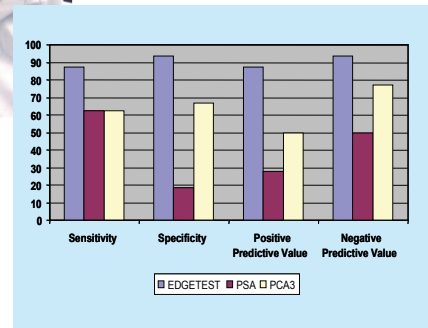
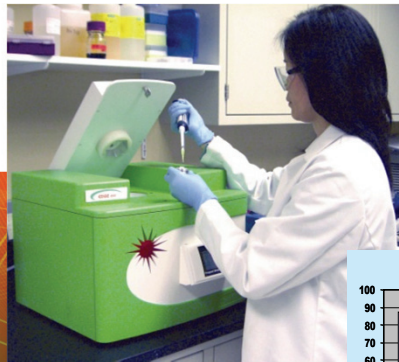




Prospect Biosystems



Biomarker Solutions for

Drug Discovery

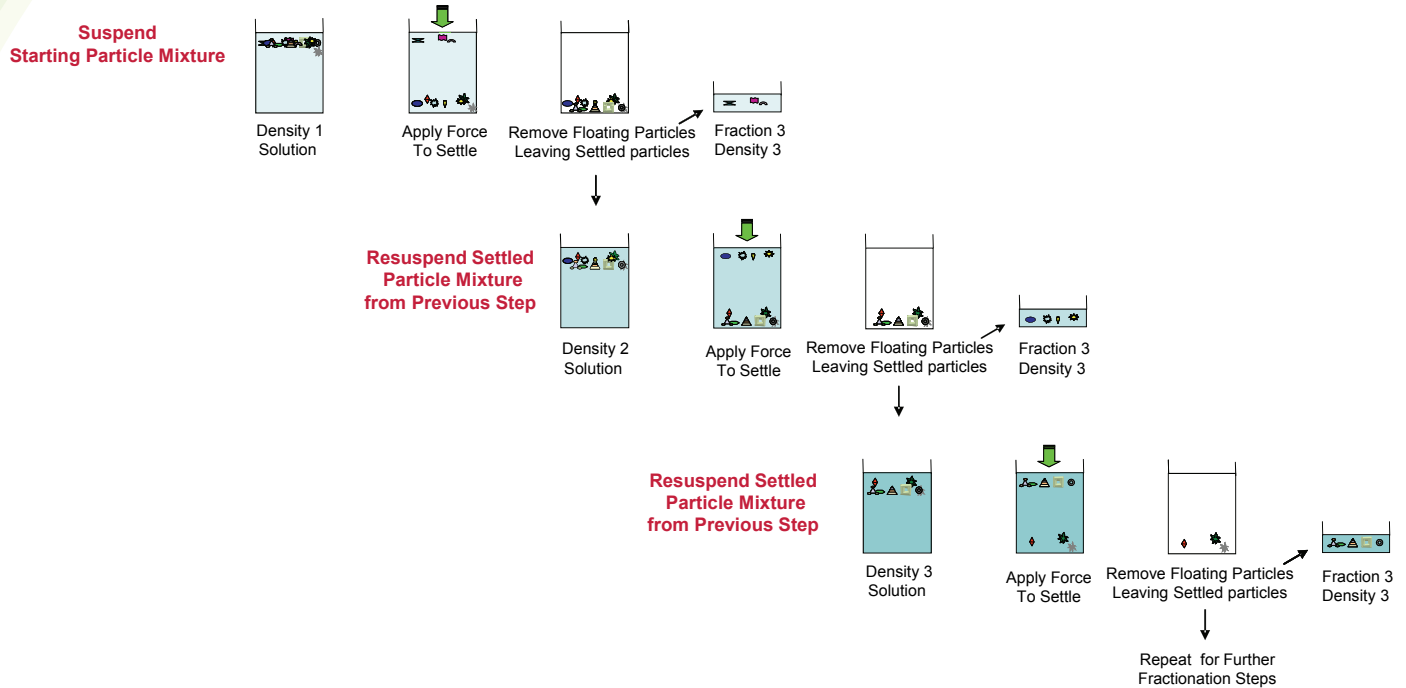
Diagnostics

Disease Management

Edge™ Technology

Edge technology, is a breakthrough, non-denaturing front-end sample fractionation and separation methodology developed by Prospect Biosystems, comprising a stepwise extraction of a particle mixture using extraction media of increasing densities. It forms the basis of a broad-range biomarker discovery capability and an instrumentation platform, providing rapid biology-based fractionation and separation of complex tissue homogenate and cell lysate samples.

Edge technology provides a powerful, well defined, and reproducible fractionation method for biomarker discovery and proteomics analysis. Edge fractionation is compatible with and complements all down stream analyses including gel electrophoresis, HPLC, mass spectrometry, and microarrays, etc. The following steps describe how the technology works:

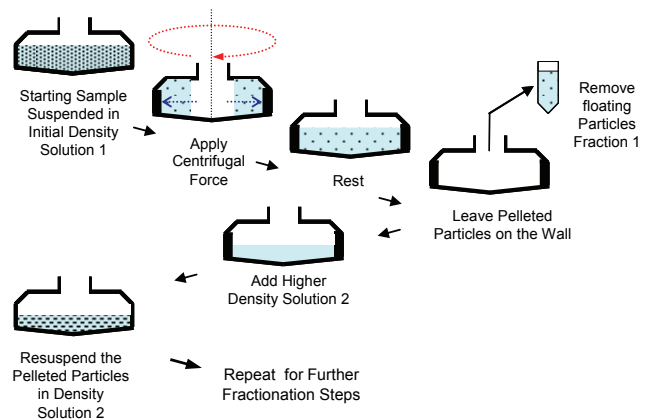


Centricollation™

Edge technology can be accelerated and enhanced by various external forces, such as centrifugal, magnetic and electrical force. When the process is driven by centrifugal force for the rapid, ordered extraction of substances, the process is called **Centricollation**.

Centricollation is implemented on the **Edge 200 Separation System**. The Edge 200 is an easy to use air-driven ultra speed centrifugal system. Its patent-pending design provides distinct benefits.

Prospect's unique rotor design, combined with its fixed density GradiSpec™ extraction media provides robust, well defined fractionation of all types of tissue homogenate and cell lysate samples. Sample handling and cross contamination are minimized through the use of sterile disposable inserts. Rapid air driven acceleration up to 150,000 x g force in less than 60 seconds, in a temperature-controlled environment, assures that individual fraction steps are completed in 5-6 minutes.



The Edge 200 System does not use vacuum or high speed bearings, and does not rely on complex software. With starting sample volumes of 3 ml or less, and user-defined extraction volumes as low as 100 µl, recovery yields are routinely >90%.

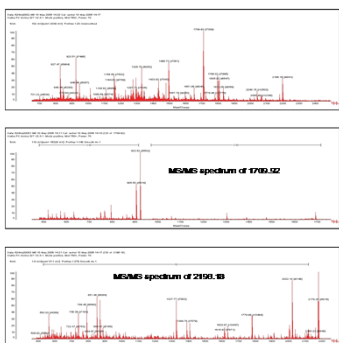
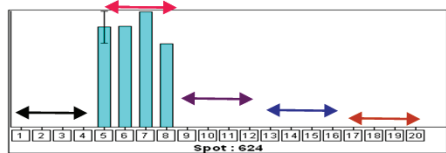
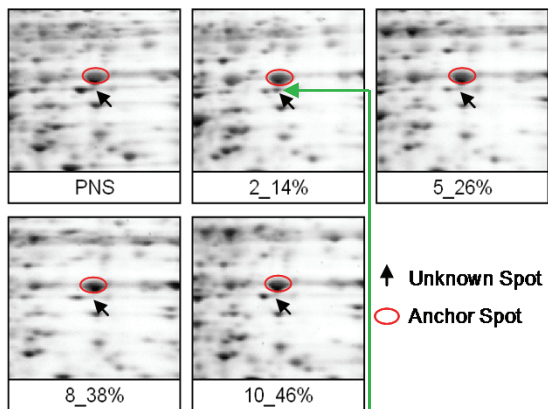
By fractionating based upon biologically relevant compartments, Edge technology and Centricollation can significantly enhance proteomics' analytics by reducing the complexity of biological samples 10-20 fold.

The Power of Selective Fractionation and Enrichment

In the search for and isolation of low abundance proteins, up-stream fractionation of a tissue or cell culture sample plays a key role in reducing the complexity of the sample. The ability to enrich selective fractions with proteins of interest can significantly expedite the identification of those proteins. Since Edge technology fractionates intact biological compartments without denaturing those compartments, additional biological information about subcellular location may also be learned.

Analytical processes such as gel electrophoresis are limited by the amount of sample which may be analyzed at one time. Fractionation allows proteins of low abundance to be visualized within fractions, when they normally would be obscured by higher abundance proteins in the total sample, or not be visible at all due to the large dynamic range of protein concentration within the sample.

Edge technology's ability to enrich low abundance proteins and enhance their identification is shown by the fractionation of the post-nuclear supernatant (PNS) of a rat liver homogenate. Proteins not visible in the PNS may be easily identified following fractionation:



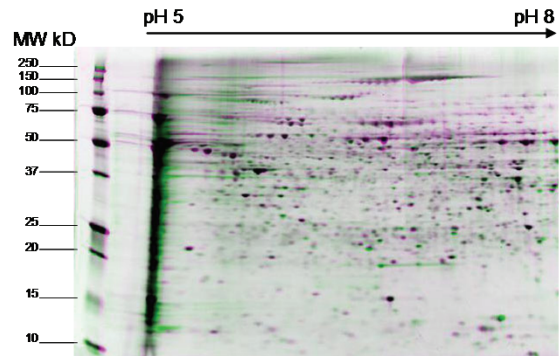
Hypothetical Protein
(gi|34869622)

Speeding Up Selective Fractionation with FastExtract™

Edge technology provides a powerful, selective sample fractionation tool for proteomics analysis, including low abundance protein isolation and enrichment, as well as information on subcellular location. Furthermore, the Edge fractionation method separates complex samples at the level of individual compartments, retaining information about functional and low abundance proteins inside the compartments in their biological context.

In investigations focused on certain biological events, for example translocation, it can be beneficial to compare protein distribution within two or more distinct fractions. Since Edge fractionation comprises a series of discrete extractive steps, rather than a separation continuum such as in chromatography or electrophoresis, extraction may start at, or jump to any density step of interest without the need for going through the intervening steps.

This process, termed **FastExtract**, provides significant time and reagent savings when comparing biological processes. Additionally, the Fast Extract process produces near-identical results to the normal multiple step extraction without the need for further optimization.



A comparison of **FastExtract** results with typical step-wise extraction of the same sample is shown in the 2D gel overlay above. Two 22% sucrose fractions obtained by the following methods were compared on Sypro Ruby stained gels. Spot similarity was 93.6%.

Method 1: Sucrose extraction using density steps 8.5%, 10%, 14%, 18%, 22% (w/v).

Method 2: Fast Extract using sucrose density steps 8.5%, 18%, 22% (w/v).

Prospect ... "explore for useful or valuable things or substances"



Folate Deficiency and Oxidative Stress

Folate cannot be synthesized biologically in humans and most mammals, and thus must be obtained from food and dietary supplements. Dietary folate is required for normal development, particularly for pregnant women and the elderly. Epidemiological and experimental studies have demonstrated that folate deficiency (FD) is not only linked to neurodegenerative and psychological disorders, but also contributes to cancer and cardiovascular diseases. Numerous reports suggest that oxidative stress can be induced by folate deficiency, which will cascade DNA damage, alter one-carbon metabolism, and trigger multiple organ damage and neurodegenerative disorders.

In animals, the liver is an important organ for folate storage and metabolism. Little is known about folate deficiency induced oxidative stress at the subcellular level in a single tissue. In addition, more data are still required to clearly explain the cellular and molecular mechanisms connecting folate deficiency and various diseases.

Edge technology has been applied in a proteomic approach to better understand potential subcellular components of folate deficiency in liver. Some unique proteins and potential biomarkers have been identified which may lead to a better understanding, diagnosis and treatment of various diseases associated with folate deficiency.

Healthy adult rats were fed either a diet deficient in folate or a control diet. Rat liver tissue from both groups was homogenized and fractionated using the Edge 200 separation system. Samples from each fraction were subjected to Western Blot analyses using the markers GPx1 and GRP75. The 30% and 40% fractions were selected for comparative analysis by 2DE, based upon significant differences between the control and FD samples.

Spots differentially expressed by comparison of corresponding 2DE gels were excised, digested and identified by MALDI-TOF/TOF.

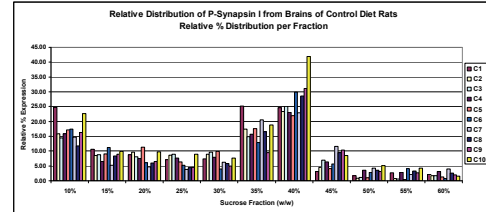
	Spot	Protein	Relative Expression
Fr 30%	0012	similar to NADH dehydrogenase:Ubiquinone Fe-S protein 8	Down
	4305	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10	Down
	3305	fructose-1,6-biphosphatase 1	Unique to Control
	5001	Calreticulin	Unique to Control
	0201	Annexin A5	Unique to FD
	3607	similar to alpha-1 major acute phase protein prepeptide	Up
Fr 40%	1202	regucalcin	Down
	2214	estrogen sulfotransferase isomer 3	Down
	4008	chain D, rat transthyretin	Down
	4505	4-trimethylaminobutyraldehyde dehydrogenase	Unique to Control
	8507	similar to 6-phosphogluconate dehydrogenase,decarboxylating	Unique to Control
	9611	fibrinogen, beta polypeptide	Unique to FD
	3702	Hpx protein	UP
	2411	unknown	UP
	8208	haptoglobin	UP

Biomarker Evaluation and Validation

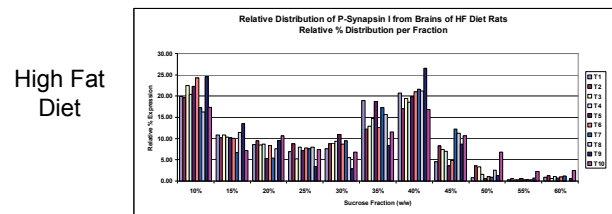
The identification of biomarkers is an essential element for early prediction of diseases and development of personalized medicine in the future. Biomarker discovery, evaluation and validation are the key steps in biomarker development processes. The on-going development of high-throughput proteomics, which includes high sensitivity mass spectrometry and automation of protein identification, has significantly increased the database of potential biomarkers. However, the evaluation and validation steps of the biomarker development process remain a bottleneck.

Rat models of high fat diet (HF) have shown that a diet high in fat has a profound impact on brain function. Phosphosynapsin (p-synapsin) has been described as a marker of synaptic dysfunction. To evaluate the utility of p-synapsin as a marker of synaptic dysfunction arising from high fat diet, brains of rats fed high fat diets were compared with brains of rats fed a control diet.

Brain tissue from each animal was fractionated into 11 fractions using Edge technology. Fractions from each animal were analyzed by Western blot analysis for p-synapsin, and relative percentage distributions for the marker were calculated and plotted across all fractions for all animals:



Control



High Fat Diet

Comparison of changes in relative distribution of the marker between the control and high fat diet groups revealed that the ratio of the marker in the 10% versus the 40% fraction was significantly different in each group, thus providing a means for evaluation:

	Ratio of p-Synapsin I Expression in Frx 10% vs Frx 40%										Mean	SD
Control	1.01	0.68	0.58	0.69	0.78	0.58	0.64	0.41	0.52	0.54	0.64	0.165
HF	0.96	1.15	1.16	1.10	1.13	1.15	0.80	0.79	1.03	0.93	1.02	0.144

Since the evaluation method relies on the ratio between internal fractions of the sample, the method is independent of sample amount and does not require internal or external standards.

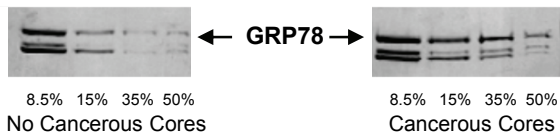
Prospect ... "a prediction of the course of a disease"

Rapid Screening for Prostate Cancer in Tissue Samples

Prostate cancer (PCA) is one of the major health problems in the developed world and is the second leading cause of cancer death in men in the United States. Currently, major problems inherent in the management of prostate cancer include the lack of specificity and sensitivity of diagnostic tests such as the prostate-specific antigen (PSA) and the PCA3plus tests, and the lack of a method to distinguish between progressive and indolent diseases. With the aging of the population, more sensitive and specific markers than PSA and PCA3 are critically needed to improve and change the management of prostate cancer.

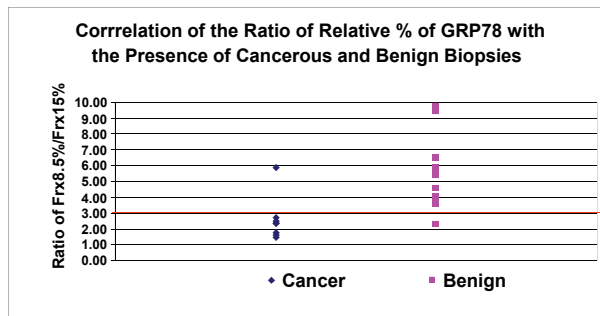
Glucose-regulated protein (GRP78) is reported to be related to several human cancers. A recent study demonstrated that GRP78 plays a crucial role in prostate cancer development by promoting cancer cell proliferation, mediating oncogenic signaling and protecting cancer cells against cell death resulting from the stress of tumor development.

GRP78 was evaluated as a potential prostate cancer biomarker in needle core biopsy samples using Edge technology and the **EDGE*TEST™** algorithm, to further develop a diagnostic test for prostate cancer screening.



Two blinded prostate needle core biopsy samples, taken in addition to the normal twelve from patients during routine prostate biopsy, were fractionated into four fractions using Edge technology which were then subjected to Western Blot analysis for GRP78 (see above). **EDGE*TEST** analysis indicated that ratios of GRP78 in the 8.5% fraction versus the 15% fraction were significantly different in patients found to have cancer in one or more biopsy cores than in patients found to have no cancer in any cores.

Ratio data from 24 patients was compiled and correlated with biopsy findings:



EDGE*TEST specificity and sensitivity for GRP78 were 87.5% and 93.75%, respectively, indicating that preliminary screening of two needle biopsies with GRP78 may be effective in predicting whether or not cancer will be found in the prostate.

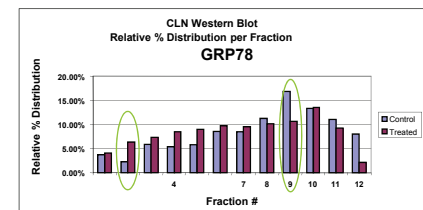
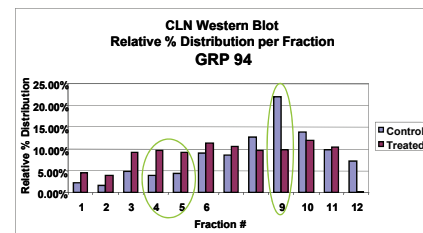
Improved Identification of Possible Translocation Events

Autoimmune type 1 diabetes (T1D) can result from an imbalance between regulatory and autoreactive T lymphocytes in genetically predisposed individuals. Recently, the differentiation of T lymphocytes into cytokine-secreting effector cells was shown to initiate an endoplasmic reticulum (ER) stress response, including the upregulation of heat shock protein (HSP) “chaperones” found in the ER that help to fold newly synthesized proteins.

The **Edge 200** was used to investigate the role of ER chaperones, and other heat shock protein family members, in the T cell changes that occur with the induction of T1D.

A control group of BBDR rats was injected with phosphate-buffered saline, while a treated group was injected with anti-ART2 mAb plus polyinosinic: polycytidylic acid (poly I:C). Untreated BBDR rats do not develop diabetes, whereas 100% of those treated with anti-ART2 mAb plus poly I:C become diabetic over a time course of 14-18 days.

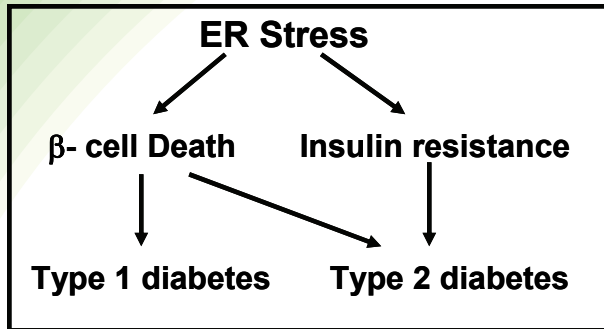
Cervical lymph nodes were collected from both the control and treated groups. Tissues were homogenized, fractionated using the Edge 200 separation system and samples from each fraction were subjected to Western analyses for the markers GRP 78 and GRP 94.



Changes in profiles of relative percentage distribution of markers per fraction indicate their potential translocation in the cell.



Following the Biology

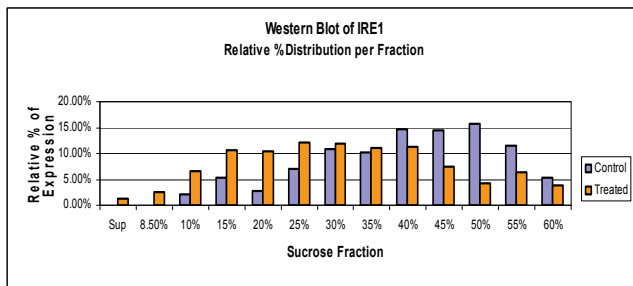


ER Stress and Diabetes

Information exists regarding key proteins that are part of specific biochemical pathways. To the extent that such proteins are reflective of activity within those pathways, following their movement and location can be used to identify additional proteins which may be markers of that pathway.

IRE1 is a known pathway marker for ER stress. Research has shown that ER stress plays a role in the pathology of diabetes. Edge-fractionated biological samples provide a method to quickly and efficiently track pathway biomarkers. Relative percentage distribution of the IRE1 marker within fractions is expected to differ between normal and treated (or diseased) states of pathologies involving the ER stress pathway.

Probing the distribution of known pathway biomarkers such as IRE1 within defined fractions, other differentially expressed proteins related to the specific pathway may be easily found. A fraction, or fractions, can be identified which have the highest difference in relative percentage distribution between normal and treated states. Fractions 25% and 50%, below, represent two such fractions.



If proteomic analysis, such as 2DE, is performed on those fractions, in both groups, more differentially expressed proteins will be identified in these fractions than in others. These proteins are likely to be related to the known markers through networks or pathways.

Following 2DE of fractions of control and treated samples that showed significant differences in IRE1 expression, gels were compared to find spots having large differences in expression. The table below shows, as expected, that the 25% and 50% fractions contain the largest number of differentially expressed proteins.

Fr	Relative Change in IRE1 Expression*	Number of Differentially Expressed Proteins
25%	0.71	37
30%	0.10	31
35%	0.08	18
45%	-0.93	31
50%	-2.73	55

Differentially expressed spots were identified using mass spectrometry, and then those identities were searched for relationships with ER stress and diabetes using the PubMed database. The following table, showing a representative sampling of proteins found, demonstrates the high degree of correlation between those proteins found in fractions containing the largest amount of IRE1 differential expression and diabetes and ER stress.

Fr	Protein	Relative Expression	ER Stress Pathway**	Diabetes**
25%	expressed in non-metastatic cell 1, protein (NM23A) (nucleoside diphosphate kinase)	Up	✓	✓
	N-ethylmaleimide sensitive fusion protein	Up	✓	✓
	similar to Eukaryotic translation initiation factor 5A	Up	Unkown	Unkown
	Eno 1 protein	Up	✓	✓
	unnamed protein product	Up	Unkown	Unkown
	arginase 1	Up	✓	✓
	guanine nucleotide-binding protein, beta-1 subunit	Up	✓	✓
	expressed in non-metastatic cell 2	Up	✓	✓
	signal sequence receptor 4	Up	✓	✓
	unnamed protein product	Down	Unkown	Unkown
	aconitase 2, mitochondrial	Down	✓	✓
	50%	isovaleryl Coenzyme A dehydrogenase	Up	✓
Eef1g protein		Up	✓	✓
similar to prohibitin (BAP 32)		Up	Unkown	Unkown
unnamed protein product		Up	Unkown	Unkown
ATP synthase, H+ transporting, mitochondrial F1 complex, beta subunit		Up	✓	✓
mitochondrial aconitase		Up	✓	✓
glycerol-3-phosphate dehydrogenase 2		Up	✓	✓
dnaK-type molecular chaperone grp 75 precursor		Down	✓	✓
glucose regulated protein, 58 kDa		Down	✓	✓
insulin I		Down	✓	✓

Prospect's technology provides a robust, yet gentle, non-denaturing fractionation and separation of subcellular components. The separation of the components is based on the individual density of their compartments, as opposed to individual proteins or specific organelles. As such, groups of markers may be identified within their biologically relevant environments allowing the researcher to follow the biology throughout the whole disease process from diagnosis through treatment. **The ability of the Edge technology to "follow the biology" makes it ideally suited to biomarker discovery.**

Edge 200 Separation System



Prospect's **Edge 200**, is an automated, easy-to-use, bench-top air-driven centrifugation system, specifically designed to implement the Edge technology. Using "house air", or an external compressor, the **Edge 200 rotor** can reach speeds of 95,000-100,000 RPM in less than a minute, providing 120,000 to 150,000 x g force. The system provides well defined, non-denaturing, and reproducible fractionation of complex biological samples by their densities, maintaining the biological context necessary for further analysis and biomarker discovery.

The **Edge 200** addresses the market's needs in the following areas:

- Low abundance protein isolation and enrichment
- Biomarker discovery
- Subcellular biology, subcellular proteomics, cell biology
- Biological information on subcellular location
- Drug target discovery and characterization
- Binding studies and protein-protein interactions



CentriCol™ 105



An integral component of the **Edge 200** is Prospect's **Air Prep™ 205** unit (APU) which provides a unique combination of controlled pre-dried and low-temperature air. Throughout each extraction step, the APU conditions the inlet air to assure a clean, dry and temperature-controlled environment.

Additionally, Prospect has developed its **GradiSpec™** line of fixed density extraction media to provide robust, well-defined fractionations. These reagents facilitate the use of the **Edge 200** and are available in kits which:

- contain highly accurate, defined sucrose concentrations in buffered solutions
- are sterilized and are DNase, RNase and pyrogen free
- have extended shelf lifetimes and are stable at room temperature
- provide easy instructions for making additional highly accurate density solutions from solutions provided



The **Edge 200** uses Prospect's **SteriLiner™** disposable liners placed within the rotor to ensure the integrity of the sample during fractionation. These liners, available in bulk:



- protect the sample from contacting the rotor's surface
- are optimized for the Edge fractionation process
- retain their integrity under multiple spins at high centrifugal force
- ensure easy loading and removal of samples and fractions
- provide a cost effective approach to single sample use and the elimination of sample cross-contamination
- are available sterilized, in sealed packs

Prospect ... "the possibility of future success"



Prospect Biosystems, Inc. engages in the development, manufacture, and marketing of bioanalytical instrumentation, reagents and a broad-range of biomarker applications to accelerate life sciences research; pharmaceutical product discovery, development, and manufacturing; clinical diagnostics; and surgical pathology. The Company's patent-pending **Edge™** discovery platform is a breakthrough bioanalytical fractionation and separation technology that allows scientists, researchers, medical experts, including physicians and surgeons involved in:

- Analytical Proteomics to significantly enrich low-abundance proteins from their crude cell or tissue extracts
- Biomarker Discovery to rapidly characterize differences between diseased and healthy tissue
- Clinical Diagnostics to easily identify and evaluate predictive, diagnostic and prognostic disease biomarkers
- Surgical Pathology to facilitate outcome prediction through the use of tissue biomarkers
- Clinical Trials to accelerate and improve outcome prediction through the use of surrogate biomarkers

The ability to rapidly and efficiently identify biomarkers and quickly analyze complex biological components of tissue and cell samples will accelerate biomarker discovery; reduce false positive margins and improve outcomes for cancer and other surgical procedures; facilitate and advance pharmaceutical product development; accelerate many life sciences research; and improve efficiency and reduce costs of many healthcare delivery problems.

The Company markets and distributes its products directly and through distributors and agents worldwide. The Company was founded in January 2006, and currently operates out of the NJIT Enterprise Development Center, in Newark, NJ, USA.

Prospect Biosystems, Inc.

Enterprise Development Center
211 Warren Street
Newark, NJ 07103
USA
Phone (973) 242-6500
Fax (973) 215-2558
www.prospectbiosys.com