



NextGen Sciences Report

Mass Spectrometry Report

To: Dr. Smith
Company: X

Samples: Band 1
NGS# 12345

Date: April 29th, 2009

Methods

Samples received:

NGS	X
12345	Band 1

Each sample was digested using trypsin according to the protocol described at the end of this report. Gel digests were analyzed using LC/MS/MS with a 30min gradient on a LTQ Orbitrap XL mass spectrometer. Product ion data were searched against the concatenated forward and reverse IPI human v3.53 database using the Mascot search engine. The database was appended with commonly observed background proteins ([cRAP](#)) to prevent false assignment of peptides derived from those proteins. Mascot output files were parsed into the Scaffold program (www.proteomesoftware.com) for filtering to assess false discovery rates and allow only correct protein identifications.

The Scaffold file used to generate this data is available for download from your account (<https://prsonline.spartacus-consulting.com/prs/guest/login.php?msg=goodbye>) and can be used to visualize much more data than can be described in this report. This includes viewing product ion data, coverage maps, for example. You will need to download the free viewer for Scaffold version 2.2 from www.proteomesoftware.com. It will be necessary to use a PC with at least 1GB of RAM to view the file.

Results

The table below list the proteins identified in the sample. We require at least 2 unique peptides per protein and use the Scaffold Protein and Peptide Prophet thresholds described at the end of the report. The list of proteins is also provided in the accompanying Excel spreadsheet.

Identified Proteins (159)	Accession Number	Molecular Weight	Spectral Count	Unique Peptides
ACTB Actin, cytoplasmic 1	IPI00021439 (+1)	42 kDa	277	36
ALDOA Fructose-bisphosphate aldolase A	IPI00465439	39 kDa	86	31
HNRNPA3 Isoform 1 of Heterogeneous nuclear ribonucleoprotein A3	IPI00419373	40 kDa	45	27
ACTR2 Actin-related protein 2	IPI00005159	45 kDa	32	19
CAPG Macrophage-capping protein	IPI00027341	39 kDa	29	14
HNRNPD Isoform 1 of Heterogeneous nuclear ribonucleoprotein D0	IPI00028888 (+1)	38 kDa	25	13
PCBP1 Poly(rC)-binding protein 1	IPI00016610	37 kDa	22	13
ZMPSTE24 CAAX prenyl protease 1 homolog	IPI00027180	55 kDa	19	11
HNRNPA2B1 Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1	IPI00396378 (+2)	37 kDa	18	14
GOT2 Aspartate aminotransferase, mitochondrial	IPI00018206	47 kDa	17	15
ERLIN2 Isoform 1 of Erlin-2	IPI00026942	38 kDa	17	15
PRMT1 HMT1 hnRNP methyltransferase-like 2 isoform 3	IPI00382516	41 kDa	17	14
MAPK1 Mitogen-activated protein kinase 1	IPI00003479	41 kDa	16	13
GNAI2 Galphai2 protein	IPI00465121 (+1)	42 kDa	16	12
STRAP cDNA FLJ51909, highly similar to Serine-threonine kinase receptor-associatedprotein	IPI00294536	40 kDa	14	13
TMPO Isoform Beta of Lamina-associated polypeptide 2, isoforms beta/gamma	IPI00030131 (+2)	51 kDa	14	12
ACTA1 Actin, alpha skeletal muscle	IPI00021428 (+1)	42 kDa	138	2
DRG1 Developmentally-regulated GTP-binding protein 1	IPI00031836	41 kDa	13	11
SEPT2 Septin-2	IPI00014177 (+1)	41 kDa	13	10
HLA-A HLA class I histocompatibility antigen, A-69 alpha chain	IPI00760554 (+6)	41 kDa	13	9
ENO1 Isoform alpha-enolase of Alpha-enolase	IPI00465248	47 kDa	13	6
PSAT1 cDNA FLJ56437, highly similar to Phosphoserine aminotransferase	IPI00001734	45 kDa	12	12
SAE1 SUMO-activating enzyme subunit 1	IPI00033130	38 kDa	12	8
TWF2 Twinfilin-2	IPI00550917	40 kDa	12	9
CHORDC1 Isoform 1 of Cysteine and histidine-rich domain-containing protein 1	IPI00015897 (+1)	37 kDa	12	8
sp_TRYP_PIG	IPIsp_TRYP_PIG	24 kDa	12	4
PLEK Pleckstrin	IPI00306311	40 kDa	10	8
CHI3L1 Chitinase-3-like protein 1	IPI00002147	43 kDa	9	9
TOMM40 Isoform 1 of Mitochondrial import receptor subunit TOM40 homolog	IPI00014053	38 kDa	9	8
TTC1 Tetratricopeptide repeat protein 1	IPI00016912	34 kDa	9	8
AHSA1 Activator of 90 kDa heat shock protein ATPase homolog 1	IPI00030706	38 kDa	9	8
EEF1A1 Elongation factor 1-alpha	IPI00025447 (+3)	48 kDa	9	3
GALK1 cDNA FLJ56840, highly similar to Galactokinase	IPI00019383	45 kDa	8	8
PSMD7 26S proteasome non-ATPase regulatory subunit 7	IPI00019927	37 kDa	8	6
NANS Sialic acid synthase	IPI00147874	40 kDa	8	7

ACAT2 Acetyl-CoA acetyltransferase, cytosolic	IPI00291419	41 kDa	8	7
DCPS Scavenger mRNA-decapping enzyme Dcp5	IPI00335385	39 kDa	8	8
PSMD13 proteasome 26S non-ATPase subunit 13 isoform 2	IPI00375380 (+1)	43 kDa	8	8
HNRNPA1 Isoform A1-B of Heterogeneous nuclear ribonucleoprotein A1	IPI00215965 (+2)	39 kDa	13	6
KRT1 Keratin, type II cytoskeletal 1	IPI00220327 (+1)	66 kDa	8	7
SERPINB1 Leukocyte elastase inhibitor	IPI00027444	43 kDa	7	6
ACAT1 Acetyl-CoA acetyltransferase, mitochondrial	IPI00030363	45 kDa	7	6
CAP1 Isoform 1 of Adenylyl cyclase-associated protein 1	IPI00008274 (+1)	52 kDa	7	7
H2AFY H2A histone family, member Y isoform 2	IPI00059366 (+2)	39 kDa	7	7
LAIR1 Isoform 2 of Leukocyte-associated immunoglobulin-like receptor 1	IPI00028015 (+3)	30 kDa	7	6
PTPN7 Isoform 1 of Tyrosine-protein phosphatase non-receptor type 7	IPI00017338 (+3)	41 kDa	7	6
ALDOC Fructose-bisphosphate aldolase C	IPI00418262	39 kDa	14	6
SSB Lupus La protein	IPI00009032	47 kDa	7	5
CHID1 Isoform 3 of Chitinase domain-containing protein 1	IPI00045536 (+1)	42 kDa	6	6
BUB3 Mitotic checkpoint protein BUB3	IPI00013468 (+2)	37 kDa	6	6
DDRGK1 Isoform 1 of Uncharacterized protein C20orf116	IPI00028387	36 kDa	6	6
SERPINB6 Putative uncharacterized protein DKFZp686I04222	IPI00413451 (+1)	46 kDa	6	6
ATP6V1C1 V-type proton ATPase subunit C 1	IPI00007814 (+1)	44 kDa	6	6
SERPINB10 Serpin B10	IPI00010304	45 kDa	6	6
SET Isoform 1 of Protein SET	IPI00072377	33 kDa	6	5
EIF3H Eukaryotic translation initiation factor 3, subunit 3 gamma, 40kDa, isoform CRA_b	IPI00647650	42 kDa	6	5
SUGT1 Isoform 2 of Suppressor of G2 allele of SKP1 homolog	IPI00791573 (+1)	38 kDa	6	5
HNRNPK Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	IPI00216049 (+4)	51 kDa	6	5
CKB Creatine kinase B-type	IPI00022977	43 kDa	6	5
TMOD3 Tropomodulin-3	IPI00005087	40 kDa	6	6
PURA Transcriptional activator protein Pur-alpha	IPI00023591	35 kDa	6	5
WDR77 Methylosome protein 50	IPI00012202	37 kDa	6	4
FCGRT IgG receptor FcRn large subunit p51 (Fragment)	IPI00026646	40 kDa	6	4
RCN1 Reticulocalbin-1	IPI00015842	39 kDa	6	4
PPT1 Palmitoyl-protein thioesterase 1	IPI00002412	34 kDa	5	5
ACADS Short-chain specific acyl-CoA dehydrogenase, mitochondrial	IPI00027701	44 kDa	5	5
CAMK1 Calcium/calmodulin-dependent protein kinase type 1	IPI00028296	41 kDa	5	5
GNAI3 Guanine nucleotide-binding protein G(k) subunit alpha	IPI00220578	41 kDa	11	5
ZYX Zyxin	IPI00020513 (+1)	67 kDa	5	5
RTCD1 Isoform 1 of RNA 3'-terminal phosphate cyclase	IPI00011726	39 kDa	5	5
CLEC11A C-type lectin domain family 11 member A	IPI00033466	36 kDa	5	5
EIF3M Eukaryotic translation initiation factor 3 subunit M	IPI00102069	43 kDa	5	5
LUC7L2 Isoform 1 of Putative RNA-binding protein Luc7-like 2	IPI00006932	47 kDa	5	4
HNRNPC Isoform C1 of Heterogeneous nuclear ribonucleoproteins C1/C2	IPI00216592 (+2)	32 kDa	5	4
MAPK14 Isoform CSBP2 of Mitogen-activated protein kinase 14	IPI00002857 (+3)	41 kDa	5	4
WDR5 WD repeat-containing protein 5	IPI00005492	37 kDa	5	4
CSNK2A2 Casein kinase II subunit alpha'	IPI00020602	41 kDa	5	5
HLA-B;HLA-C HLA class I histocompatibility antigen, B-15 alpha chain	IPI00646083 (+1)	40 kDa	11	3

HM13 Isoform 1 of Minor histocompatibility antigen H13	IPI00152441 (+1)	41 kDa	5	3
TMEM43 Transmembrane protein 43	IPI00301280	45 kDa	5	4
DNAJB1 DnaJ homolog subfamily B member 1	IPI00015947	38 kDa	4	4
GMPPB Isoform 2 of Mannose-1-phosphate guanyltransferase beta	IPI00002496 (+1)	43 kDa	4	4
SORD Sorbitol dehydrogenase	IPI00216057	38 kDa	4	4
PCBP2 poly(rC) binding protein 2 isoform b	IPI00012066 (+3)	38 kDa	7	4
PTER Phosphotriesterase-related protein	IPI00100933 (+1)	39 kDa	4	4
STOML2 Stomatin-like protein 2	IPI00334190 (+1)	39 kDa	4	4
VPS26A Vacuolar protein sorting-associated protein 26A	IPI00411426 (+1)	38 kDa	4	4
SLC16A1 Monocarboxylate transporter 1	IPI00024650	54 kDa	4	4
HNRNPR Heterogeneous nuclear ribonucleoprotein R	IPI00012074 (+3)	71 kDa	4	4
RG9MTD1 RNA (guanine-9-) methyltransferase domain containing 1	IPI00099996	47 kDa	4	4
ACOT7 Isoform 1 of Cytosolic acyl coenzyme A thioester hydrolase	IPI00010415 (+2)	42 kDa	4	4
IVD Isovaleryl-CoA dehydrogenase, mitochondrial	IPI00645805 (+1)	46 kDa	4	4
ACAA1 3-ketoacyl-CoA thiolase, peroxisomal	IPI00012828	44 kDa	4	4
SMARCA5 SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5	IPI00297211	122 kDa	4	4
KRT10 Keratin, type I cytoskeletal 10	IPI00009865 (+2)	60 kDa	4	4
NUCKS1 Isoform 1 of Nuclear ubiquitous casein and cyclin-dependent kinases substrate	IPI00022145 (+1)	27 kDa	4	4
IMPACT Isoform 1 of Protein IMPACT	IPI00020451	36 kDa	4	4
ARPC1B Actin-related protein 2/3 complex subunit 1B	IPI00005160	41 kDa	4	4
HNRNPAB Isoform 2 of Heterogeneous nuclear ribonucleoprotein A/B	IPI00334587 (+2)	36 kDa	6	4
PSMC6 proteasome 26S ATPase subunit 6	IPI00021926	46 kDa	4	4
AZU1 Azurocidin	IPI00022246	27 kDa	4	3
CALU Calumenin, isoform CRA_c	IPI00789155	38 kDa	4	3
ADK Isoform Short of Adenosine kinase	IPI00234368 (+1)	39 kDa	4	3
DFFA Isoform DFF45 of DNA fragmentation factor subunit alpha (Fragment)	IPI00010882	37 kDa	3	3
SFRS5 Isoform SRP40-1 of Splicing factor, arginine/serine-rich 5	IPI00012341	31 kDa	3	3
SH3GLB1 Isoform 1 of Endophilin-B1	IPI00006558 (+1)	41 kDa	3	3
RP2 Protein XRP2	IPI00026627	40 kDa	3	3
VPS26B Vacuolar protein sorting-associated protein 26B	IPI00059264	39 kDa	3	3
GNAQ Guanine nucleotide binding protein (G protein), q polypeptide, isoform CRA_c	IPI00288947	42 kDa	3	3
DHCR7 7-dehydrocholesterol reductase	IPI00294501	54 kDa	3	3
CECR5 Isoform 2 of Cat eye syndrome critical region protein 5	IPI00011511 (+1)	46 kDa	3	3
THOC3 THO complex subunit 3	IPI00063729 (+1)	42 kDa	3	3
UCHL5 Isoform 2 of Ubiquitin carboxyl-terminal hydrolase isozyme L5	IPI00219512 (+6)	36 kDa	3	3
YBX1 Nuclease-sensitive element-binding protein 1	IPI00031812 (+1)	36 kDa	3	3
DRG2 Developmentally-regulated GTP-binding protein 2	IPI00022697 (+1)	41 kDa	3	3
TUBA4A Tubulin alpha-4A chain	IPI00007750 (+8)	50 kDa	3	3
PAAF1 Isoform 1 of Proteasomal ATPase-associated factor 1	IPI00743862 (+1)	42 kDa	3	3
ANXA1 Annexin A1	IPI00218918 (+1)	39 kDa	3	3
FAM50A Protein FAM50A	IPI00030098 (+2)	40 kDa	3	3
CRYZL1 Quinone oxidoreductase-like protein 1	IPI00438923 (+3)	39 kDa	3	3

GLRX3 Glutaredoxin-3	IPI00008552	37 kDa	3	3
BXDC2 Brix domain-containing protein 2	IPI00181728	41 kDa	3	3
CPOX Coproporphyrinogen III oxidase, mitochondrial	IPI00093057	50 kDa	3	3
RPL4 60S ribosomal protein L4	IPI00003918 (+1)	48 kDa	3	3
UROD Uroporphyrinogen decarboxylase	IPI00301489 (+1)	41 kDa	3	3
HNRNPA3 Isoform 2 of Heterogeneous nuclear ribonucleoprotein A3	IPI00455134	37 kDa	38	2
HNRNPH1 Heterogeneous nuclear ribonucleoprotein H	IPI00013881 (+2)	49 kDa	3	2
NUP43 cDNA FLJ38675 fis, clone IMR322000243, highly similar to Nucleoporin Nup43	IPI00171664 (+1)	48 kDa	3	2
PRKACA Isoform 1 of cAMP-dependent protein kinase catalytic subunit alpha	IPI00396630	41 kDa	3	2
SNRNP40 cDNA FLJ56825, highly similar to WD repeat protein 57	IPI00006723	45 kDa	2	2
MGC3207 Isoform 1 of Translation initiation factor eIF-2B subunit alpha/beta/delta-like protein	IPI00005948 (+1)	39 kDa	2	2
QTRT1 cDNA, FLJ95065, highly similar to Homo sapiens queuine tRNA-ribosyltransferase 1 (tRNA-guanine transglycosylase) (QTRT1), mRNA	IPI00215974	44 kDa	2	2
M6PRBP1 Isoform B of Mannose-6-phosphate receptor-binding protein 1	IPI00303882	47 kDa	2	2
PCID2 Isoform 3 of PCI domain-containing protein 2	IPI00072541 (+3)	43 kDa	2	2
PIGK GPI-anchor transamidase	IPI00022543 (+2)	45 kDa	2	2
MAN2B1 Lysosomal alpha-mannosidase	IPI00012989 (+1)	114 kDa	2	2
QKI Isoform 6 of Protein quaking	IPI00385562 (+7)	36 kDa	2	2
GNA13 Guanine nucleotide-binding protein subunit alpha-13	IPI00290928 (+1)	44 kDa	3	2
GAPDH Glyceraldehyde-3-phosphate dehydrogenase	IPI00219018 (+4)	36 kDa	2	2
RAE1 mRNA export factor	IPI00019733 (+1)	41 kDa	2	2
RPL3 60S ribosomal protein L3	IPI00550021 (+3)	46 kDa	2	2
RPL6 60S ribosomal protein L6	IPI00329389 (+5)	33 kDa	2	2
ADAP1 Centaurin-alpha-1	IPI00009992 (+5)	43 kDa	2	2
DAP3 28S ribosomal protein S29, mitochondrial	IPI00018120 (+1)	46 kDa	2	2
TUBB2C Tubulin beta-2C chain	IPI00007752 (+18)	50 kDa	2	2
LRPPRC Leucine-rich PPR motif-containing protein, mitochondrial	IPI00783271	158 kDa	2	2
RRP15 RRP15-like protein	IPI00007004	31 kDa	2	2
POLR1C Isoform 1 of DNA-directed RNA polymerases I and III subunit RPAC1	IPI00005179 (+2)	39 kDa	2	2
ASNA1 Arsenical pump-driving ATPase	IPI00013466	39 kDa	2	2
BCAT1 Branched-chain-amino-acid aminotransferase, cytosolic	IPI00382412 (+1)	43 kDa	2	2
AUP1 Isoform Long of Ancient ubiquitous protein 1	IPI00001891 (+2)	53 kDa	2	2
C20orf43 cDNA FLJ52798	IPI00218962 (+2)	38 kDa	2	2
TEX264 Testis-expressed sequence 264 protein	IPI00006372 (+1)	34 kDa	2	2
UBC;RPS27A;UBB ubiquitin and ribosomal protein S27a precursor	IPI00179330 (+26)	18 kDa	2	2
TBCC Tubulin-specific chaperone C	IPI00019924	39 kDa	2	2
PRPF38A Isoform 1 of Pre-mRNA-splicing factor 38A	IPI00171390	37 kDa	2	2
IGBP1 Immunoglobulin-binding protein 1	IPI00019148	39 kDa	2	2
SCAMP3 Isoform 1 of Secretory carrier-associated membrane protein 3	IPI00306382 (+1)	38 kDa	2	2
PDHA1 Mitochondrial PDHA1	IPI00306301	48 kDa	2	2

Additional descriptions of methods

In-gel digestion

Samples were subjected to proteolytic digestion on a ProGest (Genomic Solutions) workstation as follows:

- Samples were reduced with DTT at 60°C.
- Samples were allowed to cool to room temperature.
- Samples were alkylated with iodoacetamide.
- Samples were incubated at 37°C for 4h in the presence trypsin.
- Formic acid was added to stop the reaction.
- The supernatant was analyzed directly.

LC/MS/MS

- Samples were analyzed by nano LC/MS/MS on a ThermoFisher LTQ Orbitrap XL.
- 30µl of hydrolysate was loaded onto a 5mm 75µm ID C12 (Jupiter Proteo, Phenomenex) vented column at a flow-rate of 10µL/min. Gradient elution was over a 15cm 75µm ID C12 column at 300nL/min (NanoAcquity, Waters). A 30min gradient was employed.
- The mass spectrometer was operated in data-dependent mode, the six most abundant ions were selected for MS/MS. The Orbitrap MS scan was performed at 60,000 FWHM resolution.
- MS/MS data were searched using a local copy of Mascot (www.matrixscience.com).
- The parameters for all database searches were as follows:

Type of search : MS/MS Ion Search

Enzyme : Trypsin

Fixed modifications : Carbamidomethyl (C)

Variable modifications : Oxidation (M), Acetyl (N-term), Pyro-glu (N-term Q)

Mass values : Monoisotopic

Protein Mass : Unrestricted

Peptide Mass Tolerance : ± 5 ppm (Orbitrap)

Fragment Mass Tolerance: ± 0.5 Da (LTQ)

Max Missed Cleavages : 2

SCAFFOLD

Samples were processed in the Scaffold algorithm (www.proteomesoftware.com) using DAT files generated by Mascot. Parameters for LTQ Orbitrap XL data require a minimum of 2 peptides matching per protein with minimum probabilities of 90% at the protein level and 50% at the corresponding peptide level. Please note that NGS does not support peptides or protein identifications in Scaffold below these threshold limits without *de novo* sequencing.

NOTE: Detailed protocols for each of these methods can be found in the technical information section of our website (www.nextgensciences.com).

cRAP

All of our databases are now appended with the common Repository of Adventitious Proteins, or cRAP database. Many of these proteins occur in every protein identification experiment and unless the product ion spectra are accounted for can lead to increased false discovery rate (through being allowed to match to incorrect proteins in a database not containing the cRAP sequences). The cRAP sequences have the SwisProt accession number format; their accession number is also listed as the description. More information on cRAP can be found at <http://www.thegpm.org/crap/index.html>