Comparison of Protein Composition in Stimulated vs. Unstimulated Whole Human Saliva

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A thesis presented by Chad D. Carver, D.D.S.
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Abstract:

Introduction: Saliva is an easily accessible biofluid with diagnostic potential. Essential to saliva-based diagnostics is a complete catalog of proteins in saliva, as well as an understanding of the effect of collection method on its composition. For example, little is known of the changes to the whole human salivary proteome caused by stimulation. **Purpose**: The purpose of our study was to compare the proteome of stimulated vs. unstimulated whole human saliva. **Methods and materials**: Two samples of whole saliva, one unstimulated, one stimulated with citric acid, were collected from five healthy male subjects age 27-32. Saliva samples were analyzed using highly sensitive 2-dimensional-liquid chromatography/mass spectrometry (2D-LC/MS) to identify both major and minor proteins present. Relative protein amounts were estimated from the 2D-LC/MS data utilizing spectral counting. Changes in protein relative abundance were compared using paired two-tailed Student's t-test. **Results:** The 2-DLC study was able to identify, with high confidence, 509 non-redundant proteins. 288 proteins found in at least 3 of 5 subjects were compared to detect changes in relative abundance. 36 proteins were found to change significantly ($p<0.05$) following stimulation (14 increased, 22 decreased). **Conclusions:** Stimulation with citric acid alters the proteome of human whole saliva, inducing changes in protein relative abundance. Future biomarker and proteome studies of whole saliva will need to account for the effect of stimulation on protein composition when considering collection protocols.

Introduction:

In the post-genomics world, direct study of the effector molecules of genes, proteins, has led to a rapidly growing field known as proteomics. One promising area of proteomics is the discovery of protein biomarkers that can diagnose diseases at early stages, monitor disease progress, or measure the response of disease to treatment (Hofman 2001, Jacobs et al. 2005, Vitzhum el at., 2005).

Serum is the most commonly used biological fluid for proteomic studies (Jacobs et al. 2005), but easier fluids, such as saliva, are beginning to receive considerable attention (Alaiya et al. 2005, Marko-Varga et al. 2005, Vitzhum 2005, Wong 2006). Since saliva contains many possible serum-derived proteins in addition to secretions from major and minor salivary glands (Wilmarth et al. 2004), it is conceivable that saliva may be an alternative to serum for some diagnostic tests.

Many different collection techniques have been used in proteomic saliva studies in the past, some using mechanical stimulation (Yao et al. 2003, Huang 2004) or no stimulation (Ghafouri et al. 2003, Vitorino et al. 2004, Wilmarth et al. 2004, Hu et al. 2005, Xie et al. 2005, Walz et al. 2006). While several studies have examined the effect of stimulation on selected proteins (Johnson et al. 2000, Francis et al. 2001, Becerra et al. 2003, Seeman et al. 2005, Hoek et al. 2005, Seeman et al. 2005, Neyraud et al. 2006), large scale proteomic studies of multiple biological subjects and differences associated with collection techniques have not yet been performed.

The purpose of this study was to determine how stimulation of flow alters the protein composition of whole saliva. The results of this study will suggest the best strategy to collect whole saliva when performing diagnostic tests. For example, unstimulated whole saliva is expected to yield the highest concentration of serum-derived proteins (from crevicular fluid) (Hoek 2002), while stimulated whole saliva is expected to have a greater concentration of glandular derived proteins. In addition, the multiple pathways involved in protein secretion may yield different proteins under stimulated compared to unstimulated conditions (von Zastow and Castle 1987, Gorr et al. 2005). It is also well documented that glandular contribution by volume changes with stimulation (Mandel 1972, Shannon 1974, Edgar 1990). Since the individual salivary glands secrete different protein mixtures (Walz et al. 2006), a concomitant change in whole saliva protein composition with changing glandular contributions would be expected.

This study extends previous cataloging studies by comparing whole saliva during unstimulated and stimulated flow with citric acid from five healthy male subjects. A mass spectrometer with a high sensitivity linear ion trap with fast scan speed was used to identify 509 proteins. This greatly increased the number of proteins detected in saliva over previous efforts. The new technique of estimating protein abundances by MS/MS spectral counting identified 36 proteins that changed in relative abundance during stimulated saliva flow.

Literature Review:

Saliva

Origin and Anatomy

Saliva is a clear, slightly acidic, mucoserous, exocrine secretion in the oral cavity. It is a complex mixture of fluids from the major and minor salivary glands, crevicular fluid, bacteria, and food. The major salivary glands are paired, and include the parotid, sublingual, and submandibular glands, as shown in Figure 1. The minor glands include the labial glands, lingual glands, palatine glands, buccal glands, molar glands, incisive glands and the glands of the nasopharynx. The minor glands secrete many protective components, while the major glands produce most of the volume of saliva (Humphry and Williamson 2001).

The parotids are the largest salivary glands. They are wedge-shaped and situated in front of the ear and behind the ramus of the mandible and are intimately associated with the peripheral branches of the facial nerve (CN VII). The duct of the parotid (Stenson's duct) emerges at the anterior border of the gland and opens into the oral cavity in a papilla on the buccal mucosa opposite the second upper molar tooth (Whelton 2004). The parotid secretions are serous.

The submandibular glands (SM) are variable in size, about half the size of the parotid. Located in the floor of the mouth, the submandibular gland has a thin walled duct that runs forward between the side of the tongue and mylohyoid. It opens into the floor of the mouth underneath the anterior part of the tongue on the summit of the

sublingual papilla lateral to the lingual frenum (Wharton's duct). The secretions are a mixture of mucous and serous fluids (Whelton 2004).

The sublingual glands (SL) are the smallest of the paired major salivary glands, about one fifth the size of the submandibular glands. They are situated in the floor of the mouth beneath the sublingual folds of mucous membrane. Predominantly mucous secretions enter the oral cavity through 8-20 small ducts on the summit of the sublingual fold (Whelton 2004).

Minor salivary glands are located throughout the oral cavity, including the lateral border of the tongue, the posterior part of the palate and in the buccal and labial mucosa. Their secretions are primarily mucous (Roth and Calmes 1981).

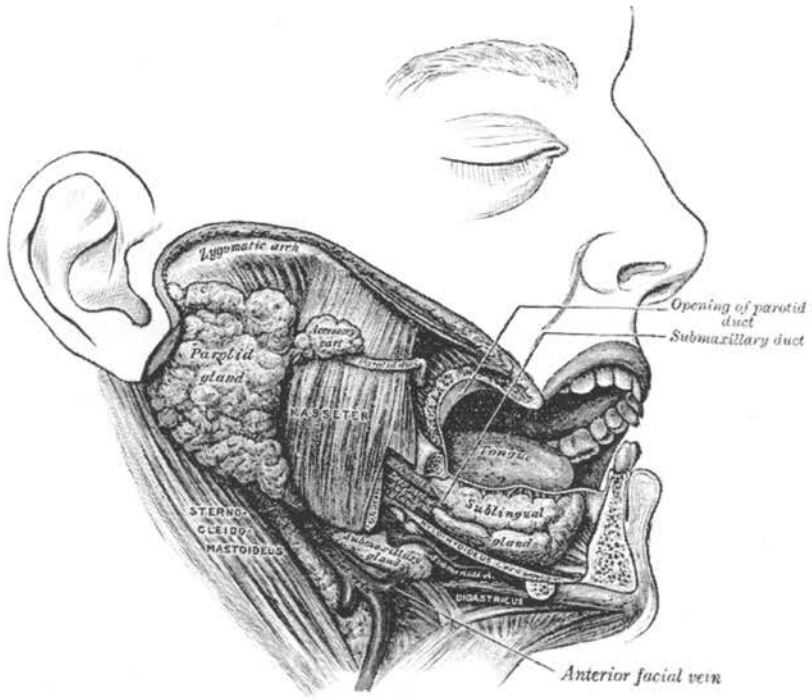

Figure 1: *Dissection, showing salivary glands of right side. Note: "submaxillary" refers to present day submandibular gland. (Taken from Wikipedia.org, from the 20th U.S. edition of* Gray's Anatomy of the Human Body, *originally published in 1918.*

Structure of salivary glands

The working parts of the salivary glandular tissue consist of the secretory end pieces (acini) and the branched ductal system, depicted in Figure 2a. The cells in the end pieces are arranged in a spherical form in serous glands or in a tubular configuration with a large central lumen in mucous glands. In both gland types, the cells in the end piece surround a lumen which is the start of the ductal system (Whelton 2004).

Types of cells found in salivary glands incude acinar cells, various duct system cells, and myoepethelial cells. Saliva is first secreted from the end piece acinar cells, which determine the type of secretion from the various glands (Humphry and Williamson 2001). For example, secretions from the parotid gland are serous or watery in consistency; those from the SM and SL glands, and particularly from the minor mucous glands, are much more viscous due to their glycoprotein content. Acinar cells exhibit histological polarity, with a high density of secretory vesicles at the apical pole (Fig. 2b). The secretions are unidirectionally produced into the duct system (von Zastrow and Castle 1987, Smith 2004).

The three types of duct system cells include the intercalated, striated and excretory duct cells. The intercalated cells line the first duct connecting acinar cells to the rest of the gland. Intercalated cells are not involved in electrolyte modification. The second type of ductal cell in the network is the striated cell, which is involved in sodium reuptake and electrolyte regulation (Humphrey and Williamson, 2001). Striated duct cells also exhibit polarity, with basal infoldings and a high density of mitochondria

(Smith 2004). The final part of the ductal system before exodus into the oral cavity consists of excretory duct cells.

The last cell types found in salivary glands are the myoepithelial cells. These cells wrap around acinar cells, and contract to constrict acinar cells during regulation of secretion (Humphrey and Williamson, 2001).

Figure 2:

a. b.

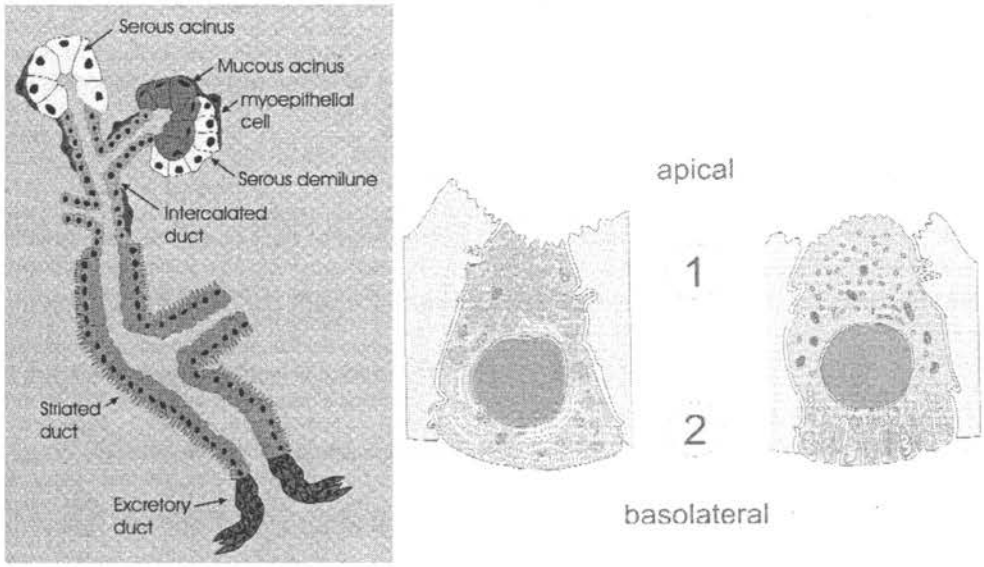

Figure 2: Structure of the salivary glands. a.) Diagram of the secretory end piece (acini) and the branched ductal system (Whelton 2004). b.) Diagram showing histological polarity of an acinar cell on the left with high density of secretory vesicles at the apical pole, and a striated duct cell with a high density of mitochondria at the basolateral pole (Smith 2004).

Composition

Saliva is a dilute fluid, consisting of 99% water, and is a mix of various electrolytes (Na, K, Ca , Mg, bicarbonate, phosphate), immunoglobulins, proteins, enzymes, mucins, and nitrogenous products (urea and ammonia) (Dawes 2004, Schenkels et al. 1995). The major glands contribute most of the volume and most of the electrolytes, while the minor glands contribute most of the blood-group substances (Humphey and Williamson, 2001). These components occur in small amounts and vary with flow rates.

The components of saliva interact to perform various functions. For example, bicarbonates and phosphates provide buffering action and modulate the pH of saliva. Macromolecules, proteins, and mucins serve to cleanse the oral cavity, aggregate microorganisms, and are involved in dental plaque metabolism. For example, Ca^{+2}, phosphate and proteins interact to modulate demineralization and remineralization of the tooth surfaces (Humphey and Williamson, 2001).

Saliva is isotonic as it forms in the acini, and becomes hypotonic as it passes through the ducts. The hypotonicity of saliva serves many functions. The low levels of glucose, urea, and bicarbonate, and sodium allow for better taste perception in comparison to the high levels of these components found in plasma. Hypotonic saliva also allows for the expansion and hydration of mucin glycoproteins (Humphrey and Williamson 2001).

The pH of saliva is slightly acidic, normally falling between 6.5-7.4 (Pedersen et al. 2002), and is flow dependent. Bicarbonate, the most important buffer in saliva, is

taken up by the striated duct cells. As stimulated flow increases, proportionately less bicarbonate is removed from saliva, increasing its concentration and the resultant pH of saliva (Whelton 2004). Saliva can range from pH 5.3 (low flow) to pH 7.8 (highest flow) (Humphrey and Williamson 2001).

Many factors affect salivary composition. These include glandular source, flow rate, duration of stimulation, previous stimulation, biological rhythms, nature of stimulus, plasma composition, hormones, pregnancy, genetic polymorphisms, antigenic stimulus, exercise, drugs, and various diseases (Dawes 2004). The influence of these factors needs to be considered in evaluating the diagnostic capabilities of saliva and determining collection protocols.

Functions of Saliva

The functions of saliva can be organized into 5 major categories: 1) taste and digestion, 2) lubrication and protection, 3) buffering action and clearance, 4) maintenance of tooth integrity, and 5) antibacterial activity (Mandel 1987). Salivary components involved in these functions tend to be redundant, multifunctional, and amphifunctional (for and against the host). The multiple functions of many salivary components are summarized in Figure 3.

The first functional category of saliva is the enhancement of taste and the beginning of the digestive process. As mentioned above, the hypotonicity of saliva enhances the tasting capacity of salty foods and nutrients. Various proteins, such as carbonic anhydrase (gustin), which bind zinc, enhance the sensation of taste. Saliva is

also involved in early digestion by breaking down various food products. For example, amylase, the most abundant parotid protein, breaks down starch and dissolves sugar. Other enzymes in saliva initiate fat digestion. Another important digestive function provided by saliva is the lubrication of the food bolus, which is provided by salivary mucins (Humphrey and Williamson 2001).

The second category of saliva function is that of lubrication and protection of oral cavity surfaces. The seromucous coating secreted from the minor glands contains a high abundance of mucin. This coating aids in mastication, speech, and swallowing and provides protection from irritants such as proteolytic enzymes, chemicals, and dessication (Amerongen and Veerman 2002).

Buffering and clearance are the third function of saliva. Components involved in this function include bicarbonate, histidine-rich peptides, urea, and phosphate. Bicarbonate is the most important buffering system in saliva. It diffuses into plaque to neutralize acidic assaults on tooth structure, and also generates ammonia to form amines which add to the buffering capacity. The majority of the nonbicarbonate buffering is provided by histidine-rich proteins and peptides. Additional buffering is provided by ammonia release from urea and by phosphate, which is likely to be important as a buffer only during unstimulated flow (Humphrey and Williamson 2001).

The fourth functional category of saliva is the maintenance of tooth integrity, or regulation of the demineralization and remineralization process. Saliva contains supersaturated concentrations of calcium and phosphate ions which provide a reservoir for remineralization (Amerongen and Veerman 2002, Pedersen et al. 2002). Pellicle

forming salivary components, such as statherins, cystatins, histatins, and proline-rich proteins (Vitorino et al. 2006), aid in controlling crystalline growth of the enamel by allowing the penetration of minerals into the enamel for remineralization and by limiting the egress of minerals. Finally, fluoride in saliva forms caries-resistant flouroapetite crystals on the tooth surface, inhibiting demineralization.

The fifth category of saliva function is to form the first line of defense against bacterial and viral attack. Saliva contains secretory IgA, IgM, and IgG immunoglobulin complexes. IgA, the predominant immunologic component of saliva, is produced by plasma cells and translocated through the duct cells of major and minor salivary glands. Other immunoglobins in saliva are found in low quantities and are likely derived from gingival crevicular fluid. Nonimmunologic defense components are mostly produced by acinar cells, and include mucins, peptides, and enzymes. Mucins, such as MG2, complex with IgA and produce a higher affinity for pathogen binding than either MG2 or IgA alone (Amerongen and Veerman 2002). Bound pathogens are cleared during swallowing. Lactoferrin, likely secreted by ductal cells, binds iron which would otherwise be used as a food source for microbes. Lysozymes inhibit bacterial growth by lysing bacterial cell walls. Acinar cells secrete peroxidase, which catalyzes bacterial metabolic by-products. Cystatins, in addition to the regulation of calcium, inhibit cysteine-proteinase activity. Finally, salivary proteins such as glycoproteins, statherins, agglutinins, histidine-rich proteins, and proline-rich proteins work by aggregating proteins and other compounds. This action reduces the pathogen binding to tissues and facilitates their clearance from the oral cavity (Humphrey and Williamson 2001).

Figure 3: Summary of salivary component functions (Bailey 2006).

Mechanisms of Salivary Secretion

Salivary secretion is the unidirectional movement of fluid, electrolytes, and macromolecules into saliva in response to appropriate stimulation. The major stimulus for salivation is taste, although input from mastication and from other senses, such as smell, sight and thought are also thought to be integrated into the neural control of secretion. Afferent input is carried to the solitary nucleus in the medulla via the facial (VII) and glossopharyngeal (IX) nerves. Parasympathetic efferent pathways of the SMSL glands arise from the facial nerve and for the parotid gland from the glossopharyngeal nerve. Fluid secretion is regulated in these pathways by releasing acetylcholine (Ach) at the surface of the salivary gland acinar cells. Protein secretion is regulated by release of norepinephrine from sympathetic nerves (Smith 2004).

The secretory stimulus is carried from the nerves into the acinar cells via second messengers, which provide large intracellular amplification in response to a small extracellular stimulus. Binding of Ach to muscarinic M3 receptors activates fluid secretion, while the binding of norepinephrine to B-adrenergic receptors activates macromolecule secretion. Both of these receptors cause activation of a G-protein, which in turn activates a target enzyme. The target enzyme for fluid secretion is phospholipase C, and for protein secretion it is adenylate cyclase (Smith 2004).

Fluid Secretion

Phospholipase C (PLC) activation produces inositol 1,4,5 trisphosphate (IP3), which binds to receptors located on endosomes and the endoplasmic reticlulum, releasing calcium (Ca^{+2}). This creates a positive feedback release of Ca^{+2} into the cytoplasm (Smith, 2004) and opens basolateral K^+ channels and apical Cl^- channels. These Ca^{+2} associated changes in K^+ and Cl^- conductance allow KCL to flow out of the cell, resulting in the accumulation of Cl^- ions and their associated negative electrical charge in the acinar lumen. To maintain electroneutrality, Na^+ follows Cl^- into the lumen by leaking from the interstitium through the tight junctions between the cells. The resulting osmotic gradient for NaCl causes a transepithelial movement of water from interstitium to the lumen (Turner and Sugiya, 2002). Downregulation of the Ca^{+2} signal is mainly controlled by calcium ATPase activity to pump the Ca^{+2} back into the stores or out of the cell, which results in the closing of the K^+ and Cl^- channels.

Protein Secretion

Adenylate cyclase is activated following binding of norepinephrine to adrenergic receptors and activation of G-protein. Adenylate cyclase converts ATP into cAMP, the 2nd messenger responsible for subsequent steps in macromolecule secretion. Binding of cAMP to protein kinase A results in the separation of its catalytic subunits, which, in turn, phosphorylates (and thereby upregulates) cellular proteins responsible for the synthesis and secretion of secretory macromolecules. In addition, an increase in cAMP stimulates post-translational modification (eg glycosylation), migration of secretory vesicles to the apical membrane, and exocytosis (Smith, 2004).

More than 90% of the protein synthetic activity of salivary acinar cells is devoted to the production of secretory proteins (von Zastrow and Castle 1987), of which 80-90% are stored in secretory granules (Gorr et al. 2005) (Fig. 2b). Secretory proteins start with a signal sequence directing them to the ER where they are N-glycosylated and folded into proper 3-D structures. Salivary proteins exhibit vectorial transport from the ER to successive membrane-bounded compartments. These include the Golgi complex, condensing vacuoles, and secretion granules where they are post-translationally modified, packaged and stored prior to discharge (von Zastrow and Castle 1987). Pulse-chase labeling experiments have shown that protein drainage from the ER through the successive vesicles to the final secretory granule begins at 30 minutes and peaks at 60 minutes (von Zastrow and Castle 1987).

Acinar cell secretory pathways

Many pathways of protein secretion in salivary acinar cells have been identified. Much of what is known has used the parotid as a model and most experiments utilize either direct collection of saliva from the ducts of the major salivary glands of humans, salivary cell lines, or animal models (Gorr et al. 2005). Data obtained from these experiments have provided insight into the complex secretory patterns of salivary glands and the multiple pathways for protein secretion. The seven secretory pathways that have been defined include: 1) major regulated secretory pathway, 2) minor regulated secretory pathway, 3) apical constitutive secretory pathway, 4) constitutive-like secretory pathway, 5) basolateral constitutive secretory pathway, 6) basolateral secretion of secretory granules, and 7) alternate basolateral constitutive secretory pathway (Gorr et al. 2005). Figure 4 summarizes acinar cell secretory pathways.

The major and minor regulated secretory pathways arise from the maturation of large secretory granules and are expressed with stimulation. The major regulated pathway is considered the classic secretory pathway in exocrine cells, and accounts for 80-90% of total protein secretion from parotid acinar cells. The major regulated pathway depends on the retention of proteins in large granules which are eventually secreted into the lumen from the apical side of the cell (von Zastrow and Castle 1987). The minor regulated pathway consists of some of the proteins removed during maturation of secretory granules. These distinct smaller transport vesicles are secreted in response to pilocarpine and low dose of isoproterenol, conditions that do not elicit the secretion of proteins from the mature granules (Gorr et al. 2005).

Basal secretion refers to secretion that does not depend on strong extracellular stimulation of the acinar cells. This 'resting' secretion occurs between meals and during sleep. Basal secretion includes exocytosis from the minor regulated secretory pathways, in addition to the constitutive and constitutive-like pathways (Gorr et al. 2005). Constitutive secretion is the pathway that describes the small continuous output of salivary proteins in the absence of stimulation. Constitutive secretion may be the only secretion from parotid acinar cells that does not originate in granules, but instead originates in the trans-Golgi network. The constitutive-like secretory pathway allows secretion of proteins from maturing secretory granules in the absence of stimulation. It provides the slow phase of basal protein secretion seen in pulse-chase experiments with parotid cells (von Zastrow and Castle, 1987).

While much of salivary acinar cell protein secretion is directed towards apical cell membranes for exocrine activity, there also exist multiple basolateral protein secretion pathways. Basolateral secretion of proteins into circulation is thus far poorly defined, but may involve a separate vesicle pathway that originates in the trans-Golgi network or secretory granules that have been redirected from the apical granule-derived pathway (Gorr et al. 2005). The ability of salivary glands to deliver proteins into the circulation coupled with easy access for transfection or infection via the salivary ducts allows their consideration as targets for gene therapy protocols aimed at expressing secretory proteins. For example, significant amounts of secretory proteins such as insulin and hGH can be delivered to circulation using parotid glands, most likely via basolateral secretory pathways (Baum et al. 2004).

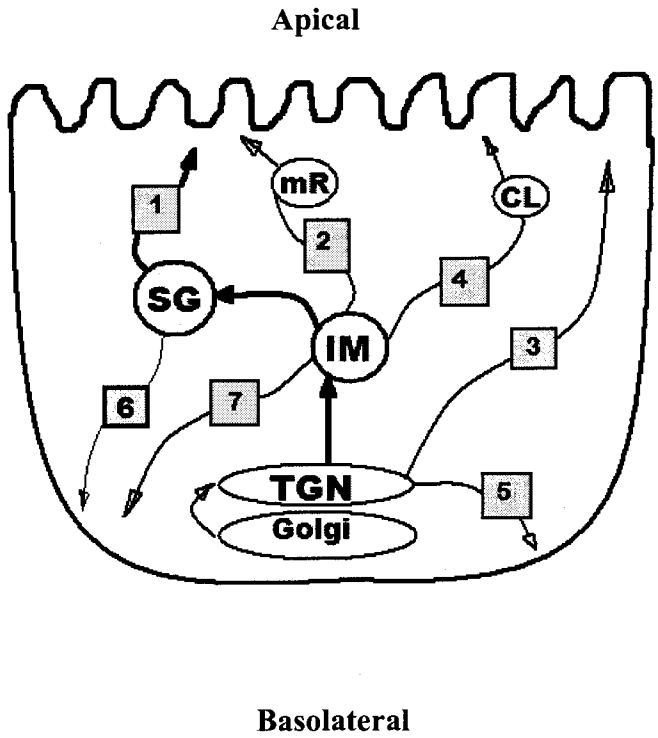

Figure 4: Secretory pathways in parotid acinar cells. TGN, trans-Golgi network; IM, immature secretory granule: SG, mature secretory granules; CL, constitutive-like secretory vesicle; mR minor regulated pathway vesicle. The arrows indicate transport pathways: 1) major regulated secretory pathway, 2) minor regulated secretory pathway, 3) apical constitutive secretory pathway, 4) constitutive-like secretory pathway, 5) basolateral constitutive secretory pathway, 6) basolateral secretion of secretory granules, and 7) alternate basolateral constitutive secretory pathway. (Adapted from Gorr et al., 2004)

While individual proteins can be secreted via multiple pathways, many proteins are secreted preferentially by specific pathways. For example, amylase is secreted prominently by both the regulated and constitutive-like secretory pathways, while parotid secretory protein (PSP) is found in abundance only in the regulated secretory pathway. Therefore, PSP is a better marker for large secretory granules than amylase (von Zastrow and Castle 1987).

Non-acinar cell protein secretion pathways

Not all secretory proteins are synthesized by the acinar cells. Some proteins secreted from salivary glands arise from the serum, either by endocytosis into acinar cells or perhaps from diffusion through dilated tight junctions between acinar cells. For example, IgA binds to receptors on the basolateral side of the salivary acinar cell and is taken into the cell by endocytosis. Following fusion with existing secretory vesicles, IgA is then secreted from the apical side by exocytosis (Smith 2004).

In salivary glandular tissues, a junctional complex consisting of a tight junction, adhering junction, and desmosomes joins adjacent cells and separates the luminal space from the intercellular and interstitial spaces. Studies of rat parotid and SMSL glands have shown that some proteins can flow through tight junctions between the basolateral and luminal spaces following stimulation. Mazariegos et al. (1984) administered protein tracers of various molecular weights via the main excretory duct of resting and isoproterenol-stimulated glands. It was concluded that tight junctions in the resting rat parotid gland were impermeable to tracers of molecular weight >1.9 kDa, but stimulation with isoproterenol resulted in a transient increase in junctional permeability allowing passage of tracers of molecular weight <34.5 kDa. Another study of the rat submandibular gland found that electrical stimulation of parasympathetic and sympathetic nerves causes an increase in tight junctional permeability of acinar cells to microperoxidase (1.63 kDa) and horseradish peroxidase (40 kDa), but not lactoperoxidase (82 kDa) (Kawabe and Takai 1990). Structural changes in rat salivary gland tight junctions during secretion have also been identified (Hashimoto et al. 2000,

Mazariegos et al. 1984). It is thus possible that stimulation-induced changes in salivary glands allow passage of smaller plasma proteins into glandular secretions.

Proteins not originating from secretory glands

While many proteins found in whole saliva are derived from glandular secretion, saliva composition also reflects a passive contribution of serum-derived proteins. For example, crevicular fluid, especially in the presence of inflammation, is a known contributor of many serum-derived proteins, such as albumin and Zinc-alpha2-glycoprotein (Schenkels et al. 1995). In addition, proteins secreted from cells found in the oral cavity, such as endothelial cells and neutrophils, contribute to the composition of proteins in whole saliva.

Stimulated vs. Unstimulated Saliva

Many differences exist between stimulated and unstimulated saliva, including flow rate, percentage contribution from various glands, and protein composition. Flow rate of saliva greatly increases with stimulation. Whole saliva flow rate increases from an average of 0.3 mL/min unstimulated to as much as 7 mL/min with stimulation (Edgar 1990, Grand et al. 1988). During unstimulated flow, percentage contributions from the different salivary glands are 20% from parotid, 65% from submandibular, 7% to 8% from sublingual, and less than 10% from numerous minor glands. Volume contributions from each gland change drastically with stimulated high flow rates, with the parotid contributing more than 50% of total salivary secretions (Edgar 1990). Stimulation

increases concentrations of proteins, sodium, chloride, bicarbonate, and calcium, and decreases concentrations of magnesium phosphate, urea, ammonia, and uric acid. pH also increases with stimulation (Amerongen 2002, Becerra 2003).

Factors affecting flow rates

Three types of local stimulus can elicit increased salivary flow rates, including mechanical (chewing), gustatory (with acids the most stimulating and sweet the least), and olfactory (the least stimulating of the three). Several factors have deleterious effects on salivary flow. For example, increased age (Navasesh 1992), systemic or localized diseases such as Sjogren's, and many medications can decrease salivary flow (Roth and Calmes 1981, Shannon 1974, Grand et al. 1988).

Circadian rhythms and diurnal variation also affect flow rate and protein composition (Dawes 1975, Oberg 1982, Hardt et al. 2005). Dawes reported that the body temperature and flow rate of saliva peak during the late afternoon, but the flow rate drops to almost zero during sleep (Edgar 2004). Hardt et al., 2005 monitored diurnal changes in the abundance of native peptide species found in parotid saliva using isotope labeling and MALDI TOF/MS. In several cases, abundance during the day changed dramatically. Therefore, standardizing the time of day at which saliva is collected is an important variable to control.

Saliva as a Diagnostic Biofluid

There has been considerable interest in saliva for use as a diagnostic biofluid (Alaiya 2005, Marko-Varga 2005, Vitzhum 2005, Wong 2006) since variable amounts of blood, serum, serum products, gingival crevicular fluid, electrolytes, epithelial and immune cells, microorganisms, bronchial products and other foreign substances are present in whole saliva. This varied and rich mixture of substances makes saliva a likely source of identifying unique biomarkers that may identify oral and systemic health changes (Hirtz 2005, Wong 2006). In comparison to other diagnostic biofluids, such as blood serum, saliva has some advantages: it is easier to collect, store and transport. Collection of saliva creates few risks, making it an attractive alternative to serum for diagnostic purposes, particularly for children, the elderly, the mentally handicapped, and IV drug users. The ease of collection decreases expense, and makes self-administered, frequent diagnostic testing possible. For example, due to diurnal and monthly variations, several hormone samples need multiple samples at various times during the day. This type of testing is difficult, if not impossible, with analogous blood diagnosis (Hofman 2001).

One disadvantage of saliva as a diagnostic biofluid is that interpretation of saliva assays is still difficult. Few studies have been published that control for known variables such as pH, time of day and month, and medications. In addition, variable amounts of contamination may arise from bleeding gingiva, skewing the abundance of serum derived proteins. Saliva to serum ratios of steroid hormones, for example, are approximately

1:200 (Hofman 2001). Collection methods have not been clearly defined, and many questions remain unanswered. Examples include; should unstimulated or stimulated saliva be collected, what amount is needed, what pretreatment of saliva is appropriate before assaying and storage, and how to collect from patients with disease or medications causing xerostomia. Other considerations include the metabolism of hormones, post-translational modification of proteins, and diurnal timing (Hofman 2001).

Salivary biomarkers have already shown promise in the diagnosis of many diseases, including oral cancer, periodontitis (Hormia et al. 1993, Miller et al. 2006), HIV (Hodinka et al. 1998), Hep B (Fisker et al. 2002), breast cancer (Steckfus et al. 2006), and measles (Nigatu et al. 2001). Immunoassays have been developed to detect both secretory IgA and serum-derived IgG (from crevicular fluid) found in saliva for various diseases. In addition, saliva has shown potential for hormone and drug screening (Hofman 2001, Cone et al. 2002, Kaufman and Lamster 2002).

Serum remains one of the most utilized diagnostic biofluids. Many of the proteins found in serum are also found in saliva (Wilmarth et al. 2004), suggesting diagnostic capability for saliva. Many mechanisms exist for transport of proteins and ions from serum into salivary gland ducts. These include active transport of selected compounds, passive diffusion of lipid soluble compounds, and simple filtration from capillaries through ductal membranes (Schenkels et al. 1995, Turner and Sugiya 2002).

There are many potential types of biomarkers that might be utilized in salivary diagnosis. These biomarkers range in purpose from categorization of disease, prediction of future disease, detection of disease, and monitoring of treatment outcomes (Vitzhum et al. 2005). These categories contain biomarkers that are qualitative, quantitative, or both, and some examples are summarized in Table 1.

Table 1: Biomarker Types		
Biomarker type	**Description**	**Example**
Acute markers	Acute disease event, provides information for specific treatment	B-type natriuretic peptide to rule out heart failure in patients with acute dyspnea
Screening markers	Identify diseased within a population to begin treatment as soon as possible to ensure high treatment success	Albumin in urine for renal disease
Primary risk assessment markers	Assess risk of disease in future	Cholesterol for cardiovascular disease
Secondary risk assessment	Assess how disease may develop, risk to suffer other disease, etc.	Cardiac troponins in myocardial infarction patients as indicator for morbidity and mortality
Disease staging or classification	Classify disease states	
Treatment response stratification	Predict response to pharmaceutical tx before its application	Hemostasis markers prior to anticoag therapy, viral and bacterial resistance
Treatment or therapeutic monitoring	Effectiveness of treatment	Blood lipids track impact of exercise, nutrition, etc.
Therapeutic drug monitoring	Determine pharmaca administered	Immunosuppressive drugs have to be monitored to prevent graft rejection
Compliance	Track treatment compliance	Glycosylated hemoglobin A used to monitor insulin therapy compliance

Table 1: Biomarker types, descriptions, and examples. (Adapted from Vitzhum et al., 2005)

Often the specificity and sensitivity can be greatly improved if more than one biomarker can be identified for a given diagnostic purpose. For example the sensitivity of a diagnostic test for bladder carcinoma increased from 43% using individual markers, to 87% when combinations were used (Vlahou et al. 2001). The improvement derived from adjunctive biomarkers stresses the advantages of considering global changes for diagnostics. Only recently have new techniques allowed the simultaneous analysis of great numbers of proteins.

Diseases show a continuum ranging from completely genetic in origin, such as Down's syndrome, to completely environmental, such as scurvy. Most diseases lie somewhere in the middle, and people with different genes will react differently to the same stressors. Because the expression levels of proteins, the effector molecules created from genes, are sometimes partially controlled by the environment, proteomics can shed useful information on a wide spectrum of diseases.

Salivary Proteomics

Mapping proteomes, the protein complements to genomes, from tissues, cells and organisms is being used to identify new protein targets, to explore mechanisms of action of toxicology, and to discover disease biomarkers for clinical and diagnostic applications. Once the proteins expressed in saliva are known, their global changes in expression can be studied under changing environmental conditions.

Several recent studies have used proteomic techniques to separate the proteins present in saliva and used mass spectrometry to identify those proteins. Two-dimensional

gel electrophoresis (2-DE) is one method to separate the protein components, and mass spectrometry is used to identify the peptides produced from in-gel digests of the proteins of interest. 2-DE has the greatest protein resolving power of any available separation technique, provides information about modifications and proteolysis, and provides quantitative information. However, 2-DE has difficulty in resolving large or small molecular weight proteins, highly acidic or highly basic proteins, and hydrophobic proteins. There have been several 2-DE studies of whole saliva (Ghafouri et al 2003, Yao et al 2003, Vitorino et al 2004, Huang 2004, Hu 2005), parotid gland secretions (Hardt et al. 2005), and 2-DE comparisons of whole saliva to parotid and submandibular/sublingual secretions (Walz et al. 2005). These studies detected predominantly well-known salivary proteins; however, they lacked the sensitivity required to characterize most of the low-abundance proteins present in secreted saliva.

Multiplexed proteomics techniques, which often consist of prefractionation, multidimensional separation, and MS/MS techniques, are required to achieve a more comprehensive analysis. Several salivary proteomic studies have utilized such methods as 2-DE + liquid chromatography (LC) (Hu et al. 2005), strong cation exchange (SCX) chromatography + reverse phase liquid chromatography (RPLC) (Wilmarth et al. 2004), free-flow electrophoresis (Xie et al. 2004) and transient capillary isotachophoresis/capillary zone electrophoresis (CITP/CZE) (Fang et al. 2007) to expand the list of identified salivary proteins.

A summary of whole saliva proteome studies organized by methodology follows:

2-DE MS studies:

Multiple Glands:

Yao et al. (2003), compared protein components in enamel pellicle, whole saliva, and individual glands using peptide mass fingerprinting and MALDI-TOF tandem MS. The samples were collected from several individuals under stimulated flow then pooled. They found significant differences between the protein patterns observed for glandular secretions and whole saliva (WS), even though electrophoretograms from both parotid saliva (PS) and submandibular/sublingual saliva (SMSL) were simple and similar to one another.

Walz et al. (2006) collected unstimulated WS, PS, and SMSL from four individuals (two female, two male). Their aim was to compare the protein map of WS to parotid and SMSL secretions, with an emphasis on proline rich proteins (PRPs) and mucins. Acidic PRPs were found equally in parotid and SMSL secretions, whereas basic PRPs and proline-rich glycoprotein were found primarily in parotid secretion. Salivary mucin MUC7 was identified in SMSL secretion. A MALDI-TOF MS approach was used. They concluded that, with little intra-and inter-individual variations, most components in WS could be explained by mixed contributions of the two major salivary glands. Some spots apparent in WS, but not in glandular salivas included calgranulin A, calgranulin B, and transferrin. Conversely, some spots seen in glandular secretions that could not be detected in WS included histatin 1, cystatin S, or immunoglobulins. Little intra-individual variation at different time points was noted. Differences among

individuals were seen in WS only, and may have reflected the momentary status of the local environment of the individual's oral cavity during collection.

Whole Saliva only:

Ghafouri et al. (2003) characterized unstimulated WS from 5 pooled individuals. Their aim was to map proteins in saliva by 2-DE and to identify abundant proteins by peptide mass fingerprinting using trypsin cleavage and MALDI –TOF MS. In-gel digestion of 150 spots resulted in protein identifications of 101 spots from only 20 different proteins. This list did not contain a number of well-known salivary proteins (lactoferrin, cystatin C, mucins, histatins, peroxidase, and carbonic anyhydrase). Possible explanations were that those proteins were from the spots that could not be identified or that the p*I* of the missing proteins was outside the range of pH 4-7 used in the first dimension separation.

Huang (2004) collected WS from 2 males and 2 females with no overt signs of gingivitis or caries and compared them to 2 males and 2 females with at least 2 sites that bled after gentle periodontal probing and showed at least 4 mm of pocket depth. Samples were collected by expectoration following 3 min of tooth brushing without toothpaste. The tooth brushing caused noticeable bleeding in the tooth decay group. Fifty-four spots, from 26 different proteins were identified using N-terminal sequencing and mass-spectrometry. Ten proteins, alpha-1-antitrypsin, apolipoprotein A-1, cystatin A, SA, SA-III, and SN, enolase I, hemoglobin B-chain, thioredoxin peroxiredoxin B, and prolactin-

inducible protein, showed significant abundance changes when bleeding occurred in the oral cavity.

Vitorino et al. (2004) collected unstimulated saliva from a single subject. Proteins were separated using a pH range between 3-10, digested, then analyzed by MALDI-TOF MS/MS. 100 spots were analyzed and 28 proteins were identified.

2DE-MS, LC-MS/MS:

Hu et al. (2005), collected unstimulated WS from one individual and claimed to identify 309 proteins using a combination of 2-DE-MS and LC-MS/MS. The WS proteins were prefractionated into 3 fractions based on molecular weight prior to trypsin digest and LC-MS/MS (Q-TOF electrospray). A database search using MASCOT in which trypsin was specified yielded 266 nonredundant protein identifications. However, only 122 proteins (46%) were matched with two or more peptides. The remaining 54% were identified based on only a single peptide hit, and 82 of those proteins had sequence coverage of less than 2%. 2-DE followed by fluorescent SYPRO staining resolved more than 300 protein spots. Among the putative 64 proteins identified by 2-DE-MS, only 21 proteins overlapped with proteins identified in the LC-MS experiments.

Free Flow Electrophoresis-MS/MS:

Xie et al. (2005) collected unstimulated WS from one healthy female. This study utilized free flow electrophoresis for first dimension peptide fractionation followed by LTQ MS/MS. In addition to peptide separation, free flow electrophoresis adds peptide pI information which can significantly improve the confidence of the identifications. 437 proteins were identified with claimed high confidence (peptide false positive rate below 1%). Single peptide per protein identifications were allowed and no estimated protein false positive rate was reported.

2-DLC MS/MS (MudPIT):

Wilmarth et al. (2004) collected unstimulated whole saliva from one healthy male. The WS sample was digested with trypsin, and separated by strong cation exchange followed by reverse-phase liquid chromatography electrospray MS/MS (LCQ). Search criteria and peptide match scores were clearly defined for validating matches. Manual validation was utilized for all protein matches for all protein identifications based on two or more distinct peptide matches. To identify proline-rich proteins (which have few tryptic cleavage sites) no enzyme specificity was used in the SEQUEST search. This study identified 102 non-redundant proteins with an estimated protein false positive rate of less than 1%. Parsimony filtering of the proteins and removal of spurious immunoglobulin matches were used so that results were not over-inflated.

CITP/CZE + LC-MS/MS

Fang et al. (2007) used electrokinetic separation techniques to selectively deplete high abundance peptide from proteins such as amylase, mucins, PRPs, and secretory IgA to enhance detection of peptides from low abundance proteins. Using whole unstimulated saliva from one male subject, they were able to identify 1479 proteins (962 with 2 or more distinct peptides), a great increase over previous studies. While they reported a peptide false discovery rate (FDR) of 1%, their protein FDR was much higher at 8.8%. Quantification of protein abundance becomes skewed using this method, however, due to the relative enhancement of lower abundance peptidess.

Comparison of the catalogues of salivary proteins from these studies is difficult due to the lack of standards in publishing mass spectrometry-derived proteomic datasets (American Society for Biochemistry and Molecular Biology, 2007). Many of the studies did not describe the criteria for determining correct peptide and protein matches. In addition, no estimate of false positive rates or peptide sequence match scoring was provided in most of the studies. Finally, there was inconsistency in the databases used for searches, and many used larger databases having high effective sequence redundancy which can inflate results lists. All of these factors make comparison of salivary proteomic data sets difficult. Table 2 provides a summary of the whole salivary proteome studies discussed in this section.

Table 2: Summary of Whole Saliva Proteomic Studies

Author	Type of Study	Algorithm software	Protein Database	Stimulated?	Number of Subjects	Number of Identified Proteins
Ghafouri et al., 2003	2-DE MS	Peptident, MS-fit, MASCOT	NCBI or Swiss-prot	N	5	20
Huang, 2004	2-DE MS	MASCOT	Swiss-Prot	Y (Tooth brushing)	4	26
Vitorino et al., 2004	2-DE MS	MASCOT	NCBI nonredundant	N	1	28
Wilmarth et al., 2004	2-DLC MS/MS	SEQUEST, DTASelect	Swiss-Prot, NCBI	N	1	102
Yao et al., 2004	2-DE MS	MASCOT, Protein Prospector, PepSea and PeptideSearch	Not listed	Y (Paraffin)	Multiple (exact # not disclosed)	7
Hu et al., 2005	2-DE MS + LC-MS/MS	MASCOT, Pro ID	Not specified- "human protein sequence database"	N	1	309
Xie et al., 2005	Free-flow electrophoresis-MS/MS	TurboSEQUEST	Non-redundant human International Protein Index	N	1	437
Walz et al., 2006	2-DE MS	ProFound, SEQUEST, MASCOT	PMF-NCBInr without taxonomy restriction PFF- "a protein database"	N	4	29
Fang et al., 2007	CITP/CZE + LC-MS/MS	Open mass spectrometry search algorithm	Swissp-Prot	N	1	1479

Stimulated vs. Unstimulated Salivary Protein Studies.

Few publications exist analyzing the effect of stimulation on human whole saliva protein composition. Most studies have focused on individual proteins under specific conditions, with the type of stimulation varying greatly. Studies looking at protein changes in human saliva have typically analyzed samples from individual glands, not whole saliva. Many of the studies did not account for variables such as age, health, diurnal considerations, or sex of individuals. Protein abundances were influenced by type of stimulation, glandular source, and amount of time a stimulant was applied.

In considering the effect of stimulation on salivary protein composition, it is important to consider the type of stimulation, since compositional changes may be stimulant-dependant. For example, one study found that toothbrushing had a significant effect on the protein composition of saliva by contamination with serum components; specifically an increase in albumin (Hoek et al. 2002). In another study of parotid saliva, citric acid stimulation produced a decrease in albumin (Oberg et al. 1982). The toothbrush stimulation likely increased the crevicular fluid components in saliva, while increased flow due to citric acid stimulation likely diluted serum components in saliva.

The most comprehensive comparison of whole saliva protein composition using different tastants was offered by Neyraud et al. (2006). This study analyzed protein abundance changes from four healthy subjects in whole and parotid saliva following stimulation with different tastants at low concentration and high concentration using 2DE and MS. In whole saliva, the number of proteins affected by an increase in concentration of taste stimulation increased in the order sweet<umami<bitter<acid. The respective

numbers of differentially expressed proteins were 2, 9, 10, and 16. All of the differentially expressed proteins were low in abundance in comparison to total spot volumes and the vast majority of the salivary proteome was, in fact, unchanged by different concentrations of stimulants. Unfortunately, only the few differently expressed spots were analyzed so it is difficult to assess the validity of their conclusions.

Other studies have examined the effect of changes in the oral environment on unstimulated vs. stimulated protein secretion. Seeman et al. (2004) examined the levels of parotid and SMSL salivary IgA in response to experimental gingivitis in humans. They found a statistically significant increase in the IgA secretion rate in stimulated parotid saliva after 6 and 12 days without oral hygiene. This change was not seen in resting saliva, or in any of the SMSL samples. Interestingly, the salivary gland output increased during the development of experimental gingivitis. A proposed reason was perhaps that the accumulation of plaque-derived substances or inflammatory products triggered salivary secretion via neural pathways.

Seeman's study also shows that different glands responded differently to the same stimulus. Johnson et al., 2000 found histatin concentrations in resting saliva to be much higher for parotid than SMSL saliva, and upon stimulation the histatin concentrations decreased in parotid and increased in SMSL saliva. Flow rates increased significantly for SMSL and parotid glands during stimulation. However, there was only a 4.5-fold increase in flow in SMSL glands as compared to the 14.5-fold increase for parotid saliva. Protein concentration of SMSL was not affected by gland stimulation. Both Seeman's and Johnson's study reveal a differing glandular response to the same stimulus.

Other protein abundances may not be affected by stimulation, however. Francis et al. (2001) determined effects of stimulation on SMSL and parotid kallikrein and pre-kallikrein concentrations. Using both mechanical and citric acid stimulation for parotid and citric acid only for SMSL, they found that the proportions of pre-kallikrein and active kallikerein were similar in salivas secreted at rest and during stimulation. This variable response may complicate efforts to determine glandular origin of proteins in whole saliva.

Salivary protein composition in response to stimulation exhibits individual variability. Oberg et al., 1982 in a SDS-PAGE study, monitored changes in relative concentration of proteins in parotid saliva samples obtained under a variety of collection protocols. Six donors were analyzed. Individual relative protein concentrations differed under unstimulated and stimulated conditions but were at least partially independent from circadian and feeding effects. This study showed that differences in composition between resting and stimulated salivas varied greatly between individuals. For example, while one donor showed a definite decrease in protein concentration with stimulation, another donor showed little change. One donor also had a greater change in IgA concentration than the other donor. These differences were reproducible over a 12-month period. Certain common change patterns, however, were noted with stimulation. The IgA concentration decreased in every instance, and the albumin and lysozyme content decreased in all but one donor. In contrast, no consistent changes were observed in amylase and total protein content with stimulation.

The duration of stimulation may affect some protein abundances. In Oberg's study, changes in parotid saliva protein composition that were dependent on the duration of stimulation were observed in four of six subjects. Some protein bands became undetectable with continued citric acid stimulation from 5 to 20 min. Specific assays indicated that salivary albumin, IgA and lysozyme concentrations decreased, whereas peroxidase and amylase concentrations were unchanged. The concentration changes with stimulation were suggested to be indicative of different secretory mechanisms for the proteins. For example, albumin probably entered saliva as a by-product of serum transudation. Therefore, decreases in its concentration with stimulation may have reflected permeability changes in vascular or glandular structures or relative increases in outputs of proteins of glandular origin. Lysozyme and secretory IgA are thought to be secreted primarily from the ducts. Therefore, the fact that their concentrations decreased relative to amylase concentration on prolonged stimulation may suggest that secretion of proteins from ductal origin do not increase in proportion with the secretion of proteins from acinar origin.

In contrast to Oberg's study, Becerra et al. (2003) found no significant time effect during resting and stimulated conditions. Becerra's study characterized the influence of gustatory stimulation (fruit flavored candies) and duration of stimulation on the secretion pattern of salivary mucins MG1 and MG2 and non-mucin glycoproteins in SMSL secretion. Samples were analyzed using SDS-PAGE, followed by Western analysis using polyclonal antibodies against MG1, MG2, lactoferrin, amylase, and carbonic anhydrase. They recorded a significant increase in stimulated total protein concentration over

unstimulated conditions. Image analyses revealed that the level of MG1 increased and the level of MG2 remained nearly the same after stimulation. However, after stimulation the intensity of lactoferrin, peroxidase, and amylase decreased whereas the intensity of carbonic anhydrase, proline-rich glycoprotein, and basic glycosylated proline-rich protein increased. It was suggested these patterns may reflect secretion from preformed vesicles since de novo synthesis would be unexpected within the time frame of their experiment. The variable patterns observed suggest that mucins and non-mucin glycoproteins in SMSL derive from subsets of secretory vesicles, some originating in mucous and others in serous acini, as well as ductal cells.

Schipper et al. (2007), analyzed how various methodological aspects and pre-analytical variables affect saliva SELDI-TOF-MS profiling. While the main thrust of this study was to compare these methodological variables, it was determined that unstimulated vs. stimulated saliva sampling critically affect the amount and composition of detected salivary proteins. The proteins were neither identified nor quantified in this study.

In summary, it is evident from previous studies that stimulation has significant effect on the protein composition of saliva. Variables such as type of stimulation, duration of stimulation, gland studied, individual variability, and specific protein appear to influence protein abundances found in unstimulated vs. stimulated saliva. Table 3 provides a summary of the studies discussed in this section.

Table 3: Summary of Studies Comparing Stimulated vs. Unstimulated Protein secretion			
Study	**Saliva**	**Stimulant**	**Protein abundance change with stimulation**
Bacerra et al.	SMSL	Fruit flavored candies	MG1 (+) MG2 (N/C) Lactoferrin (-) Peroxidase (-) Amylase (-) Carbonic anhydrase (+) Proline-rich glycoprotein (+) Basic glycosylated proline-rich protein (+)
Francis et al.	SMSL	Mechanical and citric acid	Pre-kallikrein/kallikrein ratio (N/C)
Hoek et al.	Whole	Tooth brushing	Albumin (+)
Johnson el al.	Parotid and SMSL	Mechanical and citric acid	Histatin, parotid (-) Histatin, SMSL (+)
Neyraud et al.*	Whole	Citric acid (sour)	PRPH2 (+) Alpha amylase (+) Calgranulin (+) Annexin A1 (+) Annexin A2 (+) Beta-2- microglobulin (-) 9 unidentified proteins (+,-)
		Sweetness	2 unidentified proteins (-)
		Umami	Calgranulin A (+) Annexin A1 (+) 6 unidentified proteins (+,-)
		Bitterness	Calgranulin A (+) Annexin A1 (+) Cystatin S (-) Enolase 1 (-) 4 unidentified proteins (+,-)
Oberg et al.	Parotid	Citric acid	IgA (-) Albumin (-) Lysozyme (-) Amylase (N/C)
Schipper et al.	Whole	Mechanical (paraffin chewing)	Noted change in amount and composition of unidentified proteins
Seeman et al.	Whole	Parotid and SMSL (with experimental gingivitis)	IgA, parotid (+) IgA, SMSL (N/C)

*Compared low concentration stimulus to high concentration
(+)- increase with stimulation
(-)- decrease with stimulation
(N/C)- no change with stimulation

Specific Aims:

Aim 1: Identify major and minor secretory components present in human whole saliva by large scale proteomic analysis of biological replicates.

Whole saliva will be collected and analyzed using highly sensitive 2D-LC/MS to identify both major and minor proteins present. Unstimulated and stimulated saliva from 5 different subjects will be collected and large numbers of peptides sequenced using a new linear ion trap mass spectrometer. High scan rates, high sensitivity, high degree of peptide separation, and 10 replicates (5 subjects x 2 samples) will increase depth of proteome coverage.

Aim 2: Compare protein content and abundance of stimulated vs. unstimulated human whole saliva.

Unstimulated whole saliva and whole saliva following gustatory stimulation with citric acid will be collected from 5 subjects and similar 2D-LC/MS analyses will be performed. Relative protein amounts will be estimated from MS/MS data utilizing a new technique known as spectral counting and the relative protein contents compared.

Materials and Methods:

Saliva Sample Collection:

Saliva samples were collected from five individuals with the following inclusion criteria to ensure homogeneity among individuals: all subjects were healthy male between the ages 27-35, with relatively good periodontal health and taking no medications. Each individual contributed an unstimulated and a stimulated sample. Health status was determined by interview and completion of a health history form. Periodontal health was determined by periodontal examination including measuring probing depths and recording bleeding points. Subjects were excluded if any medications had been taken within the previous week, if any known disease or illness was present, or if periodontal conditions existed beyond localized minor gingivitis.

Saliva collected from each individual at mid-morning (10 a.m.), between May 8, 2006 and May 19, 2006 to minimize diurnal variation (Dawes 1975, Hardt et al. 2005). Unsitmulated and stimulated samples were taken from the same individual, mid-morning, two hours after eating and tooth brushing. The volunteer was asked not to drink any fluids other than water during the two hours proceeding collection. 2 ml samples of whole saliva, parotid secretions, and submandibular/sublingual secretions were collected independently under two conditions, first unstimulated, and then stimulated (2 samples per subject). Samples of parotid and submandibular/sublingual secretions collected concomitantly will be analyzed in separate studies. Stimulated samples were obtained by application of Real Lemon juice (citric acid) to the tongue at periodic intervals, 3 to 4 times per minute.

Whole saliva was collected by expectoration. *(For use by other studies, parotid gland saliva was also collected using a Lashley cup (Stone Machine Company, Colton, CA) placed over Stenson's duct to eliminate any contamination from other saliva glands and from gingival fluid. Submandibular/sublingual (SMSL) saliva was collected while isolating the area under the tongue with cotton rolls and collecting pooled secretions with a sterile syringe fitted with a silicon tube).* All collected samples were kept on ice before processing.

Saliva Sample Preparation:

Immediately after collection and temporary storage on ice, saliva was centrifuged at 20,000 x g for 15 min at 4° C. The supernatant was pipetted off and the pellet discarded to remove cellular debris and epithelial contaminants. The final retentate volume and protein concentration was measured by performing a Bicinchoninic Acid (BCA) protein assay (Pierce Biotechnology, Inc. Rockville, IL) in triplicate. Aliquots of 1 mg protein were lyophilized and stored at -80° C until use.

The protein samples were dissolved in 100 µl digestion buffer containing: 8 M electrophoresis grade urea (in deionized water), 0.8 M Tris, 0.08 M methylamine, and 8 mM $CaCl_2$. The sample was reduced by the addition of 12.5 µl 0.9 M DTT and incubation at 50° C for 15 minutes. Cysteine alkylation was performed by the addition of 12.5 µl 1.0 M iodoacetamide (IAA) solution and incubation in the dark for 30 min. An additional 12.5 µl DTT solution was added to scavenge any remaining IAA before adding 210 µl of water. Ten µl of the solution was analyzed by SDS-PAGE to provide a pre-

digestion assay. Forty µl of 1 µg/µl trypsin gold (1:25 enzyme to substrate ratio), which had previously been prepared by dissolving a 100 µg vial of trypsin gold (ProMega) in 100 µl of 1mM HCl, was then added. The solution was vortexed, centrifuged, and incubated at 37 ° C for approximately 16 hours. Ten µl of the digested solution was analyzed by SDS-PAGE, and compared to the pre-digestion assay to determine digestion completeness. 20 µl of 88% formic acid was added to end digestion.

A peptide clean up step was performed on the digest using a Sep Pak (Waters/Millipore part #20515) cartridge with a C-18 Plus column to remove the salts and reagents remaining from the previous steps.

Strong Cation Exchange (1st dimension of separation)

Strong cation exchange (SCX) separates peptides based on charge. SCX was a 100 x 2.1 mm column of polysulfoethyl A (The Nest Group, Inc. Southborough, MA, USA). The mobile phase A was 10mM sodium phosphate (pH 3.0) and 25% acetonitrile. Mobile phase B was identical except that it contained 350 mM KCl. After 5 minutes to load and wash, the gradient was a 45 min linear gradient from 0% to 50% B, then a 20 min gradient from 50% to 100% B. One minute fractions were collected.

Fractions were combined according to peptide quantity estimated from UV tracings. Fractions with low quantity of peptide were combined every 4 minutes, while the remaining fractions were combined every 2 minutes to reduce the number of samples from 80 to about 34. The samples were lyophilized and reconstituted in 100 µl of 5% formic acid.

Reverse Phase Liquid Chromatography (2nd dimension of separation)

All SCX fractions of whole saliva digests were analyzed by LC/MS using an Agilent 1100 series capillary LC system and an LTQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA). Samples (roughly one quarter of the SCX fractions) were applied at 20 µl/min to a trap cartridge (Michrom Bioresources, Inc. Auburn, CA), and then switched onto a 0.5 X 250 mm Zorbax SB-C18 column (Agilent Technologies, Palo Alto, CA) using a mobile phase containing 0.1% formic acid. The gradient was 7-30% acetonitrile over 90 minutes at a 10 µl/min flow rate.

Mass Spectrometry

The linear ion trap was configured to automatically obtain tandem mass spectra of peptides as they eluted from the reverse phase column. Data dependent collection of MS/MS spectra used the dynamic exclusion feature of the instrument control software (exclusion mass width: low 1.0, high 4.0 m/z; repeat count, 1; exclusion list size, 50 ions; and exclusion duration, 30 sec to obtain MS/MS spectra of the three most abundant ions following each survey scan. The dynamic exclusion procedure limited analysis of abundant peptides, allowing a greater number of minor components to be analyzed. DTA files were generated from MS/MS spectra using extract_msn software (ThermoFinnigan) with a molecular weight range of 400 - 4000 Daltons, a minimum of 25 ions and a low TIC threshold of 500. Charge state analysis (ZSA) was performed following DTA

creation, and each 2-DLC run produced about 340,000 spectra. The total number of MS/MS spectra in the entire 10-MudPIT data set was 3,444,365.

Protein Identification

The 3.44 million tandem mass spectra were searched with SEQUEST (Eng at al. 1994) to identify peptides and proteins present in the whole saliva samples. Previous mass spectrometry studies of saliva have demonstrated that there are significant numbers of partial tryptic peptides, and that faster full-tryptic peptide searches may not identify all proteins (Wilmarth et al. 2004). "No enzyme specificity" searches of a SwissProt, human-only protein database (with sequence-reversed entries and common contaminants appended to the database) were performed. The relevant parameters used in the SEQUEST searches are listed in Table 4.

Table 4: Parameters used in SEQUEST[a] searches	
Parameter	
parent ion mass tolerance	2.5 Da
parent ion masses	Average
fragment ion tolerance	Default
fragment ion masses	Monoisotopic
enzymatic cleavage specificity	None
static modifications	+57.02@C
variable modifications	None
no. of database entries[b]	15,961

[a]Version 2.7 rev 12
[b]Number of forward entries. An equal number of sequence-reversed entries were also present

The large number of MS/MS spectra in these LTQ datasets could not be directly analyzed by most software. Therefore, a discriminant function analysis of SEQUEST

score was used to "filter" out correct peptide identification and reduce data set sizes. This analysis allowed peptide thresholds to be set for each charge state (1+, 2+, 3+) and tryptic status (full, half, or non-tryptic). An Institute for Systems Biology discriminant score (Keller et al. 2002) modified by an alternate DeltaCN score (difference between top score and averaged 4-10 scores) was computed. Reversed sequence matches were used to adjust LTQ normalizations by charge state.

The discriminant scores were histogrammed by charge-state and tryptic status. The histograms were fit with a normal distribution for the correct distribution, and a functional form used in Peptide Prophet (Keller et al. 2002) for the incorrect distribution. Least squares minimization was performed using the Solver feature of EXCEL (Microsoft Corporation, Redmond, WA). A peptide probability of 0.80 was used in nearly all cases to determine the score cutoffs for each charge state and tryptic status. The MS/MS spectra that passed the discrimant function thresholds were written to new SCT and MS2 files (McDonald et al. 2004).

DTASelect was used to map the identified peptides to a parsimonious list of proteins. Each MudPIT run was processed separately. Completely non-tryptic peptides were excluded and a minimum peptide length of 8 amino acids was also required to control false positive matches. The filtering process negated the need for XCorr or DeltaCN thresholds, but Occam's razor function (parsimony filtering) and a 2 peptides/protein requirement were used. A PERL Program was used to non-redundantly count spectra and create a results table. An EXCEL pivot table analysis was then used

generate a union of identified protein results. Peptide and protein false positive rates were estimated using sequence-reversed matches.

Protein lists were manually checked to reconcile primary protein identifications. Occasionally, Occam's razor function of DTASelect switches reported proteins. Therefore, for each primary protein, additional redundant proteins were manually grouped together. Multiple, essentially redundant, immunoglobulin sequences (eg. Ig Kappa light chains) were also grouped together to avoid inflating the results list. In addition, contaminant proteins (trypsin, BSA, carryover proteins) were identified and removed from the final protein lists.

Stimulated vs. Unstimulated Comparison

Spectral Counting

Relative abundances of proteins were determined using a new technique known as spectral counting. This new technique (Liu et al. 2004, Colinge et al. 2005, Gao et al. 2005) allows abundance estimates to be derived from 2D-LC/MS data previously used only for protein identification. High abundance proteins produce a larger number of tryptic peptides. These abundant peptides are more likely to be detected in multiple charge states, and abundant peptides may overload columns and elute in multiple fractions over a greater time. This means that more MS/MS spectra are acquired for more abundant proteins. Furthermore, it has been shown that totalling the number of MS/MS

spectra assigned to a protein is directly related to its abundance in the sample (Liu et al. 2004). This allows abundance estimates to be made directly from 2D-LC/MS data without having to perform more involved quantitative experiments.

Using DTASelect, proteins with significant numbers of shared peptides were identified and spectral counts combined accordingly. Proteins were grouped if they shared more than 20% of the identified peptides. The sums of the protein family counts were used for comparisons instead of individual counts. In addition, contaminant peptide counts (trypsin, carryover, etc.) were identified and removed before normalizations.

As a minimal filter, inclusion of a protein for comparative analysis required presence in at least 3 of the 5 human subjects. This helped reduce "missing" data points which are problematic for statistical analysis. This produced a list of 288 proteins for comparing relative abundance changes.

No minimum average count values were used prior to statistical testing. An overall normalization factor (Net Total) for each MudPIT run was determined. Net Total is the total number of peptides passing filters less the contaminant counts. All spectral count numbers were scaled to the average Net Total for the 10 MudPIT runs. To reduce the distortion of "missing" data during comparisons, 0.2 was added to all spectral counts (including zeros) before scaling.

Statistical Analysis

The normalized spectral count data of the 288 proteins from the two saliva flow states of the 5 subjects were analyzed with Student's paired t-test to identify any relative protein abundance differences. A recent publication evaluated several statistical tests to assess sample differences using spectral counts (Zhang et al. 2006) and found the best results utilizing a Student's t-test for experiments with more than 4 replicates. Because the data was skewed, a log (base 2) transformation of the counts was performed prior to testing (Zybailov et al. 2006). Fold changes and p-values were calculated. A protein was considered to have a significant relative abundance change if $p<0.05$.

Results:

In this investigation, whole saliva was collected from 5 Caucasian males (ages 28 to 34 years) during unstimulated and stimulated (citric acid) saliva flow. Each sample was digested with trypsin, and the peptides were separated using two-dimensional liquid chromatography (strong cation exchange/reverse phase). A linear quadrapole ion trap mass spectrometer was used to perform tandem mass spectrometry to identify peptides and proteins. A net total of 509 non-redundant whole saliva proteins were identified from the 3,444,365 MS/MS spectra. Table 4 summarizes the samples that were collected and processed as part of this investigation.

Table 5: Sample summary

Subject	Age	Whole Saliva Flow	Total Protein Concentration in whole saliva (mg/ml)	No. of identified MS/MS spectra
A	30y 10mo	unstim	1.18	19,603
		stim	0.70	14,955
B	31y 11mo	unstim	0.89	17,998
		stim	0.97	18,872
C	30y 2mo	unstim	1.88	11,768
		stim	1.37	8367
D	27y 11mo	unstim	1.05	10,205
		stim	2.50	15,233
E	30y 2mo	unstim	1.60	12,016
		stim	1.40	9020
		Mean unstim	1.32	
		Mean stim	1.38	
			Total spectra	138,037

(For comparison avg. parotid concentrations in these subjects were unstim-3.10 mg/ml, stim-1.54 mg/ml for the same subjects)

Protein Identification

Discriminant analysis yielded an estimated 138,037 identified peptides with a false discovery rate (FDR) of 0.74% from which an initial 620 non-redundant proteins were identified with a FDR of 0.33%. (*A comparison of the discriminant analysis to previous DTASelect criteria is included in Appendix 1).* Any matches to common contaminants, such as trypsin autolysis products or BSA (used for instrument quality control and present as a carryover), were excluded (35 contaminants), and immunoglubins collapsed into categories, which yielded a final list of 509 non-redundant proteins. The complete list of redundant proteins identified by 2 or more distinct peptides (excluding contaminants) in the 10 samples is given in Appendix 2. Immunoglobulins can have many distinct protein entries in databases and inflate the number of identified proteins. The study used the minimally redundant SwissProt database, but 76 of the 585 proteins (14%) were still immunoglobulin sequences. Individual immunoglobulin variability inflates the number of these proteins in protein sequence databases, and should not be included in proteomic results.

A total of 452 proteins were identified in unstimulated samples and 402 in stimulated samples. 372 common proteins were found in both states and the overlap is shown in Figure 5. There were 107 proteins identified only in unstimulated saliva, 57 observed only in stimulated saliva, and 345 common proteins found in both states. Since the protein results require 2 peptides per protein, many of the proteins detected in one flow state only might be present in the other flow state but at a single peptide level. Therefore, the non-overlap in the two proteomes is probably over estimated.

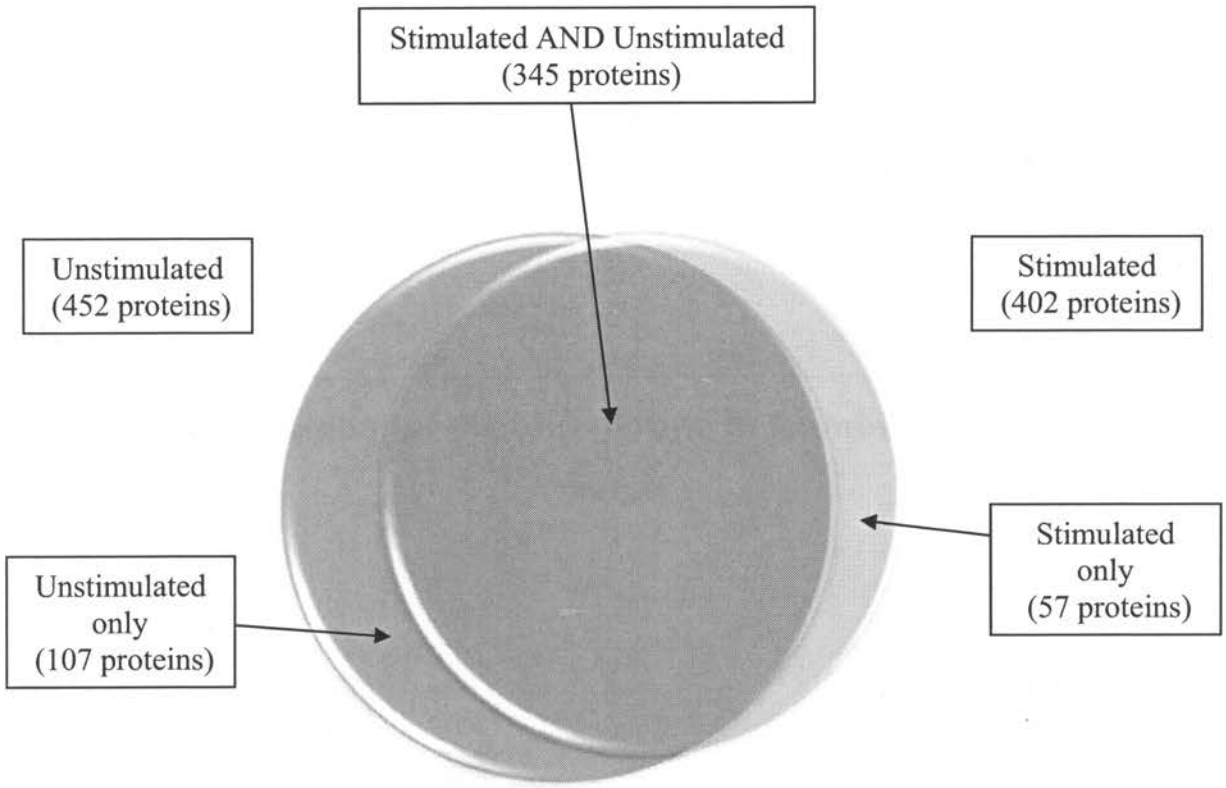

Figure 5. Venn diagram showing number of proteins identified and overlap in the two flow states.

Figure 6 shows the number of proteins found in 1/5 subjects, 2/5 subjects, etc. for unstimulated and stimulated saliva. The number of proteins found in 5 subjects is greater than any of the other categories. This would imply that proteins found in all 5 subjects are relatively abundant in both states of whole saliva (i.e. major salivary gland proteins). The next largest number of proteins was found in one subject alone. This pattern of protein detection is typical of replicate 2D-LC experiments (Liu et al. 2004).

Technical replicate analyses of 2D-LC samples resulted in an asymptotic increase in identified proteins (Liu et al. 2004), and Figure 7 demonstrates that repeated analyses of biological replicates of two related samples (unstimulated and stimulated saliva) exhibited similar behavior. Multiple samples in this study increased the number of identified proteins by nearly 3-fold over analyses of single samples.

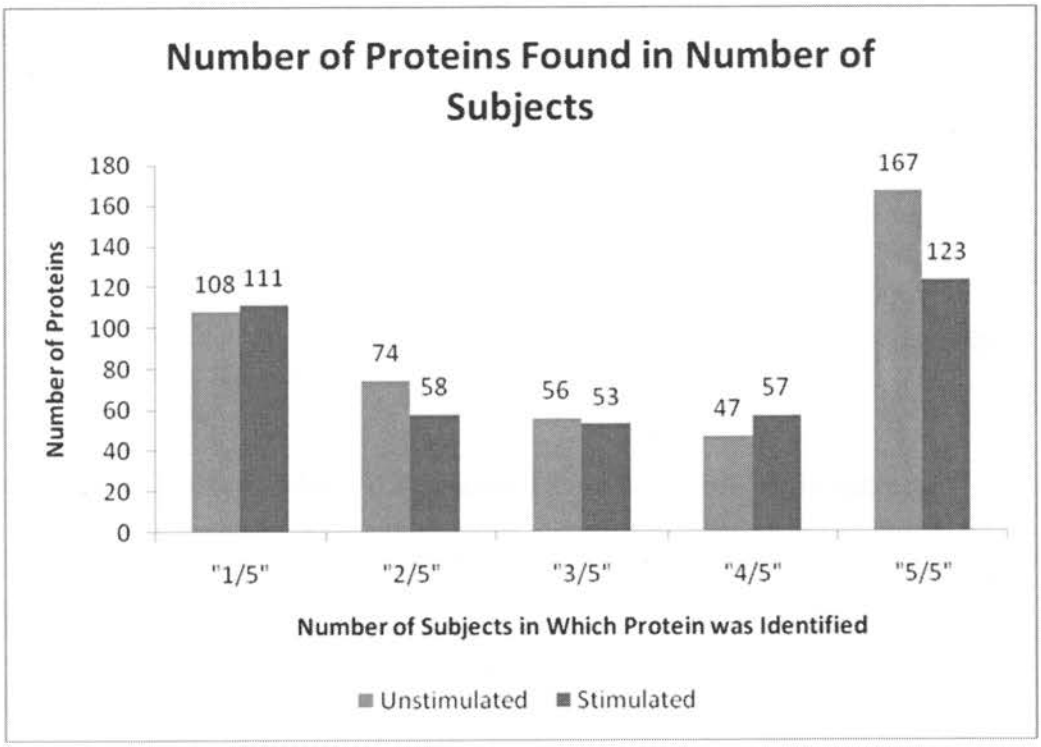

Figure 6. Number of proteins found in one subject only, 2 subjects only, etc.

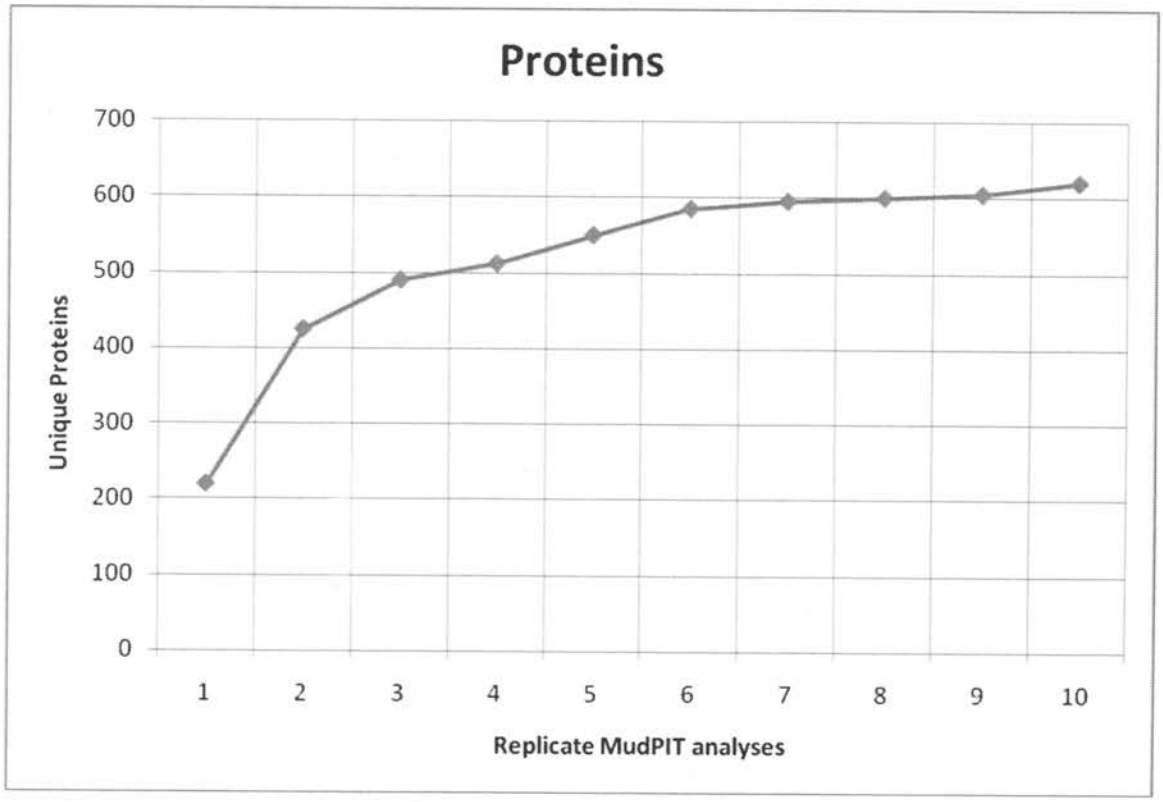

Figure 7. The cumulative number of unique non-redundant protein identifications as a function of replicate analyses.

Comparison of Unstimulated vs. Stimulated Protein Relative Abundance

The relative abundance changes of 288 proteins/groups meeting the criteria detailed in the methods section were tested against an $p<0.05$ using a two-tailed Student's paired t-test (Appendix 3). 36 proteins (12.5% of 288, 7% of total 509) showing significant change are presented in Table 6. Of the significant proteins, 14 proteins showed an increase in relative abundance with stimulation and 22 showed a decrease. Fold changes for all proteins tested are depicted in Figure 8, and the subset of proteins with $p<0.05$ are depicted in Figure 9.

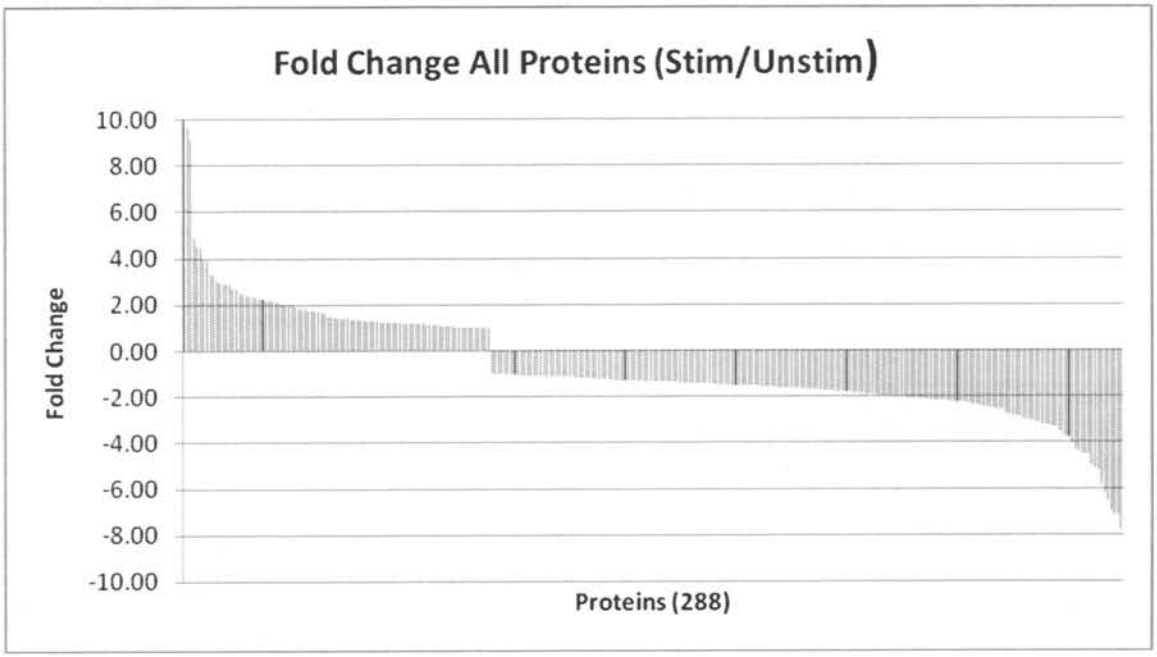

Figure 8: Fold changes of averaged spectral counts (Stimulated/Unstimulated) from 5 biological subjects for 288 whole saliva proteins. Proteins with positive values increased in relative abundance with stimulation (94 proteins), while proteins with negative values decreased (194 proteins).

Table 6. Proteins showing statistically significant ($p<0.05$) relative abundance change in unstimulated vs. stimulated whole saliva. Greater relative abundances in stimulated saliva have positive fold-changes.

Locus_ID	Locus Description	Mean Diff.	Std Error	Fold Change	P-value
MYH9_HUMAN	Myosin-9 (Myosin heavy chain 9)	-4.37	1.26	-7.84	0.0258
PDC6I_HUMAN	Programmed cell death 6-interacting protein	-3.43	0.73	-7.16	0.0092
WDR1_HUMAN	WD repeat protein 1 (Actin-interacting protein 1)	-3.54	1.23	-6.55	0.0453
6PGL_HUMAN	6-phosphogluconolactonase	-3.34	0.7	-6.25	0.0091
ACTIN GROUP	Actins	-4.84	1.06	-5.26	0.0102
IDHC_HUMAN	Isocitrate dehydrogenase [NADP] cytoplasmic	-2.41	0.86	-5.13	0.0482
TBAK_HUMAN	Tubulin alpha-ubiquitous chain	-3.54	1.13	-5.01	0.0349
G6PI_HUMAN	Glucose-6-phosphate isomerase	-3.96	1.34	-4.60	0.0419
HXK3_HUMAN	Hexokinase-3	-2.19	0.79	-4.51	0.0500
ARP3_HUMAN	Actin-like protein 3	-1.70	0.55	-2.56	0.0365
HEAT SHOCK PROTEIN GROUP	Heat Shock Proteins	-3.67	1.08	-2.52	0.0271
PRB3_HUMAN	Basic salivary proline-rich protein 3 precursor	-1.49	0.49	-2.31	0.0390
IVINC_HUMAN	Vinculin	-2.81	0.97	-2.27	0.0447
GELS_HUMAN	Gelsolin precursor (Actin-depolymerizing factor)	-1.28	0.42	-2.27	0.0371
HEAVY GROUP	IG Heavy Chains	-0.82	0.16	-1.76	0.0076
HSPB1_HUMAN	Heat-shock protein beta-1	-0.76	0.2	-1.72	0.0176
LAMBDA GROUP	Lambda_GROUP	-0.63	0.18	-1.60	0.0234
TRFE_HUMAN	Serotransferrin precursor	-0.65	0.22	-1.58	0.0425
DEFENSIN GROUP	Defensinsm (DEF1 and DEF3)	-0.68	0.18	-1.57	0.0196
WFDC2_HUMAN	WAP four-disulfide core domain protein 2 precursor	-0.57	0.14	-1.47	0.0148
IGA GROUP	IGA_GROUP (IGA1 andIGA2)	-0.56	0.19	-1.43	0.0436
KAPPA GROUP	Kappa_GROUP	-0.48	0.16	-1.38	0.0355
CRIS3_HUMAN	Cysteine-rich secretory protein 3 precursor	0.23	0.07	1.17	0.0285
FCGBP_HUMAN	IgGFc-binding protein precursor	0.41	0.09	1.23	0.0122
BPIL1_HUMAN	Bactericidal/permeability-increasing protein-like 1 precursor	0.48	0.09	1.34	0.0059
U773_HUMAN	Protein UNQ773/PRO1567 precursor	0.98	0.33	1.70	0.0412
CAH6_HUMAN	Carbonic anhydrase 6 precursor	0.83	0.28	1.72	0.0410
CYTD_HUMAN	Cystatin-D precursor (Cystatin-5)	1.01	0.29	1.83	0.0260
CYTC_HUMAN	Cystatin-C precursor (Cystatin-3))	1.04	0.3	1.99	0.0246
CYSTATIN GROUP	Cystatins (S, SA,SN)	1.18	0.26	2.15	0.0107
NUCB2_HUMAN	Nucleobindin-2 precursor	1.22	0.35	2.23	0.0246
RNT2_HUMAN	Ribonuclease T2 precursor	2.88	0.98	2.39	0.0360
TSP1_HUMAN	Thrombospondin-1 precursor	2.66	0.8	2.92	0.0295
SPLC2_HUMAN	Short palate, lung and nasal epithelium carcinoma-associated protein 2 precursor (Parotid secretory protein)	1.68	0.42	2.97	0.0166
CBPE_HUMAN	Carboxypeptidase E	2.39	0.85	3.37	0.0487
RNAS4_HUMAN	Ribonuclease 4 precursor	3.47	1.01	4.90	0.0266

Mean Difference = Mean difference of log2 counts between stimulated and unstimulated saliva.
Std. Error = Standard error of the mean difference
Fold change = Stimulated counts/Unstimulated counts
p-value determined from two-tailed, Student's paired t-test

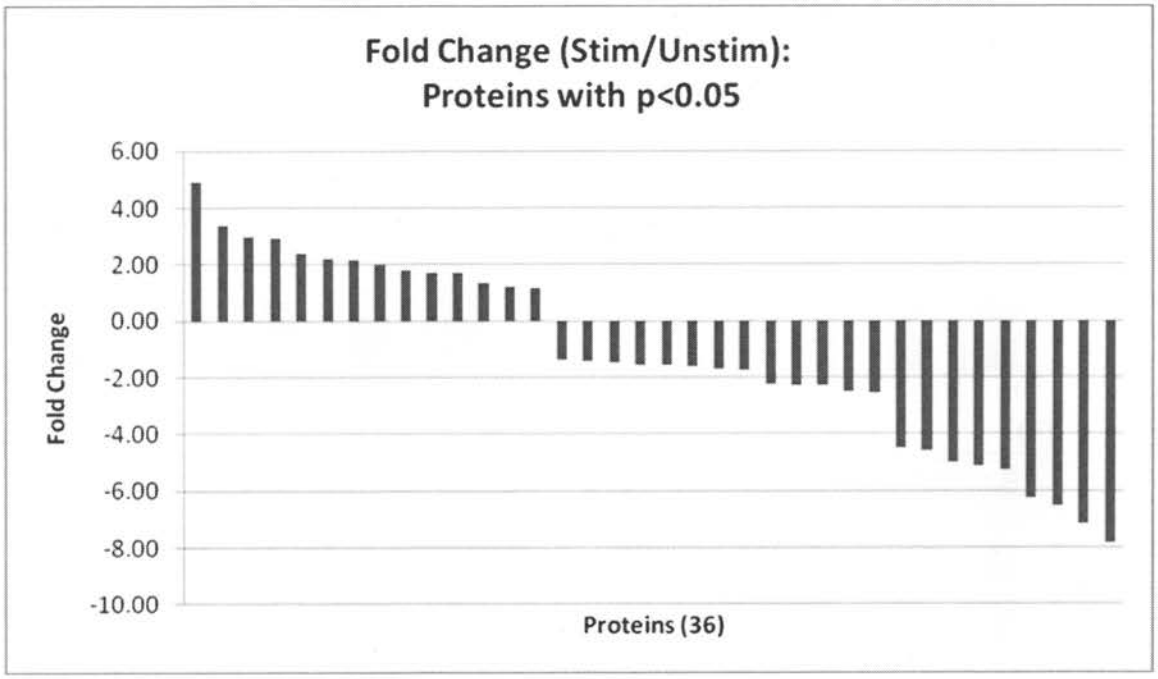

Figure 9. Fold change of 36 proteins with $p<0.05$. Proteins with positive values increased in relative abundance with stimulation, while proteins with negative values decreased.

The normalized spectral counts of 4 well established major salivary gland proteins in unstimulated and stimulated salivas were compared across the 5 biological subjects, and are shown on the left of Figure 10 (Panels A-D). One known minor salivary gland protein (Von Ebner gland protein) is shown in panel E of Fig. 10. Similar data are shown for 3 common serum proteins on the right side of Fig. 10 (panels F-H). With the frequent exception of subject D, salivary gland proteins were increased in stimulated saliva in most cases. Many serum proteins of possible leakage origin showed decreased

abundances in stimulated saliva. The minor salivary gland protein (panel E) also showed a decrease in abundance during stimulated flow.

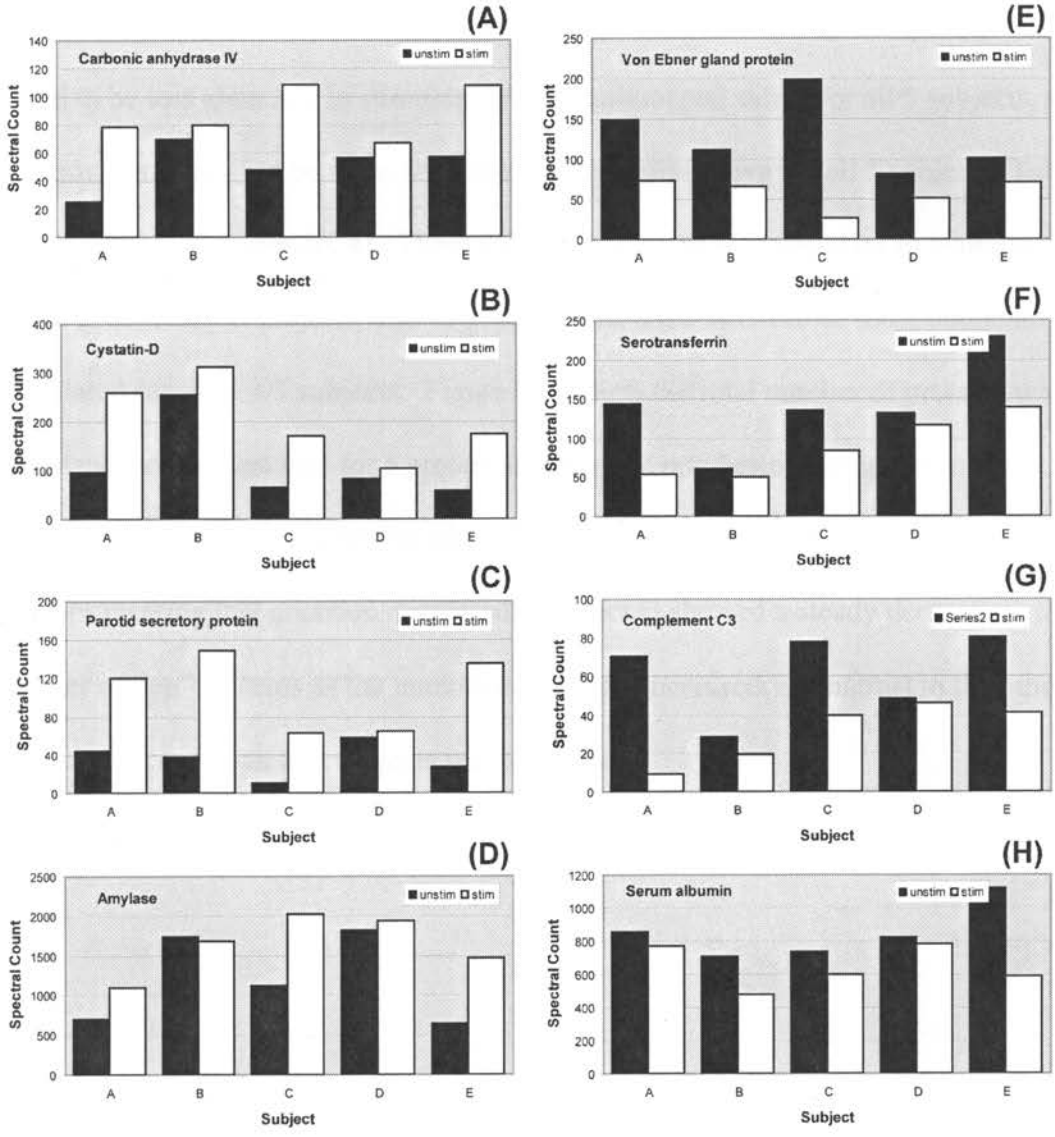

Figure 10. Normalized spectral counts in unstimulated saliva (black bars) compared to stimulated saliva (white bars) across the 5 biological subjects. Four well known major

salivary gland proteins are shown on the left. A minor salivary gland protein is shown in panel E. Panels F-G are three putative serum derived proteins.

Considerable individual variations in relative abundance changes were found among the 5 subjects. Of the 288 proteins/groups tested for change, 44 proteins were found to be less abundant in stimulated than unstimulated saliva for all 5 subjects, and 14 proteins were found to be more abundant in stimulated saliva for all 5 subjects (Tables A4_1 and A4_2, Appendix 4). There were 123 proteins less abundant in stimulated saliva in at least 4/5 subjects, and 48 proteins that were found to be more abundant in stimulated saliva in 4/5 subjects. Figure 11 depicts the total number of proteins where the abundance trend increased for a protein in the given number of biological subjects. As the number of biological subjects where the trend was "up" increased, the total number of proteins meeting that criterion decreased. Subject D showed a steady decrease in the number of "up" proteins as the number of subjects increased, in contrast to the other 4 subjects where a peak at 3 subjects was observed.

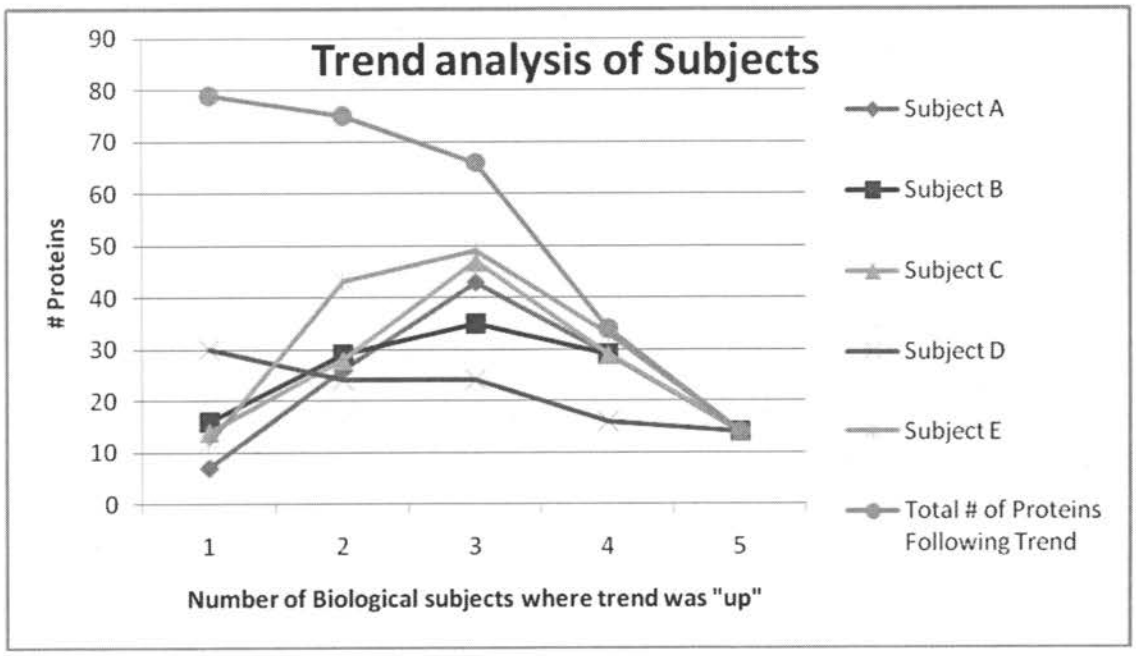

Figure 11. Trend analysis showing the number of proteins where the abundance trend increased in a given number of biological subjects. Subject D differed from the overall trend seen in the other 4 subjects.

A common criterion to evaluate protein abundance changes in 2-DE experiments is a greater than 2-fold abundance change under different experimental conditions and this criterion has also been used with spectral counting (Liao et al. 2007). Figure 12 shows a scatter-plot of the calculated p-values and fold change for the 288 proteins. The p-values are aligned on the horizontal axis. There were 70 proteins that had a greater than 2-fold decrease in abundance and 32 that were more than 2-fold increased. Any p-value exceeding 0.05 was rejected as being significant by our analysis. This plot shows that 81 of the 102 proteins with > 2-fold change did not have a statistically significant change. Thus, the 2-fold criterion is not useful where n=5.

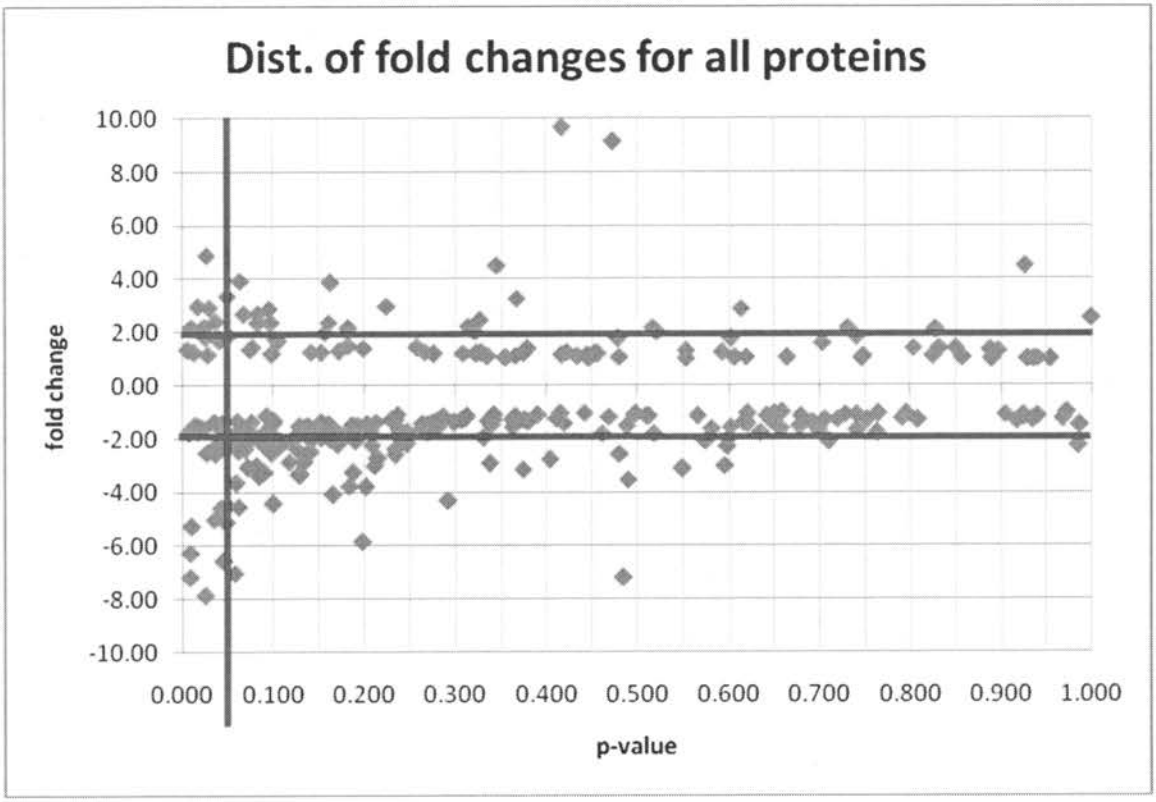

Figure 12. Scatterplot of p-values and fold changes for the 288 proteins. Proteins with a p-value <0.05 were considered to show significant change (vertical red line). Horizontal red lines demarcate 2-fold change levels. 102 proteins showed showed a fold change > 2 up or down.

Figure 13 shows the distribution of fold changes for the proteins having $p<0.05$. 21 of these proteins had a greater than 2-fold abundance change. However, 15 statistically significant proteins did not. A simple 2-fold expression difference is overly simplistic if variability (either technical or biological) is large. Of the 102 proteins having a greater than 2-fold expression difference, only 21% had statistically significant abundance changes. Conversely, 42% of the 36 statistically significant proteins had fold changes that were less than 2. The median p-value of the proteins having greater than 2-

fold differences was 0.132. If we assume that the subset of proteins with the same direction of expression change in all 5 subjects (either all up of all down) have less biological variability, we would expect the p-values to decrease. Indeed, the median p-value decreased to 0.048 for those 58 proteins.

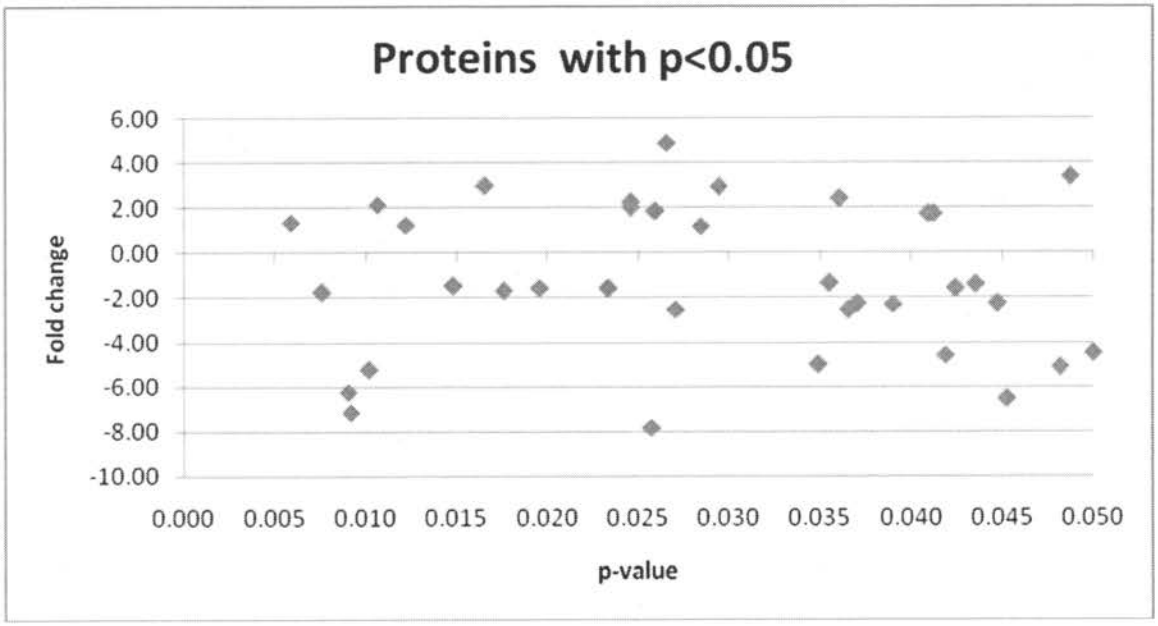

Figure 13. Distribution of fold changes for 36 proteins with p<0.05. 21 proteins had both a p-value <0.05 and a fold change > 2 up or down.

Functional categorization of proteins showing abundance change

Proteins that showed statistically significant abundance change were categorized into functional groups based on annotations from the Swissprot expasy database (Apweiler et al. 2004). Although many proteins are multifunctional, attempts were made to categorize based on primary function. There were noticeable differences between those proteins that increased in relative abundance with stimulation and those that

decreased (Figures 14 and 15). Proteins involved in host defense (31%) comprised the largest functional category in the increased relative abundance group, including the major salivary cystatins and two other proteins. The largest functional category of the decreased relative abundance group contained those proteins involved in the cytoskeleton and actin-myosin regulation (33%). Proteins such as actin, mysosin, tubulin, and vinculin, were included in this category. The next largest category in the decreased proteins consisted of proteins involved in defense. However, 4/5 proteins in this group were immunoglobin groups. Also of note, is that the enzymes that increased with stimulation were proteases, while those that decreased were mostly involved in metabolism.

Proteins showing significant change were also categorized by their probable source based on annotations from Swissprot database. The categories were secreted, cytoplasm, membrane, or unknown. A few proteins were categorized into multiple categories. The proteins that increased with stimulation were largely secreted proteins (59%), followed by unknown (29%), membrane (6%), and cytoplasm (6%). In contrast, the proteins that decreased with stimulation were largely cytoplasmic (71%) in origin, followed by secreted (23%), and unknown (6%), with no membrane proteins.

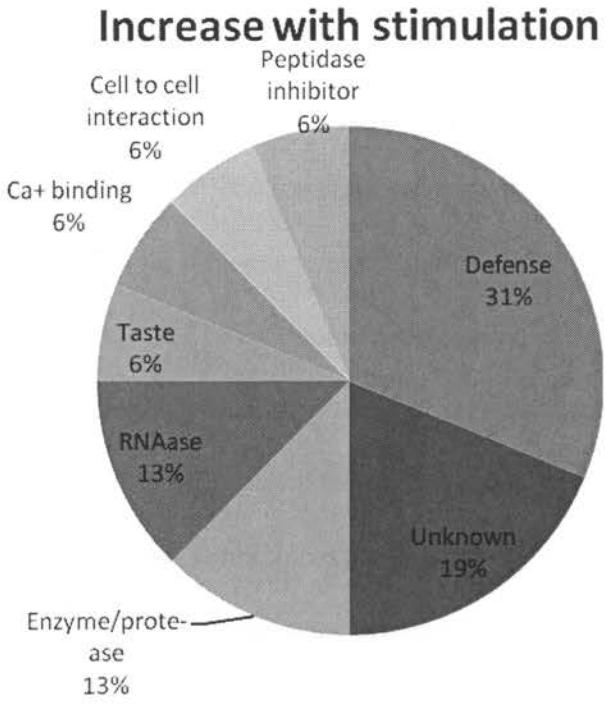

Figure 14. Functional distribution of significantly increased proteins.

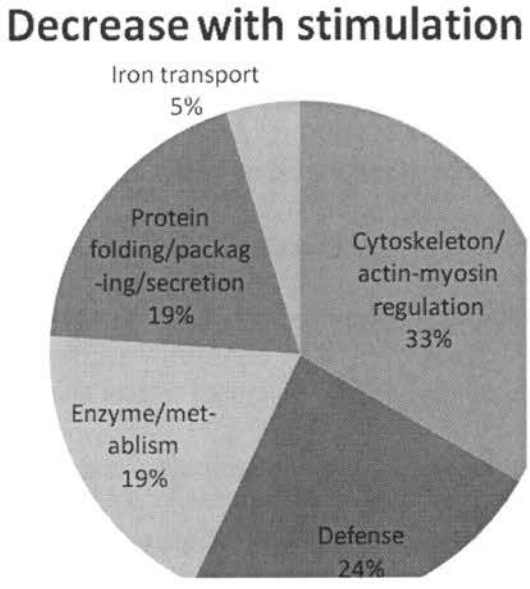

Figure 15. Functional distribution of significantly decreased proteins.

Discussion

The present study identified 509 distinct, non-redundant proteins in whole human saliva. Nearly 3.5 million MS/MS spectra were acquired using a highly efficient discriminant analysis, and rigorous identification criteria to keep the overall protein false discovery rate at 0.3%. The discriminant analysis also resulted in an increased number of identified MS/MS spectra which were used to compare the relative protein abundances between unstimulated and stimulated saliva flow. Statistical analysis was used to identify 36 salivary proteins whose abundances were significantly (alpha= 0.05) altered by stimulation. This is the first large-scale proteomic comparison of stimulated saliva to unstimulated saliva.

There were also some shortcomings of the investigation. First the small sample size coupled with the individual variation found in the subjects limited the statistical power of our study. In a previous study of parotid samples obtained from these subjects, biological variability was found to be greater than technical variability (Bailey 2006). The tremendous amount of data generated in the present study greatly expanded the time required for analysis over previous experiments, which made the acquisition and analysis of additional subjects and/or technical replications impractical.

Many variables, including age, health, sex, diurnal considerations, and oral health were controlled for in this study. In spite of this, unknown conditions and variables that may affect proteins composition were not accounted for, such as diet, masked diseases, and level of hydration. Also, the collection method of the saliva could have introduced

some variability. Studies have shown that the duration of stimulation can affect protein concentrations in saliva (Oberg et al. 1982). Small amounts of mechanical stimulation induced during collection of concomitant samples of unstimulated parotid and SMSL saliva prior to collection of stimulated whole saliva could have affected whole saliva protein composition. However, this small amount of mechanical stimulation did not visibly alter the flow rates seen prior to citric acid stimulation, and were likely inconsequential.

In contrast to parotid saliva collection, citric acid stimulated saliva also contains the citric acid used to stimulate saliva flow. The acidification of the whole saliva could affect its composition. Basic proline-rich proteins, previously found to increase in stimulated parotid (Bailey, 2006) flow, were found to decrease in stimulated whole saliva in most subjects (Fig A5_1, Appendix A5). Because parotid contribution to whole saliva increases with stimulation (Mandel 1972, Shannon 1974, Edgar 1990), this finding was unexpected. A possible explanation is that the lower pH of citric acid stimulated saliva caused some precipitation of basic PRPs that were lost during sample clean up. A previous study found an increase in basic proline-rich proteins in SMSL flow stimulated with fruit flavored candies (Becerra 2003). It is likely that the SMSL saliva collected in Becerra's study also contained the fruit flavored candies, but a comparison of the pH changes induced with stimulation with the present study is not possible. The effect of acidification by citric acid on protein composition introduced during stimulation warrants further investigation.

The subjective nature of assigning functional categories to proteins also hindered the interpretation of the quantitative comparison. Many proteins are not only multifunctional, but may also change function depending on the tissue in which they are expressed. In addition, some proteins are less studied than others, and have uncategorized functions. Efforts were made, however, to assign proteins to categories that were well known.

Common contaminants, including trypsin autolysis products, BSA, and lens proteins were identified in some of the samples. The discovery of trypsin autolysis products was unsurprising as this was the enzyme used to digest the proteins. BSA was used to quality control the SCX column prior to loading the sample and was thus an expected contaminate. Lens proteins, contaminants from previous SCX separations, were also detected. However, the contaminating lens proteins were present in very insignificant quantities, and could be ignored.

Whole saliva proteome

Previous studies have identified from 7 to 1479 proteins using various methods to study the whole saliva proteome (Ghafouri et al. 2003, Yao et al. 2003, Huang et al. 2004, Vitorino et al. 2004, Walz et al. 2004, Wilmarth et al. 2004, Hu et al. 2005, Xie et al. 2005, Fang et al. 2007) (Table 2), many not reporting a false positive rate. To our knowledge, no prior investigation has studied the human salivary proteome under both unstimulated and stimulated flow states. Of the six studies that analyzed unstimulated

saliva without abundant protein depletion (Ghafouri et al., 2003, Vitorino el al. 2004, Wilmarth et al. 2004, Hu et al. 2005, Xie et al. 2005, Walz et al. 2006), Xie's study identified the most proteins (437). However, they included proteins identified by a single peptide. The criterion of 2 peptides per protein greatly reduces protein false positive rates and they identified only 221 proteins using this criterion. The present study was able to identify 452 proteins in unstimulated whole saliva, twice as many proteins at a 2 peptide per protein level. Only two studies reported findings using stimulated saliva (Yao et al. 2003, Huang et al. 2004). Of these, Huang identified the most proteins (26), while Yao identified 7 proteins. In saliva stimulated with citric acid applied to the tongue, the present study identified 402 proteins.

167 proteins and 123 proteins were identified in all 5 subjects for unstimulated and stimulated saliva, respectively (Fig. 6). The next largest number of subjects in which a given protein was found was 1 subject. This distribution follows that of proteomes analyzed from cell lysates (Liu et al. 2004) in which the largest number of proteins were represented in all samples. The proteins found in all 5 subjects likely represent more common and abundant salivary proteins, such as amylase. The distribution of proteins found in just one subject may reflect less abundant proteins or individual variability.

Unstimulated vs. stimulated flow

This study identified 36 proteins that changed in relative abundance during stimulation. This represents 12.5% of the 288 proteins/groups that were compared.

Because few studies have compared protein relative abundance changes with stimulation in whole saliva, and none have compared the entire proteome, comparisons to the present study are difficult. One study reported that stimulated vs. unstimulated collection of saliva critically affect the amount and composition of detected salivary proteins (Schipper et al. 2007). However, their study did not identify the detected proteins. Neyraud's study found many proteins that changed with increased concentration of tastants, but the vast majority of gel spots did not show appreciable change (Neyraud et al. 2006). This is in agreement with our results, where 87.5% of the proteins compared showed no statistically significant change. However, Neyraud's study did not actually compare unstimulated to stimulated saliva, instead testing the affect of different concentrations of tastants. It is difficult to know if the low concentrations used in Neyraud's study reached thresholds that triggered stimulated saliva flow.

One might have expected a larger number of proteins to show change upon stimulation. With stimulation, the percent contributions of the different glands changes drastically (Mandel 1972, Shannon 1974, Edgar 1990). Each gland's unique set and concentration of proteins would therefore be expected to alter the composition of whole saliva with changes in flow. One might also expect serum leakage proteins to become diluted with increased flow of glandular proteins and decrease in abundance. Multiple secretory pathways (Gorr et al. 2005) and permeability changes (Mazariegos et al. 1984, Hashimoto et al., 2000) during stimulation combined with the changes in flow rate from different glands create a multitude of interactions that complicate the prediction of protein composition in saliva.

Some protein relative abundance changes might have failed statistical tests due to the small sample size and the large variation seen among individuals. 58 proteins showed consistent up or down changes in all five subjects (Tables A4_1 and A4_2, Appendix 4). These 58 proteins overall had much lower p-values than the entire group illustrating the influence individual variability had on statistical tests to measure change in relative abundance. In particular, Subject D's response to stimulation differed compared to the trend established by the other four subjects, in either direction or degree of response (Figs. 10 and 11, and Fig. A5_1 in Appendix 5). This differing response may simply reflect individual biological variability in stimulation threshold. It has been well established that individuals possess different levels of taste perception, and can be categorized into nontasters, medium-tasters, and supertasters based on their taste response to 6-*n*-propylthiouracil (PROP) (Fox 1932, Chang et al. 2005). It is estimated that 15-30% of people are known to be nontasters genetically, requiring a much higher threshold for taste perception (Bartoshuk 2000). Subject D may not have received an inadequate amount of citric acid to trigger the level of response seen in the other four subjects. It is also possible other variables described above, such as level of hydration, affected the stimulatory response of this subject. While it is possible methodological errors occurred during collection or processing of subject D samples, no evidence supporting this contention was identified.

Of the well known major salivary gland proteins depicted in Fig. 10, carbonic anhydrase, cystatin-D and parotid secretory protein (PSP) increased with stimulation, while amylase showed little change. Carbonic anhydrase, or gustin, is thought to be

involved in taste perception and bicarbonate regulation. Becerra (2003) also found carbonic anhydrase to increase, but in SMSL saliva stimulated with fruit flavored candies. Amylase, a well known digestive enzyme, did not show a statistically significant change, although it was slightly increased in 4/5 subjects with stimulation. Oberg's study of parotid saliva (1982) also found no change in amylase with stimulation. In contrast, Neyraud's study (2006) showed an increase of amylase in whole saliva following stimulation with higher concentration of sour tastant.

The abundance of the well known salivary gland proteins following stimulation likely reflects underlying secretory mechanisms (Figure 4). PSP, for example, is known to be secreted almost exclusively via large secretory granules in the major regulated pathway, a pathway upregulated following stimulation. In contrast, amylase is known to be prominently secreted via both the major regulated pathway as well as the constitutive-like pathway (basal secretion) (von Zastrow and Castle 1987, Gorr et al. 2004). PSP therefore would be expected to increase only following stimulation, while amylase would be expected to be found in both flow states.

The minor salivary gland protein, Von Ebner gland protein (lipocalin-1 precursor), is thought to be involved in taste perception (Fig. 10, panel E). In contrast to carbonic anhydrase, however, Von Ebner gland protein decreased in abundance in all 5 subjects, missing detection as a protein showing significant change by a small amount (p-value=0.059). While no statistical difference was found, the trend shown by this minor salivary gland protein with stimulation may be due to dilution from the increased contribution to whole saliva from the major glands. This dilution may also contribute to

the overall decreases seen in the three putative serum derived proteins shown in Fig. 10, panels F-H.

The functional composition differed between those proteins that increased with stimulation and those that decreased (Figs. 14 and 15). Of those proteins that increased with stimulation, the largest category included proteins known to be involved in host defense, including cystatins and bactericidal/permeability-increasing protein-like 1 precursor. The increase in these non-specific defense oriented proteins is perhaps not surprising, as stimulation is usually accompanied by an abundance of pathogens contained in food (Amerongen and Veerman, 2002). In contrast, four immunoglobin groups, including IgA, heavy, lambda, and kappa along with the defensin group, all decreased with stimulation. The decrease in IgA with citric acid simulation is in agreement with Oberg's study of parotid saliva (1982), and may reflect the unique mechanism for entrance of IgA into the salivary gland. The other three immunoglobin groups are likely serum-derived proteins that may also have been diluted with increased glandular flow.

The largest category of those proteins that decreased with stimulation included proteins involved in the cytoskeleton and actin/myosin regulation. Many proteins involved with protein folding/packaging/secretion also decreased. Proteins having roles in protein preparation and vesicle transport pathways (via cytoskeleton/microtubules) in acinar cells might be expected to increase during stimulated flow due to the release of secretory vesicle contents. A proposed explanation is that these proteins might reflect a particular acinar secretory pathway (Gorr et al. 2005) that is preferentially expressed

during unstimulated flow (i.e. constitutive-like pathway). During stimulation proteins involved in this pathway may then become diluted or down regulated, resulting in a decrease in relative abundance. The decrease of these proteins may warrant further investigation.

Understanding the functional significance of whole saliva, as well as its value for serving as a diagnostic fluid, is highly dependent on our ability to establish its composition. Protein relative abundance changed in whole saliva upon stimulation with citric acid in our experiments, demonstrating the need to standardize collection protocols in future salivary proteomic studies. A recent study found stimulation of whole saliva with pilocarpine partially restored the levels and numbers of some proteins in whole saliva in patients with Sjogren's Syndrome compared to controls (Peluso et al. 2007). Peluso's study corroborates the notion that the dynamic proteome of saliva may be manipulated by stimulation to yield varying abundances of proteins for the use of diagnostic testing. Proteins that showed abundance changes following stimulation in the present study may be used as "markers" of stimulation in developing saliva collection protocols. While this study identified 36 proteins that changed in abundance, the majority of the 102 proteins with a greater than 2-fold change escaped statistical detection due, in part, to individual variability. Future studies using more subjects may further elucidate the degree of individual response, and increase the number of proteins found to change in abundance with stimulation. As previous studies have shown, protein composition changes with different stimulants (Oberg et al. 1982, Hoek et al. 2002, Neyraud et al. 2006). Large scale proteomic studies utilizing different stimulants may

have the potential to greatly increase the number of proteins undergoing changes upon stimulation. Studies to determine the origins and functions of proteins found in whole saliva are also needed. It is evident that further investigations are necessary to characterize the dynamic salivary proteome and realize the full potential of saliva as a diagnostic fluid.

Conclusions

The present study identified 509 proteins in whole human saliva with a false positive rate of 0.33%. The development of a high-throughput analysis technique allowed discriminate function filtering to be applied to large LTQ datasets. The data analysis greatly increased the numbers of identified MS/MS spectra while simultaneously increasing the accuracy of the peptide assignments. This enabled a proper statistical treatment of spectral counts to compare protein abundances in unstimulated and stimulated saliva for the first time. It was found that the relative abundance of 36 proteins significantly changed following stimulation with citric acid (alpha=0.05), which represents 12.5% of the proteins tested for change. This study shows that stimulation with citric acid alters the human whole saliva proteome, and that future human salivary proteome studies should take into consideration the effects of stimulation when collecting saliva for the identification of biomarker candidates.

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Appendix 1: Discriminant Analysis Comparison to DTASelect thresholds

In previous LCQ analyses, slightly higher than default DTASelect thresholds (2.0, 2.5, 3.8 for 1+, 2+, and 3+ ion, respectively) were used with a DeltaCN threshold of 0.10. Only fully or semi tryptic peptides were allowed and protein identification required at least two peptides. Occam's razor filtering was also used to provide as minimal a list of proteins as the experimental evidence supported. Table A1 compares the numbers of peptides and proteins identified using the discriminant function analysis to the previously-used DTASelect thresholds.

Identification thresholds of different analysis methods can be directly compared using the false-positive rate (FPR), defined as the number of incorrect identifications as a percentage of the total number of identifications. The numbers of incorrect identifications are typically estimated using sequence-reversed decoy databases concatenated to the original forward databases. The discriminant analysis identified 29.5% more peptides than DTASelect with a peptide FPR that was 2.4 times lower. At the protein level, the discriminant analysis identified 12.7% more proteins with a 4.5 times lower protein FPR. Salivary proline-rich proteins have sequence regions that are essentially palindromic with respect to fragment ion masses. The matches to the reversed sequences of these proteins are not strictly incorrect and are artifacts of the method. The adjusted reversed protein match numbers are given in parentheses and the protein FPR is actually over 8 times lower for the discriminant analysis. Therefore, the discriminant analysis dramatically increased sensitivity while also improving specificity.

Table A1_1.	Discriminant Analysis	DTASelect
Total peptides	138,037	108,820
Reversed peptides	1,017	1,894
Estimated correct peptides	136,003	105,032
Peptide FPR	0.74%	1.8%
Total NR proteins	620	602
Reversed NR proteins	6 (2)	25 (23)
Estimated correct proteins	612	552
Protein FPR	0.98% (0.33%)	4.3% (4.0%)

Appendix 2 Table A2_1: Redundant list of identified human whole saliva proteins.

No.	Acc.	Cov.	Pep	Rank	Description (40 characters)
1	1433B	39.8	8	194	14-3-3 protein beta/alpha (Protein kinas
2	1433E	22.7	5	301	14-3-3 protein epsilon (14-3-3E) - Homo
3	1433F	16.3	5	345	14-3-3 protein eta (Protein AS1) - Homo
4	1433G	18.6	5	367	14-3-3 protein gamma (Protein kinase C i
5	1433S	50.0	15	104	14-3-3 protein sigma (Stratifin) (Epithe
6	1433Z	38.0	10	144	14-3-3 protein zeta/delta (Protein kinas
7	2AAA	5.4	2	548	Serine/threonine-protein phosphatase 2A
8	6PGD	38.5	18	92	6-phosphogluconate dehydrogenase, decarb
9	6PGL	26.7	4	323	6-phosphogluconolactonase (EC 3.1.1.31)
10	A1AG1	27.9	7	179	Alpha-1-acid glycoprotein 1 precursor (A
11	A1AG2	13.4	2	465	Alpha-1-acid glycoprotein 2 precursor (A
12	A1AT	30.1	15	93	Alpha-1-antitrypsin precursor (Alpha-1 p
13	A1BG	24.2	8	196	Alpha-1B-glycoprotein precursor (Alpha-1
14	A2GL	29.7	6	188	Leucine-rich alpha-2-glycoprotein precur
15	A2MG	51.9	80	48	Alpha-2-macroglobulin precursor (Alpha-2
16	AACT	13.2	5	309	Alpha-1-antichymotrypsin precursor (ACT)
17	AATC	8.0	2	369	Aspartate aminotransferase, cytoplasmic
18	AATM	7.4	2	531	Aspartate aminotransferase, mitochondria
19	ACBP	64.4	6	180	Acyl-CoA-binding protein (ACBP) (Diazepa
20	ACTB	82.1	86	23	Actin, cytoplasmic 1 (Beta-actin) - Homo
	ACTG				Actin, cytoplasmic 2 (Gamma-actin) - Hom
21	ACTA	51.2	35	46	Actin, aortic smooth muscle (Alpha-actin
	ACTC				Actin, alpha cardiac muscle 1 (Alpha-car
22	ACTN1	18.7	13	227	Alpha-actinin-1 (Alpha-actinin cytoskele
23	ACTN4	22.7	14	147	Alpha-actinin-4 (Non-muscle alpha-actini
24	ACTS	40.6	30	174	Actin, alpha skeletal muscle (Alpha-acti
25	ADH7	23.5	7	296	Alcohol dehydrogenase class 4 mu/sigma c
26	AFAM	7.8	3	404	Afamin precursor (Alpha-albumin) (Alpha-
27	AK1A1	12.6	3	425	Alcohol dehydrogenase [NADP+] (EC 1.1.1.
28	AK1BA	31.3	7	359	Aldo-keto reductase family 1 member B10
29	AL3A1	21.2	10	231	Aldehyde dehydrogenase, dimeric NADP-pre
30	AL9A1	4.9	2	557	4-trimethylaminobutyraldehyde dehydrogen
31	ALBU	85.9	232	2	Serum albumin precursor - Homo sapiens (
32	ALDOA	71.7	25	55	Fructose-bisphosphate aldolase A (EC 4.1
33	ALDOC	18.7	7	143	Fructose-bisphosphate aldolase C (EC 4.1
34	AMBP	17.3	3	508	AMBP protein precursor [Contains: Alpha-
35	AMD	8.0	4	290	Peptidyl-glycine alpha-amidating monooxy
36	AMPB	5.7	2	579	Aminopeptidase B (EC 3.4.11.6) (Ap-B) (A
37	AMYC	69.1	119	28	Alpha-amylase 2B precursor (EC 3.2.1.1)
38	AMYP	69.7	167	21	Pancreatic alpha-amylase precursor (EC 3
39	AMYS	88.1	273	1	Salivary alpha-amylase precursor (EC 3.2
40	AN32A	14.9	2	553	Acidic leucine-rich nuclear phosphoprote
41	ANGT	6.2	2	532	Angiotensinogen precursor [Contains: Ang
42	ANT3	11.9	4	308	Antithrombin-III precursor (ATIII) - Hom
43	ANXA1	63.3	31	65	Annexin A1 (Annexin I) (Lipocortin I) (C
44	ANXA2	43.4	18	230	Annexin A2 (Annexin II) (Lipocortin II)
45	ANXA3	41.2	12	244	Annexin A3 (Annexin III) (Lipocortin III
46	ANXA5	6.6	2	538	Annexin A5 (Annexin V) (Lipocortin V) (E
47	APM2	71.1	2	475	Adipose most abundant gene transcript 2
48	APOA1	55.4	24	71	Apolipoprotein A-I precursor (Apo-AI) (A
49	APOA2	32.0	5	334	Apolipoprotein A-II precursor (Apo-AII)
50	APOA4	13.1	4	310	Apolipoprotein A-IV precursor (Apo-AIV)
51	APOC3	33.3	2	407	Apolipoprotein C-III precursor (Apo-CIII
52	APOD	6.3	3	509	Apolipoprotein D precursor (Apo-D) (ApoD
53	APOE	13.6	3	403	Apolipoprotein E precursor (Apo-E) - Hom
54	APOH	22.6	7	242	Beta-2-glycoprotein 1 precursor (Beta-2-
55	APT	18.3	2	460	Adenine phosphoribosyltransferase (EC 2.
56	ARC1B	19.6	6	347	Actin-related protein 2/3 complex subuni
57	ARGI1	16.8	3	375	Arginase-1 (EC 3.5.3.1) (Type I arginase
58	ARMET	17.9	2	497	ARMET protein precursor (Arginine-rich p
59	ARP2	18.5	8	321	Actin-like protein 2 (Actin-related prot
60	ARP3	25.1	9	217	Actin-like protein 3 (Actin-related prot
61	ARPC2	15.3	4	385	Actin-related protein 2/3 complex subuni
62	ARPC3	13.5	2	478	Actin-related protein 2/3 complex subuni
63	ARPC4	11.3	2	559	Actin-related protein 2/3 complex subuni
64	ARPC5	46.4	4	400	Actin-related protein 2/3 complex subuni
65	ARTS1	2.8	2	581	Adipocyte-derived leucine aminopeptidase
66	ASPG	12.4	2	443	N(4)-(beta-N-acetylglucosaminyl)-L-aspar
67	B2MG	57.1	10	88	Beta-2-microglobulin precursor [Contains
68	B4GT1	5.0	2	540	Beta-1,4-galactosyltransferase 1 (EC 2.4
69	BASP	23.3	3	339	Brain acid soluble protein 1 (BASP1 prot
70	BD01	33.8	2	560	Beta-defensin 1 precursor (BD-1) (Defens
71	BD02	34.4	2	526	Beta-defensin 2 precursor (BD-2) (hBD-2)
72	BPI	14.7	5	313	Bactericidal permeability-increasing pro
73	BPIL1	40.4	32	44	Bactericidal/permeability-increasing pro
74	BST1	10.7	2	501	ADP-ribosyl cyclase 2 precursor (EC 3.2.
75	C4BP	4.0	2	535	C4b-binding protein alpha chain precurso
76	CAB45	8.3	2	477	45 kDa calcium-binding protein precursor
77	CADH1	4.0	2	442	Epithelial-cadherin precursor (E-cadheri
78	CAH1	23.8	4	448	Carbonic anhydrase 1 (EC 4.2.1.1) (Carbo
79	CAH6	71.8	28	37	Carbonic anhydrase 6 precursor (EC 4.2.1
80	CALL3	54.4	6	232	Calmodulin-like protein 3 (Calmodulin-re
81	CALL5	43.8	8	221	Calmodulin-like protein 5 (Calmodulin-li
82	CALR	17.3	6	326	Calreticulin precursor (CRP55) (Calregul
83	CAMP	34.7	5	269	Cathelicidin antimicrobial peptide precu
84	CAP1	32.4	15	125	Adenylyl cyclase-associated protein 1 (C
85	CAP7	35.9	8	126	Azurocidin precursor (Cationic antimicro
86	CAPG	33.6	7	214	Macrophage capping protein (Actin-regula
87	CAPZB	37.2	6	349	F-actin capping protein subunit beta (Ca
88	CASPE	9.9	2	549	Caspase-14 precursor (EC 3.4.22.-) (CASP
89	CATA	32.1	15	137	Catalase (EC 1.11.1.6) - Homo sapiens (H
90	CATB	31.6	9	163	Cathepsin B precursor (EC 3.4.22.1) (Cat
91	CATC	19.9	8	295	Dipeptidyl-peptidase 1 precursor (EC 3.4
92	CATD	36.7	11	117	Cathepsin D precursor (EC 3.4.23.5) [Con
93	CATG	30.6	7	268	Cathepsin G precursor (EC 3.4.21.20) (CG
94	CATH	9.6	2	434	Cathepsin H precursor (EC 3.4.22.16) [Co
95	CATL	15.0	4	396	Cathepsin L precursor (EC 3.4.22.15) (Ma
96	CATZ	21.5	4	325	Cathepsin Z precursor (EC 3.4.22.-) (Cat
97	CAZA1	31.1	5	266	F-actin capping protein subunit alpha-1
98	CAZA2	9.8	2	556	F-actin capping protein subunit alpha-2
99	CBPD	5.8	3	406	Carboxypeptidase D precursor (EC 3.4.17.
100	CBPE	21.4	8	264	Carboxypeptidase E precursor (EC 3.4.17.

Pep	No.	Acc.	Cov.	Rank	Description (40 characters)
101	CCL28	26.0	3	388	Small inducible cytokine A28 precursor (
102	CD14	28.0	9	172	Monocyte differentiation antigen CD14 pr
103	CD44	3.0	2	426	CD44 antigen precursor (Phagocytic glyco
104	CD59	36.7	5	236	CD59 glycoprotein precursor (Membrane at
105	CDC42	16.8	3	417	Cell division control protein 42 homolog
106	CDD	55.5	7	200	Cytidine deaminase (EC 3.5.4.5) (Cytidin
107	CERU	19.6	14	138	Ceruloplasmin precursor (EC 1.16.3.1) (F
108	CF058	50.0	20	76	Uncharacterized protein C6orf58 precurso
109	CFAB	14.0	7	220	Complement factor B precursor (EC 3.4.21
110	CFAH	8.5	6	219	Complement factor H precursor (H factor
111	CH10	25.5	2	498	10 kDa heat shock protein, mitochondrial
112	CH3L1	11.5	3	409	Chitinase-3-like protein 1 precursor (Ca
113	CH3L2	7.4	2	438	Chitinase 3-like protein 2 precursor (YK
114	CHAD	10.3	3	503	Chondroadherin precursor (Cartilage leuc
115	CHIT1	9.7	3	374	Chitotriosidase-1 precursor (EC 3.2.1.14
116	CLIC1	43.2	8	182	Chloride intracellular channel protein 1
117	CLUS	29.2	11	134	Clusterin precursor (Complement-associat
118	CO2	3.3	2	510	Complement C2 precursor (EC 3.4.21.43) (
119	CO3	36.3	60	47	Complement C3 precursor [Contains: Compl
120	CO4A	13.6	15	155	Complement C4-A precursor (Acidic comple
	CO4B				Complement C4-B precursor (Basic complem
121	CO6A1	4.8	3	462	Collagen alpha-1(VI) chain precursor - H
122	CO6A2	6.0	4	419	Collagen alpha-2(VI) chain precursor - H
123	COEA1	7.0	9	294	Collagen alpha-1(XIV) chain precursor (U
124	COF1	62.0	13	127	Cofilin-1 (Cofilin, non-muscle isoform)
125	COR1A	31.2	15	128	Coronin-1A (Coronin-like protein p57) (C
126	COTL1	24.6	2	570	Coactosin-like protein - Homo sapiens (H
127	CQ025	11.5	3	439	Uncharacterized protein C17orf25 - Homo
128	CRIS3	69.8	20	39	Cysteine-rich secretory protein 3 precur
129	CYTA	53.1	4	329	Cystatin-A (Stefin-A) (Cystatin-AS) - Ho
130	CYTB	94.9	19	40	Cystatin-B (Stefin-B) (Liver thiol prote
131	CYTC	69.2	22	42	Cystatin-C precursor (Cystatin-3) (Neuro
132	CYTD	72.5	58	19	Cystatin-D precursor (Cystatin-5) - Homo
133	CYTN	74.5	82	4	Cystatin-SN precursor (Cystatin-1) (Sali
134	CYTS	81.6	113	3	Cystatin-S precursor (Cystatin-4) (Saliv
135	CYTT	76.6	62	12	Cystatin-SA precursor (Cystatin-S5) - Ho
136	DAG1	5.4	3	437	Dystroglycan precursor (Dystrophin-assoc
137	DBNL	6.3	2	521	Drebrin-like protein (SH3 domain-contain
138	DCD	43.6	3	387	Dermcidin precursor (Preproteolysin) [Co
139	DEF1	25.5	6	75	Neutrophil defensin 1 precursor (HNP-1)
	DEF3				Neutrophil defensin 3 precursor (HNP-3)
140	DESP	3.7	6	428	Desmoplakin (DP) (250/210 kDa paraneopla
141	DHCA	20.6	3	432	Carbonyl reductase [NADPH] 1 (EC 1.1.1.1
142	DMBT1	18.7	48	16	Deleted in malignant brain tumors 1 prot
143	DNJC3	9.1	2	446	DnaJ homolog subfamily C member 3 (Inter
144	DOPD	19.5	2	547	D-dopachrome decarboxylase (EC 4.1.1.84)
145	DSC2	25.1	16	101	Desmocollin-2 precursor (Desmosomal glyc
146	DSG1	14.0	13	149	Desmoglein-1 precursor (Desmosomal glyco
147	DSG3	25.0	26	78	Desmoglein-3 precursor (130 kDa pemphigu
148	ECHA	6.0	2	554	Trifunctional enzyme subunit alpha, mito
149	ECM1	30.7	9	225	Extracellular matrix protein 1 precursor
150	ECP	26.2	3	402	Eosinophil cationic protein precursor (E
151	EF1A1	16.9	5	240	Elongation factor 1-alpha 1 (EF-1-alpha-

No.	Acc.	Cov.	Pep	Rank	Description (40 characters)
152	EF2	5.5	4	449	Elongation factor 2 (EF-2) - Homo sapien
153	EGF	2.6	2	533	Pro-epidermal growth factor precursor (E
154	EHD1	18.5	5	418	EH domain-containing protein 1 (Testilin
155	ELAF	35.9	3	373	Elafin precursor (Elastase-specific inhi
156	ELNE	35.2	8	223	Leukocyte elastase precursor (EC 3.4.21.
157	ENOA	80.0	52	33	Alpha-enolase (EC 4.2.1.11) (2-phospho-D
158	ENOB	13.6	4	271	Beta-enolase (EC 4.2.1.11) (2-phospho-D-
159	ENOG	7.8	2	365	Gamma-enolase (EC 4.2.1.11) (2-phospho-D
160	ERO1A	13.0	4	283	ERO1-like protein alpha precursor (EC 1.
161	ERP29	17.2	3	436	Endoplasmic reticulum protein ERp29 prec
162	EST1	4.1	2	543	Liver carboxylesterase 1 precursor (EC 3
163	EST2	13.1	6	281	Carboxylesterase 2 precursor (EC 3.1.1.1
164	EZRI	21.7	13	162	Ezrin (p81) (Cytovillin) (Villin-2) - Ho
165	FABPE	78.5	25	53	Fatty acid-binding protein, epidermal (E
166	FAM25	33.7	4	316	Protein FAM25 - Homo sapiens (Human)
167	FAM3B	17.0	4	354	Protein FAM3B precursor (Cytokine-like p
168	FAM3C	12.8	2	576	Protein FAM3C precursor (Protein GS3786)
169	FAM3D	54.0	11	141	Protein FAM3D precursor - Homo sapiens (
170	FCG3B	14.2	2	529	Low affinity immunoglobulin gamma Fc reg
171	FCGBP	24.2	101	27	IgGFc-binding protein precursor (Fcgamma
172	FETUA	22.3	7	157	Alpha-2-HS-glycoprotein precursor (Fetui
173	FIBA	9.8	10	184	Fibrinogen alpha chain precursor [Contai
174	FIBB	52.3	26	77	Fibrinogen beta chain precursor [Contain
175	FIBG	48.1	18	97	Fibrinogen gamma chain precursor - Homo
176	FINC	2.7	5	395	Fibronectin precursor (FN) (Cold-insolub
177	FKB1A	25.0	3	485	FK506-binding protein 1A (EC 5.2.1.8) (P
178	FLNA	5.2	8	302	Filamin-A (Alpha-filamin) (Filamin-1) (E
179	FMOD	7.4	2	564	Fibromodulin precursor (FM) (Collagen-bi
180	FOLR1	22.2	4	315	Folate receptor alpha precursor (FR-alph
181	FOLR3	31.3	6	415	Folate receptor gamma precursor (FR-gamm
182	FSTL1	6.8	2	565	Follistatin-related protein 1 precursor
183	FURIN	14.5	8	238	Furin precursor (EC 3.4.21.75) (Paired b
184	FUT8	2.4	2	577	Alpha-(1,6)-fucosyltransferase (EC 2.4.1
185	G3P	67.5	41	38	Glyceraldehyde-3-phosphate dehydrogenase
186	G6PD	30.3	11	253	Glucose-6-phosphate 1-dehydrogenase (EC
187	G6PI	39.4	20	136	Glucose-6-phosphate isomerase (EC 5.3.1.
188	GANAB	6.1	3	473	Neutral alpha-glucosidase AB precursor (
189	GDIA	6.9	2	484	Rab GDP dissociation inhibitor alpha (Ra
190	GDIB	36.2	12	216	Rab GDP dissociation inhibitor beta (Rab
191	GDIR	22.5	6	246	Rho GDP-dissociation inhibitor 1 (Rho GD
192	GDIS	76.1	23	108	Rho GDP-dissociation inhibitor 2 (Rho GD
193	GELS	37.6	27	80	Gelsolin precursor (Actin-depolymerizing
194	GLRX1	24.5	2	495	Glutaredoxin-1 (Thioltransferase-1) (TTa
195	GMFG	15.5	2	528	Glia maturation factor gamma (GMF-gamma)
196	GP73	18.2	6	265	Golgi phosphoprotein 2 (Golgi membrane p
197	GRB2	5.5	2	563	Growth factor receptor-bound protein 2 (
198	GRN	14.0	7	181	Granulins precursor (Proepithelin) (PEPI
199	GRP78	28.3	17	121	78 kDa glucose-regulated protein precurs
200	GSHR	12.1	4	479	Glutathione reductase, mitochondrial pre
201	GSLG1	3.5	2	393	Golgi apparatus protein 1 precursor (Gol
202	GSTP1	71.9	20	63	Glutathione S-transferase P (EC 2.5.1.18
203	H14	22.8	8	344	Histone H1.4 (Histone H1b) - Homo sapien
	H12				Histone H1.2 (Histone H1d) - Homo sapien

Pep	No.	Acc.	Cov.	Rank	Description (40 characters)
	H13				Histone H1.3 (Histone H1c) - Homo sapien
204	H15	22.1	5	377	Histone H1.5 (Histone H1a) - Homo sapien
205	H2A1	35.4	6	260	Histone H2A type 1 (H2A.1) - Homo sapien
	H2A1A				Histone H2A type 1-A - Homo sapiens (Hum
	H2A1D				Histone H2A type 1-D (H2A.3) - Homo sapi
	H2A1H				Histone H2A type 1-H - Homo sapiens (Hum
	H2A1J				Histone H2A type 1-J - Homo sapiens (Hum
	H2A2A				Histone H2A type 2-A (H2A.2) - Homo sapi
	H2A2B				Histone H2A type 2-B - Homo sapiens (Hum
	H2A2C				Histone H2A type 2-C (H2A-GL101) (H2A/r)
	H2AX				Histone H2A.x (H2a/x) - Homo sapiens (Hu
206	H4	39.8	7	333	Histone H4 - Homo sapiens (Human)
207	HBA	83.8	9	152	Hemoglobin subunit alpha (Hemoglobin alp
208	HBB	89.8	21	82	Hemoglobin subunit beta (Hemoglobin beta
209	HBD	59.9	12	222	Hemoglobin subunit delta (Hemoglobin del
210	HCLS1	7.0	2	512	Hematopoietic lineage cell-specific prot
211	HEBP2	17.1	3	411	Heme-binding protein 2 (Protein SOUL) (P
212	HEMO	34.2	16	103	Hemopexin precursor (Beta-1B-glycoprotei
213	HIS1	45.6	26	18	Histatin-1 precursor (Histidine-rich pro
214	HIS3	39.2	3	167	Histatin-3 precursor (Histidine-rich pro
215	HMGB1	17.7	4	276	High mobility group protein B1 (High mob
	HMG1X				High mobility group protein 1-like 10 (H
216	HMGB2	19.6	4	324	High mobility group protein B2 (High mob
217	HPRT	10.6	2	530	Hypoxanthine-guanine phosphoribosyltrans
218	HPT	59.6	27	64	Haptoglobin precursor [Contains: Haptogl
219	HPTR	35.9	16	166	Haptoglobin-related protein precursor -
220	HRG	14.3	5	348	Histidine-rich glycoprotein precursor (H
221	HS70L	19.8	14	150	Heat shock 70 kDa protein 1L (Heat shock
222	HS90A	15.3	10	212	Heat shock protein HSP 90-alpha (HSP 86)
223	HS90B	11.3	7	358	Heat shock protein HSP 90-beta (HSP 84)
224	HSP71	40.1	26	67	Heat shock 70 kDa protein 1 (HSP70.1) (H
225	HSP74	4.6	3	468	Heat shock 70 kDa protein 4 (Heat shock
226	HSP76	13.7	10	318	Heat shock 70 kDa protein 6 (Heat shock
227	HSP7C	35.3	23	105	Heat shock cognate 71 kDa protein (Heat
228	HSPB1	41.5	7	164	Heat-shock protein beta-1 (HspB1) (Heat
229	HTRA1	15.2	3	422	Serine protease HTRA1 precursor (EC 3.4.
230	HV101	19.7	4	319	Ig heavy chain V-I region EU - Homo sapi
	HV106				Ig heavy chain V-I region SIE - Homo sap
231	HV102	32.5	4	261	Ig heavy chain V-I region HG3 precursor
232	HV103	23.9	2	494	Ig heavy chain V-I region V35 precursor
233	HV107	19.2	4	300	Ig heavy chain V-I region Mot - Homo sap
234	HV207	13.7	3	288	Ig heavy chain V-II region NEWM - Homo s
	HV209				Ig heavy chain V-II region ARH-77 precur
235	HV301	41.0	6	234	Ig heavy chain V-III region TRO - Homo s
236	HV302	36.0	6	198	Ig heavy chain V-III region WEA - Homo s
237	HV303	54.7	11	85	Ig heavy chain V-III region VH26 precurs
238	HV304	42.6	8	145	Ig heavy chain V-III region TIL - Homo s
239	HV305	28.3	12	66	Ig heavy chain V-III region BRO - Homo s
240	HV306	35.7	5	177	Ig heavy chain V-III region BUT - Homo s
241	HV307	28.7	4	390	Ig heavy chain V-III region CAM - Homo s
242	HV308	24.6	4	430	Ig heavy chain V-III region GA - Homo sa
243	HV309	21.0	2	492	Ig heavy chain V-III region NIE - Homo s
244	HV310	22.3	3	304	Ig heavy chain V-III region HIL - Homo s

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245	HV311	19.0	2	380	Ig heavy chain V-III region KOL - Homo s
246	HV312	25.2	3	507	Ig heavy chain V-III region BUR - Homo s
247	HV313	33.6	7	258	Ig heavy chain V-III region POM - Homo s
248	HV314	33.6	4	361	Ig heavy chain V-III region LAY - Homo s
249	HV315	41.0	8	153	Ig heavy chain V-III region WAS - Homo s
250	HV316	28.6	10	118	Ig heavy chain V-III region TEI - Homo s
251	HV318	36.2	7	106	Ig heavy chain V-III region TUR - Homo s
252	HV319	35.7	8	161	Ig heavy chain V-III region JON - Homo s
253	HV320	34.5	6	178	Ig heavy chain V-III region GAL - Homo s
254	HV322	22.9	3	427	Ig heavy chain V-III region GAR - Homo s
255	HXK3	5.0	4	386	Hexokinase-3 (EC 2.7.1.1) (Hexokinase ty
256	IDHC	9.9	4	363	Isocitrate dehydrogenase [NADP] cytoplas
257	IDUA	3.1	2	551	Alpha-L-iduronidase precursor (EC 3.2.1.
258	IF5A1	19.5	2	292	Eukaryotic translation initiation factor
	IF5A2				Eukaryotic translation initiation factor
259	IGHA1	90.9	107	7	Ig alpha-1 chain C region - Homo sapiens
260	IGHA2	80.3	85	10	Ig alpha-2 chain C region - Homo sapiens
261	IGHG1	63.0	35	36	Ig gamma-1 chain C region - Homo sapiens
262	IGHG2	62.9	33	31	Ig gamma-2 chain C region - Homo sapiens
263	IGHG3	49.3	19	49	Ig gamma-3 chain C region (Heavy chain d
264	IGHG4	58.7	24	52	Ig gamma-4 chain C region - Homo sapiens
265	IGJ	66.4	28	35	Immunoglobulin J chain - Homo sapiens (H
266	IGLL1	18.3	7	187	Immunoglobulin lambda-like polypeptide 1
267	IL16	4.9	2	569	Interleukin-16 precursor (IL-16) (Lympho
268	IL1F6	23.4	3	355	Interleukin-1 family member 6 (IL-1F6) (
269	IL1RA	41.8	8	151	Interleukin-1 receptor antagonist protei
270	ILEU	41.4	19	96	Leukocyte elastase inhibitor (LEI) (Serp
271	IMB1	3.1	2	571	Importin beta-1 subunit (Karyopherin bet
272	INVO	23.8	9	280	Involucrin - Homo sapiens (Human)
273	IQGA1	3.6	4	392	Ras GTPase-activating-like protein IQGAP
274	ISK5	16.8	12	170	Serine protease inhibitor Kazal-type 5 p
275	ISK7	32.9	3	370	Serine protease inhibitor Kazal-type 7 p
276	ITAM	4.8	4	398	Integrin alpha-M precursor (Cell surface
277	ITB2	4.8	4	481	Integrin beta-2 precursor (Cell surface
278	ITIH1	4.9	3	435	Inter-alpha-trypsin inhibitor heavy chai
279	ITIH2	12.9	6	328	Inter-alpha-trypsin inhibitor heavy chai
280	ITIH4	5.3	3	472	Inter-alpha-trypsin inhibitor heavy chai
281	K1C10	10.6	5	257	Keratin, type I cytoskeletal 10 (Cytoker
282	K1C13	66.2	52	57	Keratin, type I cytoskeletal 13 (Cytoker
283	K1C15	16.7	13	156	Keratin, type I cytoskeletal 15 (Cytoker
284	K1C17	19.2	8	336	Keratin, type I cytoskeletal 17 (Cytoker
285	K1C20	6.1	4	414	Keratin, type I cytoskeletal 20 (Cytoker
286	K22E	16.1	8	160	Keratin, type II cytoskeletal 2 epiderma
287	K22O	33.1	14	267	Keratin, type II cytoskeletal 2 oral (Cy
288	K2C3	11.9	8	299	Keratin, type II cytoskeletal 3 (Cytoker
289	K2C4	70.2	42	94	Keratin, type II cytoskeletal 4 (Cytoker
290	K2C5	23.7	14	183	Keratin, type II cytoskeletal 5 (Cytoker
291	K2C6A	38.7	29	110	Keratin, type II cytoskeletal 6A (Cytoke
292	K2C6B	36.5	23	116	Keratin, type II cytoskeletal 6B (Cytoke
293	K2C6C	38.7	29	122	Keratin, type II cytoskeletal 6A (Cytoke
	K2C6D				Keratin, type II cytoskeletal 6D (Cytoke
	K2C6E				Keratin, type II cytoskeletal 6E (Cytoke
294	K2C8	5.2	3	383	Keratin, type II cytoskeletal 8 (Cytoker

Pep	No.	Acc.	Cov.	Pep	Rank	Description (40 characters)
	K2C7					Keratin, type II cytoskeletal 7 (Cytoker
295	KAC	90.6	58		15	Ig kappa chain C region - Homo sapiens (
296	KCRB	18.9	6		171	Creatine kinase B-type (EC 2.7.3.2) (Cre
297	KLK1	33.2	9		142	Kallikrein-1 precursor (EC 3.4.21.35) (T
298	KLK10	10.5	3		454	Kallikrein-10 precursor (EC 3.4.21.-) (P
299	KLK11	25.5	7		218	Kallikrein-11 precursor (EC 3.4.21.-) (H
300	KLK12	31.0	4		453	Kallikrein-12 precursor (EC 3.4.21.-) (K
301	KLK13	25.3	6		252	Kallikrein-13 precursor (EC 3.4.21.-) (K
302	KLK6	17.6	3		423	Kallikrein-6 precursor (EC 3.4.21.-) (Pr
303	KNG1	9.2	4		340	Kininogen-1 precursor (Alpha-2-thiol pro
304	KPYM	64.8	47		50	Pyruvate kinase isozymes M1/M2 (EC 2.7.1
305	KV101	42.6	5		148	Ig kappa chain V-I region AG - Homo sapi
306	KV102	42.6	7		159	Ig kappa chain V-I region AU - Homo sapi
307	KV105	43.5	7		129	Ig kappa chain V-I region DEE - Homo sap
308	KV106	32.4	8		146	Ig kappa chain V-I region EU - Homo sapi
	KV104					Ig kappa chain V-I region CAR - Homo sap
309	KV109	34.2	5		277	Ig kappa chain V-I region HK101 precurso
310	KV110	34.2	4		272	Ig kappa chain V-I region HK102 precurso
311	KV113	36.1	3		210	Ig kappa chain V-I region Lay - Homo sap
312	KV114	22.2	2		458	Ig kappa chain V-I region OU - Homo sapi
313	KV117	27.8	4		293	Ig kappa chain V-I region Scw - Homo sap
314	KV118	43.5	6		356	Ig kappa chain V-I region WEA - Homo sap
	KV108					Ig kappa chain V-I region Hau - Homo sap
	KV125					Ig kappa chain V-I region WAT - Homo sap
315	KV119	22.2	4		206	Ig kappa chain V-I region Wes - Homo sap
316	KV120	16.5	4		307	Ig kappa chain V-I region Mev - Homo sap
317	KV121	30.4	4		237	Ig kappa chain V-I region Ni - Homo sapi
318	KV122	27.8	4		273	Ig kappa chain V-I region BAN - Homo sap
319	KV201	51.3	10		131	Ig kappa chain V-II region Cum - Homo sa
320	KV203	34.8	4		203	Ig kappa chain V-II region MIL - Homo sa
321	KV204	41.6	7		204	Ig kappa chain V-II region TEW - Homo sa
	KV205					Ig kappa chain V-II region GM607 precurs
322	KV205	59.8	12		74	Ig kappa chain V-II region GM607 precurs
323	KV206	72.9	13		120	Ig kappa chain V-II region RPMI 6410 pre
324	KV301	31.5	2		441	Ig kappa chain V-III region B6 - Homo sa
325	KV302	65.1	12		111	Ig kappa chain V-III region SIE - Homo s
	KV312					Ig kappa chain V-III region HAH precurso
	KV313					Ig kappa chain V-III region HIC precurso
326	KV303	41.0	6		226	Ig kappa chain V-III region NG9 precurso
327	KV304	56.9	12		243	Ig kappa chain V-III region Ti - Homo sa
328	KV305	56.9	12		168	Ig kappa chain V-III region WOL - Homo s
329	KV306	24.8	2		197	Ig kappa chain V-III region POM - Homo s
330	KV307	45.9	6		305	Ig kappa chain V-III region GOL (Rheumat
331	KV308	33.3	6		135	Ig kappa chain V-III region CLL precurso
332	KV309	67.8	8		133	Ig kappa chain V-III region VG precursor
333	KV310	23.3	3		190	Ig kappa chain V-III region VH precursor
334	KV311	28.9	3		364	Ig kappa chain V-III region IARC/BL41 pr
335	KV401	44.6	6		191	Ig kappa chain V-IV region precursor (Fr
336	KV402	47.4	7		193	Ig kappa chain V-IV region Len - Homo sa
337	KV403	40.6	7		291	Ig kappa chain V-IV region JI precursor
338	KV404	44.8	9		115	Ig kappa chain V-IV region B17 precursor
339	KV405	35.8	2		486	Ig kappa chain V-IV region STH (Fragment
340	LAC	94.3	34		24	Ig lambda chain C regions - Homo sapiens

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341	LACRT	23.2	4	284	Extracellular glycoprotein lacritin prec
342	LASP1	16.9	3	516	LIM and SH3 domain protein 1 (LASP-1) (M
343	LCN1	81.8	45	29	Lipocalin-1 precursor (Von Ebner gland p
344	LDHA	44.9	11	173	L-lactate dehydrogenase A chain (EC 1.1.
345	LDHB	21.3	6	274	L-lactate dehydrogenase B chain (EC 1.1.
346	LEG3	19.6	8	255	Galectin-3 (Galactose-specific lectin 3)
347	LEG7	55.1	5	228	Galectin-7 (Gal-7) (HKL-14) (PI7) (p53-i
348	LG3BP	33.2	16	79	Galectin-3-binding protein precursor (Le
349	LKHA4	28.8	15	140	Leukotriene A-4 hydrolase (EC 3.3.2.6) (
350	LPLC1	38.0	16	98	Long palate, lung and nasal epithelium c
351	LSP1	12.1	2	550	Lymphocyte-specific protein 1 (Protein p
352	LSR	9.7	3	429	Lipolysis-stimulated lipoprotein recepto
353	LV001	15.4	2	431	Ig lambda chain V region 4A precursor -
354	LV101	10.8	2	534	Ig lambda chain V-I region VOR - Homo sa
355	LV102	25.9	5	312	Ig lambda chain V-I region HA - Homo sap
356	LV104	18.9	2	459	Ig lambda chain V-I region NIG-64 - Homo
	LV107				Ig lambda chain V-I region BL2 precursor
357	LV105	31.1	2	491	Ig lambda chain V-I region NEWM - Homo s
358	LV106	36.7	4	401	Ig lambda chain V-I region WAH - Homo sa
359	LV301	25.0	4	251	Ig lambda chain V-III region SH - Homo s
360	LV302	37.8	7	132	Ig lambda chain V-III region LOI - Homo
361	LV403	17.8	4	165	Ig lambda chain V-IV region Hil - Homo s
362	LV405	23.6	2	382	Ig lambda chain V-IV region MOL - Homo s
363	LV501	31.5	3	480	Ig lambda chain V-V region DEL - Homo sa
364	LYPD3	22.0	6	195	Ly6/PLAUR domain-containing protein 3 pr
365	LYSC	62.8	47	20	Lysozyme C precursor (EC 3.2.1.17) (1,4-
366	M4K4	3.0	2	505	Mitogen-activated protein kinase kinase
367	M6PBP	23.5	5	289	Mannose-6-phosphate receptor-binding pro
368	MA1A1	6.3	3	467	Mannosyl-oligosaccharide 1,2-alpha-manno
369	MANS1	9.7	2	520	MANSC domain-containing protein 1 precur
370	MCFD2	23.3	2	574	Multiple coagulation factor deficiency p
371	MDHC	26.6	6	233	Malate dehydrogenase, cytoplasmic (EC 1.
372	MDHM	35.8	10	306	Malate dehydrogenase, mitochondrial prec
373	MGP	25.2	3	278	Matrix Gla-protein precursor (MGP) (Cell
374	MIF	37.4	4	248	Macrophage migration inhibitory factor (
375	MLRM	17.5	2	464	Myosin regulatory light chain 2, nonsarc
376	MMP8	13.9	6	360	Neutrophil collagenase precursor (EC 3.4
377	MMP9	28.3	20	86	Matrix metalloproteinase-9 precursor (EC
378	MNDA	10.1	3	410	Myeloid cell nuclear differentiation ant
379	MOES	33.6	23	109	Moesin (Membrane-organizing extension sp
380	MSLN	15.9	8	285	Mesothelin precursor (Pre-pro-megakaryoc
381	MSMB	23.7	4	371	Beta-microseminoprotein precursor (Prost
382	MTPN	30.5	3	381	Myotrophin (Protein V-1) - Homo sapiens
383	MUC	62.3	32	41	Ig mu chain C region - Homo sapiens (Hum
384	MUC2	0.5	4	279	Mucin-2 precursor (Intestinal mucin-2) -
385	MUC5B	20.7	209	5	Mucin-5B precursor (Mucin-5 subtype B, t
386	MUC7	18.3	21	34	Mucin-7 precursor (MUC-7) (Salivary muci
387	MUCB	58.6	23	62	Ig mu heavy chain disease protein (BOT)
388	MYH9	20.4	29	123	Myosin-9 (Myosin heavy chain 9) (Myosin
389	MYL6	42.4	7	245	Myosin light polypeptide 6 (Smooth muscl
390	MYO1F	1.8	2	524	Myosin-If (Myosin-Ie) - Homo sapiens (Hu
391	NACA	13.0	2	567	Nascent polypeptide-associated complex s
392	NAGK	7.6	2	582	N-acetylglucosamine kinase (EC 2.7.1.59)

Pep	No.	Acc.	Cov.	Rank	Description (40 characters)
393	NAMPT	15.9	6	287	Nicotinamide phosphoribosyltransferase (
394	NCF1	22.8	5	413	Neutrophil cytosol factor 1 (NCF-1) (Neu
395	NCF4	6.5	2	517	Neutrophil cytosol factor 4 (NCF-4) (Neu
396	NDKB	31.6	5	378	Nucleoside diphosphate kinase A (EC 2.7.
	NDKA				Nucleoside diphosphate kinase B (EC 2.7.
	NDK8				Putative nucleoside diphosphate kinase (
397	NDRG2	7.8	2	583	Protein NDRG2 (Protein Syld709613) - Hom
398	NGAL	48.0	8	211	Neutrophil gelatinase-associated lipocal
399	NHERF	11.2	2	389	Ezrin-radixin-moesin-binding phosphoprot
400	NPC2	38.4	4	330	Epididymal secretory protein E1 precurso
401	NUCB1	28.4	10	224	Nucleobindin-1 precursor (CALNUC) - Homo
402	NUCB2	61.9	24	61	Nucleobindin-2 precursor (DNA-binding pr
403	OS9	9.6	3	433	Protein OS-9 precursor (Amplified in ost
404	OST4	6.1	2	487	Heparan sulfate glucosamine 3-O-sulfotra
405	OXRP	2.1	2	584	150 kDa oxygen-regulated protein precurs
406	PA2GA	18.1	2	493	Phospholipase A2, membrane associated pr
407	PARK7	36.5	6	286	Protein DJ-1 (Oncogene DJ1) (Parkinson d
408	PCBP1	14.0	3	474	Poly(rC)-binding protein 1 (Alpha-CP1) (
409	PCP	4.4	2	555	Lysosomal Pro-X carboxypeptidase precurs
410	PDC6I	8.6	5	314	Programmed cell death 6-interacting prot
411	PDIA1	30.9	18	107	Protein disulfide-isomerase precursor (E
412	PDIA3	11.7	5	397	Protein disulfide-isomerase A3 precursor
413	PDIA4	3.4	2	539	Protein disulfide-isomerase A4 precursor
414	PDIA6	10.9	2	572	Protein disulfide-isomerase A6 precursor
415	PDXK	6.7	2	525	Pyridoxal kinase (EC 2.7.1.35) (Pyridoxi
416	PEBP1	65.8	7	185	Phosphatidylethanolamine-binding protein
417	PEDF	6.0	2	552	Pigment epithelium-derived factor precur
418	PEPL	2.1	2	490	Periplakin (195 kDa cornified envelope p
419	PERE	4.8	3	511	Eosinophil peroxidase precursor (EC 1.11
420	PERL	55.8	46	26	Lactoperoxidase precursor (EC 1.11.1.7)
421	PERM	41.6	39	60	Myeloperoxidase precursor (EC 1.11.1.7)
422	PGAM1	44.9	12	158	Phosphoglycerate mutase 1 (EC 5.4.2.1) (
	PGAM2				Phosphoglycerate mutase 2 (EC 5.4.2.1) (
	PGAM4				Probable phosphoglycerate mutase 4 (EC 5
423	PGK1	64.3	31	72	Phosphoglycerate kinase 1 (EC 2.7.2.3) (
424	PGM1	5.9	3	469	Phosphoglucomutase-1 (EC 5.4.2.2) (Gluco
425	PGRP	27.6	3	297	Peptidoglycan recognition protein precur
426	PIGR	63.7	168	8	Polymeric-immunoglobulin receptor precur
427	PIP	77.4	76	11	Prolactin-inducible protein precursor (P
428	PKP1	4.8	2	568	Plakophilin-1 (Band-6 protein) (B6P) - H
429	PLMN	5.2	2	506	Plasminogen precursor (EC 3.4.21.7) [Con
430	PLOD1	7.0	3	421	Procollagen-lysine,2-oxoglutarate 5-diox
431	PLSL	42.4	23	95	Plastin-2 (L-plastin) (Lymphocyte cytoso
432	PLST	13.6	8	275	Plastin-3 (T-plastin) - Homo sapiens (Hu
433	PLTP	17.6	6	235	Phospholipid transfer protein precursor
434	PLUNC	22.7	4	391	Protein Plunc precursor (Palate lung and
435	PNPH	18.3	4	379	Purine nucleoside phosphorylase (EC 2.4.
436	PP11	14.1	3	513	Placental protein 11 precursor (EC 3.4.2
437	PPIA	74.5	13	73	Peptidyl-prolyl cis-trans isomerase A (E
438	PPIB	50.5	13	113	Peptidyl-prolyl cis-trans isomerase B pr
439	PPIC	22.2	2	496	Peptidyl-prolyl cis-trans isomerase C (E
440	PRB2	56.3	36	30	Basic salivary proline-rich protein 2 (S
441	PRB3	34.0	22	54	Basic salivary proline-rich protein 3 pr

No.	Acc.	Cov.	Pep	Rank	Description (40 characters)
442	PRB4M	38.2	11	83	Basic salivary proline-rich protein 4 al
	PRB4L				Basic salivary proline-rich protein 4 al
443	PRB4S	43.3	20	70	Basic salivary proline-rich protein 4 al
444	PRDX1	31.2	8	262	Peroxiredoxin-1 (EC 1.11.1.15) (Thioredo
445	PRDX2	21.2	3	341	Peroxiredoxin-2 (EC 1.11.1.15) (Thioredo
446	PRDX4	29.2	5	353	Peroxiredoxin-4 (EC 1.11.1.15) (Prx-IV)
447	PRDX5	43.0	6	282	Peroxiredoxin-5, mitochondrial precursor
448	PRDX6	34.4	6	208	Peroxiredoxin-6 (EC 1.11.1.15) (Antioxid
449	PRIO	8.7	2	536	Major prion protein precursor (PrP) (PrP
450	PROF1	65.7	24	56	Profilin-1 (Profilin I) - Homo sapiens (
451	PROL4	32.8	10	99	Proline-rich protein 4 precursor (Lacrim
452	PROM1	4.6	2	455	Prominin-1 precursor (Prominin-like prot
453	PRP1	70.2	77	9	Basic salivary proline-rich protein 1 pr
454	PRPC	90.4	100	17	Salivary acidic proline-rich phosphoprot
455	PRPE	95.1	14	51	Basic proline-rich peptide P-E (IB-9) -
456	PRSS8	13.1	4	405	Prostasin precursor (EC 3.4.21.-) (Serin
457	PRTN3	30.9	6	202	Myeloblastin precursor (EC 3.4.21.76) (L
458	PSA	8.1	3	335	Puromycin-sensitive aminopeptidase (EC 3
459	PSA2	15.4	2	544	Proteasome subunit alpha type 2 (EC 3.4.
460	PSA5	16.2	2	545	Proteasome subunit alpha type 5 (EC 3.4.
461	PSA6	9.3	2	515	Proteasome subunit alpha type 6 (EC 3.4.
462	PSB6	9.2	3	514	Proteasome subunit beta type 6 precursor
463	PTN6	9.9	4	483	Tyrosine-protein phosphatase non-recepto
464	PURA2	22.8	8	357	Adenylosuccinate synthetase isozyme 2 (E
465	PYGL	14.6	10	213	Glycogen phosphorylase, liver form (EC 2
466	PZP	2.9	2	542	Pregnancy zone protein precursor - Homo
467	QSCN6	14.9	8	189	Sulfhydryl oxidase 1 precursor (EC 1.8.3
468	RAB7	30.0	4	450	Ras-related protein Rab-7 - Homo sapiens
469	RABP2	44.9	4	362	Cellular retinoic acid-binding protein 2
470	RAC1	22.9	5	384	Ras-related C3 botulinum toxin substrate
471	RAC2	34.9	8	207	Ras-related C3 botulinum toxin substrate
472	RADI	11.8	8	343	Radixin - Homo sapiens (Human)
473	RAN	8.8	2	499	GTP-binding nuclear protein Ran (GTPase
474	RETBP	30.8	4	327	Plasma retinol-binding protein precursor
475	REV	17.9	6	124	REVERSED PRP1
476	REV	54.2	5	346	REVERSED PRP5
477	REV	14.9	2	522	REVERSED PRB4L
	REV				REVERSED PRB4S_HUMAN
478	REV	1.1	2	585	REVERSED GP112
479	REV	0.2	3	523	REVERSED TITIN
480	RHOA	26.9	6	270	Transforming protein RhoA precursor (H12
481	RHOG	24.1	3	471	Rho-related GTP-binding protein RhoG pre
482	RHOQ	10.2	2	463	Rho-related GTP-binding protein RhoQ pre
483	RINI	21.9	7	247	Ribonuclease inhibitor (Ribonuclease/ang
484	RNAS2	19.9	2	461	Nonsecretory ribonuclease precursor (EC
485	RNAS4	34.0	6	201	Ribonuclease 4 precursor (EC 3.1.27.-) (
486	RNAS7	19.2	2	519	Ribonuclease 7 precursor (EC 3.1.27.-) (
487	RNT2	28.5	7	241	Ribonuclease T2 precursor (EC 3.1.27.-)
488	ROA2	13.0	4	482	Heterogeneous nuclear ribonucleoproteins
489	S100P	17.9	3	466	Protein S100-P (S100 calcium-binding pro
490	S10A2	17.3	2	546	Protein S100-A2 (S100 calcium-binding pr
491	S10A4	19.8	3	408	Protein S100-A4 (S100 calcium-binding pr
492	S10A7	22.8	3	416	Protein S100-A7 (S100 calcium-binding pr

Pep	No.	Acc.	Cov.	Rank	Description (40 characters)
493	S10A8	44.1	11	87	Protein S100-A8 (S100 calcium-binding pr
494	S10A9	65.8	21	59	Protein S100-A9 (S100 calcium-binding pr
495	S10AB	47.6	5	254	Protein S100-A11 (S100 calcium-binding p
496	S10AC	48.9	9	176	Protein S100-A12 (S100 calcium-binding p
497	S10AE	25.0	2	580	Protein S100-A14 (S100 calcium-binding p
498	S11Y	20.2	2	440	Putative S100 calcium-binding protein H_
499	SAA	41.0	5	366	Serum amyloid A protein precursor (SAA)
500	SAHH	9.3	3	399	Adenosylhomocysteinase (EC 3.3.1.1) (S-a
501	SAP	37.6	14	112	Proactivator polypeptide precursor [Cont
502	SCYB5	22.8	2	445	Small inducible cytokine B5 precursor (C
503	SDCB1	17.8	2	489	Syntenin-1 (Syndecan-binding protein 1)
504	SFRP1	17.5	7	350	Secreted frizzled-related protein 1 prec
505	SG1D1	48.9	6	337	Lipophilin-A precursor (Secretoglobin fa
506	SG2A1	51.6	7	259	Mammaglobin-B precursor (Mammaglobin-2)
507	SG3A1	31.7	3	256	Uteroglobin-related protein 2 precursor
508	SH3L1	27.2	2	504	SH3 domain-binding glutamic acid-rich-li
509	SH3L3	32.3	4	331	SH3 domain-binding glutamic acid-rich-li
510	SIP	38.5	4	376	Steroidogenesis-inducing protein (Fragme
511	SLPI	72.7	15	58	Antileukoproteinase precursor (ALP) (Sec
512	SLUR1	37.9	2	451	Secreted Ly-6/uPAR-related protein 1 pre
513	SMR3A	45.5	7	199	Submaxillary gland androgen-regulated pr
514	SMR3B	69.6	63	6	Submaxillary gland androgen-regulated pr
515	SODC	57.8	4	368	Superoxide dismutase [Cu-Zn] (EC 1.15.1.
516	SODE	10.0	2	537	Extracellular superoxide dismutase [Cu-Z
517	SPB10	8.1	2	470	Serpin B10 (Bomapin) (Protease inhibitor
518	SPB13	13.3	4	317	Serpin B13 (Hurpin) (HaCaT UV-repressibl
519	SPB3	39.5	16	102	Serpin B3 (Squamous cell carcinoma antig
520	SPB4	25.6	9	229	Serpin B4 (Squamous cell carcinoma antig
521	SPB5	19.7	5	263	Serpin B5 precursor (Maspin) (Protease i
522	SPIT2	8.7	2	527	Kunitz-type protease inhibitor 2 precurs
523	SPLC2	60.2	34	32	Short palate, lung and nasal epithelium
524	SPR1A	78.7	11	119	Cornifin-A (Small proline-rich protein I
525	SPR1B	78.7	10	130	Cornifin-B (Small proline-rich protein I
526	SPR2A	61.1	4	250	Small proline-rich protein 2A (SPR-2A) (
527	SPR2B	79.2	5	239	Small proline-rich protein 2B (SPR-2B) -
528	SPR2D	79.2	5	322	Small proline-rich protein 2D (SPR-2D) (
529	SPR2E	72.2	4	175	Small proline-rich protein 2E (SPR-2E) (
530	SPRL1	29.5	17	90	SPARC-like protein 1 precursor (High end
531	SPRR3	94.1	51	43	Small proline-rich protein 3 (Cornifin b
532	STAT	67.7	53	14	Statherin precursor - Homo sapiens (Huma
533	TAGL2	31.2	4	320	Transgelin-2 (SM22-alpha homolog) - Homo
534	TALDO	28.8	10	154	Transaldolase (EC 2.2.1.2) - Homo sapien
535	TBAK	29.7	10	205	Tubulin alpha-ubiquitous chain (Alpha-tu
	TBA1				Tubulin alpha-1 chain (Alpha-tubulin 1)
	TBA2				Tubulin alpha-2 chain (Alpha-tubulin 2)
	TBA3				Tubulin alpha-3 chain (Alpha-tubulin 3)
	TBA6				Tubulin alpha-6 chain (Alpha-tubulin 6)
536	TBB4	22.1	4	352	Tubulin beta-4 chain (Tubulin 5 beta) -
	TBB2A				Tubulin beta-2A chain - Homo sapiens (Hu
	TBB2B				Tubulin beta-2B chain - Homo sapiens (Hu
	TBB2C				Tubulin beta-2C chain (Tubulin beta-2 ch
537	TBB5	43.5	13	249	Tubulin beta chain (Tubulin beta-5 chain
538	TBCA	36.1	3	372	Tubulin-specific chaperone A (Tubulin-fo

No.	Acc.	Cov.	Pep	Rank	Description (40 characters)
539	TCO1	23.3	12	91	Transcobalamin-1 precursor (Transcobalam
540	TERA	2.5	2	558	Transitional endoplasmic reticulum ATPas
541	TETN	18.3	4	412	Tetranectin precursor (TN) (C-type lecti
542	TF3C2	4.8	2	575	General transcription factor 3C polypept
543	TFF3	67.5	12	81	Trefoil factor 3 precursor (Intestinal t
544	TGM3	22.2	13	169	Protein-glutamine gamma-glutamyltransfer
545	THIC	12.8	2	502	Acetyl-CoA acetyltransferase, cytosolic
546	THIO	88.6	12	100	Thioredoxin (Trx) (ATL-derived factor) (
547	THRB	8.4	3	457	Prothrombin precursor (EC 3.4.21.5) (Coa
548	TIMP1	58.9	11	114	Metalloproteinase inhibitor 1 precursor
549	TITIN	0.2	3	476	Titin (EC 2.7.11.1) (Connectin) (Rhabdom
550	TKT	52.5	25	69	Transketolase (EC 2.2.1.1) (TK) - Homo s
551	TLN1	16.4	26	192	Talin-1 - Homo sapiens (Human)
552	TM11D	15.1	5	351	Transmembrane protease, serine 11D precu
553	TNF13	10.4	3	456	Tumor necrosis factor ligand superfamily
554	TPIS	78.3	19	68	Triosephosphate isomerase (EC 5.3.1.1) (
555	TPM3	34.9	7	215	Tropomyosin alpha-3 chain (Tropomyosin-3
	TPM1				Tropomyosin-1 alpha chain (Alpha-tropomy
	TPM2				Tropomyosin beta chain (Tropomyosin 2) (
	TPM4				Tropomyosin alpha-4 chain (Tropomyosin-4
556	TPP1	10.7	4	298	Tripeptidyl-peptidase 1 precursor (EC 3.
557	TRFE	69.2	80	25	Serotransferrin precursor (Transferrin)
558	TRFL	80.0	151	13	Lactotransferrin precursor (EC 3.4.21.-)
559	TRFM	6.2	3	424	Melanotransferrin precursor (Melanoma-as
560	TSP1	6.9	6	303	Thrombospondin-1 precursor - Homo sapien
561	TTHY	31.3	5	394	Transthyretin precursor (Prealbumin) (TB
562	TWF2	10.6	3	518	Twinfilin-2 (Twinfilin-1-like protein) (
563	TXNL5	22.8	2	447	Thioredoxin-like protein 5 (14 kDa thior
564	TYB10	31.8	2	500	Thymosin beta-10 - Homo sapiens (Human)
565	TYB4	88.6	10	139	Thymosin beta-4 (T beta 4) (Fx) [Contain
566	TYPH	5.6	2	541	Thymidine phosphorylase precursor (EC 2.
567	U773	66.3	41	45	Protein UNQ773/PRO1567 precursor - Homo
568	UB2V1	8.6	2	566	Ubiquitin-conjugating enzyme E2 variant
569	UBE1	12.2	9	311	Ubiquitin-activating enzyme E1 (A1S9 pro
570	UBE2N	17.1	2	561	Ubiquitin-conjugating enzyme E2 N (EC 6.
571	UFM1	58.8	2	562	Ubiquitin-fold modifier 1 precursor - Ho
572	UGPA1	6.9	3	488	UTP--glucose-1-phosphate uridylyltransfe
	UGPA2				UTP--glucose-1-phosphate uridylyltransfe
573	URP2	9.0	4	452	Unc-112-related protein 2 (Kindlin-3) (M
574	UTER	50.5	5	332	Uteroglobin precursor (Secretoglobin fam
575	VAS1	3.0	2	573	Vacuolar ATP synthase subunit S1 precurs
576	VASP	11.6	3	420	Vasodilator-stimulated phosphoprotein (V
577	VAT1	13.2	4	342	Synaptic vesicle membrane protein VAT-1
578	VATA1	5.5	2	444	Vacuolar ATP synthase catalytic subunit
579	VINC	10.7	8	209	Vinculin (Metavinculin) - Homo sapiens (
580	VTDB	51.9	21	84	Vitamin D-binding protein precursor (DBP
581	VTNC	16.5	5	338	Vitronectin precursor (Serum-spreading f
582	WDR1	37.0	17	186	WD repeat protein 1 (Actin-interacting p
583	WFDC2	67.7	7	89	WAP four-disulfide core domain protein 2
584	ZA2G	70.8	55	22	Zinc-alpha-2-glycoprotein precursor (Zn-
585	ZF106	2.9	2	578	Zinc finger protein 106 homolog (Zfp-106

Appendix 3 Table A3_1: Comparison of Stimulted vs Unstimulated Normalized Spectral Counts (Protein Relative Abundance) (288 proteins)

No.	Locus	Description	A(u)	A(s)	B(u)	B(s)	C(u)	C(s)	D(u)	D(s)	E(u)	E(s)	p	fold
1	SMR3A	Submaxillary gland androgen-regu	2.2	2.0	0.2	11.4	2.5	44.7	0.3	0.2	0.2	13.9	0.101	13.30
2	ANXA3	Annexin A3 (Annexin III) (Lipoco	1.5	0.2	0.2	3.9	0.2	28.2	3.0	0.2	0.2	17.0	0.417	9.66
3	H14	Histone H1.4 (Histone H1b) - Hom	0.1	0.2	1.7	0.1	0.2	18.4	0.3	0.2	0.2	4.8	0.473	9.13
4	RNAS4	Ribonuclease 4 precursor (EC 3.1	3.6	10.4	4.9	8.4	0.2	11.8	0.3	3.7	0.2	10.9	0.027	4.90
5	GSLG1	Golgi apparatus protein 1 precur	0.1	3.0	1.7	0.1	0.2	3.6	0.3	0.2	0.2	4.8	0.346	4.51
6	ANXA2	Annexin A2 (Annexin II) (Lipocor	0.1	0.2	7.3	0.1	0.2	48.0	3.0	0.2	0.2	0.3	0.926	4.51
7	CBPD	Carboxypeptidase D precursor (EC	0.1	2.0	0.2	2.4	0.2	3.6	0.3	0.2	2.5	4.8	0.063	3.95
8	HTRA1	Serine protease HTRA1 precursor	0.1	3.9	1.7	2.4	0.2	0.3	0.3	0.2	0.2	3.3	0.164	3.89
9	CBPE	Carboxypeptidase E precursor (EC	0.1	3.0	1.7	9.9	0.2	0.3	0.3	4.6	4.8	6.4	0.049	3.37
10	CATL	Cathepsin L precursor (EC 3.4.22	1.5	2.0	2.5	0.1	0.2	3.6	0.3	2.0	0.2	7.9	0.368	3.27
11	MGP	Matrix Gla-protein precursor (MG	1.5	10.4	2.5	2.4	2.5	11.8	0.3	0.2	2.5	3.3	0.225	3.00
12	SPLC2	Short palate, lung and nasal epi	44.6	120.5	38.0	148.8	10.6	62.7	58.3	65.2	27.6	135.1	0.017	2.97
13	TSP1	Thrombospondin-1 precursor - Hom	0.1	3.0	0.2	3.1	4.9	11.8	0.3	2.0	2.5	3.3	0.029	2.92
14	FURIN	Furin precursor (EC 3.4.21.75) (	2.2	10.4	2.5	3.9	2.5	8.5	0.3	0.2	3.7	9.4	0.096	2.89
15	MNDA	Myeloid cell nuclear differentia	2.2	0.2	0.2	0.1	0.2	3.6	0.3	0.2	0.2	4.8	0.613	2.88
16	LYSC	Lysozyme C precursor (EC 3.2.1.1	79.4	205.6	110.5	244.4	88.2	576.8	79.9	68.8	150.9	272.8	0.067	2.69
17	NUCB1	Nucleobindin-1 precursor (CALNUC	0.1	3.0	0.2	7.6	3.7	10.2	3.0	2.9	7.1	13.9	0.083	2.67
18	S10AC	Protein S100-A12 (S100 calcium-b	4.3	36.3	5.7	3.1	2.5	0.3	4.3	2.9	2.5	6.4	0.999	2.53
19	CADH1	Epithelial-cadherin precursor (E	0.1	2.0	0.2	0.1	0.2	3.6	0.3	2.0	2.5	0.3	0.327	2.44
20	RNT2	Ribonuclease T2 precursor (EC 3.	0.1	3.9	0.2	5.4	4.9	10.2	0.3	2.0	9.4	13.9	0.036	2.39
21	PROL4	Proline-rich protein 4 precursor	12.0	10.4	12.8	27.8	14.1	57.8	9.7	7.3	11.6	39.7	0.161	2.37
22	SLPI	Antileukoproteinase precursor (A	12.6	52.0	8.8	33.0	14.1	77.5	38.1	19.8	35.6	74.5	0.098	2.35
23	AMD	Peptidyl-glycine alpha-amidating	0.1	3.9	0.2	3.1	0.2	6.9	5.7	2.9	3.7	6.4	0.084	2.35
24	NUCB2	Nucleobindin-2 precursor (DNA-bi	12.0	40.9	17.5	53.9	10.6	31.5	31.3	28.7	28.8	68.4	0.025	2.23
25	NPC2	Epididymal secretory protein E1	0.1	0.2	1.7	1.6	2.5	10.2	0.3	0.2	3.7	6.4	0.314	2.22
26	CAP7	Azurocidin precursor (Cationic a	20.3	0.2	0.2	8.4	9.5	43.0	13.8	24.2	0.2	20.0	0.516	2.18
27	FAM3B	Protein FAM3B precursor (Cytokin	2.2	4.8	1.7	0.1	0.2	3.6	0.3	0.2	2.5	6.4	0.731	2.17
28	KLK11	Kallikrein-11 precursor (EC 3.4.	2.9	2.0	3.3	9.9	0.2	6.9	7.0	6.4	2.5	9.4	0.183	2.16
29	*Cystatin	Cystatin_GROUP	888.7	3008.8	1489.8	2318.1	357.0	1026.8	978.3	1352.0	424.0	1189.9	0.011	2.15
30	*Hemo	Hemo_GROUP	6.4	0.2	8.8	83.0	14.1	13.5	13.8	13.5	25.3	33.6	0.828	2.10
31	ANXA1	Annexin A1 (Annexin I) (Lipocort	11.3	28.9	41.1	15.1	22.2	125.1	13.8	7.3	18.5	39.7	0.521	2.02
32	HEBP2	Heme-binding protein 2 (Protein	0.1	0.2	0.2	2.4	3.7	0.3	0.3	2.9	0.2	3.3	0.321	2.02
33	GP73	Golgi phosphoprotein 2 (Golgi me	0.1	0.2	1.7	6.9	3.7	3.6	5.7	7.3	0.2	4.8	0.157	1.99
34	CYTC	Cystatin-C precursor (Cystatin-3	37.7	110.3	54.6	72.6	11.8	41.4	30.0	37.6	25.3	54.8	0.025	1.99
35	PERL	Lactoperoxidase precursor (EC 1.	73.1	142.7	63.2	115.2	81.2	158.0	90.7	76.8	71.0	221.4	0.051	1.88
36	CYTD	Cystatin-D precursor (Cystatin-5	95.6	259.9	256.6	311.1	65.8	171.1	83.5	104.4	56.9	174.9	0.026	1.83
37	S10A7	Protein S100-A7 (S100 calcium-bi	0.1	0.2	0.2	1.6	2.5	0.3	0.3	0.2	2.5	7.9	0.741	1.82
38	RNAS2	Nonsecretory ribonuclease precur	1.5	0.2	0.2	1.6	0.2	0.3	0.3	2.9	2.5	3.3	0.479	1.77
39	CATC	Dipeptidyl-peptidase 1 precursor	2.2	0.2	0.2	1.6	10.6	18.4	0.3	0.2	0.2	3.3	0.603	1.75
40	PPIB	Peptidyl-prolyl cis-trans isomer	6.4	15.9	2.5	12.8	9.5	10.2	21.9	18.9	16.2	41.2	0.102	1.75
41	CAH6	Carbonic anhydrase 6 precursor (	25.2	78.8	70.3	80.1	47.7	108.7	57.0	67.0	57.3	107.8	0.041	1.72
42	U773	Protein UNQ773/PRO1567 precursor	14.0	22.4	14.3	61.4	28.0	66.0	89.4	96.4	43.6	76.0	0.041	1.70

No.	Locus	Description	A(u)	A(s)	B(u)	B(s)	C(u)	C(s)	D(u)	D(s)	E(u)	E(s)	p	fold
43	LEG7	Galectin-7 (Gal-7) (HKL-14) (PI7	0.1	3.0	12.0	15.1	0.2	0.3	0.3	2.9	0.2	0.3	0.106	1.68
44	HBA	Hemoglobin subunit alpha (Hemogl	3.6	0.2	2.5	33.0	8.3	3.6	4.3	2.9	12.8	10.9	0.704	1.60
45	*Perox	Perox1_GROUP	1.2	2.0	1.7	3.1	6.0	8.5	10.4	6.4	3.7	13.9	0.183	1.48
46	PIP	Prolactin-inducible protein prec	196.1	528.6	564.6	684.2	194.6	300.9	274.4	267.5	202.2	306.2	0.077	1.46
47	*Keratin2	Keratin2_GROUP	2.2	89.5	138.8	65.9	16.4	62.7	35.4	28.7	25.3	68.4	0.258	1.45
48	PERM	Myeloperoxidase precursor (EC 1.	55.0	5.7	10.4	20.3	10.6	90.7	34.0	29.6	31.0	53.3	0.804	1.41
49	CATB	Cathepsin B precursor (EC 3.4.22	2.9	8.5	5.7	3.9	9.5	13.5	4.3	10.0	13.9	15.4	0.200	1.41
50	MUC7	Mucin-7 precursor (MUC-7) (Saliv	22.4	109.4	110.5	134.6	56.9	80.8	54.3	31.4	64.1	73.0	0.379	1.39
51	MIF	Macrophage migration inhibitory	5.0	0.2	2.5	4.6	4.9	5.3	0.3	4.6	3.7	7.9	0.850	1.38
52	SH3L3	SH3 domain-binding glutamic acid	1.5	0.2	1.7	0.1	2.5	3.6	0.3	4.6	4.8	6.4	0.832	1.37
53	*Amylase	Amylase_GROUP	704.1	1105.1	1731.3	1691.7	1122.5	2025.3	1816.4	1935.7	647.2	1470.5	0.075	1.37
54	CATG	Cathepsin G precursor (EC 3.4.21	4.3	0.2	3.3	1.6	3.7	13.5	0.3	2.0	3.7	3.3	0.888	1.35
55	BPIL1	Bactericidal/permeability-increa	25.9	35.3	75.8	90.5	21.1	34.8	46.2	58.1	31.0	48.8	0.006	1.34
56	S10A4	Protein S100-A4 (S100 calcium-bi	1.5	0.2	0.2	0.1	2.5	3.6	0.3	2.0	3.7	4.8	0.897	1.32
57	*HMGB	HMGB_GROUP	0.1	3.9	0.2	2.4	8.3	6.9	8.4	7.3	5.9	9.4	0.172	1.30
58	H2A1	Histone H2A type 1 (H2A.1) - Hom	0.1	7.6	8.8	2.4	2.5	5.3	0.3	2.9	2.5	0.3	0.553	1.29
59	SPRR3	Small proline-rich protein 3 (Co	10.6	6.7	8.0	20.5	135.6	90.7	63.7	83.9	42.4	129.0	0.422	1.27
60	TCO1	Transcobalamin-1 precursor (Tran	12.0	10.4	12.8	24.8	30.3	33.2	7.0	11.8	28.8	35.1	0.142	1.27
61	*Keratin1	Keratin1_GROUP	5.7	87.2	152.3	43.5	15.3	44.7	23.2	26.0	10.5	59.4	0.267	1.26
62	GRN	Granulins precursor (Proepitheli	1.5	4.8	1.7	3.1	7.2	13.5	11.1	9.1	12.8	12.4	0.153	1.25
63	CD14	Monocyte differentiation antigen	9.2	11.3	4.1	6.1	4.9	6.9	4.3	2.9	9.4	12.4	0.329	1.24
64	TRFL	Lactotransferrin precursor (EC 3	461.9	196.4	123.9	171.9	294.1	499.6	241.3	244.3	259.8	592.3	0.592	1.23
65	FCGBP	IgGFc-binding protein precursor	5.5	7.0	49.6	79.2	82.4	110.4	188.0	201.6	17.3	24.5	0.012	1.23
66	SG3A1	Uteroglobin-related protein 2 pr	2.9	3.9	1.7	4.6	0.2	3.6	4.3	2.9	8.2	6.4	0.309	1.22
67	PLTP	Phospholipid transfer protein pr	2.2	3.9	1.7	6.1	3.7	5.3	7.0	3.7	4.8	4.8	0.375	1.22
68	ZA2G	Zinc-alpha-2-glycoprotein precur	98.8	159.3	152.3	177.9	183.0	227.0	167.7	153.4	187.4	230.5	0.099	1.20
69	APOH	Beta-2-glycoprotein 1 precursor	2.2	0.2	1.7	3.9	6.0	13.5	4.3	6.4	5.9	0.3	0.457	1.20
70	ISK7	Serine protease inhibitor Kazal-	0.1	0.2	0.2	0.1	4.9	5.3	0.3	2.0	3.7	3.3	0.323	1.20
71	SAP	Proactivator polypeptide precurs	7.8	8.5	8.0	12.1	18.7	21.7	16.5	12.7	20.8	30.6	0.277	1.19
72	H4	Histone H4 - Homo sapiens (Human	0.1	3.0	5.7	1.6	0.2	3.6	0.3	2.0	2.5	0.3	0.454	1.19
73	TPP1	Tripeptidyl-peptidase 1 precurso	2.9	3.9	1.7	3.1	3.7	3.6	3.0	2.0	4.8	6.4	0.445	1.18
74	CRIS3	Cysteine-rich secretory protein	45.3	50.2	53.8	71.1	45.4	52.9	55.6	70.6	73.3	74.5	0.028	1.17
75	A2GL	Leucine-rich alpha-2-glycoprotei	5.7	5.7	4.9	8.4	3.7	3.6	4.3	6.4	8.2	6.4	0.417	1.14
76	*Histatin	Histatin_GROUP	140.5	382.4	231.1	254.8	110.1	62.7	186.6	171.3	165.7	73.0	0.826	1.13
77	OS9	Protein OS-9 precursor (Amplifie	1.5	2.0	3.3	1.6	0.2	0.3	0.3	2.0	0.2	0.3	0.366	1.13
78	*Serpins	Serpin_GROUP	11.6	3.9	10.4	26.8	15.7	5.3	25.5	31.4	11.6	16.2	0.749	1.12
79	FAM3D	Protein FAM3D precursor - Homo s	2.9	3.0	9.6	12.1	10.6	10.2	4.3	11.8	21.9	17.0	0.434	1.09
80	*Mucins	Mucin_GROUP	287.2	381.4	566.9	747.8	369.3	346.9	657.9	605.2	417.9	428.8	0.336	1.09
81	RABP2	Cellular retinoic acid-binding p	0.1	0.2	2.5	4.6	2.5	0.3	0.3	0.2	2.5	3.3	0.619	1.08
82	SMR3B	Submaxillary gland androgen-regu	1010.6	892.2	634.7	673.0	540.5	708.2	706.5	593.7	603.9	901.2	0.480	1.08
83	CYTA	Cystatin-A (Stefin-A) (Cystatin-	1.5	0.2	0.2	0.1	4.9	0.3	3.0	3.7	3.7	9.4	0.355	1.05
84	A1AT	Alpha-1-antitrypsin precursor (A	14.0	21.5	15.1	21.1	16.4	21.7	4.3	3.7	40.2	26.0	0.607	1.04
85	SODC	Superoxide dismutase [Cu-Zn] (EC	0.1	0.2	0.2	1.6	2.5	3.6	4.3	5.5	3.7	0.3	0.858	1.04
86	QSCN6	Sulfhydryl oxidase 1 precursor (	2.9	3.0	3.3	6.1	4.9	8.5	9.7	5.5	10.5	9.4	0.664	1.04
87	INVO	Involucrin - Homo sapiens (Human	0.1	0.2	0.2	1.6	13.0	3.6	5.7	2.0	0.2	12.4	0.446	1.03
88	CYTB	Cystatin-B (Stefin-B) (Liver thi	19.6	14.1	27.7	67.4	84.7	52.9	92.1	71.5	80.1	104.8	0.941	1.02

No.	Locus	Description	A(u)	A(s)	B(u)	B(s)	C(u)	C(s)	D(u)	D(s)	E(u)	E(s)	p	fold
89	TFF3	Trefoil factor 3 precursor (Inte	6.4	9.4	18.3	26.3	24.5	29.9	35.4	30.5	32.2	23.0	0.552	1.02
90	*NDKs	NDK_GROUP	2.2	0.2	0.2	0.1	6.0	0.3	0.3	5.5	3.7	6.4	0.746	1.02
91	FAM25	Protein FAM25 - Homo sapiens (Hu	0.1	0.2	0.2	3.9	4.9	3.6	3.0	0.2	5.9	6.4	0.930	1.01
92	FABPE	Fatty acid-binding protein, epid	21.7	21.5	45.1	31.5	36.1	38.1	40.8	43.0	53.9	65.4	0.890	1.01
93	TIMP1	Metalloproteinase inhibitor 1 pr	9.9	9.4	15.1	12.8	9.5	11.8	15.1	14.4	23.1	24.5	0.936	1.01
94	LG3BP	Galectin-3-binding protein precu	22.4	20.5	12.8	18.1	29.2	23.3	23.2	18.9	29.9	36.6	0.954	1.00
95	S10A9	Protein S100-A9 (S100 calcium-bi	42.5	10.4	28.5	14.3	40.7	61.1	35.4	31.4	21.9	51.8	0.658	-1.00
96	*Contams	Contaminants	264.2	480.4	829.4	735.8	257.1	383.0	421.6	215.8	353.4	299.3	0.972	-1.01
97	IL1RA	Interleukin-1 receptor antagonis	2.9	2.0	9.6	9.1	8.3	6.9	16.5	15.3	8.2	10.9	0.498	-1.03
98	LEG3	Galectin-3 (Galactose-specific l	0.1	0.2	0.2	1.6	9.5	3.6	3.0	4.6	10.5	12.4	0.442	-1.03
99	ACBP	Acyl-CoA-binding protein (ACBP)	0.1	0.2	2.5	4.6	15.3	6.9	7.0	10.9	11.6	12.4	0.650	-1.05
100	CALL3	Calmodulin-like protein 3 (Calmo	2.9	2.0	1.7	4.6	4.9	0.3	4.3	8.2	10.5	7.9	0.621	-1.05
101	*PRP3	PRP3_GROUP	9.2	9.4	13.6	9.9	51.1	31.5	25.9	18.0	19.6	44.2	0.795	-1.06
102	CD59	CD59 glycoprotein precursor (Mem	1.5	3.0	0.2	2.4	8.3	6.9	5.7	5.5	8.2	4.8	0.415	-1.06
103	COF1	Cofilin-1 (Cofilin, non-muscle i	23.1	12.2	4.9	8.4	4.9	6.9	16.5	18.9	11.6	10.9	0.765	-1.06
104	PRPC	Salivary acidic proline-rich pho	386.5	121.4	190.1	412.4	411.0	250.0	359.4	366.4	309.5	397.0	0.729	-1.07
105	CHIT1	Chitotriosidase-1 precursor (EC	1.5	0.2	0.2	0.1	3.7	3.6	3.0	3.7	0.2	0.3	0.497	-1.07
106	ECM1	Extracellular matrix protein 1 p	4.3	0.2	2.5	0.1	7.2	3.6	7.0	9.1	4.8	10.9	0.237	-1.08
107	B2MG	Beta-2-microglobulin precursor [	9.9	23.3	33.3	17.3	19.9	23.3	17.8	11.8	20.8	18.5	0.905	-1.08
108	ELNE	Leukocyte elastase precursor (EC	9.9	0.2	4.1	3.9	6.0	6.9	0.3	7.3	4.8	4.8	0.924	-1.08
109	PRDX2	Peroxiredoxin-2 (EC 1.11.1.15) (	1.9	2.0	2.5	3.1	0.2	0.3	3.6	2.0	0.2	0.3	0.741	-1.10
110	CATZ	Cathepsin Z precursor (EC 3.4.22	2.9	3.9	1.7	0.1	2.5	5.3	4.3	0.2	2.5	3.3	0.343	-1.10
111	CDD	Cytidine deaminase (EC 3.5.4.5)	7.8	0.2	2.5	1.6	7.2	10.2	4.3	3.7	9.4	12.4	0.390	-1.11
112	IL1F6	Interleukin-1 family member 6 (I	0.1	0.2	1.7	0.1	3.7	3.6	0.3	0.2	5.9	6.4	0.367	-1.12
113	APOA4	Apolipoprotein A-IV precursor (A	2.2	0.2	2.5	3.9	0.2	0.3	4.3	3.7	3.7	3.3	0.510	-1.13
114	VATA1	Vacuolar ATP synthase catalytic	1.5	0.2	0.2	0.1	2.5	0.3	0.3	0.2	0.2	3.3	0.679	-1.13
115	CATD	Cathepsin D precursor (EC 3.4.23	9.9	7.6	8.8	8.4	18.7	20.0	16.5	13.5	21.9	17.0	0.093	-1.14
116	PEBP1	Phosphatidylethanolamine-binding	5.0	2.0	7.3	5.4	9.5	5.3	4.3	10.0	7.1	6.4	0.512	-1.14
117	KLK13	Kallikrein-13 precursor (EC 3.4.	0.1	0.2	2.5	3.1	9.5	5.3	4.3	2.0	8.2	10.9	0.641	-1.15
118	CF058	Uncharacterized protein C6orf58	4.3	6.7	11.2	25.5	34.9	18.4	43.5	36.7	36.7	26.0	0.941	-1.15
119	THIO	Thioredoxin (Trx) (ATL-derived f	9.9	9.4	14.3	18.1	18.7	11.8	21.9	19.8	23.1	17.0	0.313	-1.16
120	BPI	Bactericidal permeability-increa	5.7	0.2	0.2	0.1	2.5	5.3	5.7	6.4	0.2	0.3	0.565	-1.16
121	*IGG	IGG_GROUP	167.6	42.8	66.4	56.9	129.8	190.8	121.8	156.3	209.1	147.2	0.469	-1.17
122	SPB13	Serpin B13 (Hurpin) (HaCaT UV-re	0.1	0.2	0.2	2.4	5.2	0.3	3.4	3.7	3.7	4.1	0.925	-1.18
123	*PRP2	PRP2_GROUP	3.6	0.2	4.1	5.8	17.6	8.5	12.4	5.5	17.3	26.0	0.288	-1.19
124	PRTN3	Myeloblastin precursor (EC 3.4.2	7.1	2.0	3.3	2.4	8.3	10.2	5.7	7.3	5.9	3.3	0.286	-1.20
125	NAMPT	Nicotinamide phosphoribosyltrans	4.3	0.2	0.2	0.1	3.7	0.3	5.7	6.4	2.5	6.4	0.312	-1.22
126	EF1A1	Elongation factor 1-alpha 1 (EF-	5.0	2.0	8.0	3.9	4.9	5.3	0.3	2.9	4.8	4.8	0.792	-1.22
127	HRG	Histidine-rich glycoprotein prec	5.7	0.2	0.2	3.1	0.2	0.3	0.3	0.2	2.5	3.3	0.968	-1.24
128	HEMO	Hemopexin precursor (Beta-1B-gly	18.9	0.2	7.3	9.1	17.6	21.7	17.8	21.6	32.2	23.0	0.411	-1.24
129	PRDX6	Peroxiredoxin-6 (EC 1.11.1.15) (	5.0	3.0	4.1	6.1	6.0	0.3	7.0	6.4	5.9	6.4	0.362	-1.27
130	TXNL5	Thioredoxin-like protein 5 (14 k	0.1	0.2	1.7	0.1	0.2	0.3	3.0	0.2	0.2	3.3	0.720	-1.27
131	VAT1	Synaptic vesicle membrane protei	2.9	0.2	0.2	0.1	3.7	3.6	3.0	0.2	3.7	6.4	0.235	-1.28
132	FOLR1	Folate receptor alpha precursor	0.1	0.2	2.5	1.6	6.0	3.6	0.3	2.9	5.9	3.3	0.705	-1.28
133	KNG1	Kininogen-1 precursor (Alpha-2-t	2.9	0.2	0.2	1.6	2.5	0.3	0.3	2.9	4.8	3.3	0.934	-1.28
134	FETUA	Alpha-2-HS-glycoprotein precurso	12.0	4.8	9.6	5.4	8.3	11.8	5.7	4.6	12.8	10.9	0.230	-1.29

No.	Locus	Description	A(u)	A(s)	B(u)	B(s)	C(u)	C(s)	D(u)	D(s)	E(u)	E(s)	p	fold
135	PDIA1	Protein disulfide-isomerase prec	4.3	5.7	4.9	11.4	30.3	5.3	20.5	20.7	31.0	27.6	0.752	-1.29
136	*RAC	RAC_GROUP	9.5	0.2	0.2	0.1	9.5	3.6	7.5	4.2	5.9	17.0	0.342	-1.30
137	GDIR	Rho GDP-dissociation inhibitor 1	5.0	0.2	1.7	2.4	4.9	3.6	3.0	2.9	9.4	9.4	0.376	-1.30
138	AACT	Alpha-1-antichymotrypsin precurs	2.2	2.0	0.2	1.6	6.0	5.3	3.0	0.2	4.8	3.3	0.809	-1.30
139	S10A8	Protein S100-A8 (S100 calcium-bi	29.3	9.4	12.0	9.9	34.9	25.0	19.2	20.7	17.3	21.5	0.308	-1.30
140	ALBU	Serum albumin precursor - Homo s	856.3	771.0	709.6	478.9	737.2	598.1	824.0	783.5	1119.1	590.1	0.060	-1.32
141	PGRP	Peptidoglycan recognition protei	2.2	0.2	2.5	0.1	7.2	8.5	3.0	0.2	3.7	4.8	0.101	-1.33
142	MSLN	Mesothelin precursor (Pre-pro-me	3.6	3.0	1.7	2.4	0.2	0.3	0.3	0.2	10.5	6.4	0.647	-1.34
143	IGJ	Immunoglobulin J chain - Homo sa	60.6	93.6	79.8	42.7	105.5	54.5	43.5	47.4	104.1	53.3	0.281	-1.35
144	*Caza	Caza_GROUP	5.7	0.2	0.2	0.1	4.9	3.6	3.0	2.9	8.2	9.4	0.337	-1.35
145	HS70L	Heat shock 70 kDa protein 1L (He	7.5	0.2	0.2	2.3	8.2	0.3	5.8	4.7	0.2	8.6	0.917	-1.35
146	VTDB	Vitamin D-binding protein precur	27.2	8.5	13.6	18.8	17.6	15.1	28.6	25.1	40.2	26.0	0.275	-1.36
147	ILEU	Leukocyte elastase inhibitor (LE	22.7	2.0	7.3	13.1	19.1	10.2	28.6	23.3	21.9	24.5	0.380	-1.36
148	G3P	Glyceraldehyde-3-phosphate dehyd	83.5	19.6	34.8	28.5	92.8	67.7	73.2	88.4	92.7	71.5	0.213	-1.37
149	*Ig Kappa	Kappa_GROUP	564.4	465.6	428.2	256.3	689.8	471.7	408.1	407.4	469.2	259.2	0.036	-1.38
150	CALL5	Calmodulin-like protein 5 (Calmo	1.5	0.2	2.5	5.4	13.0	8.5	8.4	3.7	5.9	4.8	0.299	-1.38
151	PIGR	Polymeric-immunoglobulin recepto	433.1	519.3	727.8	431.1	534.8	348.5	435.1	429.7	621.0	256.2	0.153	-1.39
152	IQGA1	Ras GTPase-activating-like prote	2.2	0.2	0.2	0.1	0.2	0.3	0.3	3.7	3.7	0.3	0.692	-1.39
153	STAT	Statherin precursor - Homo sapie	659.6	402.7	335.2	201.0	225.8	263.1	526.9	433.3	310.6	175.9	0.076	-1.39
154	LPLC1	Long palate, lung and nasal epit	16.1	3.9	3.3	16.6	38.4	8.5	13.8	10.0	32.2	35.1	0.616	-1.40
155	*Plastin	Plastin_GROUP	38.4	0.2	4.1	4.6	26.8	21.7	16.5	24.2	24.2	27.6	0.419	-1.40
156	GRP78	78 kDa glucose-regulated protein	11.0	3.9	8.3	6.7	27.7	12.4	9.2	13.5	12.4	12.2	0.264	-1.41
157	FIBG	Fibrinogen gamma chain precursor	16.1	5.7	10.4	14.3	21.1	15.1	20.5	18.9	34.5	18.5	0.203	-1.41
158	CERU	Ceruloplasmin precursor (EC 1.16	14.0	3.9	11.2	10.6	10.6	11.8	11.1	6.4	12.8	9.4	0.161	-1.42
159	GSTP1	Glutathione S-transferase P (EC	35.6	16.8	33.3	20.3	34.9	15.1	25.9	43.0	39.0	23.0	0.155	-1.43
160	*IGA	IGA_GROUP	1195.4	983.8	704.1	340.0	825.2	560.4	493.1	490.3	817.3	437.9	0.044	-1.43
161	*IgMu	IgMu_GROUP	57.1	62.2	49.8	28.5	112.5	80.8	34.0	30.5	93.8	39.7	0.099	-1.44
162	NHERF	Ezrin-radixin-moesin-binding pho	1.5	0.2	0.2	1.6	3.7	0.3	0.3	0.2	2.5	3.3	0.620	-1.44
163	APOA1	Apolipoprotein A-I precursor (Ap	32.1	7.6	23.8	22.6	30.3	31.5	19.2	17.1	45.9	24.5	0.187	-1.46
164	WFDC2	WAP four-disulfide core domain p	30.0	25.2	17.5	10.6	24.5	18.4	11.1	8.2	28.8	13.9	0.015	-1.47
165	6PGD	6-phosphogluconate dehydrogenase	25.2	2.0	5.7	11.4	33.8	5.3	24.6	28.7	28.8	32.1	0.340	-1.48
166	A1AG2	Alpha-1-acid glycoprotein 2 prec	0.1	2.0	0.2	0.1	0.2	0.3	3.0	2.0	3.7	0.3	0.986	-1.50
167	FIBB	Fibrinogen beta chain precursor	31.4	5.7	23.8	10.6	19.9	18.4	16.5	34.0	41.3	20.0	0.269	-1.50
168	KLK1	Kallikrein-1 precursor (EC 3.4.2	5.7	2.0	7.3	6.1	25.7	13.5	5.7	7.3	23.1	15.4	0.140	-1.52
169	DMBT1	Deleted in malignant brain tumor	55.8	55.1	66.2	27.4	153.0	89.0	70.5	84.8	205.6	104.8	0.130	-1.53
170	M6PBP	Mannose-6-phosphate receptor-bin	2.2	0.2	2.5	1.6	3.7	3.6	4.3	2.0	5.9	4.8	0.151	-1.53
171	CFAH	Complement factor H precursor (H	6.4	0.2	3.3	2.4	6.0	10.2	7.0	6.4	7.1	0.3	0.193	-1.53
172	ERO1A	ERO1-like protein alpha precurso	2.2	0.2	3.3	3.9	6.0	0.3	4.3	2.0	3.7	6.4	0.190	-1.54
173	*Enolase	Enolase_GROUP	114.1	24.2	57.7	49.4	89.3	64.4	101.5	98.2	64.1	41.2	0.138	-1.54
174	SPB5	Serpin B5 precursor (Maspin) (Pr	0.1	0.2	1.7	3.9	7.2	0.3	5.7	6.4	7.1	3.3	0.488	-1.54
175	ERP29	Endoplasmic reticulum protein ER	1.5	0.2	0.2	0.1	3.7	0.3	0.3	2.9	0.2	0.3	0.678	-1.54
176	PSA	Puromycin-sensitive aminopeptida	2.2	0.2	0.2	2.4	3.7	0.3	3.0	2.0	3.7	3.3	0.602	-1.55
177	GDIS	Rho GDP-dissociation inhibitor 2	30.0	3.9	5.7	6.9	13.0	18.4	16.5	13.5	25.3	15.4	0.363	-1.56
178	*DEFs	DEF1_GROUP	26.5	10.4	13.6	9.1	66.2	39.7	30.0	23.3	32.2	24.5	0.020	-1.57
179	TRFE	Serotransferrin precursor (Trans	144.3	53.9	60.9	50.9	135.6	84.1	131.9	116.9	229.0	139.6	0.042	-1.58
180	ELAF	Elafin precursor (Elastase-speci	0.1	0.2	2.5	1.6	3.7	0.3	0.3	0.2	4.8	4.8	0.281	-1.59

No.	Locus	Description	A(u)	A(s)	B(u)	B(s)	C(u)	C(s)	D(u)	D(s)	E(u)	E(s)	p	fold
181	*Ig Lambda	Lambda_GROUP	169.0	119.6	157.0	75.2	231.6	158.0	109.6	103.5	318.0	160.8	0.023	-1.60
182	AL3A1	Aldehyde dehydrogenase, dimeric	1.5	0.2	0.2	1.6	15.3	0.3	5.7	5.5	7.1	10.9	0.581	-1.60
183	CATA	Catalase (EC 1.11.1.6) - Homo sa	16.1	0.2	3.3	1.6	25.7	16.8	16.5	10.0	9.4	15.4	0.264	-1.61
184	SPRL1	SPARC-like protein 1 precursor (	2.9	13.1	23.0	12.1	28.0	11.8	24.6	18.0	48.2	23.0	0.657	-1.62
185	CLUS	Clusterin precursor (Complement-	16.1	15.0	13.6	4.6	10.6	8.5	12.4	10.0	15.1	3.3	0.095	-1.64
186	PPIA	Peptidyl-prolyl cis-trans isomer	27.2	3.9	17.5	15.8	44.2	20.0	31.3	24.2	35.6	30.6	0.135	-1.65
187	TALDO	Transaldolase (EC 2.2.1.2) - Hom	9.9	0.2	0.2	2.4	25.7	13.5	7.0	6.4	17.3	13.9	0.699	-1.65
188	CAPG	Macrophage capping protein (Acti	3.6	0.2	2.5	1.6	6.0	6.9	9.7	8.2	11.6	3.3	0.169	-1.65
189	*Aldolase	Aldolase_GROUP	60.6	7.6	28.5	17.3	55.8	23.3	47.5	55.4	73.3	56.3	0.134	-1.66
190	GANAB	Neutral alpha-glucosidase AB pre	0.1	0.2	0.2	2.4	2.5	0.3	0.3	0.2	2.5	0.3	0.741	-1.66
191	*HSP1	HSP1_GROUP	43.1	4.8	14.9	19.1	78.2	22.8	41.2	42.1	49.7	46.7	0.238	-1.68
192	*ACT1	ACT1_GROUP	264.6	74.2	116.0	69.6	168.0	82.4	174.5	187.3	168.0	115.4	0.063	-1.68
193	MMP9	Matrix metalloproteinase-9 precu	29.3	0.2	5.7	3.1	39.6	28.2	19.2	16.2	41.3	32.1	0.247	-1.69
194	A1AG1	Alpha-1-acid glycoprotein 1 prec	4.3	2.0	12.0	5.4	3.7	8.5	13.8	5.5	8.2	3.3	0.207	-1.69
195	HSPB1	Heat-shock protein beta-1 (HspB1	4.3	3.9	15.1	9.1	10.6	5.3	9.7	6.4	8.2	3.3	0.018	-1.72
196	PROF1	Profilin-1 (Profilin I) - Homo s	73.1	3.9	15.1	12.8	47.7	46.3	30.0	32.3	50.4	29.1	0.270	-1.74
197	PARK7	Protein DJ-1 (Oncogene DJ1) (Par	6.4	0.2	0.2	0.1	7.2	3.6	0.3	2.0	4.8	4.8	0.635	-1.75
198	*Ig Heavy	Heavy_GROUP	102.3	69.6	67.2	36.7	158.7	84.1	112.3	84.8	140.6	54.8	0.008	-1.76
199	*S100	S100_GROUP	1.5	0.2	0.2	0.1	11.8	8.5	9.7	5.5	8.2	3.3	0.092	-1.77
200	A1BG	Alpha-1B-glycoprotein precursor	8.5	0.2	1.7	0.1	11.8	6.9	5.7	7.3	9.4	6.4	0.141	-1.77
201	PP11	Placental protein 11 precursor (	0.1	0.2	0.2	2.4	2.5	0.3	3.0	0.2	0.2	0.3	0.764	-1.78
202	TGM3	Protein-glutamine gamma-glutamyl	2.2	0.2	5.7	2.4	22.2	8.5	5.7	10.9	15.1	6.4	0.143	-1.79
203	ARGI1	Arginase-1 (EC 3.5.3.1) (Type I	1.5	0.2	0.2	2.4	3.7	0.3	3.0	2.9	2.5	0.3	0.461	-1.79
204	AATC	Aspartate aminotransferase, cyto	2.9	0.2	0.2	0.1	4.9	0.3	0.3	2.0	2.5	3.3	0.518	-1.80
205	DSC2	Desmocollin-2 precursor (Desmoso	9.2	3.9	27.0	9.9	22.2	6.9	12.4	18.0	33.3	18.5	0.075	-1.82
206	PGK1	Phosphoglycerate kinase 1 (EC 2.	36.3	4.8	11.2	10.6	52.3	11.8	23.2	26.9	37.9	33.6	0.182	-1.83
207	PRDX5	Peroxiredoxin-5, mitochondrial p	5.7	0.2	4.9	1.6	2.5	3.6	3.0	0.2	5.9	6.4	0.141	-1.84
208	CO4A	Complement C4-A precursor (Acidi	5.7	0.2	8.8	4.6	13.0	6.9	11.1	14.4	18.5	4.8	0.137	-1.84
209	CAP1	Adenylyl cyclase-associated prot	23.8	5.7	3.3	2.4	17.6	3.6	17.8	17.1	19.6	15.4	0.089	-1.85
210	KPYM	Pyruvate kinase isozymes M1/M2 (	89.1	14.1	27.0	20.3	43.0	21.7	52.9	38.5	51.6	44.2	0.101	-1.90
211	TKT	Transketolase (EC 2.2.1.1) (TK)	45.3	5.7	4.9	6.9	59.2	13.5	36.7	34.9	32.2	32.1	0.239	-1.92
212	ANT3	Antithrombin-III precursor (ATII	3.6	0.2	0.2	0.1	4.9	0.3	4.3	3.7	4.8	4.8	0.161	-1.92
213	TPIS	Triosephosphate isomerase (EC 5.	27.2	5.7	17.5	15.1	53.5	15.1	38.1	32.3	44.7	26.0	0.063	-1.92
214	DSG3	Desmoglein-3 precursor (130 kDa	7.8	3.9	35.6	14.3	32.6	11.8	15.1	22.5	61.8	26.0	0.075	-1.95
215	CO3	Complement C3 precursor [Contain	70.3	9.4	28.5	19.6	77.7	39.7	48.9	46.5	80.1	41.2	0.087	-1.95
216	*PRP1	PRP1_GROUP	302.7	54.8	499.3	250.6	478.5	133.9	318.7	358.7	339.1	188.0	0.057	-1.97
217	ITIH2	Inter-alpha-trypsin inhibitor he	5.0	0.2	0.2	0.1	0.2	0.3	5.7	2.0	3.7	4.8	0.331	-1.97
218	TYB4	Thymosin beta-4 (T beta 4) (Fx)	14.0	5.7	1.7	3.1	24.5	11.8	16.5	7.3	13.9	7.9	0.153	-1.97
219	PGM1	Phosphoglucomutase-1 (EC 5.4.2.2	1.5	0.2	0.2	0.1	3.7	0.3	0.3	2.0	0.2	0.3	0.599	-2.01
220	PYGL	Glycogen phosphorylase, liver fo	2.9	0.2	0.2	0.1	14.1	0.3	8.4	10.9	10.5	6.4	0.164	-2.02
221	*Haptoglobin	Haptoglobin_GROUP	40.4	4.8	25.8	10.6	50.0	31.5	20.5	21.6	57.3	27.6	0.083	-2.02
222	*1433	1433_GROUP	23.1	5.7	15.9	19.6	45.4	3.6	36.7	24.2	60.7	36.6	0.123	-2.02
223	RINI	Ribonuclease inhibitor (Ribonucl	6.4	0.2	3.3	4.6	3.7	0.3	3.0	2.0	8.2	4.8	0.141	-2.06
224	CFAB	Complement factor B precursor (E	5.0	3.0	3.3	3.1	9.5	0.3	5.7	5.5	8.2	3.3	0.191	-2.07
225	AFAM	Afamin precursor (Alpha-albumin)	2.9	0.2	0.2	0.1	2.5	0.3	0.3	2.0	0.2	0.3	0.575	-2.09
226	LYPD3	Ly6/PLAUR domain-containing prot	4.3	0.2	4.1	3.9	9.5	3.6	12.4	6.4	9.4	4.8	0.109	-2.09

No.	Locus	Description	A(u)	A(s)	B(u)	B(s)	C(u)	C(s)	D(u)	D(s)	E(u)	E(s)	p	fold
227	TAGL2	Transgelin-2 (SM22-alpha homolog	5.0	0.2	1.7	1.6	3.7	3.6	3.0	2.9	4.8	0.3	0.169	-2.12
228	PPIC	Peptidyl-prolyl cis-trans isomer	6.4	0.2	4.9	1.6	0.2	0.3	0.3	0.2	0.2	3.3	0.710	-2.12
229	G6PD	Glucose-6-phosphate 1-dehydrogen	10.6	0.2	0.2	0.1	7.2	0.3	3.0	2.9	3.7	7.9	0.247	-2.15
230	A2MG	Alpha-2-macroglobulin precursor	115.5	9.4	11.6	14.3	26.8	16.8	36.7	40.3	100.6	53.3	0.247	-2.17
231	DSG1	Desmoglein-1 precursor (Desmosom	5.0	0.2	7.3	3.1	22.2	5.3	8.4	11.8	21.9	9.4	0.110	-2.18
232	FIBA	Fibrinogen alpha chain precursor	9.2	0.2	5.7	3.1	4.9	3.6	7.0	7.3	15.1	4.8	0.172	-2.19
233	*PRP4	PRP4_GROUP	100.0	18.7	126.8	45.7	101.3	34.8	47.5	65.2	81.6	44.2	0.069	-2.19
234	ISK5	Serine protease inhibitor Kazal-	1.5	0.2	4.9	5.0	19.9	5.3	7.0	3.7	18.5	9.4	0.059	-2.20
235	LCN1	Lipocalin-1 precursor (Von Ebner	148.9	73.3	111.3	66.6	199.2	26.6	81.3	51.0	100.6	71.5	0.058	-2.22
236	*EZR	EZR_GROUP	28.6	0.2	5.7	3.9	48.8	5.3	27.3	26.0	16.2	21.5	0.209	-2.23
237	LACRT	Extracellular glycoprotein lacri	9.2	2.0	0.2	2.4	0.2	3.6	0.3	0.2	9.4	0.3	0.986	-2.25
238	VINC	Vinculin (Metavinculin) - Homo s	7.1	0.2	1.7	0.1	6.0	0.3	9.7	6.4	12.8	9.4	0.045	-2.27
239	GELS	Gelsolin precursor (Actin-depoly	26.5	6.7	13.6	8.4	28.0	8.5	30.0	31.4	58.4	13.9	0.037	-2.27
240	LDHA	L-lactate dehydrogenase A chain	13.7	0.2	0.2	2.4	15.9	3.6	10.4	8.2	5.4	5.6	0.598	-2.27
241	TPM3	Tropomyosin alpha-3 chain (Tropo	5.7	5.7	3.3	0.1	8.3	0.3	5.7	8.2	10.5	0.3	0.091	-2.28
242	PRB3	Basic salivary proline-rich prot	161.4	64.0	61.1	26.3	206.9	28.2	132.6	140.1	79.0	18.5	0.039	-2.31
243	MYL6	Myosin light polypeptide 6 (Smoo	5.0	0.2	0.2	0.1	7.2	0.3	5.7	5.5	11.6	6.4	0.126	-2.36
244	NGAL	Neutrophil gelatinase-associated	11.3	3.0	2.5	1.6	4.9	0.3	7.0	6.4	9.4	3.3	0.069	-2.39
245	CLIC1	Chloride intracellular channel p	17.5	0.2	4.9	3.1	7.2	3.6	4.3	3.7	7.1	6.4	0.234	-2.40
246	IGLL1	Immunoglobulin lambda-like polyp	11.3	5.7	12.0	7.2	3.7	0.3	0.3	0.2	6.5	0.3	0.063	-2.44
247	LKHA4	Leukotriene A-4 hydrolase (EC 3.	16.1	0.2	5.7	2.4	24.5	3.6	15.1	14.4	9.4	7.9	0.141	-2.48
248	CAMP	Cathelicidin antimicrobial pepti	3.6	0.2	0.2	0.1	9.5	6.9	5.7	2.9	7.1	0.3	0.099	-2.50
249	*HSP2	HSP2_GROUP	8.5	0.2	6.5	0.1	6.0	0.3	9.7	12.7	3.7	0.3	0.027	-2.52
250	ARP3	Actin-like protein 3 (Actin-rela	9.9	2.0	2.5	1.6	3.7	0.3	8.4	2.9	9.4	6.4	0.037	-2.56
251	MDHC	Malate dehydrogenase, cytoplasmi	8.5	0.2	0.2	0.1	11.8	0.3	0.3	7.3	8.2	3.3	0.481	-2.56
252	ADH7	Alcohol dehydrogenase class 4 mu	0.1	0.2	1.7	1.6	7.2	0.3	4.3	2.9	8.2	3.3	0.234	-2.59
253	MTPN	Myotrophin (Protein V-1) - Homo	2.2	0.2	1.7	2.4	0.2	0.3	0.3	0.2	4.8	0.3	0.215	-2.73
254	VTNC	Vitronectin precursor (Serum-spr	2.9	0.2	0.2	1.6	7.2	3.6	0.3	0.2	5.9	0.3	0.404	-2.78
255	RETBP	Plasma retinol-binding protein p	3.6	0.2	5.7	1.6	0.2	0.3	3.0	0.2	3.7	3.3	0.118	-2.85
256	PGAM1	Phosphoglycerate mutase 1 (EC 5.	6.4	0.2	4.1	4.6	26.8	0.3	9.7	6.4	17.3	10.9	0.134	-2.87
257	TBCA	Tubulin-specific chaperone A (Tu	2.2	0.2	0.2	0.1	3.7	0.3	0.3	2.9	4.8	0.3	0.338	-2.92
258	FLNA	Filamin-A (Alpha-filamin) (Filam	5.0	0.2	0.2	0.1	0.2	0.3	5.7	2.9	9.4	3.3	0.212	-2.98
259	PRSS8	Prostasin precursor (EC 3.4.21.-	0.1	0.2	0.2	1.6	2.5	0.3	0.3	0.2	4.8	0.3	0.595	-3.00
260	RHOA	Transforming protein RhoA precur	9.9	0.2	2.5	1.6	2.5	0.3	3.0	0.2	3.7	4.8	0.082	-3.00
261	UFM1	Ubiquitin-fold modifier 1 precur	1.5	0.2	1.7	0.1	0.2	0.3	3.0	2.0	2.5	0.3	0.072	-3.07
262	SLUR1	Secreted Ly-6/uPAR-related prote	0.1	0.2	2.5	0.1	0.2	0.3	0.3	2.0	5.9	0.3	0.549	-3.11
263	*GDP	GDP_GROUP	12.0	0.2	0.2	1.6	9.5	0.3	4.3	4.6	10.5	4.8	0.375	-3.13
264	MMP8	Neutrophil collagenase precursor	5.7	0.2	1.7	0.1	3.7	3.6	3.0	0.2	0.2	0.3	0.090	-3.24
265	TLN1	Talin-1 - Homo sapiens (Human)	21.7	0.2	0.2	0.1	3.7	0.3	4.3	4.6	8.2	6.4	0.189	-3.27
266	APM2	Adipose most abundant gene trans	0.1	0.2	0.2	0.1	2.5	0.3	4.3	2.0	2.5	0.3	0.130	-3.31
267	APOA2	Apolipoprotein A-II precursor (A	2.2	2.0	1.7	1.6	4.9	0.3	0.3	0.2	5.9	0.3	0.129	-3.35
268	CORlA	Coronin-1A (Coronin-like protein	18.2	0.2	4.9	2.4	25.7	10.2	20.5	10.0	28.8	6.4	0.084	-3.37
269	PNPH	Purine nucleoside phosphorylase	3.6	0.2	0.2	0.1	6.0	0.3	0.3	2.0	0.2	0.3	0.489	-3.52
270	BASP	Brain acid soluble protein 1 (BA	4.3	0.2	0.2	0.1	4.9	3.6	4.3	0.2	2.5	0.3	0.060	-3.65
271	MDHM	Malate dehydrogenase, mitochondr	0.1	0.2	2.5	0.1	4.9	5.3	0.3	0.2	15.1	0.3	0.184	-3.76
272	TBB4	Tubulin beta-4 chain (Tubulin 5	0.1	0.2	4.9	0.1	4.9	0.3	4.3	2.9	0.2	0.3	0.202	-3.78

No.	Locus	Description	A(u)	A(s)	B(u)	B(s)	C(u)	C(s)	D(u)	D(s)	E(u)	E(s)	p	fold
273	EST2	Carboxylesterase 2 precursor (EC	0.1	0.2	1.7	1.6	9.5	0.3	5.7	3.7	8.2	0.3	0.166	-4.07
274	ARP2	Actin-like protein 2 (Actin-rela	6.4	0.2	0.2	0.1	3.7	0.3	0.3	2.9	5.9	0.3	0.292	-4.31
275	LDHB	L-lactate dehydrogenase B chain	4.7	0.2	0.2	0.1	10.1	0.3	5.0	2.0	3.1	2.6	0.101	-4.42
276	HXK3	Hexokinase-3 (EC 2.7.1.1) (Hexok	1.5	0.2	0.2	0.1	6.0	0.3	3.0	2.0	2.5	0.3	0.050	-4.51
277	ARC1B	Actin-related protein 2/3 comple	5.7	0.2	0.2	0.1	2.5	0.3	5.7	0.2	4.8	3.3	0.062	-4.52
278	G6PI	Glucose-6-phosphate isomerase (E	23.8	0.2	3.3	0.1	30.3	0.3	15.1	13.5	15.1	4.8	0.042	-4.60
279	TBAK	Tubulin alpha-ubiquitous chain (	10.6	0.2	4.9	0.1	10.6	0.3	4.3	4.6	12.8	3.3	0.035	-5.01
280	IDHC	Isocitrate dehydrogenase [NADP]	2.2	0.2	0.2	0.1	6.0	0.3	3.0	2.0	3.7	0.3	0.048	-5.13
281	*ACT2	ACT2_GROUP	10.6	0.2	4.9	0.1	19.9	0.3	28.6	17.1	31.0	0.3	0.010	-5.26
282	AK1BA	Aldo-keto reductase family 1 mem	0.1	0.2	2.5	1.6	9.5	0.3	3.0	0.2	0.2	0.3	0.198	-5.82
283	6PGL	6-phosphogluconolactonase (EC 3.	4.3	0.2	2.5	0.1	3.7	0.3	3.0	2.0	4.8	0.3	0.009	-6.25
284	WDR1	WD repeat protein 1 (Actin-inter	17.5	0.2	3.3	1.6	7.2	0.3	5.7	4.6	12.8	0.3	0.045	-6.55
285	CALR	Calreticulin precursor (CRP55) (	0.1	0.2	1.7	0.1	10.6	0.3	4.3	2.0	3.7	0.3	0.057	-7.00
286	PDC6I	Programmed cell death 6-interact	4.3	0.2	1.7	0.1	6.0	0.3	3.0	2.0	5.9	0.3	0.009	-7.16
287	TBB5	Tubulin beta chain (Tubulin beta	10.6	0.2	0.2	3.1	0.2	0.3	0.3	0.2	18.5	0.3	0.485	-7.19
288	MYH9	Myosin-9 (Myosin heavy chain 9)	28.6	0.2	8.8	0.1	17.6	0.3	7.0	5.5	48.2	7.9	0.026	-7.84

Appendix 4: Tables of proteins increased or decreased in all 5 subjects

Table A4_1: Stimulated > Unstimulated All 5 Subjects	
RNAS4_HUMAN	*Ribonuclease 4 precursor)*
CBPE_HUMAN	*Carboxypeptidase E precursor*
SPLC2_HUMAN	*Short palate, lung and nasal epithelium carcinoma-associated protein 2 precursor (Parotid secretory protein)*
TSP1_HUMAN	*Thrombospondin-1 precursor*
RNT2_HUMAN	*Ribonuclease T2 precursor*
Cystatin_GROUP	*Cystatins*
CYTC_HUMAN	*Cystatin-C precursor*
CYTD_HUMAN	*Cystatin-D precursor*
CAH6_HUMAN	*Carbonic anhydrase 6 precursor*
U773_HUMAN	*Protein UNQ773/PRO1567 precursor*
LEG7_HUMAN	Galectin-7
BPIL1_HUMAN	*Bactericidal/permeability-increasing protein-like 1 precursor*
FCGBP_HUMAN	*IgGFc-binding protein precursor*
CRIS3_HUMAN	*Cysteine-rich secretory protein 3 precursor*

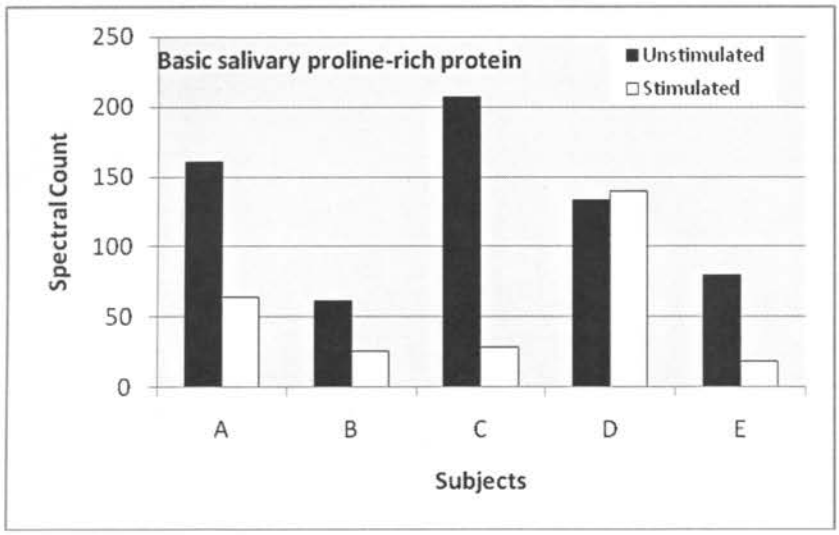
Table A4_1. 14 proteins showing an increase in relative abundance change with stimulation in all 5 subjects. (48 proteins in 4/5 subjects)

Table A4_2: Stimulated < Unstimulated All 5 Subjects	
ALBU_HUMAN	*Serum albumin precursor*
Kappa_GROUP	*IG Kappa Chains*
IGA_GROUP	*IGA group*
WFDC2_HUMAN	*WAP four-disulfide core domain protein 2 precursor*
M6PBP_HUMAN	*Mannose-6-phosphate receptor-binding protein 1*
Enolase_GROUP	*Enolases*
DEFENSIN_GROUP	*Defensins*
TRFE_HUMAN	*Serotransferrin precursor*
Lambda_GROUP	*IG Lambda Chains*
CLUS_HUMAN	*Clusterin precursor*
PPIA_HUMAN	*Peptidyl-prolyl cis-trans isomerase A*
MP9_HUMAN	*Matrix metalloproteinase-9 precursor*
HSPB1_HUMAN	*Heat-shock protein beta-1*
Heavy_GROUP	*IG Heavy Chains*
S100_GROUP	*S100 proteins*
CAP1_HUMAN	*Adenylyl cyclase-associated protein 1*
KPYM_HUMAN	*Pyruvate kinase isozymes M1/M2*
TPIS_HUMAN	*Triosephosphate isomerase*
CO3_HUMAN	*Complement C3 precursor*
CFAB_HUMAN	*Complement factor B precursor*
LYPD3_HUMAN	*Ly6/PLAUR domain-containing protein 3 precursor*
TAGL2_HUMAN	*Transgelin-2*
LCN1_HUMAN	*Lipocalin-1 precursor (Von Ebner gland protein)*
VINC_HUMAN	*Vinculin*
MYL6_HUMAN	*Myosin light polypeptide 6*
NGAL_HUMAN	*Neutrophil gelatinase-associated lipocalin precursor*
CLIC1_HUMAN	*Chloride intracellular channel protein 1*
IGLL1_HUMAN	*Immunoglobulin lambda-like polypeptide 1 precursor*
LKHA4_HUMAN	*Leukotriene A-4 hydrolase*
CAMP_HUMAN	*Cathelicidin antimicrobial peptide precursor*
ARP3_HUMAN	*Actin-like protein 3*
APOA2_HUMAN	*Apolipoprotein A-II precursor*
COR1A_HUMAN	*Coronin-1A*
BASP_HUMAN	*Brain acid soluble protein 1*
LDHB_HUMAN	*L-lactate dehydrogenase B*
HXK3_HUMAN	*Hexokinase-3*
ARC1B_HUMAN	*Actin-related protein 2/3 complex subunit 1B*
G6PI_HUMAN	*Glucose-6-phosphate isomerase*
IDHC_HUMAN	*Isocitrate dehydrogenase [NADP] cytoplasmic*
ACTIN2_GROUP	*Actins*
6PGL_HUMAN	*6-phosphogluconolactonase*
WDR1_HUMAN	*WD repeat protein*
PDC6I_HUMAN	*Programmed cell death 6-interacting protein*
MYH9_HUMAN	*Myosin-9*

Table A4_2. Forty-four proteins showing a decrease in relative abundance change with stimulation in all 5 subjects (123 proteins in 4/5 subjects)

Appendix 5: Basic proline-rich protein spectral counts across 5 subjects

Whole Saliva



Parotid Saliva

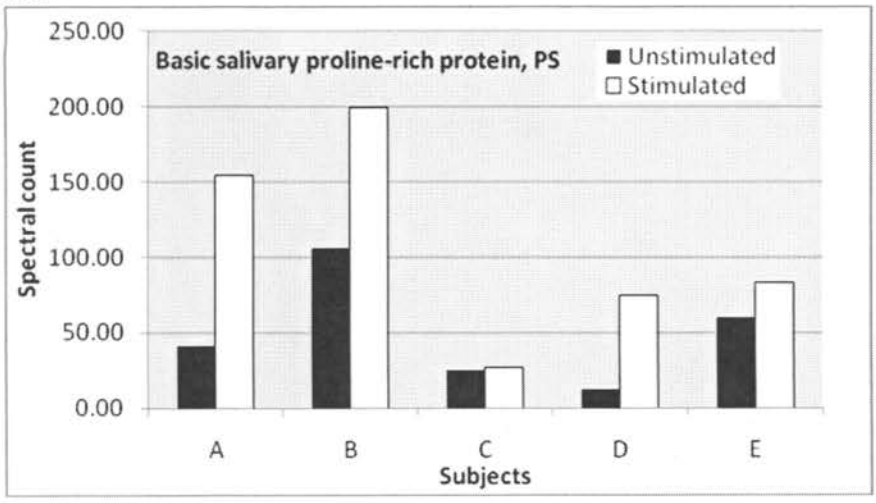


Figure A5_1. Normalized spectral counts for basic salivary proline-rich protein in stimulated saliva (white bars) compared to unstimulated saliva (black bars) across the 5 biological subjects. With the exception of subject D, basic salivary proline-rich proteins were less abundant in stimulated than unstimulated whole saliva. In contrast, abundances showed large increases in parotid saliva.