Utilizing Spectral Counting to Quantitatively Characterize Tandem Removal of Abundant Proteins (TRAP) in Human Plasma

Christopher M. Shuford[1], Adam M. Hawkridge[1], John C. Burnett, Jr.[2], and David C. Muddiman[1]*

[1]*W.M. Keck FT-ICR Mass Spectrometry Laboratory, Department of Chemistry, North Carolina State University, Raleigh, North Carolina, 27695*

[2]*Division for Cardiovascular Disease, Mayo Clinic College of Medicine, Rochester, Minnesota 55905*

Abstract

Herein are supporting information to the above titled article. The contents include specific details on the chromatographic parameters used to perform the tandem depletions, results from a 1D-SDS-PAGE analysis of the various depletion samples, additional spectral counting data analysis, as well as the raw spectral counting data for all proteins identified in these studies.

Tandem Abundant Protein Depletions

Shown in **Figure S-1** are representative chromatograms for the three depletion setups evaluated. The gradients employed in both TRAP setups were lengthened to accommodate for the increased bed volume of the system and are outline in **Table S-1**. More specifically, the length of the initial binding step was increased to allow the targeted proteins sufficient time to interact with and bind to the columns. It should be noted when connecting the columns in series a marginal pressure increase was observed for the system; however, by minimizing the total length of all connecting tubing, the total back pressure was kept well below the maximum operating pressure for these columns.

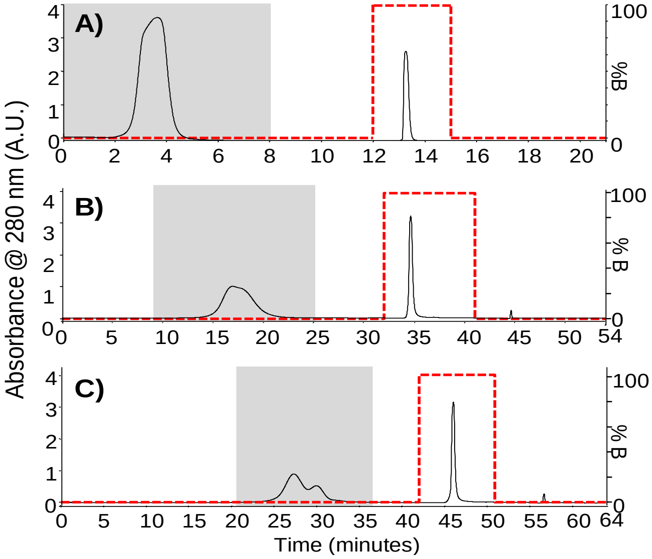

Figure S-1 - Chromatograms obtained from A) HSA depletion, B) HSA/Hu14 tandem depletion, and C) HSA/Hu14/Hu14 tandem depletion of pooled human plasma. The red line indicates the gradient profile used for each depletion setup and the grey boxes show the retention window of the unbound protein fraction that was collected for bottom-up proteomic analysis.

1D-SDS-PAGE

In order to visually inspect the quality of the protein depletions, each sample was run by 1D-SDS-PAGE. One aliquot from the raw, un-depleted plasma and each depleted sample was diluted 2-fold with Laemmli sample buffer and boiled for 5 minutes. Thirteen micrograms of total protein from each denatured sample was then loaded onto the 12.5% Tris-HCl gel and run for 55 minutes at a constant 200 V according to the manufacturer's specifications in Tris/Glycine/SDS running. The gel was briefly rinsed three times with water, stained for 60 minutes with Bio-Safe Coomassie Stain, and rinsed for 30 additional minutes with water prior to capturing the image with a ChemiDoc XRS imaging system. All supplies utilized herein for this analysis were from Bio-Rad (Hercules, CA). The gel image obtained for each sample is shown in **Figure S-2**. As expected, the bands corresponding to the abundant plasma proteins were noticeably diminished across the samples and many additional protein bands corresponding to less abundant proteins were visualized in the TRAP samples. For instance, the band corresponding to HSA (67 kDa) was prominent in the un-depleted sample and was unobserved in all depleted samples. Several target abundant proteins were also observed as dark bands in sample A, but were no longer present in the TRAP samples. Moreover, many more protein bands were visualized in samples B and C compared to the un-depleted plasma and sample A as a result of the greater protein enrichment in the TRAP samples. The protein bands in sample C, in particular, were also slightly, yet noticeably more intense than those in sample B as a result of the relative enrichment of the proteins afforded to sample C by the additional Hu14 removal column. Even though this was an expected outcome based on the Bradford assay results, it was interesting the improvement was observable in the gel image.

Table S-1 - Gradient Time Tables

A - HSA Depletion			B - HSA/Hu14 Depletion			C - HSA/Hu14/Hu14 Depltion		
time	flowrate (mL/min)	% B	time	flowrate (mL/min)	% B	time	flowrate (mL/min)	% B
0.00	0.250	0	0.00	0.125	0	0.00	0.125	0
10.00	0.250	0	30.00	0.125	0	40.00	0.125	0
10.01	1.000	0	30.01	1.000	0	40.01	1.000	0
12.00	1.000	0	32.00	1.000	0	42.00	1.000	0
12.01	1.000	100	32.01	1.000	100	42.01	1.000	100
15.00	1.000	100	41.00	1.000	100	51.00	1.000	100
15.01	1.000	0	41.01	1.000	0	51.01	1.000	0
21.00	1.000	0	54.00	1.000	0	64.00	1.000	0

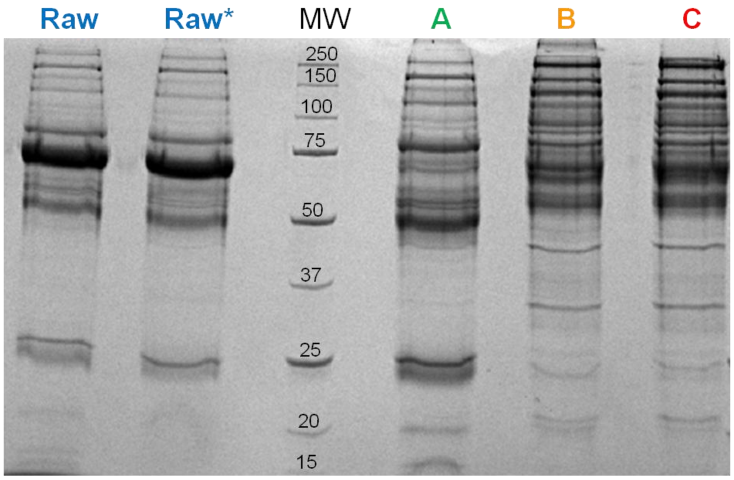

Figure S-2 - 1D-SDS-PAGE image of the raw, un-depleted plasma and unbound fractions from A) HSA depleted, B) HSA/Hu14 tandem depleted, and C) HSA/Hu14/Hu14 tandem depleted plasma. The un-depleted plasma run in the second lane (Raw*) served as a control and was produce by running an aliquot of un-depleted plasma through the entire sample work-up process (e.g., filtering, concentrating, buffer exchange, etc.) yet without depletion. The units for the molecular weight markers (MW) are in kDa.

Spectral Counting Improvements

Shown in **Figure S-3** are plots comparing the normalized spectral counts for proteins identified between successive samples. The slopes of the resulting lines give an estimate of the average improvement in spectral counts for all proteins identified between the two samples. In the first plot, the normalized spectral counts for proteins identified in the un-depleted plasma are plotted on the x-axis and the corresponding normalized spectral counts from sample A are plotted on the y-axis. Based on the slope of the regression line, the normalized spectral counts increased roughly 2.4-fold from the un-depleted plasma to the HSA depleted plasma, which is comparable with the 2.8-fold enrichment factor estimated by the Bradford assays. From sample A to sample B, the improvement in spectral counts was estimated to be 2.0-fold. In this case the increase in spectral counts did not correspond with the 4.9-fold difference in total protein mass measured between these two samples; however, the discrepancy between the two measurements was not unexpected given the substantial change in the protein matrix. Between samples B and C, the 1.1-fold increase in spectral counts was consistent with the 1.15-fold difference in total protein mass and, again, demonstrates the benefit of using multiple depletion columns in tandem. In all cases, the regression lines showing the increase in spectral counts were obtained with correlation coefficients (R^2) greater than 0.9, which suggests the improvements were real. In each case shown, the data was also subjected to the Wilcoxon Signed Rank Test to determine if the difference in the normalized spectral counts was statistically significant. This test was chosen because it is a non-parametric test and, as such, does not assume the data is normally distributed. In all cases the *p-value* of the test statistic was less than 0.001, indicating the normalized spectral counts between the respective samples were statistically different and the observed increase real.

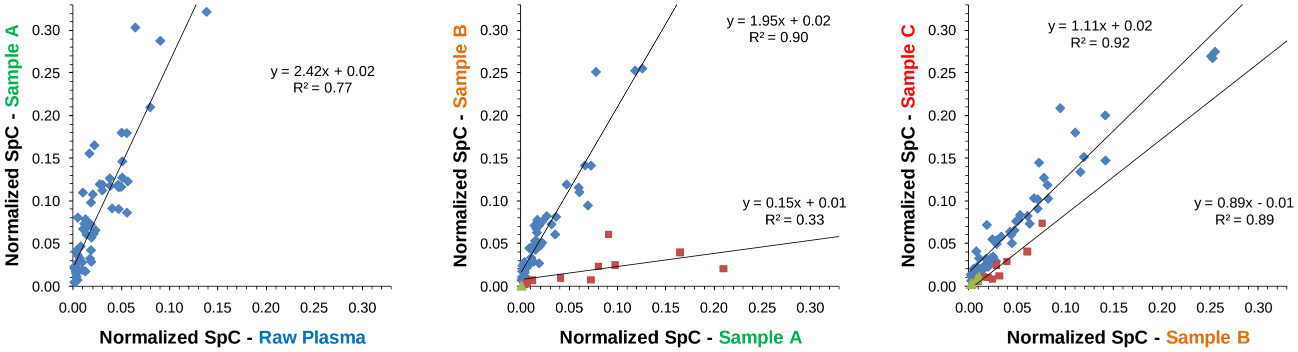

Figure S-3 - Scatter plots comparing the normalized spectral counts between A) raw, un-depleted plasma and HSA depleted plasma, B) HSA depleted plasma and HSA/Hu14 tandem depleted plasma, and C) HSA/Hu14 tandem depleted plasma and HSA/Hu14/Hu14 tandem depleted plasma. Only proteins that were identified within both samples were included in the plots. The blue markers represent proteins that experienced an increase in spectral counts between the two samples, while red markers represent those that demonstrated a decrease. The green markers represent those proteins whose spectral counts remained constant between samples. Note, only the top scoring proteins from each protein group were utilized in these plots.

Raw Spectral Counting Data

Compiled in the subsequent table is the raw spectral counting data obtained for all proteins identified in these studies across the four samples. The proteins are organized by group number and the top scoring protein in each group is indicated as such. The normalized spectral count values were calculated by dividing the total spectral counts (average × 3) obtained for a given protein and dividing it by the number of amino acids in the annotated protein sequence (*i.e.*, the protein length, L). Also reported in this table is the standard deviation of the average spectral counts obtained across the three technical replicates.

Table S-2

Sequence Information					Raw Plasma		Sample A		Sample B		Sample C	
Group #	**Top Hit ?**	**Sequence ID**	**Sequence Name**	**L (aa)**	**Avg SpC**	**SpC StDev**	**Avg SpC**	**SpC StDev**	**Avg SpC**	**SpC StDev**	**Avg SpC**	**SpC StDev**
1	**Yes**	**IPI00745872.2**	**Isoform 1 of Serum albumin**	**609**	**106.33**	**10.12**						
1	No	IPI00022434.4	Putative uncharacterized protein ALB	627	96.00	8.72						
1	No	IPI00908876.1	cDNA FLJ50830, highly similar to Serum albumin	523	88.33	9.29						
1	No	IPI00878517.1	Putative uncharacterized protein ALB	494	86.67	9.29						
1	No	IPI00216773.4	ALB protein	396	58.67	4.73						
1	No	IPI00878282.1	Putative uncharacterized protein ALB	197	28.67	3.21						
2	**Yes**	**IPI00418163.3**	**complement component 4B preproprotein**	**1744**	**2.33**	**0.58**	**15.67**	**4.04**	**47.67**	**16.56**	**59.67**	**17.79**
2	No	IPI00892604.1	Complement component C4B (Childo blood group) 2	1744	2.33	0.58	15.67	4.04	47.67	16.56	59.67	17.79
2	No	IPI00887154.2	Complement component 4B	1744	2.33	0.58	15.67	4.04	47.67	16.56	59.67	17.79
3	**Yes**	**IPI00022463.1**	**Serotransferrin**	**698**	**15.00**	**7.21**	**70.67**	**17.62**				
3	No	IPI00798216.1	12 kDa protein	105	5.00	2.65	16.00	4.36				
3	No	IPI00798430.2	17 kDa protein	155	1.67	1.53	15.00	5.20				
3	No	IPI00877763.1	Putative uncharacterized protein TF	115	3.67	1.53	10.33	1.53				
3	No	IPI00945828.1	Putative uncharacterized protein TF	120	3.67	1.53	10.33	1.53				
3	No	IPI00796467.1	11 kDa protein	102	1.33	0.58	9.00	1.00				
4	**Yes**	**IPI00029739.5**	**Isoform 1 of Complement factor H**	**1231**	**1.67**	**1.15**	**15.00**	**2.65**	**33.33**	**7.23**	**48.67**	**17.04**
4	No	IPI00942414.2	Isoform 2 of Complement factor H	449	1.67	1.15	10.67	1.15	20.67	4.04	29.67	14.01
5	**Yes**	**IPI00555812.4**	**Isoform 1 of Vitamin D-binding protein**	**474**	**2.00**	**1.00**	**12.33**	**4.16**	**39.67**	**4.73**	**42.67**	**3.51**
5	No	IPI00954102.1	Isoform 2 of Vitamin D-binding protein	352	1.00	1.00	8.33	2.52	27.33	2.31	32.67	3.06
6	**Yes**	**IPI00019591.2**	**cDNA FLJ55673, highly similar to Complement factor B**	**1266**	**1.00**	**1.00**	**8.00**	**1.73**	**30.00**	**9.17**	**43.00**	**10.82**
6	No	IPI00939824.1	141 kDa protein	1266	1.00	1.00	8.00	1.73	30.00	9.17	43.00	10.82
7	**Yes**	**IPI00947496.1**	**124 kDa protein**	**1115**	**1.00**	**1.00**	**6.00**	**1.00**	**25.00**	**7.21**	**38.33**	**8.62**
8	**Yes**	**IPI00021885.1**	**Isoform 1 of Fibrinogen alpha chain**	**866**	**6.33**	**2.31**	**47.67**	**10.69**	**11.33**	**4.93**	**8.33**	**4.73**
8	No	IPI00029717.1	Isoform 2 of Fibrinogen alpha chain	644	6.33	2.31	47.67	10.69	11.33	4.93	8.33	4.73
8	No	IPI00902755.1	FGA protein (Fragment)	250	1.33	1.15	19.33	2.08	4.33	1.53	0.67	1.15
8	No	IPI00871469.1	FGA protein	289	1.00	1.00	17.67	2.52	4.00	1.73	0.33	0.58
9	**Yes**	**IPI00641737.1**	**Haptoglobin**	**420**	**12.67**	**3.51**	**40.33**	**12.86**				
9	No	IPI00478493.3	haptoglobin isoform 2 preproprotein	347	12.00	3.61	34.00	9.54				
9	No	IPI00942787.1	42 kDa protein	382	12.00	3.00	34.00	8.72				
9	No	IPI00431645.1	HP protein	281	10.00	3.46	25.00	7.94				
10	**Yes**	**IPI00783987.2**	**Complement C3 (Fragment)**	**1663**	**10.00**	**0.00**	**40.00**	**2.00**	**4.00**	**1.00**	**3.67**	**2.08**
10	No	IPI00942927.1	cDNA FLJ57339, highly similar to Complement C3	1098	10.00	0.00	34.00	3.61	4.00	1.00	3.33	2.31
10	No	IPI00887739.3	similar to complement component C3, partial	1295	8.00	1.00	32.67	2.31	4.00	1.00	3.33	2.31
10	No	IPI00739237.1	similar to complement component 3	390	2.00	1.00	6.67	1.53			0.33	0.58
11	**Yes**	**IPI00022229.1**	**Apolipoprotein B-100**	**4563**			**4.00**	**1.73**	**13.33**	**4.04**	**12.00**	**7.55**

12	**Yes**	**IPI00022895.7**	**Alpha-1B-glycoprotein**	**495**	**1.67**	**2.08**	**11.00**	**3.00**	**23.33**	**10.69**	**24.33**	**5.77**
12	No	IPI00644018.1	41 kDa protein	373	1.00	1.73	8.00	1.73	17.00	7.21	19.67	4.73
12	No	IPI00646799.1	cDNA FLJ31323 fis, clone LIVER2000037, highly similar to Alpha-1B-glycoprotein	326	1.00	1.73	7.67	1.15	15.67	6.66	17.00	4.36
12	No	IPI00902880.1	cDNA FLJ42722 fis, clone BRAMY4000277, highly similar to Alpha-1B-glycoprotein	345	0.67	1.15	4.00	1.00	11.00	3.61	11.67	2.52
13	**Yes**	**IPI00017601.1**	**Ceruloplasmin**	**1065**			**5.00**	**5.29**	**17.33**	**4.73**	**27.00**	**6.56**
13	No	IPI00946976.1	Putative uncharacterized protein CP	188			1.00	0.00	3.67	1.53	6.33	2.52
14	**Yes**	**IPI00304273.2**	**Apolipoprotein A-IV**	**396**	**5.00**	**2.00**	**16.67**	**1.15**	**33.67**	**5.86**	**36.33**	**7.09**
15	**Yes**	**IPI00947307.1**	**cDNA FLJ58075, highly similar to Ceruloplasmin**	**946**			**4.67**	**4.73**	**16.67**	**4.04**	**26.33**	**5.03**
16	**Yes**	**IPI00847179.1**	**apolipoprotein A-IV precursor**	**396**	**4.00**	**2.00**	**15.67**	**1.15**	**33.33**	**6.43**	**35.33**	**7.09**
17	**Yes**	**IPI00019580.1**	**Plasminogen**	**810**			**5.33**	**1.15**	**12.67**	**6.66**	**17.67**	**5.51**
17	No	IPI00025327.2	plasminogen isoform 2 precursor	136			1.00	0.00	2.33	1.53	5.00	2.00
17	No	IPI00855913.1	Plasminogen	166			1.00	0.00	2.33	1.53	5.00	2.00
18	**Yes**	**IPI00218999.3**	**Putative uncharacterized protein CFH**	**439**	**1.67**	**1.15**	**10.67**	**1.15**	**20.67**	**4.04**	**29.33**	**14.50**
18	No	IPI00515041.5	Complement factor H	385	1.67	1.15	9.33	2.31	15.67	1.53	24.33	13.01
19	**Yes**	**IPI00215894.1**	**Isoform LMW of Kininogen-1**	**427**	**3.00**	**1.00**	**8.67**	**2.08**	**15.67**	**6.43**	**25.67**	**3.21**
19	No	IPI00924859.1	kininogen 1 isoform 3	391	2.67	0.58	7.67	2.08	13.67	5.51	24.33	2.08
19	No	IPI00789376.1	KNG1 protein	291	2.67	1.15	7.00	1.00	12.00	5.20	20.00	1.73
19	No	IPI00797833.4	21 kDa protein	189	0.33	0.58	1.67	1.15	3.67	1.53	5.67	1.53
20	**Yes**	**IPI00021841.1**	**Apolipoprotein A-I**	**267**	**12.33**	**2.52**	**28.67**	**7.37**				
20	No	IPI00853525.1	Apolipoprotein A1	245	12.33	2.52	28.67	7.37				
21	**Yes**	**IPI00032328.2**	**Isoform HMW of Kininogen-1**	**644**	**1.00**	**1.00**	**7.67**	**2.89**	**13.00**	**4.58**	**17.67**	**3.51**
21	No	IPI00797175.1	20 kDa protein	175	0.33	0.58	1.33	1.53	1.00	1.00	0.33	0.58
22	**Yes**	**IPI00305461.3**	**Inter-alpha-trypsin inhibitor heavy chain H2**	**946**	**0.33**	**0.58**	**7.00**	**4.36**	**16.00**	**4.36**	**24.33**	**6.03**
22	No	IPI00645038.1	Inter-alpha (Globulin) inhibitor H2	935	0.33	0.58	7.00	4.36	16.00	4.36	24.33	6.03
22	No	IPI00910636.1	cDNA FLJ53848, highly similar to Inter-alpha-trypsin inhibitor heavy chain H2	543			5.00	2.65	8.67	4.04	14.00	3.61
22	No	IPI00514159.1	Inter-alpha (Globulin) inhibitor H2	237	0.33	0.58	2.00	1.73	7.33	1.15	10.33	4.73
23	**Yes**	**IPI00896419.3**	**Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H4**	**930**	**1.00**	**1.00**	**3.33**	**1.15**	**10.33**	**5.86**	**18.00**	**4.58**
24	**Yes**	**IPI00026314.1**	**Isoform 1 of Gelsolin**	**782**	**0.33**	**0.58**	**1.00**	**1.00**	**7.33**	**5.51**	**14.00**	**3.61**
24	No	IPI00646773.2	Isoform 2 of Gelsolin	731	0.33	0.58	1.00	1.00	7.33	5.51	14.00	3.61
24	No	IPI00647556.2	gelsolin isoform c	742	0.33	0.58	1.00	1.00	7.33	5.51	14.00	3.61
24	No	IPI00796316.4	cDNA FLJ53327, highly similar to Gelsolin	705	0.33	0.58	1.00	1.00	7.33	5.51	14.00	3.61
25	**Yes**	**IPI00022431.2**	**cDNA FLJ55606, highly similar to Alpha-2-HS-glycoprotein**	**433**	**2.00**	**1.00**	**8.67**	**2.89**	**16.67**	**4.62**	**19.33**	**3.79**
25	No	IPI00953689.1	Alpha-2-HS-glycoprotein	367	2.00	1.00	8.67	2.89	16.67	4.62	19.33	3.79
25	No	IPI00795830.1	29 kDa protein	261			4.67	2.08	11.00	3.46	14.67	3.21
26	**Yes**	**IPI00553177.1**	**Isoform 1 of Alpha-1-antitrypsin**	**418**	**2.33**	**1.53**	**21.67**	**7.37**				
26	No	IPI00790784.2	Isoform 2 of Alpha-1-antitrypsin	359	2.00	1.00	16.33	4.16				

26	No	IPI00869004.1	Isoform 3 of Alpha-1-antitrypsin	306			11.00	2.00				
26	No	IPI00305457.5	PRO2275	120	2.00	1.73	9.00	4.36				
27	**Yes**	**IPI00019568.1**	**Prothrombin (Fragment)**	**622**	**1.00**	**0.00**	**3.67**	**2.08**	**10.67**	**2.89**	**16.33**	**4.04**
27	No	IPI00877967.1	Putative uncharacterized protein F2	324	1.00	0.00	2.00	1.00	3.00	0.00	6.00	2.00
28	**Yes**	**IPI00292530.1**	**Inter-alpha-trypsin inhibitor heavy chain H1**	**911**			**4.00**	**2.65**	**8.67**	**3.06**	**15.00**	**7.00**
28	No	IPI00953675.1	inter-alpha (globulin) inhibitor H1 isoform b	769			2.33	1.15	5.00	2.00	11.33	6.03
28	No	IPI00877852.2	inter-alpha (globulin) inhibitor H1 isoform c	623			2.00	1.00	5.00	2.00	11.00	5.57
28	No	IPI00383338.1	PRO2769	494			2.00	1.00	4.67	1.53	10.33	5.03
28	No	IPI00945924.1	Putative uncharacterized protein ITIH1	213			1.67	1.53	3.67	1.15	3.67	1.15
29	**Yes**	**IPI00414283.6**	**Isoform 9 of Fibronectin**	**2240**	**1.00**	**1.00**	**3.33**	**1.53**	**8.33**	**1.53**	**14.67**	**8.02**
29	No	IPI00845263.1	fibronectin 1 isoform 2 preproprotein	2421	1.00	1.00	3.33	1.53	8.33	1.53	14.67	8.02
30	**Yes**	**IPI00876888.1**	**cDNA FLJ78387**	**472**	**8.00**	**1.73**	**23.00**	**7.21**				
31	**Yes**	**IPI00794184.1**	**cDNA FLJ37971 fis, clone CTONG2009958, highly similar to CERULOPLASMIN**	**846**			**3.00**	**4.36**	**12.33**	**4.62**	**17.00**	**3.00**
31	No	IPI00946417.1	Putative uncharacterized protein CP	852			3.00	4.36	12.33	4.62	17.00	3.00
32	**Yes**	**IPI00478003.2**	**Alpha-2-macroglobulin**	**1474**	**3.33**	**2.08**	**15.67**	**7.64**				
33	**Yes**	**IPI00384938.1**	**Putative uncharacterized protein DKFZp686N02209**	**482**	**8.00**	**1.73**	**18.67**	**7.37**				
34	**Yes**	**IPI00550731.2**	**Putative uncharacterized protein**	**239**	**4.00**	**3.00**	**14.33**	**2.08**				
34	No	IPI00939470.1	26 kDa protein	240	4.00	3.00	13.67	1.53				
35	**Yes**	**IPI00940069.1**	**Ig kappa chain C region**	**234**	**4.33**	**3.51**	**14.00**	**2.00**				
36	**Yes**	**IPI00386879.1**	**cDNA FLJ14473 fis, clone MAMMA1001080, highly similar to Homo sapiens SNC73 protein (SNC73) mRNA**	**494**	**3.33**	**1.53**	**17.67**	**8.02**				
36	No	IPI00426060.3	Putative uncharacterized protein DKFZp686J11235 (Fragment)	506	3.33	1.53	15.67	8.02				
36	No	IPI00449920.1	cDNA FLJ90170 fis, clone MAMMA1000370, highly similar to Ig alpha-1 chain C region	493	3.33	1.53	15.67	8.02				
37	**Yes**	**IPI00022488.1**	**Hemopexin**	**462**			**7.33**	**1.53**	**18.33**	**6.51**	**23.33**	**6.03**
38	**Yes**	**IPI00894122.1**	**Putative uncharacterized protein APOB**	**828**			**1.00**	**1.00**	**7.67**	**2.31**	**8.00**	**4.58**
39	**Yes**	**IPI00291262.3**	**Isoform 1 of Clusterin**	**449**	**0.33**	**0.58**	**3.33**	**2.31**	**11.33**	**3.21**	**11.00**	**3.00**
39	No	IPI00400826.1	Isoform 2 of Clusterin	501	0.33	0.58	3.33	2.31	11.33	3.21	11.00	3.00
39	No	IPI00795633.1	CLU	448	0.33	0.58	3.33	2.31	11.33	3.21	11.00	3.00
39	No	IPI00793848.1	54 kDa protein	466	0.33	0.58	2.00	2.00	7.00	2.65	8.00	2.65
40	**Yes**	**IPI00032179.3**	**Antithrombin-III**	**464**	**0.67**	**0.58**	**2.67**	**0.58**	**12.00**	**7.21**	**19.67**	**2.08**
40	No	IPI00844156.2	SERPINC1 protein	259	0.67	0.58	2.33	0.58	10.00	6.56	14.33	2.89
41	**Yes**	**IPI00298828.3**	**Beta-2-glycoprotein 1**	**345**	**2.00**	**1.00**	**3.67**	**2.52**	**8.33**	**5.13**	**16.67**	**4.16**
41	No	IPI00910625.1	cDNA FLJ51265, moderately similar to Beta-2-glycoprotein 1	274	2.00	1.00	3.67	2.52	8.33	5.13	16.33	4.04

42	**Yes**	**IPI00399007.7**	**Putative uncharacterized protein DKFZp686I04196 (Fragment)**	**417**	**5.33**	**0.58**	**16.33**	**4.16**				
42	No	IPI00930072.1	Putative uncharacterized protein DKFZp686E23209	470	4.00	0.00	12.67	0.58				
43	**Yes**	**IPI00426051.3**	**Putative uncharacterized protein DKFZp686C15213**	**464**	**4.67**	**0.58**	**17.33**	**4.16**				
44	**Yes**	**IPI00298497.3**	**Fibrinogen beta chain**	**491**	**3.00**	**1.00**	**16.00**	**6.24**	**4.00**	**1.00**	**1.33**	**1.53**
44	No	IPI00816687.1	FGB protein (Fragment)	173	1.00	1.00	5.33	0.58	1.67	0.58	0.33	0.58
45	**Yes**	**IPI00944677.1**	**IGL@ protein**	**233**	**3.67**	**1.15**	**9.00**	**3.00**				
46	**Yes**	**IPI00887169.2**	**Putative uncharacterized protein**	**236**	**3.67**	**0.58**	**9.33**	**2.31**				
47	**Yes**	**IPI00658130.1**	**IGL@ protein**	**236**	**4.00**	**1.00**	**10.00**	**1.73**				
47	No	IPI00784935.1	IGL@ protein	234	4.00	1.00	10.00	1.73				
48	**Yes**	**IPI00219713.1**	**Isoform Gamma-A of Fibrinogen gamma chain**	**437**	**0.67**	**0.58**	**11.67**	**3.79**	**3.33**	**1.53**		
48	No	IPI00877792.1	Putative uncharacterized protein FGG	445	0.67	0.58	11.67	3.79	3.33	1.53		
48	No	IPI00021891.5	Isoform Gamma-B of Fibrinogen gamma chain	453	0.67	0.58	10.67	3.79	2.67	1.15		
48	No	IPI00877703.1	Putative uncharacterized protein FGG	461	0.67	0.58	10.67	3.79	2.67	1.15		
49	**Yes**	**IPI00298971.1**	**Vitronectin**	**478**	**2.00**	**1.00**	**2.67**	**0.58**	**10.00**	**3.61**	**11.67**	**2.52**
50	**Yes**	**IPI00879709.3**	**Complement component 6 precursor**	**943**			**0.33**	**0.58**	**2.33**	**2.52**	**3.67**	**2.31**
51	**Yes**	**IPI00941961.1**	**66 kDa protein**	**599**	**3.67**	**1.53**	**8.33**	**1.53**				
51	No	IPI00952640.1	Isoform 1 of Ig mu chain C region	452	3.67	1.53	8.33	1.53				
51	No	IPI00479708.6	Full-length cDNA clone CS0DD006YL02 of Neuroblastoma of Homo sapiens	375	3.67	1.53	7.67	1.53				
51	No	IPI00385264.1	Ig mu heavy chain disease protein	391	1.67	0.58	5.00	1.00				
52	**Yes**	**IPI00022420.3**	**Retinol-binding protein 4**	**201**	**1.00**	**1.00**	**4.67**	**1.53**	**6.33**	**2.52**	**14.00**	**3.61**
52	No	IPI00480192.1	Retinol binding protein 4, plasma	199	1.00	1.00	4.67	1.53	6.33	2.52	14.00	3.61
52	No	IPI00844536.2	Retinol binding protein 4, plasma, isoform CRA_b	199	1.00	1.00	4.67	1.53	6.33	2.52	14.00	3.61
53	**Yes**	**IPI00939270.1**	**25 kDa protein**	**233**	**4.33**	**0.58**	**6.67**	**3.06**				
53	No	IPI00941543.1	25 kDa protein	232	4.33	0.58	6.67	3.06				
54	**Yes**	**IPI00930442.1**	**Putative uncharacterized protein DKFZp686M24218**	**476**	**3.67**	**0.58**	**10.33**	**3.21**				
55	**Yes**	**IPI00829640.1**	**IGL@ protein**	**233**	**3.67**	**0.58**	**7.00**	**3.00**				
56	**Yes**	**IPI00829877.1**	**IGL@ protein**	**232**	**3.67**	**0.58**	**9.00**	**1.00**				
57	**Yes**	**IPI00019943.1**	**Afamin**	**599**					**3.67**	**2.31**	**14.33**	**3.21**
58	**Yes**	**IPI00550991.3**	**cDNA FLJ35730 fis, clone TESTI2003131, highly similar to ALPHA-1-ANTICHYMOTRYPSIN**	**448**	**0.67**	**0.58**	**1.00**	**0.00**	**3.67**	**2.08**	**5.00**	**1.00**
58	No	IPI00847635.1	Isoform 1 of Alpha-1-antichymotrypsin	423	0.67	0.58	1.00	0.00	3.67	2.08	5.00	1.00
59	**Yes**	**IPI00291867.3**	**Complement factor I**	**583**			**0.33**	**0.58**	**4.67**	**2.52**	**10.67**	**1.15**
59	No	IPI00935408.1	CFI protein	377			0.33	0.58	4.67	2.52	9.00	2.00
59	No	IPI00796990.3	cDNA FLJ58124, highly similar to Complement factor I	591			0.33	0.58	4.33	2.08	9.00	1.00
60	**Yes**	**IPI00220327.4**	**Keratin, type II cytoskeletal 1**	**644**			**0.67**	**0.58**				
61	**Yes**	**IPI00163207.1**	**Isoform 1 of N-acetylmuramoyl-L-alanine amidase**	**576**			**1.33**	**1.15**	**4.33**	**1.53**	**6.33**	**3.79**

61	No	IPI00394992.1	Isoform 2 of N-acetylmuramoyl-L-alanine amidase	634			1.33	1.15	4.33	1.53	6.33	3.79
62	**Yes**	**IPI00011264.2**	**Complement factor H-related protein 1**	**330**			**1.67**	**1.53**	**4.67**	**2.52**	**7.00**	**5.29**
62	No	IPI00883722.1	Complement factor H-related 1	271			0.33	0.58	3.00	1.73	3.33	2.89
65	**Yes**	**IPI00011252.1**	**Complement component C8 alpha chain**	**584**					**2.00**	**1.00**	**6.33**	**2.08**
66	**Yes**	**IPI00022426.1**	**Protein AMBP**	**352**	**0.33**	**0.58**	**1.67**	**1.15**	**8.33**	**2.08**	**10.67**	**2.08**
66	No	IPI00922298.1	cDNA FLJ51445, highly similar to AMBP protein	270					2.00	0.00	2.00	1.00
67	**Yes**	**IPI00006114.4**	**Pigment epithelium-derived factor**	**418**					**3.00**	**1.73**	**3.67**	**2.08**
67	No	IPI00796279.1	25 kDa protein	216					1.00	0.00	2.00	0.00
68	**Yes**	**IPI00028413.8**	**Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H3**	**890**					**2.33**	**4.04**	**2.33**	**2.52**
68	No	IPI00873416.2	Putative uncharacterized protein ITIH3	887					2.33	4.04	2.33	2.52
68	No	IPI00876950.1	Isoform 2 of Inter-alpha-trypsin inhibitor heavy chain H3	886					2.33	4.04	2.33	2.52
68	No	IPI00953573.1	Putative uncharacterized protein ITIH3	885					2.33	4.04	2.33	2.52
69	**Yes**	**IPI00022395.1**	**Complement component C9**	**559**					**3.67**	**0.58**	**5.33**	**1.53**
71	**Yes**	**IPI00032291.2**	**Complement C5**	**1676**			**0.33**	**0.58**	**4.00**	**1.00**	**7.00**	**2.65**
71	No	IPI00816741.1	Complement component 5 variant (Fragment)	1106			0.33	0.58	3.33	0.58	5.67	2.08
72	**Yes**	**IPI00830047.1**	**Putative uncharacterized protein ENSP00000374858 (Fragment)**	**106**	**2.00**	**0.00**	**4.33**	**0.58**				
73	**Yes**	**IPI00017696.1**	**Complement C1s subcomponent**	**688**			**0.33**	**0.58**	**4.00**	**2.65**	**7.33**	**2.08**
73	No	IPI00749179.2	Putative uncharacterized protein C1S	682			0.33	0.58	4.00	2.65	7.33	2.08
73	No	IPI00790679.1	21 kDa protein	186					0.67	1.15	2.00	1.00
76	**Yes**	**IPI00654888.4**	**Plasma kallikrein**	**638**			**1.33**	**0.58**	**1.00**	**1.00**	**4.33**	**0.58**
76	No	IPI00879984.6	cDNA FLJ51250, highly similar to Plasma kallikrein	514			0.67	0.58	0.67	0.58	3.67	1.15
79	**Yes**	**IPI00031101.1**	**Natriuretic peptides B**	**134**							**2.33**	**2.08**
80	**Yes**	**IPI00218732.3**	**Serum paraoxonase/arylesterase 1**	**355**					**2.33**	**0.58**	**2.67**	**1.15**
80	No	IPI00916980.1	Putative uncharacterized protein PON1	77					2.00	1.00	1.67	1.53
81	**Yes**	**IPI00020986.2**	**Lumican**	**338**			**1.00**	**0.00**	**5.00**	**2.00**	**5.67**	**0.58**
81	No	IPI00794403.1	23 kDa protein	202			1.00	0.00	4.67	1.53	4.33	1.53
82	**Yes**	**IPI00022417.4**	**Leucine-rich alpha-2-glycoprotein**	**347**					**3.67**	**0.58**	**1.33**	**1.15**
83	**Yes**	**IPI00019581.1**	**Coagulation factor XII**	**615**					**3.67**	**1.53**	**5.00**	**0.00**
84	**Yes**	**IPI00021842.1**	**Apolipoprotein E**	**317**	**0.33**	**0.58**	**4.33**	**2.08**	**1.00**	**0.00**	**2.00**	**1.73**
84	No	IPI00878953.1	MRNA for apolipoprotein E	288			3.00	1.00	1.00	0.00	0.67	0.58
84	No	IPI00879368.1	Apolipoprotein E	216			3.00	1.00	0.67	0.58	0.67	0.58
84	No	IPI00879456.1	25 kDa protein	219			3.00	1.00	0.67	0.58	0.67	0.58
85	**Yes**	**IPI00292950.4**	**Serpin peptidase inhibitor, clade D (Heparin cofactor), member 1**	**527**			**0.67**	**1.15**	**3.00**	**1.00**	**4.33**	**0.58**
85	No	IPI00879573.1	Heparin cofactor 2	499			0.67	1.15	3.00	1.00	4.33	0.58
86	**Yes**	**IPI00032220.3**	**Angiotensinogen**	**485**			**0.67**	**0.58**	**1.33**	**0.58**	**3.67**	**2.08**

86	No	IPI00908365.1	cDNA FLJ52255, highly similar to Angiotensinogen	403			0.67	0.58	1.33	0.58	3.33	2.31
87	**Yes**	**IPI00296608.6**	**Complement component C7**	**843**					**0.67**	**0.58**	**3.00**	**2.00**
87	No	IPI00909594.1	cDNA FLJ58413, highly similar to Complement component C7	486							1.00	0.00
88	**Yes**	**IPI00022371.1**	**Histidine-rich glycoprotein**	**525**			**3.33**	**1.53**	**4.67**	**4.04**		
90	**Yes**	**IPI00006154.1**	**Isoform Long of Complement factor H-related protein 2**	**270**					**0.67**	**0.58**	**3.67**	**2.31**
90	No	IPI00218949.1	Isoform Short of Complement factor H-related protein 2	243					0.67	0.58	3.67	2.31
91	**Yes**	**IPI00387118.1**	**Ig kappa chain V-III region WOL**	**109**	**1.00**	**1.00**	**4.33**	**0.58**				
91	No	IPI00030205.3	Ig kappa chain V-III region HAH	129	1.00	1.00	3.00	1.00				
93	**Yes**	**IPI00021727.1**	**C4b-binding protein alpha chain**	**597**			**2.33**	**0.58**	**1.33**	**1.53**		
93	No	IPI00872510.1	Putative uncharacterized protein C4BPA	408			1.33	1.15	0.67	0.58		
94	**Yes**	**IPI00021854.1**	**Apolipoprotein A-II**	**100**	**2.67**	**1.15**	**7.00**	**2.65**	**0.67**	**0.58**	**0.33**	**0.58**
95	**Yes**	**IPI00387115.1**	**Ig kappa chain V-III region SIE**	**109**			**2.00**	**1.00**				
96	**Yes**	**IPI00166729.4**	**alpha-2-glycoprotein 1, zinc precursor**	**298**					**1.00**	**1.00**		
96	No	IPI00924948.1	Putative uncharacterized protein AZGP1	227					1.00	1.00		
97	**Yes**	**IPI00021364.1**	**Properdin**	**469**					**2.33**	**0.58**	**1.67**	**1.53**
97	No	IPI00946489.1	Complement factor properdin, isoform CRA_c	415					2.33	0.58	1.67	1.53
97	No	IPI00946166.1	Putative uncharacterized protein CFP	167					1.67	0.58	1.00	1.00
98	**Yes**	**IPI00303963.1**	**Complement C2 (Fragment)**	**752**			**0.33**	**0.58**	**2.67**	**0.58**	**2.67**	**1.53**
99	**Yes**	**IPI00003351.2**	**Isoform 1 of Extracellular matrix protein 1**	**540**					**0.67**	**1.15**	**2.00**	**1.73**
99	No	IPI00645849.1	Isoform 4 of Extracellular matrix protein 1	567					0.67	1.15	2.00	1.73
100	**Yes**	**IPI00294395.1**	**Complement component C8 beta chain**	**591**					**0.33**	**0.58**	**2.67**	**1.15**
101	**Yes**	**IPI00178926.2**	**Immunoglobulin J chain**	**159**	**1.00**	**1.00**	**3.00**	**1.00**				
101	No	IPI00947235.1	Putative uncharacterized protein IGJ	70	1.00	1.00	2.00	0.00				
102	**Yes**	**IPI00296165.6**	**cDNA FLJ54471, highly similar to Complement C1r subcomponent**	**719**					**2.33**	**1.15**	**1.33**	**0.58**
102	No	IPI00923551.1	cDNA FLJ54318, highly similar to Complement C1r subcomponent	599					2.33	1.15	1.33	0.58
102	No	IPI00941440.1	Putative uncharacterized protein C1R (Fragment)	279					1.67	0.58	1.00	0.00
103	**Yes**	**IPI00022429.3**	**Alpha-1-acid glycoprotein 1**	**201**	**0.67**	**0.58**	**7.33**	**1.15**				
103	No	IPI00884926.1	orosomucoid 1 precursor	201	0.67	0.58	7.33	1.15				
104	**Yes**	**IPI00011261.2**	**Complement component C8 gamma chain**	**202**					**1.00**	**0.00**	**1.33**	**0.58**
105	**Yes**	**IPI00006662.1**	**Apolipoprotein D**	**189**			**1.00**	**1.00**				
105	No	IPI00924574.1	Putative uncharacterized protein APOD	215			1.00	1.00				
106	**Yes**	**IPI00029863.4**	**55 kDa protein**	**495**					**1.00**	**1.00**	**1.33**	**1.53**
106	No	IPI00879231.1	Alpha-2-antiplasmin	491					1.00	1.00	1.33	1.53

106	No	IPI00879608.2	alpha-2-antiplasmin isoform b precursor	427							1.33	1.53
107	**Yes**	**IPI00021857.1**	**Apolipoprotein C-III**	**99**	**1.33**	**0.58**	**3.00**	**1.00**	**2.00**	**1.00**	**1.33**	**1.53**
107	No	IPI00657670.1	Apolipoprotein C-III variant 1	117	1.33	0.58	3.00	1.00	2.00	1.00	1.33	1.53
108	**Yes**	**IPI00294004.1**	**Vitamin K-dependent protein S**	**676**			**1.33**	**1.15**	**0.67**	**1.15**	**0.67**	**1.15**
108	No	IPI00878131.1	cDNA FLJ56936, highly similar to Vitamin K-dependent protein S	545			1.00	1.00	0.33	0.58	0.67	1.15
108	No	IPI00795619.1	PROS1 protein	367			0.33	0.58	0.33	0.58	0.33	0.58
109	**Yes**	**IPI00296534.2**	**Isoform D of Fibulin-1**	**703**							**2.00**	**0.00**
110	**Yes**	**IPI00025204.1**	**CD5 antigen-like**	**347**	**0.33**	**0.58**	**2.00**	**2.00**				
111	**Yes**	**IPI00746623.2**	**Hyaluronan-binding protein 2**	**560**					**1.00**	**1.00**	**1.67**	**1.53**
112	**Yes**	**IPI00019576.1**	**Coagulation factor X**	**488**					**0.33**	**0.58**	**1.00**	**1.73**
113	**Yes**	**IPI00387120.1**	**Ig kappa chain V-IV region Len**	**114**			**1.00**	**1.00**				
114	**Yes**	**IPI00736885.1**	**Ig kappa chain V-II region TEW**	**113**			**0.67**	**1.15**				
114	No	IPI00827773.1	Cold agglutinin FS-1 L-chain (Fragment)	113			0.67	1.15				
116	**Yes**	**IPI00296537.4**	**Isoform C of Fibulin-1**	**683**							**1.67**	**0.58**
116	No	IPI00889740.1	Fibulin 1	721							1.67	0.58
117	**Yes**	**IPI00018305.4**	**Insulin-like growth factor-binding protein 3**	**291**							**2.33**	**1.15**
117	No	IPI00556155.2	insulin-like growth factor binding protein 3 isoform a precursor	297							2.33	1.15
117	No	IPI00903293.2	cDNA FLJ52568, highly similar to Insulin-like growth factor-binding protein 3	276							2.33	1.15
118	**Yes**	**IPI00890703.1**	**Cryocrystalglobulin CC1 kappa light chain variable region (Fragment)**	**107**			**1.00**	**0.00**				
120	**Yes**	**IPI00186903.4**	**Isoform 2 of Apolipoprotein L1**	**414**			**1.67**	**0.58**				
120	No	IPI00514475.5	Isoform 1 of Apolipoprotein L1	398			1.67	0.58				
120	No	IPI00914948.1	apolipoprotein L1 isoform c precursor	380			1.67	0.58				
121	**Yes**	**IPI00748998.1**	**Single-chain Fv (Fragment)**	**240**			**1.33**	**1.53**				
122	**Yes**	**IPI00477992.1**	**complement component 1, q subcomponent, B chain precursor**	**253**			**0.33**	**0.58**	**1.33**	**1.15**	**2.00**	**1.00**
122	No	IPI00643948.2	Complement component 1, q subcomponent, B chain	231			0.33	0.58	1.33	1.15	2.00	1.00
123	**Yes**	**IPI00022391.1**	**Serum amyloid P-component**	**223**					**0.33**	**0.58**	**1.00**	**0.00**
124	**Yes**	**IPI00382436.1**	**Ig lambda chain V-III region SH**	**108**	**0.33**	**0.58**	**0.67**	**0.58**				
125	**Yes**	**IPI00022394.2**	**Complement C1q subcomponent subunit C**	**245**					**2.33**	**0.58**	**2.00**	**1.00**
126	**Yes**	**IPI00410714.5**	**Hemoglobin subunit alpha**	**142**			**0.67**	**0.58**				
127	**Yes**	**IPI00783024.1**	**Myosin-reactive immunoglobulin heavy chain variable region (Fragment)**	**131**			**2.33**	**0.58**				
128	**Yes**	**IPI00019399.1**	**Serum amyloid A-4 protein**	**130**	**0.33**	**0.58**	**2.00**	**0.00**				
129	**Yes**	**IPI00915820.1**	**Anti-folate binding protein (Fragment)**	**120**			**2.00**	**0.00**				
130	**Yes**	**IPI00009028.1**	**Tetranectin**	**202**					**1.67**	**0.58**	**2.33**	**0.58**

130	No	IPI00792115.1	Putative uncharacterized protein DKFZp686H17246	160					1.67	0.58	2.33	0.58
131	**Yes**	**IPI00218413.2**	**Biotinidase**	**543**					**0.67**	**0.58**	**1.33**	**0.58**
131	No	IPI00744685.3	Putative uncharacterized protein BTD	545					0.67	0.58	1.33	0.58
131	No	IPI00927683.1	cDNA FLJ51892, highly similar to Biotinidase	545					0.67	0.58	1.33	0.58
132	**Yes**	**IPI00291866.5**	**Plasma protease C1 inhibitor**	**500**	**0.33**	**0.58**	**0.67**	**0.58**	**0.33**	**0.58**	**0.33**	**0.58**
132	No	IPI00879931.1	cDNA FLJ58826, highly similar to Plasma protease C1 inhibitor	543	0.33	0.58	0.67	0.58	0.33	0.58	0.33	0.58
133	**Yes**	**IPI00020091.1**	**Alpha-1-acid glycoprotein 2**	**201**			**1.67**	**1.15**				
134	**Yes**	**IPI00299503.2**	**Isoform 1 of Phosphatidylinositol-glycan-specific phospholipase D**	**840**							**0.67**	**0.58**
135	**Yes**	**IPI00384401.1**	**Myosin-reactive immunoglobulin kappa chain variable region (Fragment)**	**109**	**0.33**	**0.58**	**1.00**	**1.00**				
136	**Yes**	**IPI00816799.1**	**Rheumatoid factor D5 light chain (Fragment)**	**118**			**1.00**	**0.00**				
137	**Yes**	**IPI00029168.1**	**Apolipoprotein(a)**	**4548**							**0.33**	**0.58**
138	**Yes**	**IPI00024825.2**	**Isoform A of Proteoglycan 4**	**1404**							**0.33**	**0.58**
138	No	IPI00655976.1	Isoform C of Proteoglycan 4	1311							0.33	0.58
138	No	IPI00656092.1	Isoform F of Proteoglycan 4	1361							0.33	0.58
139	**Yes**	**IPI00010295.1**	**Carboxypeptidase N catalytic chain**	**458**					**0.33**	**0.58**	**0.33**	**0.58**
140	**Yes**	**IPI00030739.1**	**Apolipoprotein M**	**188**			**1.00**	**0.00**				
140	No	IPI00645213.1	Apolipoprotein M, isoform CRA_a	116			1.00	0.00				
141	**Yes**	**IPI00479116.1**	**Carboxypeptidase N subunit 2**	**545**					**0.67**	**0.58**		
142	**Yes**	**IPI00382440.1**	**Ig lambda chain V-IV region Hil**	**107**	**0.67**	**0.58**	**1.00**	**0.00**				
144	**Yes**	**IPI00387116.1**	**Ig kappa chain V-III region NG9 (Fragment)**	**100**			**0.33**	**0.58**				
145	**Yes**	**IPI00004798.4**	**cDNA FLJ75207**	**258**							**0.33**	**0.58**
145	No	IPI00942117.1	Cysteine-rich secretory protein 3	245							0.33	0.58
147	**Yes**	**IPI00007199.4**	**Protein Z-dependent protease inhibitor**	**484**							**0.33**	**0.58**
148	**Yes**	**IPI00473011.3**	**Hemoglobin subunit delta**	**147**			**0.33**	**0.58**				
148	No	IPI00654755.3	Hemoglobin subunit beta	147			0.33	0.58				
149	**Yes**	**IPI00022392.1**	**Complement C1q subcomponent subunit A**	**245**							**0.33**	**0.58**
151	**Yes**	**IPI00854644.2**	**similar to hCG1686089**	**152**			**1.00**	**0.00**				
152	**Yes**	**IPI00013933.2**	**Isoform DPI of Desmoplakin**	**2871**			**0.33**	**0.58**	**0.33**	**0.58**		
153	**Yes**	**IPI00021856.3**	**Apolipoprotein C-II**	**101**			**0.33**	**0.58**				