
4.1	6.07	P01875	48827	58
	6.85	P02789	79551	41
4.2	5.51	P19121	71868	79
4.5				
4.6	5.51	P19121	71868	207
3.5	5.51	P19121	71868	192

3.7				
4	5.51	P19121	71868	392

4.1				
4.5	5.51	P19121	71868	544

	6.41	Q5ZIJ9	108211	15
	8.74	Q5ZK14	11934	13
5	5.51	P19121	71868	27
6.1	6.85	P02789	79551	204

	5.51	P19121	71868	61
7	6.85	P02789	79551	49
7.5	6.85	P02789	79551	154

8.2
8.5
6
6.1

6.2				
6.3				
6.6	6.48	P28685	114086	35
6.8	6.85	P02789	79551	69
7	6.85	P02789	79551	44
	6.48	P28685	114086	26
7.5	6.85	P02789	79551	68
6				
6.2	5.93	O93510	86120	250

6.3	5.93	O93510	86120	867
-----	------	--------	-------	-----

	6.85	P02789	79551	266
--	------	--------	-------	-----

	6.07	P01875	48827	116
--	------	--------	-------	-----

6.8	5.93	O93510	86120	246
-----	------	--------	-------	-----

	6.85	P02789	79551	86
--	------	--------	-------	----

6.8				
7	6.85	P02789	79551	373

	5.93	O93510	86120	208
--	------	--------	-------	-----

7.2	6.85	P02789	79551	729
-----	------	--------	-------	-----

7.3	6.85	P02789	79551	1123
-----	------	--------	-------	------

7.5	6.85	P02789	79551	1760
-----	------	--------	-------	------

7.6	6.85	P02789	79551	863
-----	------	--------	-------	-----

7.9	6.85	P02789	79551	700
-----	------	--------	-------	-----

8.2				
8.6	6.85	P02789	79551	61
7.9	5.51	P19121	71868	243
8	5.51	P19121	71868	152
8.2	5.51	P19121	71868	306
5.9	6.16	P10184	54394	202
6.1	6.16	P10184	54394	132
6.2	6.16	P10184	54394	97
6.5	6.16	P10184	54394	36
6.9				
7.1	7.18	Q02020	53272	44
7.4	5.51	P19121	71868	269

7.5	6.85	P02789	79551	361
	5.51	P19121	71868	146
7.9	6.85	P02789	79551	422
	5.51	P19121	71868	61
8.1	7.29	P00548	58434	238
8.5	5.51	P19121	71868	116
	7.29	P00548	58434	28
8.4	7.18	Q02020	53272	166
	7.29	P00548	58434	28
5.5	5.51	P19121	71868	561

	6.17	P51913	47617	46
5.6	5.51	P19121	71868	427
5.9	5.51	P19121	71868	178
	5.93	P05122 / Q92061	43129	115
6.1				
6.5	5.93	P05122 / Q92061	43129	139
6.7	6.10	Q5ZJ23	47330	223
6.9	8.24	Q6QLQ9	7525	166
6.8				
6	10.36	P86346	32672	36
7.1	6.17	P51913	47617	1205

	7.28	P07322	47566	142
7.7				
7.7				
8				
8.1	8.31	P51903	45087	66
8.5	8.31	P51903	45087	400
	8.22	P00504	46134	115
8.2	8.22	P00504	46134	218
7.5	6.92	Q5ZME2	36748	100
7.8	7.07	P00337	36694	468
8.1	7.75	P00340	36776	116
9	5.43	P16075 / Q90916	33654	33
7.7				
8				
8	6.71	P00940	26832	557

	6.85	P20136	26047	82
7.5	5.58	P08250	30661	109
8	6.71	P00940	26832	297
8				
3.6				
4.9	5.11	P27731	16356	415
	5.58	P08250	30661	141
	5.51	P19121	71868	54
5	5.11	P27731	16356	49
5.6	5.60	P17153	36290	189
4.9	5.58	P08250	30661	459
	6.10	P20763	11525	76
5.5	5.58	P08250	30661	502

6	6.10	P20763	11525	76
	5.51	P19121	71868	759
6.1	6.10	P20763	11525	76
	6.10	P20763	11525	149
	5.58	P08250	30661	94
4	8.82	P41366	20677	50
4.2	5.51	P19121	71868	212
4.7	5.51	P19121	71868	131
6.1	5.93	P41263	22843	201
6.3	5.58	P08250	30661	44
	5.11	P27731	16356	410
5				
5.5	5.61	Q05423	15031	636

6.2	5.11	P27731	16356	50
	5.61	Q05423	15031	355
7				
8	7.60	P01038	15562	195
6.7	6.02	P85000	6371	208
7	6.02	P85000	6371	189
8.3	6.85	P02789	79551	71
	8.65	Q6QLR1 / Q09MS3	7616	132
	8.24	Q6QLQ9	7525	73
9.5	8.24	Q6QLQ9	7525	136
8.3	8.24	Q6QLQ9	7525	100
	8.65	Q6QLR1 / Q09MS3	7616	49
8.6	8.24	Q6QLQ9	7525	166
9.5				
3	5.51	P19121	71868	60
3.3	5.51	P19121	71868	63
4.2				
5	5.51	P19121	71868	2634

5	5.51	P19121	71868	1227
---	------	--------	-------	------

	6.85	P02789	79551	35
	6.07	P01875	48827	22
5	5.51	P19121	71868	911

5.5	5.51	P19121	71868	389
	6.85	P02789	79551	184
	6.07	P01875	48827	53
6				
5.5	6.85	P02789	79551	481
	6.07	P01875	48827	111
	5.51	P19121	71868	42
5.9	6.85	P02789	79551	453
	6.07	P01875	48827	81
	5.51	P19121	71868	56

6	6.85	P02789	79551	377
	5.93	O93510	86120	263
6.5	6.85	P02789	79551	142
6.4	6.85	P02789	79551	76
	6.48	P81475	27719	61
6.5	6.85	P02789	79551	840
7	6.85	P02789	79551	436
6.5	6.85	P02789	79551	364

	5.51	P19121	71868	40
7	5.51	P19121	71868	70
7.5	5.51	P19121	71868	309
8.5	5.51	P19121	71868	216
5				
5	5.93	O93510	86120	67
	5.51	P19121	71868	40
6	6.16	P10184	54394	129
6.5				
6.2	5.93	P05122/Q92061	43129	193
	6.1	Q5ZJ23	47330	51
6.5	6.17	P51913	47617	436
	7.28	P07322	47566	30

6.5				
4.8	5.11	P27731	16356	214
5.1				
5.6				
6.1				
6.1				
6.9	6.92	Q5ZME2	36748	54
	7.07	P00337/O93362/Q548X0	36694	28
7.1	7.07	P00337/O93362/Q548X0	36694	645
8.5	6.76	P0DJJ2	46929	149
5	8.71	00356; Q90848; Q90849; Q9892	35909	41
	5.58	P08250	30661	921
	6.1	P20763	11525	115
5.3	5.58	P08250	30661	111
7.5				
7.5	6.71	P00940	26832	84
6	5.93	P41263	22843	101

7.6	5.58	P08250	30661	86
5	5.11	P27731	16356	219
5.5	5.61	Q05423	15031	379
6				
6.2				
7.6	7.6	P01038	15562	83
5				
6.1	6.02	P85000	6371	105
6.2	6.02	P85000	6371	145
7.5				
8.7	8.65	Q6QLR1 /Q09MS3	7616	130
9				
9				
5				
5.5	5.40	Q10751	138474	131
	5.23	Q90827	96096	55
5	5.23	Q90827	96096	167
5.5				
4.6				
4.8				
5				
5.1				
5.2	8.68	Q5ZLR5	29710	27
4.7	5.51	P19121	71868	144
	5.95	Q804X6	53083	77

4.9	5.51	P19121	71868	221
	5.23	Q90827	96096	42
	10.29	Q03352	11502	35
5.7	5.51	P19121	71868	2329

4.7	4.78	P09203 / P02555	50333	912
-----	------	-----------------	-------	-----

	4.79	P09653	50395	617
--	------	--------	-------	-----

	4.95	P09644	50715	151
5	4.95	P09644	50715	300
	4.79	P09653	50395	194
5.6				
6.1				
6.2				
6.3				
6.4				
6.5				
6.7	5.93	O93510	86120	239
6.9	6.85	P02789	79551	75
	5.51	P19121	71868	36
6.9	5.93	O93510	86120	603

	5.51	P19121	71868	106
	6.07	P01875	48827	65
7				
7.1	6.48	P81475	27719	122
7.7	6.85	P02789	79551	63
8.5				
7.4				
7.6	6.85	P02789	79551	1779

	5.51	P19121	71868	105
	6.07	P01875	48827	80
7.8	6.85	P02789	79551	106
8.1				
7.9	6.85	P02789	79551	1441

8.1	6.85	P02789	79551	161
-----	------	--------	-------	-----

	8.65	Q6QLR1 / Q09MS3	7616	27
8.5	6.85	P02789	79551	300

9	6.85	P02789	79551	511
---	------	--------	-------	-----

6.5	5.51	P19121	71868	935
-----	------	--------	-------	-----

5.7	5.51	P19121	71868	182
5.8	5.51	P19121	71868	550
5.9				
7.1	5.51	P19121	71868	389
7.3				
7.6	6.85	P02789	79551	215
	5.51	P19121	71868	87
7.8	5.51	P19121	71868	155
8.1	5.51	P19121	71868	188
	6.85	P02789	79551	124
8.3	7.29	P00548	58434	98

9				
6.2	6.16	P10184	54394	387
6.3	6.16	P10184	54394	135
6.5	6.16	P10184	54394	222
6.8	6.16	P10184	54394	61
7	7.18	Q02020	53272	66
	6.16	P10184	54394	34
7.1	7.18	Q02020	53272	57
	6.16	P10184	54394	48
	5.11	P27731	16356	28
7.3	7.18	Q02020	53272	95
	5.51	P19121	71868	79
7.5	7.18	Q02020	53272	352
	5.51	P19121	71868	99
	5.69	P14448	83242	62
6	5.51	P19121	71868	68
6.1				
6.2				
6.1	5.51	P19121	71868	417
	5.93	P05122 / Q92061	43129	77
6.5	5.93	P05122/Q92061	43129	128

214

844

246

159

123

296

8	7.07	P00337 / O93362 / Q548X0	36694	498
8.3	7.07	P00337 / O93362 / Q548X0	36694	183
8.2	7.03	Q5ZLN1 / P84174	29051	171
8				
8.1	6.71	P00940	26832	342
8				
8.2				
9.2	6.85	P02789	79551	154
	8.65	Q6QLR1 / Q09MS3	7616	43
	9.12	Q5ZLC1	18192	24
4.2				
5.2	5.11	P27731	16356	582
	5.58	P08250	30661	264

5.7	5.11	P27731	16356	212
-----	------	--------	-------	-----

5.9	6.10	P20763	11525	75
-----	------	--------	-------	----

4.3

5.3	5.58	P08250	30661	825
-----	------	--------	-------	-----

	6.10	P20763	11525	87
--	------	--------	-------	----

	5.33	Q01406	63462	40
--	------	--------	-------	----

5.9	5.58	P08250	30661	478
-----	------	--------	-------	-----

	6.10	P20763	11525	106
--	------	--------	-------	-----

6.5	6.10	P20763	11525	91
-----	------	--------	-------	----

6.5	5.93	P41263	22843	176
-----	------	--------	-------	-----

	5.58	P08250	30661	85
--	------	--------	-------	----

5.3	5.11	P27731	16356	380
-----	------	--------	-------	-----

5.3	5.51	P19121	71868	930
	5.10	P08629	11978	77
6	5.61	Q05423	15031	846
6.8	5.61	Q05423	15031	235
	7.60	P01038	15562	62
7.1	5.61	Q05423	15031	48
8.2	7.60	P01038	15562	272
8.8	7.60	P01038	15562	171
	7.01	P02001 / Q549K7 / Q78AJ9	15742	49
9.8	6.85	P02789	79551	289

	9.37	P00698 / Q90884	16741	242
	6.76	P0DJJ2	46929	85
7	6.02	P85000	6371	179
	5.00	Q5ZI08	120306	38
7.5	6.02	P85000	6371	90
	6.85	P02789	79551	39
8				
9.5	8.65	Q6QLR1 / Q09MS3	7616	199
	8.24	Q6QLQ9	7525	92
8.6				

Coverage%	Peptide Count (S)	Peptide Ion Score	Peptide m/z	Peptide charge Z
32(615)	19(11)	51	643.8167	2
		93	756.4079	2
		68	499.2741	3
		48	432.7273	2
		57	874.3719	2
		47	509.6128	3
		48	782.0294	3
		89	779.4150	2
		63	965.4402	2
		84	634.8147	2
21 (816)	14 (10)	52	527.3798	2
		48	684.4836	2
		87	595.9177	2
		44	818.0701	2
		56	774.2372	3
		62	595.4487	2
		68	692.9443	2
		62	741.0199	2
24(615)	15(8)	65	643.8157	2
		45	756.4021	2
		72	499.2731	3
		59	597.7707	2
		54	773.8997	2
		42	653.3026	2
		48	562.9749	3
		50	516.7468	2
2(446)	1(1)	58	733.4302	2
11(705)	7(0)	35	551.6193	3
13(615)	8(3)	61	597.7708	2
		44	432.7248	2
		42	516.7426	2
26(615)	15(5)	79	756.4028	2
		44	499.2776	3
		41	432.7265	2
		49	782.0269	3
		70	965.4410	2
		48	413.7938	2
15(615)	10(5)	60	643.8108	2
		70	499.2749	3

		70	597.7711	2
		43	432.7269	2
		47	516.7446	2
38(615)	20(10)	61	643.8178	2
		79	756.4071	2
		68	614.3730	2
		71	499.2774	3
		46	882.3711	2
		50	773.8958	2
		75	782.0320	3
		55	562.6430	3
		43	413.7950	2
		61	642.8091	2
45(615)	23(13)	60	643.8142	2
		82	764.4067	2
		60	499.2740	3
		55	773.8872	2
		62	752.6585	3
		63	509.6155	3
		76	782.0267	3
		75	771.4168	2
		62	557.3123	3
		47	973.4414	2
		53	516.7418	2
		46	413.7934	2
		67	642.8082	2
1(954)	1(0)	15	1444.9937	1
9(102)	1(0)	13	1170.6873	1
1(615)	1(0)	27	432.7274	2
23(705)	13(6)	54	654.8207	2
		66	821.3958	2
		56	557.9259	3
		52	534.2493	2
		41	401.2029	3
		41	551.6183	3
		43	529.3006	2
12(615)	7(1)	40	643.8125	2
		43	471.9685	4
6(705)	4(1)	49	401.2066	3
14(705)	11(5)	63	654.8228	2
		46	512.2906	3
		65	612.3208	2
		42	401.2016	3
		43	551.6185	3

1(1036)	1(0)	35	554.6258	3
10(705)	6(2)	45	512.2842	3
		53	401.2078	3
7(705)	5(1)	44	457.2391	2
1(1036)	1(0)	26	554.6205	3
11(705)	7(1)	60	612.3228	2
16(778)	10(7)	61	411.2172	2
		42	639.3550	2
		55	637.3434	3
		77	438.8996	3
		74	752.3856	2
		64	666.8361	2
		57	526.2571	2
30 (778)	19(16)	59	499.7768	2
		48	642.8839	2
		45	457.7734	2
		152	1104.5549	2
		60	639.3556	2
		67	562.2766	2
		46	637.3433	3
		45	673.3321	3
		82	438.8987	3
		71	924.7696	3
		53	573.2326	2
		82	1051.5419	2
		75	610.8287	2
		90	752.3922	2
		61	666.8204	2
		55	526.2555	2
17 (705)	12(7)	58	654.8222	2
		47	512.2778	3
		46	447.2706	2
		61	848.4086	2
		66	551.6247	3
		48	666.3378	2
		49	582.9811	3
4 (446)	2(2)	60	498.7280	2
		89	733.4222	2
20(778)	12(7)	52	499.7821	2
		52	642.8848	2
		68	438.9004	3
		81	924.7762	3
		58	610.8293	2
		66	752.3889	2
		70	526.2550	2
15(705)	10(2)	43	512.2846	3
		51	534.2554	2

31(705)	21(11)	59	479.5845	3
		61	654.8251	2
		40	512.2916	3
		62	821.3993	2
		48	534.2527	2
		49	401.2037	3
		58	551.6154	3
		88	921.9135	2
		52	512.7704	4
		51	529.3013	2
		86	1000.4426	2
14(778)	9(4)	57	637.3380	3
		69	438.8925	3
		62	924.7823	3
		65	610.8276	2
44 (705)	27(17)	79	718.8795	2
		78	654.8305	2
		75	512.2891	3
		108	980.4918	2
		92	821.3989	2
		72	557.9280	3
		58	447.2747	2
		83	848.4149	2
		52	612.3203	2
		48	534.2622	2
		47	401.2029	3
		41	905.4303	3
		67	551.6181	3
		89	921.9074	2
		51	683.3630	3
		50	529.3034	2
		77	1000.4492	2
49 (705)	30 (28)	77	942.8324	3
		55	479.5776	3
		79	654.8239	2
		72	512.2844	3
		103	980.4903	2
		88	821.4016	2
		45	518.2783	2
		62	677.3193	2
		79	848.4058	2
		50	612.3137	2
		51	534.2554	2
		65	601.3055	2
		52	443.9002	3
		54	521.2853	2
		71	551.6187	3
		41	602.2862	2
		113	1275.5238	2

49 (705)	36 (27)	47	630.3019	3
		89	913.8946	2
		49	512.7651	4
		42	582.9736	3
		49	529.3053	2
		96	1008.4643	2
		131	718.8748	2
		73	654.8320	2
		72	512.2889	3
		89	980.4855	2
		96	821.4014	2
		73	557.9329	3
		66	518.2781	2
		63	848.4136	2
		58	612.3239	2
		51	534.7573	2
		64	401.2055	3
		61	443.9089	3
		46	471.2650	4
		56	697.3889	2
		57	521.2880	2
		60	551.6270	3
		77	630.3107	3
		80	913.9117	2
		51	512.7747	4
		67	809.4307	3
		108	873.9764	2
49 (705)	31 (22)	50	529.3019	2
		89	1000.4610	2
		87	942.8241	3
		118	718.9291	2
		78	654.8246	2
		82	512.2877	3
		87	980.4872	2
		80	821.3981	2
		73	557.9263	3
		53	447.2696	2
		102	848.4155	2
		49	612.3190	2
		48	534.2531	2
		58	401.2068	3
		84	930.4580	3
		70	551.6205	3
		41	602.2900	2
		46	512.7904	4
		49	582.9820	3
		50	529.3010	2
		60	1000.4441	2
47 (705)	28(15)	101	942.8282	3
		48	479.5895	3

		70	654.8328	2
		72	512.2890	3
		80	821.3997	2
		62	447.2699	2
		46	848.4064	2
		55	612.3200	2
		47	534.2587	2
		60	601.3044	2
		40	930.8045	3
		71	551.6215	3
		95	1275.5198	2
		50	529.3016	2
		81	1000.4468	2
6 (705)	4(1)	54	654.9547	2
19 (615)	10(6)	55	643.8194	2
		85	756.4050	2
		52	499.2740	3
		52	771.4164	2
		70	691.8295	2
		75	965.4385	2
24 (615)	12(5)	47	432.7266	2
		55	851.0798	3
		43	782.0326	3
		62	965.4432	2
		48	413.7935	2
		41	616.3156	2
21 (615)	11(9)	58	643.8179	2
		95	756.4124	2
		54	499.2737	3
		45	597.7664	2
		49	569.8051	2
		69	782.0243	3
		68	771.4116	2
		67	965.4385	2
		46	413.7932	2
18(472)	7(4)	57	572.9625	3
		54	568.7460	2
		62	1028.4470	3
		56	603.2683	2
17(472)	7(3)	52	578.2848	3
		53	560.7495	2
		56	603.2728	2
12(472)	6(3)	51	574.7643	2
		50	568.7442	2
		49	554.2780	2
8(472)	4(0)	36	408.7256	2
2(463)	1(1)	44	679.3367	2
35(615)	20(9)	81	756.4030	2

		50	614.3647	2
		56	499.2730	3
		41	569.8068	2
		47	882.3753	2
		49	782.0279	3
		74	965.4409	2
		54	642.8016	2
20(705)	12(8)	70	512.2839	3
		78	980.4910	2
		83	551.6261	3
		46	512.7735	4
		44	457.7768	2
		50	582.9854	3
		50	529.3031	2
		65	1000.4537	2
20(615)	12(5)	99	756.4023	2
		41	499.2770	3
		51	432.7262	2
		44	965.4523	2
35(705)	23(9)	46	479.5759	3
		76	654.8264	2
		40	494.7370	2
		79	512.2880	3
		103	980.4888	2
		40	443.9036	3
		57	551.6205	3
		51	921.9103	2
		47	512.7720	4
		50	529.2999	2
		82	1000.4482	2
10(615)	5(2)	44	499.2735	3
		40	413.7919	2
26(530)	10(4)	65	615.3224	2
		61	680.3608	2
		62	818.9464	2
		85	937.9687	2
		41	510.2569	2
10 (615)	6(2)	85	756.4044	2
		47	499.2768	3
4(530)	2(0)	28	724.4243	2
18(463)	7(4)	63	679.3319	2
		75	801.8951	2
		41	492.7065	2
		42	551.2733	2
6(530)	3(0)	28	571.3068	2
29 (615)	16(11)	52	432.7255	2
		51	509.6139	3
		58	693.8647	2
		64	782.0177	3
		49	562.6437	3

		73	779.9160	2
		68	671.3034	3
		69	965.4438	2
		43	413.7920	2
		75	634.8142	2
		73	746.6940	3
14 (434)	3(1)	46	756.4090	4
30 (615)	18(9)	47	432.7271	2
		60	516.2631	3
		48	509.6154	3
		53	562.6497	3
		73	779.4127	2
		47	671.3115	3
		49	466.8885	3
		57	965.4287	2
		43	413.7927	2
26 (615)	17(3)	55	516.2650	3
		40	782.3465	3
		47	965.4367	2
14 (381)	4(3)	51	616.8226	2
		71	679.8839	2
		63	779.4074	2
27(381)	8(4)	62	616.8123	2
		71	679.8792	2
		44	424.7517	2
		76	707.6952	3
19(416)	8(6)	55	590.8030	2
		73	668.3715	2
		46	779.3771	2
		64	493.7200	2
		53	460.7585	2
		89	779.4235	2
45(68)	2(1)	94	859.7257	3
3(298)	1(0)	36	428.7679	2
66 (434)	24(16)	86	725.3659	2
		148	902.9781	2
		65	707.8737	2
		58	556.9332	3
		68	648.7914	2
		71	760.4161	2
		120	1007.8672	3
		51	572.3112	2
		88	980.9700	2
		50	408.2495	2
		108	786.8888	2
		104	841.7252	3
		82	1031.0492	2
		40	504.2546	2

14 (434)	5(3)	90	817.4155	2
		110	771.3879	2
		48	452.7358	2
		49	707.8737	2
		71	760.4161	2
		90	817.4155	2
11 (417)	4(1)	66	545.6048	3
46 (417)	16(7)	106	906.9774	2
		90	545.6020	3
		72	590.3397	3
		43	1005.9770	2
		56	666.8757	2
		134	877.8970	2
		66	580.9772	3
10 (412)	4(2)	88	649.8546	2
		58	902.9991	2
33 (412)	12(4)	59	748.4003	2
		40	970.1986	3
		40	506.7495	2
		74	576.9842	3
		90	902.9980	2
13(334)	3(2)	56	878.4783	2
		71	697.3603	2
44 (333)	14(10)	64	995.0485	2
		84	844.9317	2
		88	498.9342	3
		42	484.7396	2
		45	457.2971	2
		96	631.8156	2
		72	646.8455	2
		60	579.8248	2
		65	644.3658	2
		74	638.8469	2
25(332)	8(3)	68	837.4262	2
		51	624.7993	2
		69	625.8375	2
6(298)	1(0)	33	757.0504	3
75 (248)	15(12)	62	541.7944	2
		100	676.8788	2
		79	1092.5367	2
		51	562.2844	2
		58	669.3468	2
		105	762.4034	2
		79	729.8693	2
		63	603.3309	3

		83	801.9528	2
		54	489.5830	3
		74	629.3148	2
		54	1023.8688	3
25 (220)	4(2)	54	945.0143	2
		62	508.3004	2
17(264)	4(2)	94	687.3686	2
		43	493.2797	2
56(248)	12(8)	44	728.6894	3
		59	669.3375	2
		51	508.6071	3
		61	486.9175	3
		77	801.9498	2
		65	733.8696	2
		83	629.3167	2
		57	663.3313	2
70 (150)	8(7)	43	472.6159	3
		52	499.2446	3
		89	684.3175	2
		104	872.0824	3
		70	631.2918	2
		92	830.3990	3
		54	866.4220	3
25 (264)	6(2)	74	661.8323	2
		88	687.3679	2
		41	603.3400	2
4 (615)	3(1)	54	499.2742	3
6 (150)	1(1)	49	631.2902	2
48 (321)	16(6)	67	634.9478	2
		64	501.4083	2
		40	500.3796	2
		44	616.9331	2
		63	885.6116	2
		52	537.4057	2
55 (264)	17(10)	54	664.3240	2
		79	661.8341	2
		92	687.3576	2
		63	441.2466	3
		44	500.2981	2
		57	493.2700	2
		48	589.2941	2
		52	596.3070	2
		52	568.7845	2
		55	603.3375	2
19 (103)	2(1)	76	635.8704	2
61 (264)	16(12)	62	878.4097	2
		66	663.8261	2
		77	661.8364	2

		98	687.3710	2
		63	441.2487	3
		49	627.2874	2
		64	493.2666	2
		53	589.2977	2
		49	596.3005	2
		53	553.2861	2
		54	568.7929	2
		45	603.3370	2
11 (103)	1(1)	76	635.8797	2
26 (615)	14(8)	68	643.8200	2
		89	756.4039	2
		67	614.3603	2
		89	1038.8342	3
		40	690.7595	2
		77	748.4039	2
		105	1026.5177	3
		88	974.5045	3
		76	597.7755	2
36 (103)	2(1)	76	635.8697	2
44 (103)	3(3)	76	636.0034	2
		54	729.2132	3
		76	980.3604	3
31 (264)	8(4)	53	661.9675	2
		44	687.5035	2
		47	568.9066	2
		41	603.4650	2
4 (183)	1(1)	50	487.8503	2
17 (615)	9(4)	65	643.8131	2
		93	764.4051	2
		44	472.2124	2
		46	499.2736	3
10 (615)	6(2)	76	764.4047	2
		59	597.7727	2
47 (196)	9(4)	52	599.8185	2
		108	1048.9492	2
		41	516.7593	2
		59	580.8111	2
3 (264)	1(1)	44	603.3416	2
54 (150)	7(5)	65	472.6164	3
		84	684.3135	2
		96	872.0815	3
		70	631.2880	2
		74	830.3983	3
69 (132)	9(9)	64	743.3176	2
		52	469.2515	2
		70	624.6753	3
		65	780.4484	3

		48	419.7206	2
		109	762.3290	2
		72	467.2506	2
		63	546.6466	3
		101	769.9072	2
8 (150)	1(1)	50	684.3157	2
53 (132)	5(4)	44	490.5538	3
		55	469.2500	2
		51	585.5839	4
		110	762.3264	2
		96	785.8959	2
58 (139)	7(5)	102	911.4720	2
		58	665.3203	2
		45	450.7876	2
		53	545.8190	2
		52	694.9491	3
92 (55)	5(4)	43	444.2158	2
		58	599.7471	2
		67	1028.9530	2
		90	934.7728	3
76 (55)	4(4)	44	444.2272	2
		48	599.7596	2
		55	1028.9514	2
		133	934.7765	3
19 (705)	8(1)	63	942.8284	3
32 (67)	2(1)	61	538.2657	2
45 (68)	2(1)	73	859.7254	3
45 (68)	2(2)	41	441.6988	2
		109	859.7216	3
45 (68)	2(1)	97	859.7217	3
32 (67)	2(1)	49	538.2725	2
45 (68)	2(1)	94	860.0636	3
615(11)	7(1)	60	752.67	3
615(9)	4(1)	63	752.66	3
615(58)	34 (27)	68	644.33	2
		55	695.62	4
		91	756.42	2
		40	472.22	2
		89	1038.84	3
		44	806.88	2
		86	748.91	2
		47	770.14	4
		51	731.11	4
		108	846.08	3
		109	1128.49	2
		67	509.61	3

		67	685.86	2
		75	782.03	3
		85	835.46	2
		73	771.42	2
		56	557.32	3
		78	671.31	3
		77	965.44	2
		44	516.64	2
		44	463.59	3
		94	634.96	2
		122	746.69	3
615(56)	31(21)	45	643.82	2
		61	695.61	4
		95	756.40	2
		101	1038.83	3
		84	748.40	2
		77	597.77	2
		43	432.73	2
		42	569.80	2
		68	752.66	3
		53	509.61	3
		81	782.03	3
		58	835.46	2
		76	771.42	2
		56	557.31	3
		65	671.31	3
		89	965.44	2
		54	516.75	2
		43	413.79	2
		58	616.32	2
		80	634.82	2
		122	746.69	3
705(3)	2(0)	34	565.94	3
446(2)	1(0)	22	733.41	2
615(45)	30 (21)	74	695.61	4
		92	718.05	3
		56	409.92	3
		74	1038.83	3
		100	846.67	4
		64	748.40	2
		74	770.14	4
		65	591.05	4
		59	690.90	2
		48	432.73	2
		49	516.27	3
		58	782.03	3
		41	562.64	3
		47	557.31	3
		76	491.84	2
		157	1119.53	2

615(31)	17(9)	56	643.82	2
		79	756.40	2
		69	499.27	3
		78	782.02	3
		88	779.42	2
		62	965.44	2
		57	516.75	2
		41	413.79	2
		74	634.82	2
705(14)	9(6)	65	512.29	3
		56	980.49	2
		46	612.32	2
		52	534.26	2
		49	401.21	3
		42	457.25	2
		39	551.62	3
446(2)	1(1)	53	733.42	2
705(33)	21(13)	55	479.59	3
		54	512.29	3
		78	980.49	2
		88	821.40	2
		56	557.93	3
		60	612.32	2
		52	534.26	2
		52	401.21	3
		61	551.62	3
		80	822.40	2
		73	921.91	2
		65	512.77	4
		50	529.30	2
		46	498.73	2
446(10)	3(3)	79	733.42	2
		49	880.45	3
		47	413.79	2
615(7)	5(1)	50	479.59	3
705(28)	20(13)	76	654.83	2
		70	512.29	3
		88	980.49	2
		54	821.40	2
		42	565.95	3
		62	612.32	2
		52	534.26	2
		62	401.21	3
		47	551.62	3
		83	822.40	2
		56	512.77	4
		50	529.30	2
		81	733.44	2
446(2)	1(1)	56	413.79	2
615(1)	1(1)			

705(21)	13(9)	54	558.95	3
		72	654.82	2
		80	512.29	3
		48	980.49	2
		73	557.93	3
		48	534.26	2
		55	443.90	3
		43	457.24	2
		92	826.93	2
		41	457.78	2
778(13)	9(8)	44	418.91	3
		59	499.78	2
		60	642.88	2
		67	639.36	2
		52	637.34	3
		46	562.31	3
		81	610.83	2
		80	752.39	2
705(10)	7(4)	69	512.29	3
		66	612.32	2
		50	401.21	3
		41	457.24	2
		43	512.77	4
705(6)	4(2)	61	821.40	2
		41	551.62	3
250(14)	4(1)	61	649.87	2
705(36)	26 (20)	110	718.88	2
		81	654.83	2
		74	767.93	2
		92	980.49	2
		72	821.40	2
		87	557.93	3
		61	518.28	2
		59	612.32	2
		62	401.21	3
		62	443.91	3
		72	826.92	2
		50	921.91	2
		42	442.70	2
		57	512.77	4
		50	529.30	2
		54	672.64	3
705(32)	22(7)	51	602.30	4
		70	654.83	2
		72	512.29	3
		96	980.49	2
		64	691.01	3
		82	826.93	2
		80	822.40	2
705(22)	15(7)	82	767.93	2

		83	980.48	2
		80	848.42	2
		62	612.32	2
		71	551.62	3
		50	512.77	4
		49	582.99	3
615(11)	7(0)	40	471.97	4
615(3)	2(2)	50	432.73	2
		57	625.86	2
615(37)	20(8)	84	756.42	2
		53	499.27	3
		49	432.73	2
		67	851.09	3
		59	782.03	3
		76	771.42	2
		54	965.44	2
		61	634.82	2
615(27)	17(8)	85	756.41	2
		46	499.28	3
		72	597.77	2
		41	460.93	3
		48	432.73	2
		50	752.66	3
		42	454.26	4
		55	516.74	2
778(2)	2(1)	67	610.82	2
615(4)	3(0)	40	432.72	2
472(20)	6(2)	104	1028.79	3
		45	721.74	5
	10(6)	56	616.81	2
		81	679.88	2
		79	779.40	2
		49	424.75	2
		44	446.25	2
		59	659.82	2
416(2)	1(0)	39	754.39	2
434(55)	19(11)	69	641.67	3
		114	902.97	2
		78	634.33	4
		77	756.15	4
		50	572.31	2
		88	919.80	3
		46	408.25	2
		76	786.89	2
		52	618.95	3
		68	519.93	3
		44	452.74	2
434(15)	4(0)	30	817.42	2

150(28)	7(5)	51	783.95	2		
		50	429.92	3		
		65	472.62	3		
		87	684.32	2		
		75	631.29	2		
334(17)	6(1)	54	697.36	2		
333(13)	3(0)	27	658.36	3		
333(57)	13(12)	83	727.92	2		
		67	663.70	3		
		86	844.93	2		
		43	493.74	4		
		91	498.93	3		
		46	457.30	2		
		44	894.80	3		
		82	631.81	2		
		72	646.85	2		
		42	485.29	2		
		54	1028.20	3		
		63	574.80	2		
		409(26)	9(5)	42	622.75	2
				48	538.79	2
				52	549.31	3
59	515.30			2		
63	691.85			2		
333(6)	2(1)	41	403.22	2		
264(61)	18(13)	50	656.33	2		
		82	661.84	2		
		102	687.37	2		
		64	441.25	3		
		50	627.30	2		
		46	500.31	2		
		57	493.28	2		
		57	589.30	2		
		49	596.31	2		
		57	545.29	2		
		61	560.80	2		
		55	603.35	2		
		103(28)	3(2)	77	635.87	2
				65	729.06	3
		264(25)	7(2)	62	661.84	2
78	687.37			2		
248(33)	7(2)	61	661.35	2		
		54	486.91	3		
196(22)	4(3)	56	599.81	2		

		71	1048.95	2
		44	516.76	2
264(23)	6(2)	64	687.37	2
		50	560.79	2
150(52)	5(3)	97	684.32	2
		91	872.09	3
		75	631.29	2
132(80)	12(8)	56	490.55	3
		58	461.25	2
		58	469.24	2
		49	585.59	4
		97	510.93	3
		48	419.72	2
		110	762.33	2
		55	546.65	3
		83	785.90	2
139(46)	5(3)	57	665.32	2
		43	450.79	2
		52	545.82	2
55(76)	3(2)	42	444.22	2
		55	599.76	2
		73	934.77	3
55(76)	3(2)	62	599.76	2
		118	934.77	3
67(14)	1(1)	62	538.26	2
2(1193)	3(1)	80	842.8748	2
2(816)	2(1)	55	684.3533	2
13 (816)	9 (6)	44	527.3690	2
		68	684.4747	2
		67	692.9279	2
		71	741.0018	2
3(272)	1(0)	27	499.2887	2
21 (615)	11(5)	46	1038.8198	3
		59	499.2779	3
		42	569.8094	2
		69	516.2676	3
		43	514.9502	3
3 (471)	1(1)	77	828.9789	2

23 (615)	11(3)	57	1038.8359	3
		77	597.7697	2
		45	413.7937	2
1 (816)	1(1)	42	527.2764	2
10(105)	1(0)	35	680.8566	2
65 (615)	39 (28)	68	643.8323	2
		41	695.6096	4
		88	756.4096	2
		87	614.3708	2
		129	1038.8364	3
		88	748.4461	2
		93	974.4708	3
		69	591.0511	4
		43	432.7206	2
		46	569.8126	2
		98	874.3769	2
		60	516.2698	3
		87	846.0706	3
		111	752.3622	3
		79	763.9162	2
		69	685.8668	2
		60	835.4627	2
		78	600.0102	2
		81	771.4370	2
		55	557.3165	3
		66	671.3064	3
		78	692.3413	2
		68	973.5501	2
		90	634.8193	2
		146	1119.5289	2
56 (445)	21(17)	48	709.3642	3
		47	608.3164	3
		90	933.5420	3
		139	980.0155	2
		91	696.3518	3
		77	903.4600	3
		75	565.8029	2
		57	629.8457	2
		45	429.9126	3
		68	580.3229	2
		62	546.6139	3
		47	731.8485	2
		46	848.9089	2
		61	514.7651	2
		111	937.5253	2
		41	677.3504	3
		52	623.3001	2
26 (446)	13(10)	67	809.4177	2
		139	980.0155	2
		91	696.3518	3

		75	565.8029	2
		57	629.8457	2
		45	429.9126	3
		68	580.3229	2
		47	854.4335	2
		46	848.9089	2
		102	937.5253	2
		40	677.3504	3
16 (448)	6(3)	53	705.8893	2
		63	573.6351	3
		80	1205.1168	2
20 (448)	6(5)	77	705.8897	2
		73	573.6366	3
		119	1205.1106	2
		44	508.2969	2
		51	777.3502	3
14 (446)	5(3)	89	653.6599	3
		71	565.8043	2
		74	580.3255	2
19(778)	15(8)	57	411.2188	2
		63	639.3536	2
		57	561.7749	2
		65	637.3400	3
		43	438.9490	3
		46	1051.5497	2
		60	666.8328	2
		47	526.2588	2
17 (705)	10(3)	41	821.4005	2
		43	534.3088	2
		43	457.3075	2
6(615)	4(0)	32	413.7892	2
35 (778)	22(16)	46	418.9009	3
		59	499.7715	2
		75	642.8794	2
		113	1104.5557	2
		79	922.4487	2
		65	411.2166	2
		52	637.3352	3
		44	673.3317	3
		81	562.3031	3
		75	438.9000	3
		72	1051.5435	2
		75	610.8237	2
		92	752.3815	2

		57	666.8347	2
		47	464.2326	3
		55	526.2634	2
9 (615)	6(3)	73	643.8195	2
		41	756.4102	2
		49	413.7904	2
2 (446)	1(1)	65	733.4259	2
13 (250)	2(2)	44	504.7746	2
		70	871.7680	3
3 (705)	3(2)	51	601.3734	2
		44	457.2372	2
54 (705)	34 (31)	129	718.8753	2
		81	654.8723	2
		92	768.0234	2
		87	980.4935	2
		90	821.4114	2
		57	557.9359	3
		58	518.4788	2
		43	677.3546	2
		116	848.5256	2
		61	612.3897	2
		51	534.8087	2
		57	401.2062	3
		58	443.9172	3
		54	471.3103	4
		51	594.4265	2
		46	649.4585	3
		40	905.4308	3
		63	521.8361	2
		72	551.7640	3
		63	630.3085	3
		84	921.9050	2
		48	546.8216	2
		108	874.0862	2
		50	529.8214	2
		90	1008.4531	2
4 (615)	3 (2)	96	756.4614	2
		46	413.8023	2
2 (446)	1 (1)	80	733.4910	2
16(705)	10(2)	42	557.9259	3
		50	444.9027	3
		40	457.7721	2
55 (705)	36 (27)	123	718.9610	2
		74	654.8900	2
		72	512.3270	3
		106	980.6179	2

		90	821.5090	2
		73	557.9578	3
		59	518.3461	2
		80	848.4218	2
		52	612.3258	2
		51	534.3115	2
		49	601.3806	2
		68	594.4100	2
		86	551.6925	3
		45	666.4261	2
		82	1275.6824	2
		76	630.3881	3
		93	922.0209	2
		65	683.4185	3
		105	873.9819	2
		50	529.3714	2
		75	1000.4611	2
18 (705)	10(3)	105	942.9947	3
		64	654.9485	2
		41	551.7212	3
14(67)	1(0)	27	537.8743	2
24 (705)	14(8)	88	942.9991	3
		63	654.9475	2
		40	512.3864	3
		100	980.6764	2
		44	612.4371	2
		50	521.3881	2
		45	551.7217	3
		69	683.5005	3
33 (705)	19(11)	88	942.8249	3
		63	654.8801	2
		46	512.2930	3
		84	980.4944	2
		77	821.4990	2
		41	557.9602	3
		64	612.3928	2
		48	534.2573	2
		40	601.3405	2
		46	551.6753	3
		49	529.2990	2
		91	1000.5303	2
41(615)	24(15)	60	643.8221	2
		80	764.4039	2
		87	614.3680	2
		76	1038.8245	3
		72	499.2738	3
		44	432.7310	2
		91	874.3745	2
		51	514.9452	3
		70	843.4622	2

		95	779.4146	2
		51	461.5616	3
		72	973.4384	2
		44	413.7910	2
		70	634.8116	2
		92	746.6886	3
26(615)	15(5)	81	614.3690	2
		51	499.2816	3
		50	432.7254	2
		50	509.6121	3
30(615)	18 (15)	91	756.4052	2
		79	614.3667	2
		64	1038.8285	3
		75	748.4022	2
		42	432.7279	2
		46	514.9458	3
		44	562.9766	3
		57	605.3437	3
		70	965.4434	2
		44	413.7941	2
		79	634.8135	2
36 (615)	18(8)	94	756.5455	2
		42	614.4816	2
		42	1038.6868	3
		77	748.5637	2
		62	974.6602	3
		107	779.5539	2
		60	973.6480	2
24(705)	13 (8)	60	512.2892	3
		56	980.4840	2
		57	557.9355	3
		49	529.3002	2
		57	1000.4597	2
13(615)	7 (5)	49	432.7277	2
		49	965.4771	2
23(615)	14 (6)	83	756.4122	2
		45	597.7695	2
		57	965.4379	2
20(615)	12 (7)	40	643.8247	2
		70	614.3732	2
		62	597.7711	2
		51	461.5615	3
		57	965.4409	2
		47	634.8518	2
10(705)	6 (3)	67	512.2888	3
		50	534.2579	2
6 (530)	3 (3)	64	680.3580	2
		52	724.4147	2

		52	571.3140	2
30(472)	12(7)	57	572.9649	3
		49	560.7526	2
		81	1028.7821	3
		57	761.3405	2
		55	603.2761	2
		88	882.3910	3
		55	783.3401	2
20(472)	10(3)	50	574.7586	2
		50	882.4033	2
		44	783.3154	2
23(472)	10(5)	54	572.9596	3
		44	560.7450	2
		49	882.4070	2
		68	877.0619	3
		62	783.3339	2
24(472)	9(1)	40	574.7591	2
		44	578.6231	3
5(463)	2(1)	59	801.8842	2
7(472)	3(0)	33	572.9537	3
2 (463)	1 (1)	46	679.3425	2
2 (472)	1 (1)	40	560.7488	2
16 (150)	1 (1)	28	1299.1354	2
4 (463)	2(2)	64	679.3450	2
		64	620.2578	2
5 (615)	3(1)	79	756.4105	2
27 (463)	11(9)	90	832.0980	3
		42	406.7708	2
		64	679.3302	2
		58	484.2309	2
		64	569.6166	3
		49	551.2789	2
		61	645.2898	2
		52	620.2662	2
		76	814.3449	2
6 (615)	4(1)	99	756.4069	2
2 (741)	1(1)	44	713.3499	3
5 (615)	3 (2)	63	614.4799	2
25 (615)	15(8)	43	569.8061	2
		77	758.0306	3
		47	509.6351	3
		45	562.7144	3
		48	771.5178	2
		70	699.8393	2
		71	965.4437	2
3 (281)	1(1)	52	779.4100	2
16(381)	6(3)	55	679.8779	2

		78	779.4035	2
		62	659.8059	2
29(381)	11(3)	40	616.8057	2
		41	679.8832	2
		50	779.4092	2
		48	424.7524	2
		128	982.9702	2
66 (434)	24(17)	78	725.3635	2
		141	902.9760	2
		40	472.2608	3
		64	640.7977	2
		41	760.4204	2
		93	756.1602	4
		52	572.3150	2
		70	980.9689	2
		48	408.2486	2
		80	786.8836	2
		64	550.7736	2
		74	618.9609	3
		46	1198.0494	2
		74	730.3997	3
		40	504.2598	2
		88	817.4158	2
		74	514.5961	3
		61	1177.0785	2
		45	452.7353	2
16 (434)	7(6)	141	902.9760	2
		41	760.4204	2
		52	572.3150	2
		64	550.7736	2
		74	618.9609	3
		57	533.6057	3
14 (434)	4(3)	41	760.4204	2
		88	817.4158	2
		61	1177.0785	2
16(417)	7(2)	87	545.6066	3
		57	666.8726	2
50 (334)	15 (12)	64	502.2704	3
		50	576.3064	2
		84	878.4780	2
		60	569.6281	3
		54	466.7564	2
		68	970.5182	3
		42	588.8204	2
		74	697.3663	2

47 (333)	14(11)	74	727.9177	2
		74	844.9247	2
		63	668.6491	3
		78	498.9350	3
		48	457.8002	2
		104	631.8160	2
		72	646.8474	2
		45	485.2909	2
		61	579.8231	2
		54	643.8713	2
		62	574.2930	2
30 (333)	8(3)	87	844.9230	2
		106	631.8107	2
		63	646.8494	2
51 (254)	8(5)	57	890.3876	2
		61	537.2911	2
		79	813.7306	3
		46	575.8348	2
		51	1025.1666	3
64 (248)	11(9)	64	676.8853	2
		64	562.2825	2
		59	669.3477	2
		43	508.6035	3
		72	729.8674	2
		96	801.9479	2
		42	489.5818	3
		79	629.3113	2
		76	1023.8698	3
10 (705)	5(3)	42	942.9452	3
		78	654.8294	2
		75	821.4211	2
17(67)	1(0)	34	465.8785	3
7(161)	1(0)	24	614.8198	2
68 (150)	7(5)	132	748.3681	2
		89	684.3212	2
		126	872.0895	3
		70	631.2913	2
		83	830.4034	3
43 (264)	11(7)	61	661.8372	2
		91	687.3716	2
		40	499.7993	2
		44	493.2710	2
		61	589.3012	2
		58	553.2901	2
		47	568.7920	2
		40	524.7878	2

		47	603.3453	2
52 (150)	5(3)	86	684.3126	2
		123	872.0810	3
		61	631.2913	2
44 (103)	3 (2)	44	635.4995	2
69 (264)	23 (15)	67	790.9200	2
		52	656.3258	2
		79	661.8337	2
		107	687.3664	2
		43	643.3205	2
		59	441.2468	3
		51	627.2949	2
		45	500.3041	2
		63	493.2724	2
		49	589.2961	2
		59	596.3062	2
		57	553.2905	2
		65	560.7933	2
		53	469.2487	3
		55	603.3361	2
19 (103)	2 (2)	72	635.8714	2
		42	729.0567	3
1 (563)	1 (1)	40	458.7567	2
69 (264)	21(14)	62	585.9412	3
		51	527.6175	3
		54	656.3242	2
		82	661.8336	2
		95	687.3660	2
		46	441.2472	3
		53	627.2924	2
		48	499.8042	2
		61	493.2715	2
		56	589.2956	2
		44	596.3052	2
		54	553.2889	2
		60	560.7931	2
		59	603.3460	2
29 (103)	3(2)	60	635.8710	2
		73	729.0612	3
44 (103)	3(2)	61	635.9982	2
		55	980.3565	3
44 (196)	6(3)	110	1056.8398	3
		60	599.8155	2
		61	901.3692	3
21 (264)	6(3)	42	589.2963	2
		44	596.3038	2
		58	603.3411	2
70 (150)	8 (7)	50	499.2431	3

		82	684.3171	2
		126	872.0899	3
		74	631.2907	2
		90	830.4010	3
		50	866.4333	3
51 (615)	27 (19)	65	643.8184	2
		84	756.4190	2
		44	614.3682	2
		103	1038.8285	3
		82	748.4089	2
		44	432.7274	2
		46	592.2836	2
		63	757.9886	3
		71	509.6111	3
		77	771.4188	2
		70	671.3066	3
		71	965.4467	2
		72	634.8113	2
		143	1119.5239	2
38 (105)	5 (2)	64	696.8558	2
		44	554.2902	2
69 (132)	9(8)	86	743.3193	2
		57	461.2580	2
		59	624.6788	3
		68	585.5910	4
		110	762.3307	2
		57	467.2511	2
		51	546.6480	3
		92	769.9083	2
53 (132)	5(2)	95	762.3311	2
		65	785.9002	2
12 (139)	1(1)	62	911.4673	2
6 (132)	1(1)	48	461.2540	2
58 (139)	6(6)	107	911.4746	2
		60	657.3229	2
		48	450.7822	2
		56	545.8185	2
		75	689.6176	3
		50	1117.0723	2
62 (139)	8(3)	97	911.4761	2
		57	657.3193	2
		42	606.0337	3
		41	545.8187	2
10 (141)	1(1)	49	539.5946	3
25 (705)	16(8)	76	654.9038	2
		70	512.3219	3
		55	980.6104	2
		59	821.4998	2
		59	612.3261	2

		41	457.2418	2
		45	529.3430	2
		59	1008.4685	2
47 (147)	5(5)	46	437.7079	2
		52	663.3895	2
		76	714.8303	2
		128	877.5216	2
		67	1254.6383	2
3 (409)	1(1)	61	691.9245	2
92 (55)	5(5)	46	444.2158	2
		50	494.2502	4
		55	599.7548	2
		64	1028.9528	2
		88	934.7783	3
1(1079)	1(0)	38	658.8801	2
41 (55)	1(1)	90	934.7844	3
3(705)	2(0)	39	551.6224	3
32 (67)	2(1)	63	538.3372	2
35 (68)	1(1)	92	859.7986	3

Peptide Sequence	Seq. start-end
YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
APFSEVSK	254-261
MMoxSNLCSQQDVFSGK	288-302
RHPEFSIQLIMR	365-376
TDNPAECYANAQEQLNQHIK	391-410
MoxPQVPTDLLLETGK	443-456
YVPPPFNPDMFSFDEK	525-540
TIADGFTAMVDK	574-585
AFLFPDTSR	57-65
ASPETAIEFFQK	69-80
YLELESSGHR	113-122
SHTEVFPYILADDK	136-149
VVEKPFMDLPVGTTFWLQGR	175-194
IMoxELQDILAK	241-250
ECQSVCFEGR	329-339
HNGQIWVLENDK	648-659
YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
ESDVGACLDTK	199-209
IKDCCEKPIVER	303-314
DCCEKPIVER	305-314
KMoxPQVPTDLLLETGK	442-456
LCSAPAEER	541-549
VITPSFVDIFISK	219-231
TDERPASYFAVAVAR	443-457
ESDVGACLDTK	199-209
APFSEVSK	254-261
LCSAPAEER	541-549
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
APFSEVSK	254-261
TDNPAECYANAQEQLNQHIK	391-410
YVPPPFNPDMFSFDEK	525-540
LLINLIK	556-562
YNDLKEETFK	38-47
VSFLGHFIYSVAR	160-172

ESDVGACLDTK	199-209
APFSEVSK	254-261
LCSAPAEER	541-549

YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
LVKDVVDLAQK	69-79
VSFLGHFIYSVAR	160-172
MoxMoxSNLCSQQDVFS GK	288-302
IKDCCEKPIVER	303-314
TDNPAECYANAQEQLNQHIK	391-410
KMoxPQVPTDLLLETGK	442-456
LLINLIK	556-562
TIADGFTAMoxV DK	574-585

YNDLKEETFK	38-47
AVAMoxITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
IKDCCEKPIVER	303-314
SFEAGHDAFMAEFVYEYSR	346-364
RHPEFSIQLIMR	365-376
TDNPAECYANAQEQLNQHIK	391-410
MPQVPTDLLLETGK	443-456
MPQVPTDLLLETGKK	443-457
YVPPPFNPD MoxFSFDEK	525-540
LCSAPAEER	541-549
LLINLIK	556-562
TIADGFTAMoxV DK	574-585
HPNIICDCCKK	86-96
TDSQDMoxNEDT	93-102
APFSEVSK	254-261
GTEFTVNDLQGK	120-131
FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
TDERPASYFAVAVAR	443-457
FYTVISSLK	680-688
YNDLKEETFK	38-47
TNCDLLHDHGEADFLK	418-433
SDFHLFGPPGK	299-309
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
VEDIWSFLSK	279-288
SDFHLFGPPGK	299-309
TDERPASYFAVAVAR	443-457

VTGPGGDGTPAEVHIVR	982-998
SAGWNIPIGTLLHR	141-154
SDFHLFGPPGK	299-309
DQLTPSPR	353-360
VTGPGGDGTPAEVHIVR	982-998
VEDIWSFLSK	279-288
AGGVASGFR	165-173
HVVPNEVTVQR	174-184
HTQVQVLPESGETPLFK	370-386
VPFDAATLHTSR	416-427
GSNSAELSGAQELLK	605-619
EPDNFWVALGGK	633-644
YIETDPASR	726-734
EPGLQIWR	61-68
IEKFDLVPVPK	69-79
FDLVPVPK	72-79
NLYGDFFTGD SYLVLTIR	80-98
HVVPNEVTVQR	174-184
VFVSEEGAER	254-263
HTQVQVLPESGETPLFK	370-386
DKDQTEGLGEAYISGHVAK	394-412
VPFDAATLHTSR	416-427
VPVDPATYGQFYGGDSYIILYDYS	454-477
EGGQTTPAQTR	550-560
AVELDPAASQLNSNDAFVLK	574-593
TPSAAYLWVGR	594-604
GSNSAELSGAQELLK	605-619
EPDNFWVALGGK	633-644
YIETDPASR	726-734
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
VAAHAVVAR	266-274
DDNKVEDIWSFLSK	275-288
TDERPASYFAVAVAR	443-457
KDSNVNWNNLK	458-468
EFLGDKFYTVISSLK	674-688
EDFEGPFR	122-129
VITPSFVDIFISK	219-231
EPGLQIWR	61-68
IEKFDLVPVPK	69-79
VPFDAATLHTSR	416-427
VPVDPATYGQFYGGDSYIILYDYS	454-477
TPSAAYLWVGR	594-604
GSNSAELSGAQELLK	605-619
YIETDPASR	726-734
SAGWNIPIGTLLHR	141-154
AQSDFGVDTK	289-298

KGTEFTVNDLQ GK	119-131
GTEFTVNDLQ GK	120-131
SAGWNIPIGTLLHR	141-154
FFSASCVPGATIEQK	174-188
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
TDERPASYFAVAVAR	443-457
NLQMoxDDFELLCTDGR	579-593
ECNLAEVPTHAVVVRPEK	602-619
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
HTQVQVLPESGETPLFK	370-386
VPFDAATLHTR	416-427
VPVDPATYGQFYGGDSYIILYD YR	454-477
TPSAAYLWVGR	594-604
KGTEFTVNDLQ GK	119-131
GTEFTVNDLQ GK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
VAAHAVVAR	266-274
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
VPSLMoxDSQLYLGF EYYS AIQSMR	329-351
TDERPASYFAVAVAR	443-457
NLQMoxDDFELLCTDGR	579-593
ECNLAEVPTHAVVVRPEK	602-619
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
LKPIAAEVYEHTEGSTTSYYAVAVVK	93-118
KGTEFTVNDLQ GK	119-131
GTEFTVNDLQ GK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
DGKGDVAFVK	219-228
HTTVNENAPDQK	229-240
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
SDFHLFGPPGKK	299-310
KDQLTPSPR	352-360
TDERPASYFAVAVAR	443-456
DSNVNWN NLK	459-468
TGTCNFDEYFSEGCAPGSPPNSR	494-516

GDVAFIQHSTVEENTGGK	554-571
NLQMDDFELLCTDGR	579-593
ECNLAEVPTHAVVVRPEK	602-619
EFLGDKFYTVISSLK	674-688
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
KGTEFTVNDLQGK	119-131
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
DGKGDVAFVK	219-228
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
SDFHLFGPPGKK	299-310
SDFHLFGPPGKKDPVLK	299-315
DLLFKDSAIMLK	316-327
KDQLTPSPR	352-360
TDERPASYFAVAVAR	443-457
GDVAFIQHSTVEENTGGK	554-571
NLQMDDFELLCTDGR	579-593
ECNLAEVPTHAVVVRPEK	602-619
EGTTYKEFLGDKFYTVISSLK	668-688
EFLGDKFYTVISSLK	674-688
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
LKPAAAEVYEHTEGSTTSYYAVAVVK	93-118
KGTEFTVNDLQGK	119-131
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
VAAHAVVAR	266-274
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
GEADAVALDGGLVYTAGVCGLVPVMAER	406-433
TDERPASYFAVAVAR	443-457
DSNVNWNNLK	459-468
ECNLAEVPTHAVVVRPEK	602-619
EFLGDKFYTVISSLK	674-688
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
LKPAAAEVYEHTEGSTTSYYAVAVVK	93-118
KGTEFTVNDLQGK	119-131

GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
FFSASCVPGATIEQK	174-188
VAAHAVVAR	266-274
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
GEADAVALDGGLVYTAGVCGLVPVMAER	406-433
TDERPASVFAYAVAR	443-457
TGTCNFDEYFSEGCAPGSPPNSR	494-516
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705

GTEFTVNDLQGK	120-131
YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
MPQVPTDLLLETGK	443-456
RPCFTAMGVDTK	513-524
YVPPFPNPDMFSFDEK	525-540
APFSEVSK	254-261
SQCIMoxEAEFDEKPADLPSLVEK	315-336
TDNPAECYANAQEQLNQHIK	391-410
YVPPFPNPDMFSFDEK	525-540
LLINLIK	556-562
KPQMTEEQIK	564-573
YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
ESDVGACLDTK	199-209
FVHDSIGVHK	262-271
TDNPAECYANAQEQLNQHIK	391-410
MPQVPTDLLLETGK	443-456
YVPPFPNPDMFSFDEK	525-540
LLINLIK	556-562
HVMIDCSPYLQVVR	89-102
DGNTMoxVACPR	103-112
ILSPVCGTDGFTYDNECGICAHNAEQR	179-205
EEVPELDCSK	349-358
HVMoxIDCSPYLQVVR	89-102
DGNTMVACPR	103-112
EEVPELDCSK	349-358
DGTSWVACPR	38-47
DGNTMoxVACPR	103-112
LEIGSVDCSK	152-161
TLVACPR	172-178

DNLDNNIPSSLR	159-170
AVAMITFAQYLQR	48-60

LVKDVVDLAQK	69-79
VSFLGHFIYSVAR	160-172
FVHDSIGVHK	262-271
MoxMoxSNLCSQQDVFS GK	288-302
TDNPAECYANAQEQLNQHIK	391-410
YVPPPFNPDMFSFDEK	525-540
TIADGFTAMoxV DK	574-585
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
TDERPASYFAVAVAR	443-457
ECNLAEVPTHAVVVRPEK	602-619
IRDLLER	623-629
EFLGDKFYTVISSLK	674-688
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
APFSEVSK	254-261
YVPPPFNPDMFSFDEK	525-540
KGTEFTVNDLQ GK	119-131
GTEFTVNDLQ GK	120-131
TSCHTGLGR	132-140
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
SDFHLFGPPGKK	299-310
TDERPASYFAVAVAR	443-457
NLQMoxDDFELLCTDGR	579-593
ECNLAEVPTHAVVVRPEK	602-619
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
VSFLGHFIYSVAR	160-172
LLINLIK	556-562
LDIDSEPTIAR	32-42
NTGIICTIGPASR	43-55
GVNLPGAAVDLPVASEK	207-223
FGVEQNVDMoxVFASFIR	230-245
GDYPLEAVR	367-375
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
IYVDDGLISLLVK	173-185
DNLDNNIPSSLR	159-170
SPCVASCNIPVVSGR	199-213
ECEDIYR	214-220
YQLSVSNYK	341-349
GDLGIEIPA EK	294-304
APFSEVSK	254-261
RHPEFSIQLIMR	365-376
HPEFSIQLIMoxR	366-376
TDNPAECYANAQEQLNQHIK	391-410
KMoxPQVPTDLLLETGK.K	442-456

MoxPQVPTDLLLETGK	443-456
MACSEGYSIVIHDTCT	474-490
YVPPPFNPDMFSFDEK	525-540
LLINLIK	556-562
TIADGFTAMVVK	574-585
QSDINTCFGEEGANLIVQSR	589-608
HIADLAGNPEVILPVPAFNVINGGSHAGNK	133-162
APFSEVSK	254-261
IKDCCEKPIVER	303-314
RHPEFSIQLIMR	365-376
KMoxPQVPTDLLLETGK	442-456
MPQVPTDLLLETGK	443-456
MACSEGYSIVIHDTCT	474-490
RPCFTAMoxGVDTK	513-524
YVPPPFNPDMFSFDEK	525-540
LLINLIK	556-562
IKDCCEKPIVER	303-314
TDNPAECYANAQEQLNQHIK	391-410
YVPPPFNPDMFSFDEK	525-540
ELFDPVIEDR	87-96
LSVEALGSLGGDLK	157-170
FCTGLTQIETLFK	253-265
ELFDPVIEDR	87-96
LSVEALGSLGGDLK	157-170
FGEVLKR	308-314
RGTGGVDTAAGGVFVDSNADR	320-341
KVYIQDVCR	59-67
HLVLIAQEEGAR	102-113
FELGCYALCPSIK	129-141
MoxPEFYQR	148-154
APLDLYTK	203-210
SQQAVAGTVHVSFK	342-356
AGACPPTFTISGQCHGGLLNCCAK	41-64
GGASIVTPR	203-211
GNPTVEVDLYTNK	16-28
AAVPSGASTGIYEALER	33-50
NVNVVEQEKIDK	81-92
IDKLMoxLEMoxDGTENK	90-103
LMLEMDGTENK	93-103
FGANAILGVSLAVCK	106-120
HIADLAGNPEVILPVPAFNVINGGSHAGNK	133-162
IGAENVHNLK	184-193
DATNVGDEGGFAPNILENK	203-221
EALLLK	222-228
VVIGMDVAASEFYR	240-253
NYPVVSIEDPFDQDDWAAWKK	286-306
FTASVGIQVVGDDLTVTNPK	307-326
SCNCLLLK	336-343

VNQIGSVTESLQACK	344-358
LAQSNWGVMSHR	359-372
IEEELGSK	413-420
KISVVEQEKIDK	81-92
FGANAILGVSLAVCK	106-120
VNQIGSVTESIQACK	344-358

LGDVYVNDAFGTAHR	157-171
ACANPANGSVILLENLR	107-123
LGDVYVNDAFGTAHR	157-171
ALESPERPFLAILGGAK	200-216
VLNNMoxQIGNSLFDEEGSK	247-264
ITLPVDFITADK	280-291
GCITIIGGGDTATCCAK	366-382
VSHVSTGGGASLELEGK	389-406
IALGDDSPAIAQK	86-98
IVATTLTSPQLFAEWK	305-320
TDEGQPWVLPVVR	42-54
KVEQLIAGDGSLNHEYLPILGLPEFR	55-80
NFGLYNER	259-266
VGNLSVVGKDEDNVQR	267-282
IVATTLTSPQLFAEWK	305-320
VVVVGNPANTNCLIASK	126-142
FVEGLPINDFSR	299-310
ITVVGVGQVGMoxACAISILGK	23-42
GLCDELALVDVLEDK	43-57
IVADKDYAVTANSK	77-90
DYAVTANSK	82-90
IVVVTAGVR	91-99
VIGSGCNLD TAR	158-169
QVVESAYEVIR	233-243
LKDDEVAQLK	308-317
LKDDEVAQLKK	308-318
KSADTLWSIQK	318-328
DLADELTLVDVVEDK	43-57
VIGSGCNLDSAR	158-169
QVVDSAYEVIK	233-243
VEILRNAIRYIESLQALLR	138-156

KFFVGGNWK	5-13
SLGELIHTLNGAK	20-32
LSADTEVVCGAPSIYLD FAR	33-52
IGVAAQNCYK	59-68
GAFTGEISPAMoxIK	72-84
DIGAAWVILGH SER	85-98
HVFGESDELIGQK	100-112
VAHALAEGLGVIA CIGEK	113-130

VVLAYEPVWAIGTGK	160-174
TATPQQAQEVHEK	175-187
SHVSDAVAQSTR	194-205
ELASQHDVDGFLVGGASLKPEFVDIINAK	219-247
LGLDFPNLPYLIDGDVK	53-69
LTQSNAILR	70-78
LADNLDLTSAAAAK	69-82
LTPVAEEAR	164-172
LSADTEVVCGAPSIYLDGAR	33-52
GAFTGEISPAMoxIK	72-84
DIGAAWVILGHSE	85-98
HVFGESDELIGQK	100-112
VVLAYEPVWAIGTGK	160-174
TATPQQAQEVHEK	175-187
SHVSDAVAQSTR	194-205
IYGGSVTGGNCK	206-218

GSPAANVAVKVFKK	45-58
KAADGTWQDFATGK	58-71
AADGTWQDFATGK	59-71
TTEFGEIHELTTTEQFVEGVYR	72-93
VEFDTSSYWK	94-103
GLGLSPFHEYADVFTANDSGHR	104-126
HYTIAALLSPFSYSTTAVVSDPQE	127-150
DAIAQFESSAVGK	51-63
LADNLDLTSAAAAK	69-82
LISFLDELQK	252-261
VSFLGHFIYSVAR	160-172
VEFDTSSYWK	94-103
GTVTAFSPFDAR	7-18
VLTEILASR	109-117
TPAEVQNIK	118-126
ITGETSGHFQR	141-151
SVPAYFAETLYYSMK	246-260
SEIDLLDIR	277-285
DMoxVDVYLETVK	36-46
DAIAQFESSAVGK	51-63
LADNLDLTSAAAAK	69-82
IRPFLDQFSAK	120-130
LTPVAQELK	142-150
LTPVAEEAR	164-172
NLAPYSDELK	185-194
GIPQASEYQAK	208-218
MoxTPLVQEFR	230-238
LISFLDELQK	252-261
VAPTITLFPPSK	4-15
SFWQHDEPQTPLDR	20-33
DMoxVDVYLETVK	36-46
DAIAQFESSAVGK	51-63

LADNLDTLSAAAAK	69-82
IRPFLDQFSAK	120-130
WTEELEQYR	131-139
LTPVAEEAR	164-172
NLAPYSDELRL	185-194
GIPQASEYQAK	208-218
VMoxEQLSNLR	219-227
MTPLVQEFLR	230-238
LISFLDELQK	252-261
VAPTITLFPPSK	4-15
YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
LVKDVVDLAQK	69-79
CVANEDAPECSKPLPSIILDEICQVEK	80-106
DSYGAMoxADCCSK	109-120
VSFLGHFIYSVAR	160-172
RHPFLYAPAILSFAVDFEHALQSCCK	173-198
HPFLYAPAILSFAVDFEHALQSCCK	174-198
ESDVGACLDTK	199-209
VAPTITLFPPSK	4-15
VAPTITLFPPSK	4-15
VAPTITLFPPSKEELNEATK	4-23
ATLVCLINDFYSPVTVDWVIDGSTR	24-49
DAIAQFESSAVGK	51-63
LADNLDTLSAAAAK	69-82
MoxTPLVQEFLR	230-238
LISFLDELQK	252-261
DDTALNGIR	66-74
YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
CSYEGLSK	61-68
VSFLGHFIYSVAR	160-172
AVAMoxITFAQYLQR	48-60
ESDVGACLDTK	199-209
YWGVASFLQK	111-120
ELNEDGTCADSYSFVFSR	143-160
QIDLCLDR	177-184
QIDLCLDRK	177-185
LISFLDELQK	252-261
GSPAANVAVKVFKK	45-58
AADGTWQDFATGK	59-71
TTEFGEIHELTTTEEQFVEGVYR	72-93
VEFDTSSYWK	94-103
GLGLSPFHEYADVFTANDSGHR	104-126
LADSHNFDEYMoxK	11-22
ALGVGFAMoxR	23-31
QVGNVTKPTVIISSEGDK	32-49
QVGNVTKPTVIISSEGDKVVIR	32-53

NTEISFK	60-66
LGEEFDETPDDR	67-79
SVVTLDGDK	83-91
SVVTLDGDKLVHVQK	83-97
MVMTLTFGDVVAVR	114-127
AADGTWQDFATGK	59-71
LADSHNFDEYMK	11-22
ALGVGFAMoxR	23-31
QVGNVTKPTVIISSEGDKVVIR	32-53
LGEEFDETPDDR	67-79
MoxVMoxTLTFGDVVAVR	114-127
LLGAPVPVDENDEGLQR	30-46
ALQFAMoxAEYNR	47-57
RQLVSGIK	75-82
YILQVEIGR	83-91
SSGDLQSCEFHDPEMoxAK	97-114
SIPPACDK	1-8
DYSPVCGTDGK	18-28
TYPNECVLCLSNSEENK	29-45
TYPNECVLCLSNSEENKNVQIYK	29-51
SIPPACDK	1-8
DYSPVCGTDGK	18-28
TYPNECVLCLSNSEENK	29-45
TYPNECVLCLSNSEENKNVQIYK	29-51
LKPIAAEVYEHTEGSTTSYYAVAVVK	93-118
APSVDIGTCR	45-54
AGACPPTFTISGQCHGGLLNCCAK	41-64
TQGNFCR	34-40
AGACPPTFTISGQCHGGLLNCCAK	41-64
AGACPPTFTISGQCHGGLLNCCAK	41-64
APSVDIGTCR	45-54
AGACPPTFTISGQCHGGLLNCCAK	41-64
SFEAGHDAFMAEFVYEYSR	346-364
SFEAGHDAFMAEFVYEYSR	346-364
YNDLKEETFK	38-47
YNDLKEETFKAVAMITFAQYLQR	38-60
AVAMITFAQYLQR	48-60
CSYEGLSK	61-68
CVANEDAPECSKPLPSIILDEICQVEK	80-106
ADPERNECFLSFK	121-133
VSFLGHFIYSVAR	160-172
RHPFLYAPAILSFAVDFEHALQSCCK	173-198
HPFLYAPAILSFAVDFEHALQSCCK	174-198
SQCIMEAEFDEKPADLPSLVEK	315-336
SFEAGHDAFMAEFVYEYSR	346-364
RHPEFSIQLIMR	365-376

HPEFSIQLIMR	366-376
TDNPAECYANAQEQLNQHIK	391-410
KMPQVPTDLLLETGK	442-456
MPQVPTDLLLETGK	443-456
MPQVPTDLLLETGKK	443-457
MACSEGYSIVIHDTCT	474-490
YVPPFPNPDMFSFDEK	525-540
LCSAPAEER	541-549
RKPQMTEEQIK	563-573
TIADGFTAMVDK	574-585
QSDINTCFGEEGANLIVQSR	589-608
YNDLKEETFK	38-47
YNDLKEETFKAVAMITFAQYLQR	38-60
AVAMITFAQYLQR	48-60
CVANEDAPECSKPLPSIILDEICQVEK	80-106
VSFLGHFIYSVAR	160-172
ESDVGACLDTK	199-209
APFSEVSK	254-261
FVHDSIGVHK	262-271
SFEAGHDAFMAEFVYEYSR	346-364
RHPEFSIQLIMR	365-376
TDNPAECYANAQEQLNQHIK	391-410
KMPQVPTDLLLETGK	442-456
MPQVPTDLLLETGK	443-456
MPQVPTDLLLETGKK	443-457
MACSEGYSIVIHDTCT	474-490
YVPPFPNPDMFSFDEK	525-540
LCSAPAEER	541-549
LLINLIK	556-562
KPQMTEEQIK	564-573
TIADGFTAMVDK	574-585
QSDINTCFGEEGANLIVQSR	589-608
DDNKVEDIWSFLSK	275-288
VITPSFVDIFISK	219-231
YNDLKEETFKAVAMITFAQYLQR	38-60
CSYEGLSKLVKDVVDLAQK	61-79
LVKDVVDLAQK	69-79
CVANEDAPECSKPLPSIILDEICQVEK	80-106
CVANEDAPECSKPLPSIILDEICQVEKLR	80-108
VSFLGHFIYSVAR	160-172
RHPFLYAPAILSFAVDFEHALQSCCK	173-198
QQYFCGILKQFGDRVFQAR	224-242
QLIYLSQKYPK	243-253
APFSEVSK	254-261
IKDCCEKPIVER	303-314
TDNPAECYANAQEQLNQHIK	391-410
KMoxPQVPTDLLLETGK	442-456
MPQVPTDLLLETGKK	443-457
LLINLIK	556-563
QSDINTCFGEEGANLIVQSR	589-608

YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
TDNPAECYANAQEQLNQHIK	391-410
MoxPQVPTDLLLETGK	443-456
YVPPFPNPDMSFDEK	525-540
LCSAPAEER	541-549
LLINLIK	556-562
TIADGFTAMVDK	574-585
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
DQLTPSPR	353-360
TDERPASYFAVAVAR	443-457
VITPSFVDIFISK	219-231

KGTEFTVNDLQ GK	119-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
TDERPASYFAVAVAR	443-457
LCQLCQGSGGIPPEK	517-531
NLQMoxDDFELLCTDGR	579-593
ECNLAEVPTHAVVVRPEK	602-619
FYTVISSLK	680-688
EDFEGPFR	122-129
VITPSFVDIFISK	219-231
ASNARPPSVYVFPPTTEQLNGNQ R	316-339
LLINLIK	556-562
KGTEFTVNDLQ GK	119-131
GTEFTVNDLQ GK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
TDERPASYFAVAVAR	443-457
LCQLCQGSGGIPPEK	517-531
ECNLAEVPTHAVVVRPEK	602-619
FYTVISSLK	680-688
VITPSFVDIFISK	219-231
LLINLIK	556-562

KCNNLRDLTQQR	38-50
GTEFTVNDLQ GK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
NAPYSGYSGAFHCLK	204-218
AQSDFGVDTK	289-298
SDFHLFGPPGKK	299-310
DQLTPSPR	353-360
TDERPASYFAVAVAR	443-457
IRDLLER	623-629
AGKEPGLQIWR	58-68
EPGLQIWR	61-68
IEKFDLVPVPK	69-79
HVVPNEVTVQR	174-184
HTQVQVLPESGETPLFK	370-386
IEKVPFDAATLHTSR	413-427
TPSAAYLWVGR	594-604
GSNSAELSGAQELLK	605-619
SAGWNIPIGTLLHR	141-154
VEDIWSFLSK	279-288
SDFHLFGPPGK	299-309
DQLTPSPR	353-360
ECNLAEVPTHAVVVRPEK	602-619
FFSASCVPGATIEQK	174-188
TDERPASYFAVAVAR	443-457
CGPGLVLLGSAVR	164-176
KGTEFTVNDLQ GK	119-131
GTEFTVNDLQ GK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
DGKGDVAFVK	219-228
VEDIWSFLSK	279-288
SDFHLFGPPGK	299-309
SDFHLFGPPGKK	299-310
TDERPASYFAVAVAR	443-457
NLQMoxDDFELLCTDGR	579-593
ANVMoxDYR	595-601
ECNLAEVPTHAVVVRPEK	602-619
FYTVISSLK	680-688
TCNPSDILQMoxCSFLEGK	689-705
KGTEFTVNDLQ GKTSCHTGLGR	119-140
GTEFTVNDLQ GK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQKLCR	174-191
TDERPASYFAVAVAR	443-457
LCQLCQGSGGIPPEK	517-531
SAGWNIPIGTLLHR	141-154

GAIEWEGIESGSVEQAVAK	155-173
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
TDERPASYFAVAVAR	443-457
ECNLAEVPTHAVVVRPEK	602-619
EFLGDKFYTVISSLK	674-688
TNCDLLHDHGEADFLK	418-433
APFSEVSK	254-261
IAKGYESLLEK	377-387
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
APFSEVSK	254-261
SQCIMoxEAEFDEKPADLPSLVEK	315-336
TDNPAECYANAQEQLNQHIK	391-410
MPQVPTDLLLETGK	443-456
YVPPPFNPDMSFSDEK	525-540
TIADGFTAMVDK	574-585
AVAMITFAQYLQR	48-60
VSFLGHFIYSVAR	160-172
ESDVGACLDTK	199-209
QLIYLSQKYPK	243-253
APFSEVSK	254-261
SFEAGHDAFMAEFVYEYSR	346-364
KMoxPQVPTDLLLETGKK	442-457
LCSAPAEER	541-549
TPSAAYLWVGR	594-604
APFSEVSK	254-261
ILSPVCGTDGFTYDNECGICAHNAEQR	179-205
ILLPVCCTDGFTYDNECGICAHNAQHGTevkk	245-276
ELFDPVIEDR	87-96
LSVEALGSLGGDLK	157-170
FCTGLTQIETLFK	253-265
FGEVLKR	308-314
LLIEMoxEK	359-365
GQSIDDLMOxPAQK	370-381
EFVEDFIWPAVR	68-79
GNPTVEVDLYTNKGLFR	16-32
AAVPSGASTGIYEALRL	33-50
AAVPSGASTGIYEALRLDNDKTR	33-56
HIADLAGNPEVILPVPAFNVINGGSHAGNK	133-162
IGAevYHNLK	184-193
DATNVGDEGGFAPNILENKEALELLK	203-228
EALELLK	222-228
VVIGMoxDVAASEFYR	240-253
DGKYDLDFKSPDDPSR	254-269
LAQSNGWGVMOxVSHR	359-372
IEEELGSK	413-420
VNQIGSVTESIQACK	344-358

VLDAVRGSPAANVAVK	39-54
GSPAANVAVKVFK	45-57
GSPAANVAVKVFKK	45-58
AADGTWQDFATGK	59-71
VEFDTSSYWK	94-103

FVEGLPINDFSR	299-310
ITVVGVGQVGMACAISILGK	23-42
LITPVAAGSTVPSNK	8-22
ITVVGVGQVGMoxACAISILGK	23-42
GLCDELALVDVLEDK	43-57
GEMMDLQHGSLFLQTHK	60-76
IVADKDYAVTANSK	77-90
IVVVTAGVR	91-99
YSPNCTILVVSNPVDILTYVTWK	127-149
VIGSGCNLD TAR	158-169
QVVESAYEVIR	233-243
VHSVSTLVK	270-278
GTYGIENDVFLSLPCVLSASGLTSVINQK	279-307
SADTLWSIQK	319-328
SADGCWSNYGK	151-161
VGGGQTISVMK	162-172
ATIVPVPDGSVHIGQR	252-262
LGLSNLDVAK	268-277
CSTIIDAAFGLK	287-299
VG VNGFGR	4-11
DMVDVYLETVK	36-46
DAIAQFESSAVGK	51-63
LADNLD TLSAAAAK	69-82
IRPFLDQFSAK	120-130
WTEELEQYR	131-139
LTPVAQELK	142-150
LTPVAEEAR	164-172
NLAPYSDELR	185-194
GIPQASEYQAK	208-218
VMEQLSNLR	219-227
MTPLVQEFR	230-238
LISFLDELQK	252-261
VAPTITLFPPSK	4-15
VAPTITLFPPSKEELNEATK	4-23
DAIAQFESSAVGK	51-63
LADNLD TLSAAAAK	69-82
GAFTGEISPAMIK	72-84
HVFGESDELIGQK	100-112
YWGVASFLQK	111-120

ELNEDGTCADSYSFVFSR	143-160
QIDLCLDR	177-184
LADNLDLSAAAAK	69-82
MTPLVQEFR	230-238
AADGTWQDFATGK	59-71
TTEFGEIHELTTEEQFVEGVYR	72-93
VEFDTSSYWK	94-103
LADSHNFDEYMK	11-22
ALGVGFAMR	23-31
ALGVGFAMoxR	23-31
QVGNVTKPTVIISSEGDKVVIR	32-53
TQSTFKNTEISFK	54-66
NTEISFK	60-66
LGEEFDETTDDR	67-79
SVVTLGDGKLVHVQK	83-97
MoxVMoxTLTFGDVVAVR	114-127

ALQFAMoxAEYNR	47-57
RQLVSGIK	75-82
YILQVEIGR	83-91

SIPPACDK	1-8
DYSPVCGTDGK	18-28
TYPNECVLCLSNSEENKNVQIYK	29-51
DYSPVCGTDGK	18-28
TYPNECVLCLSNSEENKNVQIYK	29-51
APSVDIGTCR	45-54

QFDPSDFQDETVTR	604-617
ASPETAIEFFQK	69-80
AFLFPDTSR	57-65
ASPETAIEFFQK	69-80
ECQSVCIFEGR	329-339
HNGQIWWLENDR	648-659

ESMoxSGRSAR	51-59
CVANEDAPECSKPLPSIILDEICQVEK	80-106
VSFLGHFIYSVAR	160-172
FVHDSIGVHK	262-271
IKDCCEKPIVER	303-314
RHPEFSIQLIMoxR	365-376
SAIVLQVLTVPFVDR	375-389

CVANEDAPECSKPLPSIILDEICQVEK	80-106
ESDVGACLDTK	199-209
LLINLIK	556-562
AFLFPDTSR	57-65
DIPVNPICIYR	32-42
YNDLKEETFK	38-47
YNDLKEETFKAVAMITFAQYLQR	38-60
AVAMITFAQYLQR	48-60
LVKDVVDLAQK	69-79
CVANEDAPECSKPLPSIILDEICQVEK	80-106
VSFLGHFIYSVAR	160-172
HPFLYAPAILSFAVDFEHALQSCCK	174-198
QQYFCGILKQFGDRVFAQR	224-242
APFSEVSK	254-261
FVHDSIGVHK	262-271
MMSNLCSSQQDVFSGK	288-302
IKDCCEKPIVER	303-314
SQCIMEAEFDEKPADLPSLVEK	315-336
SFEAGHDAFMAEFVYEYSR	346-364
RHPEFSIQLIMR	365-376
HPEFSIQLIMR	366-376
KMPQVPTDLLLETGK	442-456
KMPQVPTDLLLETGKK	442-457
MPQVPTDLLLETGK	443-456
MPQVPTDLLLETGKK	443-457
MACSEGYSIVIHDTCR	474-490
RPCFTAMGVDTK	513-524
YVPPFPNPFMFSDFK	525-540
TIADGFTAMVDK	574-585
QSDINTCFGEEGANLIVQSR	589-608
MoxREIVHIQAGQCGNQIGAK	1-19
EIVHIQAGQCGNQIGAK	3-19
SGPFGQIFRPDNFVFGQSGAGNNWAK	78-103
GHYTEGAELVDSVLDVVR	104-121
GHYTEGAELVDSVLDVVRK	104-122
LTTPTYGDLNHLVSATMSGVTTCLR	217-241
FPGQLNADLR	242-251
FPGQLNADLRK	242-252
KLAVNMoxVPFPR	252-262
LAVNMoxVPFPR	253-262
LHFFMoxPGFAPLTSR	263-276
EVDEQMoxLNVQNK	325-336
NSSYFVEWIPNNVK	337-350
TAVCDIPPR	351-359
MSATFIGNSTAIQELFK	363-379
MoxSATFIGNSTAIQELFKR	363-380
ISEQFTAMoxFR	381-390
AVLVDLEPGTMoxDSVR	63-77
GHYTEGAELVDSVLDVVR	104-121
GHYTEGAELVDSVLDVVRK	104-122

FPGQLNADLR	242-251
FPGQLNADLRK	242-252
KLAVNMoxVPFPR	252-262
LAVNMoxVPFPR	253-262
ALTVPELTQQMoxFDAK	283-297
NSSYFVEWIPNNVK	337-350
MASTFIGNSTAIQELFK	363-379
MoxASTFIGNSTAIQELFKR	363-380
QLFHPEQLITGK	85-96
NLDIERPTYTNLNR	216-229
FDGALNVDLTEFQTNLVPYPR	244-264
QLFHPEQLITGK	85-96
NLDIERPTYTNLNR	216-229
FDGALNVDLTEFQTNLVPYPR	244-264
DVNAAIATIK	327-336
AFVHWYVGEGMEEGEFSEAR	403-422
GHYTEGAELVDSVLDVVR	104-121
FPGQLNADLR	242-251
LAVNMoxVPFPR	253-262

AGGVASGFR	165-173
HVVPNEVTVQR	174-184
VFVSEEGAER	254-263
HTQVQVLPESGETPLFK	370-386
VPFDAATLHTSR	416-427
AVELDPAASQLNSNDAFVLK	574-593
EPDNFWVALGGK	633-644
YIETDPASR	726-734
FFSASCVPGATIEQK	174-188
AQSDFGVDTK	289-298
DQLTPSPR	353-360
LLINLIK	556-562
AGKEPGLQIWR	58-68
EPGLQIWR	61-68
IEKFDLVPVPK	69-79
NLYGDFFTGD SYLV LNTIR	80-98
GAAAIFTVQMoxDDYLQ GK	122-138
AGGVASGFR	165-173
HTQVQVLPESGETPLFK	370-386
DKDQTEGLGEAYISGHVAK	394-412
IEKVPFDAATLHTSR	413-427
VPFDAATLHTSR	416-427
AVELDPAASQLNSNDAFVLK	574-593
TPSAAYLWVGR	594-604
GSNSAELSGAQELLK	605-619

EPDNFWVALGGK	633-644
DAQEEKTEALK	710-721
YIETDPASR	726-734
YNDLKEETFK	38-47
AVAMITFAQYLQR	48-60
LLINLIK	556-562
VITPSFVDIFISK	219-231

YLLEDTVR	154-161
APLSFDTSPSDVAASFMASLSQSVER	193-217
SDFHLFGPPGK	299-309
DQLTPSPR	353-360

KGTEFTVNDLQGK	119-131
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
DGKGDVAFVK	219-228
HTTVNENAPDQK	229-240
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
SDFHLFGPPGKK	299-310
SDFHLFGPPGKKDPVLK	299-315
DPVLKDLLFK	311-320
DPVLKDLLFKDSAIMLK	311-327
VPSLMDSQLYLGFEYSAIQSMoxR	329-351
KDQLTPSPR	352-360
TDERPASYFAVAVAR	443-457
GDVAFIQHSTVEENTGGK	554-571
NLQMoxDDFELLCTDGR	579-593
DLLFKDLTK	653-661
EFLGDKFYTVISSLK	674-688
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
AVAMITFAQYLQR	48-60
LLINLIK	556-562
VITPSFVDIFISK	219-231
NAPYSGYSGAFHCLK	204-218
IQWCAVGKDEK	364-374
IRDLLER	623-629

KGTEFTVNDLQGK	119-131
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173

FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
DGKGDVAFVK	219-228
DDNKVEDIWSFLSK	275-288
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
DPVLKDLLFK	311-320
TDERPASYFAVAVAR	443-457
KDSNVNWNNLK	458-468
TGTCNFDEYFSEGCAPGSPPNSR	494-516
GDVAFIQHSTVEENTGGK	554-571
NLQMoxDDFELLCTDGR	579-593
ECNLAEVPTHAVVVRPEK	602-619
EFLGDKFYTVISSLK	674-688
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
LKPIAAEVYEHTEGSTTSYYAVAVVK	93-118
GTEFTVNDLQGK	120-131
TDERPASYFAVAVAR	443-457
APSVDIGTCR	45-54
LKPIAAEVYEHTEGSTTSYYAVAVVK	93-118
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
VEDIWSFLSK	279-288
KDQLTPSPR	352-360
TDERPASYFAVAVAR	443-457
ECNLAEVPTHAVVVRPEK	602-619
LKPIAAEVYEHTEGSTTSYYAVAVVK	93-118
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
NAPYSGYSGAFHCLK	204-218
VEDIWSFLSK	279-288
AQSDFGVDTK	289-298
SDFHLFGPPGK	299-309
TDERPASYFAVAVAR	443-457
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
YNDLKEETFK	38-47
AVAMoxITFAQYLQR	48-60
LVKDVVDLAQK	69-79
CVANEDAPECSKPLPSIILDEICQVEK	82-106
VSFLGHFIYSVAR	160-172
APFSEVSK	254-261
MMoxSNLCSQQDVFS GK	288-302
RHPEFSIQLIMoxR	365-376
KMoxPQVPTDLLLETGK	442-456

MoxPQVPTDLLLETGK	443-456
RPCFTAMGVDTK	513-524
YVPPPFNPDMoxFSFDEK	525-540
LLINLIK	556-562
TIADGFTAMVDK	574-585
QSDINTCFGEEGANLIVQSR	589-608
LVKDVVDLAQK	69-79
VSFLGHFIYSVAR	160-172
APFSEVSK	254-261
RHPEFSIQLIMR	365-376
AVAMITFAQYLQR	48-60
LVKDVVDLAQK	69-79
CVANEDAPECSKPLPSIILDEICQVEK	80-106
VSFLGHFIYSVAR	160-172
APFSEVSK	254-261
RHPEFSIQLIMoxR	365-376
KMoxPQVPTDLLLETGK	442-456
KMoxPQVPTDLLLETGKK	442-457
YVPPPFNPDMFSFDEK	525-540
LLINLIK	556-562
TIADGFTAMVDK	574-585
AVAMITFAQYLQR	48-60
LVKDVVDLAQK	69-79
CVANEDAPECSKPLPSIILDEICQVEK	80-106
VSFLGHFIYSVAR	160-172
HPFLYAPAILSFAVDFEHALQSCCK	174-198
MoxPQVPTDLLLETGK	443-456
YVPPPFNPDMoxFSFDEK	525-540
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
NAPYSGYSGAFHCLK	204-218
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
APFSEVSK	254-261
YVPPPFNPDMFSFDEK	525-540
AVAMITFAQYLQR	48-60
ESDVGACLDTK	199-209
YVPPPFNPDMFSFDEK	525-540
YNDLKEETFK	38-47
LVKDVVDLAQK	69-79
ESDVGACLDTK	199-209
RPCFTAMGVDTK	513-524
YVPPPFNPDMFSFDEK	525-540
TIADGFTAMVDK	574-585
SAGWNIPIGTLLHR	141-154
AQSDFGVDTK	289-298
NTGIICTIGPASR	43-55
IYVDDGLISLLVK	173-185

GDLGIEIPAEK	294-304
HVMIDCSPYLQVVR	89-102
DGNTMVACPR	103-112
ILSPVCGTDGFTYDNECGICAHNAEQR	179-205
CREEVPELDCSK	347-358
EEVPELDCSK	349-358
VSPICTMoxEYVPHCGSDGVTYSNR	428-450
CFFCNAYVQSNR	451-462
DGTSWVACPR	38-47
QEIPEIDCDQYPTR	218-231
CFFCNAYVQSNR	451-462
HVMIDCSPYLQVVR	89-102
DGNTMVACPR	103-112
QEIPEIDCDQYPTR	218-231
VSPICTMEYVPHCGSDGVTYSNR	428-450
CFFCNAYVQSNR	451-462
DGTSWVACPR	38-47
HVMoxIDCSPYLQVVR	89-102
SPCVASCNIPVVSGR	199-213
HVMIDCSPYLQVVR	89-102
DNLDNNIPSSLR	159-170
DGNTMVACPR	103-112
HYTIAALLSPFSYSTTAVVSDPQE	127-150
DNLDNNIPSSLR	159-170
EDGGGWWYNR	400-409
AVAMITFAQYLQR	48-60
HPLDELGVLCPTGCELQTLLK	71-92
TVKPVLR	96-102
DNLDNNIPSSLR	159-170
AWDEYKR	269-275
VSALYGGFTIHNEGNK	325-340
YQLSVSNYK	341-349
DNDGWLTTDPR	384-394
EDGGGWWYNR	400-409
YYWGGTYSWDMAK	419-431
AVAMITFAQYLQR	48-60
LLPDLESFFTHDSVSTSSR	367-385
LVKDVVDLAQK	69-79
FVHDSIGVHK	262-271
SFEAGHDAFMoxAEFVVEYSR	346-364
RHPEFSIQLIMR	365-376
KMoxPQVPTDLLLETGK	442-456
MPQVPTDLLLETGK	443-456
RPCFTAMoxGVDTK	513-524
YVPPFPNPDMMFSFDEK	525-540
FCTGLTQIETLFK	253-265
LSVEALGSLGGDLK	157-170

FCTGLTQIETLFK	253-265
GQSIDDLMOXPAQK	370-381

ELFDPVIEDR	87-96
LSVEALGSLGGDLK	157-170
FCTGLTQIETLFK	253-265
FGEVLKR	308-314
GTGGVDTAAVGGVFDVSNADR	321-341
GNPTVEVDLYTNK	16-28
AAVPSGASTGIYEALER	33-50
NVNVVEQEKIDK	81-92
LMLEMDGTENK	93-103
FGANAILGVSLAVCK	106-120
HIADLAGNPEVILPVPAFNVINGGSHAGNK	133-162
IGAENVYHNLK	184-193
DATNVGDEGGFAPNILENK	203-221
EAELELLK	222-228
VVIGMDVAASEFYR	240-253
DGKYDLDFK	254-262
DGKYDLDFKSPDDPSR	254-269
NYPVVSIEDPFDQDDWAAWK	286-305
KFTASVGIQVVGDDLTVTNPK	306-326
SCNCLLLK	336-343
VNQIGSVTESLQACK	344-358
LAQSNGWGVMSHR	359-372
SGETEDTFIADLVVGLCTGQIK	373-394
IEEELGSK	413-420
AAVPSGASTGIYEALER	33-50
FGANAILGVSLAVCK	106-120
IGAENVYHNLK	184-193
DGKYDLDFK	254-262
DGKYDLDFKSPDDPSR	254-269
LAQENGWGVMOXVSHR	359-372
FGANAILGVSLAVCK	106-120
VNQIGSVTESIQACK	344-358
SGETEDTFIADLVVGLCTGQIK	373-394

LGDVYVNDAFGTAHR	157-171
ITLPVDFITADK	280-291

EVIPTDKEEVAFK	67-79
SQGAALDKYAK	111-121
VVVVGNPANTNCLIASK	126-142
SAPSIPKENFSCLTR	143-157
LGVTSDNDVK	171-179
EVGVYEAIKDDSWLKGDFILTVQQR	206-230
GDFILTVQQR	221-230
FVEGLPINDFSR	299-310

LITPVAAGSTVPSNK	8-22
GLCDELALVDVLEDK	43-57
GEMoxMoxDLQHGSFLQTHK	60-76
IVADKDYAVTANSK	77-90
IVVVTAGVR	91-99
VIGSGCNLD TAR	158-169
QVVESAYEVIR	233-243
VHSVSTLVK	270-278
LKDDEVAQLK	308-317
LKDDEVAQLKK	308-318
SADTLWSIQK	319-328
GLCDELALVDVLEDK	43-57
VIGSGCNLD TAR	158-169
QVVESAYEVIR	233-243
DAGYEFDICFTSVQK	47-61
HYGALTGLNK	91-100
YADLTEDQLPTCESLKDTIAR	142-162
VLIAAHGNSLR	181-191
HLEGMSEEAIMoxELNLPTGIPIVYELDK	196-222
SLGELIHTLNGAK	20-32
IGVAAQNCYK	59-68
GAFTGEISPAMoxIK	72-84
DIGAAWVILGHSE	85-98
HVFGESDELIGQK	100-112
VVLAYEPVWAIGTGK	160-174
TATPQQAQEVHEK	175-187
SHVSDAVAQSTR	194-205
ELASQHDVDGFLVGGASLKPEFVDIINAK	219-247
LKPIAAEVYEHTEGSTTSYYAVAVVK	93-118
GTEFTVNDLQ GK	120-131
FFSASCVPGATIEQK	174-188
QSHGSCSFVACR	33-44
MoxAAAIGLRVVGR	1-12
KAADGTWQDFATGK	58-71
AADGTWQDFATGK	59-71
TTEFGEIHELTTTEEQFVEGVYR	72-93
VEFDTSSYWK	94-103
GLGLSPFHEYADVFTANDSGHR	104-126
DAIAQFESSAVGK	51-63
LADNLDLTSAAAAK	69-82
LTPVAQELK	142-150
LTPVAEEAR	164-172
NLAPYSDEL R	185-194
VMoxEQLSNLR	219-227
MoxTPLVQEFR	230-238
LTPYAENLK	241-249

LISFLDELQK	252-261
AADGTWQDFATGK	59-71
TTEFGEIHELTTTEEQFVEGVYR	72-93
VEFDTSSYWK	94-103
VAPTITLFPPSK	4-15
IRDMVDVYLETVK	34-46
DMVDVYLETVK	36-46
DAIAQFESSAVGK	51-63
LADNLDLTSAAAAK	69-82
LREDMAPYYK	83-92
IRPFLDQFSAK	120-130
WTEEEQYR	131-139
LTPVAQELK	142-150
LTPVAEEAR	164-172
NLAPYSDEL R	185-194
GIPQASEYQAK	208-218
VMEQLSNLR	219-227
MTPLVQEFR	230-238
MTPLVQEFRER	230-240
LISFLDELQK	252-261
VAPTITLFPPSK	4-15
VAPTITLFPPSKEELNEATK	4-23
LEEQAKAK	400-407
SFWQHDEPQTPLDR	20-33
IRDMVDVYLETVK	34-46
DMVDVYLETVK	36-46
DAIAQFESSAVGK	51-63
LADNLDLTSAAAAK	69-82
IRPFLDQFSAK	120-130
WTEEEQYR	131-139
LTPVAQELK	142-150
LTPVAEEAR	164-172
NLAPYSDEL R	185-194
GIPQASEYQAK	208-218
VMEQLSNLR	219-227
MTPLVQEFR	230-238
LISFLDELQK	252-261
VAPTITLFPPSK	4-15
VAPTITLFPPSKEELNEATK	4-23
VAPTITLFPPSK	4-15
ATLVCLINDFYSPVTVDWVIDGSTR	24-49
KDPEGLFLQDNVVAQFTVDENGQMoxSATAK	51-79
YWGVASFQK	111-120
GNDDHWVVDTDYDTYALHYSCR	121-142
NLAPYSDEL R	185-194
GIPQASEYQAK	208-218
LISFLDELQK	252-261
KAADGTWQDFATGK	58-71

AADGTWQDFATGK	59-71
TTEFGEIHELTTTEEQFVEGVYR	72-93
VEFDTSSYWK	94-103
GLGLSPFHEYADVFTANDSGHR	104-126
HYTIAALLSPFSYSTTAVVSDPQE	127-150
YNDLKEETFk	38-47
AVAMITFAQYLQR	48-60
LVKDVVDLAQK	69-79
CVANEDAPECSKPLPSIILDEICQVEK	80-106
VSFLGHFIYSVAR	160-172
APFSEVSK	254-261
YIEDKEVCK	337-345
SFEAGHDAFMAEFVYEYSR	346-364
RHPEFSIQLIMR	365-376
MPQVPTDLLLETGK	443-456
MACSEGYSIVIHDTCR	474-490
YVPPFPNPDMSFDEK	525-540
TIADGFTAMVDK	574-585
QSDINTCFGEEGANLIVQSR	589-608
SVGNLADFEAEK	4-16
KVQEFSGANK	85-94
LADSHNFDEYMK	11-22
ALGVGFAMR	23-31
QVGNVTKPTVIISSEGDK	32-49
QVGNVTKPTVIISSEGDKVVIR	32-53
LGEEFDETPDDR	67-79
SVVTLGDGK	83-91
SVVTLGDGKLVHVQK	83-97
MVMTLTFGDVVAVR	114-127
LGEEFDETPDDR	67-79
MVMTLTFGDVVAVR	114-127
LLGAPVPVDENDEGLQR	30-46
ALGVGFAMR	23-31
LLGAPVPVDENDEGLQR	30-46
ALQFAMAENR	47-57
RQLVSGIK	75-82
YILQVEIGR	83-91
SSGDLQSCEFHDPEMAK	97-114
YTTCTFVVYSIPWLNQIK	115-132
LLGAPVPVDENDEGLQR	30-46
ALQFAMAENR	47-57
QLVSGIKYILQVEIGR	76-91
YILQVEIGR	83-91
AASHQEEFGAEALTR	17-31
GTEFTVNDLQGK	120-131
SAGWNIPIGTLLHR	141-154
GAIEWEGIESGSVEQAVAK	155-173
FFSASCVPGATIEQK	174-188
VEDIWSFLSK	279-288

DQLTPSPR	353-360
FYTVISSLK	680-688
TCNPSDILQMCSFLEGK	689-705
HGLDNYR	33-39
GYS LGN WVCAAK	40-51
FESNFNTQATNR	52-63
NTDGSTDY GILQINSR	64-79
NLCNIPCSALLSSDITASVNCAK	92-114
CSTIIDAAFGLK	287-299
SIPPACDK	1-8
SIPPACDKYSRLPGCPR	1-17
DYSPVCGTDGK	18-28
TYPNECVLCLSNSEENK	29-45
TYPNECVLCLSNSEENKNVQIYK	29-51
SVTGGMoxCSVYLK	998-1009
TYPNECVLCLSNSEENKNVQIYK	29-51
TDERPASYFAVAVAR	443-457
APSVDIGTCR	45-54
AGACPPTFTISGQCHGLLNCCAK	41-64

Supplementary table 2A. Differentially expressed proteins in the seminal fluid of a Red Junglefowl (RJF) compared with White Leghorn (WL) male chicken was identified by 2D SDS-PAGE image comparison followed by mass spectrometry analysis.

Spot no.	Proteins identified	Fold change	Average normalized volumes	
			RJF	WL
108	Serum albumin	2.0	1.153e+007	5.707e+006
109	Serum albumin	1.9	1.301e+008	6.815e+007
122	Ovotransferrin	1.9	4.099e+007	7.758e+007
123	Ovotransferrin, Serum albumin	2.1	1.939e+007	4.097e+007
124	Serum albumin	2.4	2.460e+007	5.790e+007
125	Serum albumin	3.2	1.331e+007	4.223e+007
126	Serum albumin	4.3	2.379e+006	1.033e+007
129	Ovoinhibitor	1.9	1.277e+007	2.452e+007
131	Creatinine kinase B type	2.6	1.938e+006	5.076e+006
132	A-enolase, β -enolase	5.0	2.118e+006	1.060e+007
139	Malate dehydrogenase	1.7	2.752e+007	1.604e+007
140	L-lactate dehydrogenase B chain	5.3	7.507e+006	3.941e+007
141	Glyceraldehyde-3-phosphate dehydrogenase, Astacin-like metalloendopeptidase	3.0	5.260e+007	1.731e+007
145	Triosephosphate isomerase	9.1	1.637e+006	1.493e+007
146	Retinol-binding protein 4	3.4	8.781e+006	2.987e+007
147	Apolipoprotein A-I	3.8	1.032e+006	3.972e+006
152	Cystatin	2.7	3.704e+007	1.397e+007
157	Gallinacin-9	1.9	2.800e+008	1.508e+008

Supplementary table 2B. Differentially expressed proteins in the seminal fluid of Red Junglefowl (RJF) compared with an Advanced Intercross Line (AIL) male chicken was identified by 2D SDS-PAGE image comparison followed by mass spectrometry analysis.

Spot no.	Proteins identified	Fold change	Average normalized volumes	
			RJF	AIL
162	Protein NEL	2.3	2.15e+006	9.42e+005
172	Tubulin beta- 1 chain	2.0	7.50e+005	1.50e+006
192	Ovotransferrin	4.0	1.73e+005	6.95e+005
193	Ovotransferrin	3.6	7.12e+005	2.56e+006
214	Serum albumin	3.2	6.99e+005	2.26e+006
230	Phosphoglycerate mutase 1	2.1	4.19e+005	8.84e+005
238	Transthyretin	2.6	1.99e+006	7.68e+005
243	Ig lambda chain C region	2.0	3.49e+006	1.71e+006
18	Ovotransferrin	2.9	8.87e+005	3.11e+005
		2.6	7.10e+005	2.75e+005
		3.4	2.52e+006	7.42e+005
62	Argininosuccinate synthase	3.1	1.22e+006	4.00e+005
85	Transthyretin	2.9	1.59e+006	5.53e+005
89	Serum albumin	2.1	5.87e+005	2.78e+005