# Differential expression of proteins in renal cortex and medulla: A proteomic approach<sup>1</sup>

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#### Differential expression of proteins in renal cortex and medulla: A proteomic approach.

*Background.* Western blotting has previously been used to identify changes in protein expression in renal tissue. However, only a few proteins can be studied in each experiment by Western blot. We have used proteomic tools to construct protein maps of rat kidney cortex and medulla.

*Methods.* Expression of proteins was determined by silver stain after two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Protein spots were excised and digested with trypsin. Peptide masses were identified by MALDI-TOF mass spectrometry. The Mascot search engine was used to analyze the peptide masses and identify the proteins.

*Results.* Seventy-two proteins were identified (54 unique proteins) out of approximately 1000 spots visualized on each gel. Most of the spots were expressed both in cortex and medulla. Of the identified proteins, three were expressed only in medulla and one only in cortex. Nine proteins were expressed in both regions but to a greater extent in cortex and three proteins were expressed more in medulla. Differential expression was confirmed for three proteins by Western blot.

*Conclusions.* A large group of proteins and their relative expression levels from cortical and medullary portions of rat kidneys were found. Sixteen proteins are differentially expressed. Proteomics can be used to identify differential expression of proteins in the kidney on a large scale. Proteomics should be useful to detect changes in renal protein expression in response to a large range of physiological and pathophysiological stimuli.

Renal cortex and medulla serve different functions. Structures in the cortex filter fluid and electrolytes from proteins and formed elements, reabsorb glucose, amino acids, water and electrolytes, and produce hormones that

<sup>1</sup>See Editorial by Bonventre, p. 1470.

Received for publication August 15, 2001 and in revised form March 14, 2002 Accepted for publication May 28, 2002

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modulate blood pressure, hematopoiesis and calcium homeostasis. The medulla is largely responsible for concentrating urine and as a consequence medullary cells must survive in an environment where the osmolality is much higher than the rest of the body. Since specific functions of cortex and medulla are mediated by proteins, it would be expected that protein expression would differ between cortex and medulla. Techniques such as Western blotting are able to identify differences in protein expression, but these techniques are limited to identification of a single protein at a time and are only available for proteins for which antibodies exist. Recently, methods have become available that allow comparison of expression of many proteins simultaneously. In 1975 O'Farrell developed a technique for resolution of proteins using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) [1]. Proteins are separated using isoelectric focusing in the first dimension and polyacrylamide gel electrophoresis in the second dimension, resulting in a unique spot for each protein that differs in molecular size and/or pI. Using this technique O'Farrell was able to resolve 1100 different components from Escherichia coli. More recently, technical modifications including large format gels, complex ampholyte sets, use of ready-made gel solutions and improvements in staining have resulted in resolution of as many as 10,000 polypeptide spots [2]. The analysis of separated proteins by mass spectrometry has permitted analysis of proteins on a "genomic" scale [3]. Analysis of proteins on a genomic scale has acquired the name "proteomics." The most common means of proteomic analysis uses high-resolution 2D-PAGE, peptide mass fingerprinting and bioinformatics to identify proteins in a high-throughput fashion. Peptide mass fingerprinting is done by excising specific protein spots from the gel, digesting them with trypsin and measuring the sizes of the peptide fragments by mass spectrometry. The group of peptide sizes obtained from a specific protein is then compared to the predicted fragment sizes that would be

**Key words:** proteomics, kidney, cortex, medulla, peptide mass fingerprinting, two-dimensional gel electrophoresis.

obtained by cleavage of all proteins in a large database. A match between actual peptide fragments obtained from digestion of the protein spot and the expected fragments obtained from a specific database allows a prediction that the proteins are the same [4]. Databases of proteins expressed in specific tissues have been established and published. Fifty protein spots have been identified from rat liver [5]. Many of these proteins were identified from several different spots. For instance six spots were identified as peroxisomal bifunctional enzyme, so the number of unique proteins identified is considerably smaller. Proteomic analysis has been used to identify eighteen proteins that were previously not shown to be present in that species [6]. Relatively large databases of protein spots have been identified from yeast (401 spots representing 279 different proteins) [7]. Protein databases for cerebrospinal fluid, heart, kidney and multiple cell lines are available on the Internet at the Expert protein analysis system (ExPASy) site www.expasy.ch that is maintained by the Swiss Institute of Bioinformatics [8]. Limited databases of proteins expressed in renal tissue have been compiled [9]. Once proteins are identified in a specific tissue, a map can be generated that links individual spots with the protein identification of that spot. As these maps are developed, they can be used to compare changes in protein expression within that tissue for multiple interventions. We have prepared protein from rat renal cortex and medulla and analyzed a number of proteins by two-dimensional gel electrophoresis, excision and tryptic digestion of the spots and peptide mass fingerprinting using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. A map of renal protein expression in the cortex and medulla was generated. Once the protein spots were identified, we determined protein quantity based on spot intensity in a series of gels from cortex and medulla. Relative expression of all proteins identified was then calculated for cortex and

#### **METHODS**

#### Rat kidney protein preparation

kidney regions were determined.

All studies using rats have been approved by the University of Louisville Institutional Animal Care and Use Committee. Sprague-Dawley rats (150 to 200 g) were fed standard rat chow obtained from PMI Nutrition International (Richmond, IN, USA). Rats were sacrificed using a  $CO_2$  chamber. The kidney was dissected and the capsule removed. The cortex was separated from the medulla and cut into several pieces and washed in icecold saline. The pieces of renal tissue were frozen in liquid nitrogen and ground to a powder using a chilled mortar and pestle. Tissue was resuspended in a buffer containing 50 mmol/L Tris, 0.3% sodium dodecyl sulfate

medulla, and statistically significant differences between

(SDS) and 200 mmol/L dithiothreitol (DTT), incubated at 100°C for five minutes and transferred to ice. One tenth volume of a buffer containing 500 mmol/L Tris, 50 mmol/L MgCl<sub>2</sub>, 1 mg/mL DNAse I and 0.25 mg/mL RNAse A was added and incubated for an additional 10 minutes. The 12,000  $\times$  g supernatant was obtained, trichloroacetic acid (TCA) added to 10% and the 12,000  $\times$ g pellet obtained. After several washes with acetone, the pellet was suspended in a sample buffer containing 40 mmol/L Tris, 7.92 mol/L urea, 0.06% SDS, 1.76% ampholytes, 120 mmol/L DTT and 3.2% Triton X-100.

#### **Two-dimensional gel electrophoresis**

Proteins obtained from rat kidney cortex (75 µg/sample) were separated by two-dimensional gel electrophoresis using a two-dimensional electrophoresis system (Genomic Solutions, Ann Arbor, MI, USA). Proteins were separated by isoelectric focusing over a pH range of 3 to 10 using precast first dimension gels for 17.5 hours at 110  $\mu$ A/gel. The first dimension gel was then loaded on a large format  $(22 \times 22 \text{ cm})$  second-dimension slab gel to separate proteins by molecular weight. After electrophoresis, proteins were silver stained using a modified Morrissey stain without glutaraldehyde according to the manufacturer's specifications. For identification of proteins using peptide mass fingerprinting, gels were stained with European Molecular Biology Laboratory (EMBL) silver stain [10] or Brilliant Blue G-colloidal concentrate (Sigma Chemical Co. St. Louis, MO, USA) according to the manufacturer's specifications, except that gels were fixed for one hour in a solution of 7% glacial acetic acid in 40% methanol.

#### **In-gel tryptic digestion**

Samples were prepared using a modification of the technique described by Jensen et al [11]. The stained gel slabs were washed with dI water (18 mega Ohm) four to five times. Spots on the gel were excised with a clean scalpel into one mm cubes. The gel pieces were transferred to clean 0.5 mL microfuge tubes. Ammonium bicarbonate (0.1 mol/L) was added to the gel pieces to twice the volume of the gel and incubated at room temperature for 15 minutes. Acetonitrile was added to the gel pieces and incubated at room temperature for 15 minutes. The solvent was removed and the gel pieces were dried using a vacuum centrifuge. The gel pieces were rehydrated with 20 µL of 20 mmol/L DTT in 0.1 mol/L NH<sub>4</sub>HCO<sub>3</sub> and incubated at 56°C for 45 minutes to reduce the protein. The tubes were chilled at room temperature and the DTT solution was removed and replaced with 20 µL of 55 mmol/L iodoacetamide in 0.1 mol/L NH<sub>4</sub>HCO<sub>3</sub> and incubated at room temperature in the dark for 30 minutes. The iodoacetamide was removed and replaced with 0.2 mL of 50 mmol/L NH<sub>4</sub>HCO<sub>3</sub> and incubated at room temperature for 15 minutes. Acetonitrile (0.2 mL) was added and the sample was incubated at room temperature for 15 minutes. The solvent was removed and the gel pieces were dried with a vacuum centrifuge. The gel pieces were rehydrated with 5  $\mu$ L of 20 ng/ $\mu$ L modified trypsin (Promega, Madison, WI, USA) in 50 mmol/L NH<sub>4</sub>HCO<sub>3</sub>. After the gel pieces were rehydrated, 50 mmol/L NH<sub>4</sub>HCO<sub>3</sub> was added to cover the gel pieces and they were incubated overnight at 37°C.

# Sample preparation for matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry

A nitrocellulose solution was made by dissolving a nitrocellulose membrane in 1:1 acetone/isopropanol solvent. Alpha-cyano-4-hydroxycinnamic acid ( $\alpha$ -CN) was washed with 50 µL of acetone and acetone phase was discarded. The  $\alpha$ -CN was dissolved in acetone to a concentration of 10 mg/mL, and the nitrocellulose and  $\alpha$ -CN solutions were mixed to 1:4 ratio; 1 µL of this mixture was deposited onto the 96-well MALDI target plate. The sample was prepared for addition to the plate by adding  $2 \,\mu L$  of sample to  $2 \,\mu L$  of a solution of acetone washed  $\alpha$ -CN dissolved in 0.1% trifluoroacetic acid and added to a 1:1 H<sub>2</sub>O/acetonitrile to a final concentration of 10 mg/mL  $\alpha$ -CN. One microliter of the sample mixture was loaded onto each thin film. After the sample mixture was dried, 1.5 µL of 2% formic acid in dI water was added to each spot. The formic solution was removed by gentle blotting. This washing step was performed twice. The samples were then dried at room temperature. Fragment size was determined by MALDI-TOF mass spectrometry.

## **MALDI-TOF mass spectrometry**

Mass spectral data were obtained using a Micromass Tof-Spec 2E instrument equipped with a 337 nm  $N_2$  laser at 20-35% power in the positive ion reflectron mode. Spectral data were obtained by averaging 10 spectra each of which was the composite of 10 laser firings. The mass axis was calibrated using known peaks from tryptic autolysis.

# Analysis of peptide sequences

Protein identification from tryptic fragment sizes was made using the Mascot search engine (*www.matrixscience. com*) based on the entire NCBI protein database using the assumption that peptides are monoisotopic, oxidized at methionine residues and carboxamidomethylated at cysteine residues. Up to one missed trypsin cleavage was allowed, although most matches did not contain any missed cleavages. A mass accuracy tolerance of a maximum of 100 ppm was used for matching tryptic peptides. Probability based Mowse score defined as  $-10*\log$ (P), where P was the probability that the observed match was a random event were determined [12]. Scores greater than 70 were considered significant (P < 0.05). All protein identifications were in the expected size range based on position in the gel.

# Identification of proteins with changes in quantity

Silver stained gels were scanned using a high-resolution 12-bit camera. Scanned gel files were analyzed using Bioimage 2-D gel software. A composite gel was formed from five cortical and five medullary gels. Bioimage software was initially used to match the same spots together from each tissue according to the manufacturer's documentation. This software uses easily identified protein spots or "anchors" to serve as a starting point for spot matching and then matches additional spots based on pattern recognition. In addition, the accuracy of the protein spot matching was determined manually for each spot on each gel. Protein intensity was compared for each protein identified from cortex and medulla. The integrated intensity of each spot was determined from each of 10 gels obtained from 10 different rats. Mean and SEM was calculated and a two-tailed, non-paired t test was done on each to determine differences.

# Western blot

Proteins were prepared from four rat kidneys using the same protocol and buffers as those described for 2-D gel electrophoresis. Western blotting was done as previously described using polyclonal antibodies against GRP-78 (1:2000; Accurate Chemical, Westbury, NY, USA) and retinol binding protein (1:1000; Cortex Biochem, San Leandro, CA, USA) or a monoclonal antibody against fatty acid binding Protein (1:1000; Chemicon, Temecula, CA, USA).

# RESULTS

Two-dimensional gel electrophoresis was performed on proteins extracted from cortical and medullary tissue derived from Sprague-Dawley rat kidney. Digital images of stained spots were obtained with a CCD camera and individual images were matched together to form composite images of renal cortex and medulla. Each spot on the composite represented the appropriate spot within each individual gel. The initial matching process was done by the Bioimage software program. Accuracy of the matches was confirmed by manually comparing each gel image to the composite and to other individual gels. Composites of silver stained gels were generated from both regions, and 1095 spots were visualized by spot picking software in the cortex and 885 in the medulla (Fig. 1). Protein spots were picked, digested with trypsin and identified by peptide mass fingerprinting. A typical mass spectrum from the protein calbindin is shown in Figure 2. The peptide masses determined by mass spectrometry were used to identify the protein spot. In many cases additional peptides were seen after digestion that



Fig. 1. Composite images of two-dimensional gels from renal cortex (A) and medulla (B). A total of 1095 spots were visualized in the cortex and 885 in the medulla by spot picking software. Numbers represent proteins that were identified by tryptic digestion, MALDI-TOF mass spectrometry and peptide mass fingerprinting. The numbers correspond to the peptides listed in Table 1.

did not correspond to the identified protein. These peptides likely represented other proteins present in the same spot, but we were not able to identify these other proteins with confidence. From the 180 spots picked, 72 spots representing 54 unique proteins were identified (Table 1 and Fig. 1). Predicted pI and molecular size were determined for each identified protein and compared to the pI and molecular size predicted from the location of the spot on the gel. Predicted and actual pI and size were nearly identical for all spots. Several horizontal rows of spots were seen on the gels. Within these rows analysis of multiple spots identified the same protein. These rows of proteins spots, which typically had similar molecular sizes but slightly different isoelectric points, probably were due to posttranslational modifications which cause changes in the protein charge but little

change in the protein mass as seen on the gel. The protein identities from all spots were entered in the composite gel database so that changes in protein expression could be determined for each protein spot. Integrated protein intensity was determined for each identified spot in ten gels each from cortex and medulla. Mean intensity of each spot was calculated by silver stain intensity and compared between cortex and medulla. Protein concentration within each region was calculated by silver stain intensity and relative intensities between cortex and medulla were compared. The average coefficient of variation for multiple replicates of a single spot was relatively large (65%). Table 2 lists the proteins that were differentially expressed. Only proteins identified by peptide mass fingerprinting were analyzed for differential expression. Alpha enolase was expressed only in cortex while aflatoxin B1 aldehyde reductase, alpha B crystallin and BH3 interacting death domain were expressed only in medulla. Nine proteins were expressed to a greater extent in cortex than medulla. They were 3-mercaptopyruvate sulfurtransferase, alpha 2u globulin, aldehyde dehydrogenase, contraception associated protein 1, heat shock 60 kD protein, isocitrate dehydrogenase, NADH-ubiquinone oxidoreductase 24 kD subunit precursor, ornithine aminotransferase and retinol binding protein. Among the proteins identified only albumin, glucose-regulated protein precursor (78 kD) and fatty acid binding protein were found to have a statistically significant increase in expression in the medulla. Four adjacent spots were identified as albumin and only one of them differed between cortex and medulla, probably representing a difference in posttranslational modification between cortex and medulla.

In order to confirm the differences in expression found

tide mass fingerprinting of a gel spot. After separation of renal cortical tissue by twodimensional gel electrophoresis, the gel spot was excised and digested with trypsin. Peptides obtained from the digestion were analyzed by MALDI-TOF mass spectrometry. The abscissa represents the mass/charge ratio of ions detected in the MALDI-TOF experiment. Peaks are almost always for the protonated molecular ion  $[M + H^+]$ . The ordinate represents the relative intensity of the averaged ion current in arbitrary units.

Fig. 2. Typical spectrum obtained from pep-

by the proteomics approach, we performed Western blots on several proteins found to be differentially expressed. Expression as determined by densitometry was found to be qualitatively similar to that determined using twodimensional gels. Figure 3 shows the expression of alpha-B crystallin, GRP78 and fatty acid binding protein by Western blot.

#### DISCUSSION

We have created a map of renal protein expression consisting of a 2-dimensional representation of renal protein expression based on pI and molecular size. This map permits consistent identification of a protein following multiple interventions since the protein location can be reproducibly identified on a two-dimensional gel. We have visualized about 1000 protein spots and identified the protein by peptide mass fingerprinting from 72. Using this technique to look at protein expression will allow protein expression to be determined for multiple proteins simultaneously instead of the small number possible with Western blotting. This technique is useful in hypothesis testing as well as for generating new hypotheses and screening for proteins involved in a specific physiological pathway.

While two-dimensional gels provide high resolution separation, they have a number of shortcomings. It is difficult to identify proteins of certain types. In particular, proteins of low abundance, membrane proteins, proteins at the extremes of molecular size and basic proteins have been difficult to identify using classical techniques. Fortunately, refinements in technique have improved the ability to identify some of these difficult proteins by two-dimensional gel analysis. Our current study found



Table	1.	Differentially	expressed	proteins

1         3-hydroxyntrauliae 3.4-dioxygenase         98/91/0250         NP (0+64).           2         3-meczep/opwartae sulfurmaterise         98/31/2930         P 7532           3         Alpha 2u globulin         98/31/381         P 3532           4         Acidae mades phosphoprotein 32         98/731/381         P 3532           7         Alphasenolsas         98/732         P 3532           8         Ahminin         98/13/581         P 1267/141         AAK01310           8         Ahminin         98/13/581         P 12700           8         Ahminin         91/13/581         P 102701           11         Albumin         91/13/581         P 102701           12         Aldehyde dehydrogenase         91/13/581         P 02701           13         Aldehyde dehydrogenase         91/13/581         P 02701           14         Apha B 2ryallain         NP 05520         AAA 4723           15         Aldehyde dehydrogenase         91/13/581         P 02701           16         Apha B 2ryallain         NP 05520         AAA 4723           17         Alpha - 1401         92/23/315         C 23/435           18         Apolipoprotein A-1         92/23/315         C 23/435	Spot no.	Proteins	GenInfo identifier	Accession no.
2         3-incregiopyravate sulfurtaniferase         µ1312290         P 7532           3         Apha 2a jobuin         µ204261         A A A1108           4         Acida nuclex phosphortein 32         µ173318         P 3067           6         Aphase modes         µ173818         P 3067           7         Allatoxin B1 aldehyde reductase         µ173819         P 3918           8         Albumin         µ111380         P 02770           9         Albumin         µ111380         P 02770           10         Albumin         µ11380         P 02770           12         Aldehyde reductase 1         µ11380         P 02770           13         Aldehyde reductase 1         µ10789         P 02770           14         Apha 1.anitrypin preumor         µ103633         AA 40788           15         Apha-1.anitrypin preumor         µ102433         AA 40788           16         Apha-1.anitrypin preumor         µ102433         AA 40788           17         Apha-1.anitrypin preumor         µ102435         24305           18         Apoliopyrotein A-1         µ102435         24305           21         Actin, het         µ1024365         24305           22 <td< td=""><td>1</td><td>3-hydroxyantranilate 3,4-dioxygenase</td><td>gi 9910256</td><td>NP 064461</td></td<>	1	3-hydroxyantranilate 3,4-dioxygenase	gi 9910256	NP 064461
3       Apha 2x globulin       pi2124251       A AA 41798         5       Apha-renuise phosphoprotein 32       pi730118       P 39687         5       Apha-renuise phosphoprotein 32       pi730118       P 39687         7       Aflarxins Bi aldehyde reductise       pi720118       P 39618         8       Albumin       pi113580       P 102770         9       Albumin       pi113580       P 102770         10       Albumin       pi113580       P 102770         11       Albumin       pi113580       P 102770         12       Albumin       pi113580       P 102770         13       Albumin       pi113580       P 102770         14       Alpha 8-rystalin       pi113580       P 102770         15       Alpha-4-nitryspin precursor       pi2189053       AAA 44788         16       Alpha-4-nitryspin precursor       pi2189053       AAA 44788         17       Alpha-4-nitryspin precursor       pi2189053       AAA 44788         18       Archin, heta       pi224055       224055         19       Aphiproprotein A-V       pi7778       LFRTA4         20       Actin, heta       pi224055       224045         21       A	2	3-mercaptopyruvate sulfurtransferase	gi 3122930	P 97532
4         Acidic nuclear phosphorptoin 32         pi73038         P 30887           5         Alpha-enolase         pi12067141         AAK0139           6         Alpha-enolase         pi12067141         AAK0139           7         Albumin         pi12067141         AAK0139           9         Albumin         pi113580         P 02710           9         Albumin         pi113580         P 02770           10         Albumin         pi113580         P 02770           11         Albumin         pi113580         P 02770           12         Aklohyde dehydrogenase         pi11478447         XP07526           13         Albumin         pi11380         P 02770           14         Albuhydrogenase         pi1078038         AAA 40788           15         Alpha-abutitryskip precursor         gi103963         AAA 40723           16         Alpha-abutitryskip precursor         gi103963         AAA 40723           17         Alpha-abutitryskip precursor         gi103963         AAA 40723           18         Apolipoprotein A-I         gi103963         AAA 40723           17         Alpha-institryskip precursor         gi113450         224305           18 <td< td=""><td>3</td><td>Alpha 2u globulin</td><td>gi 204261</td><td>AAA 41198</td></td<>	3	Alpha 2u globulin	gi 204261	AAA 41198
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11         Audum         []]         []]         []]         []]         []]         []]]<	10	Albumin	gi 113580	P 02770
14         Analysis         Biology (1)         NP (06:0)           14         Alpha Crystallin         Biology (2)         CA 33095           15         Alpha - I-antitrypsin precursor         Biology (2)         CA 33095           15         Alpha - I-antitrypsin precursor         Biology (2)         CA 34075           16         Alpha - I-matroglobulin         Biology (2)         CA A40788           17         Alpha - I-matroglobulin         Biology (2)         CA A40788           18         Apoliopyrotein A-I         Biology (2)         CA 3405         C2 34365           21         Actin, beta         Biology (2)         C2 34365         C2 34365           22         Bibl Sinteracting domain death agonist         Biology (2)         NP 01570           23         Bibl Sinteracting domain death agonist         Biology (2)         A5113           24         Tubulin, beta         Biology (2)         A5213           25         Tubulin, beta         Biology (2)         A5213           26         Ca-independent phospholipase A2         Biology (2)         A6323           27         Calbindin - dannel protein 1         Biology (2)         A63232           28         Calcrictiulin         Biology (2)         A740930	11	Aldahuda dahudroganasa	gi 113380 cil14785467	P 02770 VP007526
14         Appla B. crystalin         pill 112         CAC 3309           15         Appla Iantitrysin precursor         pil20363         AAA 40788           16         Appla Iantitrysin precursor         pil20287         AAA 40788           17         Appla Iantitrysin precursor         pil20287         AAA 40728           18         Apolipoprotein A-I         pil214517         AAB5840           20         Actin, heta         gil224305         224405           21         Actin, heta         gil224305         224405           22         Artin, heta         gil224305         224405           22         Artin, heta         gil224305         224405           22         Artin, heta         gil224305         224405           23         Thybin beta         gil205907291         OS3244           24         Chindependent phosphilpase A2         gil20502791         OS3244           25         Thybin beta         gil205683         AAA 7220           26         Choride intracellular channel protein 1         gil6680782         Qil2055           27         Cabindin-Gabe         gil58453         AAA 242121           28         Contraception associated protein 10 (IISC71)         gil180807	12	Aldehyde reductase 1	gi[14783407 gi[6978491	NP 036630
15         Alpha-1-antitrysin procursor         #2124983         AAA 40788           16         Alpha-1-matroglobulin         #21245147         AAA 40788           17         Alpha-1-matroglobulin         #21245147         AAA 40788           19         Apolipoprotein A-1         #2124517         AAA 40788           20         Actin, beta         #2124305         224405           21         Actin, beta         #2124305         224405           22         Hin, beta         #2124807         224405           23         Hin, beta         #2124807         224405           24         Tubulin, beta         #1029900         A25113           25         Tubulin, beta         #1099390         A25113           26         Calindependent phospholipase A2         #1099390         A25113           27         Calindin-128K         #10442         #10442           28         Calcriculur         #107559         #1A44733           30         Contraception associated protein 1         #10555394         #AAA 40798           313         Supperoxide dismutase         #2121954         #A44973           32         Optochrome 5         #1554593         #AAA 40798           33	13	Alpha B crystallin	gil13162243	CAC 33095
In         Alphal-l-mittrypsin precursor         g1220383         A.A.A. 40723           17         Alphal-l-mittrypsin precursor         g1202837         A.A.A. 40723           18         Apolipoprotein A-I         g1214317         A.AB5840           20         Actin, beta         g1224305         224305           21         Actin, beta         g1224305         224405           22         Actin, beta         g1224305         224405           22         Actin, beta         g102500         Actin           23         BH3 interacting domain death agonist         g106030782         NP 01570           24         Calindendent phospholipase A2         g105902791         OS5244           25         Calindendent phospholipase A2         g105902791         OS5244           26         Calindendent phospholipase A2         g10742894         JE 0544           27         Calindendent phospholipase A2         g10742894         JE 0544           28         Calindendent phospholipase A2         g10742894         JE 0544           29         Choirde intracellular channel protein 1         g166853283         OS2104           21         Calindendent phospholipase A2         g10742894         JE 0544           22 <td< td=""><td>15</td><td>Alpha-1-antitrypsin precursor</td><td>gi 203063</td><td>AAA 40788</td></td<>	15	Alpha-1-antitrypsin precursor	gi 203063	AAA 40788
17       Alpha-1-macróglobalin       pil 2028877       AAA 40723         18       Apolipoprotein A-IV       pil 2145147       AABS430         19       Apolipoprotein A-IV       pil 2145147       AABS430         20       Actin, beta       pil 224305       224305         21       Actin, beta       pil 224305       224305         22       Actin, beta       pil 224305       224305         23       BH3 interacting domain death agonist       pil 202930       A25113         24       Tubulin, beta       pil 202930       A25113         25       Tubulin, beta       pil 202930       A25113         26       Calriciculin       pil 20394       E 0344         27       Calriciculin       pil 203954       E 0342         28       Calriciculin       pil 203954       E 0344         29       Chloride intracellular channel protein 1       pil 203954       E 0344         21       Dismutase       pil 213954       F 044         22       Dismutase       pil 213958       AA 21205         23       Dismutase       pil 213955       AA 40996         24       Dismutase       pil 213930       C AA 2121         25       H	16	Alpha-1-antitrypsin precursor	gi 203063	AAA 40788
18       Apolipoprotein A-1       pi2143147       AABSM30         19       Apolipoprotein A-V       pi214305       L24305         20       Actin, beta       pi224305       L24305         21       Actin, beta       pi224305       L24305         22       Actin, beta       pi224305       L24305         23       BH3 interacting domain death agonist       gi09230       A25113         24       Tubulin, beta       gi09230       A25113         25       Tubulin, beta       gi09230       A25131         26       Cal-independent phospholipase A2       gi09230       A25131         27       Calinind-a28K       gi173532       P18418         28       Caloriani-a28K       gi173532       P18418         29       Chloride intracellular channel protein 1       gi173532       P18418         31       Cynchromon Sociated protein 1       gi153539       AAA 742020         32       Dismutase       gi03119726       CA-56581         33       Superoxide dismutase       gi03119726       L56581         34       Dismutase       gi1119804       P1643         35       Heat shock 70 kD protein 10 (HSC71)       gi1339212       P46763	17	Alpha-1-macroglobulin	gi 202857	AAA 40723
19         Apolipoprotein A-IV         pil/1798         LPRTA4           20         Actin, beta         pil/24305         224305           21         Actin, beta         pil/24305         224305           22         Actin, beta         pil/24305         224305           23         BH5 interacting domain death agonist         pil/24305         224305           24         Tubulin, beta         pil/2530         A25113           25         Tubulin, beta         pil/2530         A25113           26         Calendep and pospholipase A2         pil/2530         A25142           27         Calendep and pospholipase A2         pil/2530         AA7           28         Calendep and pospholipase A2         pil/25308         O2442           29         Chorick intracellular channel protein 1         pil/25308         O2442           30         Contraception associated protein 1         pil/254539         AAA 79206           31         Cytochrome b5         pil/254539         AAA 40996           32         Dismutase         pil/254539         CAA 29/21           33         Superoxide dismutase         pil/23302         CAA 29/21           34         Dismutase         pil/43302         CAA 29/21 </td <td>18</td> <td>Apolipoprotein A-I</td> <td>gi 2145147</td> <td>AAB58430</td>	18	Apolipoprotein A-I	gi 2145147	AAB58430
20         Actin, beta         gil22405         224305           21         Actin, beta         gil22405         224305           22         Actin, beta         gil22405         224305           23         BH3 interacting domain death agonist         gil6680782         NP 03157           24         Tubulin, beta         gil22930         A25113           25         Tubulin, beta         gil22930         A2513           26         Caindependent phospholipase A2         gil2119348         154042           27         Cabindin-d28K         gil17058         P.02105           20         Corotick inten abociated protein 1         gil170583         P.02105           21         Dimutase         gil81802         CAA 29121           23         Dimutase         gil81802         CAA 29121           24         Dimutase         gil81802         CAA 29121           25         Heat shock 70 kD protein 10 (HSC71)         gil7107863         AAA 7420           26         DNAK type molecular chaperone         gil119761         156581           37         Vimentin         gil10802         CAA 29121           36         DNAK type molecular chaperone         gil210976         156581	19	Apolipoprotein A-IV	gi 71798	LPRTA4
21       Actin, beta       gil22405       224305         22       Actin, beta       gil22405       224305         23       BH3 interacting domain death agonist       gil92930       A25113         24       Tubulin, beta       gil92930       A25113         25       Tubulin, beta       gil92930       A25113         26       Cainedpendent phospholipase A2       gil5902791       O35244         27       Calbindin-425K       gil117505       P 18418         28       Caireicubin associated protein 1       gil6485324       O92105         30       Contraception associated protein 1       gil7429394       JE 0344         31       Cytochrome b5       gil578077       CAA 24121         33       Superoxide dismutase       gil7429394       JE 0344         34       Dismutase       gil7429374       JE 0344         35       Homatose       gil72077       NP 065832         36       Dotextine 04 D protein 10 (HSC71)       gil720870       CAA 24121         37       Vimentin       gil720877       NP 065881         38       Fatty acid binding protein       gil720870       CA 440121         39       Ferritin light chain       gil2119695       L 5477	20	Actin, beta	gi 224305	224305
22         Actin, beta         gil23405         224305           23         BH3 interacting domain death agonist         gil6680782         NP 03157           24         Tubulin, beta         gil92930         A25113           25         Tubulin, beta         gil92930         A25113           26         Ca-independent phospholipase A2         gil719348         I 54042           28         Calbrindin-d28K         gil7197553         P 18418           29         Chloride intracellular channel protein 1         gil759537         AAA 74203           30         Contraception associated protein 1         gil759573         AAA 74204           31         Cytochrone b5         gil759573         AAA 74204           32         Dismutase         gil705675         CAA 2121           33         Superoxide dismutase         gil705877         NP 006584           34         Dismutase         gil719726         I 56581           35         Heat shock 70 kD protein 10 (HSC71)         gil732877         NP 006585           36         DNAK type molecular chaperone         gil199312         P 48670           37         Vimenti         gil7302312         P 48670           36         Farty acid binding protein	21	Actin, beta	gi 224305	224305
23         BH3 interacting domain death agonst         gift230         A25113           24         Tubulin, beta         gift2300         A25113           25         Tubulin, beta         gift2300         A25113           26         Ca-independent phospholipase A2         gift3002791         O35244           27         Calbindin-d25K         gift3002791         O35244           28         Calreticulin         gift3002791         O35244           29         Chloride intracellular channel protein 1         gift302394         JE 0344           20         Contraception associated protein 1         gift30279         ACA 7021           31         Symmitse         gift30279         CA 72121           32         Oynchrone b5         gift30287         CA 72121           33         Bymittase         gift30287         CA 72121           34         Symmitase         gift302377         PA 06583           35         Heat shock 70 kD protein 10 (HSC71)         gift30287         CA 72121           36         DNAK type molecular chaperone         gift119804         P 07483           37         Vimentin         gift19804         P 048570           38         Fatty acid binding protein         gift197040	22	Actin, beta	gi 224305	224305
24         I ubuln, beta         gib2930         A25113           25         Tubuln, beta         gib2930         A25113           26         Ca-independent phospholipase A2         gib2930         A25113           27         Calindin-d28K         gib2103948         I 54042           28         Calinticular         gib685328         O921O5           29         Chloridie intracellular channel protein 1         gib685328         O921O5           30         Contraception associated protein 1         gib7429594         JE         O44           22         Dismutase         gib7429594         JE         O44         A4 A9996           31         Cytochrome b5         gib728677         NP 006588         AAA 7420           33         Superroxide dismutase         gib729877         NP 006588         AAA 49996           34         Dismutase         gib729877         NP 006588         AAA 49996           35         Heat shock 70 kD protein 10 (HSC71)         gib729877         NP 006588         AAA 29121           36         DNAK type molecular chaptone         gib711966         1 54670         P144           40         Fraction inglace         gib8393466         NP 05901         gib76203         Q AC 64392 <td>23</td> <td>BH3 interacting domain death agonist</td> <td>g1 6680782</td> <td>NP 031570</td>	23	BH3 interacting domain death agonist	g1 6680782	NP 031570
25         I ubulin, beta         gli90230         A25113           26         Ca-independent phospholipase A2         gli502791         O35244           27         Calbindin-d28K         gli171705         P18418           28         Caltrictuulin         gli171705         P18418           29         Chloride intracellular channel protein 1         gli67685328         O927105           30         Contraception associated protein 1         gli720594         IE 0344           31         Cytochrome b5         gli818029         CAA 2121           33         Superoxide dismutase         gli818029         CAA 24212           34         Dismutase         gli1729877         NP 006588           35         Heat shock 70 kD protein 10 (HSC71)         gli1752877         NP 00658           36         DNAK type molecular chaperone         gli2119095         I 54774           37         Vimentin         gli11535212         P 48670           38         Farty acid binding protein         gli117076         I 55581           39         Ferritu light chain         gli1170038         P 46413           41         Glutamate cysteine ligase         gli8239346         NP 059001           42         Glutamitoe synthetase bet	24	Tubulin, beta	g1 92930	A25113
20         Cal-Independent prospinolipase A.2         gli21902/391         O52244           27         Calindin-d28K         gli2190348         154042           28         Calreticulin         gli668528         O921Q5           30         Contraception associated protein 1         gli6685328         O921Q5           31         Cytochrome b5         gli54559         AAA 72420           32         Dismutase         gli203658         AAA 74240           33         Superoxide dismutase         gli203658         AAA 74240           34         Dismutase         gli2119726         15651           35         Heat shock 70 kD protein 10 (HSC71)         gli352321         P 48670           36         DNAK type molecular chaperone         gli2119726         15651           37         Vimentin         gli119804         P 07483           38         Fatty acid binding protein         gli119704         P 19112           44         Glutathione synthetase         gli33922         Q 90593           45         Glutase-regulated protein precursor (78 kD)         gli4033392         Q 90593           44         Glucase-regulated protein precursor (78 kD)         gli4033320         Q 90593           45         Grue-specifie	25	Tubulin, beta	g192930	A25113
24Calmini-Lockgli11/19-461/14-4228Calreticuliagli11/5-46P144829Chloride intracellular channel protein 1gli6685323Q92/10530Contraception associated protein 1gli7429544JE 1634431Cytochrome b5gli18029CAA 7242032Dismutasegli18029CAA 2012133Superoxide dismutaegli18029CAA 2012134Dismutasegli18029CAA 2012135Heat shock 70 kD protein 10 (HSC71)gli17226877NP 00658836DNAK type molecular chaperonegli11972615558137Vimentingli183212P 4867038Fatty acid binding proteingli183212P 4867039Ferritin light chaingli119740P 1911241Glutamate cysteine ligasegli119740P 1911242Glutathone synthetasegli119740P 1911243Glucose-regulated protein precursor (78 kD)gli4033392Q 9059344Glucose-regulated protein precursor (78 kD)gli4033392Q 9059345GTP-specific succinyl-CoA synthetase beta subunitgli13766203A AC 6439946H* transporting ATP synthasegli170755HSXL 3247Heterogeneous nuclear ribonucleoprotein Fgli18226760N P0495748High mobility group protein 1gli1334284CAA 3765445Heat shock 60 kD proteingli1334284CAA 3765445Heat shock 60 kD proteing	20	Calhindin dogr	g115902791 ~:12110248	U35244
50         Chinoride intracellular channel protein 1         glidS23         [112:03)         [112:03]         [112:03]         [112:03]         [112:03]         [112:03]         [112:03]         [112:03]         [112:03]         [112:03]	21 28	Calenticulin	gil2119546 gil117505	I 34042 D 18/18
50Contraception associated protein 1gif7229594JE 034/31Cytochrome b5gif534539AAA 7242032Dismutasegif518029CAA 2912133Superoxide dismutaegif818029CAA 2912134Dismutasegif818029CAA 2912135Heat shock 70 kD protein 10 (HSC71)gif5729877NP 00658836DNAK type molecular chaperonegif135212P 4867037Vimentingif135212P 4867038Fatty acid binding proteingif19964P 0748339Ferritin light chaingif170038P 1911241Glutamate cysteine ligasegif170038P 4641342Glutacione s-regulated protein precursor (78 kD)gif4033392Q 9059344Glucose-regulated protein precursor (78 kD)gif4033392Q 9059345GTP-specific succinyl-CoA synthetase beta subunitgif3766203A 2C 6439946H <sup>+</sup> transporting ATP synthasegif1334284CAA 3765447Heterogencous nuclear riboncicoprotein Fgif134284CAA 3765448High mobility group protein 1gif134284CAA 3765449Histone H3.2gif7737351XP 01218041Heat shock 60 kD proteingif134284CAA 3765442Heat shock 60 kD proteingif134284CAA 3765443Isocirtat dehydrogenasegif134284CAA 3765444Hat shock 60 kD proteingif134284CAA 3765445Isocirtat dehydrogenase </td <td>20</td> <td>Chloride intracellular channel protein 1</td> <td>gil6685328</td> <td>097105</td>	20	Chloride intracellular channel protein 1	gil6685328	097105
31         Cytochrome b5         gli55439         AAA 72420           32         Dismutase         gli818029         CAA 29121           33         Superoxide dismutase         gli818029         CAA 29121           34         Dismutase         gli818029         CAA 29121           35         Heat shock 70 kD protein 10 (HSC71)         gli7572877         NP 00558           36         DNAK type molecular chaperone         gli2119726         I 56581           37         Vimentin         gli119804         P 07483           39         Ferritin light chain         gli119740         P 19112           40         Fructose-1,6-bisphosphatase         gli8393446         NP 059001           42         Glutathione synthetase         gli110708         P 46413           43         Glucose-regulated protein precursor (78 kD)         gli4033392         Q 90593           44         Harsporting ATP synthase         gli3766203         AAC 64399           45         GTP-specific succinyl-CoA synthetase beta subunit         gli3766203         AAC 64399           46         H <sup>+</sup> transporting ATP synthase         gli376203         AAC 64399           47         Heterogeneous nuclear ribonucleoprotein F         gli4826760         NP 004957      <	30	Contraception associated protein 1	gil0085528 gil7429594	UF 0344
32         Dimutase         Bit Bacop         CAA 29121           33         Superoxide dismutase         git03658         AAA 4096           34         Dismutase         git020857         NAA 4096           35         Heat shock 70 kD protein 10 (HSC71)         git572877         NP 006588           36         DNAK type molecular chaperone         git155212         P 48670           37         Vimentin         git1355212         P 48670           38         Fatty acid binding protein         git119804         P 07483           39         Ferritin light chain         git119700         P 19112           41         Glutamate crysteine ligase         git8393446         NP 059001           42         Glutathione synthetase         git1033392         Q 90593           43         Glucose-regulated protein precursor (78 kD)         git4033392         Q 90593           44         Glucose-regulated protein precursor (78 kD)         git14033392         Q 90593           45         GTP-specific succinyl-CoA synthetase beta subunit         git376600         NP 049977           47         Heterogeneous nuclear ribonucleoprotein F         git14236760         NP 049987           48         High mobility group protein         git1234284         CAA 37	31	Cytochrome b5	gil554539	AAA 72420
33Superoxide dismutaseintroductorintroductorAA A 4099634DismutasegilS1209CAA 21912135Heat shock 70 kD protein 10 (HSC71)gilS729877NP 00658836DNAK type molecular chaperonegil21197261 5658137Vimentingil133212P 4867038Fartty acid binding proteingil119804P 0748339Ferritin light chaingil1196951 5477440Fructose-1,6-bisphosphatasegil10740P 1911241Glutamate cysteine ligasegil10740P 1911242Glutathione synthetasegil170038P 4641343Glucose-regulated protein precursor (78 kD)gil4033392Q 9059344Glucose-regulated protein precursor (78 kD)gil02350A 2K70147Heterogeneous nuclear ribonucleoprotein Fgil8236760NP 00495748High mobility group protein 1gil123677P 1010349Histone H3.2gil70755HSXL 3250Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil1334284CAA 3765452Hay shock 60 kD proteingil1334284CAA 3765453Isocitrate dehydrogenasegil120367P 1012354Lamini-binding proteingil34284CAA 3765455Myosin, light chain, smooth musclegil12373751XP 01218056NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil28867P 1923457 </td <td>32</td> <td>Dismutase</td> <td>gi 818029</td> <td>CAA 29121</td>	32	Dismutase	gi 818029	CAA 29121
34         Dismutase         jitilis029         CAA 29121           35         Heat shock 70 kD protein 10 (HSC71)         git3729877         NP 006588           36         DNAK type molecular chaperone         git3135312         P 48670           37         Vimentin         git119726         156581           38         Farty acid binding protein         git119695         154774           39         Ferritin light chain         git119695         154774           40         Fructose-1.6-bisphosphatase         git3939446         NP 059001           41         Glutamate cysteine ligase         git3939446         NP 059001           42         Glutathione synthetase         git303392         Q 90593           43         Glucose-regulated protein precursor (78 kD)         git4033392         Q 90593           44         Glucose-regulated protein precursor (78 kD)         git376600         NP 004957           45         GTP-specific succiny-LocA synthetase beta subunit         git376601         NP 004957           46         H <sup>+</sup> transporting ATP synthase         git3728767         P 10103           47         Heter shock 60 kD protein         git374284         CAA 37654           48         High mobility group protein 1         git372787	33	Superoxide dismutase	gi 203658	AAA 40996
$35$ Heat shock 70 kD protein 10 (HSC71) $\mu$ ijS729877NP 006588 $36$ DNAK type molecular chaperone $\mu$ ilJ353212P 48670 $37$ Vimentin $\mu$ ilJ353212P 48670 $38$ Fatty acid binding protein $\mu$ ilJ19695I 56581 $39$ Ferritin light chain $\mu$ ilJ19695I 54774 $40$ Fructose-1,6-bisphosphatase $\mu$ ilJ19740P 19112 $41$ Glutamate cysteine ligase $\mu$ ilJ19740P 19112 $42$ Glutathione synthetase $\mu$ ilJ170038P 46413 $43$ Glucose-regulated protein precursor (78 kD) $\mu$ ild033392Q 90593 $44$ Glucose-regulated protein precursor (78 kD) $\mu$ ild033392Q 90593 $45$ GTP-specific succinyl-CoA synthetase beta subunit $\mu$ ild033392Q 90593 $45$ GTP-specific succinyl-CoA synthetase beta subunit $\mu$ ild023392Q 90593 $46$ H* transporting ATP synthase $\mu$ ild023392Q 90593 $45$ GTP-specific succinyl-CoA synthetase beta subunit $\mu$ ild236760N 2804957 $47$ Hetergencous nuclear ribonucleoprotein F $\mu$ ild236760N P004957 $48$ High mobility group protein $\mu$ ild324284CAA 37654 $51$ Heat shock 60 kD protein $\mu$ ild324284CAA 37654 $52$ Heat shock 60 kD protein $\mu$ ild2342CAA 37654 $53$ Isocitrate dehydrogenase $\mu$ ill7174785P 10234 $54$ Laminin-binding protein $\mu$ ill23424CAA 37654 $55$ Myosin, light chain, smooth muscl	34	Dismutase	gi 818029	CAA 29121
36       DNAK type molecular chaperone       gil13726       1 56581         37       Vimentin       gil1353212       P 48670         38       Fatty acid binding protein       gil119804       P 07483         39       Ferritin light chain       gil2119695       1 54774         40       Fructose-1.6-bisphosphatase       gil8393446       NP 059001         41       Glutathione synthetase       gil8393446       NP 059001         42       Glutathione synthetase       gil170038       P 46413         43       Glucose-regulated protein precursor (78 kD)       gil4033392       Q 90593         44       Glucose-regulated protein precursor (78 kD)       gil4033392       Q 90593         45       GTP-specific succinyl-CoA synthetase beta subunit       gil736203       A AC 6439         46       H <sup>+</sup> transporting ATP synthase       gil23567       A 28701         47       Heterogeneous nuclear ribonucleoprotein F       gil134284       CAA 37654         48       High mobility group protein 1       gil134284       CAA 37654         50       Heat shock 60 kD protein       gil1314284       CAA 37654         51       Heat shock 60 kD protein       gil134234       CAA 43469         55       Myosin, light chain, smoot	35	Heat shock 70 kD protein 10 (HSC71)	gi 5729877	NP 006588
37       Vimentin       gil1353212       P 48670         38       Fatty acid binding protein       gil119804       P 07483         39       Ferritin light chain       gil119700       P 19112         40       Fructose-1,6-bisphosphatase       gil19740       P 19112         41       Glutamite cysteine ligase       gil8393446       NP 059001         42       Glutathicone synthetase       gil1003382       Q 90593         44       Glucose-regulated protein precursor (78 kD)       gil366203       AAC 64399         45       GTP-specific succinyl-CoA synthetase beta subunit       gil3766203       AAC 64399         46       H* transporting ATP synthase       gil1334257       P 10103         47       Heterogeneous nuclear ribonucleoprotein F       gil423367       P 10103         48       High mobility group protein 1       gil1334284       CAA 37654         50       Heat shock 60 kD protein       gil1334284       CAA 37654         51       Heat shock 60 kD protein       gil1234284       CAA 37654         52       Heat shock 60 kD protein       gil1334284       CAA 37654         53       Isocitrat dehydrogenase       gil170478       P 19234         54       Laminin-binding protein       gil8237	36	DNAK type molecular chaperone	gi 2119726	I 56581
38Fatty acid binding proteingill 19804P 0748339Ferritin light chaingill 19695I. 5477440Fructose-1,6-bisphosphatasegill 19740P 1911241Glutamate cysteine ligasegill 393446NP 05900142Glutathione synthetasegill 170038P 4641343Glucose-regulated protein precursor (78 kD)gild 033392Q 9059344Glucose-regulated protein precursor (78 kD)gild 033392Q 9059345GTP-specific succinyl-CoA synthetase beta subunitgil3766203A AC 6439946H <sup>+</sup> transporting ATP synthasegild 2250A 2870147Heterogeneous nuclear ribonucleoprotein Fgill 23367P 1010349Histone H3.2gill 70755HSXL 3250Heat shock 60 kD proteingill 334284CAA 3765451Heat shock 60 kD proteingill 34284CAA 3765452Heat shock 60 kD proteingill 273751XP 01218054Laminn-binding proteingill 2273751XP 01218055Myosin, light chain, smooth musclegill 2019P 0418261Phosphotiquetase 24 kD subunit precursorgill 831527O 0855759Ornithica aminotransferasegill 9019P 0418261Phosphotiquetane Seasegill 9019P 0418262Protein disulfide isomerasegill 52733CAA 3440764Arbora seasegill 90030980930965Serine protease inhibitor 2gill 57233CAA 3	37	Vimentin	gi 1353212	P 48670
39Ferruin light chaingill 11995 $154/14$ 40Fructose-1.6-bisphosphatasegill 19740P 1911241Glutamate cysteine ligasegill 393446NP 05900142Glutathione synthetasegill 170038P 4641343Glucose-regulated protein precursor (78 kD)gil4033392Q 9059344Glucose-regulated protein precursor (78 kD)gil303392Q 9059345GTP-specific sucinyl-CoA synthetase beta subunitgil766203AAC 6439946H <sup>+</sup> transporting ATP synthasegil92350A 2870147Heterogeneous nuclear ribonucleoprotein Fgil4033392Q 9059348High mobility group protein 1gil123367P 1010349Histone H3.2gil1334284CAA 3765450Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil134284CAA 3765452Heat shock 60 kD proteingil12737351XP 01218054Laminin-binding proteingil12737351XP 01218055Myosin, light chain, smooth musclegil12019P 0418256NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil831527O 0855759Ornithine aminotransferasegil8039910NP 08893261Phosphotidylethanolamine binding proteingil8393910NP 08893262Protein disulfide isomerasegil681324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding	38	Fatty acid binding protein	gi 119804	P 07483
40Fractose-1,6-ospinospinategli 119/40F 19/1241Glutamate cysteine ligasegil 393446NP 05900142Glutamate cysteine ligasegil 393446NP 05900143Glucose-regulated protein precursor (78 kD)gil 4033392Q 9059344Glucose-regulated protein precursor (78 kD)gil 4033392Q 9059345GTP-specific succiny1-CoA synthetase beta subunitgil 3766203AAC 6439946H' transporting ATP synthasegil 22350A 2870147Heterogeneous nuclear ribonucleoprotein Fgil 4225760NP 00495748High mobility group protein 1gil 70755HSXL 3250Heat shock 60 kD proteingil 1334284CAA 3765451Heat shock 60 kD proteingil 134284CAA 3765452Heat shock 60 kD proteingil 12737351XP 01218053Isocitrate dehydrogenasegil 12737351XP 01218054Laminin-binding proteingil 28857P 1950955Myosin, light chain, smooth musclegil 29019P 0418256NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil 2893910NP 05893260Ornithine aminotransferasegil 6981324NP 03713061Phosphotidylethanolamine binding proteingil 57233CAA 3440762Protein disulfide isomerasegil 6981324NP 03713063Protein disulfide isomerasegil 6981324NP 03713064Retinol binding proteingil 57233CAA 34407 <td>39</td> <td>Ferritin light chain</td> <td>gi 2119695</td> <td>I 54//4 D 10112</td>	39	Ferritin light chain	gi 2119695	I 54//4 D 10112
1Oltidatione Cystenic figasegilassisticgilassisticNP 0.594042Glutathione SynthetasegilatolisaseQ 9059343Glucose-regulated protein precursor (78 kD)gilatolisasisticQ 9059344Glucose-regulated protein precursor (78 kD)gilatolisasisticQ 9059345GTP-specific succinyl-CoA synthetase beta subunitgil3766203AAC 6439946H <sup>+</sup> transporting ATP synthasegil2350A 2870147Heterogeneous nuclear ribonucleoprotein Fgil123367P 1010348High mobility group protein 1gil123367P 1010349Histone H3.2gil70755HSXL 3250Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil134284CAA 3765452Heat shock 60 kD proteingil34234CAA 3765453Isocitrate dehydrogenasegil1170478P 4156254Laminin-binding proteingil32342Q 0855755Myosin, light chain, smooth musclegil129019P 0418260Ornithine aminotransferasegil6831527O 0855761Phosphotidylethanolamine binding proteingil8393910NP 0893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inh	40	Clutemete systeme lieses	gii119740 cii8202446	P 19112 ND 050001
12Ontainone syntheticate $g(111)(05)$ $g(140)(33)$ $g(140)(33)$ 43Glucose-regulated protein precursor (78 kD) $g(140)(33)$ $g(140)(33)$ $Q$ <t< td=""><td>41 42</td><td>Glutathione synthetase</td><td>gil0595440 gil1170038</td><td>NF 039001 P 46413</td></t<>	41 42	Glutathione synthetase	gil0595440 gil1170038	NF 039001 P 46413
Glucose-regulated protein precursor (78 kD)gil403332Q 9059345GTP-specific succinyl-CoA synthetase beta subunitgil3766203AAC 6439946H* transporting ATP synthasegil92350A 2870147Heterogeneous nuclear ribonucleoprotein Fgil42367P 1010348High mobility group protein 1gil123367P 1010349Histone H3.2gil70755HSXL 3250Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil1334284CAA 3765452Heat shock 60 kD proteingil123473P 4156253Isocitrate dehydrogenasegil1170478P4156254Laminin-binding proteingil223737351XP 01218055Myosin, light chain, smooth musclegil128867P 1923456NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil28867P 1923457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil2881527O 0855759Ornithine aminotransferasegil6981324NP 03713061Phosphotidylethanolamine binding proteingil8393910NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhib	43	Glucose-regulated protein precursor (78 kD)	gil1170050 gil4033392	O 90593
45GTP-specific succinyl-CoA synthetase beta subunitgil3766203AAC 6439946H <sup>+</sup> transporting ATP synthasegil92350A 2870147Heterogeneous nuclear ribonucleoprotein Fgil4826760NP 00495748High mobility group protein 1gil123367P 1010349Histone H3.2gil70755HSXL 3250Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil134284CAA 3765452Heat shock 60 kD proteingil34234CAA 3765453Isocitrate dehydrogenasegil1170478P4156254Lamini-binding proteingil34234CAA 4346955Myosin, light chain, smooth musclegil12737351XP 01218056NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil128867P 1923457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil128019P 0418258N <sup>6</sup> , NG-dimethylarginine dimethylaminohydrolase 1gil681327O 0857759Ornithine aminotransferasegil6981324NP 03713061Phosphotidylethanolamine binding proteingil89930080930962Protein disulfide isomerasegil57233CAA 3440764Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine	44	Glucose-regulated protein precursor (78 kD)	gi 4033392	O 90593
46 $H^+$ transporting ATP synthasegil92350A 2870147Heterogeneous nuclear ribonucleoprotein Fgil92360NP 00495748High mobility group protein 1gil123367P 1010349Histone H3.2gil70755HSXL 3250Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil1334284CAA 3765452Heat shock 60 kD proteingil1334284CAA 3765453Isocitrate dehydrogenasegil142434CAA 3765454Laminin-binding proteingil34234CAA 4346955Myosin, light chain, smooth musclegil12825P 1569056NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil128825P 1569058N <sup>G</sup> , NG-dimethylarginine dimethylaminohydrolase 1gil6831527O 0855759Ornithine aminotransferasegil129019P 0418261Phosphotidylethanolamine binding proteingil8393910NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxin peroxidase 1gil8394432<	45	GTP-specific succinvl-CoA synthetase beta subunit	gi 3766203	AAC 64399
47Heterogeneous nuclear ribonucleoprotein Fgil4826760NP 00495748High mobility group protein 1gil23367P 1010349Histone H3.2gil70755HSXL 3250Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil1334284CAA 3765452Heat shock 60 kD proteingil1334284CAA 3765453Isocitrate dehydrogenasegil1170478P4156254Laminin-binding proteingil34234CAA 4346955Myosin, light chain, smooth musclegil28867P 1923456NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil28867P 1923457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil2881527O 0855759Ornithine aminotransferasegil29019P 0418260Ornithine aminotransferasegil6981324NP 03713061Phosphotidylethanolamine binding proteingil8393910NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil35723CAA 3440768Serine protease inhibitor 2gil35723CAA 3440769Thioredoxingil35776P 11232	46	H <sup>+</sup> transporting ATP synthase	gi 92350	A 28701
48High mobility group protein 1gil123367P 1010349Histone H3.2gil70755HSXL 3250Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil1334284CAA 3765452Heat shock 60 kD proteingil134284CAA 3765453Isocitrate dehydrogenasegil170478P4156254Laminin-binding proteingil34234CAA 4346955Myosin, light chain, smooth musclegil128867P 1923456NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil128825P 1569058N <sup>6</sup> , NG-dimethylarginine dimethylaminohydrolase 1gil6831527O 0855759Ornithine aminotransferasegil129019P 0418260Ornithine aminotransferasegil6981324NP 03713062Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil57233CAA 3440764Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil834432NP 05886571Tubulin, alpha chaingil822542S 33517	47	Heterogeneous nuclear ribonucleoprotein F	gil4826760	NP 004957
49Histone H3.2gi/70755HSXL 3250Heat shock 60 kD proteingi/1334284CAA 3765451Heat shock 60 kD proteingi/1334284CAA 3765452Heat shock 60 kD proteingi/134284CAA 3765453Isocitrate dehydrogenasegi/170757P4156254Laminin-binding proteingi/24234CAA 4346955Myosin, light chain, smooth musclegi/12737351XP 01218056NADH-ubiquinone oxidoreductase 24 kD subunit precursorgi/188877P 1923457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgi/80831527O 0855758N <sup>6</sup> , NG-dimethylarginine dimethylaminohydrolase 1gi/80831527O 0855759Ornithine aminotransferasegi/129019P 0418260Ornithine aminotransferasegi/8039301NP 05893262Protein disulfide isomerasegi/6981324NP 03713063Protein disulfide isomerasegi/6981324NP 03713064Retinol binding proteingi/57233CAA 3440765Serine protease inhibitor 2gi/57233CAA 3440766Serine protease inhibitor 2gi/57233CAA 3440767Serine protease inhibitor 2gi/57233CAA 3440768Serine protease inhibitor 2gi/57233CAA 3440770Thioredoxingi/8394432NP 05886571Tubulin, alpha chaingi/422542S 33517	48	High mobility group protein 1	gi 123367	P 10103
50Heat shock 60 kD proteingil1334284CAA 3765451Heat shock 60 kD proteingil1334284CAA 3765452Heat shock 60 kD proteingil1334284CAA 3765453Isocitrate dehydrogenasegil170478P4156254Laminin-binding proteingil123867P 1923455Myosin, light chain, smooth musclegil128867P 1923456NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil128857P 1023457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil128825P 1569058N <sup>6</sup> , NG-dimethylarginine dimethylaminohydrolase 1gil6831527O 0855759Ornithine aminotransferasegil129019P 0418261Phosphotidylethanolamine binding proteingil8393910NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil57233CAA 3440764Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil3834432NP 05886571Tubulin, alpha chaingil422542S 33517	49	Histone H3.2	gi 70755	HSXL 32
51Heat shock 60 kD proteingill $334284$ CAA $37654$ 52Heat shock 60 kD proteingill $334284$ CAA $37654$ 53Isocitrate dehydrogenasegill $170478$ P4156254Laminin-binding proteingil $2337351$ XP 01218055Myosin, light chain, smooth musclegil $2737351$ XP 01218056NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil $28825$ P 1923457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil $28825$ P 169058N <sup>6</sup> , NG-dimethylarginine dimethylaminohydrolase 1gil $6831527$ O 0855759Ornithine aminotransferasegil $129019$ P 0418261Phosphotidylethanolamine binding proteingil $893910$ NP 05893262Protein disulfide isomerasegil $6981324$ NP 03713063Protein disulfide isomerasegil $57233$ CAA $34407$ 64Retinol binding proteingil $57233$ CAA $34407$ 65Serine protease inhibitor 2gil $57233$ CAA $34407$ 66Serine protease inhibitor 2gil $57233$ CAA $34407$ 68Serine protease inhibitor 2gil $57233$ CAA $34407$ 69Thioredoxingil $35776$ P 1123270Thioredoxin peroxidase 1gil $8394432$ NP 05886571Tubulin, alpha chaingil $422542$ S $33517$	50	Heat shock 60 kD protein	gi 1334284	CAA 37654
52Heat shock 60 kD proteingil1334284CAA 3/65453Isocitrate dehydrogenasegil170478P4156254Laminin-binding proteingil34234CAA 4346955Myosin, light chain, smooth musclegil12737351XP 01218056NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil128867P 1923457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil28852P 169058N <sup>6</sup> , NG-dimethylarginine dimethylaminohydrolase 1gil6831527O 0855759Ornithine aminotransferasegil129019P 0418260Ornithine aminotransferasegil6981324NP 03713062Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxin peroxidase 1gil894322NP 05886571Tubulin, alpha chaingil422542S 3351772Tubulin, alpha chaingil422542S 33517	51	Heat shock 60 kD protein	gi 1334284	CAA 37654
5.5Isocirate denyarogenasegill 1/04/78P4156254Lamiin-binding proteingil34234CAA 4346955Myosin, light chain, smooth musclegil12737351XP 01218056NADH-ubiquinone oxidoreductase 24 kD subunit precursorgil128867P 1923457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgil6831527O 0855758N <sup>6</sup> , NG-dimethylarginine dimethylaminohydrolase 1gil6831527O 0855759Ornithine aminotransferasegil129019P 0418260Ornithine aminotransferasegil6981324NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil57233CAA 3440765Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil135776P 1123270Thioredoxin peroxidase 1gil8934432NP 05886571Tubulin, alpha chaingil422542S 33517	52	Heat shock 60 kD protein	gi 1334284	CAA 3/654
54Damm-binding proteingli12234CAA 430955Myosin, light chain, smooth musclegli12737351XP 01218056NADH-ubiquinone oxidoreductase 24 kD subunit precursorgli128867P 1923457NADH-ubiquinone oxidoreductase 75 kD subunit precursorgli128825P 1569058N <sup>6</sup> , NG-dimethylarginine dimethylaminohydrolase 1gli6831527O 0855759Ornithine aminotransferasegli129019P 0418260Ornithine aminotransferasegli129019P 0418261Phosphotidylethanolamine binding proteingli83393100NP 05893262Protein disulfide isomerasegli6981324NP 03713063Protein disulfide isomerasegli80930980930965Serine protease inhibitor 2gli57233CAA 3440766Serine protease inhibitor 2gli57233CAA 3440767Serine protease inhibitor 2gli57233CAA 3440768Serine protease inhibitor 2gli57233CAA 3440769Thioredoxingli8394432NP 05886571Tubulin, alpha chaingli422542S 3351772Tubulin, alpha chaingli422542S 33517	33 54	I socitrate denydrogenase	gi 11/04/8 gi 24224	P41302
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58NG, NG-dimethylarginine dimethylaminohydrolase 1gil6831527O 0855759Ornithine aminotransferasegil129019P 0418260Ornithine aminotransferasegil129019P 0418261Phosphotidylethanolamine binding proteingil8393010NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil80930980930965Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil33776P 1123270Thioredoxin peroxidase 1gil8394432NP 05886571Tubulin, alpha chaingil422542S 3351772Tubulin, alpha chaingil422542S 33517	57	NADH-ubiquinone oxidoreductase 75 kD subunit precursor	gi 128825	P 15690
59Ornithine aminotransferasegil129019P 0418260Ornithine aminotransferasegil129019P 0418261Phosphotidylethanolamine binding proteingil8393910NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil80930980930965Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil33776P 1123270Thioredoxin peroxidase 1gil8394432NP 05886571Tubulin, alpha chaingil422542S 3351772Tubulin, alpha chaingil422542S 33517	58	N <sup>G</sup> . NG-dimethylarginine dimethylaminohydrolase 1	gi 6831527	O 08557
60Ornithine aminotransferasegil129019P 0418261Phosphotidylethanolamine binding proteingil8393910NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil80930980930965Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil33776P 1123270Thioredoxin peroxidase 1gil8394432NP 05886571Tubulin, alpha chaingil422542S 3351772Tubulin, alpha chaingil422542S 33517	59	Ornithine aminotransferase	gi 129019	P 04182
61Phosphotidylethanolamine binding proteingil8393910NP 05893262Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil80930980930965Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil3776P 1123270Thioredoxin proxidase 1gil8394432NP 05886571Tubulin, alpha chaingil422542S 3351772Tubulin, alpha chaingil422542S 33517	60	Ornithine aminotransferase	gi 129019	P 04182
62Protein disulfide isomerasegil6981324NP 03713063Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil6981324NP 03713064Retinol binding proteingil80930980930965Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil35776P 1123270Thioredoxin peroxidase 1gil8394432NP 05886571Tubulin, alpha chaingil422542S 3351772Tubulin, alpha chaingil422542S 33517	61	Phosphotidylethanolamine binding protein	gi 8393910	NP 058932
63Protein disulfide isomerasegil6981324NP 03713064Retinol binding proteingil80930980930965Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil135776P 1123270Thioredoxin peroxidase 1gil8394432NP 05886571Tubulin, alpha chaingil422542S 3351772Tubulin, alpha chaingil422542S 33517	62	Protein disulfide isomerase	gi 6981324	NP 037130
64Retinol binding proteingil80930980930965Serine protease inhibitor 2gil57233CAA 3440766Serine protease inhibitor 2gil57233CAA 3440767Serine protease inhibitor 2gil57233CAA 3440768Serine protease inhibitor 2gil57233CAA 3440769Thioredoxingil135776P 1123270Thioredoxin peroxidase 1gil8394432NP 05886571Tubulin, alpha chaingil422542S 3351772Tubulin, alpha chaingil422542S 33517	63	Protein disulfide isomerase	gil6981324	NP 037130
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	72	Tubulin, alpha chain	gi 422542	S 33517

		Cortex			Medulla			
Spot no.	Proteins	Mean	SEM	N	Mean	SEM	N	P value
2	3-Mercaptopyruvate sulfurtransferase	1.18	0.18	10	0.59	0.13	10	0.016
3	Alpha 2u globulin	9.85	3.38	8	1.98	0.43	9	0.033
5	Alpha enolase	3.84	1.08	10	0.00	0.00	0	0.004
7	Aflatoxin B1 aldehyde reductase	0.00	0.00	10	1.79	0.30	8	0.000
10	Albumin	5.35	1.14	10	7.27	0.87	10	0.046
12	Aldehyde dehydrogenase	2.94	0.53	10	1.28	0.35	10	0.040
14	Alpha B crystallin	0.00	0.00	10	1.30	0.46	7	0.002
23	BH3 interacting domain death agonist	0.00	0.00	10	0.44	0.12	10	0.004
30	Contraception associated protein 1	5.38	0.57	10	3.06	0.42	10	0.005
38	Fatty acid binding protein	10.62	1.56	9	14.43	2.95	6	0.047
44	Glucose-regulated protein precursor (78 kD)	1.15	0.29	8	2.15	0.43	8	0.040
52	Heat shock 60 kD protein	2.39	0.43	8	1.20	0.22	10	0.042
53	Isocitrate dehydrogenase	4.79	0.91	7	2.56	0.36	9	0.045
56	NADH-ubiquinone oxidoreductase 24 kD subunit precursor	3.77	0.46	10	2.20	0.44	10	0.035
60	Omithine aminotransferase	2.01	0.30	8	1.05	0.21	9	0.019
64	Retinol binding protein	3.65	0.46	10	1.87	0.38	10	0.016

Table 2. Differentially expressed proteins





Fig. 3. Western blot of proteins determined to be differentially expressed by two-dimensional gel analysis. Alpha-B crystallin (N = 4), GRP78 (N = 4) and fatty acid binding protein (N = 3) were all determined to be differentially expressed between cortex and medulla in a manner similar to that seen by two-dimensional gel.

a positive identifications from 72 out of 180 spots analyzed or 40%. Our data demonstrate that some of the spots likely contained multiple proteins since unmatched peptides remained after identification of a first protein. We were not able to identify a second protein with statistical confidence from any spot in this study, however.

Using the two-dimensional gel technique we have identified a number of renal proteins. Many of the proteins are expected to be present in renal tissue. For instance alpha 2u globulin binds pheromones in rat urine and allows for their release during drying [13]. Calbindin is a vitamin D-dependent calcium binding protein that is found primarily in the distal nephron and the intestine [14]. A number of proteins that are widely distributed were identified including cytoskeletal and mitochondrial proteins. Cytoskeletal proteins like actin, myosin and tubulin were present in relatively high abundance. Several mitochondrial proteins were found like the H<sup>+</sup> transporting adenosine 5'-triphosphate (ATP) synthase [15], isocitrate dehydrogenase [16] and heat shock protein 60 kD (HSP 60) [17]. Some of the proteins are somewhat unexpected to find in the kidney. Alpha B-crystallin is abundant in lens and skeletal muscle but has also been identified in kidney [18]. 3-Mercaptopyruvate sulfurtransferase is an enzyme responsible for transfer of a sulfur ion to thiols or cyanide. It has been identified in the liver but not previously seen in the kidney [19]. While the preparation of the tissue should have removed most blood proteins, we cannot exclude the possibility that some of the proteins present were derived from residual blood in the tissue.

Cytosolic proteins from rat kidney and medulla have previously been compared using proteomics by Witzman and colleagues [9]. They resolved 727 protein spots in cortex and 716 in medulla (compared to 1095 and 885, respectively, in the current study). One hundred and twenty-seven of these protein spots were found to differ in abundance between cortex and medulla, and 30 were found to be unique to one region or the other. They used mass spectrometry, Western blotting and comparison of homologous spot positions from other databases to identify 20 of these spots representing 14 different proteins and variants. Eight of these spots representing seven different proteins were identified by mass spectrometry. We have expanded on these findings to use whole kidney homogenates rather than cytosolic fractions and to identify a much larger group of expressed proteins. Seventy-two spots representing 54 different proteins were identified. In addition, all of the identifications in the current study were done with peptide mass fingerprinting using MALDI-TOF mass spectrometry, a technique that is more amenable to high-throughput operation. Matching of large numbers of gels with many proteins can be time consuming and error prone. Recent improvements in software promise to make this task much easier and more accurate, but great care must be taken to ensure accurate spot matching.

Some differences between our findings and those of Witzman and colleagues [9] exist for the relative expression of cortical and medullary proteins. They found that actin was expressed to a greater extent in the medulla. We identified three actin spots, all of which had a numerically greater expression in the medulla but none reached statistical significance. The previous study had identified aflatoxin B1 aldehyde reductase expressed in both cortex and medulla but to a greater extent in medulla, while our study found it only in medulla. These differences can probably be accounted for at least in part by differences in technique. The primary difference was that the previous study had looked only at cytosolic proteins while we used whole cell homogenates.

A targeted proteomic approach has been used to quantify expression of specific transport proteins in  $Na^+$ -/H<sup>+</sup> exchanger and thiazide-sensitive  $Na^+Cl^-$  cotransporter knockout mice [20]. This technique uses antibodies against known proteins to determine abundance of proteins. It is more sensitive than looking at protein expression using silver stain but is limited to detection of known proteins for which antibodies exist.

Proteomics was used here to identify a large component of renal proteins and compare expression of these proteins between cortex and medulla. This technique provides a mechanism to characterize changes in large numbers of proteins simultaneously. Once the proteins are identified, this technique can be used to monitor changes in protein expression in response to any intervention. Two-dimensional gel electrophoresis can be done by investigators using identical protocols to identify protein spots that change their expression. These spots can then be compared to known maps of proteins such as the ones in our study to determine the identity of those spots. Further work will need to be done to fully characterize the renal proteome. Separation using another characteristic of proteins such as solubility will add a third dimension of separation, which will greatly increase both the usefulness and the number of proteins that can be identified. Eventually a nephron specific or cell type specific map will be necessary to gain the full value of proteomics for the renal community. We anticipate that this technique will be widely and effectively used in renal research to characterize physiological processes and disease.

#### ACKNOWLEDGMENTS

This work was supported by a Carl W. Gottschalk Research Scholar award to Dr. Arthur from the American Society of Nephrology. Dr. Thongboonkerd is a recipient of the International Fellowship Training Award from the International Society of Nephrology and from the Kidney Foundation of Thailand.

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