# Large-Scale Identification by Shotgun Proteomics of Proteins Expressed in Porcine Liver and Salivary Gland

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Protein catalogs containing a large number of proteins expressed in a variety of organs can be powerful tools for stem-cell research, because this requires accurate knowledge about how cells differentiate. Salivary gland progenitor (SGP) cells are somatic stem cells isolated from the salivary gland that can differentiate into hepatic or pancreatic cell lineages. Their differentiation state has been assessed by the expression of major protein markers, but to use these cells in regenerative medicine, it will be necessary to establish additional means of quality assessment. We examined the use of shotgun proteomics for porcine salivary gland (a source of SGP cells) and liver (a destination of differentiated SGP cells) for determining the state of SGP cell differentiation. Protein complexes from each organ were digested into peptides and separated by two-dimensional liquid chromatography involving strong cation-exchange chromatography followed by reversed-phase liquid chromatography. The separated peptides were analyzed by on-line electrospray ionization tandem mass spectrometry using a quadrupole-time of flight mass spectrometer (ESI Q-TOF MS/ MS), and the spectra obtained were processed to search peptides against a mammalian database for protein identification. Using this method, we identified 117 proteins in porcine salivary gland and 154 proteins in porcine liver. Of these, 72 and 109 were specific to salivary gland and liver, respectively, and some of these were previously shown to be organ specific. The current study can be utilized in the future as a basis to study the pattern of differentiation in protein expression by stem cells.

Key words: porcine, shotgun proteomics, SGP, SCX-RPLC separation, tandem mass spectrometry

# INTRODUCTION

The liver is one of the most complex organs in animals, and it plays critical roles during development, including the detoxification of xenobiotics, the metabolism of lipids and sugars, and the purification of blood (Kellum et al., 2003; Naruse, 2005; Sarlis and Gourgiotis, 2005). Although many liver-related diseases have been reported, liver transplantation has for many years been the only way to effectively treat severe liver diseases (Brown and Moonka, 2004; Shimada et al., 2005); however, the lack of enough donors for a large number of patients and immunorejection represents major challenges.

One idea to alleviate these problems is to identify somatic stem cells that can differentiate into hepatic lineages and use them for cell transplantation therapy. Recent studies have shown that somatic stem cells derived from the salivary gland, which are known as salivary gland progenitor

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(SGP) cells, can differentiate into hepatic or pancreatic cell lineages in rats and mice (Okumura et al., 2003; Hisatomi et al., 2004) as well as in pigs (Matsumoto et al., 2007). It is hoped that the isolation of SGP cells from patients with severe liver disease and their transplantation back into the patients will enable regeneration of their livers without immunorejection and the time-consuming search for matching donors.

To use SGP cells in human patients, it is important to establish protocols for their quality control, such as determining whether the cells are pure and whether they can differentiate appropriately. We are currently examining the use of SGP cells in regenerative medicine in pigs, which are widely used as a model of the human immune system due to the similar size and function of their organs (Kurome et al., 2005). A useful approach to use pigs to establish a model system for self-transplantation regenerative medicine would be to transfer the SGP nucleus to recipient cells, in order to replicate the pig genome and to artificially create disease states in the pig (Dwyer et al., 2002). So far, only major hepatic (e.g., albumin and alfa-fetoprotein) and pancreatic (e.g., glucagon and insulin) markers have been used to assess the differentiation state (Matsumoto et al., 2007), but the differentiation-dependent expression and function of many proteins remain to be characterized. Furthermore, in the pig, even proteins expressed in organs have been poorly characterized. Therefore, a first step is to catalog the proteins expressed in organs, especially the salivary gland (source of SGP cells) and liver (destination of differentiated SGP cells). After that, protein expression profiles can be used to determine the differentiation state of SGP cells.

Traditionally, protein expression profiling has been performed by two-dimensional electrophoresis followed by mass spectrometry (MS) (Unwin et al., 2003). This approach, however, is not effective for identifying very acidic or basic, or very small or large, proteins. Recently, a highthroughput gel-free alternative called "shotgun proteomics" has been developed. This method involves enzymatic digestion of a complex mixture of proteins, followed by sorting the peptide fragments by one or more steps of chromatography followed by data dependent MS-MS analysis, and, finally, identification of the origin of the peptides by a database search (Kang et al., 2005; Kubota et al., 2005). This strategy has shown impressive capabilities for high-throughput protein identification in a variety of biological systems such as in human proteome analysis (Kang et al., 2005).

In this study, we analyzed the proteins expressed in porcine liver and salivary gland by using on-line twodimensional nanoflow liquid chromatography/tandem mass spectrometry (2D LC-LC-MS-MS) based on the dual-trap method (Kang et al., 2005). The on-line 2D-LC method utilized a homemade pulled-tip capillary column (C18) and a dualpurpose trap packed with strong cation-exchange (SCX) resins. Here we present the results of this study and also discuss the usefulness of protein catalogs like those we obtained.

# MATERIALS AND METHODS

#### Animals

Tissues were isolated from a crossbred (Large White/Landrace ×Duroc) barrow (52 d; 10.4 kg). After an 18-h fast, the barrow was anesthetized with atropine (0.2 mg/kg), azaperone (4 mg/kg), and ketamine (20 mg/kg) and killed by bleeding. Liver and salivary gland were rapidly collected, divided into approximately 1-g portions, frozen in liquid nitrogen, and stored at  $-80^{\circ}$ C. All procedures were carried out in accordance with the Regulation of Experiments of the National Agricultural Research Center for Kyushu Okinawa Region (KONARC) and approved by the Animal Experiments Committee of KONARC.

#### Protein extraction from porcine liver and salivary gland

A piece of porcine liver or salivary gland was homogenized in extraction buffer (7 M urea, 2 M thiourea, 4% CHAPS, 50 mM dithiothreitol, and 0.1% SDS) containing a protease inhibitor cocktail (Sigma). The liver homogenates were centrifuged at  $17,000 \times g$  for 30 min. at 4°C to remove cell debris, and the supernatant lysate was transferred to an Amicon YM-3 centrifugal filter unit (15 mL) having a membrane filter (3,000 molecular mass cutoff) from Millipore (Bedford, MA), to wash off the extraction buffer. The lysate was the retrieved by reverse filtration with 0.1 M phosphate buffer solution. Protein concentration was measured by the Bradford method (Bradford, 1976). Proteins extracted were stored at  $-80^{\circ}$ C until use.

#### Sample clean up and protein digestion

Protein extracts from both porcine liver and salivary gland were digested and prepared by the following procedure. Approximately 100  $\mu$ g of each lyophilized protein extract was dissolved in 0.1 M phosphate buffer containing 8 M urea and 10 mM dithiothreitol. After incubation of the solution at 37°C for 2 h, 20 mM iodoaceta-

mide was added to alkylate the reduced thiol groups in the dark at 0°C for 2 h. Excess cystein (~40X) was added to remove the remaining iodoacetamide, and the mixture was diluted with phosphate buffer at a total concentration of 1.0 M urea. For digestion, proteomics-grade trypsin (Sigma; St. Louis, MO, USA) was added to the protein solution at 1:50 (protein:trypsin) and the mixture was incubated for 18 h at 37°C. At the end of digestion, alpha-tosyl-Llysine chloromethyl ketone (TLCK) was added at a slight excess to the estimated number of moles of peptides. The final solution was cleaned up with an Oasis HLB cartridge (Waters; Milford, MA, USA) using acetonitrile, and the remaining organic solvent was evaporated in a vacuum centrifuge. The powdered peptides were redissolved in 5% CH<sub>3</sub>CN in water containing 0.1% formic acid for 2D-LC-LC-MS-MS analysis.

#### 2D-LC-LC-MS-MS

The on-line 2D-LC-LC-MS-MS system utilized in this study was based on the experimental setup reported previously (Kang et al., 2005). The 2D-LC consisted of an analytical column (C18-150 mm×75  $\mu$ m i.d. (inner diameter)) and a dual trap column (SCX-15 mm and C18-10 mm×200 µm i.d.), as shown in Fig. 1. Both columns were made in-house with fused-silica capillaries (Polymicro Technology LLC; Phoenix, AZ, USA). Before packing the column, one end of the analytical column was pulled by flame into a tip diameter of about 10 µm, and the end tip was filled with frit about 2 mm in length, as explained by Kang et al. (2005). One end of the dual trap column was filled in the same way with frit about 2 mm in length. Both columns were packed in house: the analytical column was packed with Magic C18AQ^TM resins having 5.0  $\mu m$ -100 Å pore size (Michrom BioResources Inc.; Auburn, CA, USA), and the dual trap was packed with Magic C18AQ<sup>™</sup> resins having 5.0 µm-200 Å in the first 1.0 cm, followed by packing for 1.5 cm with Polysulfoethyl A<sup>™</sup> SCX resins having 5.0 µm-300 Å pore size (Nest Group Inc.; Southboro, MA, USA). The 2D-LC system was constructed by connecting both the dual trap column and analytical column with a PEEK microcross, and by directly interfacing the analytical column to MS via ESI as shown in Fig. 1. For the supply of electric voltage for ESI, a Pt wire was connected to the PEEK microcross. The 2D-LC-LC-MS-MS was carried out using the CapLC system (Waters; Milford, MA, USA), which consisted of three syringe pumps: two pumps for the binary gradient and another for delivering sample solution from the autosampler to the trap column, as shown in Fig. 1.

A sample of peptide mixture was initially loaded from the autosampler to the SCX part of the dual trap (the solid-line configuration of Fig. 1), and the bound peptides were eluted from SCX with NH<sub>4</sub>HCO<sub>3</sub> solution. The SCX separation was made with a step gradient by increasing the concentration of the NH<sub>4</sub>HCO<sub>3</sub> solution (0, 5, 10, 15, 20, 50, and 500 mM). Delivery of the NH<sub>4</sub>HCO<sub>3</sub> solution, which was stored in a microvial of the autosampler, was made by pump C. The solution delivered by pump C was 5% CH<sub>3</sub>CN with 0.1% formic acid. Only 8 µL of each salt solution was delivered to the SCX trap to elute bound peptides, and the desorbed peptides were readily absorbed by the C18 trap next to the SCX resins in the dual trap. When desorbed peptides were transferred to the C18 trap, desalting was made by delivery by pump C of the same solution used for sample delivery. During this process, all flow was directed toward the waste line via the PEEK microcross, due to the pressure from the analytical column. After desalting, the 10-port valve was turned to 36 degrees so that the binary gradient flow from pumps A and B could enter the analytical column with the vent line closed but with the split outlet open. In this configuration, only a small portion (180 nL/min) of pump flow was directed to the analytical column, and all remaining flow exited through the micro-Tee.

Binary gradient RPLC separation was carried out by varying the mobile phase composition of (A) 5% acetonitrile (ACN) in water and (B) 95% ACN. Both mobile phases contained 0.1% (v/v) formic acid. The gradient began with an increase from 0% B to 10% B over



Fig. 1. A schematic illustration of the 2D-LC-LC-MS-MS experimental setup employed in this study. The homemade column configurations are explained in detail in Materials and Methods. The 10-port valve operation shown in the Fig. is for sample loading and salt step elution. During the RPLC run, the valve position needs to be turned to 36 degrees.

10 min and ramped to 18% for 10 min, to 30% B for 70 min, and to 80% B for 3 min. It was then maintained at 80% for 10 min to wash the column, decreased to 0% for 3 min, and maintained for at least 10 min for column reconditioning. The flow rate during the gradient separation was kept at 200 nL/min, and the eluted peptides were electrosprayed directly into the mass spectrometer.

MS analysis was made with a Q-TOF Ultima mass spectrometer from Waters using direct ESI from the nanoflow LC separation. ESI of peptides was achieved by applying a voltage of 1.8 kV through the Pt wire connected at the PEEK microcross. Eluting peptide ions were analyzed first by precursor scan (200~2000 amu), followed by three data-dependent tandem mass spectrometry runs using collision-induced dissociation (CID).

#### **Data processing**

MS-MS spectra obtained from Q-TOF MS were analyzed with the Mascot Search program against the Swiss-Prot database and MSDB. A protein search was initially made with the porcine database and then followed by one with the mammalian database. Some proteins identified as other than porcine were selected in the following limited cases. Only other mammalian proteins identified by more than two peptide hits were selected as equivalent porcine proteins. The mass tolerance between the measured monoisotopic mass and the selected mass was 1.5 u for the molar mass of a precursor peptide ion and 1.0 u for CID fragments. From the searched protein/peptide lists, only those peptides having a Mascot score larger than 30 were selected as showing extensive similarity at the 95% confidence level (Yang et al., 2004; Wan et al., 2006).

# RESULTS

Samples of both peptide mixtures digested from the porcine liver and salivary gland proteomes were analyzed by 2D-LC-LC-MS-MS. For each sample, six different concentrations of salt solution (NH<sub>4</sub>HCO<sub>3</sub>) were applied for the salt step gradient of SCX separation, and after each elution of salt solution, a nanoflow LC run was carried out with datadependent tandem MS spectrometric analysis. Including the breakthrough LC run made right after loading the sample onto the SCX resins of the dual trap, seven different RPLC runs were made for each proteome sample.

Fig. 2 shows a two-dimensional map of base peak chromatograms (BPCs) from nanoflow LC runs of porcine liver peptide mixture (5 µg) which were obtained at every salt step gradient along with each individual BPC. After sample loading onto the SCX part of the dual trap, a breakthrough run (RPLC) was carried out to detect some peptides that were neutral or not retained in the cation exchange resins. The breakthrough RPLC run in Fig. 2 shows a significant number of peaks. The broad peak at the end of elution (around 85 min) appeared to originate partly from the transition of the mobile phase composition of the binary gradient to the organic rich phase and partly from elution of some remaining surfactants used for cell lysis, but the contribution from the latter seemed to disappear in subsequent RPLC runs. However, not many proteins/peptides were identified from this breakthrough run, which represented numerous peaks apparently originating from some neutral small molecules or impurities.

After the breakthrough run, 8  $\mu$ L of 5 mM NH<sub>4</sub>HCO<sub>3</sub> solution were delivered to the SCX trap from the autosampler via pump C (see Fig. 1), and some peptides eluted from the SCX trap were transferred to the C18 part of the dual trap. A second RPLC gradient elution was then accomplished. The salt step elution-RPLC run cycle was repeated by increasing the salt concentration to 10, 15, 20, 25 mM NH<sub>4</sub>HCO<sub>3</sub>, and finally 500 mM solution was used to wash off all peptides remaining in the SCX resin. As shown in Fig. 2, many peptides peaks were observed at the 5 mM NH<sub>4</sub>HCO<sub>3</sub> solution step, with very few apparent peaks in the rest of the runs. While not many peptide peaks were apparent in the RPLC run of 20 mM NH<sub>4</sub>HCO<sub>3</sub>, it was possible to obtain a number of peptide peaks solidly identified to proteins.

Fig. 3 shows the MS precursor scan at the retention time of 92.1 min of the 500 mM  $NH_4HCO_3$  salt step elution (shown with the dotted line in Fig. 2) along with the datadependent MS-MS spectrum of the ion having an m/z of 852.55. While the BPC after the 500 mM salt step elution



**Fig. 2.** Two-dimensional map of base peak chromatograms (RPLC) of porcine liver protein digests at different salt step cycles (different concentrations of NH<sub>4</sub>HCO<sub>3</sub> solution). Peak intensities are represented by the brightness of white lines in the map, along with seven RPLC chromatograms obtained at each salt step elution. The breakthrough run was obtained right after the peptide mixtures were loaded into the dual trap. See text for experimental conditions of the binary gradient RPLC runs.



**Fig. 3.** Precursor MS scan of nanoflow LC effluent at 92.1 min of the salt step cycle at 500 mM  $NH_4HCO_3$  from Fig. 2, along with the datadependent collision induced fragmentation (CID) spectrum of the selected peptide ion, m/z=852.55 (M+2H<sup>+</sup>)<sup>2+</sup>. A database search resulted in the identification of UDP-glucose pyrophosphorylase 2 containing the peptide sequence K.IFNTNNLWISLAAVK.R.

shows a noisy baseline at around 92.1 min, the precursor scan shown in Fig. 3 shows numerous peaks. The datadependent CID spectrum of m/z=852.55  $(M+2H^+)^{2+}$  followed by a database search resulted in identification of a peptide having sequence K.IFNTNNLWISLAAVK.R, originating from UDP- glucose pyrophosphorylase 2, which is a characteristic protein expressed in porcine liver.

The reproducibility of CID experiments on different batches of sample injection, in which a single injection included an entire salt step elution with each RPLC run, was fairly good, as shown previously (Kang et al., 2005). From seven RPLC-ESI-MS-MS experiments, we identified a total of 154 proteins, including 81 proteins from multiple peptide hits. The identified proteins are listed in Table 1. Some of the proteins in Table 1 are listed as from human or some other mammalian source, since the database search was carried out first against the porcine and then against the mammalian database. However, only proteins identified by multiple peptides were selected as equivalent to porcine proteins.

Fig. 4 shows the results obtained from the porcine

Table 1. Proteins identified in po	orcine	liver.
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	Identified	prot	teins in porcine live	r		-
Acc. No.	Identified protein	#	Acc. No	Identified protein	#	-
P61603	10 kDa beat shock protein mitochondrial Bovine	3	O94PW4	ATPase 8 Pig	1	-
P28037	10-formvltetrahvdrofolate dehvdrogenase. Bat	4	AAN15276	AY138843.mitochondrial Bos taurus	. 9	
O97509	14.3 kDa perchloric acid soluble protein Goat	1	BHMT PIG	Betaine-homocysteine S-methyltransferase Pig	5	
P62261	14-3-3 protein epsilon Bovine	1	CRTC PIG	Calreticulin Pig	1	
Q28956	17beta-estradiol dehydrogenase_Pig	2	Q8WN73	Carbamoyl-phosphate synthetase 1_Pig+Rat	6+1	1
1DUBA	2-enoyl-coa hydratase, chain A_Rat	1	S19307	* Carboxylesterase_Pig	6	
O02691	3-hydroxyacyl-CoA dehydrogenase type II_Bovine	1	CATA_PIG	Catalase_Pig	8	
DEPGC	3-hydroxyacyl-CoA dehydrogenase, short chain_Pig	1	COMT_PIG	Catechol-O-methyltransferase, soluble form_Pig	6	
HPPD_PIG	4-hydroxyphenylpyruvate dioxygenase_Pig	1	KHPGD	Cathepsin D_Pig	1	
Q9NZT7	4-trimethylaminobutyraldehyde dehydrogenase_Human	4	HHMS60	Chaperonin groEL_Mouse	4	
P05386	60S acidic ribosomal protein P1_Rat	1	A29240	* Cofilin_Pig	1	
RLA2_PIG	60S acidic ribosomal protein P2_Pig	1	Q9GKP1	Complement component C3_Pig	1	
1KAY	70kd heat shock cognate protein atpase domain mutant K71A_Bovine	5	CYB5_PIG	Cytochrome B5_Pig	3	
P11021	78 kDa glucose-regulated protein_Human	1	Q28977	Cytochrome P450 2C34v3_Pig	1	-
Q29092	^ 94 KDa glucose-regulated protein_Pig	4	Q8SQ65	Cytochrome P450 2C49_Pig	1	
ATROP	* Actin beta, Rovino	10	JC2019	Cytochromo P450 2D_Fig	1	
P70273	Acul-CoA dehydrogenase short-chain specific mitochondrial Pig	1	002563	Cytosolic NADP+-dependent isocitrate debydrogenase. Bovine	1	
AAD02918	AF020038 Human	1	002338	D-beta-bydroxybutyrate dehydrogenase mitochondrial Human	2	
AAF06698	AF047489 Human	1	Q9TV69	Dimeric dihydrodiol dehydrogenase. Pig	1	
AAD25332	AF104312 Mouse	1	Q9DBT9	Dimethylalycine dehydrogenase, mitochondrial Mouse	1	
AAD38072	AF154830 Human	2	P13804	* Electron transfer flavoprotein alpha-subunit, mitochondrial Human	4	
Q9BYV1	Alanineglyoxylate aminotransferase 2, mitochondrial_Human	1	P68103	Elongation factor 1-alpha 1_Bovine	11	
ALDX_PIG	Alcohol dehydrogenase [NADP+]_Pig	2	P58252	Elongation factor 2_Mouse	2	
ADHP_HUMAN	Alcohol dehydrogenase class II pi chain_Human	1	Q29092	Endoplasmin_Pig	3	
S02302	Aldehyde dehydrogenase (NAD) 1, cytosolic_Horse	1	S06477	Enoyl-CoA hydratase, mitochondrial_Rat	1	
1A4ZA	Aldehyde dehydrogenase (NAD+) 2, mitochondrial_Bovine	3	S57651	Enoyl-CoA hydratase/ 3-hydroxyacyl-CoA dehydrogenase, peroxisomal	1	
Q8MI17	Aldehyde dehydrogenase 1A1_Rabbit	7	P79381	Epoxide hydrolase_Pig	1	
Q9R146	Alpha actin_Pig	1	S45379	Fatty acid-binding protein, hepatic_Pig	1	_
NOA_HUMAN	Alpha enolase (2-phospho-D-glycerate hydro-lyase)_Human	3	Q923D2	Flavin reductase_Mouse	1	
A54731	* Alpha-1 acid glycoprotein_Pig	1	P53603	Formimidoyltransferase-cyclodeaminase_Pig	3	
FAHUAA	Alpha-actinin 1_Human	1	PAPGF	Fructose-bisphosphatase_Pig	5	
ACY1_PIG	Aminoacylase-1_Pig	1	Q91Y97	Fructose-bisphosphate aldolase B_Mouse	5	
P04272	Annexin A2_Bovine	1	P35505	Fumarylacetoacetase_Mouse	1	
AUP2_BUVIN	Antioxidant protein 2_Bovine	1	P79315	GDP dissociation innibitor beta_Pig	1	
B46018	Apolipoprotein C-III. Pig	1	NUFG \$39010	Glucose-o-priospitale isomerase_rig	1	1
A IHUBS	Argininosuccinate synthese. Human	3	GTA1 PIG	Glutathione S-transferase alpha M14. Pig	6	' .
XNPGDM	Aspartate transaminase mitochondrial Pig	2	029581	Glutathione transferase Pig	1	
ATPK BOVIN	ATP synthase f chain, mitochondrial, Bovine	1	Q8BH04	Phosphoenolpyruvate carboxykinase, mitochondrial Mouse	2	
G3P PIG	Glyceraldehyde 3-phosphate dehydrogenase Pig	4	AAA31120	PIGSCOA Pig	1	
Q9Y6B6	GTP-binding protein SAR1b Human	1	1QPWB	Poricine hemoglobin (beta subunit), chain B Pig	7	
Q9T2U6	H+-ATPase subunit, OSCP=OLIGOMYSIN sensitivity conferring protein_Pig	1	Q92620	Pre-mRNA splicing factor ATP-dependent RNA helicase PRP16_Human	1	
PWBOB	H+-transporting two-sector ATPase beta chain, mitochondrial_Bovine	6	1AWIA	* Profilin, chain A_Human	2	
PWBOA	H+-transporting two-sector ATPase, alpha chain precursor, cardiac_Bovine	4	FABO	* Profilin_Bovine	2	
Q96FZ6	Heat shock 60kD protein 1_Human	9	A39682	Prohibitin_Rat	1	
Q9DC41	Heat shock 70kD protein 5_Mouse	4	A25516	Propionyl-CoA carboxylase, beta chain_Rat	1	
HHHU27	Heat shock protein 27_Human	1	A28396	Prostaglandin-F synthase_Bovine	1	
HS9A_PIG	* Heat shock protein HSP 90-alpha_Pig	9	Q15084	Protein disulfide-isomerase A6_Human	1	
1NGB	Heat-shock cognate 70kd protein mutant with glu 175	2	JC2385	Protein disulfide-isomerase ER60_Bovine	4	
HAPG	Hemoglobin alpha chain_Pig	4	ISBOSS	Protein disulfide-isomerase_Bovine	3	
HBB_PIG	^ Hemoglobin beta chain_Pig	1	1BOH	Rhodanese_Bovine	1	
	t Histore HOA 1. Human	1		Riboprionini I_Pig	1	
HSBO2A	Histone H2A. I_Human	2	SAP PIG	S-adenosylmethonne synthetase gamma form_numan	2	
HSBO22	Histone H2B Bovine	3	S72173	Senescence marker protein 30 Mouse+Rabbit	3	
P62802	* Histope H4 Pig	3	Q9UBB1	Sepianterin reductase	1	
Q8R3H3	Hypothetical 14.5 kDa protein	1	ABPGS	* Serum albumin Pig	7	
Q8TED6	Hypothetical protein FLJ23617 Human	1	Q9MYP6	Short-chain dehydrogenase/reductase Bovine	2	
CAC86339	Immunoglobulin gamma 3 heavy chain constant region_Horse	1	Q969Z8	Solute carrier family 1, member 7_Human	1	
Q95M34	Immunogobulin gamma 1 heavy chain_Horse	2	DHSO_RAT	Sorbitol dehydrogenase_Rat +Sheep	5	
O75874	Isocitrate dehydrogenase [NADP] cytoplasmic_Human	2	Q9HCU6	Spermatogenic cell-specific glyceraldehyde 3-phosphate dehydrogenase-2_Human	1	_
IDI1_HUMAN	Isopentenyl-diphosphate delta-isomerase_Human	2	P48721	Stress-70 protein, mitochondrial_Rat	2	
3HDHA	L-3-hydroxyacyl coa dehydrogenase, chain A_Pig	3	JC2258	Substrate protein of mitochondrial ATP-dependent proteinase SP-22_Bovine	2	
Q9UQF9	Lysophospholipase isoform_Human	1	P31040	* Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial_ Human	1	
Q9TUM7	Macrophage migration inhibitory factor_Pig	2	A44529	Succinate-CoA ligase (GDP-forming), beta chain_Pig	1	
MDHC_PIG	* Malate dehydrogenase, cytoplasmic_Pig	2	DSPGCZ	* Superoxide dismutase_Pig	3	
MDHM_PIG	Malate denydrogenase, mitochondrial_Pig	9	THIO_PIG	Inioredoxin_Pig	1	
3MDDA	Medium chain acyl-coa dehydrogenase, chain A_Pig	3	IHIR_HUMAN	I niosuitate sulturtransterase_Human	1	
	Nitembrane associated progesterone receptor component_Pig	1	301384 VPPC	Transietiun_rig	5	
		4	029554	* Trifunctional enzyme alpha subunit mitochondrial Dia	2	
099497	Oncogene D.I1 Human	1	TPIS RAT	* Triosenhosnhate isomerase. Bat	3	
O19072	Ornithine carbamovItransferase, mitochondrial Pig	2	UBPGA	* Tubulin alpha chain Pig	1	
P62936	* Peptidyl-prolyl cis-trans isomerase A Pig	4	P99024	Tubulin beta-5 chain Mouse	5	
Q9GLW8	* Peroxiredoxin 5_Pig	2	P12378	UDP-glucose 6-dehydrogenase Bovine	2	-
1A44	Phosphatidylethanolamine-binding protein_Bovine+Human	1+1	I UDP2 PIG	UTPglucose-1-phosphate uridylyltransferase 2 Pig	3	

\*Those proteins identified in both organs are marked in both tables. # indicates the number of peptides used to identify each protein.



**Fig. 4.** Two-dimensional map of base peak chromatograms (RPLC) of porcine salivary gland protein digests at seven different salt step cycles, the BPC after the salt step cycle of 15 mM  $NH_4HCO_3$  solution, a precursor MS scan at 89.5 min (represented by the dotted line in the BPC), and the corresponding data-dependent CID spectrum of the peptide ion having m/z of 819.01 (M+2H<sup>+</sup>)<sup>2+</sup>. A database search of the CID spectrum resulted in identification of the peptide sequence K.IAGEWYSILLASDAK.A originating from porcine salivary lipocalin.

salivary gland proteome: a 2D map of BPCs at different concentrations of NH<sub>4</sub>HCO<sub>3</sub> solution, an example of BPC at the 15 mM NH<sub>4</sub>HCO<sub>3</sub> cycle, a characteristic MS scan at 89.5 min (expressed by the dotted line in the BPC) of the salt cycle of 15 mM NH<sub>4</sub>HCO<sub>3</sub>, and the corresponding data-dependent CID spectrum of m/z 819.01 (M+2H<sup>+</sup>) from the precursor scan. The peptide sequence identified from the

database search was K.IAGEWYSILLASDAK.A [*m*/*z* 819.01, (M+2H<sup>+</sup>)<sup>2+</sup>], from porcine salivary lipocalin. Database searches for all LC-MS-MS experiments for porcine salivary gland ended up with a total of 117 proteins, including 37 proteins having multiple peptide hits. These are listed in Table 2.

Table 2. Identified proteins list in porcine salivary gland.

Accession     Meeting protein     #     Accession     Meeting protein     #       PR1504     10.00000     10.0000     10.0000	Identified proteins in porcine salivary gland									
Petfol     10 Xba heti ance protein, Indecrook Juluman     10 WH73     Hydrothical protein, Juluman     2       QB0714     34-3 protein zelekiske, Human     10 Statistic     12 Statistic	Accession	Identified protein	#	Accession	Identified protein	#				
P6104     1 + 53 point seaksistis, Human     1 P3198     Norta di divisiones (PG, PG, PG, PG, PG, PG, PG, PG, PG, PG,	P61604	10 kDa heat shock protein, mitochondrial_Human	1	Q8N473	Hypothetical protein_Human	2				
02071     31/pticoxisper, CoA, darly drogsnase pipe IL, Human     1     02550     Live catoro jestimase, Pag     1       0311     PR0     0550-00     Linatia diriy discurse, maintal YEB, Pig     1       04074     Akaba diriy discurse, maintal YEB, Pig     1     Maintal diriy discurse, maintal YEB, Pig     1       04104     Akaba diriy discurse, maintal YEB, Pig     1     Maintal diriy discurse, maintal YEB, Pig     1       04105     Akaba diriy discurses, maintal YEB, Pig     1     Maintal diriy discurse, Pig     4       04107     Akaba diriy discurses, maintal YEB, Pig     1     Maintal diriy discurses, maintal YEB, Pig     4       04107     Akaba diriy di	P63104	14-3-3 protein zeta/delta_Human	1	P33198	Isocitrate dehydrogenase [NADP], mitochondrial_Pig	2				
III.1.P.0     05.8 Hoberman probain LT. Pg     1     DEFCLH     Landtack delyardogenes, hand H. Pg     1       180.4     Acortals byratase, maar VES. Pg     1     MF. PG     Marce delyardogenes, hotpaamie, Pg     1       180.4     Acortals byratase, maar VES. Pg     1     MF. PG     Marce delyardogenes, hotpaamie, Pg     2       060549     ADP.ATP carm probin 3, Pg     2     DEFCMM     Malas delyardogenes, hotpaamie, Pg     3       040578     Akota chardogenes, hotpaamie, Pg     3     302515     Mysin hardy delyardogenes, hotpaamie, Pg     3       04077     Akhara - Add delyardogenes (MADP-1)     9     1     04061     1       04077     Akhara - Add delyardogenes (MADP-1)     0     1     04061     1     04061     1       04077     Akhara - Add delyardogenes (MADP-1)     0     04061     1     04061     1     04061     1     04061     1     04061     1     04061     1     04061     1     04061     1     04061     1     04061     1     04061     1     04061     1     040	Q99714	3-hydroxyacyl-CoA dehydrogenase type II Human	1	Q29550	Liver carboxylesterase Pig	1				
GPR79UNAM     7 b Da globox-regulated position. Jubiana (Jubiana )     1     MF P5164     Linican, Jubiana )       ATBOB     *Actin betta, Jubianas	RL11_PIG	60S ribosomal protein L11_Pig	1	DEPGLH	L-lactate dehydrogenase, chain H_Pig	1				
10.0.0.     Acordiac hydraxer, matar YES, Pig     1     NIE, Pig     Macker dytrogenase, nytoptamic, Pig     2       0.000000     ADP, ATC struter protein 3, Pig     2     DERTMM     Matter dytrogenase, nytoptamic, Pig     3       0.000000     Adpha endase, Human     3     XXX11     *Allela add Stycoptom, Pig     3       0.000000     Apha endase, Human     1     XXX15     Morein addytic glut add struter dytrogenase, nytoptamic, Pig     1       0.000000     Apha endase, Human     1     XXX15     Morein Alley, Balachin 4, Human     1       0.000000     Antexin Fig     1     XXX25     NavK-exchanging ATPises, balaching, Pig     2       0.000000     Antexin Fig     1     0.000000     NavK-exchanging ATPises, balaching, Pig     2       0.000000     Antexin minictranel Resc, organizamic, Booine     1     0.00000     Pigotisje/Pig/O di strani Biomeriane A.Pig     2       0.000000     Appatala atranel minictranel Resc, organizamic, Booine     1     0.000000     Pigotisje/Pig/O di strani Biomeriane A.Pig     1       0.000000     Appatala atranel minictranelance, organizamic, Biomonn-Alley, Pig     2     0.000000	GRP78_HUMAN	78 kDa glucose-regulated protein_Human	5	P51884	Lumican_Human	1				
A180.6     * Actin bez, Sovie     7     MbHC, Pio     Maias edrybrogenase, nick-border, Pig     2       A0001     Appl. Art partier proteins a. Pig     1     70309     Maias edrybrogenase, nick-border, Pig     4       P00578     Aphen ackate, Human     1     3.0021     Myesin ackate, Birk for anticipation antic	1B0JA	Aconitate hydratase, mutant YES_Pig	1	MIF_PIG	Macrophage migration inhibitory factor_Pig	1				
OCORNY     APP ATP carrie protein 3, Pig     2     DPTOM     Maleas dehydrogeness, michochordial, Pig     4       P00733     Alpha and dy gycoproten J/g     1     3X0215     Morein calelyic igit functional. ICTD, Pig     1       P00730     Alpha and dy gycoproten J/g     1     83482     Nau-K-texchangin ATP-ass, bate durin. Pig     8       P00730     Alpha-Sdr164 (Jycoproten J/g     1     84482     Nau-K-texchangin ATP-ass, bate durin. Pig     8       ANCZ PG     Ameain L Pig     1     942058     Nuch-texchangin ATP-ass, bate durin. Pig     1       ANCZ PG     Ameain L Pig     1     942058     Nuch-texchangin ATP-ass, bate durin. Pig     2       ANCZ PG     Ameain L Pig     1     942058     Nuch-texchangin ATP-ass, bate durin. Pig     2       ANCZ PG     Ameain L Pig     1     942048     Payetabase L Pig     2       P3007     Asparatas inscinatoriateras, orphasine. Burin machiness, basin function durin. Pig     1     2       P30049     ATP syntase subt durin. Indicton durin. Pig     1     1     PMAG     Physian durin. Pig     1       P30444     Payetabase L	ATBOB	* Actin beta_Bovine	7	MDHC_PIG	*Malate dehydrogenase, cytoplasmic_Pig	2				
P60578     Alcoh darburdgenese [NADP.L.P.g.     1     100006     Mucin. submaker, P.g.     3     XXXX       A4731     *alpha andise, Human     1     P05576     Morain haway chain, nonzuke type A, Human     1       A4737     Alpha-adition A, Human     1     PA3526     Nar-K-texchanging ATPase, alpha chain. Pig     2       AVX2_PIG     Antexit V, Human     1     Ad4822     Nar-K-texchanging ATPase, alpha chain. Pig     2       AD5140     Annesit V, Human     1     Ad489     P050764 (pote) chains iterations and the second sec	Q6QRN9	ADP,ATP carrier protein 3_Pig	2	DEPGMM	Malate dehydrogenase, mitochondrial_Pig	4				
P6753     Apla andiase, Human     3     JX0215     Myaan catalysic ignt an, normuck by poly Aluman     1       P62700     Apla-2.418 - Jack Jycoprotein, Pig     1     B24822     Na-K+-exchanging P12Pass, Leta Lain, Pig     8       P62700     Apla-2.418 - Jycoprotein, Pig     1     B24822     Na-K+-exchanging P12Pass, Leta Lain, Pig     8       ANX2_P16     Arresin IL-Pig     1     P42028     Na-K+-exchanging P12Pas, Leta Lain, Pig     1       OK3044     Arresin VL-Imm     1     P42028     Na-K+-exchanging P12Pas, Leta Lain, Pig     2       OK5064     Arresin VL-Imm     1     P42028     Na-K-exchanging P12Pas, Leta Lain, Pig     2       P50307     Apartate transmismes, mitch chordial, Pig     1     P42028     Paptidy-inpul is dirara is comerase A_Pig     2       P50309     TP syntase Leig     000032     Paptidy-inpul is dirara is comerase A_Pig     2       S04482     Babs-Salatosid-Jurnan     1     P20304     Paptidy-inpul is dirara is comerase A_Pig     2       S04482     Babs-Salatosid-Jurnan     1     P20304     Paptidy-inpul is dirara is comerase A_Pig     2       S0	P50578	Alcohol dehydrogenase [NADP+]_Pig	1	T03099	Mucin, submaxillary_Pig	3				
AL713     * alpha-size digvoprotein. Pig     1     P3570     Alpha-size digvoprotein. Pig     1       OL4370     Alpha-size digvoprotein. Pig     1     AlA482     Nai-K-exchanging ATPasa. alpha chain. Pig     2       ANX2.PIG     Annexin L.Fig     1     AlA482     Nai-K-exchanging ATPasa. alpha chain. Pig     2       ADVL2.PIG     Annexin L.Fig     1     AdVL2.PIG     Non-sidenung diadVinome oxidoredized so 20. Sta suburd. "Introdocided.Devine 1     3       OBS/GA     Arpinase L.Fig     1     DASS     Poprotech.L.Fuman     2       OBS/GA     Apprates trainformaterises, optoplasming. Bown     1     P26386     Poprotech.Puman     2       OBS/GA     Apprates trainformaterises, optoplasming. Bown     1     P26387     Poprotech.Puman     2       VARDEM     Apprates trainformaterises, optoplasming. Bown     1     POSSA     Prostoplayeesta trainformaterises.     2       VARDEM     A DAST     Poprotech.Puman     1     2       VARDEM     T Postoplase back abscride/-into-thordial_Liman     1     2       VARDEM     T Postoplase bacins onthon-thordial_Liman     1     2	P06733	Alpha enolase_Human	3	JX0215	Myosin catalytic light chain LC17b_Pig	1				
P29700     Alpha -Athira J. Pig-proprietePig     I     B24622     Na-t/N-exchanging APTesa, bith achinPig     B2       ANX2_PIG     Annexin IPig     I     PA2028     Nu-H-lucing/more LabrainPig     B2       ANX2_PIG     Annexin IPig     I     PA2028     Nu-H-lucing/more LabrainPig     B2       OMULP     Annexin IPig     I     PA2028     Nu-H-lucing/more LabrainPig     B2       OMULP     Annexin IPig     I     PA2038     PeptidePignana LPig     B2       OMULP     Appriate a minicranferaseopolamicBovine     I     PA2038     Peptidepignana LPig     I       P30307     Appriate a minicranferaseopolamicBovines     I     PA2048     Propriate Cating PropriatePig     I       P30404     ATP syntase bita chain, michochondialBurnan     I     PA0048     Propriate Cating PropriatePig     I       P30307     CatanodulinPig     I     FADO     Propriate Cating PropriatePig     I       P30404     ATP syntase dista chain, michochondialPig     I     FADO     Propriate	A54731	* alpha-1 acid glycoprotein_Pig	1	P35579	Myosin heavy chain, nonmuscle type A_Human	1				
OASTOR     Apha-actini A, Human     1     A24822     NaKacchanging APtaea, beta chain, Pig     2       ARX2_PIG     Amesin L, Pig     1     PAGEN     Non-selenium glutanticoma di Lagona     1       ADHAP     Amesin L, Juman     1     OFTSM     Non-selenium glutantico partocimatico partocimatico di partocidase. Pig     1       ADHAP     Amesin L, Juman     1     OFTSM     Non-selenium glutantico partocimatico di partocimatico di partocidase. Pig     1       PARTO A, Appartate aminoranderesso, ortopisamic. Borine     1     PAGEN     * Partocidado T, Antochondrial. Pig     2       STRPE, ART     Appartate aminoranderesso, ortopisamic. Borine     1     PAGEN     * Partocidado T, Antochondrial. Pig     1       PAGEN     APE partata Senti chain, michochodrial. Pig     2     OFTSM     Protein chain. Antochondrial. Pig     1       PAGEN     APE partata Senti chain, michochodrial. Pig     1     AVAR     * Protein chain. Antochondrial. Pig     1       PAGEN     APE partata Senti chain, michochodrial. Pig     1     AVAR     * Protein chain. Antochondrial. Pig     1       PAGEN     Calino Diriti     Calino Diriti     1	P29700	Alpha-2-HS-glycoprotein_Pig	1	B24862	Na+/K+-exchanging ATPase, alpha chain_Pig	8				
AM22_PIO     Ameson L, Horg     1     PA2028     NADH-L-icingiance actionation at 28 Ma aubunit, micchondrial, Edware 1     1       OAG604     Anterior zyndient protein 2 homolog Human     1     JCA569     PS protein _Human     2       OBG504     Anterior zyndient protein 2 homolog Human     1     JCA569     PS protein _Human     2       P30307     Apparatite transmissiones and homolog Human     1     P23048     Paparatite transmissiones A. Pig     2       P30407     ArtP synthase bite chain, mitchondrial Human     1     P20048     Prosphate carter protein, mitchondrial Muman     1       P3041     ATP synthase bite chain, mitchondrial Human     1     PA0040     Prosphate carter protein, mitchondrial Muman     1       P30421     Carterotain Pig     1     1     NAVA     * Profilm_Bovine     1       P30431     Carterotain Pig     1     JCS500     Protein disulfor-isonacian carterotain synthese carterotain sy	O43707	Alpha-actinin 4_Human	1	A24862	Na+/K+-exchanging ATPase, beta chain_Pig	2				
Annesin     Annesin     J     OBTSX3     Non-selectinu glutathione phospholip hydroperoxide peroxidesae. Pig     I       OBS504     Anpirase LPig     C     SCAS99     Paprids-prolip clansmical, periodinal, Lonsmic     I       OBS504     Anpirase LPig     Paprids-prolip clastrans isomerase A, Pig     I       SVAPCIM     Apartata transmisse, mitochondrial, Pig     I     OGS256     Phosphate clastra isomerase A, Pig     I       PS0404     Apartata transmisse, mitochondrial, Pig     I     OGS256     Phosphate clastra isomerase A, Pig     I       PS0404     APP synthase belic chain, mitochondrial, Human     I     PMOR HUMAN     Phosphate clastra isometane binding potenin, Pig     I       PS0404     Calponin L, Pig     I     AVX140     Phosphate clastra isometane binding potenin, Pig     I       PS0404     Calponin L, Pig     I     AVX760     Phosphate clastra isometane binding potenin, Pig     I       PS0404     Calponin L, Pig     I     AVX760     Phosphate clastra isometane binding potenin, Pig     I       PS0404     Calponin L, Pig     I     AVX760     Phosphate clastra isometane binding potenin, Pig     I	ANX2_PIG	Annexin II_Pig	1	P42028	NADH-ubiquinone oxidoreductase 23 kDa subunit, mitochondrial_Bovine	1				
06959     Anterior gradient protein 2: human     1     JC4369     P Sprotein, Human     2       06951C8     Anginase L, Pig     1     P26309     Peptidy-proty Los-trans isomenase A, Pig     2       P3009     Appratus transmasme, mitochondrial, Pig     1     P26309     Pepridy-proty Los-trans isomenase A, Human     1       ATPS, ATP synthase beta chain, mitochondrial, Human     1     P0014W     Peroxindoxin 5, mitochondrial, Human     1       AS6785     Calinodulin, Pig     2     OH061     Phosphato perioding protein 3, Human     1       AS6785     Calenodulin, Pig     1     1 AVWA     * Polinin, Bovine     1       P36401     Calenodulin, Pig     1     JC5704     Protein disuffici-licomenase, Effed, Human     4       A26002     Calenon 1: Ging, Li-Human     1     OBM150     Plateine GTP-Oelissociation inhibitor, Bovine     1       A26004     * Colling, Pig     3     A5000     Protein disuffici-licomenase, Effed, Human     1       A26004     * Colling, Pig     3     A5000     Protein disuffici-licomenase, Line, Pig     1       A26004     * Colling, Pig	AQHUP	Annexin V_Human	1	Q9TSX9	Non-selenium glutathione phospholipid hydroperoxide peroxidase_Pig	1				
OBSIG     Anginase (Pig     Petrods-provide-strans isomerase A_Pig     Petrods-provide-strans isomerase A_Pig     Petrods-provide-strans isomerase A_Pig     Petrods-provide-strans isomerase B_Pig     Petrods-P	O95994	Anterior gradient protein 2 homolog_Human	1	JC4369	P5 protein_Human	2				
P3307     Appatiale aminotraniferase, optoplasmic, Boyne     1     P2284     Peptoplayroly cisi-trans isomersome B_Human     2       XPROEDM     Appatiale transmisse, mitochondrial, Piq     0     0     Peostoplayorised mutase, binal norm, Human     1       P30049     ATP synthase belia chain, mitochondrial, Human     1     PM0B, HUMAN     Phosphopytoprate mutase, brain form, Human     1       P30049     Bate Galactosido-bindring lectin, Pig     2     049430     Phosphopytoprate mutase, brain form, Human     1       P28491     Calmodulin, Pig     1     1, VIA     *Phosin, And A, Human     1       P28491     Calmodulin, Pig     1     1, OS704     Protein disulfide-isomer membrane binding protein, Pig     1       P38404     Castomy cress: a bunkit, Boyne     1     0, OS715     Putate for D004-Human     1       A28204     *Colling, Pig     3     A6009     Phosphopin LPig     1       A28204     *Colling, Pig     3     A6009     Phosphopin LPig     1       A28204     *Colling, Pig     3     A6009     Phosphopin LPig     1       A28400     Calling eni	Q95JC8	Arginase I_Pig	1	P62936	* Peptidyl-prolyl cis-trans isomerase A_Pig	2				
XINEQD     Asparate transminuse, michochondial, Pig     I     OdS100     * Peorytake transminuse, michochondial, Puman     I       P30044     ATP synthase data chain, michochondial, Human     I     PM06, HUMAN     Phosphate carrier proteins, Michochondial, Human     I       P30047     Bate-Galactoside chain, michochondial, Human     I     PM06, HUMAN     Phosphate carrier proteins, Human     I       AS6775     Calmodulin, Pig     I     I AWNA     * Profilin, chain A_Human     I       Q50642     Calunchin, Pig     I     J CS704     Profilin, Chain A_Human     I       Q50642     Calunchin, Pig     I     J CS704     Profilin, Chain A_Human     I       Q50640     Calunchin, Pig     I     J CS704     Profilin Chain Cha	P33097	Aspartate aminotransferase, cytoplasmic_Bovine	1	P23284	Peptidyl-prolyl cis-trans isomerase B_Human	2				
ATPE     ATP synthase beta chain, mitochondrial_Human     1     Poospha carrier protein, mitochondrial_Human     1       SP6494     ATP synthase beta chain, mitochondrial_Human     1     PhodB, HUMAN     PhodB, HUMAN     1       SP6492     Beta-Galactoside-binding lectin_Pig     1     AWIA     PhodIm, chain A, Human     1       SP6492     Calabodin_Pig     1     FABO     PhodIm, Bovine     1       SP6494     Calabodian_Pig     1     JCS300     Pootein conting protein TPDO4, Human     1       SP6404     Colabory Settin Suburit_Bovine     1     ORKTS     Patefield protein famp-1A_Human     1       SP6044     Colabory Settin Suburit_Bovine     1     PR6234     Raserelated protein famp-1A_Human     1       A29240     *Collagen alpha S(V) chain, Human     1     PR6234     Salavaratemic molennic Pig     1       A29241     Crelation stands and the intechnodial, Human     1     PR6234     Salavaratemic Pig     1       A29240     *Collagen alpha suburit, mitochondrial, Human     1     PG6245     Sandaratemic Pig     1       A29247     Colabory setain suburit, Bivo a	XNPGDM	Aspartate transaminase, mitochondrial_Pig	1	Q9GLW8	* Peroxiredoxin 5, mitochondrial_Pig	1				
P3004     ATP synthase data chain, mitochondrial, Human     I     PMORE, HUMAN     Propriory control proving provin	ATPB_RAT	ATP synthase beta chain, mitochondrial_Rat	4	Q00325	Phosphate carrier protein, mitochondrial_Human	1				
S06432     Beta-Galactosicb-inding lockin_Pig     2     OH3611     Profilm, chain A_Human     1       066092     Calmorduin_Pig     1     NAWA     Profilm, Boine     1       078092     Calmorduin_Pig     1     J.CS740     Profilm, Boine     1       078092     Caltorosticlin_Pig     1     J.CS740     Protein disulfici-sonrense, ER60 Human     4       078092     Cattorosticlestresse.Pig     1     J.CS740     Protein disulfici-sonrense, ER60 Human     4       078004     Coatomer zeta-t subunit_Bovine     1     PR0504     Rostorest Bornes APE-1A_Human     1       026104     Coationer zeta-t subunit_Bovine     1     PR0504     Coationer zeta-t subunit_Bovine     1       026114     Creatine Kinase, mitochondrial_Human     1     PR0507     Salvary loocatin_Fig     9       P13040     * Elocaton transfer flavoprotein alph-seubnit_mitochondrial_Human     1     O41425     Short hans-Shy droteose, mitochondrial_Muman     1       026942     * Elongation factor _Human     1     O41425     Short hans-Shy drotoxy ap-f CoA dishydrogenase, mitochondrial_Muman     1       026	P30049	ATP synthase delta chain, mitochondrial_Human	1	PMGB_HUMAN	Phosphoglycerate mutase, brain form_Human	2				
A66780     Calmovin-Pig     1     NAWA     * Profilia, naña A_Human     1       060802     Calponin-LPig     1     JCS260     Progesterone membrane binding profein_Pig     1       91807     * Carboxylesterase_Pig     1     JCS704     Profein. Sevine ass. FR0_Human     4       82800     Chaperonin Grobe-L.Human     1     ORMYKS     Putative GTP-Diod-Human     1       92864     Coatomer zeta-1 subunit.Bovine     1     PR6800     Rhoppotin CDO-discociation inhibitor.Bovine     1       0261124     Coflin_Pig     3     A65080     Rhoppotin CD-discociation inhibitor.Bovine     1       0261143     Containe encounter zeta-1 subunit.Bovine     1     PR6808     Salivary (pocalin_Pig     2       0261141     Containe encounter zeta-subunit.Indicohondrial_Human     1     PR6913     Salivary (pocalin_Pig     1       126042     Chichorane e oxidase polypeptide Va, mitochondrial_Human     1     O491425     Short china' Shiftor_Shiftoray yetain SM2_Human     1       126042     Steorota mater favorotroin alpha-subunit, mitochondrial_Human     1     O491425     Short china' Shiftoray yetain SM2_Human	S06492	Beta-Galactoside-binding lectin_Pig	2	Q9H361	Polyadenylate-binding protein 3_Human	1				
C088022     Calponin - JPig     1 FABO     * PROB     1 JCS200     Progestorno membrane binding protein PSID.     1       S19307     * Carboxylesterase _ Pig     1 JCS704     Protein disulfide-isomerase, ER60_Human     4       A28200     Chaperonin GreL_, Human     1 OPKTKS     Putative GTP-binding protein PTD04, Human     1       A28200     * Coltinn_, Pig     3 A5600     Rse-related protein Rp1-L, Human     1       A28240     * Colling- a piha 3(VI) chain, Human     1 Q45000     * Ribophorn L, Pig     2       ORNE11     Greatine kinase, mitochondrial_ Human     1 Q45000     * Ribophorn L, Pig     2       ONEX type molecular chaperone, Human     1 P61608     Salivary lpocalin Pig     9       P13634     * Electron transfer flavoprotein alpha-subunt, mitochondrial_Human     1 Q48448     Sialic acid synthese_Human     1       Q30922     * Endoplasmic-reticulum-Human Byte Status     1 Q48448     Sialic acid synthese_Human     1       Q30923     * Endoplasmic-reticulum-Human Byte Status     1 Q48448     Sialic acid synthese_Human     1       Q30924     * Endoplasmic-reticulum-Human Byte Status     1 Q47498     Sodium/ptotessum-Hranspor	A56785	Calmodulin_Pig	1	1AWIA	* Profilin, chain A_Human	1				
P28491     Carlcoulin_Pig     1 JCS20     Propertione membrane binding proteine_Pig     1       S19307     * Carboxylesterse_Pig     J JCS704     Protein disulfide-isomerses, ER60_Human     1       S19307     * Carboxylesterse_Pig     J OPNTK5     Putative GTP-binding protein Rap-1A_Human     1       P36604     Coatomer zeta-1 subunit_Bovine     1     P62834     Ras-related protein Rap-1A_Human     1       CGHU3A     Colagen alpha 3(VI) chain_Human     1     P0674     Salivary lipocalin_Pig     2       CBN1E1     Creatine kinase, mitochondrial 2 Human     1     P0674     Salivary lipocalin_Pig     1       P30674     Cytochrome c oxidase polypeptide Va, mitochondrial_Human     1     P0671     Saritarasterin_Pig     1       P13804     * Electon transfer flavoprotein alpha-subunit, mitochondrial_Human     1     O41725     Short chain 3-HydroxyacyLoa dehydrogenase, mitochondrial_Human     1       P05809     * Electon transfer flavoprotein alpha-subunit, mitochondrial_Human     1     O41725     Short chain 3-Human     1       P03804     * Electon transfer flavoprotein subunit, mitochondrial_Human     1     O41725     Subunita/Buman	Q08092	Calponin-1_Pig	1	FABO	* Profilin_Bovine	1				
S19307     * Catooxylesterase_Pig     1     JCS704     Protein disulfide-isomerase, ER60_Human     4       A32800     Chaperonic Roite_L-Human     1     QBM126     Plactatev GTP-Inding protein P70004_Human     1       A32800     * Collin, Pig     3     A45009     Rho protein GDP-dissociation inhibitor_Bovine     1       A29240     * Colling, Pig     3     A45009     Rho protein GDP-dissociation inhibitor_Bovine     1       CGNUE1     Creatine kinase, mitochondrial_Human     1     QPG714     Serotransform Pig     5       Q20674     Cytochrone codase polypeptide Va, mitochondrial_Human     1     QPG74     Serotransform Pig     9       P13694     * Electron transfer flavoprotein alpha-subuni, mitochondrial_Human     1     QPK745     Sinci chain 3-hydroxyacy/CoA dehydrogenase, mitochondrial_Human     1       T09549     Endoplasmic-reliculur-Humenal protein 28_Human     1     QPK745     Sinci chain 3-hydroxyacy/CoA dehydrogenase, mitochondrial_Human     1       T09549     Endoplasmic-reliculur-Humenal protein 28_Human     1     QPK256     Socium/posci havacy chain SM2_Human     1       T09540     Endoplasmic-reliculur-Human     1	P28491	Calreticulin_Pig	1	JC5260	Progesterone membrane binding protein_Pig	1				
A32800     Chaperonin GroEL_Human     1     OPNTKS     Putative GTP-binding protein PTD04_Human     1       P35804     Coatomer zuba- subunit_Bovine     1     P68244     Ras-related protein Rap-1A_Human     1       CGHU3A     Collagen alpha 3(VI) chain_Human     1     Q90MB0     *Ribophorin _Pig     2       CGHU3A     Creatine Kinase, mitochondrial_Human     1     P06771     Serotransferrin_Pig     1       A27077     DnaK-type molecular chaperone_Human     1     Q61425     Short chain 3-hydroxyacyl-CoA dehydrogenase, mitochondrial_Muse     1       P13804     * Electron transfer flavoprotein alpha-subuni, mitochondrial_Human     1     Q61425     Short chain 3-hydroxyacyl-CoA dehydrogenase, mitochondrial_Muse     1       P13804     * Electron transfer flavoprotein alpha-subuni, mitochondrial_Human     1     Q61425     Short chain 3-hydroxyacyl-CoA dehydrogenase, mitochondrial_Human     1       P13804     * Electron transfer flavoprotein alpha-subuni, mitochondrial_Human     1     Q61425     Short chain 3-hydroxyacyl-CoA dehydrogenase, mitochondrial_Human     1       P05809     Endoplasmic-reliculum-Human     1     Q64729     Socium/potasubuni, mitochondrial_Human     1 <td>S19307</td> <td>* Carboxylesterase_Pig</td> <td>1</td> <td>JC5704</td> <td>Protein disulfide-isomerase, ER60_Human</td> <td>4</td>	S19307	* Carboxylesterase_Pig	1	JC5704	Protein disulfide-isomerase, ER60_Human	4				
P35604Coatomer zeta-1 subunit_Bovine1P62834Ras-related protein Rap-1A, Human1A28240* Colfilin_Pig3A45009Pho protein GDP-dissociation inhibitor_Bovine1CAUSACollagen alpha 3(VI) chain_Human1096MB0* Ribophorin LPig2CRN1E1Creatine kinase, mitochondrial 2_Human1P08571Serotransferrin_Pig1A27077Dnak-type molecular chaperone_Human109671Serotransferrin_Pig9P13804* Electon transfer Ravoprotein alpha-subunit, mitochondrial_Human1061425Siatic acid synthase_Human1P13839Elongation factor 2_Human1014729Smooth muscle myosin heavy chain SM2_Human1029092* Endoplasmic-reticulum-lumenal protein 28_Human1014729Smooth muscle myosin heavy chain SM2_Human1029092* Endoplasmic-reticulum-lumenal protein 28_Human1014729Smooth muscle myosin heavy chain SM2_Human1029092* Endoplasmic-reticulum-lumenal protein 28_Human1014729Smooth muscle myosin heavy chain SM2_Human1029092* Endoplasmic-reticulum-dumentorinsferase_Human1014729Smooth muscle myosin heavy chain SM2_Human1029092* Endoplasmic reticulum reticulum and protein solutin, mitochondrial_Human1024729Sister and status1029092* Endoplasmic reticulum and protein solutin, mitochondrial_Human1024729Sister and status1029052Foluin_Fig11P37602	A32800	Chaperonin GroEL_Human	1	Q9NTK5	Putative GTP-binding protein PTD004_Human	1				
A2920*Colling_Pig3A45009Pho protein GDP-dissociation inhibitor_Bovine1CGHU3ACollagen alpha 3(V) chain_Human109GMB0* Ribophorin L/Pig2QBN1E1Creatire kinase, mitochondrial 2-Human1P81608Salivary lipocalin_Pig5P20674Cytochrome c oxidase polypeptide Va, mitochondrial_Human1P09571Seortransferrin_Pig9P13804* Electron transfer flavoprotein alpha-subunit, mitochondrial_Human1O61425Short chain 3-hydroxyacy/CoA dehydrogenase, mitochondrial_Mouse1P13839Elongation factor 2_Human1O14729Smooth muscle myosin heavy chain SM2_Human1Op9549Endoplasmic-reticulum-Humenal protein 28_Human1O14729Smooth muscle myosin heavy chain SM2_Human122395Fetuin_Pig1P11600* Sucinate dehydrogenase flavoprotein subunit, mitochondrial_Human2O9V480Tain 1_Human1ALFA_RATFructose-bisphosphate aniontactor_Human2O9V490Tain 1_Human11AGFA1_HUMANGlucocorticid receptor AF-1 specific elongation factor_Human2O9V490Tain 1_Human11GFA1_HUMANGlucocorticid receptor AF-1 specific elongation factor_Human2O9V490Tain 1_Human11GFA1_HUMANH-transporting two-sector ATPase, alpha chain_Bovine1VPRG* Transitional endoplasmic reticulum ATPase_Pig1Glucocorticid receptor AF-1 specific elongation factor_Human1VPRG* Transitional endoplasmic reti	P35604	Coatomer zeta-1 subunit_Bovine	1	P62834	Ras-related protein Rap-1A_Human	1				
CGHU3ACollagen alpha 3(V) chain_Human1O9GMB0*Ribophorin LPig208N1E1Creatine kinase, mitochondrial_Human1P8608Salivary lipocalin_Pig570074Cytochrome c oxidase polyspitde Va, mitochondrial_Human1P8608Salivary lipocalin_Pig1A27077DnaK-type molecular chaperone_Human3ABPGS*serum albumin_Pig9P13804*Elector thrasfer flavoprotein alpha-subunit, mitochondrial_Human1061425Shot chain 3-Hydroxyacy-CGA dehydrogenase, mitochondrial_Mouse1P13639Elongation factor 2_Human1091475Sialic acid synthase_Human SM2_Human1029092*Endoplasmic-reticulum-humenal protein 28_Human1014729Smooth muscle myosin heavy chain SM2_Human1029092*Endoplasmic-reticulum-humenal protein 28_Human10947260*Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human1042092*Endoplasmic-reticulum-humenal protein 28_Human2DSPGC2*Superoxide dismutas_Pig2050305Glucocsamine-fructose-6-phosphate aimotransferase_Human1P82460Thioredoxin_Pig1050306Glucosamine-fructose-6-phosphate aimotransferase_Human1P82460Thioredoxin_Pig10517Glutatinice F-tansporting XPrase, alpha chain_Bovine7EFHU1Transitional endoplasmic reticulum ATPase_Pig10517Glucasamine-fructose-6-phosphate adminotransferase_Human1P82460Thioredoxin_Pig10517Glucasamine-fructose-	A29240	* Cofilin_Pig	3	A45009	Rho protein GDP-dissociation inhibitor_Bovine	1				
QBN1E1Creatine kinase, mitochondrial 2, Human1P10805Salivary lipocalin, Pig5P20674Oytochrome c oxidase polypeptide Va, mitochondrial, Human1P09571Serotransferrin_Pig1AZ7077DnaK-type molecular chaperone, Human3ABPQS* serum albumin, Pig9P13804* Electron transfer flavoprotein alpha-subunt, mitochondrial_Human1O61425Short chain 3-hydroxyaoy-ICoA dehydrogenase, mitochondrial_Mouse1P13639Elongation factor 2, Human1O14729Smooth muscle myosin heavy chain SM2_Human1O29020* Endoplasmin-Pig3P05027Sodium/potassium-transporting ATPase beta-1 chain, Pig1S22395Fetuin_Pig1P31040* Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human1O4FA1_HUMANGlucocorticoid receptor AF-1 specific elongation factor_Human2O9Y490Talin 1, Human1OFA256Glucocorticoid receptor AF-1 specific elongation factor_Human1P37802Transgein-2, Human1OFA1_HUMANGlucose-chosphate aliolase A_Pat1P37802Transgein-2, Human1OFFC36Glucocorticoid receptor AF-1 specific elongaton factor_Human2O9Y490Talin 1, Human1OFFC36Glucocorticoid receptor AF-1 specific elongaton factor_Human1P37802Transgein/a, Human1OFFC36Glucocorticoid receptor AF-1 specific elongaton factor_Human1P42460Thioredoxin_Pig1DFFC36Glucose-chynosphate adinytogenase_P	CGHU3A	Collagen alpha 3(VI) chain_Human	1	Q9GMB0	* Ribophorin I_Pig	2				
P2067Cytochrome c oxidase polypeptide Va, mitochondrial_Human1P0571Serotransferrin_Pig1A27077Dnak-type molecular chaperone_Human3ABPGS*serum albumin.Pig9P13804*Electron transfer flavoprotein alpha-subunit, mitochondrial_Human1O61425Shot chain 3-hydroxyay-ICOA dehydrogenase, mitochondrial_Mouse1P13839Endoplasmic-reticulum-lumenal protein 28_Human1O4N445Sialic acid synthase_Human1029092*Endoplasmic-reticulum-lumenal protein 28_Human1O1572Sodum/otoasisum-transportein ATPase beta 1 chain, Pig122335Fetuin_Pig1P31040*Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human1029092Glucocordici receptor AF-1 specific elongation factor_Human2D97407Talin 1_Human10FA1_HUMANGlucose-d-phosphate alidolase A_Flat209/490Talin 1_Human110FA1_HUMANGlucose-d-phosphate aliongation factor_Human1P82460Transiedoladoplasmic reticulum ATPase_Pig10F1P_PIGGlucase-d-phosphate isomerase_Pig1P7960"Transiedolagoplasmic reticulum ATPase_Pig10F1P_PIGGlucase-d-phosphate isomerase_Pig2292401Transiedolage_Human10F1P_PIGGlucase-driende-glubin dactor ATPase, alpha chain_Bovine7ErHumaTransiedolage_Human10F1P_PIGHeat shock protein Bab-1, Bovine3029554"Trifunctional enzyme alpha subunit, mitochondrial_Pig10F19 <t< td=""><td>Q8N1E1</td><td>Creatine kinase, mitochondrial 2_Human</td><td>1</td><td>P81608</td><td>Salivary lipocalin_Pig</td><td>5</td></t<>	Q8N1E1	Creatine kinase, mitochondrial 2_Human	1	P81608	Salivary lipocalin_Pig	5				
AZ707DnaK-type molecular chaperone_Human3APES* serum alburnin_Pig9P13804* Electron transfer flavoprotein alpha-subunit, mitochondrial_Human1O61425Short chain 3-hydroxyacyl-CoA dehydrogenase, mitochondrial_Mouse1P13839Elongation factor 2_Human1O47425Sinalic acid synthase_Human1109549Endoplasmic-reticulum-Humenal protein 28_Human1O14729Smooth muscle myosin heavy chain SM2_Human1029092* Endoplasmic-reticulum-Humenal protein 28_Human1O14729Smooth muscle myosin heavy chain SM2_Human1029093* Endoplasmic-reticulum-Humenal protein 28_Human1P31040* Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human1ALFA_RATFructose-bisphosphate aldolase A_Rat2DSPGCZ* Superoxide dismutase_Pig2Q9NZS6Glucoser-forbosphate almotransferase_Human1P82460Thioredoxin_Pig1P50309Glucoser-6-phosphate isomerase_Pig1P78020Transitional endoplasmic reticulum ATPase_Pig1GTP_PIGGlutathione S-transferase P.Pig1VPPG* Transitional enlogation factor eEF-1 alpha-1 chain_Human1DEPGG3Glyceraldehyde-3-phosphate alpha chain_Bovine7EFHU1Transitional enographia bantin, mitochondrial_Pig1P19120Heat shock oprotein TP Bo o.alpha_Pig1TPIS_RAT* Trifunctional enzymosin alpha 4 chain_Pig1P04792Heat shock protein befa-1_Human1O98C33Tubulin alpha 2_Human3 <td>P20674</td> <td>Cytochrome c oxidase polypeptide Va, mitochondrial_Human</td> <td>1</td> <td>P09571</td> <td>Serotransferrin_Pig</td> <td>1</td>	P20674	Cytochrome c oxidase polypeptide Va, mitochondrial_Human	1	P09571	Serotransferrin_Pig	1				
P13804* Electron transfer flavoprotein alpha-subunit, mitochondrial_Human1061425Shot chain 3-hydroxyacyl-CoA dehydrogenase, mitochondrial_Mouse1P13639Elongatini factor 2_Human109NR45Sialic acid synthase_Human1019549Endoplasmic-reticulum-lumenal protein 28_Human1014729Smooth muscle myosin heavy chain SM2_Human1029092*Endoplasmic-Pig3P05027Sodium/potassium-transporting ATPase beta-1 chain_Pig1S22395Fetuin_Pig1P31040*Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human104FA.RATFructose-bisphosphate aldolase A_Rat2DSPGCZ*Superoxide dismutase_Pig2Q9NZS6Glucosartine-fructose-6-phosphate aninotransferase_Human1P37802Transgelin-2_Human1050309Glucosartine-fructose-6-phosphate aninotransferase_Hig1VPPG*Transitional endoplasmic reticulum ATPase_Pig10EPPGG3Glyceraldehyde-3-phosphate isomerase_Pig2P24011Transketolase_Human10EPFG3Glyceraldehyde-3-phosphate dehydrogenase_nig3Q29554*Trifunctional enzyme alpha subunit, mitochondrial_Pig1191202Heat shock cognate 71 kDa protein_Bovine7EFHU1Transketolase_Human10EPFG3Glyceraldehyde-3-phosphate isomerase_Aat2Q29554*Trifunctional enzyme alpha subunit, mitochondrial_Pig119120Heat shock protein HSP 90-alpha_Pig1TPIS_RAT*Trosephosphate isomerase_Rat219132	A27077	DnaK-type molecular chaperone_Human	3	ABPGS	* serum albumin_Pig	9				
P13639Elongation factor 2_Human1O PNR45Sialic acid synthase_Human1T09549Endoplasmin_reig1014729Smooth muscle myosin heavy chain SM2_Human1020092* Endoplasmin_reig3P05027Sodium/potassium+ransporting ATPase beta-1 chain_Pig1S22395Fetuin_Pig1P31040* Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human1ALFA_RATFructose-bisphosphate aldolase A_Rat2DSPGCZ* Suceinate dehydrogenase flavoprotein subunit, mitochondrial_Human1GFA1_HUMANGlucosamine-fructose-6-phosphate aminotransferase_Human1P82460Thioredoxin_Pig1GTP_PIGGlutose-6-phosphate isomerase_Pig1VPR6* Transitional endoplasmic reticulum ATPase_Pig1DEPGG3Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transletion elongation factor eEF-1 alpha-1 chain_Human1GRP78_HUMANH+transporting two-sector ATPase, alpha chain_Bovine7EFHU1Transletion elongation factor eEF-1 alpha-1 chain_Human1GRP78_HUMANHetrasporting two-sector ATPase, alpha chain_Bovine3C29554* Trifunctional enzyme alpha subunit, mitochondrial_Pig1P19120Heat shock protein HSP 90-alpha_Pig1TPIS_RAT* Triosephosphate isomerase_Rat2P04792Heat shock protein beta-1_Human1O8WU19Tubulin alpha 4 chain_Pig3P19166* Heurogenous nuclear ribonucleoprotein F_Human1OBBCB3Tubulin alpha 4 chain_Pig1<	P13804	* Electron transfer flavoprotein alpha-subunit, mitochondrial_Human	1	Q61425	Short chain 3-hydroxyacyl-CoA dehydrogenase, mitochondrial_Mouse	1				
T09549Endoplasmic-reticulum-lumenal protein 28_Human10 14729Smooth muscle myosin heavy chain SM2_Human1Q29092* Endoplasmin_Pig3P05027Sodium/potassium-transporting ATPase beta-1 chain_Pig1S22395Fetuin_Pig1P31040* Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human2Q9M2S6Glucocostnice/dreceptor AF-1 specific elongation factor_Human2Q9Y490Talin 1_Human1P50309Glucosamine-fructose-6-phosphate aimotoransferase_Human1P82460Thioredoxin_Pig1P50309Glucose-6-phosphate isomerase_Pig1P37802Transglin-2_Human1GTP_PIGGlutathione S-transferase P.Pig1P37802Transketolase_Human1DEPGG3Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transketolase_Human1P19120Heat shock cognate 71 kDa protein_Bovine7EFHU1Translation elongation factor eEF-1 alpha-1 chain_Human1P19120Heat shock protein hSP 90-alpha_Pig1P67937Triosephosphate isomerase_Rat2P04792Heat shock protein beta-1_Human1P67937Trubulin alpha chain_Pig3P01985Hemoglobin beta chain_Pig5UBPGA* Tubulin alpha chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein K_Human1UBPG3Tubulin alpha chain_Pig2P2537Heterogeneous nuclear ribonucleoprotein K_Human1UBPG4* Tubulin alpha chain_Pig4P14866<	P13639	Elongation factor 2_Human	1	Q9NR45	Sialic acid synthase_Human	1				
C29092*Endoplasmin_PigS P05027Sodium/potassium-transporting ATPase beta-1 chain_Pig1S22395Fetuin_Pig1P31040*Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human1S22395Fetuin_Pig2DSPGCZ*Superxide dismutase_Pig2Q9NZS6Glucose-bisphosphate aldolase A_Rat2DSPGCZ*Superxide dismutase_Pig1GFA1_HUMANGlucose-6-phosphate aminotransferase_Human1P82460Thioredoxin_Pig1P50309Glucose-6-phosphate isomerase_Pig1VPPG*Transitional endoplasmic reticulum ATPase_Pig1DEPGG3Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transletonal endoplasmic reticulum ATPase_Pig1DEPG33Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transletonal endoplasmic reticulum ATPase_Pig1P19120Heat shock cognate 71 KDa protein_Bovine3Q29554*Trifunctional enzyme alpha subunit, mitochondrial_Pig1P04792Heat shock protein hSP 90-alpha_Pig1TPIS_RAT*Trosephosphate isomerase_Rat2P04792Heat shock protein beta-1_Human1P67937Tropomyosin alpha 4 chain_Pig3P01955Hemoglobin alpha chain_Pig5UBPGA*Tubulin alpha-6 chain_Human3P19180Heterogeneous nuclear ribonucleoprotein F_Human1Q98023"tubulin alpha-6 chain_Human4P61978Heterogeneous nuclear ribonucleoprotein F_Human1Q98023Tubulin alpha-6 chain_Human1 </td <td>T09549</td> <td>Endoplasmic-reticulum-lumenal protein 28_Human</td> <td>1</td> <td>O14729</td> <td>Smooth muscle myosin heavy chain SM2_Human</td> <td>1</td>	T09549	Endoplasmic-reticulum-lumenal protein 28_Human	1	O14729	Smooth muscle myosin heavy chain SM2_Human	1				
S22395Fetuin_Pig1P31040* Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human1ALFA_RATFructose-bisphosphate aldolase A_Rat2DSPGCZ* Superoxide dismutase_Pig2GIucocorticoid receptor AF-1 specific elongation factor_Human2OSP490Talin 1_Human1GFA1_HUMANGlucosamine-fructose-6-phosphate aminotransferase_Human1P82460Thioredoxin_Pig1P50309Glucose-6-phosphate isomerase_Pig1P37802Transglein-2_Human1GTP_PIGGlutathione S-transferase P_Pig2P29401Transktolase_Human1GRP78_HUMANH+-transporting two-sector ATPase, alpha chain_Bovine7EFHU1Translation elongation factor eEF-1 alpha-1 chain_Human1P19120Heat shock cognate 71 KDa protein_Bovine3Q29554* Trifunctional enzyme alpha subunit, mitochondrial_Pig1P19120Heat shock protein heta-1_Human1P67937Trospomyosin alpha 4 chain_Pig1P04792Heat shock protein beta-1_Human1P67937Tropomyosin alpha 4 chain_Pig2P04793Heterogeneous nuclear ribonucleoprotein F_Human1Q9BOG3Tubulin alpha-chain_Pig4P19486Heterogeneous nuclear ribonucleoprotein K_Human1Q9BOG3Tubulin alpha-chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein SA2B1_Human1Q9BOG3Tubulin alpha-chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein SA2B1_Human1Q9BOG3Tubulin beta	Q29092	* Endoplasmin_Pig	3	P05027	Sodium/potassium-transporting ATPase beta-1 chain_Pig	1				
ALFA_FATFructose-bisphosphate aldolase A_Rat2DSPGCZ*Superoxide dismutase_Pig2Q9NZS6Glucocorticoid receptor AF-1 specific elongation factor_Human1ZQ9Y490Talin 1_Human1GFA1_HUMANGlucosenime-fructose-6-phosphate aminotransferase_Human1PS2460Thioredoxin_Pig1P50309Glucase-6-phosphate isomerase_Pig1P37802Transglin-2_Human1GTP_PIGGlutathione S-transferase P_Pig1VPPG*Transitonal endoplasmic reticulum ATPase_Pig1DEPGG3Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transketolase_Human1GRP78_HUMANH+-transporting two-sector ATPase, alpha chain_Bovine7EFHU1Translation elongation factor eEF-1 alpha-1 chain_Human1GRP78_HUMANH+-transporting two-sector ATPase, alpha chain_Bovine3Q29554*Trifunctional enzyme alpha subunit, mitochondrial_Pig1HS9A_PIG*Heat shock protein HSP 90-alpha_Pig1TPIS_RAT*Trosephosphate isomerase_Rat2P04792Heat-shock protein beta-1_Human1P67937Tropomyosin alpha 4 chain_Pig1P01965Hemoglobin alpha chain_Pig5UBPGA*Tubulin alpha-6 chain_Human1P61978Heterogeneous nuclear ribonucleoprotein F_Human1Q98CE3Tubulin alpha-6 chain_Human1P19180Heterogeneous nuclear ribonucleoprotein K_Human1UBPGBTubulin alpha-6 chain_Human1P22626Heterogeneous nuclear ribonucleoprotein L_Human	S22395	Fetuin_Pig	1	P31040	* Succinate dehydrogenase flavoprotein subunit, mitochondrial_Human	1				
C9NZS6Glucocorticoid receptor AF-1 specific elongation factor_Human2C9Y490Talin 1_Human1GFA1_HUMANGlucosen-6-phosphate aminotransferase_Human1P82460Thioredoxin_Pig1P50309Glucose-6-phosphate isomerase_Pig1P37802Transgelin-2_Human1GTP_PIGGlutathione S-transferase P_Pig1VPPG* Transitional endoplasmic reticulum ATPase_Pig1DEPGG3Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transketolase_Human1GRP78_HUMANH+-transporting two-sector ATPase, alpha chain_Bovine7EFHU1Translation elongation factor eEF-1 alpha-1 chain_Human1P19120Heat shock cognate 71 kDa protein_Bovine3Q29554* Trifunctional enzyme alpha subunit, mitochondrial_Pig1HS9A_PIG* Heat shock protein hSP 90-alpha_Pig1PPIS_RAT* Tropomyosin alpha 4 chain_Pig1P01965Hemoglobin alpha chain_Pig1Q8WU19Tubulin alpha 4 chain_Pig1P01965Hemoglobin beta chain_Pig5UBPGA* Tubulin alpha 4 chain_Pig2P52597Heterogeneous nuclear ribonucleoprotein F_Human1Q96023Tubulin alpha-6 chain_Human1P61978Heterogeneous nuclear ribonucleoprotein K_Human1D96037Tubulin beta-2 chain_Human1P14866Heterogeneous nuclear ribonucleoprotein K_Human1D96137Tubulin beta-2 chain_Human1P14866Heterogeneous nuclear ribonucleoprotein K_Human1D96371Tubul	ALFA_RAT	Fructose-bisphosphate aldolase A_Rat	2	DSPGCZ	* Superoxide dismutase_Pig	2				
GFA1_HUMANGlucosamine-fructose-6-phosphate aminotransferase_Human1P82460Thioredoxin_Pig1P50309Glucose-6-phosphate isomerase_Pig1P37802Transgelin-2_Human1GTP_PIGGlutathione S-transferase P_Pig1VPPG*Transitional endoplasmic reticulum ATPase_Pig1DEPGG3Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transketolase_Human1GR78_HUMANH+-transporting two-sector ATPase, alpha chain_Bovine7EFHU1Translation elongation factor eEF-1 alpha-1 chain_Human1P19120Heat shock cognate 71 KDa protein_Bovine3Q29554* Trifunctional enzyme alpha subunit, mitochondrial_Pig1HS9A_PIG* Heat shock protein HSP 90-alpha_Pig1TPIS_RAT* Triosephosphate isomerase_Rat2P04792Heat-shock protein beta-1_Human1P67937Tropomyosin alpha 4 chain_Pig3P01965Hemoglobin beta chain_Pig5UBPGA* Tubulin alpha 2_Human3HBB_PIG* Heerogeneous nuclear ribonucleoprotein F_Human1Q38QE3Tubulin alpha-6 chain_Human1P61978Heterogeneous nuclear ribonucleoprotein K_Human1UBPGBTubulin beta-chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein K_Human1O68371Tubulin beta-2 chain_Human1P22626Heterogeneous nuclear ribonucleoprotein K_2/B1_Human1O75396Vesicle trafficking protein SEC22b_Human1Q00839Heterogeneous nuclear ribonucleoprotein L_Human	Q9NZS6	Glucocorticoid receptor AF-1 specific elongation factor_Human	2	Q9Y490	Talin 1_Human	1				
P50309Glucose-6-phosphate isomerase_Pig1P37802Transglin-2_Human1GTP_PIGGlutathione S-transferase P_Pig1VPPG* Transitional endoplasmic reticulum ATPase_Pig1DEPGG3Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transketolase_Human1GRP78_HUMANH+-transporting two-sector ATPase, alpha chain_Bovine7EFHU1Translation elongation factor eEF-1 alpha-1 chain_Human1P19120Heat shock corate 71 kDa protein_Bovine3Q29554* Trifunctional enzyme alpha subunit, mitochondrial_Pig1HS9A_PIG* Heat shock protein hSP 90-alpha_Pig1TPIS_RAT* Triosephosphate isomerase_Rat2P04792Heat shock protein beta-1_Human1P67937Tropomyosin alpha 4 chain_Pig1P01965Hemoglobin alpha chain_Pig5UBPGA* Tubulin alpha 2_Human3HBB_PIG* Heterogeneous nuclear ribonucleoprotein F_Human1Q9BQE3Tubulin alpha chain_Pig2P52597Heterogeneous nuclear ribonucleoprotein K_Human1UBPGBTubulin beta-chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein L_Human1P68371Tubulin beta-chain_Pig4P26262Heterogeneous nuclear ribonucleoprotein U_Human1O75396Vesicle trafficking protein SEC22b_Human1Q29579Histone H28.nd H2A_Pig1P45880Voltage-dependent anion-selective channel protein 2_Human1Q29579Histone H28.nd H2A_Pig1P45880Voltag	GFA1_HUMAN	Glucosamine-fructose-6-phosphate aminotransferase_Human	1	P82460	Thioredoxin_Pig	1				
G1H_PIGGlutatione S-transterase P_Pig1VPPGTransitional endoplasmic reticulum ATPase_Pig1DEPGG3Glyceraldehyde-3-phosphate dehydrogenase_Pig2P29401Transitional endoplasmic reticulum ATPase_Pig1GRP78_HUMANH+-transporting two-sector ATPase, alpha chain_Bovine7EFHU1Transitional endoplasmic reticulum ATPase_Pig1P19120Heat shock cognate 71 kDa protein_Bovine3Q29554* Trifunctional enzyme alpha subunit, mitochondrial_Pig1HS9A_PIG* Heat shock protein HSP 90-alpha_Pig1TPIS_RAT* Triosephosphate isomerase_Rat2P04792Heat shock protein beta-1_Human1P67937Tropomyosin alpha 4 chain_Pig1P01965Hemoglobin alpha chain_Pig5UBPGA* Tubulin alpha 2_Human3HBB_PIG* Heterogeneous nuclear ribonucleoprotein F_Human1Q9BQE3Tubulin alpha-6 chain_Human1P61978Heterogeneous nuclear ribonucleoprotein K_Human1UBPGBTubulin beta chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein L_Human1P68371Tubulin beta chain_Human1P22626Heterogeneous nuclear ribonucleoprotein A2/B1_Human1O75396Vesicle trafficking protein SEC22b_Human1Q00839Heterogenous nuclear ribonucleoprotein U_Human1P02543Vimentin_Pig1Q29579Histone H28_1_Human1P45880Voltage-dependent anion-selective channel protein 2_Human1Q29579Histone H28_1_Human1 <td>P50309</td> <td>Glucose-6-phosphate isomerase_Pig</td> <td>1</td> <td>P37802</td> <td>Iransgelin-2_Human</td> <td>1</td>	P50309	Glucose-6-phosphate isomerase_Pig	1	P37802	Iransgelin-2_Human	1				
DEPGG3   Gipceraidenyade-3-phosphate denyadrogenase_Pig   2   P24401   Transketolase_Human   1     GRP78_HUMAN   H+-transporting two-sector ATPase, alpha chain_Bovine   7   EFHU1   Translation elongation factor eEF-1 alpha-1 chain_Human   1     HS9A_PIG   Heat shock cognate 71 kDa protein_Bovine   3   C29554   *Trifunctional enzyme alpha subunit, mitochondrial_Pig   1     HS9A_PIG   *Heat shock protein hSP 90-alpha_Pig   1   TPIS_RAT   *Triosephosphate isomerase_Rat   2     P04792   Heat shock protein beta-1_Human   1   P67937   Tropomyosin alpha 4 chain_Pig   1     P01965   Hemoglobin beta chain_Pig   1   Q8WU19   Tubulin alpha 2_Human   3     HBB_PIG   *Hemoglobin beta chain_Pig   5   UBPGA   *Tubulin alpha 6 chain_Pig   2     P52597   Heterogeneous nuclear ribonucleoprotein K_Human   1   Q9BQE3   Tubulin beta chain_Pig   1     P14866   Heterogeneous nuclear ribonucleoprotein L_Human   1   UBPG8   Tubulin beta chain_Human   1     P22626   Heterogeneous nuclear ribonucleoprotein A2/B1_Human   2   O60701   UDP-glucose 6-dehydrogenase_Human   2     Q	GTP_PIG	Glutathione S-transferase P_Pig	1	VPPG	1 ransitional endoplasmic reticulum A l Pase_Pig	1				
CH-PYS_HUMAN   H+-transporting two-sector A1Pase, appa chain_Bovine   / EH-U1   Translation elongation tactor eE1 appa-1 chain_Human   1     P19120   Heat shock cognate 71 kDa protein_Bovine   3   Q29554   * Trifunctional enzyme alpha subunit, mitochondrial_Pig   1     HS9A_PIG   * Heat shock protein HSP 90-alpha_Pig   1   TPIS_FART   * Triosephosphate isomerase_Rat   2     P04792   Heat-shock protein beta-1_Human   1   P67937   Tropomyosin alpha 4 chain_Pig   1     P01965   Hemoglobin beta chain_Pig   1   Q8WU19   Tubulin alpha 2_Human   3     HBB_PIG   * Hemoglobin beta chain_Pig   5   UBPGA   * Tubulin alpha 6 chain_Pig   2     P52597   Heterogeneous nuclear ribonucleoprotein F_Human   1   QB9DE3   Tubulin alpha-6 chain_Human   1     P61978   Heterogeneous nuclear ribonucleoprotein K_Human   1   UBPGB   Tubulin beta chain_Pig   4     P14866   Heterogeneous nuclear ribonucleoprotein L_Human   1   P68371   Tubulin beta chain_Pig   1     P22626   Heterogeneous nuclear ribonucleoprotein A2/B1_Human   2   O60701   UDP-glucose 6-dehydrogenase_Human   2     Q00839	DEPGG3	Glyceraldenyde-3-phosphate denydrogenase_Pig	2	P29401	I ransketolase_Human	1				
Pri 120Heat shock cognate / i kDa protein_Bovine3C29554Titulnchona enzyme apria subunit, mitochonona_Prig1HS9A_PIG* Heat shock protein hSP 90-alpha_Pig1TPIS_RAT* Triosephosphate isomerase_Rat2P04792Heat-shock protein bEta-1_Human1P67937Tropomyosin alpha 4 chain_Pig1P01965Hemoglobin alpha chain_Pig1Q8WU19Tubulin alpha 2_Human3HBB_PIG* Hemoglobin beta chain_Pig5UBPGA* Tubulin alpha 2_Human1P61978Heterogeneous nuclear ribonucleoprotein F_Human1UBPGBTubulin beta chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein L_Human1P68371Tubulin beta chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein L_Human2O60701UDP-glucose 6-dehydrogenase_Human2Q00839Heterogeneous nuclear ribonucleoprotein U_Human1075396Vesicle trafficking protein SEC22b_Human1Q2579Histone H28.n_Human1P02543Vimentin_Pig1Q2579Histone H28.n_Human1H28680Voltage-dependent anion-selective channel protein 2_Human1HSHUB1Histone H28.n_Human1H286L_HUMANHistone H28.c_Human1P62802* Histone H28 H2911	GRP/8_HUMAN	H+-transporting two-sector A Pase, alpha chain_Bovine	/	EFHU1	Translation elongation factor eEF-1 alpha-1 chain_Human	1				
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Port yo2Heat-shock protein beta-1-builtantPort yo37Hopomyosin alpha 4 chain_rigIP01965Hemoglobin alpha chain_Pig1Q8WU19Tubulin alpha 2_Human3HBB_PIG* Hemoglobin beta chain_Pig5UBPGA* Tubulin alpha 6_chain_Pig2P52597Heterogeneous nuclear ribonucleoprotein F_Human1Q9BQC3Tubulin alpha 6_chain_Pig4P14866Heterogeneous nuclear ribonucleoprotein K_Human1UBPGBTubulin beta-6 chain_Human1P22626Heterogeneous nuclear ribonucleoprotein A2/B1_Human2O60701UDP-glucose 6-dehydrogenase_Human2Q00839Heterogenous nuclear ribonucleoprotein U_Human1P75396Vesicle trafficking protein SEC22b_Human1HSHUA1* Histone H2A.1_Human1P05433Vimentin_Pig1Q29579Histone H2B and H2A_Pig1P45880Voltage-dependent anion-selective channel protein 2_Human1HSHUB1Histone H2B.1_Human1H2BC_HUMANHistone H2B.c_Human1P62802* Histone H2B.1_Human1H2BC_HUMANHistone H2B.c_Human1	R04700	Heat shock protein HSP 90-alpha_Pig	1	1PIS_RA1	Tropomuosin slobe 4 shoin. Dia	2				
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Hebrogeneous nuclear ribonucleoprotein F_Human   1   Q9BQE3   Tubulin alpha 6 chain_Human   1     P52597   Heterogeneous nuclear ribonucleoprotein K_Human   1   Q9BQE3   Tubulin alpha 6 chain_Human   1     P61978   Heterogeneous nuclear ribonucleoprotein K_Human   1   UBPGB   Tubulin beta chain_Pig   4     P14866   Heterogeneous nuclear ribonucleoprotein L_Human   1   P68371   Tubulin beta chain_Pig   4     P22626   Heterogeneous nuclear ribonucleoprotein L_Human   2   O60701   UDP-glucose 6-dehydrogenase_Human   2     Q00839   Heterogenous nuclear ribonucleoprotein U_Human   1   075396   Vesicle trafficking protein SEC22b_Human   1     NSHUA1   * Histone H2A.1_Human   1   P02543   Vimentin_Pig   1     Q29579   Histone H2B.and H2A_Pig   1   P45880   Voltage-dependent anion-selective channel protein 2_Human   1     HSHUB1   Histone H2B.1_Human   1   H2BC_HUMAN   Histone H2B.c_Human   1		* Hemeslehin hete ehein. Dis	5		* Tubulin alpha abaia Dia	0				
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P14866   Heterogeneous nuclear ribonucleoprotein L-Human   1   P68371   Tubulin beta chain_rug   1     P22626   Heterogeneous nuclear ribonucleoprotein L-Human   1   P68371   Tubulin beta -2 chain_Human   2     Q00839   Heterogeneous nuclear ribonucleoprotein S A2/B1_Human   2   O60701   UDP-glucose 6-dehydrogenase_Human   2     Q00839   Heterogenous nuclear ribonucleoprotein U_Human   1   O75396   Vesicle trafficking protein SEC22b_Human   1     HSHUA1   * Histone H2A.1_Human   1   P02543   Vimentin_Pig   1     Q29579   Histone H2B and H2A_Pig   1   P45880   Voltage-dependent anion-selective channel protein 2_Human   1     HSHUB1   Histone H2B.1_Human   1   H2BC_HUMAN   Histone H2B.c_Human   1	P61978	Heterogeneous nuclear ribonucleoprotein K. Human	1	LIBPGB	Tubulin beta chain. Pin	1				
P22626   Heterogeneous nuclear ribonucleoproteins Az/B1_Human   2   O60701   UDP-glucose 6-dehydrogenase_Human   2     Q00839   Heterogeneous nuclear ribonucleoprotein U_Human   1   O75396   Vesicle trafficking protein SEC22b_Human   1     HSHUA1   * Histone H2A.1_Human   1   P02543   Vimentin_Pig   1     Q29579   Histone H2B and H2A_Pig   1   P45880   Voltage-dependent anion-selective channel protein 2_Human   1     HSHUB1   Histone H2B.1_Human   1   H2BC_HUMAN   Histone H2B.c_Human   1	P1/866		1	D68371	Tubulin beta-2 chain Human	4				
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Pé2802 * Histone H4 Pig 1	HSHUB1	Histone H2B.1 Human	1	H2BC HUMAN	Histone H2B.c. Human	1				
	P62802	* Histone H4 Pig	1		·····	•				

#### DISCUSSION

In this study, we performed a large-scale protein identification by shotgun proteomics of the porcine salivary gland (a source of SGP cells) and liver (a destination for differentiated SGP cells), and identified 117 and 154 proteins, respectively. To our knowledge, this is the first report of large-scale protein catalogs for porcine organs.

Of the proteins identified in the two proteomes, 45 were redundant, whereas many others were specific to each organ (109 specific to liver and 72 specific to salivary gland). Among liver-specific proteins, there were some housekeeping proteins, and there were several others already reported to be abundantly expressed in liver, including catalase, cytochrome P450, and ornithine carbamoyltransferase (Lin et al., 1997; Baranova et al., 2005; Koger and Jones, 1997). Likewise, among the salivary gland-specific proteins, we identified some predicted to function in the salivary gland or saliva, such as submaxillary apomucin and salivary lipocalin precursor (Eckhardt et al., 1997; Loebel et al., 2000). Although we did not identify a high number of organ-specific proteins, comparison of the proteomes of salivary gland, liver, and differentiated SGP may allow determination of the state of SGP differentiation. Analysis of the SGP proteome remains to be carried out, and it will be necessary to expand the proteome map for both liver and salivary gland, because the accuracy of quality-control analysis is enhanced by identification of increased numbers of tissue-specific proteins. Because cells are predicted to contain thousands of proteins across a wide concentration range, prefractionation and additional protein extraction methods may be required.

The quality control of SGP differentiation (i.e., to determine whether SGPs are differentiating appropriately) can also be enhanced by comparison with the proteomes of additional organs. For this purpose, proteome maps should be created for all porcine organs using the method described in the current study to select markers for each organ. Identification of useful marker proteins could be improved by performing not only the qualitative analysis described here but also quantitative analysis, for example by using ICAT or iTRAQ reagents (Dunkley et al., 2004; DeSouza et al., 2005).

Although we identified expected tissue-specific proteins, we observed some unexpected expression patterns. For instance, alpha-2-HS-glycoprotein precursor was previously known to be expressed mostly by the liver and to be secreted into the serum (Brown et al., 1992), but we identified it in the salivary gland proteome. Other liver markers, such as albumin, alcohol dehydrogenase, and arginase I, were also identified in the salivary gland proteome. These unexpected findings of liver-specific proteins in the salivary gland suggest two possibilities: (i) the proteins are delivered to the salivary gland by the blood stream or are contaminants of the salivary tissue with blood components, or (ii) the proteins are normally expressed in the salivary gland. With respect to the latter possibility, it is possible that proteins are expressed in both organs because these organs are both derived from the endoderm. This also is consistent with the fact that somatic stem cells isolated from the salivary gland can transform into a hepatic lineage.

In addition to the usefulness of proteome maps for

assessing the state of SGP differentiation, they are also useful for comparison with the human proteome. Porcine organs have been extensively studied for use in xenotransplantation to humans because they have similar sizes and physiological functions (Kurome et al., 2005). Most of the previous work in this area has focused on the elimination of hyperacute immunorejection and porcine endogenous retroviruses (Takeuchi et al., 2005). To achieve xenotransplantation from pig to human, however, it will be necessary to compare the expression and function of proteins in both species. The protein catalogs obtained here should help in this regard.

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