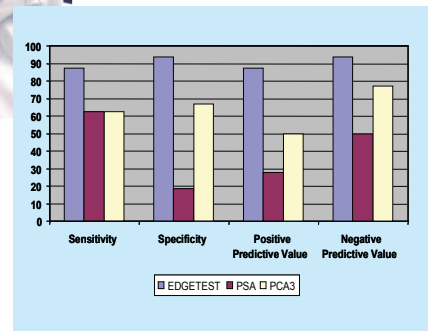
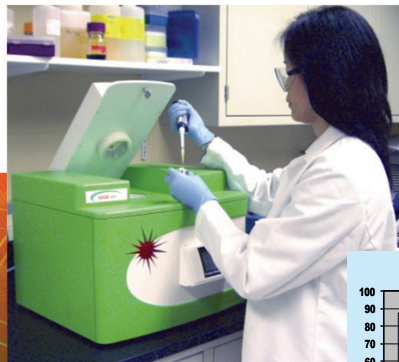




Edge Technology and EDGE*TEST



Biomarker Solutions for

Drug Discovery

Diagnostics

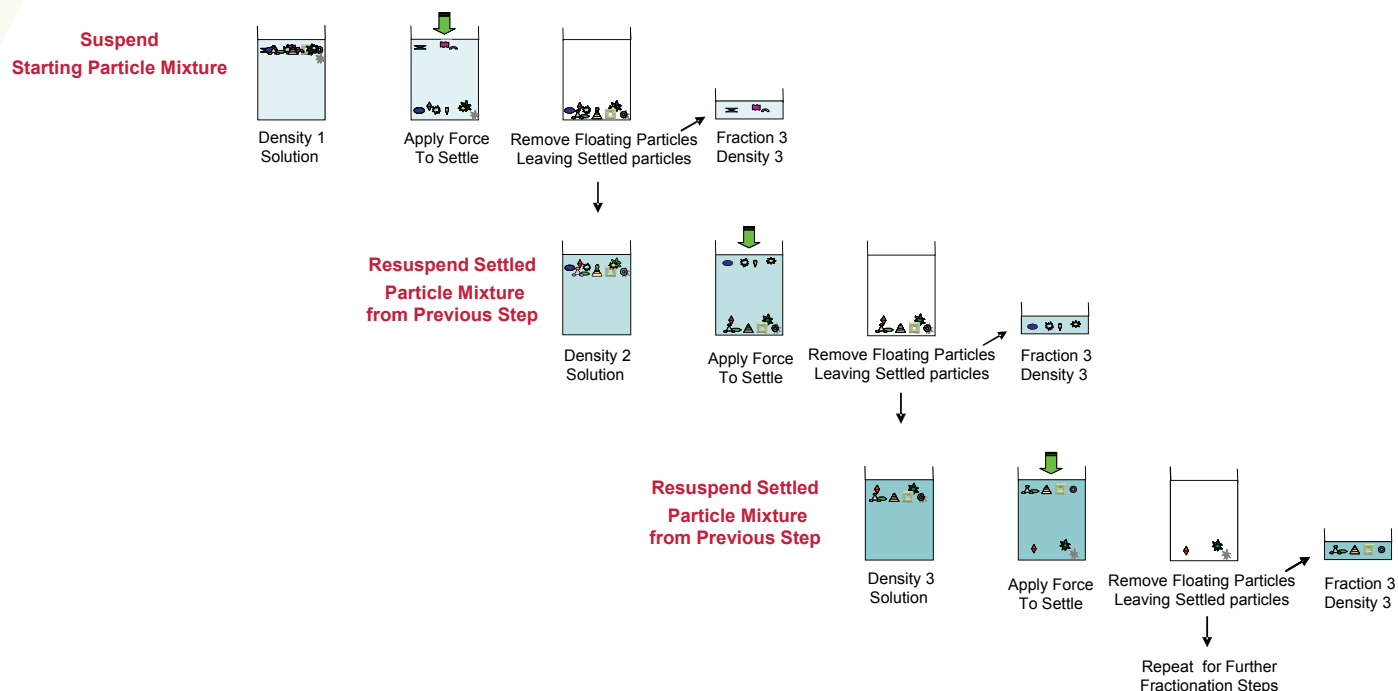
Disease Management

Prospect Biosystems, Inc

Edge™ Technology

Edge technology, is a breakthrough, non-denaturing biological sample fractionation, separation and characterization methodology. It comprises 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 biomarker development platform.

Edge technology provides a powerful, well defined, and reproducible cellular fractionations, which are 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:



EDGE*TEST

EDGE*TEST relies on **Edge** technology and Prospect Biosystems' proprietary platform to statistically differentiate between diseased and healthy (or control and treated) tissue and cell samples. **EDGE*TEST** distinguishes between diseased and healthy samples by defining a pair of density fractions whose biomarker content varies inversely in diseased versus healthy states. The ratio of relative marker content in these fractions can be directly correlated with diseased or healthy samples. The **EDGE*TEST** method has been shown to be applicable to a broad range of diseases and dysfunctions with significant benefits:

- **Rapid characterization of differences between diseased and healthy tissue**
- **Identification and evaluation of predictive, diagnostic and prognostic disease biomarkers**
- **The method is INDEPENDENT of sample amount**
- **The method DOES NOT require internal or external standards**
- **Each sample is analyzed in its ENTIRETY for a defined marker**

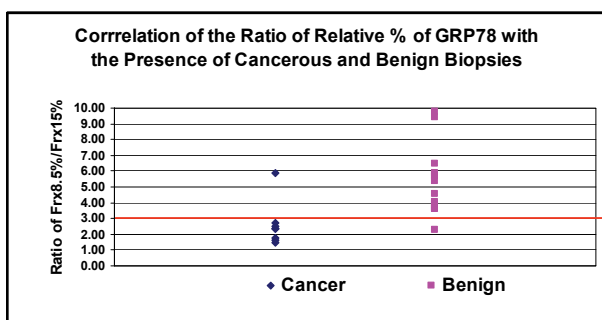
Disease Diagnostics - Application to Prostate Cancer

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.

Prospect Biosystems has demonstrated the utility of EDGE*TEST in prostate cancer (PCa) screening for a group of 24 patients, showing **increased specificity and sensitivity compared with traditional tests (PSA and PCA3 plus)**. Negative PCa biopsy patients were followed up for 18 months to compare predictive value of EDGE*TEST to traditional screening and biopsy.

- **EDGE*TEST predictability based on the initial biopsy is significantly superior to traditional tests**
- **After 18 months follow-up, EDGE*TEST still shows high predictive data of benign and not cancer**
- **EDGE*TEST provides well-correlated data on whether a prostate will contain cancerous tissue**
- **The method only needs 2 biopsy cores vs. 10-12 normally used**



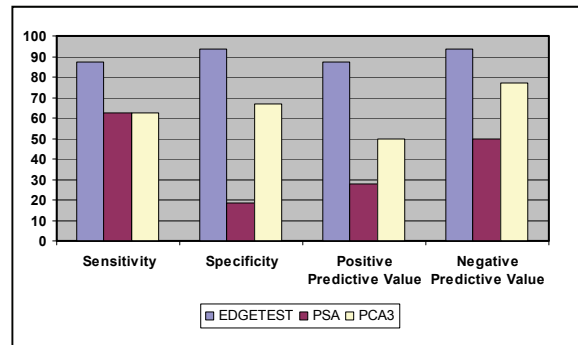
EDGE*TEST Predictability

Comparison of EDGE*TEST with PSA and PCA3 plus

Results Based on Initial Biopsy

EDGE*TEST vs. Traditional Tests	EDGE*TEST	PSA	PCA3+
Sensitivity	87.50	62.50	62.50
Specificity	93.75	18.75	66.66
Positive Predictive Value	87.50	27.77	50.00
Negative Predictive Value	93.75	50.00	76.92
Likelihood Ratio Positive Test	14.00	0.76	1.87
Likelihood Ratio Negative Test	0.133	2.000	0.562

Diagnostic Review
Guideline: EDGE*TEST <3.00, cancer
PSA: >4, cancer
PCA3: >35, cancer



Comparison of EDGE*TEST Predictability at 18 Months vs. at Initial Biopsy

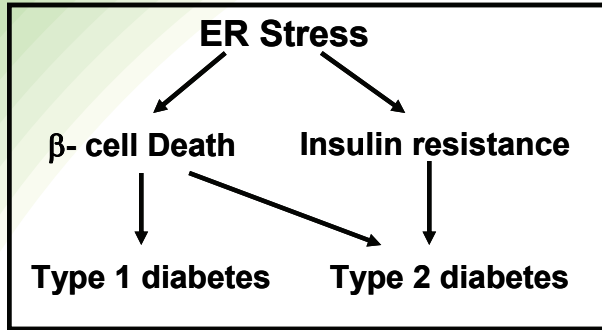
	Initial	18 months after
Sensitivity	87.50	77.78
Specificity	93.75	93.33
Positive Predictive Value	87.50	87.50
Negative Predictive Value	93.75	87.50

Sensitivity = TP/(TP+FN);
Specificity = TN/(TN+ FP)
Positive Predictive Value = TP/(TP+ FP)
Negative Predictive Value = TN/(TN+ FN)

TP = True Positive
FP = False Positive
TN = True Negative
FN = False Negative



Pathway Biomarker Discovery - Application to Diabetes

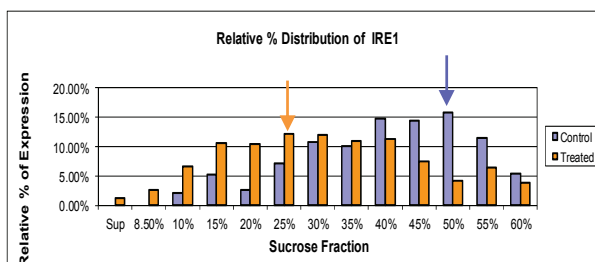


ER Stress and Diabetes

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	Unknown	Unknown
	Eno 1 protein	Up	✓	✓
	unnamed protein product	Up	Unknown	Unknown
	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	Unknown	Unknown
50%	aconitase 2, mitochondrial	Down	✓	✓
	isovaleryl Coenzyme A dehydrogenase	Up	✓	✓
	Eef1g protein	Up		
	similar to prohibitin (BAP 32)	Up	Unknown	Unknown
	unnamed protein product	Up	Unknown	Unknown
	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.**

Biomarker Evaluation - Application to Brain Dysfunction

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.

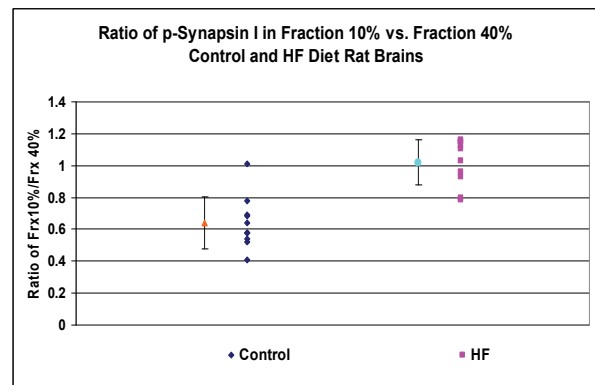
Rat models of high fat diet (HF) have shown that a diet high in fat has a profound impact on brain function. Phosphosynapsin I (p-synapsin I) has been described as a marker of synaptic dysfunction. To evaluate the utility of p-synapsin I as a marker of synaptic dysfunction arising from high fat diet,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

EDGE*TEST was used to evaluate p-synapsin I as a biomarker 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 (10 per group).

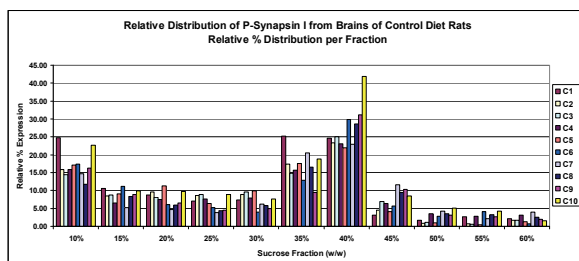
Comparison of changes in relative distribution of the marker between the control and high fat diet groups revealed that the ratio of the marker in the 10% versus the 40% fraction was significantly different in each group, thus providing a means for evaluation:

	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

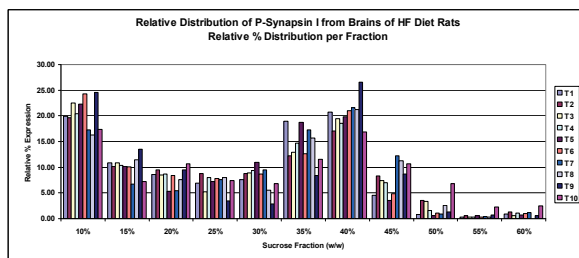
Statistical presentation of the results of analysis from 10 control and 10 HF rat brains showing a specific ratio of relative percentage distribution of p-synapsin I expression (Frax 10% / Frax 40%) . Also shown is the mean and SD for each group.



Graphical representation of ratio data in Table 1 above. Ratios (Frax 10% / Frax 40%) of RPD of p-synapsin I expression cluster into two specific groups unique to either HF or control rat brain origin. Error bars indicate mean +/- 1 SD.



Control



High Fat Diet



Prospect ... “explore for useful or valuable things or substances”

Prospect ... “a prediction of the course of a disease”

Prospect ... “the possibility of future success”

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