

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
22 July 2004 (22.07.2004)

PCT

(10) International Publication Number
WO 2004/061410 A2

(51) International Patent Classification⁷: **G01N**
(21) International Application Number:
PCT/US2003/037090
(22) International Filing Date:
16 December 2003 (16.12.2003)
(25) Filing Language: English
(26) Publication Language: English
(30) Priority Data:
60/434,075 18 December 2002 (18.12.2002) US

(71) Applicants (for all designated States except US): **CIPHERGEN BIOSYSTEMS, INC.** [US/US]; 6611 Dumbarton Circle, Fremont, CA 94555 (US). **QUEEN ELIZABETH HOSPITAL** [CN/CN]; 30 Gascoigne Road, Kowloon, Hong Kong SAR (CN).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **YIP, Timothy, Tak, Chun** [CN/CN]; 1, Kapok Path, Westwood, Palm Springs, Yuen Long N.T., Hong Kong SAR (CN). **CHO, Chi, Shing**

[—/CN]; Flat 9E, Block 4, The Tolo Place, Sunshine City, Ma On Shan, N.T., Hong Kong SAR (CN). **AU, Siu, Kie** [—/CN]; c/o Department of Clinical Oncology, Queen Elizabeth Hospital, 30 Gascoigne Road, Kowloon, Hong Kong SAR (CN). **YIP, Tai-Tung** [US/US]; 1532 Aster Court, Cupertino, CA 95014 (US). **YIP, Christine, L.** [US/US]; 1532 Aster Court, Cupertino, CA 95014 (US). **YIP, Victor, F.** [US/US]; 33008 Compton CT, Union City, CA 94587 (US).

(74) Agents: **BENT, Stephen, A.** et al.; Foley & Lardner, Washington Harbour, 3000 K Street, N.W. Suite 500, Washington, DC 20007-5101 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),

[Continued on next page]

(54) Title: SERUM BIOMARKERS IN LUNG CANCER

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-1	2011	A	IM-37	3893	A	IM-72	54026	A	IM-109	2882	B
IM-2	2030	A	IM-38	3960	A	IM-73	60170	A	IM-110	2987	B
IM-3	2069	A	IM-39	3972	A	IM-75	74372	A	IM-111	2977	B
IM-4	2128	A	IM-40	3984	A	IM-76	75545	A	IM-112	2994	B
IM-5	2146	A	IM-41	4066	A	IM-77	77543	A	IM-113	3031	B
IM-6	2186	A	IM-42	4178	A	IM-78	79507	A	IM-114	3048	B
IM-7	2232	A	IM-43	4287	A	IM-79	89854	A	IM-115	3148	B
IM-8	2277	A	IM-44	4297	A	IM-80	101831	A	IM-116	3166	B
IM-9	2295	A	IM-45	4309	A	IM-81	104301	A	IM-117	3283	B
IM-10	2318	A	IM-46	4484	A	IM-82	125160	A	IM-118	3308	B
IM-11	2411	A	IM-47	4649	A	IM-83	132976	A	IM-119	3332	B
IM-12	2434	A	IM-48	4798	A	IM-84	149099	A	IM-120	3432	B
IM-13	2467	A	IM-49	5104	A	IM-85	2016	B	IM-121	3450	B
IM-14	2482	A	IM-50	5918	A	IM-86	2029	B	IM-122	3561	B
IM-15	2498	A	IM-51	6122	A	IM-87	2144	B	IM-123	3615	B
IM-16	2565	A	IM-52	6192	A	IM-88	2130	B	IM-124	3714	B
IM-17	2574	A	IM-53	6452	A	IM-89	2168	B	IM-125	3730	B
IM-18	2586	A	IM-54	6660	A	IM-90	2184	B	IM-126	3834	B
IM-19	2605	A	IM-55	7766	A	IM-91	2200	B	IM-127	3899	B
IM-20	2722	A	IM-56	8145	A	IM-92	2284	B	IM-128	3969	B
IM-21	2746	A	IM-57	8954	A	IM-93	2299	B	IM-129	3966	B
IM-22	2788	A	IM-58	9312	A	IM-94	2314	B	IM-130	3997	B
IM-23	2855	A	IM-59	9449	A	IM-95	2414	B	IM-131	4013	B
IM-24	2871	A	IM-60	10272	A	IM-96	2428	B	IM-132	4181	B
IM-25	2984	A	IM-61	11663	A	IM-97	2451	B	IM-133	4297	B
IM-26	3030	A	IM-62	13376	A	IM-98	2466	B	IM-134	4311	B
IM-27	3144	A	IM-63	14698	A	IM-99	2483	B	IM-135	4465	B
IM-28	3243	A	IM-64	15190	A	IM-100	2565	B	IM-136	4484	B
IM-29	3273	A	IM-64	66758	A	IM-101	2583	B	IM-137	4579	B
IM-30	3290	A	IM-65	15951	A	IM-102	2597	B	IM-138	4608	B
IM-31	3369	A	IM-66	15172	A	IM-103	2697	B	IM-139	4669	B
IM-32	3445	A	IM-67	15925	A	IM-104	2715	B	IM-140	4747	B
IM-33	3483	A	IM-68	23436	A	IM-105	2740	B	IM-141	4862	B
IM-34	3676	A	IM-69	39794	A	IM-106	2752	B	IM-142	4891	B
IM-35	3779	A	IM-70	44166	A	IM-107	2767	B	IM-143	5033	B
IM-36	3793	A	IM-71	46890	A	IM-108	2865	B	IM-144	5077	B

(57) Abstract: Certain biomarkers and biomarker combinations are useful in a qualifying lung cancer status in a subject. A diagnostic methodology employing these biomarkers and combinations can detect whether a subject has lung cancer.



European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

- *without international search report and to be republished upon receipt of that report*

SERUM BIOMARKERS IN LUNG CANCER

BACKGROUND OF THE INVENTION

[0001] The present invention relates generally to the field of serum biomarkers in lung carcinoma. More particularly, the invention relates to serum biomarkers that can distinguish lung cancer from normal.

[0002] Lung cancer is the leading cause of cancer death worldwide, resulting in 150,000 deaths per year in the United States. The mortality rate from lung cancer is greater than the combined mortality from breast, prostate and colorectal cancers. On the basis of morphology, lung cancer can be broadly classified into four main categories namely, adenocarcinoma, squamous cell carcinoma, large cell undifferentiated carcinoma and small cell carcinoma. In Hong Kong from 1990 to 1996, the proportions for adenocarcinoma, squamous cell carcinoma, large cell undifferentiated carcinoma and small cell carcinoma are 45.5%, 27.5%, 4.7% and 10.3% respectively. Both squamous cell carcinoma and small cell carcinoma are strongly associated with a smoking history.

[0003] Adenocarcinoma, squamous cell carcinoma, and large cell undifferentiated carcinoma are usually referred as "non-small cell carcinoma." They are relatively chemo-resistant, and hence the mainstay of treatment is surgery. By contrast, small cell carcinoma has a higher propensity for distant metastases and is mainly treated by chemotherapy.

[0004] Biopsy can be used to diagnose lung cancer, but it is an invasive procedure and, therefore, less than desirable. Other diagnostic methods for lung cancer include ultrasound and computed tomography (CT) scan.

[0005] It would be highly desirable to have a biomarker or combination of biomarkers capable of distinguishing between lung cancer and normal cells. In addition, a simple test could aid in tracking treatment progress and even identify molecular targets for therapy. The literature on lung cancer diagnosis has not disclosed heretofore such a biomarker or combination of biomarkers, however.

SUMMARY OF THE INVENTION

[0006] In accordance with the present invention, biomarkers and combinations of biomarkers are used to identify lung cancer. The method successfully distinguishes between lung cancer and normal states, and can be used to identify the particular type of lung cancer. In one embodiment, a method for qualifying lung carcinoma status in a subject (e.g., a patient) comprises analyzing a biological sample from the subject for one or more of the top 50 biomarkers as shown in Figure 2 or Figures 4A and 4B. Thus, to assess overall lung cancer risk versus normal, a biomarker is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310,

[0007] wherein the biomarker is differentially present in samples of a subject with lung cancer and a so-called "normal" subject that is free of lung cancer.

[0008] More preferably, one or more of the top 15 biomarkers as shown in Figure 2 or Figures 4A and 4B is used to qualify lung cancer status. Thus, for assessing overall lung cancer status versus normal, the protein is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-471, IM-510, IM-544, IM-474, and IM-155, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70.

[0009] Still more preferably, one or more of the top 5 biomarkers as shown in Figure 2 or Figures 4A and 4B is used to qualify lung cancer status. In this instance, for overall lung cancer status versus normal, the biomarker is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, and IM-454, or

(B) WM-61, WM-447, WM-446, WM-133, and WM-119.

[0010] In one embodiment, the method measures a plurality of biomarkers. The plurality of biomarkers can be measured simultaneously.

[0011] Biomarkers that, by themselves, are able to identify lung cancer include the WM-446 and WM-447 protein biomarkers, and these are particularly preferred.

[0012] The present invention also provides a method for qualifying lung cancer status in a subject (e.g., a patient), comprising (A) providing a spectrum generated by subjecting a biological sample from said subject to mass spectroscopic analysis that includes profiling on a chemically-derivatized affinity surface, and (B) putting the spectrum through pattern-recognition analysis that is keyed to at least one peak selected from the top 50 biomarkers as shown in Figure 2 or Figures 4A and 4B.

Thus, for qualifying overall lung cancer status, the biomarker is selected from the, group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268 or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

[0013] For assessing the overall lung cancer status, the pattern-recognition analysis may, for example, be paired to a pair of peaks selected from the group consisting of (A) IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-266 and IM-522, IM-266 and IM-544, IM-266 and IM-471, IM-474 and IM-151, IM-474 and IM-156, IM-474 and IM-544, IM-474 and IM-38, IM-522 and IM-507, IM-522 and IM-156, and IM-522 and IM-440;

or

(B) WM-447 and WM-59, WM-447 and WM-19, WM-447 and WM-118, WM-447 and WM-473, WM-19 and WM-59, WM-19 and WM-473, WM-19 and WM-369, WM-61 and WM-154, WM-61 and WM-369, WM-118 and WM-59 and WM-282 and WM-127.

[0014] More preferably, for assessing overall lung cancer status, the pattern-recognition analysis is keyed to a pair of peaks selected from the group consisting of (A) IM-266 and IM-474, IM-266 and IM-544, and IM-156 and IM-522;

or

(B) WM-447 and WM-59, WM-447 and WM-19, and WM-19 and WM-59.

[0015] Alternatively, the pattern-recognition analysis for assessing overall lung cancer status may be keyed to a triplet of peaks selected from the group consisting of (A) IM-266, IM-454 and IM-474; and IM-266, IM-474 and IM-544;

or

(B) WM-447, WM-19 and WM-473.

[0016] In other embodiments, the pattern-recognition analysis may be keyed to a combination of more than three peaks, more particularly to a combination of 4, 5 or 6 peaks, where the combination is selected from among the combinations shown in Tables 1 and 2 herein.

[0017] In each case, the biomarker is differentially present in samples of a subject with lung cancer and a normal subject.

[0018] The invention also contemplates a kit for detecting and diagnosing lung cancer, thereby to assess lung cancer status. Kits within the invention comprise, for example, (i) an adsorbent attached to a substrate that retains one or more of the biomarkers shown in Figure 2 or Figures 4A and 4B, and (ii) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent. An inventive kit may further comprise a washing solution and/or instructions for making a washing solution. The kits may include more than type of adsorbent, each present on a different substrate, *e.g.*, on a WCX and IMAC biochip. In addition, the kits may comprise one or more containers with biomarker samples, to be used as standard(s) for calibration. The substrate comprising the adsorbent may be designed to engage a probe interface and, hence, function as a probe in gas phase ion spectrometry, preferably mass spectrometry. Alternatively, the kit may further comprise a second substrate adapted to engage the probe interface, on which the substrate comprising the adsorbent is mounted.

[0019] The method and kit according to the invention produce an article of manufacture in which one or more biomarkers according to the invention are bound to an adsorbent, optionally contacted with a matrix or energy absorbing molecule.

[0020] The present invention also provides software for qualifying lung carcinoma status in a subject, comprising an algorithm for analyzing data extracted from a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, wherein said data relates to one or more biomarkers according to the invention. In one embodiment, the algorithm carries out a pattern-recognition analysis that is keyed to data relating to at least one of the biomarkers. In another embodiment, the algorithm comprises classification tree analysis that is keyed to data relating to at least one of the biomarkers. In yet another embodiment, the algorithm

comprises an artificial neural network analysis that is keyed to data relating to at least one of the biomarkers.

[0021] In certain embodiments, the present invention provides methods and kits that use serum amyloid A protein or a fragment thereof to qualify lung carcinoma status in a subject. In one of these embodiments, the serum amyloid A biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons. In another embodiment, the serum amyloid A biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons. In yet another embodiment, the serum amyloid A biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figures 1A-1D show all biomarkers identified with a Cu(II) IMAC3 ProteinChip® array format.

[0023] Figure 2 shows the top 50 biomarkers identified with a Cu(II) IMAC3 ProteinChip® array format.

[0024] Figures 3A-3O show all biomarkers identified with a WCX ProteinChip® array format.

[0025] Figures 4A and 4B show the top 50 biomarkers identified with a WCX ProteinChip® array format.

[0026] Figure 5 shows fragments of serum amyloid A (SAA) that are biomarkers according to the present invention.

[0027] Figure 6 shows identification of SAA biomarkers with an anti-SAA antibody.

[0028] Figures 7-16 are spectra from WCX chips in which all of the top 15 WCX marker peaks are labeled, along with various other peaks from among the top 50 WCX peaks. Red shows spectra from lung cancer patients and gray shows normals.

[0029] Figures 17-28 are spectra from IMAC chips in which all of the top 15 WCX marker peaks are labeled, along with various other peaks from among the top 50 IMAC peaks. Blue shows spectra from lung cancer patients and gray shows normals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0030] In accordance with the present invention, a series of biomarkers associated with lung cancer has been discovered. In the present context, a biomarker is an organic biomolecule, particularly a polypeptide or protein, which is differentially present in a sample taken from a subject having lung cancer as compared to a comparable sample taken from a normal subject. A biomarker also may be differentially present in a sample taken from a subject with one type of lung cancer, *e.g.*, small cell carcinoma, as compared to a comparable sample taken from a subject with a different type of lung cancer, *e.g.*, adenocarcinoma or squamous cell carcinoma, or differentially present at different stages of a type of lung cancer. A biomarker is differentially present in samples taken from two groups of subjects if it is present at an elevated level or a decreased level in samples of the first group as compared to samples of the second group. More particularly, a biomarker is a polypeptide that is characterized by an apparent molecular weight, as determined by mass spectrometry, and that is present in samples from lung cancer subjects in an elevated or decreased level, as compared to subjects that do not have lung cancer. A biomarker is differentially present between two sets of samples if the amount of the biomarker in one sample set differs in a statistically significant way ($p < 0.01$) from the amount of biomarker in the other sample set.

[0031] The biomarkers of the invention can be used to assess lung cancer status in a subject. For example, they are capable of identifying lung cancer and successfully distinguishing it from normal subjects, thereby providing a way of diagnosing the presence or absence of lung cancer, including the presence or absence of a particular kind of lung cancer. In addition, the biomarkers are useful in assessing the risk of developing lung cancer, in staging of lung cancer and in assessing the effectiveness of treatment. Thus, "lung cancer status" in the context of the present invention includes, *inter alia*, the presence or absence of disease, the risk of developing disease, the stage of the disease, and the effectiveness of treatment of disease. Based on this status, further procedures may be indicated, including additional diagnostic tests or therapeutic procedures or regimens, such as endoscopy, biopsy, surgery, chemotherapy, immunotherapy, and radiation therapy.

[0032] In some instances, a single biomarker is capable of identifying lung cancer with a sensitivity or specificity of at least 85%, whereas, in other instances, a combination or plurality of biomarkers is used to obtain a sensitivity or specificity of at least 85%. The biomarkers and combinations of biomarkers thus can be used to qualify lung cancer status in a subject or patient.

[0033] The biomarkers according to the invention are present in serum. The biological sample used according to the present invention, however, need not be a serum sample. Thus, a biological sample for qualifying lung cancer status may be a serum, plasma or blood sample, although serum samples are preferred.

[0034] All of the biomarkers are characterized by molecular weight. A list of all the biomarkers obtained with the Cu(II) IMAC3 ProteinChip® array (Ciphergen Biosystems, Inc., Fremont, California, USA) is provided in Figures 1A-1D, and Figure 2 lists the top 50 biomarkers that distinguish between lung cancer and normal subjects that are identified by Cu(II) IMAC3 protocol described herein. Figures 3A-3O comprise a list of all the biomarkers obtained with the WCX2 ProteinChip® array, and Figures 4A and 4B comprise a ranking of the top 50 biomarkers that distinguish between (i) lung cancer and normal subjects, (ii) subjects with each of four types of lung cancer and normal subjects, and (iii) two types of lung cancer, *e.g.*, adenocarcinoma versus squamous cell carcinoma, as identified by WCX2 protocol described herein.

[0035] The top 50 biomarkers were determined by decision tree analysis using Biomarker Patterns™ software from Ciphergen Biosystems, Inc. Biomarkers other than those within the top 50 also are useful in distinguishing between subjects with lung cancer and normal subjects and may, in particular, appear in decision trees with multiple nodes. In preferred embodiments, one or more of the top 15 biomarkers are used, and in even more preferred embodiments, one or more of the top 5 biomarkers are used.

[0036] In each of Figures 1A-1D and 3A-3O, the number in the first column is the biomarker identifier. Thus, the first row in Figures 1A-1D relates to biomarker IM-1, the second row relates to biomarker IM-2, and so forth ("IM-" denoting biomarkers identified with the IMAC chip). Similarly, the first row in Figures 3A-3O relates to

biomarker WM-1 and the second row relates to biomarker WM-2 ("WM-" denoting biomarkers identified with the WCX2 chip). The number in the second column in Figures 1A-1D is the apparent molecular weight of the biomarker in daltons, as determined by mass spectrometry. In Figures 3A-3O, the apparent molecular weights for the biomarkers identified in the first column are reported in columns 3 through 11. The letter in the second column of Figures 1A-1D and the third column of Figures 3A-3O denotes the fraction in which the biomarker elutes in the protocol described herein; that is, biomarkers with an "A" elute in the first fraction, biomarkers with a "B" elute in the second fraction, and so forth. The fraction in which the biomarker elutes correlates with its pI, which biomarkers eluting at higher pH having a higher pI, and biomarkers eluting at lower pH having a lower pI.

[0037] Presenting the mass and affinity characteristics of a given biomarker within the invention, as in this description, characterizes that biomarker so as allow one to obtain and measure it, in accordance with the teachings herein. If desired, any of the biomarkers can be sequenced, in order to obtain an amino acid sequence, but this is not required to practice the present invention.

[0038] For example, a biomarker can be peptide mapped with a number of enzymes, such as trypsin and V8 protease, and the molecular weights of the digestion fragments can be used to search databases for sequences that match the molecular weights of the digestion fragments generated by the various enzymes. Alternatively, if the biomarkers are not proteins included in known databases, degenerate probes can be made based on the N-terminal amino acid sequence of the biomarker, which then are used to screen a genomic or cDNA library created from a sample from which the biomarker was initially detected. The positive clones can be identified, amplified, and their recombinant DNA sequences can be subcloned using techniques which are well known. Finally, protein biomarkers can be sequenced using protein ladder sequencing. Protein ladders can be generated by fragmenting the molecules and subjecting fragments to enzymatic digestion or other methods that sequentially remove a single amino acid from the end of the fragment. The ladder is then analyzed by mass spectrometry. The difference in masses of the ladder fragments identifies the amino acid removed from the end of the molecule.

[0039] Several biomarkers identified in accordance with the teachings of the present invention fit to serum amyloid A (SAA) or to a fragment of SAA. SAA is a well-known acute phase inflammatory marker. A number of the SAA biomarkers are identified in Figure 5 by both molecular mass and amino acid sequence. Most of these markers bound anti-SAA antibodies, as shown in Figure 6. The intact mass of SAA is 11.5 to 11.7 kD, and these biomarkers also have been identified by the present methodology. Fragments preferably have a molecular mass of at least about 200 Daltons, more preferably at least about 500 Daltons. In even more preferred embodiments, fragments have a molecular mass of at least about 800 Daltons, and most preferably at least about 1 Kilodalton.

[0040] In one embodiment, the fragments of SAA include a sequence of amino acids that is recognized by an epitope of an anti-SAA antibody. One way of identifying suitable fragments for use in the present invention is to enzymatically digest SAA and test the resulting fragments for the ability to bind to an anti-SAA antibody. Fragments that bind anti-SAA antibody can be sequenced using techniques well-known in the art, although the sequence of the fragment is not needed to practice the invention. In order to practice the invention with a fragment from the enzymatic digest that is identified as binding anti-SAA antibody, all that is required is to subject the fragment to mass spectrometry to determine its mass.

[0041] The serum biomarkers according to the present invention were identified by comparing mass spectra of samples derived from sera from two groups of newly-diagnosed subjects, subjects with lung cancer and normal subjects. The subjects were diagnosed according to standard clinical criteria. Lung cancer subjects were histologically confirmed, and subjects without lung cancer were followed for at least 18 months following serum collection for any sign of lung cancer, to exclude subjects with asymptomatic lung cancer.

[0042] Sera from each group of subjects was collected, and fractionated with Q Ceramic HyperDF ion exchange resin (Biosepra SA, France) into six fractions which eluted at different pH. Fraction A comprised the flow through plus pH 9 eluant, Fraction B comprised the pH 7 eluant, Fraction C comprised the pH 5 eluant, Fraction D comprised the pH 4 eluant, Fraction E comprised the pH 3 eluant, and Fraction F

comprised isopropyl alcohol/acetonitrile TFA eluant. Fractions A through F are identified on Figures 7-28 as Fractions 1 through 6, respectively.

[0043] Each fraction was diluted and applied to a ProteinChip® array, either a Cu(II) IMAC3 or WCX2 chip array. Both of these chip arrays are produced by Ciphergen Biosystems, Inc. (Fremont, CA).

[0044] The Cu(II) IMAC3 is an “immobilized metal affinity-capture” chip, with a nitrilotriacetic acid surface for high-capacity copper binding and subsequent affinity capture of proteins with metal binding residues. Imidazole may be used in binding and washing solutions to moderate protein binding, including binding of non-specific proteins. Increasing the concentration of imidazole in the washing buffers reduces the binding of the target proteins. It is produced by photopolymerizing 5-methylacylamido-2-(N,N-bis(carboxymethyl)amino)pentanoic acid (7.5 wt%) and N,N'-methylenebisacrylamide (0.4 wt%) using (-) riboflavin (0.02 wt%) as a photoinitiator. The monomer solution is deposited onto the chip substrate and irradiated to photopolymerize. The chip then is activated with Cu(II).

[0045] The WCX2 is a weak cation exchange array with a carboxylate surface to bind cationic proteins. The negatively charged carboxylate groups on the surface of the WCX2 chip interact with the positive charges exposed on the target proteins. The binding of the target proteins is reduced by increasing the concentration of salt or by increasing the pH of the washing buffers.

[0046] Following application of the eluant fraction, the chips were incubated to allow the polypeptides in the eluant to bind to the sites on the chip by an affinity interaction. After incubation, each chip array was washed to remove polypeptides that bind non-specifically and buffer contaminants. That chip then was dried, and an energy absorbing molecule or matrix was applied to it, to facilitate desorption and ionization in a mass spectrometer.

[0047] In the mass spectrometer, retained polypeptides were desorbed from the chip array by laser desorption and ionization in a ProteinChip® Reader, which is integrated with ProteinChip® Software and a personal computer to analyze proteins captured on chip arrays. The ion optic and laser optic technologies in the ProteinChip® Reader detects proteins ranging from small peptides of less than 1000 Da up to proteins of

300 kilodaltons or more, and calculates the mass based on time-of-flight. Ionized polypeptides were detected and their mass accurately determined by this Time-of-Flight (TOF) Mass Spectrometry.

[0048] The mass spectra obtained for each group were subjected to scatter plot analysis, to eliminate run-to-run variation. Protein clusters on the scatter plot that had the same pattern for both lung cancer and normal subjects, *i.e.*, protein clusters that were either elevated in both groups of subjects or depressed in both groups of subjects, were eliminated as potential biomarkers. The remaining polypeptides were further analyzed for their ability to accurately identify subjects with lung cancer. Because the molecular weights were derived from scatter plot analysis, and because of limits on the ability of mass spectrometry to resolve molecular weights, the “absolute” molecular weight values given in Figures 1A-1D and 3A-3O actually represent approximate molecular weights.

[0049] The biomarkers of this invention are characterized by their mass-to-charge ratio as determined by mass spectrometry. The mass-to-charge ratio of each biomarker is provided in Figures 1A-1D and 3A-3O. For example, IM-1 in Figure 1A has a measured mass-to-charge ratio of 2011. The mass-to-charge ratios were determined from mass spectra generated on a Ciphergen Biosystems, Inc. PBS II mass spectrometer. This instrument has a mass accuracy of about +/- 0.15 percent. Additionally, the instrument has a mass resolution of about 400 to 1000 m/dm, where m is mass and dm is the mass spectral peak width at 0.5 peak height. The mass-to-charge ratio of the biomarkers was determined using Biomarker WizardTM software (Ciphergen Biosystems). Biomarker Wizard assigns a mass-to-charge ratio to a biomarker by clustering the mass-to-charge ratios of the same peaks from all the spectra analyzed, as determined by the PBSII, taking the maximum and minimum mass-to-charge-ratio in the cluster, and dividing by two. Accordingly, the masses provided reflect these specifications.

[0050] The biomarkers of this invention are further characterized by the shape of their spectral peak in time-of-flight mass spectrometry. Mass spectra showing peaks representing the biomarkers are presented in Figures 7-28. The biomarker identifier numbers from Figures 2 and 4A-4B, respectively, are shown next to the peak, along

with their rank, which is indicated in parentheses below the biomarker identifier number.

[0051] The biomarkers of this invention are further characterized by their binding properties on chromatographic surfaces. Most of the biomarkers bind to IMAC (Cu) or WCX adsorbents (e.g., the CIPHERGEN® IMAC (Cu) or WCX ProteinChip® arrays) after washing as described herein.

[0052] Thus, a given molecular weight for a biomarker herein should be interpreted as the midpoint of a molecular-weight range. The accuracy of the mass spectrometer is $\pm 0.15\%$, and the actual molecular weight for a biomarker is therefore the value given, $\pm 0.15\%$. For example, the actual molecular weight for biomarker IM-273 is 11705 $\pm 0.15\%$, or between 11687 and 11722. Often, the range surrounding the “absolute” value given in the figure is no more than ± 5 daltons (2006 to 2016 for IM-1), generally no more than ± 3 daltons (2008 to 2014 for IM-1), and often as small as ± 1 dalton (2010 to 2012 daltons for IM-1).

[0053] CART® (Salford Systems, San Diego, CA), a classification and regression tree software, was used to determine whether a potential biomarker had predictive value in assessing lung cancer. A software macro randomly selected a subset of 15% of the peaks from Figures 1A-1D or Figures 3A-3O. The peaks and peak heights from each sample were provided to the CART® software for analysis. The software performed an iterative analysis until a single decision tree was generated that was capable of distinguishing between cancerous and non-cancerous. Each node in the resulting decision tree sorted based on the peak height of a single biomarker. A tree may contain any number of nodes, but generally contains from 1 to 6 nodes. From a practical standpoint in a commercial diagnostic test, a decision tree with fewer nodes is preferred. A total of 2000 decision trees, each based on a different 15% subset of the peaks from Figures 1A-1D or Figures 3A-3O, were generated.

[0054] The CART® software assigned a score to each biomarker in the subset, based on its relative importance. A score of 100 is very high and a score of 0 is very low. The CART® software also determined the sensitivity and specificity of each decision tree.

[0055] The data generated by the decision tree analysis was subjected to further analysis. The biomarkers were ranked based on their average scores, which were determined by adding up a biomarker's scores for each decision tree in which it appeared, and dividing by the total number of decision trees in which the biomarker appeared. Approximately 500 of the potential biomarkers showed up in at least one tree, and most of the biomarkers showed up in about 150 to 400 of the two thousand trees. The top 50 biomarkers for the IMAC and WCX chip arrays as determined by this method are shown in Figures 2 and 4A-4B, respectively.

[0056] All of the trees having sensitivities and specificities greater than 85% also were identified. All trees capable of distinguishing lung cancer from normal and having from 1 to 6 nodes that meet the 85/85 criterion are shown in Tables 1 and 2.

TABLE 1. Decision trees with IMAC Biomarkers.

2 Nodes				
474	151			
474	156			
522	507		2 trees	
522	440		2 trees	
3 Nodes				
266	454	474		
474	156	153		
474	40	156		
520	276	113		
520	265	401		
522	151	474		
522	478	153		
522	156	474		
4 Nodes				
148	521	508	251	

266	544	474	493	
266	157	126	420	
266	544	474	482	
266	471	474	38	
266	544	474	38	
266	514	471	203	
522	58	266	474	
5 Nodes				
266	544	473	151	437
266	454	474	153	264
273	143	544	401	199

TABLE 2. Decision Trees with WCX Biomarkers.

1 Node					
446					
447					
2 Nodes					
282	127				
3 Nodes					
61	16	27			
61	119	154			
61	120	154			
61	369	184			
61	184	129			
61	19	282			
133	282	319			
282	59	218			
282	111	65			

446	19	16			
4 Nodes					
61	369	282	184		
61	48	203	3		
446	369	111	67		
446	466	58	120		
446	19	59	113		
446	282	19	47		
447	118	59	417		
447	118	59	473		
447	65	59	275		
447	19	59	282		
447	369	59	206		
447	19	59	253		
447	19	47	70		
5 Nodes					
61	369	128	184	197	
61	17	425	366	341	
133	139	363	216	273	
282	133	48	19	253	
369	310	19	109	384	
446	282	15	319	66	
447	19	71	473	31	
447	19	17	473	438	
447	47	31	365	59	
6 Nodes					
369	366	192	471	19	439

[0057] Each of the biomarker combinations of Tables 1 and 2 are preferred combinations for distinguishing lung cancer subjects from normal subjects in accordance with the present invention.

[0058] All biomarkers that appeared in at least two of the trees that met the 85/85 criterion were identified. For these biomarkers, Tables 3 and 4 provide the number of times the biomarker occurred in a trees that met the criterion, as well as the ranking of that biomarker on the top 50 lists of Figures 2 and 4A-4B.

TABLE 3. Correlation of IMAC biomarker decision tree frequencies and ranking.

Peak		# times		Rank
266		9		9
522		8		1
474		4		14
520		2		3
148		1		8
273		1		2

TABLE 4. Correlation of WCX biomarker decision tree frequencies and ranking.

Peak		# times		Rank
447		11		2
61		10		1
446		7		3
282		4		9
369		2		8
133		2		4

[0059] Biomarkers that occurred frequently in the highly discriminatory trees occurred among the top 50 ranked biomarkers, and typically had a top 10 ranking. In addition, certain pairs of biomarkers reappear, *e.g.*, WM-447 and WM-59, WM-447 and WM-19, WM-19 and WM-59, IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-522 and IM-266. There also are repeats among triplets of biomarkers, such as IM-266, IM-266 and IM-38, and WM-447, WM-19 and WM-473. Other repeating pairs and trios of biomarkers can be seen in Tables 3 and 4, and are preferred.

[0060] Biomarkers and combinations of biomarkers identified in accordance with the present description may be used to qualify lung cancer status in a subject. In particular, a biomarker or combination of biomarkers can be used to distinguish lung cancer patients from normal patients with a high degree of specificity or sensitivity, *i.e.*, greater than at least 85%, preferably greater than at least 90%, and more preferably greater than 95%.

[0061] According to one aspect of the invention, therefore, the detection of biomarkers for diagnosis of lung cancer status entails contacting a sample from a subject with a substrate, *e.g.*, a SELDI probe, having an adsorbent thereon, under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, for example, mass spectrometry. Other detection paradigms that can be employed to this end include optical methods, electrochemical methods (voltametry and amperometry techniques), atomic force microscopy, and radio frequency methods, *e.g.*, multipolar resonance spectroscopy. Illustrative of optical methods, in addition to microscopy, both confocal and non-confocal, are detection of fluorescence, luminescence, chemiluminescence, absorbance, reflectance, transmittance, and birefringence or refractive index (*e.g.*, surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry).

[0062] In one aspect, the markers of this invention are detect by gas phase ion spectrometry, which refers to the use of a gas phase ion spectrometer to detect gas phase ions. A gas phase ion spectrometer is an apparatus that detects gas phase ions. Gas phase ion spectrometers include an ion source that supplies gas phase ions. Gas

phase ion spectrometers include, for example, mass spectrometers, ion mobility spectrometers, and total ion current measuring devices.

[0063] “Mass spectrometer” refers to a gas phase ion spectrometer that measures a parameter which can be translated into mass-to-charge ratios of gas phase ions. Mass spectrometers generally include an ion source and a mass analyzer. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these. “Mass spectrometry” refers to the use of a mass spectrometer to detect gas phase ions. “Laser desorption mass spectrometer” refers to a mass spectrometer which uses laser as a means to desorb, volatilize, and ionize an analyte.

[0064] “Mass analyzer” refers to a sub-assembly of a mass spectrometer that comprises means for measuring a parameter which can be translated into mass-to-charge ratios of gas phase ions. In a time-of flight mass spectrometer the mass analyzer comprises an ion optic assembly, a flight tube and an ion detector.

[0065] “Ion source” refers to a sub-assembly of a gas phase ion spectrometer that provides gas phase ions. In one embodiment, the ion source provides ions through a desorption/ionization process. Such embodiments generally comprise a probe interface that positionally engages a probe in an interrogatable relationship to a source of ionizing energy (e.g., a laser desorption/ionization source) and in concurrent communication at atmospheric or subatmospheric pressure with a detector of a gas phase ion spectrometer.

[0066] Forms of ionizing energy for desorbing/ionizing an analyte from a solid phase include, for example: (1) laser energy; (2) fast atoms (used in fast atom bombardment); (3) high energy particles generated via beta decay of radionuclides (used in plasma desorption); and (4) primary ions generating secondary ions (used in secondary ion mass spectrometry). The preferred form of ionizing energy for solid phase analytes is a laser (used in laser desorption/ionization), in particular, nitrogen lasers, Nd-Yag lasers and other pulsed laser sources. “Fluence” refers to the laser energy delivered per unit area of interrogated image. Typically, a sample is placed on the surface of a probe, the probe is engaged with the probe interface and the probe

surface is struck with the ionizing energy. The energy desorbs analyte molecules from the surface into the gas phase and ionizes them.

[0067] Other forms of ionizing energy for analytes include, for example: (1) electrons which ionize gas phase neutrals; (2) strong electric field to induce ionization from gas phase, solid phase, or liquid phase neutrals; and (3) a source that applies a combination of ionization particles or electric fields with neutral chemicals to induce chemical ionization of solid phase, gas phase, and liquid phase neutrals.

[0068] A preferred mass spectrometric technique for use in the invention is Surface Enhanced Laser Desorption and Ionization (SELDI), as described, for example, in U.S. patents No. 5,719,060 and No. 6,225,047, both to Hutchens and Yip, in which the surface of a probe that presents the analyte (here, one or more of the biomarkers) to the energy source plays an active role in desorption/ionization of analyte molecules. In this context, "probe" refers to a device adapted to engage a probe interface and to present an analyte to ionizing energy for ionization and introduction into a gas phase ion spectrometer, such as a mass spectrometer. A probe typically includes a solid substrate, either flexible or rigid, that has a sample-presenting surface, on which an analyte is presented to the source of ionizing energy.

[0069] One version of SELDI, called "Surface-Enhanced Affinity Capture" or "SEAC," involves the use of probes comprised of a chemically selective surface ("SELDI probe"). A "chemically selective surface" is one to which is bound either the adsorbent, also called a "binding moiety" or "capture reagent," or a reactive moiety that is capable of binding a capture reagent, *e.g.*, through a reaction forming a covalent or coordinate covalent bond.

[0070] The phrase "reactive moiety" here denotes a chemical moiety that is capable of binding a capture reagent. Epoxide and carbodiimidazole are useful reactive moieties to covalently bind polypeptide capture reagents such as antibodies or cellular receptors. Nitriloacetic acid and iminodiacetic acid are useful reactive moieties that function as chelating agents to bind metal ions that interact non-covalently with histidine containing peptides. A "reactive surface" is a surface to which a reactive moiety is bound. An "adsorbent" or "capture reagent" can be any material capable of

binding a biomarker of the invention. Suitable adsorbents for use in SELDI, according to the invention, are described in U.S. patent No. 6,225,047, *supra*.

[0071] One type of adsorbent is a “chromatographic adsorbent,” which is a material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators, immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (e.g., nucleotides, amino acids, simple sugars and fatty acids), mixed mode adsorbents (e.g., hydrophobic attraction/electrostatic repulsion adsorbents).

“Biospecific adsorbent” is another category, for adsorbents that contain a biomolecule, e.g., a nucleotide, a nucleic acid molecule, an amino acid, a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (e.g., a glycoprotein, a lipoprotein, a glycolipid). In certain instances the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Illustrative biospecific adsorbents are antibodies, receptor proteins, and nucleic acids. A biospecific adsorbent typically has higher specificity for a target analyte than a chromatographic adsorbent.

[0072] Another version of SELDI is Surface-Enhanced Neat Desorption (SEND), which involves the use of probes comprising energy absorbing molecules that are chemically bound to the probe surface (“SEND probe”). The phrase “Energy absorbing molecules” (EAM) denotes molecules that are capable of absorbing energy from a laser desorption ionization source and, thereafter, contributing to desorption and ionization of analyte molecules in contact therewith. The EAM category includes molecules used in MALDI, frequently referred to as “matrix,” and is exemplified by cinnamic acid derivatives, sinapinic acid (SPA), cyano-hydroxy-cinnamic acid (CHCA) and dihydroxybenzoic acid, ferulic acid, and hydroxyaceto-phenone derivatives. The category also includes EAMs used in SELDI, as enumerated, for example, by U.S. 5,719,060 and U.S. 60/351,971 (Kitagawa), filed January 25, 2002.

[0073] Another version of SELDI, called Surface-Enhanced Photolabile Attachment and Release (SEPAR), involves the use of probes having moieties attached to the surface that can covalently bind an analyte, and then release the analyte through breaking a photolabile bond in the moiety after exposure to light, e.g., to laser light.

For instance, see U.S. 5,719,060. SEPAR and other forms of SELDI are readily adapted to detecting a biomarker or biomarker profile, pursuant to the present invention.

[0074] The detection of the biomarkers according to the invention can be enhanced by using certain selectivity conditions, *e.g.*, adsorbents or washing solutions. The phrase “wash solution” refers to an agent, typically a solution, which is used to affect or modify adsorption of an analyte to an adsorbent surface and/or to remove unbound materials from the surface. The elution characteristics of a wash solution can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength, and temperature.

[0075] Pursuant to one aspect of the present invention, a sample is analyzed by means of a “biochip,” a term that denotes a solid substrate, having a generally planar surface, to which a capture reagent (adsorbent) is attached. Frequently, the surface of a biochip comprises a plurality of addressable locations, each of which has the capture reagent bound there. A biochip can be adapted to engage a probe interface and, hence, function as a probe in gas phase ion spectrometry preferably mass spectrometry. Alternatively, a biochip of the invention can be mounted onto another substrate to form a probe that can be inserted into the spectrometer.

[0076] A variety of biochips is available for the capture of biomarkers, in accordance with the present invention, from commercial sources such as Ciphergen Biosystems (Fremont, CA), Perkin Elmer (Packard BioScience Company (Meriden CT), Zyomyx (Hayward, CA), and Phylos (Lexington, MA). Exemplary of these biochips are those described in U.S. patents No. 6,225,047, *supra*, and No. 6,329,209 (Wagner *et al.*), and in PCT publications WO 99/51773 (Kuimelis and Wagner) and WO 00/56934 (Englert *et al.*).

[0077] More specifically, biochips produced by Ciphergen Biosystems have surfaces, presented on an aluminum substrate in strip form, to which are attached, at addressable locations, chromatographic or biospecific adsorbents. The surface of the strip is coated with silicon dioxide.

[0078] Illustrative of Ciphergen ProteinChip® arrays are biochips H4, SAX-2, WCX-2, and IMAC-3, which include a functionalized, cross-linked polymer in the

form of a hydrogel, physically attached to the surface of the biochip or covalently attached through a silane to the surface of the biochip. The H4 biochip has isopropyl functionalities for hydrophobic binding. The SAX-2 biochip has quaternary ammonium functionalities for anion exchange. The WCX-2 biochip has carboxylate functionalities for cation exchange. The IMAC-3 biochip has nitriloacetic acid functionalities that adsorb transition metal ions, such as Cu^{++} and Ni^{++} , by chelation. These immobilized metal ions, in turn, allow for adsorption of biomarkers by coordinate covalent bonding. Thus, CIPHERGEN's IMAC ProteinChip® arrays are sold with reactive moieties that become adsorbent upon the addition by the user of a metal solution.

[0079] In keeping with the above-described principles, a substrate with an adsorbent is contacted with the sample, containing serum, for a period of time sufficient to allow biomarker that may be present to bind to the adsorbent. In one embodiment of the invention, more than one type of substrate with adsorbent thereon is contacted with the biological sample. For example, a sample may be applied to both a WCX and an IMAC chip. This technique can allow for even more definitive assessment of cancer status. After the incubation period, the substrate is washed to remove unbound material. Any suitable washing solutions can be used; preferably, aqueous solutions are employed.

[0080] An energy absorbing molecule then is applied to the substrate with the bound biomarkers. As noted, an energy absorbing molecule is a molecule that absorbs energy from an energy source such as a laser, thereby assisting in desorption of biomarkers from the substrate. Exemplary energy absorbing molecules include, as noted above, cinnamic acid derivatives, sinapinic acid and dihydroxybenzoic acid. Preferably sinapinic acid is used.

[0081] The biomarkers bound to the substrates are detected in a gas phase ion spectrometer such as a time-of-flight mass spectrometer. The biomarkers are ionized by an ionization source such as a laser, the generated ions are collected by an ion optic assembly, and then a mass analyzer disperses and analyzes the passing ions. The detector then translates information of the detected ions into mass-to-charge

ratios. Detection of a biomarker typically will involve detection of signal intensity. Thus, both the quantity and mass of the biomarker can be determined.

[0082] Data generated by desorption and detection of biomarkers can be analyzed with the use of a programmable digital computer. The computer program analyzes the data to indicate the number of markers detected, and optionally the strength of the signal and the determined molecular mass for each biomarker detected. Data analysis can include steps of determining signal strength of a biomarker and removing data deviating from a predetermined statistical distribution. For example, the observed peaks can be normalized, by calculating the height of each peak relative to some reference. The reference can be background noise generated by the instrument and chemicals such as the energy absorbing molecule which is set as zero in the scale.

[0083] The computer can transform the resulting data into various formats for display. The standard spectrum can be displayed, but in one useful format only the peak height and mass information are retained from the spectrum view, yielding a cleaner image and enabling biomarkers with nearly identical molecular weights to be more easily seen. In another useful format, two or more spectra are compared, conveniently highlighting unique biomarkers and biomarkers that are up- or down-regulated between samples. Using any of these formats, one can readily determine whether a particular biomarker is present in a sample.

[0084] Software used to analyze the data can include code that applies an algorithm to the analysis of the signal to determine whether the signal represents a peak in a signal that corresponds to a biomarker according to the present invention. The software also can subject the data regarding observed biomarker peaks to classification tree or ANN analysis, to determine whether a biomarker peak or combination of biomarker peaks is present that indicates lung cancer status. Analysis of the data may be "keyed" to a variety of parameters that are obtained either directly or indirectly from the mass spectrometric analysis of the sample. These parameters include, but are not limited to, the presence or absence of one or more peaks, the height of one or more peaks, the log of the height of one or more peaks, and other arithmetic manipulations of peak height data.

[0085] In another aspect, the present invention provides kits for aiding in the diagnosis of lung cancer status, which kits are used to detect biomarkers according to the invention. The kits screen for the presence of biomarkers and combinations of biomarkers that are differentially present in samples from normal subjects and subjects with lung cancer.

[0086] In one embodiment, the kit comprises a substrate having an adsorbent thereon, wherein the adsorbent is suitable for binding a biomarker according to the invention, and a washing solution or instructions for making a washing solution, in which the combination of the adsorbent and the washing solution allows detection of the biomarker using gas phase ion spectrometry, e.g., mass spectrometry. The kit may include more than type of adsorbent, each present on a different substrate.

[0087] In another embodiment, a kit of the invention may include a first substrate, comprising an adsorbent thereon, and a second substrate onto which the first substrate is positioned to form a probe, which can be inserted into a gas phase ion spectrometer, e.g., a mass spectrometer. In another embodiment, an inventive kit may comprise a single substrate that can be inserted into the spectrometer.

[0088] In a further embodiment, such a kit can comprise instructions for suitable operational parameters in the form of a label or separate insert. For example, the instructions may inform a consumer how to collect the sample or how to wash the probe. In yet another embodiment the kit can comprise one or more containers with biomarker samples, to be used as standard(s) for calibration.

[0089] In a preferred embodiment, the detection of biomarkers for diagnosis of lung cancer in a subject entails contacting a sample from a subject or patient, preferably a serum sample, with a substrate having an adsorbent thereon under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, preferably by Surface Enhanced Laser Desorption/Ionization (SELDI) mass spectrometry. The biomarkers are ionized by an ionization source such as a laser. The generated ions are collected by an ion optic assembly and accelerated toward an ion detector. Ions that strike the detector generate an electric potential that is digitized by a high speed time-array recording device that digitally captures the analog signal. CIPHERGEN's

ProteinChip® system employs an analog-to-digital converter (ADC) to accomplish this. The ADC integrates detector output at regularly spaced time intervals into time-dependent bins. The time intervals typically are one to four nanoseconds long. Furthermore, the time-of-flight spectrum ultimately analyzed typically does not represent the signal from a single pulse of ionizing energy against a sample, but rather the sum of signals from a number of pulses. This reduces noise and increases dynamic range. This time-of-flight data is then subject to data processing. In Ciphergen's ProteinChip® software, data processing typically includes TOF-to-M/Z transformation, baseline subtraction, high frequency noise filtering. Thus, both the quantity and mass of the biomarker can be determined.

[0090] The detection of the biomarkers can be enhanced by using certain selectivity conditions, *e.g.*, adsorbents or washing solutions. In one embodiment, the same or similar selectivity conditions that were used to discover the biomarkers are used in the method of detecting the biomarker in the sample. For example, immobilized metal affinity capture chips such as the Cu(II) IMAC3 and weak cationic exchange chips such as the WCX2 chips are preferred as the adsorbents for biomarker detection. However, other adsorbents can be used, as long as they have the binding characteristics suitable for binding the biomarkers.

[0091] More particularly, armed with the information regarding the biomarkers identified herein, various methods can be used to recognize patterns of doublets, triplets, and higher combinations of biomarkers according to the invention. These methods take raw data regarding which peaks are present and their intensity and provide a differential diagnosis of lung cancer versus normal for a sample.

[0092] Thus, the process can be divided into the learning phase and the classification phase. In the learning phase, a learning algorithm is applied to a data set that includes members of the different classes that are meant to be classified, for example, data from a plurality of samples diagnosed as cancer and data from a plurality of samples assigned a negative diagnosis. The methods used to analyze the data include, but are not limited to, artificial neural network, support vector machines, genetic algorithm and self-organizing maps and classification and regression tree analysis. These methods are described, for example, in WO 01/31579, May 3, 2001

(Barnhill *et al.*); WO 02/06829, January 24, 2002 (Hitt *et al.*) and WO 02/42733, May 30, 2002 (Paulse *et al.*). The learning algorithm produces a classifying algorithm. The classifier is keyed to elements of the data, such as particular markers and particular intensities of markers, usually in combination, that can classify an unknown sample into one of the two classes. The classifier is ultimately used for diagnostic testing.

[0093] Software, both freeware and proprietary software, is readily available to analyze such patterns in data, and to devise additional patterns with any predetermined criteria for success. Those biomarkers which by themselves are predictive of a differential diagnosis of lung cancer versus normal do not require pattern recognition software to analyze the data.

[0094] The following examples are offered by way of illustration, and are not limiting.

Example I. Fractionation of serum

Buffers:

1. U9 (9M urea, 2% CHAPS, 50mM Tris-HCl pH9)
2. U1 (1M urea, 0.22% CHAPS, 50mM Tris-HCl pH9)
3. wash buffer 1: 50mM Tris-HCl with 0.1% n-octyl β -D-Glucopyranoside (OGP) pH9
4. wash buffer 2: 100mM sodium phosphate with 0.1% OGP pH7
5. wash buffer 3: 100mM sodium acetate with 0.1% OGP pH5
6. wash buffer 4: 100mM sodium acetate with 0.1% OGP pH4
7. wash buffer 5: 50mM sodium citrate with 0.1% OGP pH3
8. wash buffer 6: 33.3% isopropanol / 16.7% acetonitrile / 0.1% trifluoroacetic acid in water.

[0095] Thirty microliters of U9 buffer were added to 20 μ L of serum in a tube and were mixed at 4°C for 20 minutes. Ion exchange resin (Q Ceramic HyperDF ion exchange resin, Biosepra SA, France) was washed 3 times with 5 bed volumes of 50mM Tris-HCl pH9 and stored in 50% suspension. To each well of a 96-well filter plate (96-well Silent Screen filter plate, Lonrodvne membrane, 0.45 micron pore,

Nalge Nunc International, USA), 125 μ L of ion exchange resin (50% suspension) was added on a Biomek 2000 Automation Workstation (Beckman Coulter, Fullerton, CA), washed 3 times with 150 μ L U1 buffer, and vacuum dried. Urea-treated serum was transferred to each well of ion exchange resin. The serum tube was rinsed with 50 μ L of U1 buffer, which was also transferred to the corresponding well in filter plate. The filter plate was mixed on a platform shaker at 4°C for 30 minutes. Flow-through fraction was collected in a 96-well plate by vacuum suction (Fraction 1). Then, 100 μ L of wash buffer 1 was added to each well of filter plate and mixed for 10 minutes at room temperature. Eluant was collected into the same 96-well plate (Fraction 1). Resins in the filter plate were subsequently washed two times each with 100 μ L wash buffers 2, 3, 4, 5 and 6. Each eluant (total volume of 200 μ L) was collected in a 96-well plate (Fractions 2,3,4,5 and 6).

Example 2. SELDI analysis of fractionated serum

[0096] ProteinChip® Arrays were set up in 96-well bioprocessors. Buffer delivery and sample incubation were performed on a Biomek 2000 Automation Workstation. Each serum fraction was analyzed on IMAC3 (loaded with copper) and WCX2 ProteinChip® Arrays in duplicates. IMAC3 copper and WCX2 arrays (CIPHERGEN Biosystems Inc, Fremont, CA) were equilibrated two times with 150 μ L of binding buffer (100mM sodium phosphate + 0.5M NaCl pH7 for IMAC3, 100mM sodium acetate pH4 for WCX2). Each serum fraction was diluted in the corresponding binding buffer (1/5 dilution for IMAC3 and 1/10 dilution for WCX2) and 100 μ L was applied to each ProteinChip® array. Incubation was performed on a platform shaker at room temperature for 30 minutes. Each array was washed three times with 150 μ L of corresponding binding buffer and rinsed two times with water. ProteinChip® arrays were air-dried. Sinapinic acid matrix (prepared in 50% acetonitrile, 0.5% trifluoroacetic acid) was applied to each array. ProteinChip® arrays were read on a ProteinChip® PBSII Reader (CIPHERGEN Biosystems Inc.) A total of 253 laser shots were averaged for each array.

[0097] All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if

each individual publication or patent document were so individually denoted. By their citation of various references in this document Applicants do not admit that any particular reference is "prior art" to their invention.

What we claim is:

1. A method for qualifying lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a diagnostic level of a protein selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310,

wherein the biomarker is differentially present in samples of a subject with lung cancer and a normal subject that is free of lung cancer.

2. The method according to claim 1, wherein the protein is selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, and IM-155,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, and WM-70.

3. The method according to claim 1, wherein the protein is selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, and IM-454,

or from a second group consisting

(ii) WM-61, WM-447, WM-446, WM-133, and WM-119.

4. The method according to claim 1, which uses a single biomarker selected from the group consisting of the WM-446 and WM-447.

5. A method for qualifying lung carcinoma risk in a subject, comprising
(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

6. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a pair of peaks selected either from a first group consisting of

(i) IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-266 and IM-522, IM-266 and IM-544, IM-266 and IM-471, IM-474 and IM-151, IM-474 and IM-156, IM-474 and IM-544, IM-474 and IM-38, IM-522 and IM-507, IM-522 and IM-156, and IM-522 and IM-440;

or from a second group consisting of

(ii) WM-447 and WM-59, WM-447 and WM-19, WM-447 and WM-118, WM-447 and WM-473, WM-19 and WM-59, WM-19 and WM-473, WM-19 and WM-369, WM-61 and WM-154, WM-61 and WM-369, WM-118 and WM-59 and WM-282 and WM-127.

7. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a pair of peaks selected from either a first group consisting of

(i) IM-266 and IM-474, IM-266 and IM-544, and IM-156 and IM-522;

or from a second group consisting of

(ii) WM-447 and WM-59, WM-447 and WM-19, and WM-19 and WM-59.

8. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a triplet of peaks selected from

(i) IM-266, IM-454 and IM-474; and IM-266, IM-474 and IM-544;

or wherein the analysis is keyed to

(ii) WM-447, WM-19 and WM-473.

9. A kit for detecting and diagnosing lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

10. A kit according to claim 9, further comprising a washing solution or instructions for making a washing solution.

11. A kit according to claim 9, wherein the substrate is a SELDI probe that comprises either (i) functionalities that adsorb transition metal ions by chelation or (ii) functionalities that allow for cation exchange.

12. A method for qualifying lung adenocarcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429.

13. The method according to claim 12, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, and WM-120.

14. The method according to claim 12, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290.

15. A method for qualifying status of lung adenocarcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-

389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429.

16. The method according to claim 15, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, and WM-120.

17. The method according to claim 15, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290.

18. A kit for detecting and diagnosing lung adenocarcinoma, comprising
(A) an adsorbent attached to a substrate that retains one or more of biomarkers selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

19. A kit according to claim 18, further comprising a washing solution or instructions for making a washing solution.

20. A kit according to claim 18, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

21. A method for qualifying squamous cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290,

WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128.

22. The method according to claim 21, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, and WM-70.

23. The method according to claim 21, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, and WM-133.

24. A method for qualifying status of squamous cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290, WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128.

25. The method according to claim 24, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, and WM-70.

26. The method according to claim 24, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, and WM-133.

27. A kit for detecting and diagnosing squamous cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290, WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

28. A kit according to claim 27, further comprising a washing solution or instructions for making a washing solution.

29. A kit according to claim 27, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

30. A method for qualifying small cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279.

31. The method according to claim 30, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, and WM-685.

32. The method according to claim 30, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, and WM-61.

33. A method for qualifying status of small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279.

34. The method according to claim 33, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, and WM-685.

35. The method according to claim 33, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, and WM-61.

36. A kit for detecting and diagnosing small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

37. A kit according to claim 36, further comprising a washing solution or instructions for making a washing solution.

38. A kit according to claim 36, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

39. A method for qualifying non-small cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38.

40. The method according to claim 39, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, and WM-456.

41. The method according to claim 39, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, and WM-340.

42. A method for qualifying status of non-small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38.

43. The method according to claim 42, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, and WM-456.

44. The method according to claim 42, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, and WM-340.

45. A kit for detecting and diagnosing non-small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

46. A kit according to claim 45, further comprising a washing solution or instructions for making a washing solution.

47. A kit according to claim 45, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

48. A method for qualifying large cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488.

49. The method according to claim 48, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, and WM-447.

50. The method according to claim 48, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, and WM-647.

51. A method for qualifying status of large cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488.

52. The method according to claim 51, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, and WM-447.

53. The method according to claim 51, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, and WM-647.

54. A kit for detecting and diagnosing large cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-

545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

55. A kit according to claim 50, further comprising a washing solution or instructions for making a washing solution.

56. A kit according to claim 50, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

57. A method for distinguishing lung adenocarcinoma from squamous lung carcinoma in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, WM-211, WM-362, WM-133, WM-414, WM-277, WM-141, WM-64, WM-135, WM-447, WM-383, WM-338, WM-63, WM-142, WM-446, WM-186, WM-111, WM-445, WM-455, WM-276, WM-444, WM-181, WM-35, WM-285, WM-456, WM-39, WM-82, WM-17, WM-203, WM-83, WM-412, WM-96, WM-74, WM-457, WM-431, WM-340, and WM-49.

58. The method according to claim 57, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, and WM-211.

59. The method according to claim 57, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, and WM-347.

60. A method for distinguishing lung adenocarcinoma from squamous lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group

consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, WM-211, WM-362, WM-133, WM-414, WM-277, WM-141, WM-64, WM-135, WM-447, WM-383, WM-338, WM-63, WM-142, WM-446, WM-186, WM-111, WM-445, WM-455, WM-276, WM-444, WM-181, WM-35, WM-285, WM-456, WM-39, WM-82, WM-17, WM-203, WM-83, WM-412, WM-96, WM-74, WM-457, WM-431, WM-340, and WM-49.

61. The method according to claim 60, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, and WM-211.

62. The method according to claim 60, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, and WM-347.

63. A kit for distinguishing lung adenocarcinoma from squamous lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

64. A kit according to claim 63, further comprising a washing solution or instructions for making a washing solution.

65. A kit according to claim 63, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

66. A method for distinguishing lung adenocarcinoma from small cell lung carcinoma in a subject, comprised of analyzing a biological sample from said subject

for a level of a protein selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, WM-455, WM-313, WM-456, WM-86, WM-70, WM-246, WM-360, WM-190, WM-418, WM-83, WM-257, WM-138, WM-47, WM-252, WM-282, WM-60, WM-68, WM-325, WM-402, WM-411, WM-405, WM-75, WM-417, WM-387, WM-26, WM-410, WM-420, WM-164, WM-67, WM-66, WM-391, WM-340, WM-428, WM-198, WM-312, and WM-152.

67. The method according to claim 66, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, and WM-455.

68. The method according to claim 66, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, and WM-79.

69. A method for distinguishing lung adenocarcinoma from small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, WM-455, WM-313, WM-456, WM-86, WM-70, WM-246, WM-360, WM-190, WM-418, WM-83, WM-257, WM-138, WM-47, WM-252, WM-282, WM-60, WM-68, WM-325, WM-402, WM-411, WM-405, WM-75, WM-417, WM-387, WM-26, WM-410, WM-420, WM-164, WM-67, WM-66, WM-391, WM-340, WM-428, WM-198, WM-312, and WM-152.

70. The method according to claim 69, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, and WM-455.

71. The method according to claim 69, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79.

72. A kit for distinguishing lung adenocarcinoma from small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

73. A kit according to claim 72, further comprising a washing solution or instructions for making a washing solution.

74. A kit according to claim 72, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

75. A method for distinguishing squamous cell lung carcinoma from small cell lung carcinoma in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37.

76. The method according to claim 75, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, and WM-447.

77. The method according to claim 75, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, and WM-363.

78. A method for distinguishing squamous cell lung carcinoma from small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37.

79. The method according to claim 78, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, and WM-447.

80. The method according to claim 78, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, and WM-363.

81. A kit for distinguishing squamous cell lung carcinoma from small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

82. A kit according to claim 81, further comprising a washing solution or instructions for making a washing solution.

83. A kit according to claim 81, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

84. Software for qualifying lung carcinoma status in a subject, comprising an algorithm for analyzing data extracted from a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, wherein said data relates to one or more biomarkers selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

85. Software according to claim 84, wherein said algorithm carries out a pattern-recognition analysis that is keyed to data relating to at least one of the biomarkers.

86. Software according to claim 85, wherein said algorithm comprises classification tree analysis that is keyed to data relating to at least one of the biomarkers.

87. Software according to claim 85, wherein said algorithm comprises artificial neural network analysis that is keyed to data relating to at least one of the biomarkers.

88. A method for qualifying lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a diagnostic level of a biomarker that is serum amyloid A protein or a fragment thereof.

89. A method according to claim 88, wherein said serum biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons.

90. A method according to claim 89, wherein said serum biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons.

91. A method according to claim 88, wherein said serum biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

92. A method according to claim 88, for qualifying risk of lung adenocarcinoma.

93. A method according to claim 88, for qualifying risk of squamous cell lung carcinoma.

94. A method according to claim 88, for qualifying risk of small cell lung carcinoma.

95. A method according to claim 88, for qualifying risk of non-small cell lung carcinoma.

96. A method according to claim 88, for qualifying risk of large cell lung carcinoma.

97. A kit for detecting and diagnosing lung carcinoma, comprising
(A) an adsorbent attached to a substrate that retains one or more of the biomarkers that are serum amyloid A protein or a fragment thereof.

and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

98. A kit according to claim 97, wherein said serum biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons.

99. A kit according to claim 98, wherein said serum biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons.

100. A kit according to claim 97, wherein said serum biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

101. A kit according to claim 97, further comprising a washing solution or instructions for making a washing solution.

102. A kit according to claim 97, wherein the substrate is a SELDI probe.

FIGURE 1A

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-1	2011	A	IM-37	3893	A	IM-72	54026	A	IM-109	2882	B	IM-121	3450	B
IM-2	2030	A	IM-38	3960	A	IM-73	60170	A	IM-110	2967	B	IM-122	3561	B
IM-3	2069	A	IM-39	3972	A	IM-75	74372	A	IM-111	2977	B	IM-123	3615	B
IM-4	2128	A	IM-40	3984	A	IM-76	75545	A	IM-112	2994	B	IM-124	3714	B
IM-5	2146	A	IM-41	4066	A	IM-77	77543	A	IM-113	3031	B	IM-125	3730	B
IM-6	2186	A	IM-42	4178	A	IM-78	79507	A	IM-114	3048	B	IM-126	3834	B
IM-7	2232	A	IM-43	4287	A	IM-79	89854	A	IM-115	3148	B	IM-127	3899	B
IM-8	2277	A	IM-44	4297	A	IM-80	101831	A	IM-116	3166	B	IM-128	3969	B
IM-9	2295	A	IM-45	4309	A	IM-81	104301	A	IM-117	3283	B	IM-129	3986	B
IM-10	2318	A	IM-46	4484	A	IM-82	125160	A	IM-118	3308	B	IM-130	3997	B
IM-11	2411	A	IM-47	4649	A	IM-83	132976	A	IM-119	3332	B	IM-131	4013	B
IM-12	2434	A	IM-48	4798	A	IM-84	149099	A	IM-120	3432	B	IM-132	4181	B
IM-13	2467	A	IM-49	5104	A	IM-85	2016	B	IM-121	3450	B	IM-133	4297	B
IM-14	2482	A	IM-50	5918	A	IM-86	2029	B	IM-122	3561	B	IM-134	4311	B
IM-15	2498	A	IM-51	6122	A	IM-87	2144	B	IM-123	3615	B	IM-135	4465	B
IM-16	2565	A	IM-52	6192	A	IM-88	2130	B	IM-124	3714	B	IM-136	4484	B
IM-17	2574	A	IM-53	6452	A	IM-89	2168	B	IM-125	3730	B	IM-137	4579	B
IM-18	2586	A	IM-54	6660	A	IM-90	2184	B	IM-126	3834	B	IM-138	4608	B
IM-19	2605	A	IM-55	7766	A	IM-91	2200	B	IM-127	3899	B	IM-139	4669	B
IM-20	2722	A	IM-56	8145	A	IM-92	2284	B	IM-128	3969	B	IM-140	4747	B
IM-21	2746	A	IM-57	8954	A	IM-93	2299	B	IM-129	3986	B	IM-141	4862	B
IM-22	2788	A	IM-58	9312	A	IM-94	2314	B	IM-130	3997	B	IM-142	4891	B
IM-23	2855	A	IM-59	9449	A	IM-95	2414	B	IM-131	4013	B	IM-143	5033	B
IM-24	2871	A	IM-60	10272	A	IM-96	2428	B	IM-132	4181	B	IM-144	5077	B
IM-25	2984	A	IM-61	11663	A	IM-97	2451	B	IM-133	4297	B			
IM-26	3030	A	IM-62	13376	A	IM-98	2466	B	IM-134	4311	B			
IM-27	3144	A	IM-63	14698	A	IM-99	2483	B	IM-135	4465	B			
IM-28	3243	A	IM-64	15190	A	IM-100	2565	B	IM-136	4484	B			
IM-29	3273	A	IM-64	66758	A	IM-101	2583	B	IM-137	4579	B			
IM-30	3290	A	IM-65	15951	A	IM-102	2597	B	IM-138	4608	B			
IM-31	3369	A	IM-66	15172	A	IM-103	2697	B	IM-139	4669	B			
IM-32	3445	A	IM-67	15925	A	IM-104	2715	B	IM-140	4747	B			
IM-33	3483	A	IM-68	23436	A	IM-105	2740	B	IM-141	4862	B			
IM-34	3676	A	IM-69	39794	A	IM-106	2752	B	IM-142	4891	B			
IM-35	3779	A	IM-70	44166	A	IM-107	2767	B	IM-143	5033	B			
IM-36	3793	A	IM-71	46890	A	IM-108	2865	B	IM-144	5077	B			

FIGURE 1B

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-145	5099	B	IM-181	16018	B	IM-217	2130	C	IM-253	3733	C	IM-289	3733	C	IM-289	3733	C
IM-146	5143	B	IM-182	17346	B	IM-218	2145	C	IM-254	3833	C	IM-290	3833	C	IM-290	3833	C
IM-147	5158	B	IM-183	18311	B	IM-219	2167	C	IM-255	3900	C	IM-291	3900	C	IM-291	3900	C
IM-148	5272	B	IM-184	22586	B	IM-220	2182	C	IM-256	4010	C	IM-292	4010	C	IM-292	4010	C
IM-149	5306	B	IM-185	23422	B	IM-221	2199	C	IM-257	4145	C	IM-293	4145	C	IM-293	4145	C
IM-150	5349	B	IM-186	27969	B	IM-222	2211	C	IM-258	4187	C	IM-294	4187	C	IM-294	4187	C
IM-151	5364	B	IM-187	33283	B	IM-223	2230	C	IM-259	4299	C	IM-295	4299	C	IM-295	4299	C
IM-152	5421	B	IM-188	39808	B	IM-224	2250	C	IM-260	4466	C	IM-296	4466	C	IM-296	4466	C
IM-153	5554	B	IM-189	43110	B	IM-225	2280	C	IM-261	4582	C	IM-297	4582	C	IM-297	4582	C
IM-154	5711	B	IM-190	44454	B	IM-226	2297	C	IM-262	4813	C	IM-298	4813	C	IM-298	4813	C
IM-155	5876	B	IM-191	47215	B	IM-227	2317	C	IM-263	4876	C	IM-299	4876	C	IM-299	4876	C
IM-156	5916	B	IM-192	53784	B	IM-228	2412	C	IM-264	5032	C	IM-300	5032	C	IM-300	5032	C
IM-157	5931	B	IM-193	55952	B	IM-229	2428	C	IM-265	5347	C	IM-301	5347	C	IM-301	5347	C
IM-158	5988	B	IM-194	60573	B	IM-230	2468	C	IM-266	5365	C	IM-302	5365	C	IM-302	5365	C
IM-159	6137	B	IM-195	66346	B	IM-231	2481	C	IM-267	5932	C	IM-303	5932	C	IM-303	5932	C
IM-160	6200	B	IM-196	73387	B	IM-232	2498	C	IM-268	7767	C	IM-304	7767	C	IM-304	7767	C
IM-161	6443	B	IM-197	79203	B	IM-233	2567	C	IM-269	7973	C	IM-305	7973	C	IM-305	7973	C
IM-162	6644	B	IM-198	89302	B	IM-234	2585	C	IM-270	8143	C	IM-306	8143	C	IM-306	8143	C
IM-163	6958	B	IM-199	94226	B	IM-235	2599	C	IM-271	9187	C	IM-307	9187	C	IM-307	9187	C
IM-164	7481	B	IM-200	99358	B	IM-236	2698	C	IM-272	9293	C	IM-308	9293	C	IM-308	9293	C
IM-165	7568	B	IM-201	102096	B	IM-237	2715	C	IM-273	11705	C	IM-309	11705	C	IM-309	11705	C
IM-166	7765	B	IM-202	107199	B	IM-238	2745	C	IM-274	14041	C	IM-310	14041	C	IM-310	14041	C
IM-167	7955	B	IM-203	116936	B	IM-239	2766	C	IM-275	15114	C	IM-311	15114	C	IM-311	15114	C
IM-168	8144	B	IM-204	119487	B	IM-240	2867	C	IM-276	15939	C	IM-312	15939	C	IM-312	15939	C
IM-169	8698	B	IM-205	122103	B	IM-241	2885	C	IM-277	22321	C	IM-313	22321	C	IM-313	22321	C
IM-170	8821	B	IM-206	125431	B	IM-242	2998	C	IM-278	28001	C	IM-314	28001	C	IM-314	28001	C
IM-171	8944	B	IM-207	132052	B	IM-243	3052	C	IM-279	33296	C	IM-315	33296	C	IM-315	33296	C
IM-172	9138	B	IM-208	138518	B	IM-244	3096	C	IM-280	39770	C	IM-316	39770	C	IM-316	39770	C
IM-173	9298	B	IM-209	145147	B	IM-245	3151	C	IM-281	44460	C	IM-317	44460	C	IM-317	44460	C
IM-174	9390	B	IM-210	157502	B	IM-246	3167	C	IM-282	47307	C	IM-318	47307	C	IM-318	47307	C
IM-175	9516	B	IM-211	168579	B	IM-247	3286	C	IM-283	50625	C	IM-319	50625	C	IM-319	50625	C
IM-176	11711	B	IM-212	173391	B	IM-248	3303	C	IM-284	55898	C	IM-320	55898	C	IM-320	55898	C
IM-177	11914	B	IM-213	2011	C	IM-249	3335	C	IM-285	60882	C	IM-321	60882	C	IM-321	60882	C
IM-178	14033	B	IM-214	2030	C	IM-250	3448	C	IM-286	66294	C	IM-322	66294	C	IM-322	66294	C
IM-179	15110	B	IM-215	2050	C	IM-251	3619	C	IM-287	78892	C	IM-323	78892	C	IM-323	78892	C
IM-180	15838	B	IM-216	2096	C	IM-252	3709	C	IM-288	83848	C	IM-324	83848	C	IM-324	83848	C

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-289	89081	C	IM-325	2565	D	IM-361	13857	D	IM-397	2082	E
IM-290	94147	C	IM-326	2582	D	IM-362	14056	D	IM-398	2128	E
IM-291	99324	C	IM-327	2597	D	IM-363	15108	D	IM-399	2148	E
IM-292	107163	C	IM-328	2716	D	IM-364	15844	D	IM-400	2170	E
IM-293	110350	C	IM-329	2747	D	IM-365	22243	D	IM-401	2187	E
IM-294	113339	C	IM-330	2767	D	IM-366	25465	D	IM-402	2206	E
IM-295	116291	C	IM-331	2866	D	IM-367	28022	D	IM-403	2232	E
IM-296	122769	C	IM-332	2882	D	IM-368	33272	D	IM-404	2250	E
IM-297	131908	C	IM-333	2994	D	IM-369	40149	D	IM-405	2279	E
IM-298	145248	C	IM-334	3032	D	IM-370	43113	D	IM-406	2296	E
IM-299	159252	C	IM-335	3050	D	IM-371	44219	D	IM-407	2314	E
IM-300	165164	C	IM-336	3148	D	IM-372	47196	D	IM-408	2354	E
IM-301	174928	C	IM-337	3164	D	IM-373	51062	D	IM-409	2394	E
IM-302	196003	C	IM-338	3278	D	IM-374	56082	D	IM-410	2413	E
IM-303	2007	D	IM-339	3334	D	IM-375	58239	D	IM-411	2436	E
IM-304	2016	D	IM-340	3385	D	IM-376	60285	D	IM-412	2457	E
IM-305	2030	D	IM-341	3432	D	IM-377	66148	D	IM-413	2466	E
IM-306	2052	D	IM-342	3451	D	IM-378	73668	D	IM-414	2499	E
IM-307	2099	D	IM-343	3617	D	IM-379	77433	D	IM-415	2566	E
IM-308	2130	D	IM-344	3701	D	IM-380	79986	D	IM-416	2583	E
IM-309	2144	D	IM-345	3725	D	IM-381	80844	D	IM-417	2612	E
IM-310	2154	D	IM-346	3833	D	IM-382	88962	D	IM-418	2662	E
IM-311	2166	D	IM-347	3899	D	IM-383	94399	D	IM-419	2723	E
IM-312	2184	D	IM-348	4008	D	IM-384	99419	D	IM-420	2738	E
IM-313	2204	D	IM-349	4157	D	IM-385	108395	D	IM-421	2750	E
IM-314	2231	D	IM-350	4297	D	IM-386	116433	D	IM-422	2849	E
IM-315	2252	D	IM-351	4580	D	IM-387	123337	D	IM-423	2867	E
IM-316	2275	D	IM-352	4805	D	IM-388	131977	D	IM-424	3036	E
IM-317	2299	D	IM-353	6946	D	IM-389	145658	D	IM-425	3147	E
IM-318	2316	D	IM-354	7053	D	IM-390	152603	D	IM-426	3281	E
IM-319	2412	D	IM-355	7767	D	IM-391	159524	D	IM-427	3319	E
IM-320	2435	D	IM-356	7954	D	IM-392	196072	D	IM-428	3445	E
IM-321	2466	D	IM-357	8139	D	IM-393	2010	E	IM-429	3693	E
IM-322	2480	D	IM-358	9292	D	IM-394	2029	E	IM-430	3731	E
IM-323	2499	D	IM-359	11671	D	IM-395	2050	E	IM-431	3818	E
IM-324	2518	D	IM-360	13727	D	IM-396	2068	E	IM-432	3885	E

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-433	4136	E	IM-469	86211	E	IM-505	4174	F	IM-541	95033	F	IM-546	160768	F
IM-434	4169	E	IM-470	89407	E	IM-506	4362	F	IM-542	100310	F			
IM-435	4257	E	IM-471	100270	E	IM-507	4473	F	IM-543	116889	F			
IM-436	4277	E	IM-472	109638	E	IM-508	4631	F	IM-544	132711	F			
IM-437	4355	E	IM-473	117132	E	IM-509	4822	F	IM-545	147276	F			
IM-438	4369	E	IM-474	132843	E	IM-510	5862	F	IM-546					
IM-439	4470	E	IM-475	147160	E	IM-511	6192	F						
IM-440	4486	E	IM-476	152199	E	IM-512	6941	F						
IM-441	4541	E	IM-477	166461	E	IM-513	7626	F						
IM-442	4634	E	IM-478	176635	E	IM-514	7772	F						
IM-443	4841	E	IM-479	2011	F	IM-515	7957	F						
IM-444	5862	E	IM-480	2030	F	IM-516	8150	F						
IM-445	5911	E	IM-481	2128	F	IM-517	8954	F						
IM-446	6649	E	IM-482	2149	F	IM-518	9300	F						
IM-447	6952	E	IM-483	2186	F	IM-519	11545	F						
IM-448	7769	E	IM-484	2207	F	IM-520	11717	F						
IM-449	8148	E	IM-485	2279	F	IM-521	13887	F						
IM-450	8260	E	IM-486	2299	F	IM-522	14073	F						
IM-451	8785	E	IM-487	2319	F	IM-523	15196	F						
IM-452	9301	E	IM-488	2412	F	IM-524	15903	F						
IM-453	10071	E	IM-489	2434	F	IM-525	22460	F						
IM-454	11721	E	IM-490	2467	F	IM-526	23135	F						
IM-455	13910	E	IM-491	2485	F	IM-527	28135	F						
IM-456	15919	E	IM-492	2582	F	IM-528	33577	F						
IM-457	22422	E	IM-493	2605	F	IM-529	39813	F						
IM-458	28233	E	IM-494	2697	F	IM-530	42344	F						
IM-459	33490	E	IM-495	2751	F	IM-531	43274	F						
IM-460	43121	E	IM-496	2865	F	IM-532	44345	F						
IM-461	44558	E	IM-497	3036	F	IM-533	51007	F						
IM-462	46694	E	IM-498	3151	F	IM-534	56318	F						
IM-463	50954	E	IM-499	3372	F	IM-535	60079	F						
IM-464	54478	E	IM-500	3440	F	IM-536	66690	F						
IM-465	60041	E	IM-501	3488	F	IM-537	75122	F						
IM-466	66652	E	IM-502	3717	F	IM-538	78429	F						
IM-467	75580	E	IM-503	3890	F	IM-539	81249	F						
IM-468	79463	E	IM-504	4155	F	IM-540	89384	F						

FIGURE 2

RANK	MW	MARKER ID	RANK	MW	MARKER ID
1	14073	IM-522	39	117132	IM-473
2	11705	IM-273	40	13727	IM-360
3	11717	IM-520	41	4257	IM-435
4	11545	IM-519	42	5349	IM-150
5	11721	IM-454	43	5364	IM-151
6	4473	IM-507	44	2967	IM-110
7	13887	IM-521	45	6122	IM-51
8	5272	IM-148	46	6958	IM-163
9	5365	IM-266	47	4355	IM-437
10	75122	IM-537	48	160768	IM-546
11	100270	IM-471	49	5554	IM-153
12	5862	IM-510	50	7767	IM-268
13	132711	IM-544			
14	132843	IM-474			
15	5876	IM-155			
16	5932	IM-157			
17	11711	IM-176			
18	5911	IM-445			
19	11914	IM-177			
20	4486	IM-440			
21	79463	IM-468			
22	4369	IM-438			
23	100310	IM-542			
24	11671	IM-359			
25	4277	IM-436			
26	2752	IM-106			
27	13910	IM-455			
28	5862	IM-444			
29	5988	IM-158			
30	5347	IM-265			
31	5918	IM-50			
32	6137	IM-159			
33	5916	IM-156			
34	4470	IM-439			
35	5931	IM-157			
36	4631	IM-508			
37	7772	IM-514			
38	176635	IM-478			

[illegible]

[illegible]

Figure 3D

WM-153 B	78769	78791	78804	78916	78983	78995	79003
WM-154 B	89208	89436	89215	89201	89531	89295	89585
WM-155 B	94635	94824	94657	94638	94654	94590	94583
WM-156 B	99871	100030	99922	99949	100059	100005	100082
WM-157 B	107055	107058	107066	107049	107052	107069	107061
WM-158 B	110703	110858	110369	110683	110657	110383	110601
WM-159 B	117776	117850	117925	117904	117962	117940	117940
WM-160 B	132587	132587	132591	132586	132602	132608	132612
WM-161 B	144519	144275	144694	144428	144770	144753	145063
WM-162 B	158266	158834	158780	159132	158659	158020	158971
WM-163 B	174576	174729	174503	174900	175470	175520	175363
WM-164 B	197048	197048	197425	197322	197080	197100	197052
WM-165 C	2011	2011	2032	2010	2010	2011	2010
WM-166 C	2032	2032	2032	2032	2032	2032	2032
WM-167 C	2078	2078	2077	2077	2079	2078	2079
WM-168 C	2168	2166	2166	2167	2167	2168	2185
WM-169 C	2183	2183	2183	2184	2184	2183	2210
WM-170 C	2211	2210	2212	2211	2211	2211	2228
WM-171 C	2228	2225	2226	2226	2228	2228	2278
WM-172 C	2278	2281	2275	2277	2278	2277	2297
WM-173 C	2296	2294	2302	2296	2297	2297	2353
WM-174 C	2352	2354	2354	2353	2353	2353	2430
WM-175 C	2426	2428	2431	2428	2429	2427	2474
WM-176 C	2479	2480	2487	2479	2474	2460	2538
WM-177 C	2539	2539	2542	2538	2539	2538	2582
WM-178 C	2580	2581	2585	2581	2580	2580	2637
WM-179 C	2636	2637	2639	2636	2636	2636	2751
WM-180 C	2750	2750	2757	2750	2750	2751	2866
WM-181 C	2864	2865	2864	2864	2865	2864	2935
WM-182 C	2934	2935	2933	2934	2935	2934	3083
WM-183 C	3083	3084	3080	3081	3084	3082	3153
WM-184 C	3144	3144	3144	3151	3154	3153	3282
WM-185 C	3151	3154	3158	3282	3282	3281	3445
WM-186 C	3283	3283	3280	3284	3445	3441	3554
WM-187 C	3446	3447	3460	3443	3554	3556	3686
WM-188 C	3555	3557	3565	3559	3685	3685	3813
WM-189 C	3686	3687	3686	3687	3811	3814	3837
WM-190 C	3817	3817	3813	3814	3836	3842	4130
WM-191 C	3839	3843	3844	3839	4134	4141	4211
WM-192 C	3948	3951	3954	4144	4210	4210	4287
WM-193 C	4141	4132	4204	4206	4285	4285	4410
WM-194 C	4213	4214	4287	4286	4410	4410	4801
WM-195 C	4289	4287	4413	4413	4779	4779	6632
WM-196 C	4417	4413	4795	4793	6627	6634	7775
WM-197 C	4789	4795	6630	6632	7765	7768	8138
WM-198 C	6632	6632	7765	7765	8146	8138	9185
WM-199 C	7771	7774	7751	7767	9184	9186	9288
WM-200 C	8141	8151	8149	8146	9305	9288	11705
WM-201 C	9185	9187	9227	9300	11697	11701	
WM-202 C	9306	9303	9227	9300			
WM-203 C							

WM-204	14093	14088	14080	14079	14066	14066	14086	14086	14086
WM-205	15148	15148	15146	15139	15667	15667	15130	15139	15139
WM-206	15882	15870	15882	15882	15867	15867	15864	15867	15866
WM-207	17385	17387	17387	17376	17369	17369	17389	17337	17337
WM-208	22236	22250	22258	22258	22250	22250	22252	22246	22266
WM-209	28110	28117	28123	28112	28128	28128	28140	28114	28129
WM-210	33310	33315	33323	33325	33320	33320	33316	33314	33339
WM-211	37250	37214	37276	37215	37278	37278	37293	37205	37270
WM-212	44511	44528	44513	44515	44444	44444	44503	44542	44518
WM-213	47360	47378	47424	47424	47424	47424	47391	47384	47423
WM-214	51236	51236	51280	51284	51287	51287	51297	51237	51359
WM-215	56204	56260	56240	56290	56176	56176	56212	56298	56307
WM-216	59753	59753	59764	59738	59695	59695	59710	59758	59768
WM-217	66438	66446	66446	66443	66391	66391	66463	66447	66462
WM-218	74748	74748	74685	74702	74791	74791	74780	74823	74897
WM-219	78816	78816	78689	78736	78835	78835	78892	78946	78946
WM-220	80232	80408	80450	80375	80380	80380	80275	80557	80468
WM-221	83685	83637	83699	83617	83687	83687	83674	83506	83436
WM-222	88917	88928	88973	88973	88893	88893	88893	88960	88960
WM-223	94672	94716	94702	94625	94624	94617	94736	94736	94736
WM-224	98619	98668	98713	98739	98685	98685	98738	98763	98796
WM-225	110774	110758	110684	110795	110925	110925	110629	110738	110615
WM-226	118513	118193	118236	118237	118243	118243	117858	118098	118344
WM-227	132423	132427	132440	132417	132434	132434	132426	132417	132435
WM-228	145134	145087	145761	145642	145400	145400	145157	145477	145594
WM-229	154912	154930	154787	154931	155086	154986	155075	155075	155075
WM-230	165497	165378	165442	165399	165420	165420	165374	165437	165437
WM-231	177303	177164	177360	177206	177355	177355	177339	177058	176677
WM-232	181213	181213	181213	181213	181213	181213	181213	181213	181213
WM-233	196920	196888	197088	197005	196998	196998	197049	196911	196871
WM-234	2011	2011	2011	2010	2009	2009	2010	2010	2012
WM-235	2032	2032	2032	2032	2029	2029	2030	2032	2033
WM-236	2054	2054	2052	2053	2050	2050	2052	2053	2054
WM-237	2078	2078	2078	2080	2078	2078	2077	2078	2078
WM-238	2168	2168	2167	2167	2167	2167	2167	2168	2167
WM-239	2185	2185	2185	2184	2185	2185	2183	2185	2185
WM-240	2212	2212	2211	2212	2208	2208	2210	2211	2212
WM-241	2230	2230	2230	2231	2228	2228	2229	2229	2229
WM-242	2256	2256	2255	2256	2254	2254	2254	2255	2255
WM-243	2277	2277	2278	2277	2276	2276	2278	2278	2278
WM-244	2302	2299	2298	2300	2287	2287	2298	2299	2299
WM-245	2357	2354	2353	2354	2352	2352	2354	2352	2354
WM-246	2427	2428	2428	2428	2427	2427	2427	2428	2428
WM-247	2483	2481	2480	2483	2478	2478	2479	2481	2481
WM-248	2500	2499	2489	2500	2488	2488	2500	2500	2499
WM-249	2544	2538	2543	2531	2542	2542	2542	2542	2542
WM-250	2578	2578	2637	2637	2579	2581	2579	2637	2637
WM-251	2635	2637	2637	2637	2636	2636	2637	2637	2637
WM-252	2754	2751	2749	2754	2748	2748	2749	2749	2750
WM-253	2868	2865	2863	2867	2862	2862	2864	2864	2864
WM-254	2935	2937	2935	2935	2935	2935	2936	2936	2936

WM-255	D	3084	3082	3085	3080	3082	3083	3083	3083
WM-256	D	3158	3157	3159	3155	3157	3154	3153	3155
WM-257	D	3281	3282	3283	3285	3286	3284	3283	3283
WM-258	D	3376		3368			3370	3369	3369
WM-259	D	3445	3446	3443	3442	3444	3446	3444	3444
WM-260	D	3558	3556	3558	3556	3557	3559	3557	3557
WM-261	D	3688	3688	3688	3684	3684	3688	3688	3686
WM-262	D	3815	3815	3816	3813	3814	3816	3814	3814
WM-263	D	3845	3842	3843	3839	3839	3841	3842	3841
WM-264	D	3957	3953	3953	3949	3950	3951	3950	3950
WM-265	D	4059					4052	4051	
WM-266	D	4132	4133	4131	4129	4133	4129	4129	4130
WM-267	D	4212	4207	4202	4207	4208	4208	4208	4206
WM-268	D	4417							4410
WM-269	D	4784	4795	4801	4796	4801	4797	4795	4795
WM-270	D	6436	6437	6435	6432	6436	6437	6437	6437
WM-271	D	6646	6638	6633	6632	6635	6637	6636	6630
WM-272	D	6959	6949	6947	6941	6944	6951	6948	6948
WM-273	D	7573	7574	7571	7574	7571	7572	7572	7573
WM-274	D	7776	7760	7762	7762	7771	7767	7764	7764
WM-275	D	7944	7943	7946			7944	7948	7947
WM-276	D	11503	11456				11498	11500	11497
WM-277	D	11688	11678	11673	11652	11676	11679	11679	11679
WM-278	D	13779	13778	13777	13771	13776	13774	13774	13773
WM-279	D	13889	13886	13885	13871	13875	13889	13889	13892
WM-280	D	15142	15138	15144	15126	15134	15137	15137	15140
WM-281	D	15879	15874	15885	15880	15883	15891	15888	
WM-282	D	17347	17322	17345	17341	17349	17323	17346	
WM-283	D	22275	22277	22274	22288	22277	22282	22297	22297
WM-284	D	28121	28135	28129	28130	28119	28138	28135	28135
WM-285	D	33372	33384	33330	33347	33336	33367	33367	
WM-286	D	37251	37272	37271	37381	37306	37278	37232	
WM-287	D	40261	40265	40259	40264	40251	40274	40266	
WM-288	D	41131	41135	41133	40304	40255	40320	40265	
WM-289	D	44571	44493	44493	44502	44519	44162	44190	
WM-290	D	51239	51228	51224	51224	51209	51236	51262	
WM-291	D	59648	59659	59885	59584	59614	59655	59667	
WM-292	D	66475	66487	66404	66470	66460	66446	66493	
WM-293	D	73907	73753	73556	73587	73587	73587	73596	
WM-294	D	75077	75040	75064	75060	75133	73775		
WM-295	D	78048	78098				78098	78103	
WM-296	D	80309	80529	80224	80234	80226	80448	80423	
WM-297	D	81525	81643	81600			81559	81650	
WM-298	D	88910	88990	88949	88857	88875	88967	89035	
WM-299	D	94508	94554	94451	94328	94400	94540	94543	
WM-300	D	99811	99766	99712	99757	99752	99820	99733	
WM-301	D	107098	107205	107177	107007	107003	107188	107159	
WM-302	D	110406	110412	110664	110654	110654	110341	110405	
WM-303	D	117373	117342	117259	117365	117365	117260	117271	
WM-304	D	132451	132443	132443	132441	132438	132451	132469	
WM-305	D	146222	145604	146253	145734	145982	145763	145819	

Figure 3c

WM-306	D	154336	160431	153873	153878	160347	160810
WM-307	D	160515	160442	160442	160441	165189	165663
WM-308	D	165342	165737	165648	165672	175446	175606
WM-309	D	175807	175478	174282	175447	181799	181902
WM-310	D	182482	182438	182273	181723	2010	2011
WM-311	E	2010	2011	2029	2029	2031	2039
WM-312	E	2029	2029	2029	2029	2031	2039
WM-313	E	2050	2052	2052	2051	2052	2052
WM-314	E	2067	2068	2067	2067	2066	2067
WM-315	E	2081	2081	2083	2081	2081	2081
WM-316	E	2126	2127	2166	2165	2125	2125
WM-317	E	2166	2167	2166	2165	2164	2167
WM-318	E	2186	2186	2186	2185	2165	2161
WM-319	E	2183	2212	2211	2211	2212	2212
WM-320	E	2231	2231	2231	2231	2231	2233
WM-321	E	2276	2278	2278	2276	2276	2279
WM-322	E	2295	2295	2295	2295	2295	2295
WM-323	E	2311	2312	2306	2295	2291	2311
WM-324	E	2389			2389	2305	
WM-325	E	2481	2474	2481	2481	2481	2481
WM-326	E	2499	2500	2499	2499	2500	2500
WM-327	E	2567	2568	2567	2567	2567	2567
WM-328	E	2581	2581	2580	2580	2582	2581
WM-329	E	2737	2736	2736	2736	2865	2865
WM-330	E	2862	2859	2865	2865	3147	3147
WM-331	E	3157	3158	3157	3158	3146	3147
WM-332	E	3321	3315	3315	3315	3316	3318
WM-333	E	3443	3442	3439	3439	3439	3439
WM-334	E	3818	3817	3816	3816	3817	3816
WM-335	E	3882	3884	3884	3884	3884	3886
WM-336	E	4054	4054	4055	4055	4054	4053
WM-337	E	4146			4056	4147	
WM-338	E	4211	4211	4205	4210	4214	4208
WM-339	E	4256	4254	4259	4260	4257	4256
WM-340	E	4355	4356	4356	4356	4359	4353
WM-341	E	4469	4469	4469	4472	4468	4468
WM-342	E	4548	4541	4540	4543	4540	4537
WM-343	E	4628	4628	4624	4631	4632	4625
WM-344	E	4821	4820				
WM-345	E	4856	4856				
WM-346	E	5070	5070	5068	4956	5068	4954
WM-347	E	5846	5857	5846	5069	5066	5066
WM-348	E	6461	6461		5852	5864	5849
WM-349	E	6643	6644	6642	6644	6461	
WM-350	E	6679	6681	6680	6642	6645	6643
WM-351	E	6855	6855	6855	6677	6684	6678
WM-352	E	6949	6949	6951	6863	6884	6850
WM-353	E	7193	7193	6945	6857	6847	6951
WM-354	E	7770	7770	7191	6945	6949	
WM-355	E	8148	7776	7768	7193	7778	7769
WM-356	E	8701	8701	8702	7776	8147	8147
					8704	8700	8701

Figure 3H

WM-357 E	8828	8834	8828	8827	8831	8830	8830
WM-358 E	8949	8955	8955	8952	8948	8948	8948
WM-359 E	9174	9176	9176	9176	9194	9194	9194
WM-360 E	9302	9300	9299	9301	9301	9297	9296
WM-361 E	9470	9470	9454	9452	9470	9466	9477
WM-362 E	11530	11515	11532	11520	11533	11527	11527
WM-363 E	11714	11699	11687	11687	11714	11703	11716
WM-364 E	12462	12450	12464	12452	12461	12460	12458
WM-365 E	12614	12622	12613	12622	12617	12625	12618
WM-366 E	13897	13876	13888	13896	13901	13883	13887
WM-367 E	15151	15150	15154	15154	15155	15160	15154
WM-368 E	15891	15891	15901	15899	15899	15896	15896
WM-369 E	17354	17370	17320	17381	17363	17361	17365
WM-370 E	22369	22345	22349	22322	22364	22409	22332
WM-371 E	28169	28161	28162	28185	28183	28154	28172
WM-372 E	33429	33451	33431	33426	33425	33413	33428
WM-373 E	39455	39235	44627	44591	44608	44651	39238
WM-374 E	44837	44596	51357	51429	51507	51400	44675
WM-375 E	51376	51378	59228	59847	59920	59747	59799
WM-376 E	59221	59842	66572	68568	68581	66590	66604
WM-377 E	66557	66538	75365	75201	75468	75469	75413
WM-378 E	75385	75469	79334	79836	79378	79378	79327
WM-379 E	79373	79334	79388	79836	89421	89542	89361
WM-380 E	89514	89266	89490	89370	89421	89542	89361
WM-381 E	89411	89266	89490	89370	89421	89542	89361
WM-382 E	89991	89991	89978	89552	100146	100028	100001
WM-383 E	100004	100004	109994	109816	110474	110414	109921
WM-384 E	110157	110157	117748	117739	117699	117802	117465
WM-385 E	117783	117603	132628	132627	132657	132642	132642
WM-386 E	146553	146507	146553	146206	145912	146010	146653
WM-387 E	152936	154171	165192	165727	152549	153495	152534
WM-388 E	165680	165723	182289	182289	165692	165920	165575
WM-389 E	182591	182524	182289	182289	183093	183366	183366
WM-390 F	197326	197348	197206	197254	197249	197301	197301
WM-391 F	2011	2010	2010	2012	2011	2011	2011
WM-392 F	2033	2029	2028	2030	2029	2029	2029
WM-393 F	2065	2068	2049	2053	2054	2052	2067
WM-394 F	2068	2068	2067	2071	2072	2071	2080
WM-395 F	2083	2080	2083	2084	2084	2084	2080
WM-396 F	2123	2126	2126	2125	2126	2125	2126
WM-397 F	2164	2167	2167	2167	2167	2166	2166
WM-398 F	2214	2213	2214	2214	2213	2213	2213
WM-399 F	2234	2232	2233	2234	2234	2233	2234
WM-400 F	2267	2279	2277	2280	2279	2279	2279
WM-401 F	2298	2296	2297	2297	2297	2296	2296
WM-402 F	2386	2413	2390	2389	2413	2389	2412
WM-403 F	2411	2413	2412	2412	2413	2399	2412
WM-404 F	2482	2482	2479	2482	2482	2481	2482
WM-405 F	2501	2500	2500	2501	2501	2501	2500
WM-406 F	2568	2568	2570	2569	2568	2568	2568
WM-407 F	2581	2582	2582	2582	2581	2582	2582
	2697	2697	2700	2695	2696	2696	

WM-408	F	2722	2723	2724	2725	2726	2727	2728	2729	2730	2731	2732	2733	2734	2735	2736	2737	2738	2739	2740	2741	2742	2743	2744	2745	2746	2747	2748	2749	2750	2751	2752	2753	2754	2755	2756	2757	2758	2759	2760	2761	2762	2763	2764	2765	2766	2767	2768	2769	2770	2771	2772	2773	2774	2775	2776	2777	2778	2779	2780	2781	2782	2783	2784	2785	2786	2787	2788	2789	2790	2791	2792	2793	2794	2795	2796	2797	2798	2799	2800																																																																																	
WM-409	F	2751	2752	2753	2754	2755	2756	2757	2758	2759	2760	2761	2762	2763	2764	2765	2766	2767	2768	2769	2770	2771	2772	2773	2774	2775	2776	2777	2778	2779	2780	2781	2782	2783	2784	2785	2786	2787	2788	2789	2790	2791	2792	2793	2794	2795	2796	2797	2798	2799	2800	2801	2802	2803	2804	2805	2806	2807	2808	2809	2810	2811	2812	2813	2814	2815	2816	2817	2818	2819	2820																																																																																										
WM-410	F	2866	2867	2868	2869	2870	2871	2872	2873	2874	2875	2876	2877	2878	2879	2880	2881	2882	2883	2884	2885	2886	2887	2888	2889	2890	2891	2892	2893	2894	2895	2896	2897	2898	2899	2900	2901	2902	2903	2904	2905	2906	2907	2908	2909	2910	2911	2912	2913	2914	2915	2916	2917	2918	2919	2920	2921	2922	2923	2924	2925	2926	2927	2928	2929	2930	2931	2932	2933	2934	2935	2936	2937	2938	2939	2940	2941	2942	2943	2944	2945	2946	2947	2948	2949	2950	2951	2952	2953	2954	2955	2956	2957	2958	2959	2960	2961	2962	2963	2964	2965	2966	2967	2968	2969	2970	2971	2972	2973	2974	2975	2976	2977	2978	2979	2980	2981	2982	2983	2984	2985	2986	2987	2988	2989	2990	2991	2992	2993	2994	2995	2996	2997	2998	2999	3000																									
WM-411	F	2879	2880	2881	2882	2883	2884	2885	2886	2887	2888	2889	2890	2891	2892	2893	2894	2895	2896	2897	2898	2899	2900	2901	2902	2903	2904	2905	2906	2907	2908	2909	2910	2911	2912	2913	2914	2915	2916	2917	2918	2919	2920	2921	2922	2923	2924	2925	2926	2927	2928	2929	2930	2931	2932	2933	2934	2935	2936	2937	2938	2939	2940	2941	2942	2943	2944	2945	2946	2947	2948	2949	2950	2951	2952	2953	2954	2955	2956	2957	2958	2959	2960	2961	2962	2963	2964	2965	2966	2967	2968	2969	2970	2971	2972	2973	2974	2975	2976	2977	2978	2979	2980	2981	2982	2983	2984	2985	2986	2987	2988	2989	2990	2991	2992	2993	2994	2995	2996	2997	2998	2999	3000																																						
WM-412	F	3158	3159	3160	3161	3162	3163	3164	3165	3166	3167	3168	3169	3170	3171	3172	3173	3174	3175	3176	3177	3178	3179	3180	3181	3182	3183	3184	3185	3186	3187	3188	3189	3190	3191	3192	3193	3194	3195	3196	3197	3198	3199	3200	3201	3202	3203	3204	3205	3206	3207	3208	3209	3210	3211	3212	3213	3214	3215	3216	3217	3218	3219	3220	3221	3222	3223	3224	3225	3226	3227	3228	3229	3230	3231	3232	3233	3234	3235	3236	3237	3238	3239	3240	3241	3242	3243	3244	3245	3246	3247	3248	3249	3250	3251	3252	3253	3254	3255	3256	3257	3258	3259	3260	3261	3262	3263	3264	3265	3266	3267	3268	3269	3270	3271	3272	3273	3274	3275	3276	3277	3278	3279	3280	3281	3282	3283	3284	3285	3286	3287	3288	3289	3290	3291	3292	3293	3294	3295	3296	3297	3298	3299	3300																	
WM-413	F	3373	3374	3375	3376	3377	3378	3379	3380	3381	3382	3383	3384	3385	3386	3387	3388	3389	3390	3391	3392	3393	3394	3395	3396	3397	3398	3399	3400	3401	3402	3403	3404	3405	3406	3407	3408	3409	3410	3411	3412	3413	3414	3415	3416	3417	3418	3419	3420	3421	3422	3423	3424	3425	3426	3427	3428	3429	3430	3431	3432	3433	3434	3435	3436	3437	3438	3439	3440	3441	3442	3443	3444	3445	3446	3447	3448	3449	3450	3451	3452	3453	3454	3455	3456	3457	3458	3459	3460	3461	3462	3463	3464	3465	3466	3467	3468	3469	3470	3471	3472	3473	3474	3475	3476	3477	3478	3479	3480	3481	3482	3483	3484	3485	3486	3487	3488	3489	3490	3491	3492	3493	3494	3495	3496	3497	3498	3499	3500																																
WM-414	F	3441	3442	3443	3444	3445	3446	3447	3448	3449	3450	3451	3452	3453	3454	3455	3456	3457	3458	3459	3460	3461	3462	3463	3464	3465	3466	3467	3468	3469	3470	3471	3472	3473	3474	3475	3476	3477	3478	3479	3480	3481	3482	3483	3484	3485	3486	3487	3488	3489	3490	3491	3492	3493	3494	3495	3496	3497	3498	3499	3500	3501	3502	3503	3504	3505	3506	3507	3508	3509	3510	3511	3512	3513	3514	3515	3516	3517	3518	3519	3520	3521	3522	3523	3524	3525	3526	3527	3528	3529	3530	3531	3532	3533	3534	3535	3536	3537	3538	3539	3540	3541	3542	3543	3544	3545	3546	3547	3548	3549	3550	3551	3552	3553	3554	3555	3556	3557	3558	3559	3560	3561	3562	3563	3564	3565	3566	3567	3568	3569	3570	3571	3572	3573	3574	3575	3576	3577	3578	3579	3580	3581	3582	3583	3584	3585	3586	3587	3588	3589	3590	3591	3592	3593	3594	3595	3596	3597	3598	3599	3600
WM-415	F	3484	3485	3486	3487	3488	3489	3490	3491	3492	3493	3494	3495	3496	3497	3498	3499	3500	3501	3502	3503	3504	3505	3506	3507	3508	3509	3510	3511	3512	3513	3514	3515	3516	3517	3518	3519	3520	3521	3522	3523	3524	3525	3526	3527	3528	3529	3530	3531	3532	3533	3534	3535	3536	3537	3538	3539	3540	3541	3542	3543	3544	3545	3546	3547	3548	3549	3550	3551	3552	3553	3554	3555	3556	3557	3558	3559	3560	3561	3562	3563	3564	3565	3566	3567	3568	3569	3570	3571	3572	3573	3574	3575	3576	3577	3578	3579	3580	3581	3582	3583	3584	3585	3586	3587	3588	3589	3590	3591	3592	3593	3594	3595	3596	3597	3598	3599	3600																																											
WM-416	F	3580	3581	3582	3583	3584	3585	3586	3587	3588	3589	3590	3591	3592	3593	3594	3595	3596	3597	3598	3599	3600	3601	3602	3603	3604	3605	3606	3607	3608	3609	3610	3611	3612	3613	3614	3615	3616	3617	3618	3619	3620	3621	3622	3623	3624	3625	3626	3627	3628	3629	3630	3631	3632	3633	3634	3635	3636	3637	3638	3639	3640	3641	3642	3643	3644	3645	3646	3647	3648	3649	3650	3651	3652	3653	3654	3655	3656	3657	3658	3659	3660	3661	3662	3663	3664	3665	3666	3667	3668	3669	3670	3671	3672	3673	3674	3675	3676	3677	3678	3679	3680	3681	3682	3683	3684	3685	3686	3687	3688	3689	3690	3691	3692	3693	3694	3695	3696	3697	3698	3699	3700																																							
WM-417	F	3720	3721	3722	3723	3724	3725	3726	3727	3728	3729	3730	3731	3732	3733	3734	3735	3736	3737	3738	3739	3740	3741	3742	3743	3744	3745	3746	3747	3748	3749	3750	3751	3752	3753	3754	3755	3756	3757	3758	3759	3760	3761	3762	3763	3764	3765	3766	3767	3768	3769	3770	3771	3772	3773	3774	3775	3776	3777	3778	3779	3780	3781	3782	3783	3784	3785	3786	3787	3788	3789	3790	3791	3792	3793	3794	3795	3796	3797	3798	3799	3800																																																																															
WM-418	F	3888	3889	3890	3891	3892	3893	3894	3895	3896	3897	3898	3899	3900	3901	3902	3903	3904	3905	3906	3907	3908	3909	3910	3911	3912	3913	3914	3915	3916	3917	3918	3919	3920	3921	3922	3923	3924	3925	3926	3927	3928	3929	3930	3931	3932	3933	3934	3935	3936	3937	3938	3939	3940	3941	3942	3943	3944	3945	3946	3947	3948	3949	3950	3951	3952	3953	3954	3955	3956	3957	3958	3959	3960	3961	3962	3963	3964	3965	3966	3967	3968	3969	3970	3971	3972	3973	3974	3975	3976	3977	3978	3979	3980	3981	3982	3983	3984	3985	3986	3987	3988	3989	3990	3991	3992	3993	3994	3995	3996	3997	3998	3999	4000																																															
WM-419	F	4007	4008	4009	4010	4011	4012	4013	4014	4015	4016	4017	4018	4019	4020	4021	4022	4023	4024	4025	4026	4027	4028	4029	4030	4031	4032	4033	4034	4035	4036	4037	4038	4039	4040	4041	4042	4043	4044	4045	4046	4047	4048	4049	4050	4051	4052	4053	4054	4055	4056	4057	4058	4059	4060	4061	4062	4063	4064	4065	4066	4067	4068	4069	4070	4071	4072	4073	4074	4075	4076	4077	4078	4079	4080	4081	4082	4083	4084	4085	4086	4087	4088																																																																														

Figure 3k

WM-510	A					4753
WM-511	A					4800
WM-512	A					4878
WM-513	A					5097
WM-514	A					5175
WM-515	A					5211
WM-516	A					5371
WM-517	A					5572
WM-518	A					5876
WM-519	A					5995
WM-520	A					6187
WM-521	A					6490
WM-522	A					6506
WM-523	A					6841
WM-524	A					7152
WM-525	A					7192
WM-526	A					7479
WM-527	A					7650
WM-528	A					7929
WM-529	A					8329
WM-530	A					8453
WM-531	A					9100
WM-532	A					9183
WM-533	A					9511
WM-534	A					11388
WM-535	A					11648
WM-536	A					11734
WM-537	A					12714
WM-538	A					13592
WM-539	A					14073
WM-540	A					16268
WM-541	A					16750
WM-542	A					17078
WM-543	A					17432
WM-544	A					18617
WM-545	A					19391
WM-546	A					32667
WM-547	A					46716
WM-548	A					111238
WM-549	A					117808
WM-550	A					133294
WM-551	A					135741
WM-552	A					178527
WM-553	A					2035
WM-554	A					2036
WM-555	A					2032
WM-556	B					2182
WM-557	B					2183
WM-558	B					2212
WM-559	B					2411
WM-560	B					2449

WM-561	B	2540	2540	2539	2467	4877
WM-562	B				2539	
WM-563	B		2540	2978	2638	
WM-564	B	2978	2978	3182	2978	
WM-565	B	3181	3181	3274	3182	
WM-566	B			3360	3274	
WM-567	B	3360	3360	3377	3360	
WM-568	B	3376	3376		3376	
WM-569	B				3485	
WM-570	B				3558	
WM-571	B			3771	3685	
WM-572	B	3768	3772		3770	
WM-573	B				3843	
WM-574	B				3950	
WM-575	B			4091	4080	
WM-576	B	4092	4091	4148	4092	4092
WM-577	B	4146	4143	4647	4152	
WM-578	B	4647	4647	4762	4647	
WM-579	B	4790	4794		4792	4872
WM-580	B			5443	4793	
WM-581	B	5442	5443	5988	5442	
WM-582	B	5987	5990	6184	5987	
WM-583	B	6187	6187	8605	6184	
WM-584	B				10663	
WM-585	B		16057		16065	
WM-586	B				17907	
WM-587	B					
WM-588	B			83665	53727	
WM-589	B		53728		83653	
WM-590	B	83912	83843		156495	
WM-591	B				171918	
WM-592	B				2051	
WM-593	C				2126	
WM-594	C	2126	2126		2255	
WM-595	C			2286		
WM-596	C			2416		
WM-597	C			2468		
WM-598	C					
WM-599	C		2457	2574	2410	
WM-600	C				2462	
WM-601	C				2485	
WM-602	C	2685		2566	2566	
WM-603	C	2724		2691	2679	
WM-604	C			2725	2725	
WM-605	C					
WM-606	C	2984	2980	2983	2979	
WM-607	C					
WM-608	C	3163		3174	3164	
WM-609	C	3178		3177	3182	
WM-610	C					
WM-611	C			3309		

[illegible]

Figure 30.



84106
88790
162376
166896

84125
88712
162354

161441

84472

84133



WM-714 F
WM-715 F
WM-716 F
WM-717 F

Figure 4A

Rank	Normal vs Cancer	Adeno vs Normal	Squamous vs Normal	Small Cell vs Normal	Non-small Cell vs Normal	Large Cell vs Normal	Adeno vs Squamous	Adeno vs Small Cell	Squamous vs Small Cell
1	WM-61	WM-447	WM-447	WM-70	WM-341	WM-16	WM-62	WM-457	WM-276
2	WM-447	WM-652	WM-61	WM-706	WM-342	WM-26	WM-415	WM-72	WM-277
3	WM-446	WM-61	WM-277	WM-369	WM-343	WM-499	WM-152	WM-369	WM-362
4	WM-133	WM-446	WM-446	WM-447	WM-48	WM-134	WM-385	WM-78	WM-257
5	WM-119	WM-290	WM-61	WM-447	WM-340	WM-647	WM-347	WM-79	WM-363
6	WM-278	WM-363	WM-134	WM-652	WM-346	WM-277	WM-134	WM-73	WM-347
7	WM-134	WM-133	WM-363	WM-282	WM-47	WM-310	WM-36	WM-64	WM-53
8	WM-363	WM-341	WM-362	WM-446	WM-339	WM-363	WM-108	WM-320	WM-254
9	WM-282	WM-285	WM-276	WM-456	WM-389	WM-446	WM-99	WM-419	WM-17
10	WM-362	WM-366	WM-706	WM-134	WM-669	WM-221	WM-151	WM-85	WM-252
11	WM-120	WM-282	WM-203	WM-203	WM-447	WM-648	WM-289	WM-82	WM-431
12	WM-290	WM-362	WM-466	WM-646	WM-652	WM-657	WM-363	WM-53	WM-513
13	WM-65	WM-310	WM-366	WM-455	WM-154	WM-290	WM-61	WM-412	WM-446
14	WM-277	WM-292	WM-65	WM-65	WM-587	WM-328	WM-117	WM-440	WM-355
15	WM-70	WM-120	WM-70	WM-685	WM-456	WM-447	WM-211	WM-455	WM-447
16	WM-369	WM-134	WM-341	WM-473	WM-450	WM-684	WM-362	WM-313	WM-133
17	WM-17	WM-276	WM-429	WM-343	WM-283	WM-183	WM-133	WM-456	WM-245
18	WM-473	WM-428	WM-347	WM-466	WM-207	WM-190	WM-414	WM-86	WM-52
19	WM-47	WM-277	WM-17	WM-341	WM-436	WM-686	WM-277	WM-70	WM-96
20	WM-203	WM-20	WM-47	WM-340	WM-384	WM-397	WM-141	WM-246	WM-238
21	WM-276	WM-119	WM-431	WM-363	WM-61	WM-466	WM-64	WM-360	WM-243
22	WM-279	WM-340	WM-62	WM-339	WM-167	WM-20	WM-135	WM-190	WM-138
23	WM-62	WM-48	WM-473	WM-457	WM-382	WM-17	WM-447	WM-418	WM-62
24	WM-366	WM-389	WM-384	WM-86	WM-285	WM-545	WM-383	WM-83	WM-580
25	WM-456	WM-450	WM-438	WM-506	WM-650	WM-47	WM-338	WM-257	WM-134
26	WM-428	WM-47	WM-652	WM-72	WM-203	WM-191	WM-63	WM-138	WM-240
27	WM-384	WM-343	WM-282	WM-287	WM-119	WM-147	WM-142	WM-47	WM-256
28	WM-287	WM-17	WM-389	WM-82	WM-282	WM-480	WM-446	WM-252	WM-203
29	WM-420	WM-593	WM-290	WM-528	WM-686	WM-590	WM-186	WM-282	WM-111
30	WM-292	WM-70	WM-278	WM-85	WM-383	WM-218	WM-111	WM-50	WM-95
31	WM-431	WM-706	WM-456	WM-73	WM-429	WM-285	WM-445	WM-58	WM-247
32	WM-455	WM-346	WM-673	WM-138	WM-11	WM-652	WM-455	WM-325	WM-157
33	WM-20	WM-466	WM-340	WM-384	WM-208	WM-651	WM-276	WM-402	WM-242
34	WM-340	WM-646	WM-55	WM-83	WM-451	WM-366	WM-444	WM-411	WM-556
35	WM-19	WM-384	WM-455	WM-450	WM-473	WM-403	WM-181	WM-405	WM-63
36	WM-389	WM-336	WM-645	WM-310	WM-220	WM-418	WM-35	WM-75	WM-239
37	WM-63	WM-294	WM-138	WM-277	WM-685	WM-430	WM-285	WM-417	WM-234
38	WM-438	WM-339	WM-420	WM-79	WM-338	WM-456	WM-456	WM-387	WM-274

Figure 4B

39	WM-450	WM-473	WM-450	WM-71	WM-714	WM-39	WM-26	WM-370
40	WM-466	WM-369	WM-207	WM-268	WM-646	WM-82	WM-410	WM-301
41	WM-296	WM-38	WM-278	WM-70	WM-109	WM-17	WM-420	WM-449
42	WM-343	WM-283	WM-290	WM-545	WM-302	WM-203	WM-164	WM-74
43	WM-341	WM-685	WM-366	WM-675	WM-587	WM-83	WM-67	WM-261
44	WM-339	WM-674	WM-472	WM-446	WM-375	WM-412	WM-66	WM-467
45	WM-55	WM-120	WM-420	WM-120	WM-131	WM-96	WM-391	WM-237
46	WM-66	WM-20	WM-147	WM-267	WM-706	WM-74	WM-340	WM-262
47	WM-48	WM-287	WM-55	WM-466	WM-398	WM-457	WM-428	WM-295
48	WM-38	WM-83	WM-669	WM-347	WM-309	WM-431	WM-198	WM-288
49	WM-138	WM-342	WM-357	WM-153	WM-55	WM-340	WM-312	WM-384
50	WM-310	WM-429	WM-279	WM-38	WM-488	WM-49	WM-152	WM-37

10 20 30 40 50 60 70 80 90 100
 RSFFSLGEAFDCARDMWRAYSDMREANYIGSDKYFHARGNYDAAKRGPGGVWAAEAISDARENIQORFFGHGAEDSLADQAANEWGRSGKDPNHFRPAGLPEKY

2803		
SAA 42-67 (2802.1)		
3168	5927	10300
SAA 69-97 (3167.3)	SAA 32-85 (5925.3)	SAA 6-97 (10299.1)
3277	6874	10866
SAA 39-68 (3276.6)	SAA 26-88 (6873.3)	SAA 4-101 (10871.8)
3552	7776	10851
SAA 38-70 (3552)	SAA 1-68 (7774.6)	SAA 5-102 (10853.7)
3897	7941	
SAA 64-98 (3897.2)	SAA 18-88 (7939.5)	
4300	8152	
SAA 54-93 (4302.5)	SAA 25-98 (8150)	
4490	8952	
SAA 53-93 (4489)	SAA 6-85 (8950)	
4655	9233	
SAA 5-44 (4655.0)	SAA 16-97 (9235)	

Figure 5

Figure 6

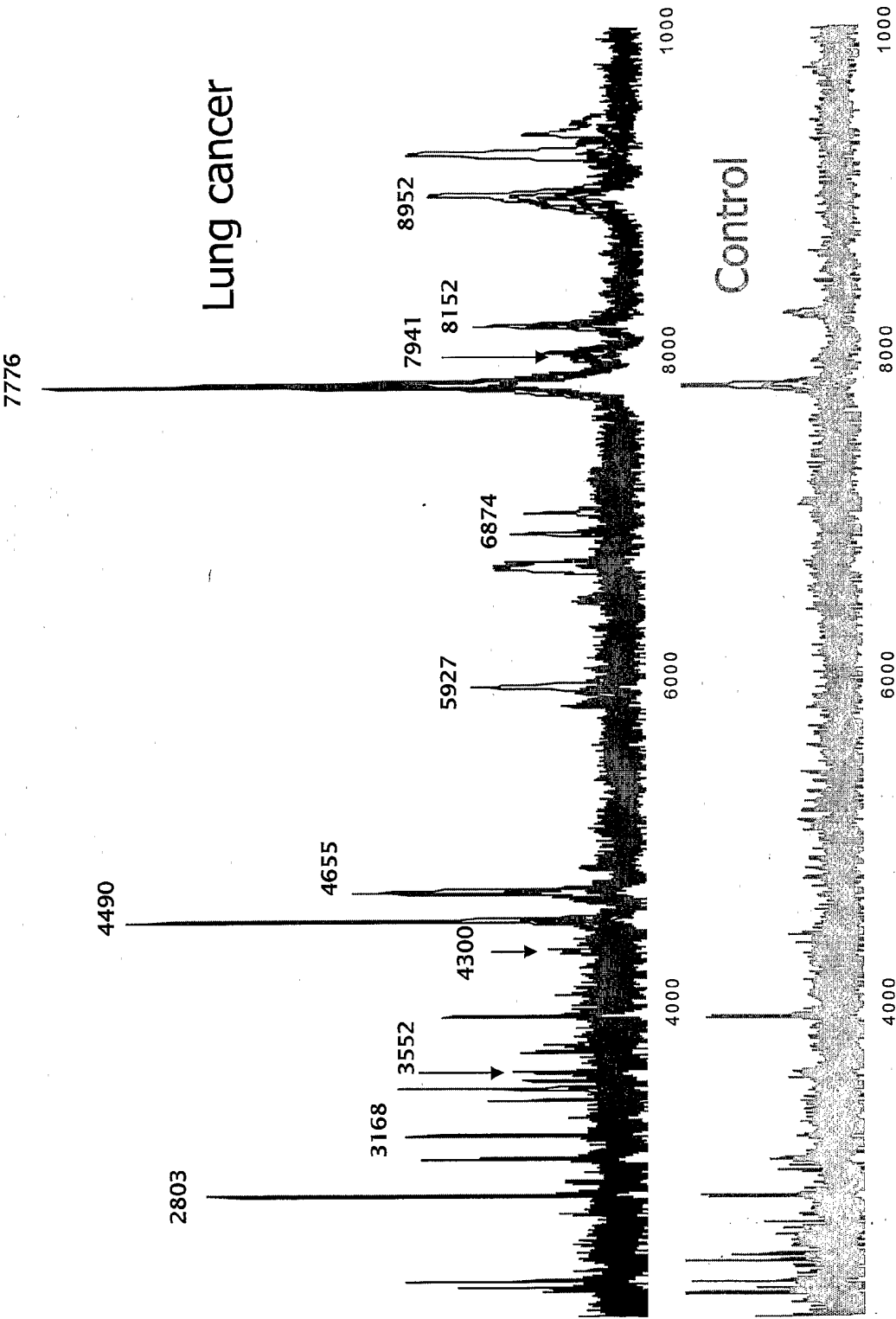


FIGURE 7
Protein Profile of Selected Samples Q Fraction 1 WCX2

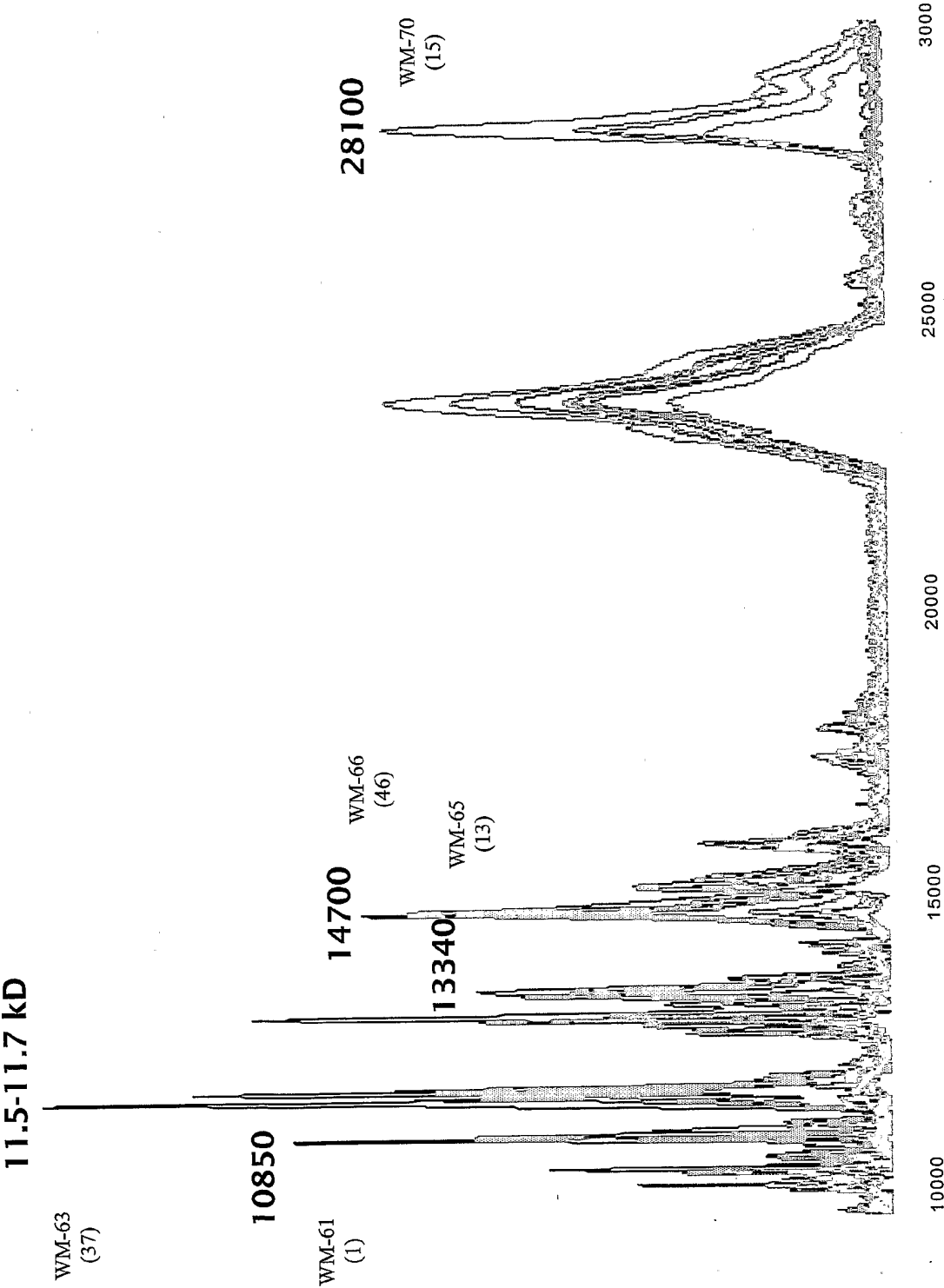


Figure 8
Protein Profile of Selected Samples
Q Fraction 1 WCX2

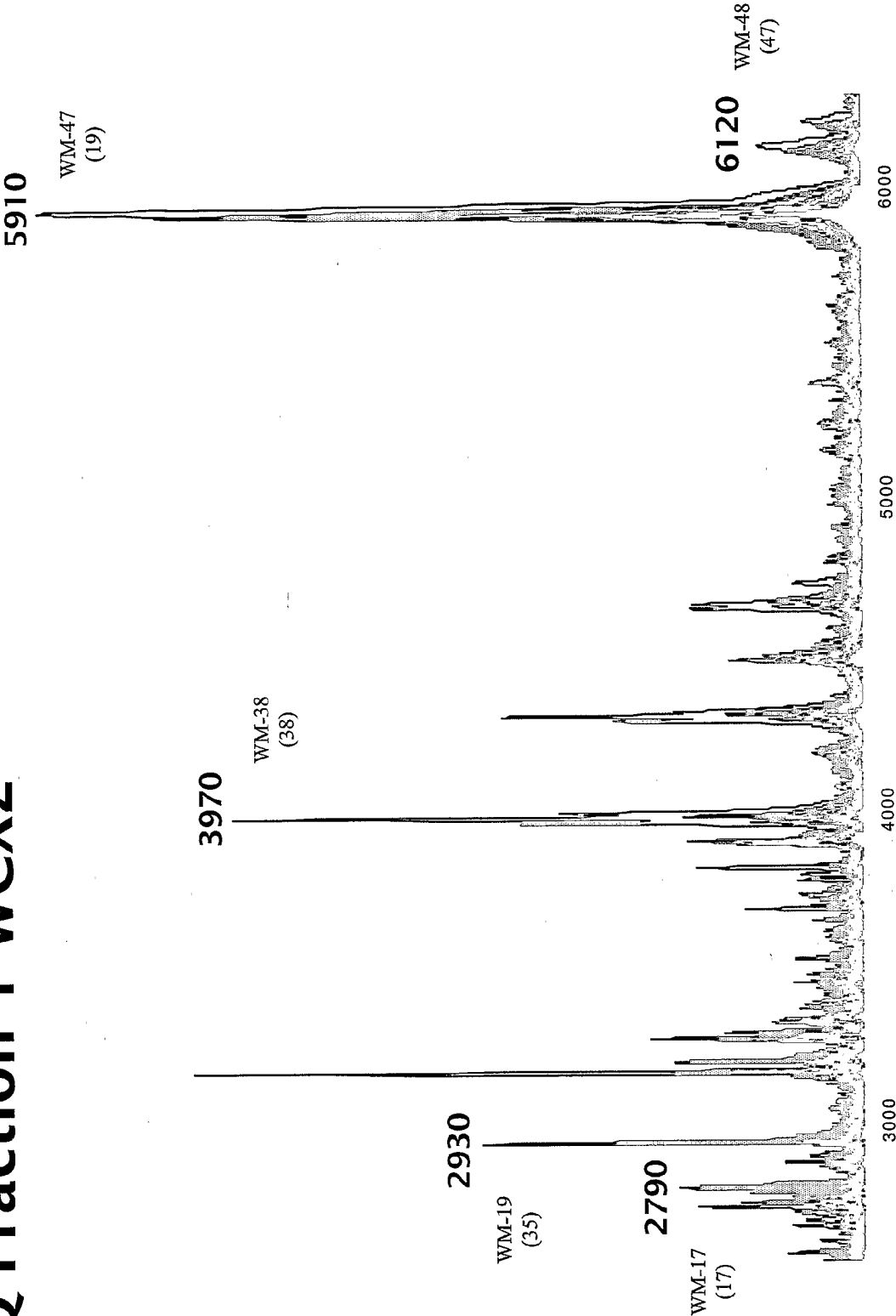


Figure 9
Protein Profile of Selected Samples
Q Fraction 2 WCX2

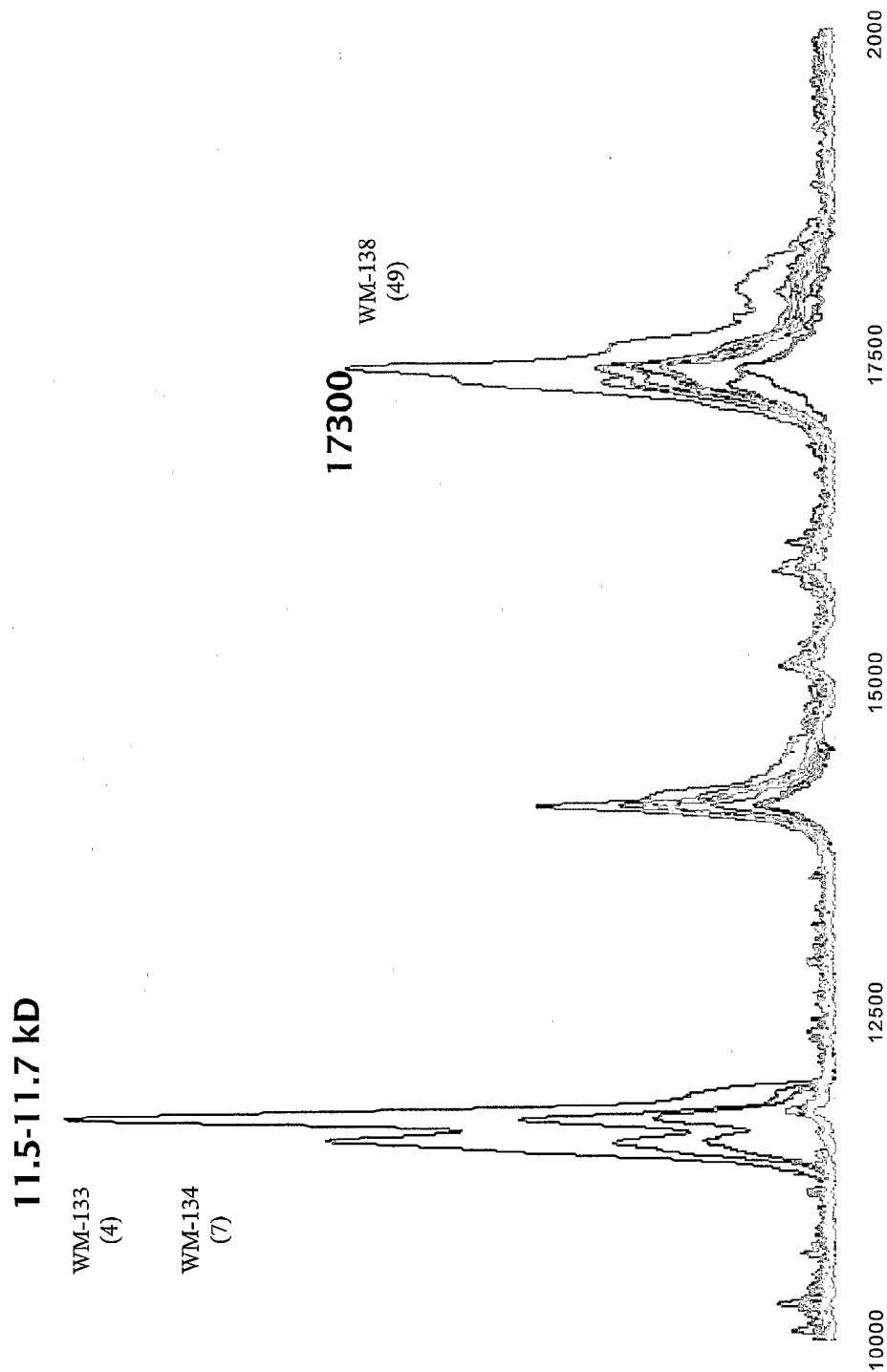


Figure 10
Protein Profile of Selected Samples
Q Fraction 2 WCX2

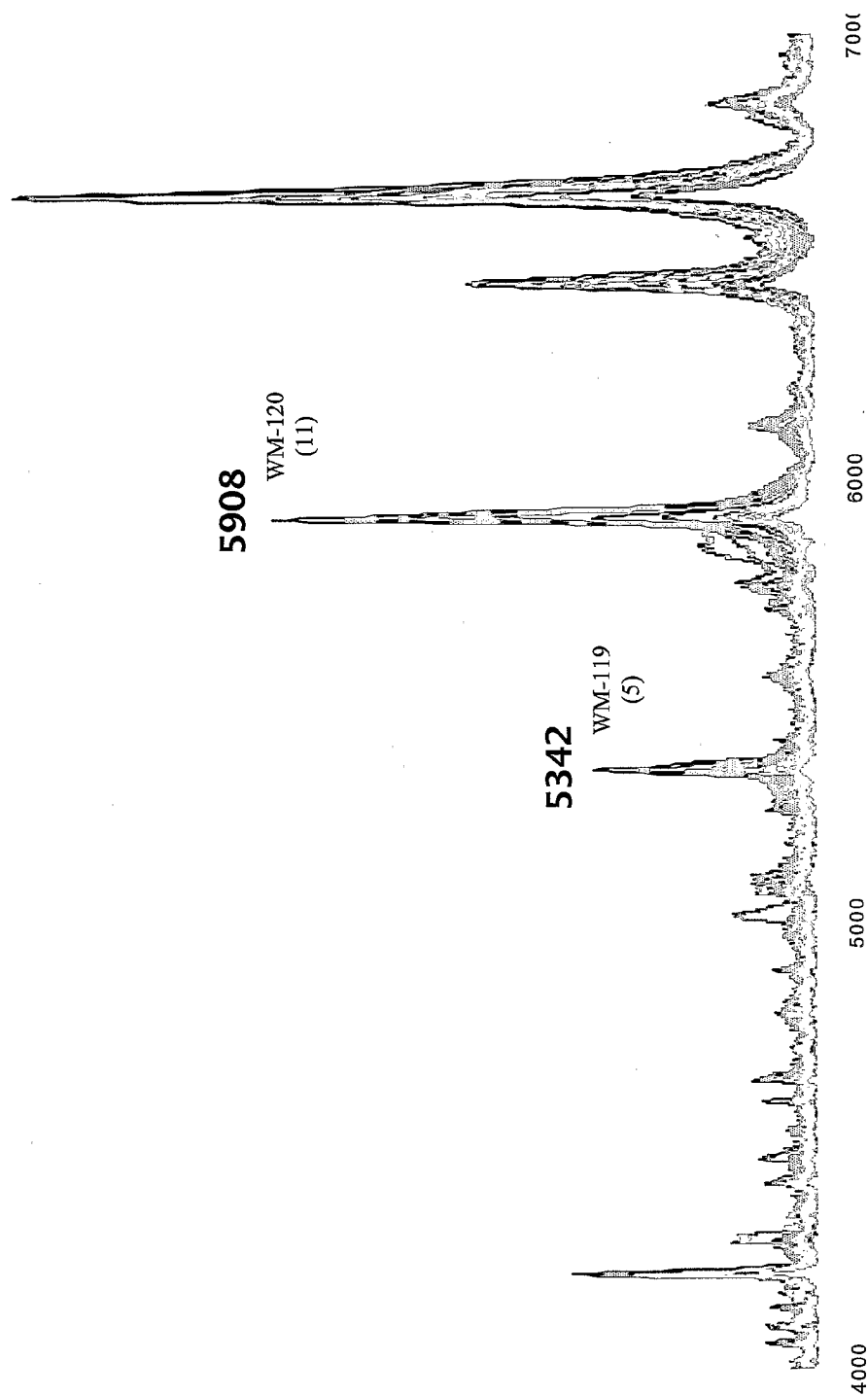


Figure 11
Protein Profile of Selected Samples
Q Fraction 4 WCX2

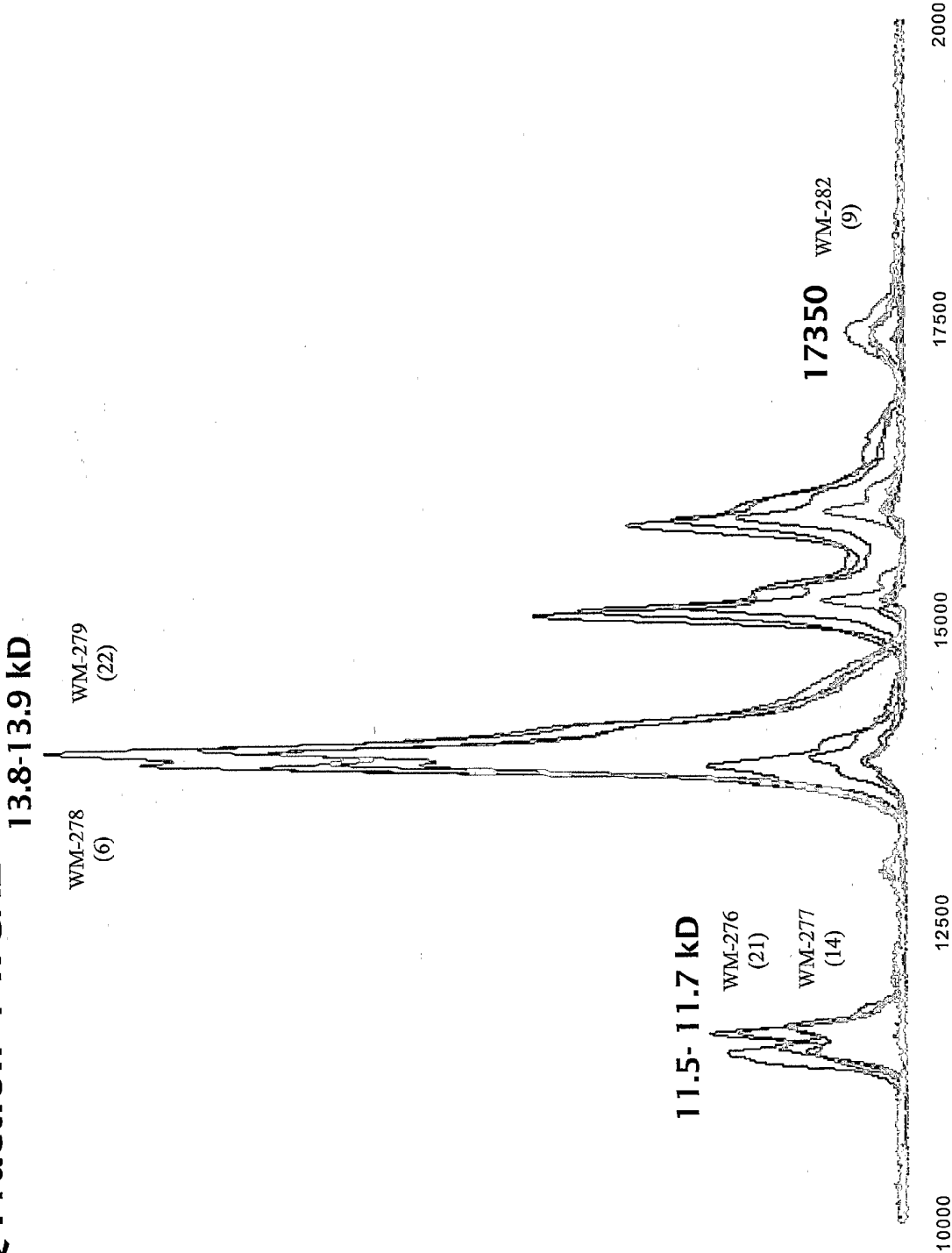


Figure 12
Protein Profile of Selected Samples
Q Fraction 4 WCX2

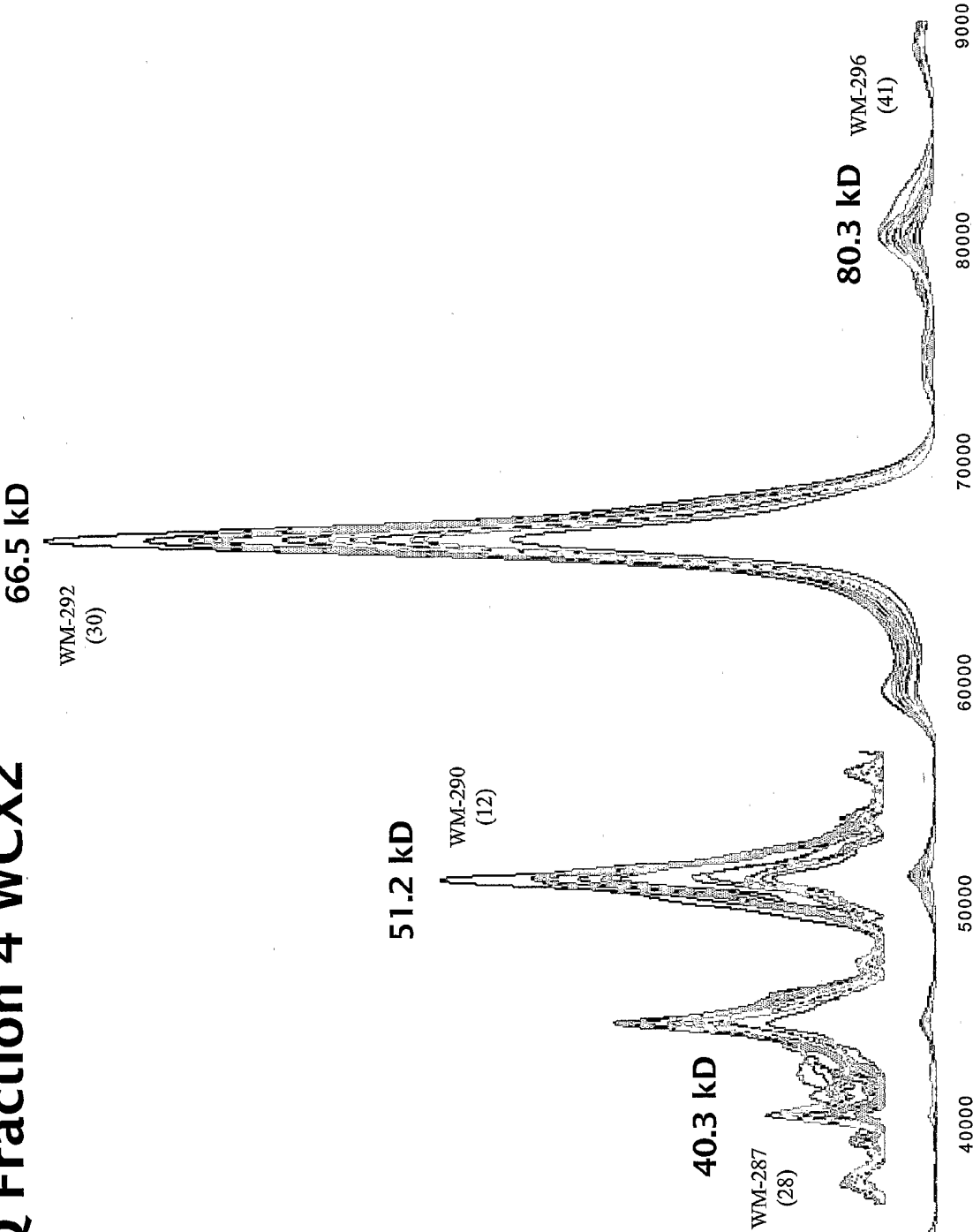


Figure 13
Protein Profile of Selected Samples
Q Fraction 5 WCX2

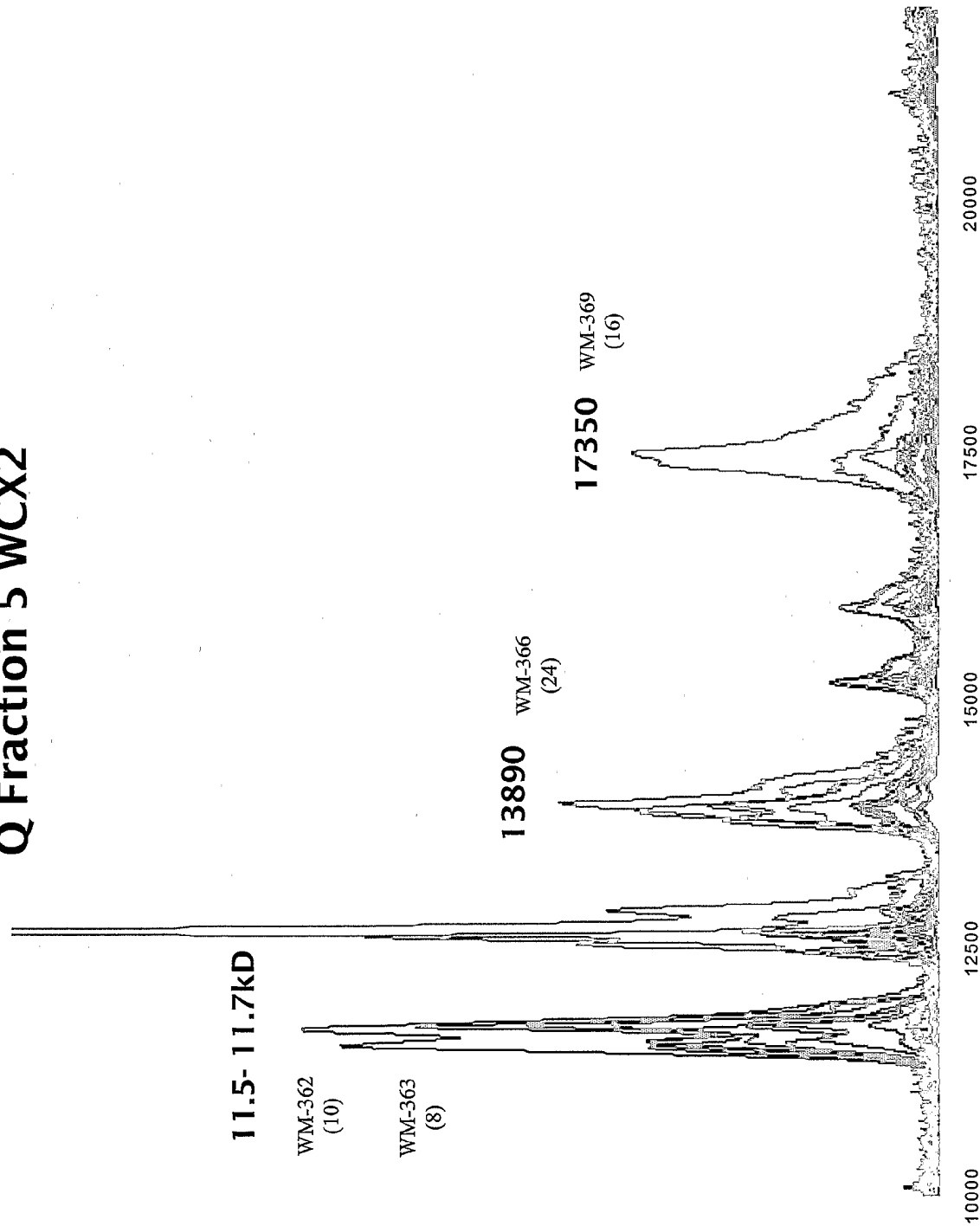


Figure 14
Protein Profile of Selected Samples
Q Fraction 5 WCX2

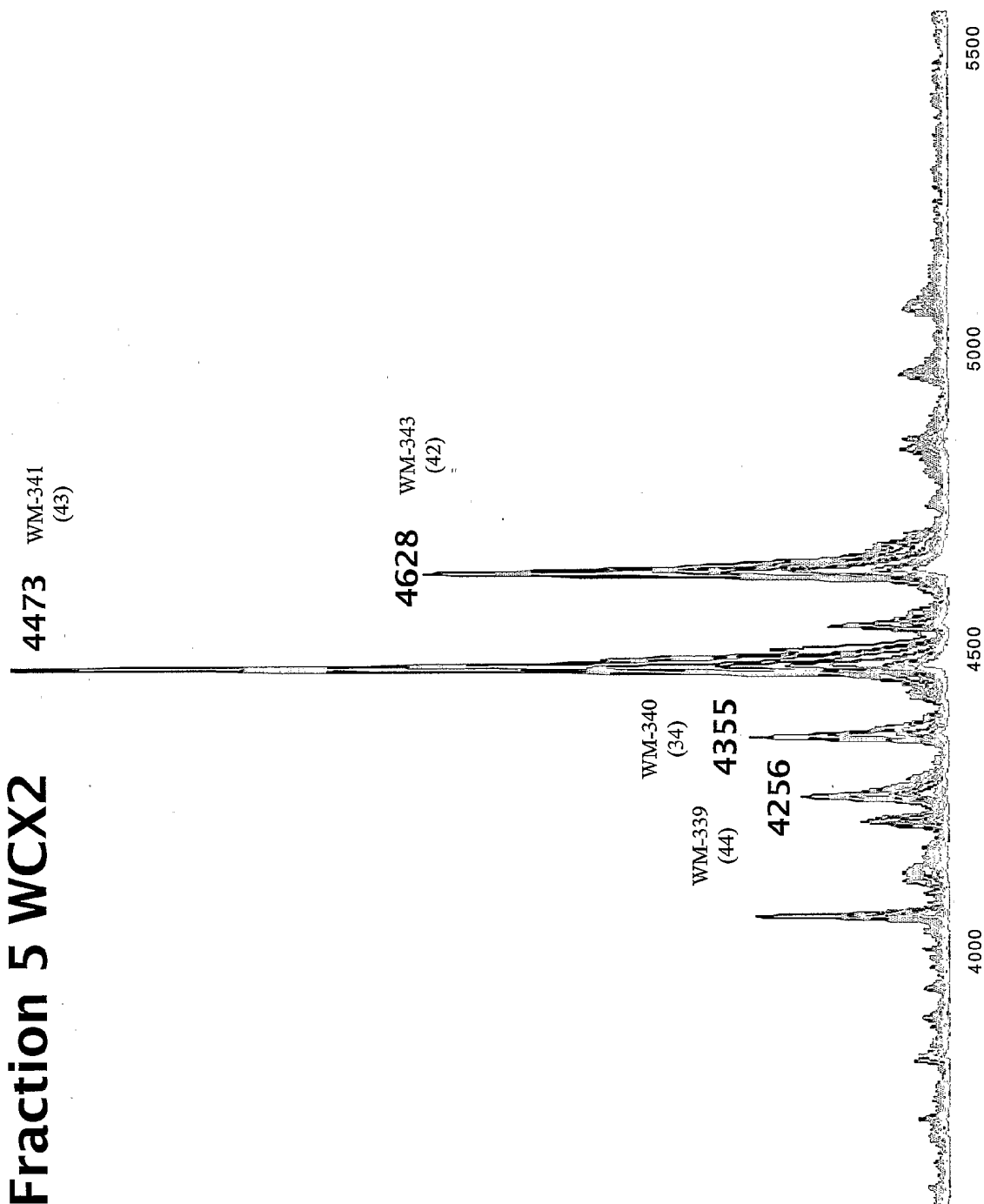


Figure 15
Protein Profile of Selected Samples
Q Fraction 6 WCX2

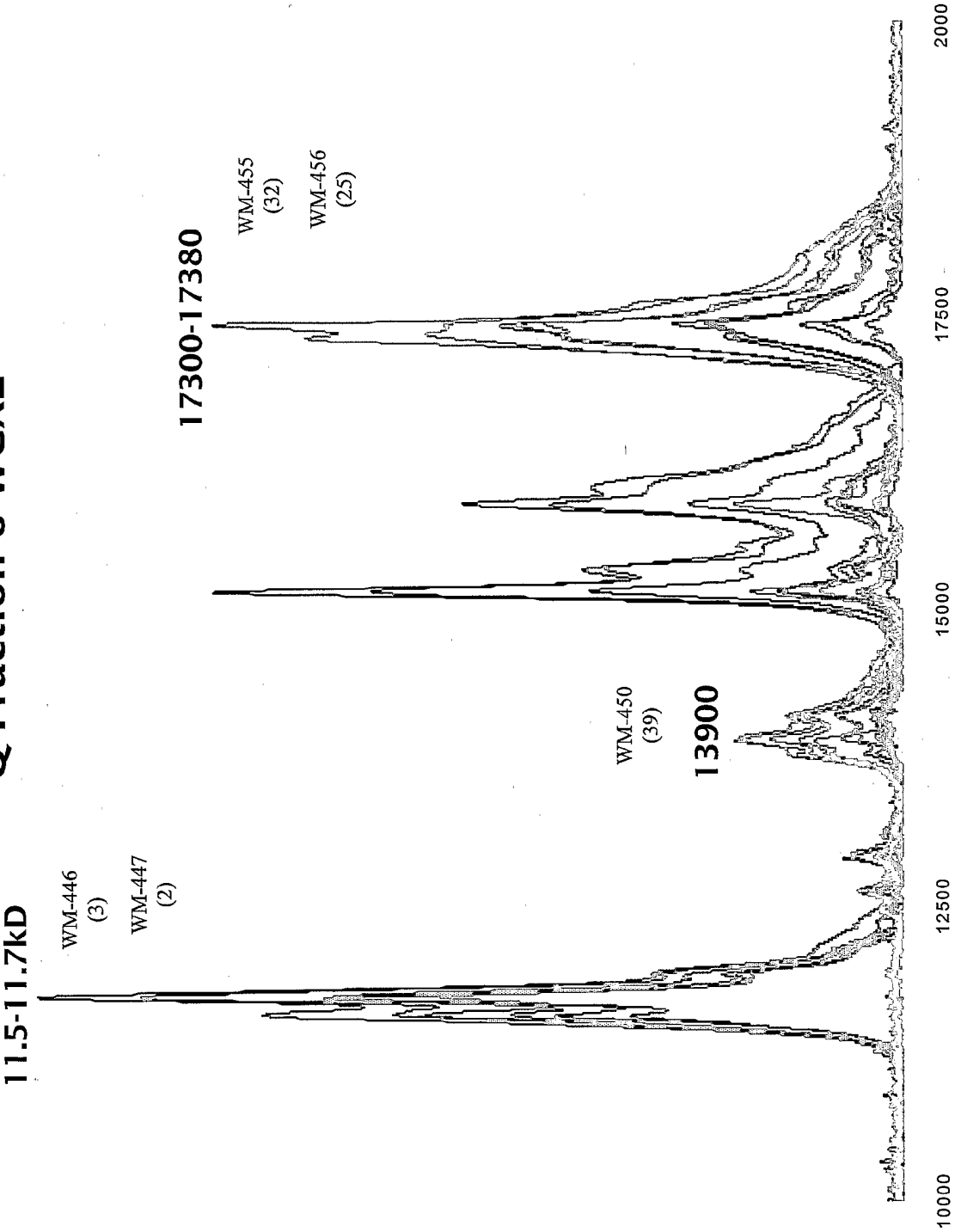


Figure 16
Protein Profile of Selected Samples
Q Fraction 6 WCX2

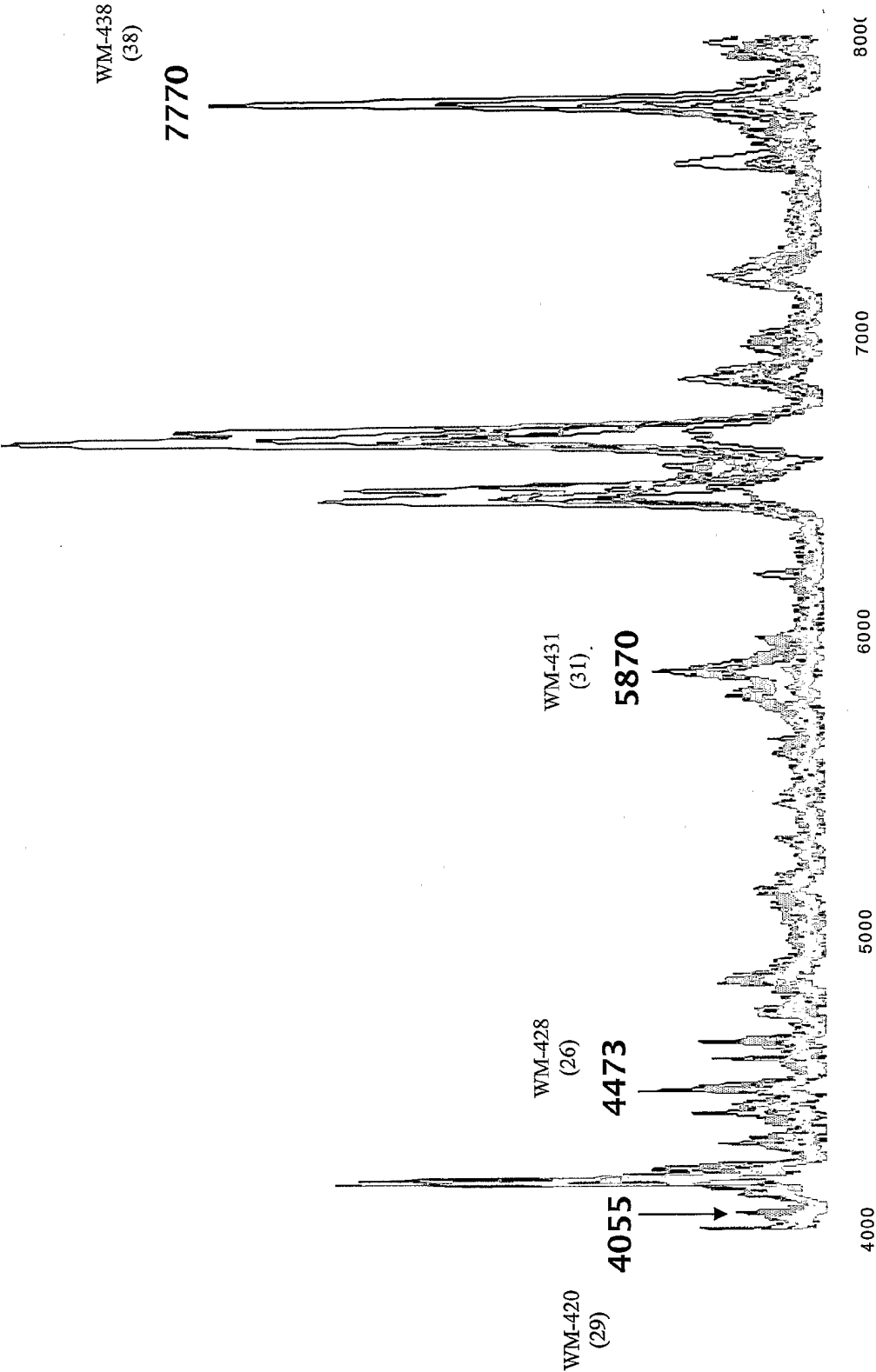


Figure 17
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

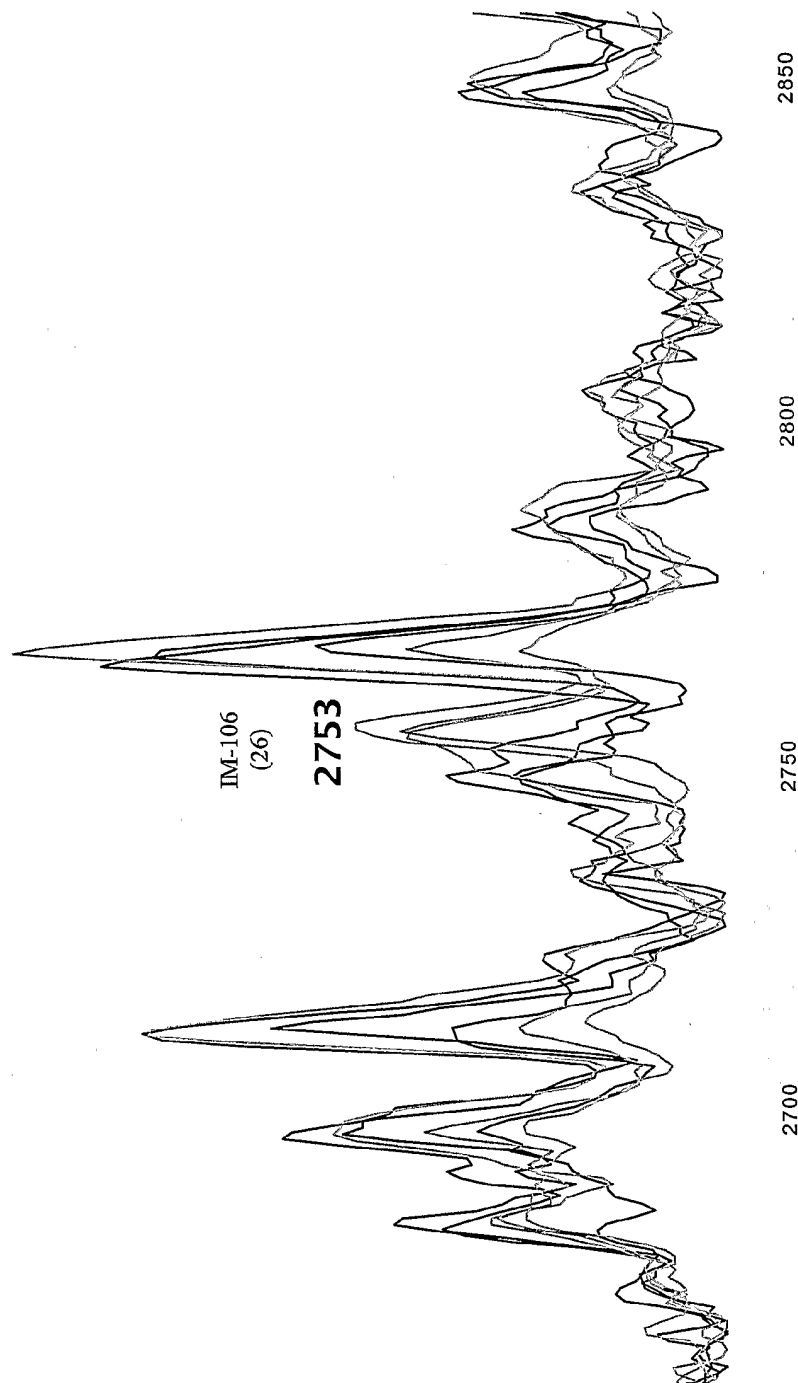


Figure 18
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

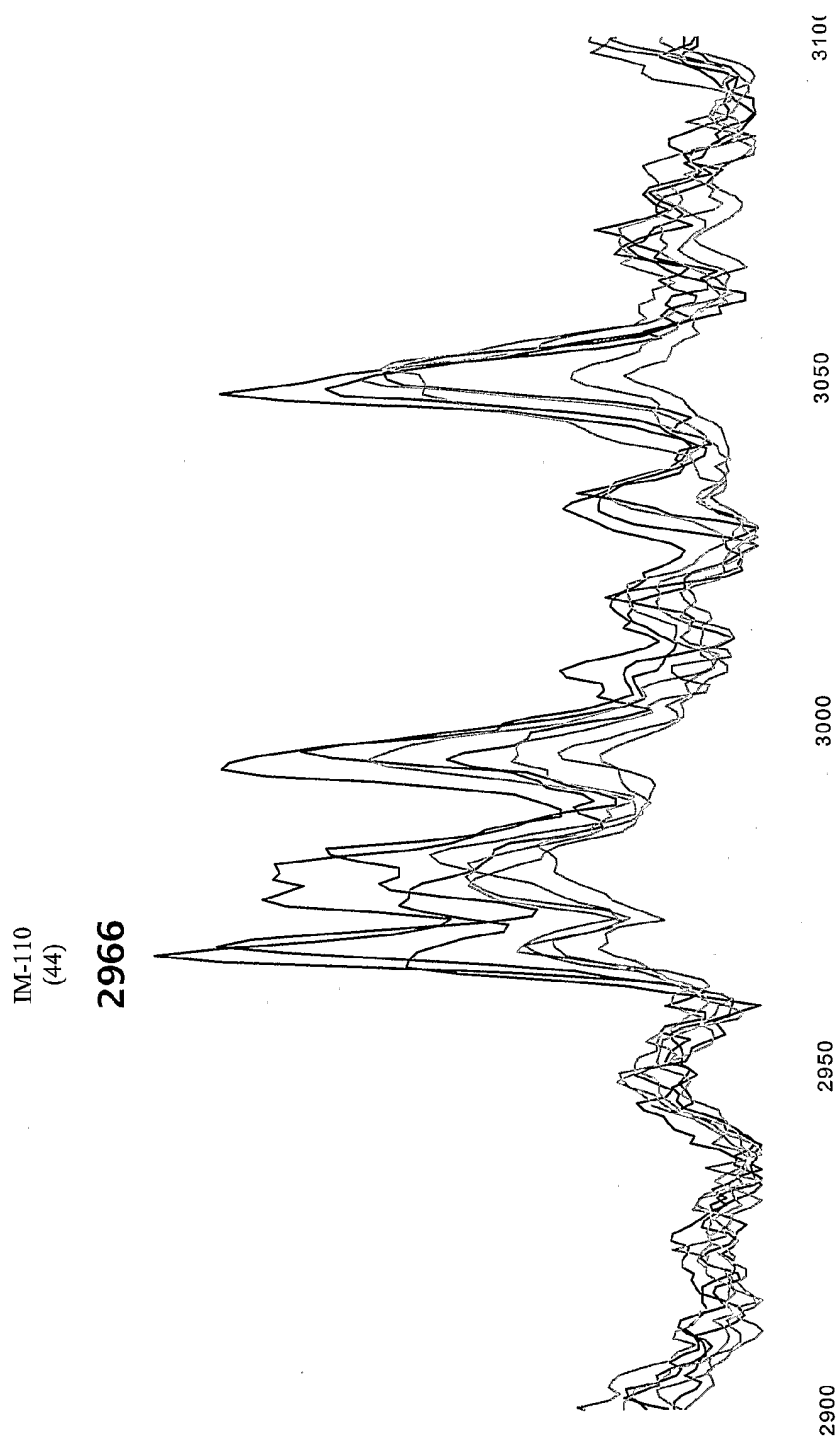


Figure 19
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

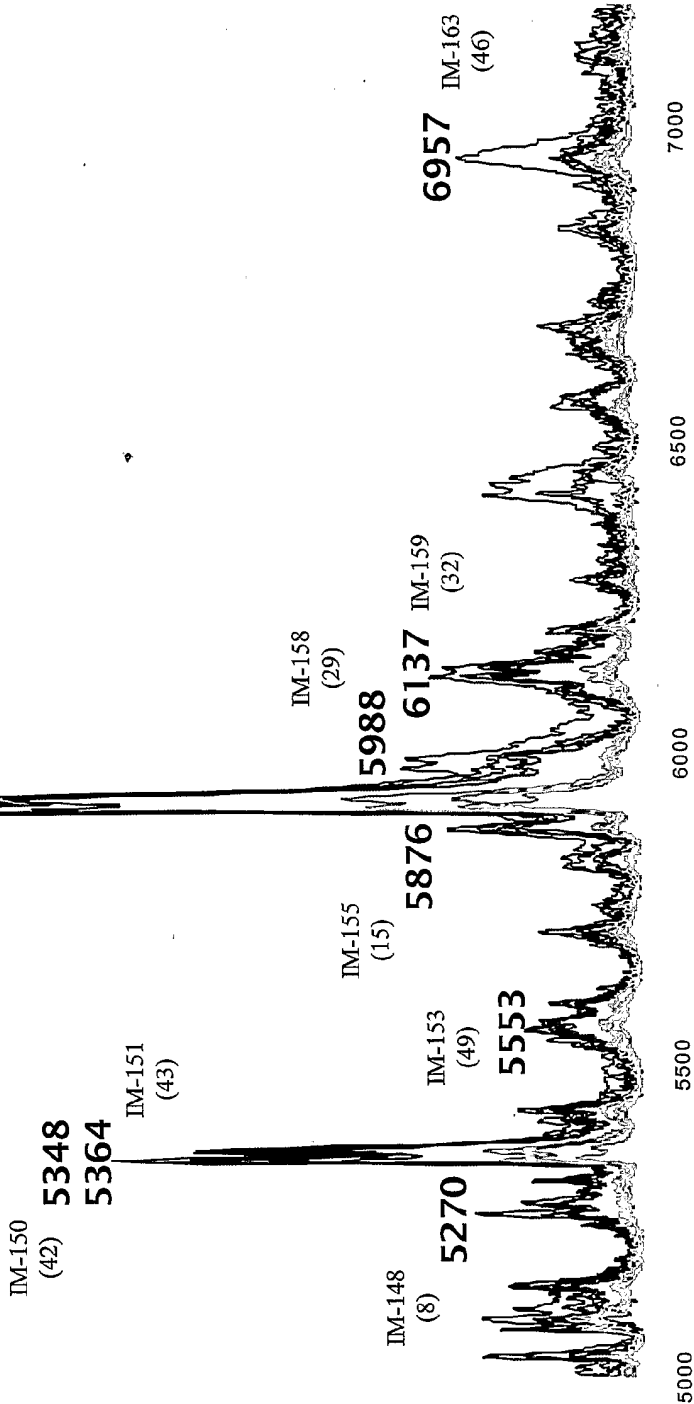
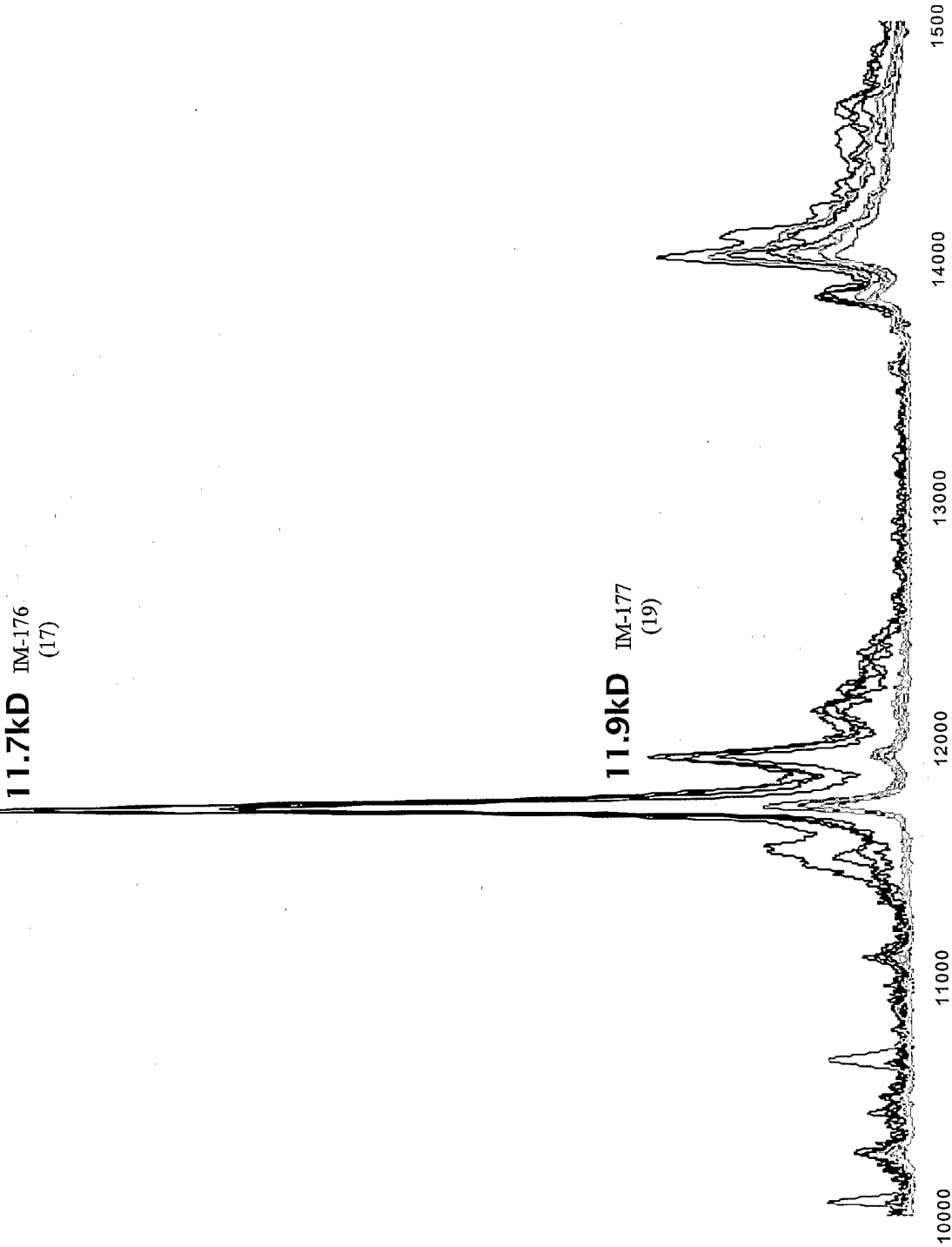


Figure 20
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)



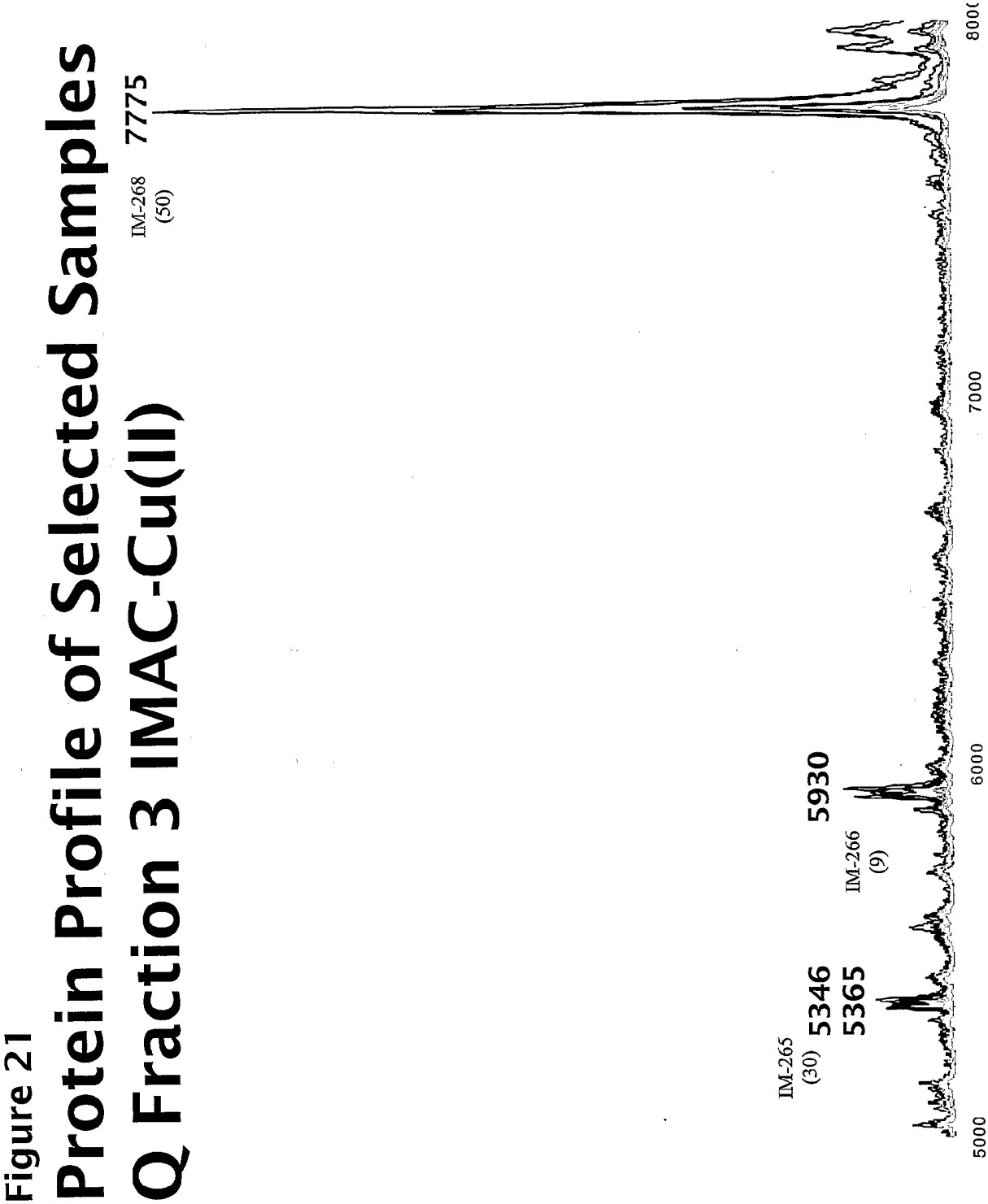


Figure 22
Protein Profile of Selected Samples
Q Fraction 3 IMAC-Cu(II)

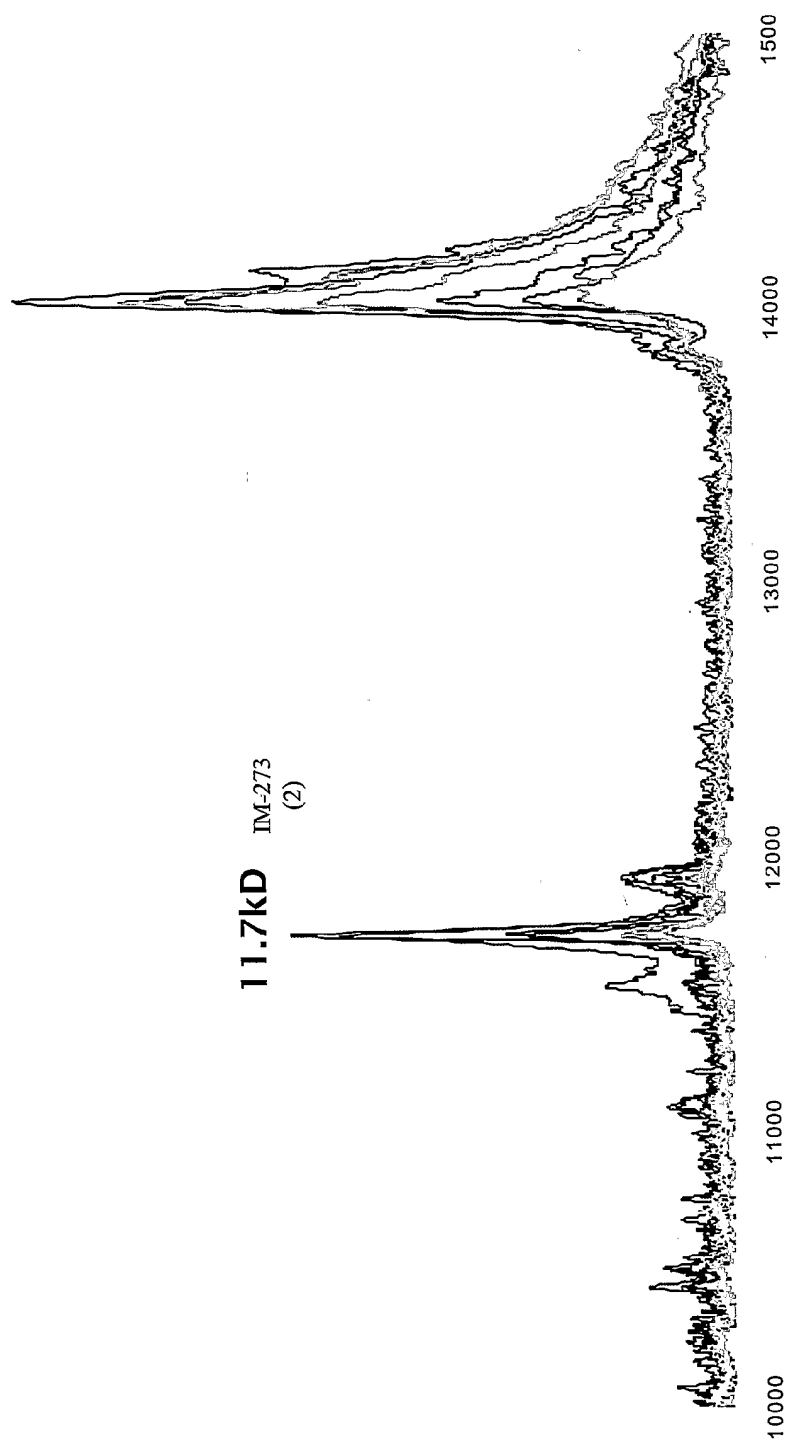


Figure 23
Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

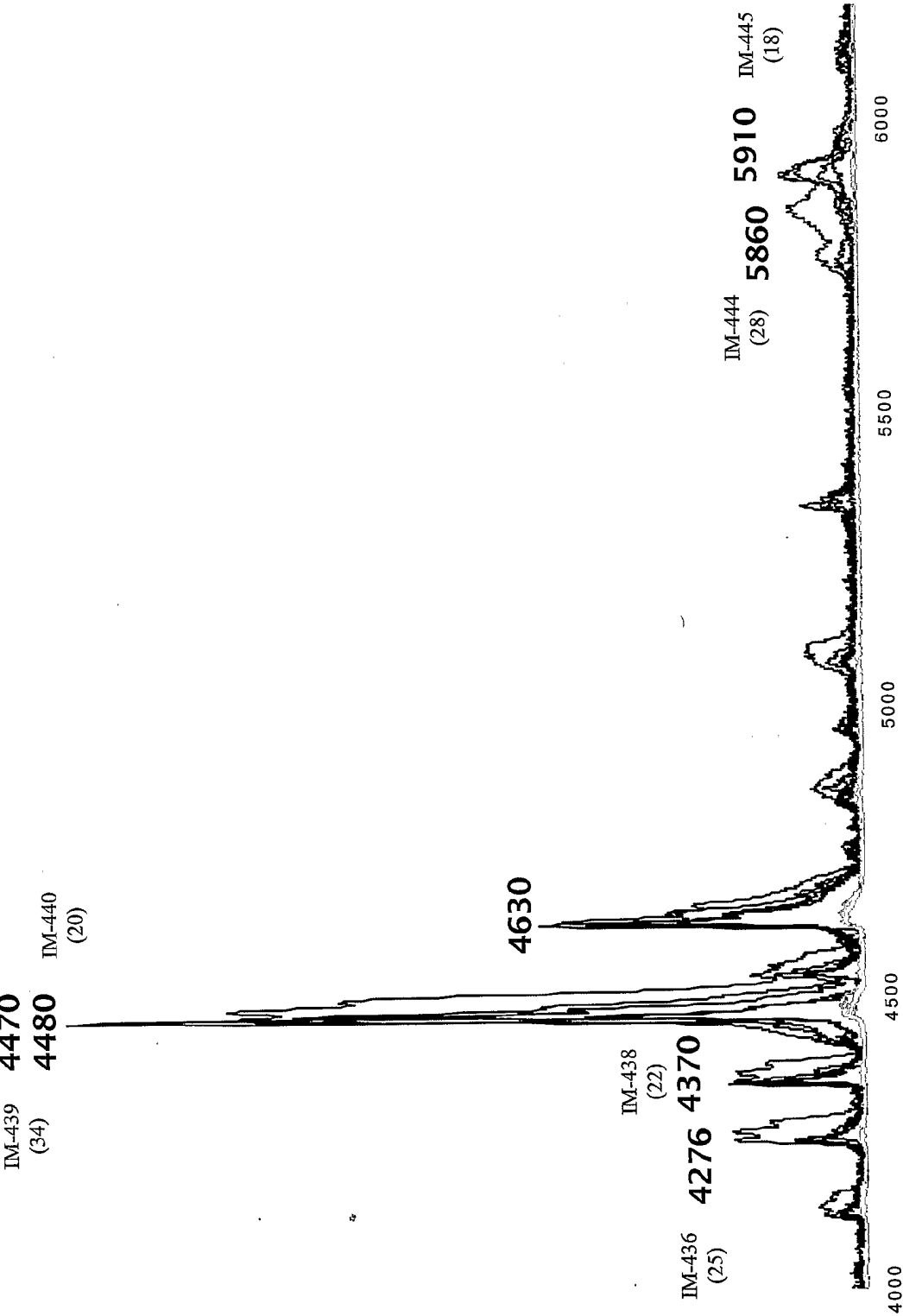


Figure 24
Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

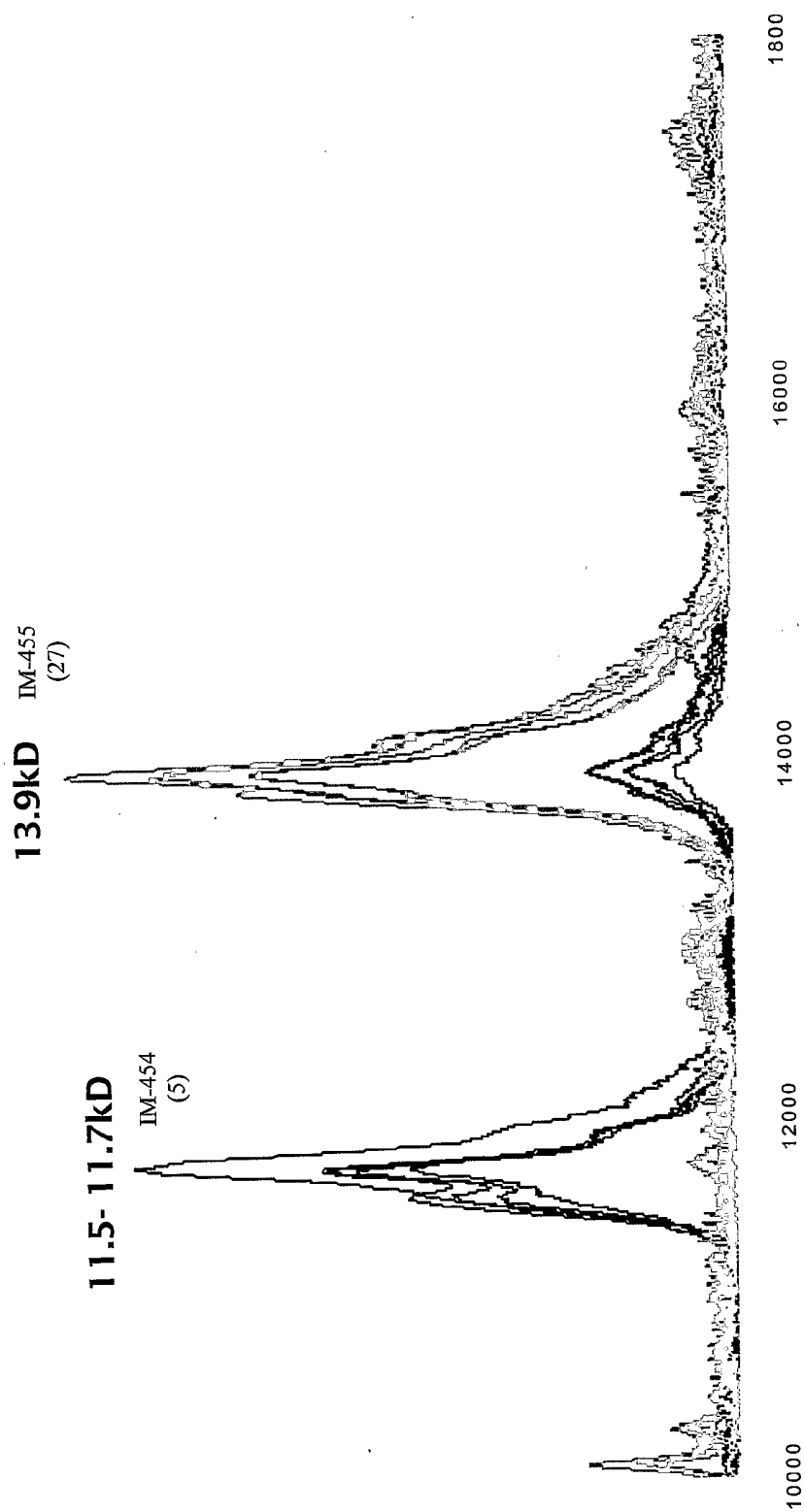


Figure 25
Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

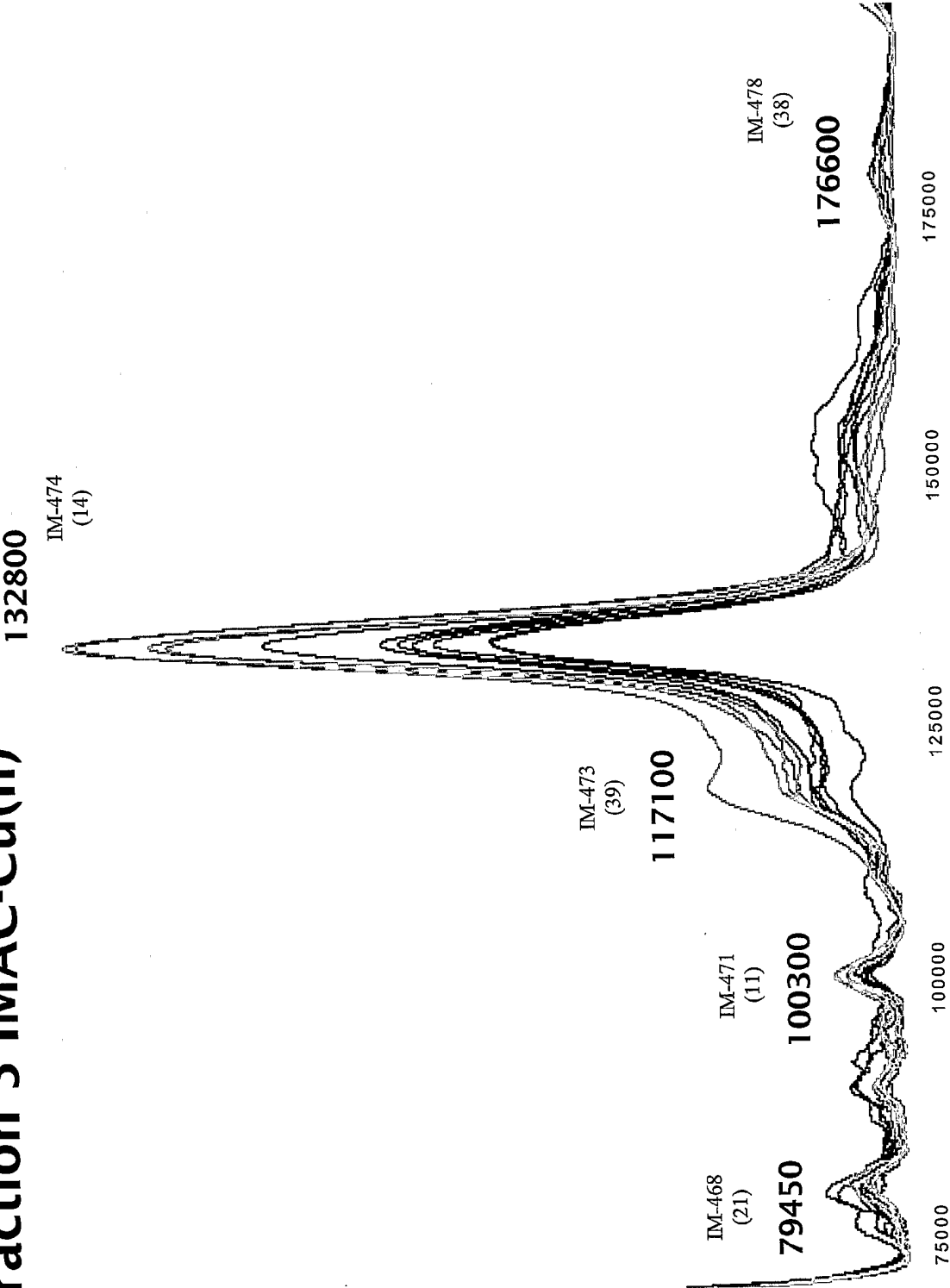


Figure 26
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)

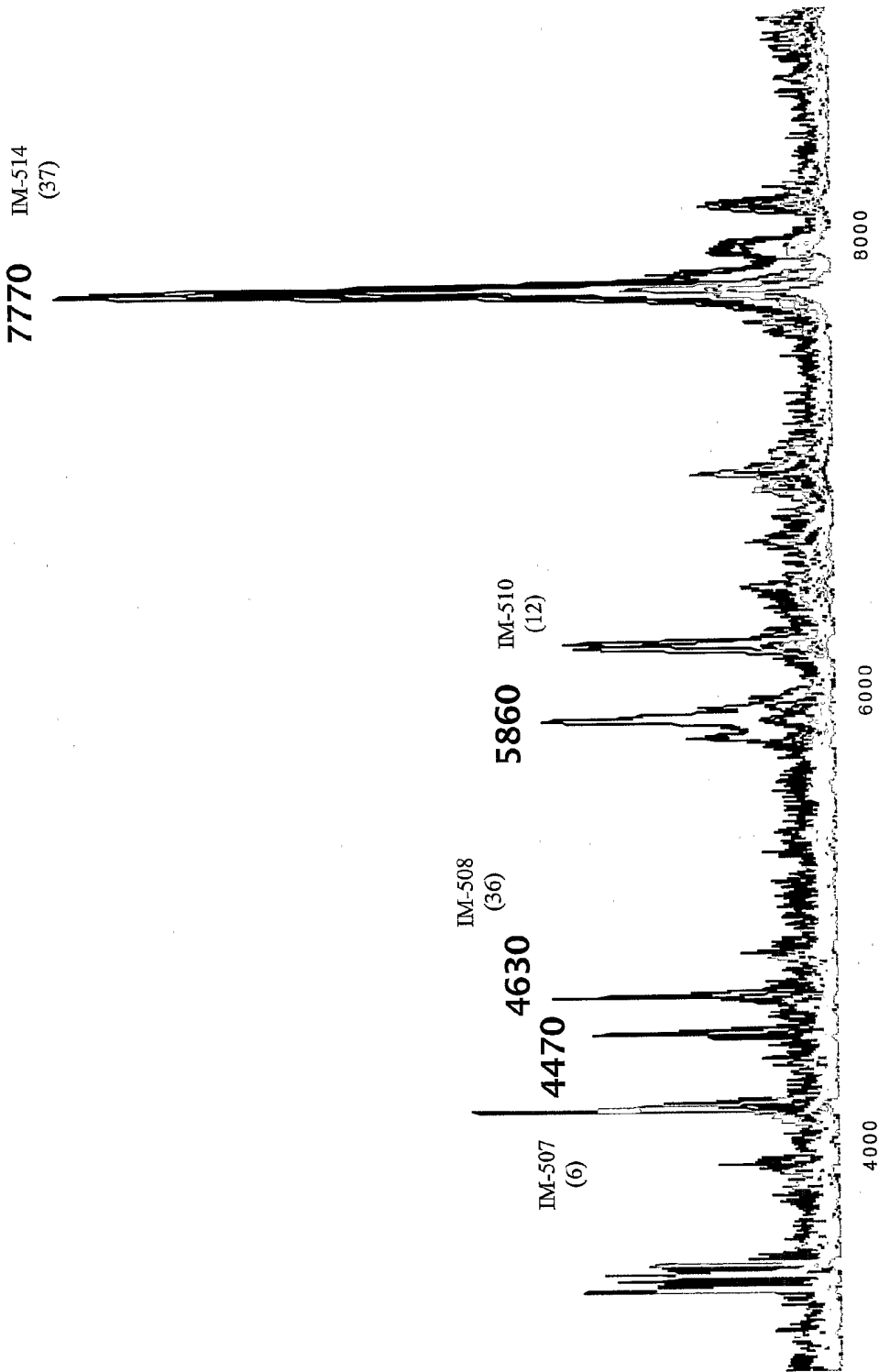


Figure 27
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)

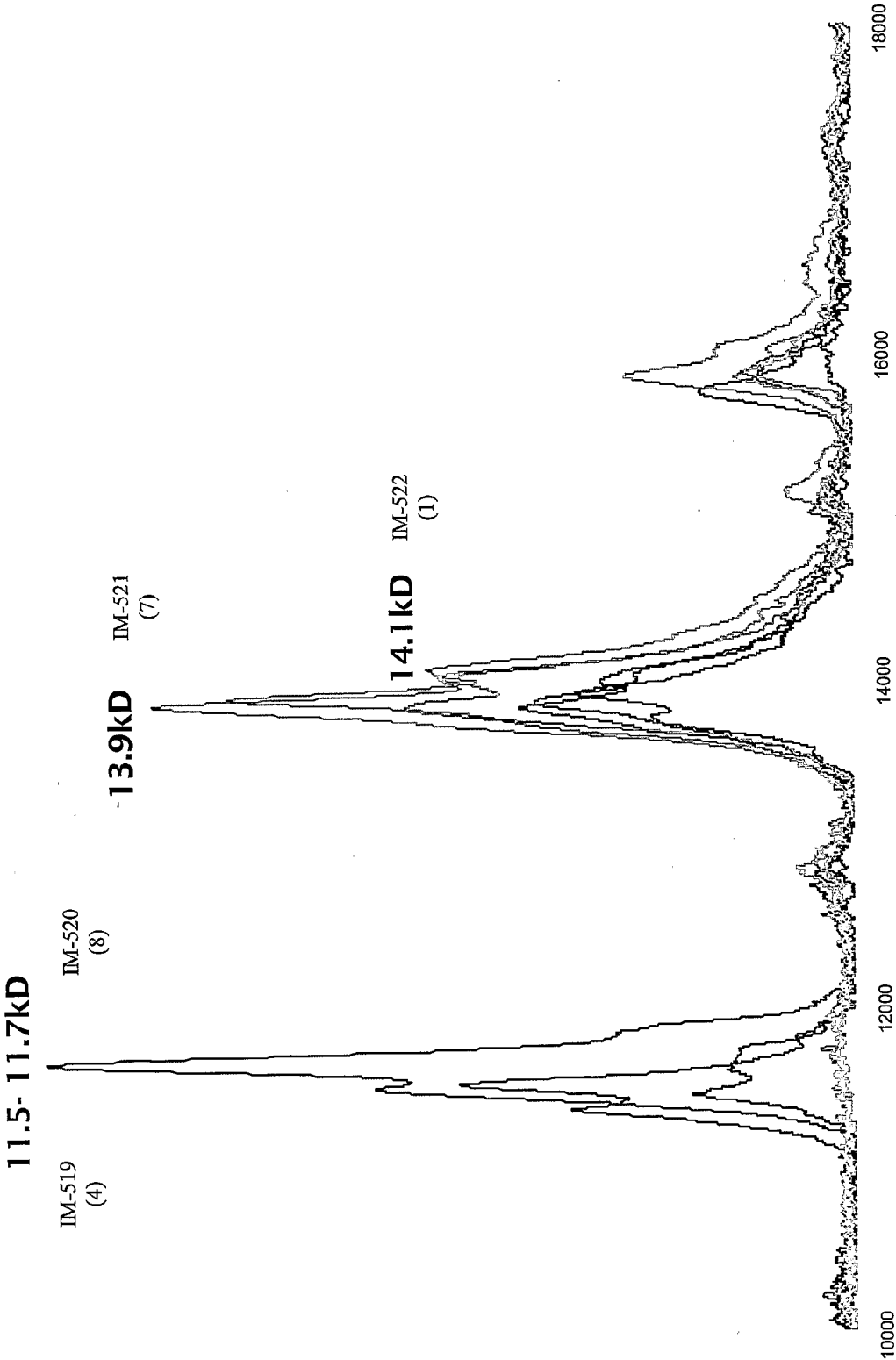


Figure 28
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)

