A Genomic Approach to Biomarker Discovery in Prostate Cancer

Case Study

A Quick Introduction: Gene Logic Programs Overview

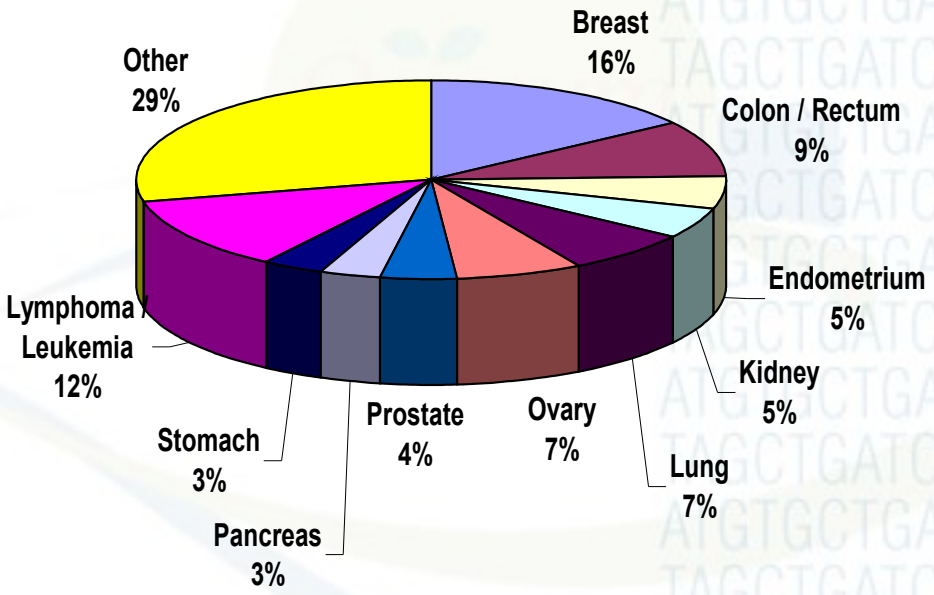
- **Gene Logic has developed one of the world's largest and most detailed knowledge bases of gene expression profiles using Affymetrix GeneChip® microarray technology.**
  - Over 36,000 human and animal samples significant to key therapeutic areas and with full clinical information are offered in our reference databases.
  - Over 200,000 microarrays have been processed in our high-throughput, GLP facility.

- **We offer a wide range of genomic products and services including:**
  - Genomic reference databases:
    - BioExpress® System
    - ToxExpress® System
    - The ASCENTA® System
  - Genomic data generation & bioinformatics analysis services:
    - Gene Expression including GeneChip® microarrays, miRNA, Exon, Q-RT PCR
    - SNP Genotyping
    - aCGH

The BioExpress® Oncology Program - Highlights

More than 6,000 samples, including:

- Primary tumors
- Secondary (metastatic) tumors
- Benign tumors
- Matched non-malignant tissue controls
- Extensive clinical annotation
- Various cell line studies
  - NCI-60 cell lines
  - Various drug-treated studies and cell culture experiments
- Human into mouse xenografts
- LCM samples



BioExpress Oncology® Program - Standard Clinical Data

- **Demographics**
  - Age
  - Gender
  - Race/Ethnicity
- **Health Risk Factors**
  - Height / weight / BMI
  - Allergies / exposures
  - Diet / supplements
  - Smoking history
  - Alcohol use
  - Recreational drug use
- **Medical History**
  - Primary disease
  - Concurrent disease(s)
  - Prior history
- **Treatment History**
  - Current and previous medications
  - Anesthetics / preoperative agents
  - Surgical procedure(s)
- **Family History**
  - Relative, disease, age of diagnosis
- **Diagnostic Tests**
  - Preoperative lab work
  - Disease-specific studies

Prostate Cancer | Epidemiology

- The most prevalent cancer in men (American Cancer Society)
- More than 220,000 new cases and nearly 30,000 deaths were reported in 2003 for the United States
- More than 40 Millions PSA tests are preformed worldwide each year and this number is expected to grow due to aging population

Prostate Cancer | PSA Testing Fact Sheet

- PSA Test is specific for prostate tissue not prostate cancer
  - Elevated PSA does not necessarily indicate prostate cancer
- Only 25% of patients found positive by PSA testing (> 4ng/ml) are confirmed by a biopsy to have cancer (high false positive rate)
- Urologists must then decide to
  - Conduct a biopsy or
  - Conduct additional PSA testing or
  - To delay follow-up
- It is being advocated to decrease the PSA threshold for biopsy in order to detect more cancer, the consequences are:
  - Increase number of expensive and uncomfortable biopsies
  - Increasing the rate of negative biopsies
- Not all prostate cancers release high levels of PSA in blood

Prostate Cancer | *In silico* Experimental Outline

- Curation of adenocarcinoma prostate, benign prostatic hypertrophy (BPH) and normal prostate sample sets
- Identifying candidate biomarkers that discriminate between prostate cancer, BPH or normal prostate
- Assess tissue specificity of selected gene in normal tissues as well as in other cancers and diseased tissues

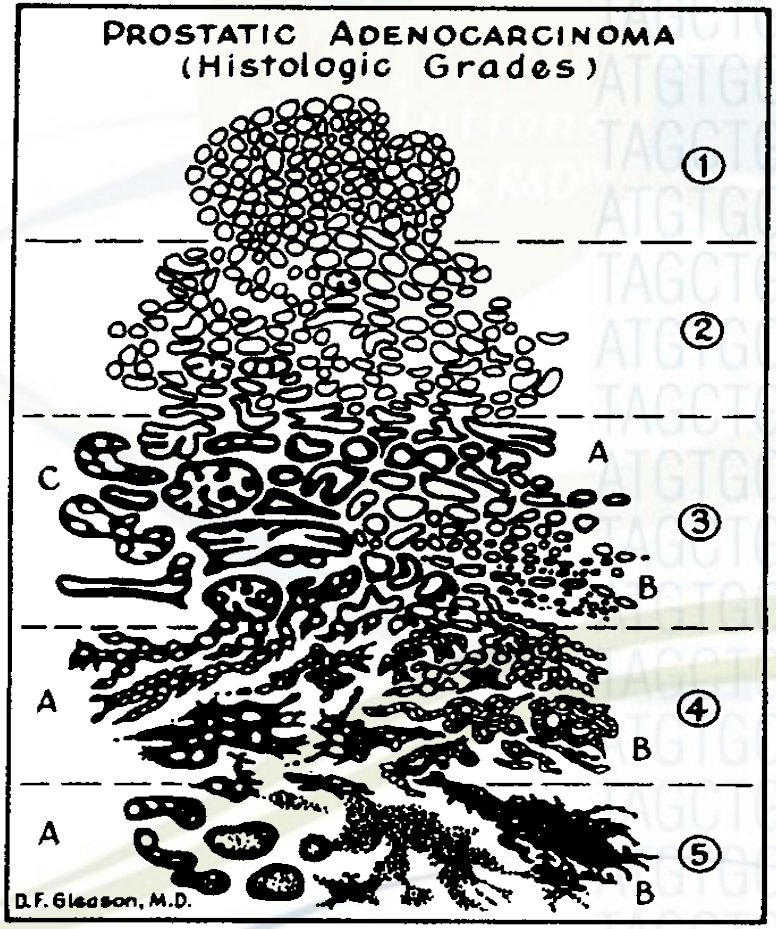
Prostate Cancer | Sample Set Curation

Sample sets defined by using specific pathological and clinical features available in the BioExpress® System.

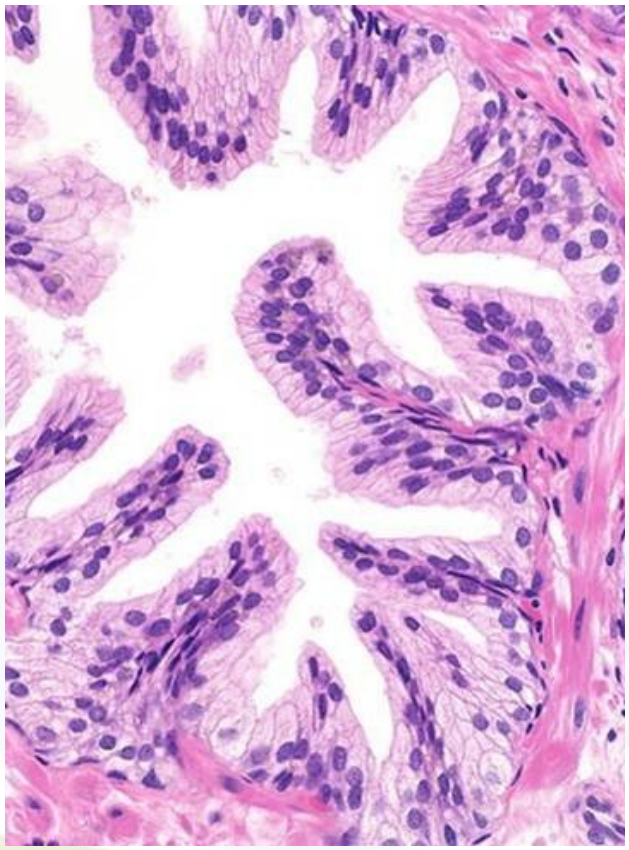
- Prostate Adenocarcinoma
  - Create sample sets based on Gleason score (5 or higher)
- Benign Prostatic Hypertrophy (BPH)
  - Select samples from patients with no malignancy in the prostate
- Normal Prostate
  - Samples from patients where the primary site of disease is not in the prostate or is elsewhere in the prostate

Gleason Grading Scheme

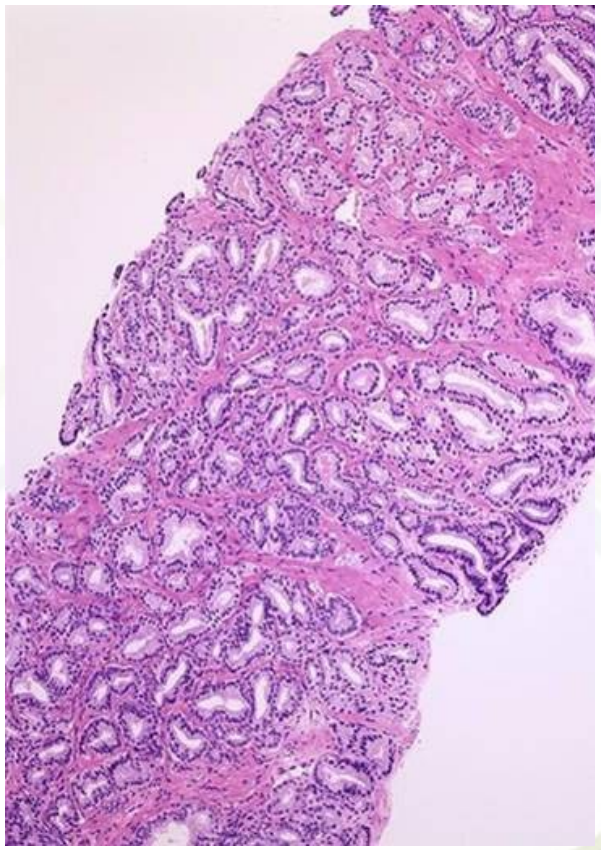
- A value from 1 to 5 is assigned to the microscopic architecture of the cancer.
- Two values are assigned to incorporate the two predominant patterns. The values are added to form a score.
- Gleason Scoring:
  - 2 to 4 is considered low grade;
  - 5 to 7 an intermediate grade; and
  - 8 to 10 a high grade.



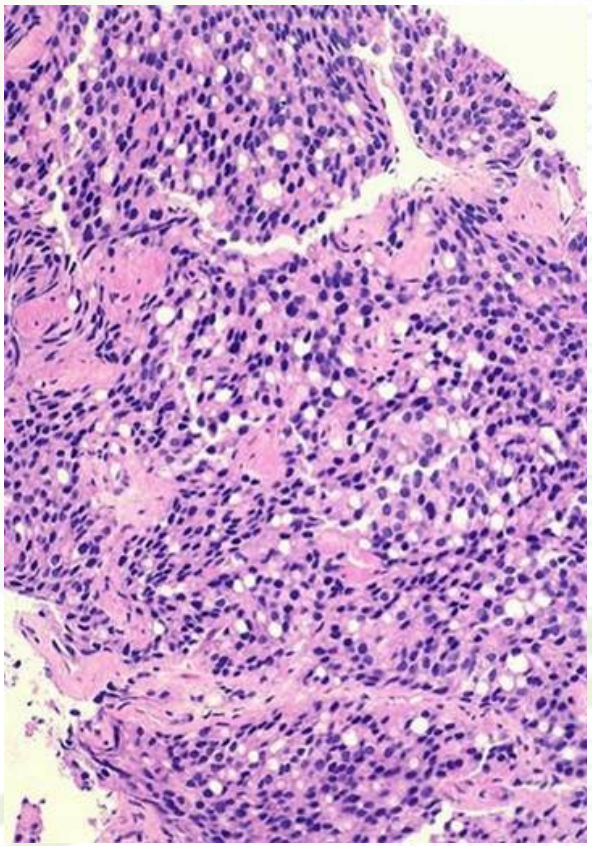
Gleason Grading Examples



Normal Prostate

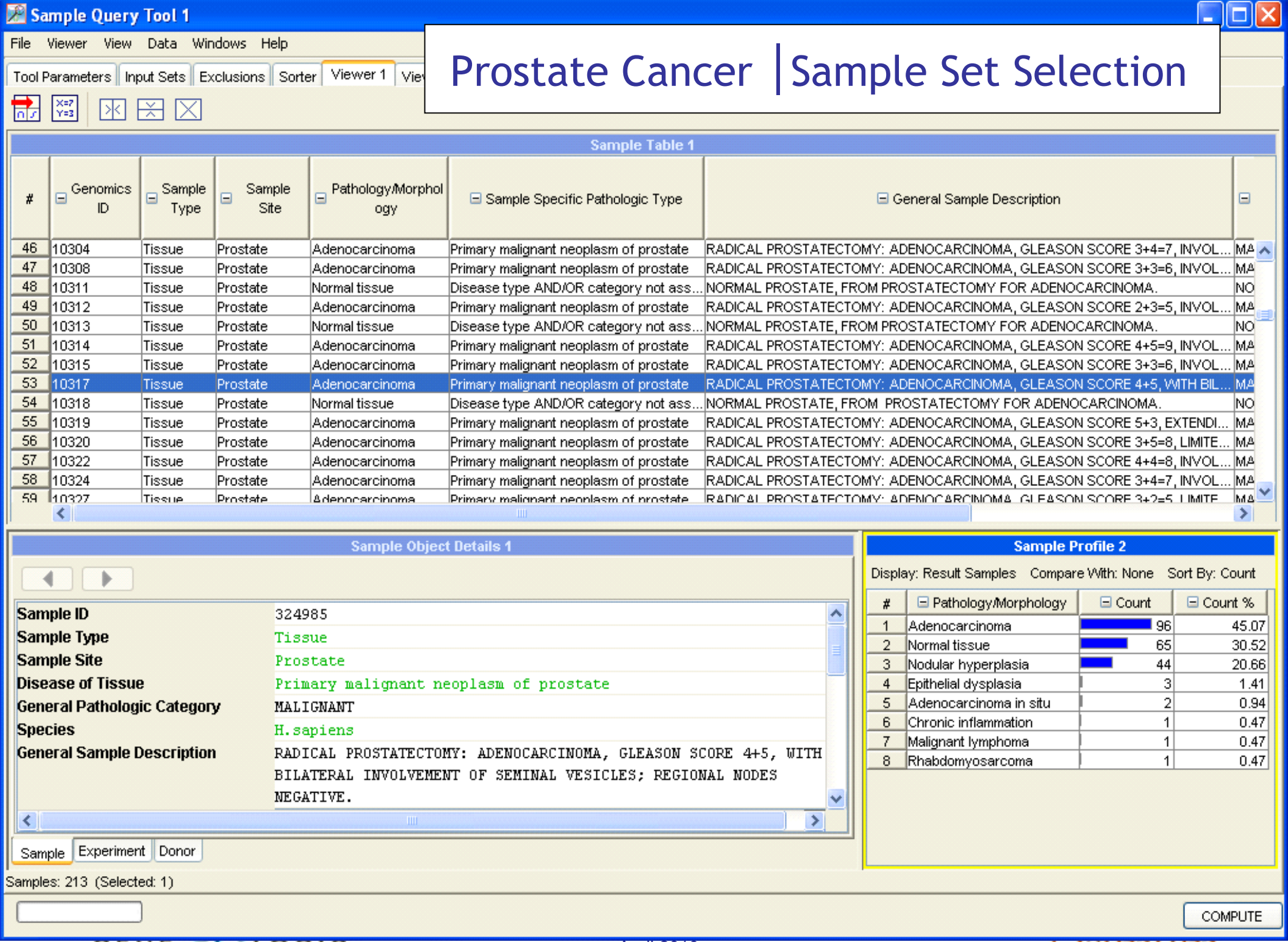


Gleason Pattern 3

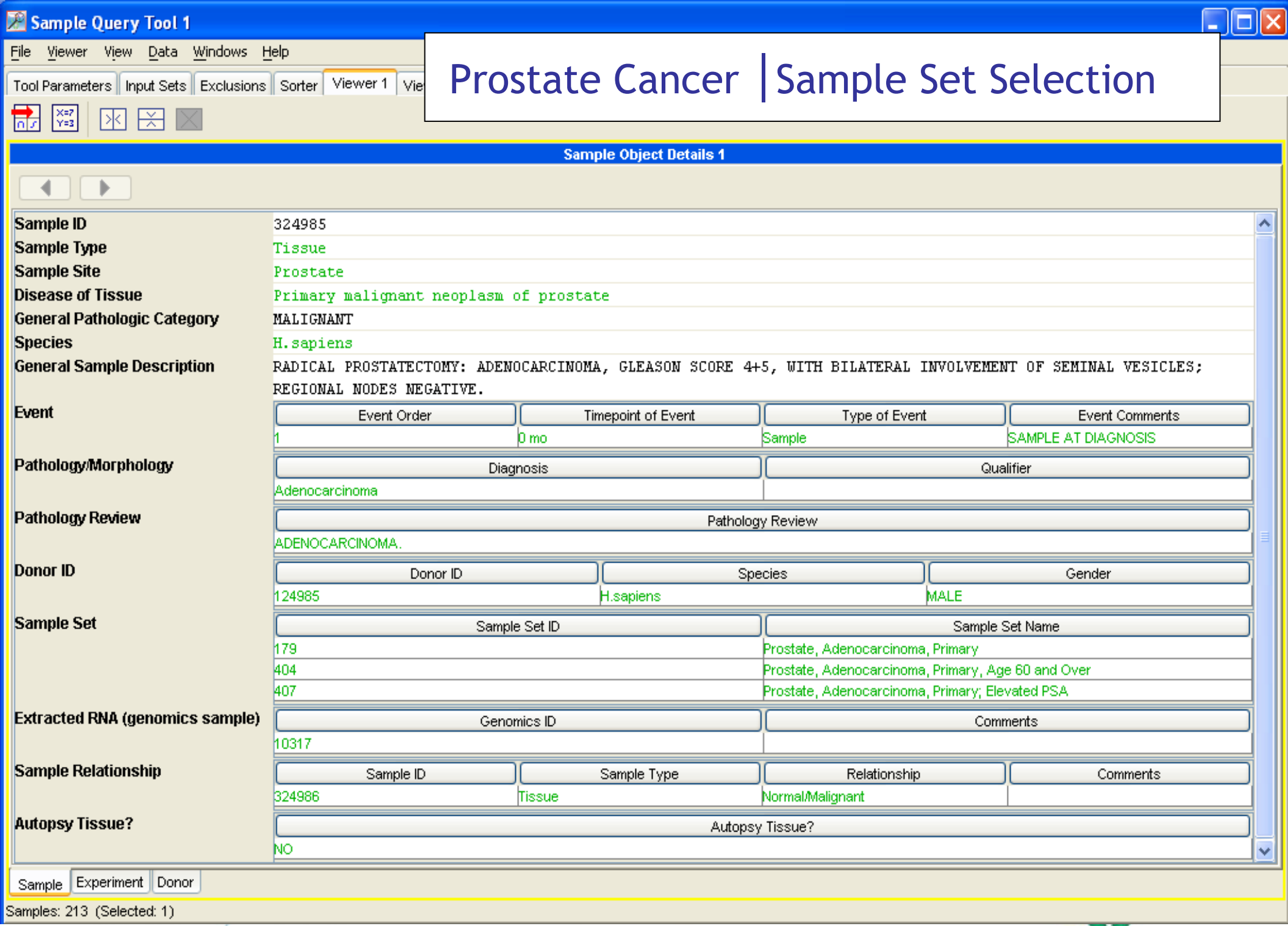


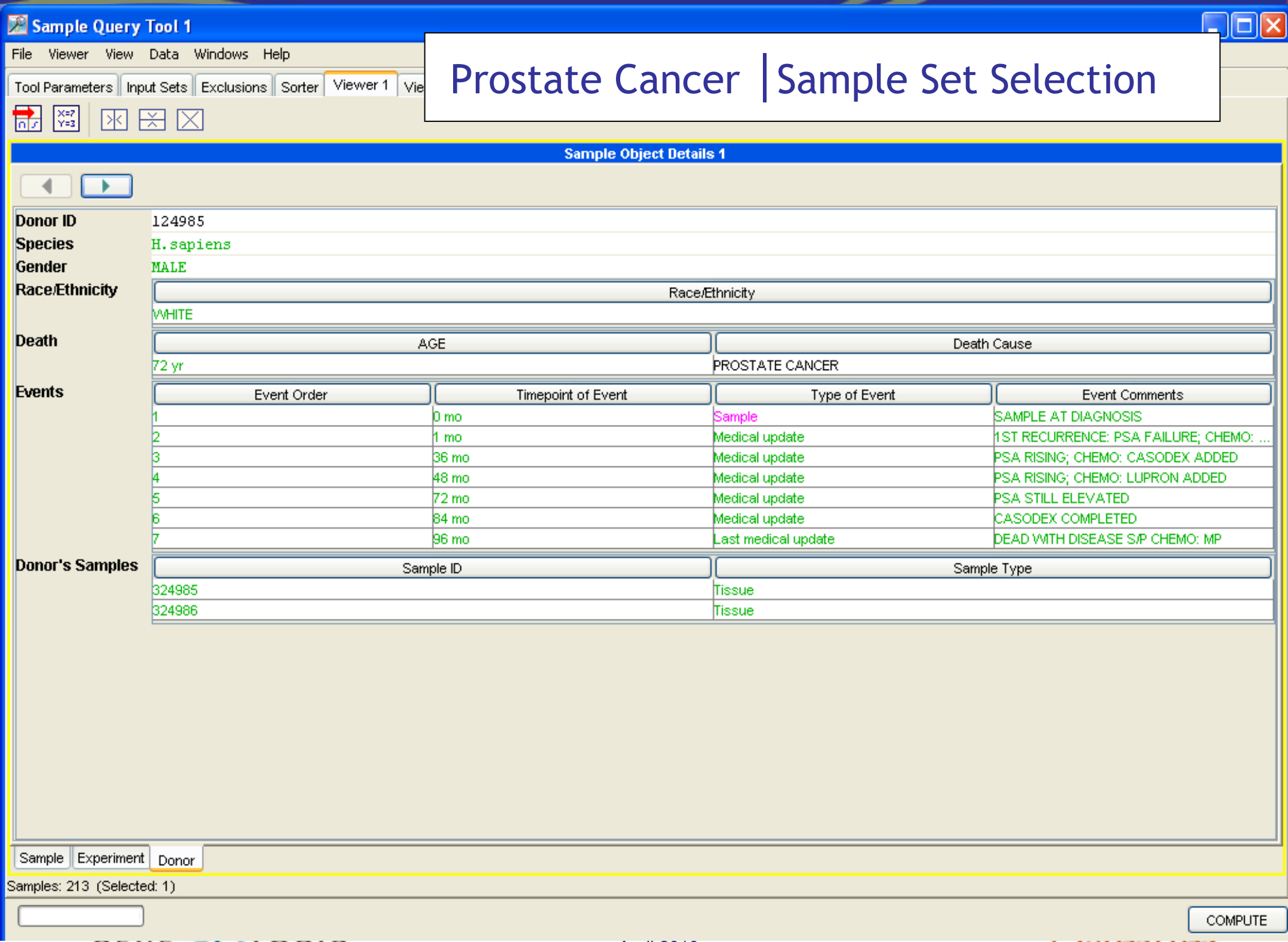
Gleason Pattern 5

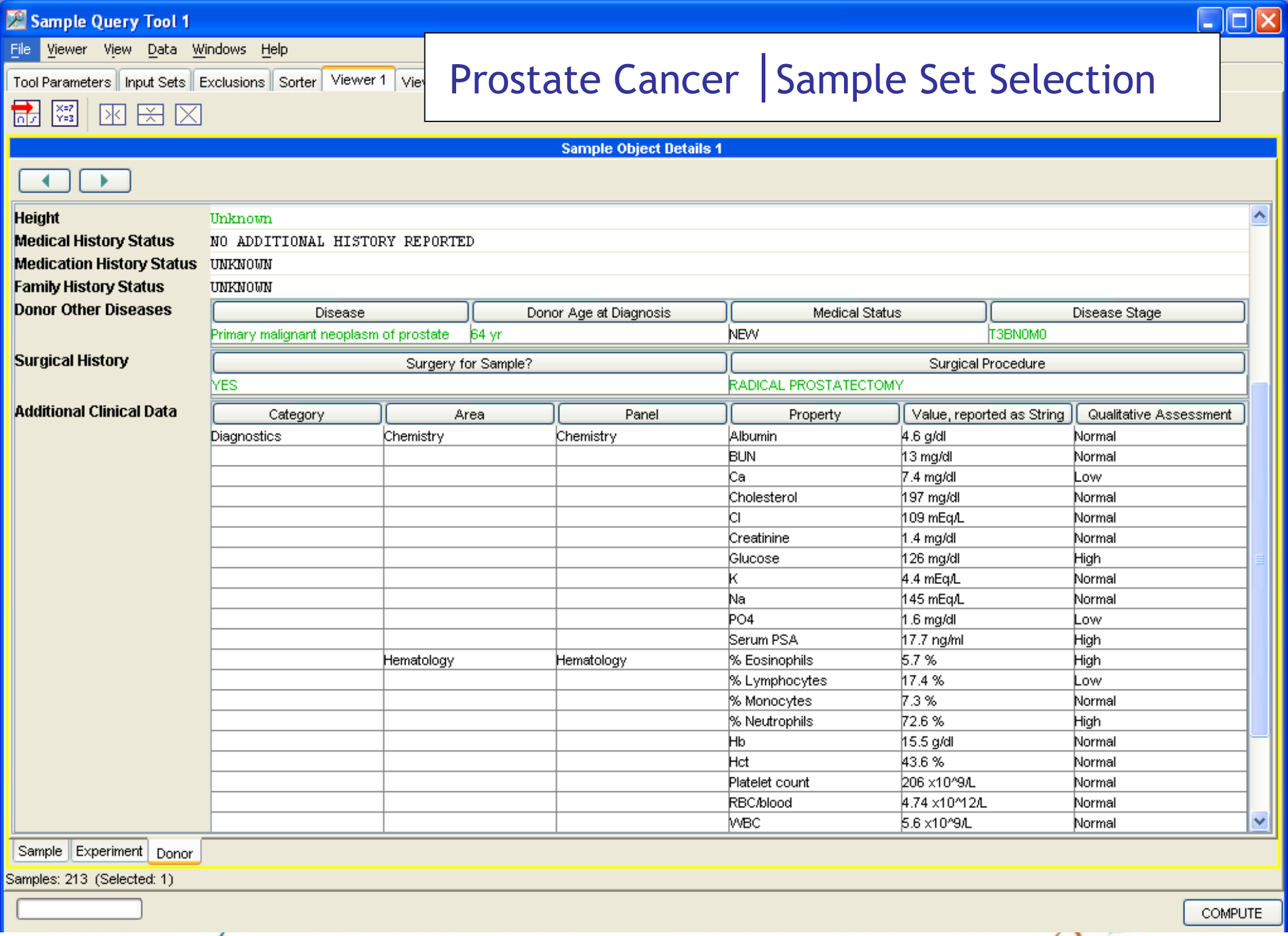
Prostate Cancer | Sample Set Selection



Prostate Cancer | Sample Set Selection







In silico Experiment | Candidate Biomarker Identification

Analysis and Visualization Methodologies

- Comparative Analysis: Differential Expression
- Contrast Analysis: Selective Pattern Matching
- Absolute Analysis: E-Northern Visualization

Comparative Analysis

- **Expression Criteria:**
  - Fold Change ≥ 5.0
  - p-value ≤ 0.05
- **Results:**
  - 28 Changing Genes

Comparative Analysis Tool 1

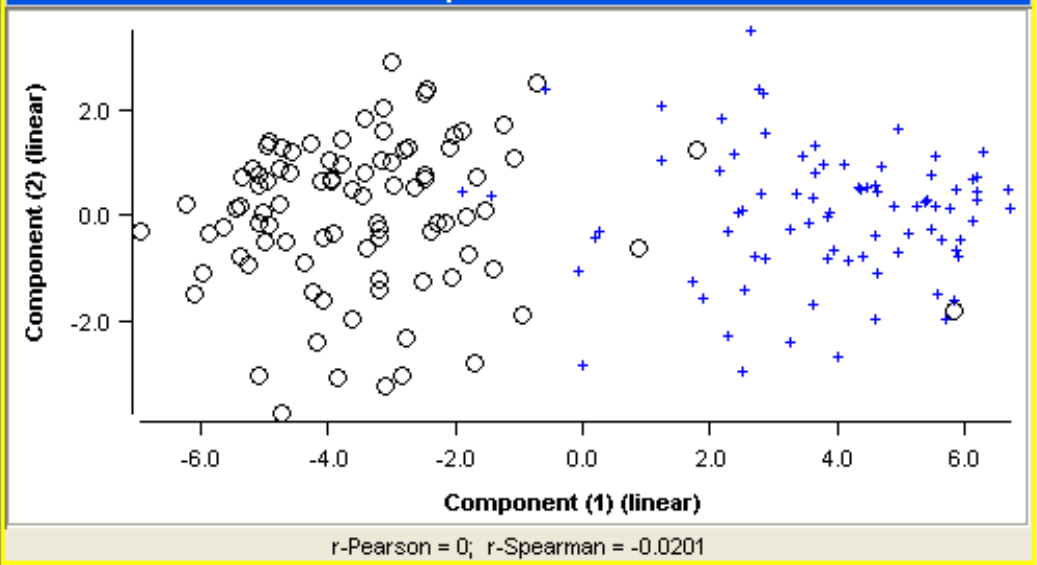
File Viewer View Data Windows Help

Tool Parameters | Analysis Filter | Gene Query Filter | Gene Sorter | Sample Sorter | Sample Exclusions | Gene Exclusions | View

Gene Table 1

#	Sequence Clusters: Cluster Title	Known Genes: Gene Symbol	t-Test p-Value (Prostate, Normal vs. Prostate, Adenocarcinoma)	FC Signed Magnitude (Prostate, Normal vs. Prostate, Adenocarcinoma)	FC Signed Magnitude (/public/SampleSets/ASCENTA/Human/Disease/Normal/Normal/Prostate/Prostate, Normal vs. Prostate, Benign Nodular Hyperplasia)	t-Test p-Value (/public/SampleSets/ASCENTA/Human/Disease/Normal/Normal/Prostate/Prostate Normal vs. Prostate, Benign Nodular Hyperplasia)
1	Alpha-methylacyl-CoA racemase	AMACR	6.6695E-33	10.62	-1.14	0.40
2	Alpha-methylacyl-CoA racemase	AMACR	3.2271E-32	8.53	-1.06	0.60
3	Alpha-methylacyl-CoA racemase	AMACR	4.2314E-30	8.52	-1.13	0.31
4	Hepsin (transmembrane protease, se...	HPN	3.2326E-26	5.08	-1.08	0.54
5	Transcribed locus		7.9979E-25	5.32	1.08	0.58
6	PDZ and LIM domain 5	PDLIM5	1.3589E-21	6.04	-1.25	0.21
7	Distal-less homeobox 1	DLX1	3.5151E-21	8.18	-1.29	0.22
8	Cytochrome P450, family 3, subfamily...	CYP3A5	6.6245E-20	-6.49	-1.12	0.51
9	Solute carrier family 2 (facilitated gluc...	SLC2A5	1.1243E-19	-6.59	-1.11	0.60
10	Prostate cancer antigen 3	PCA3	1.4915E-19	7.73	-1.18	0.39

Sample PCA 2D Plot 2



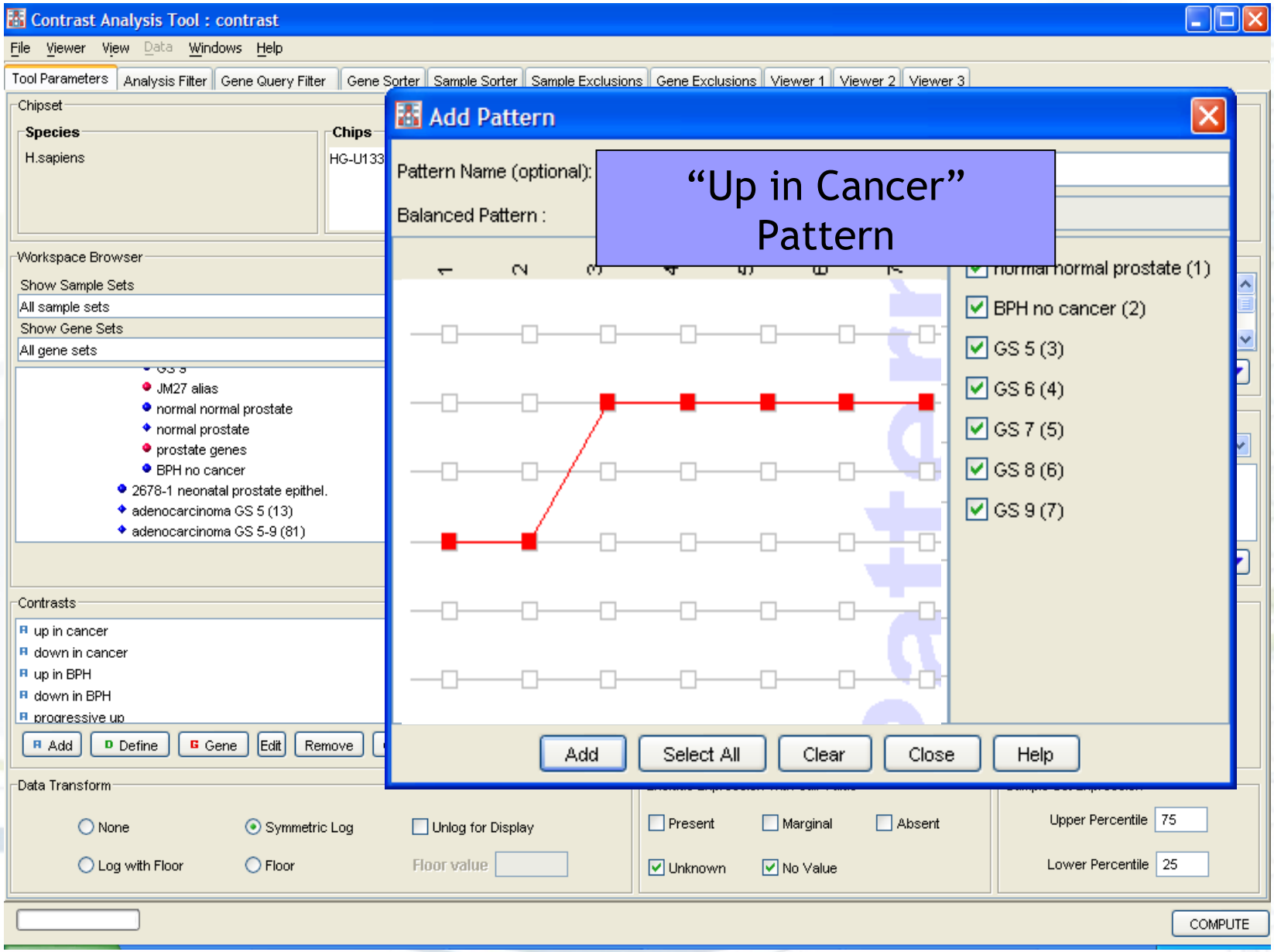
r-Pearson = 0; r-Spearman = -0.0201

Sample Profile 1

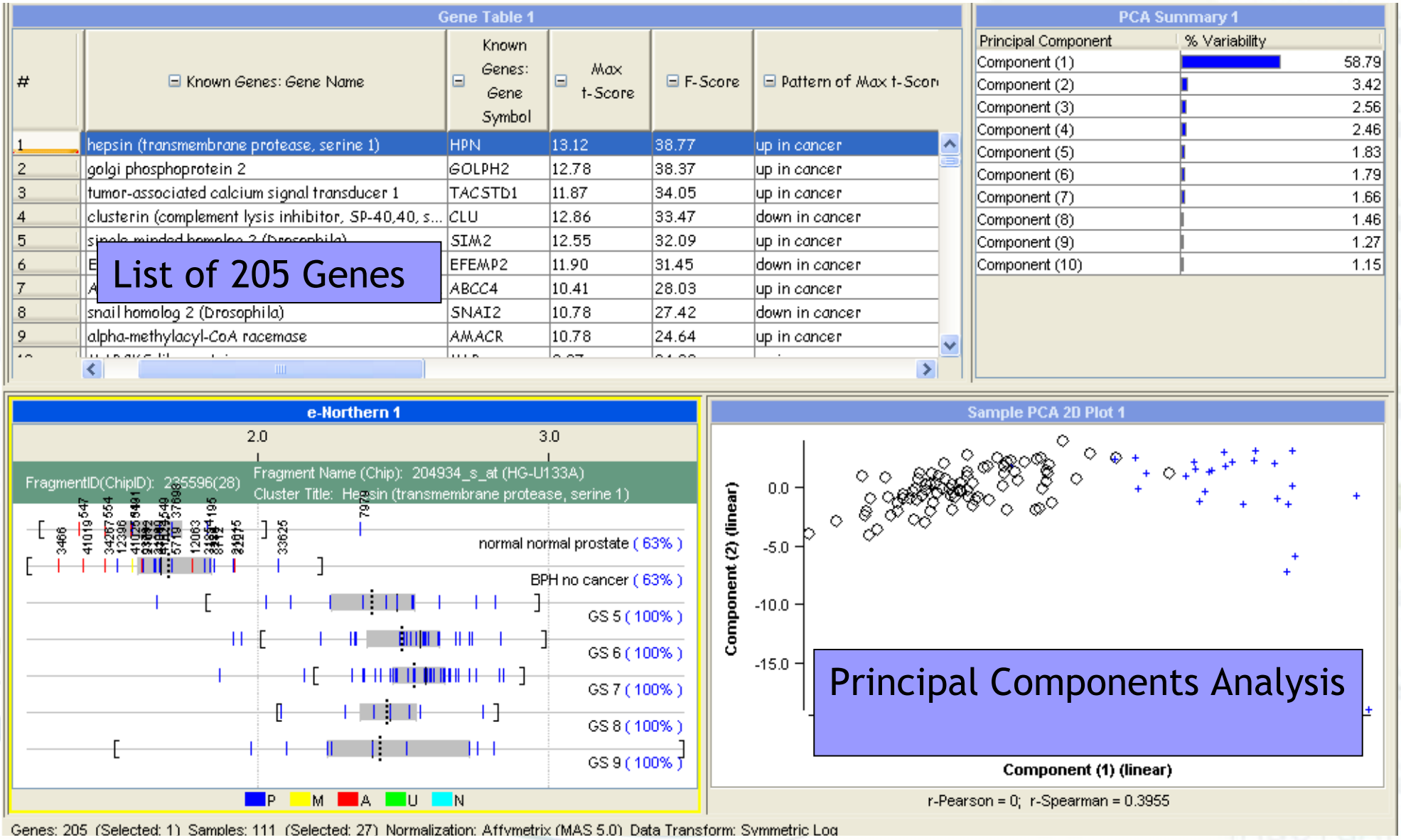
Display: Result Samples Compare With: None Sort By: Value Groups: 3 (Selected: 1)

#	Pathology/Morphology	Count	Count %
1	Adenocarcinoma	82	47.40
2	Nodular hyperplasia	34	19.65
3	Normal tissue	57	32.95

Contrast Analysis: Identifying genes by pattern matching

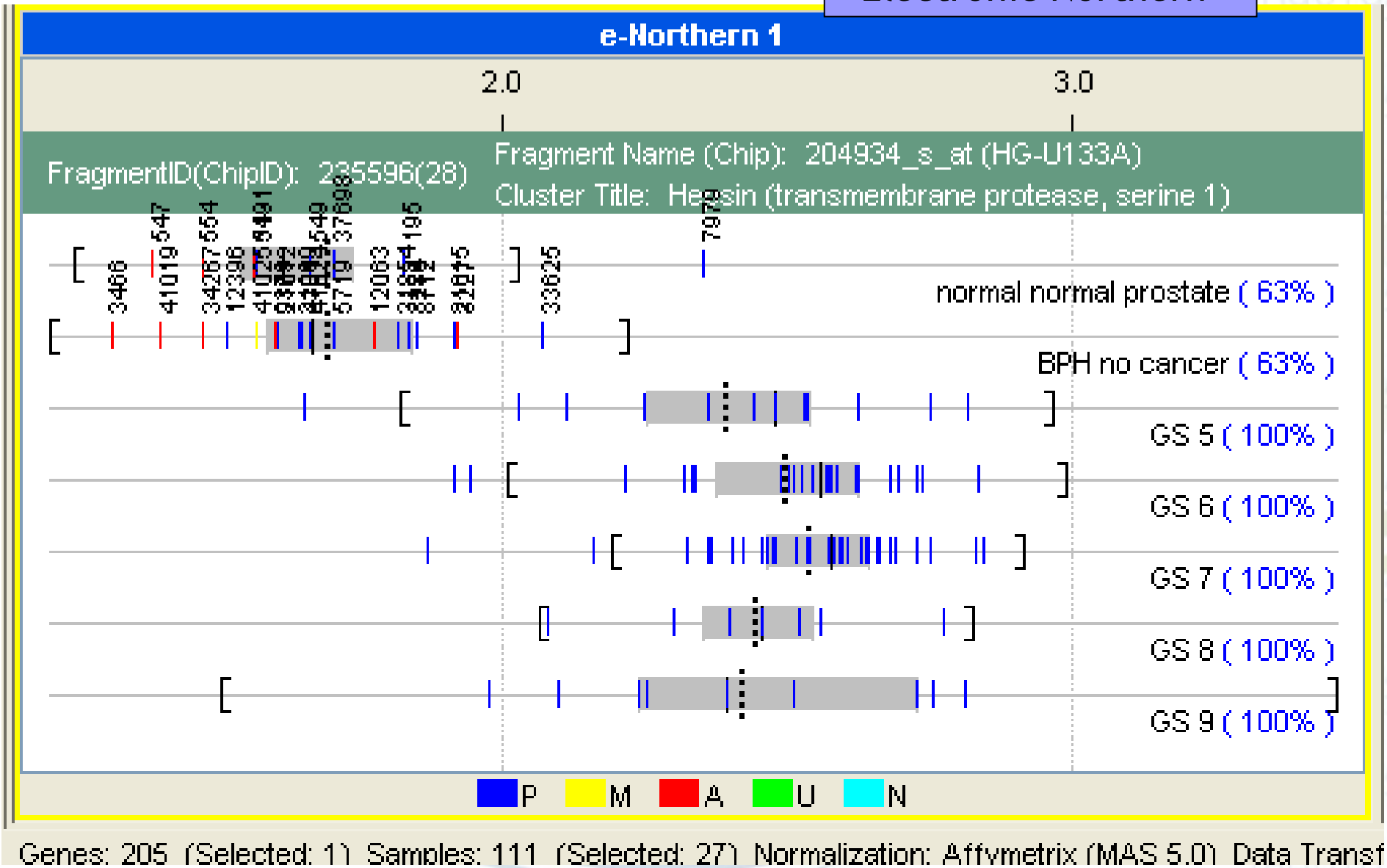


Result Example: Hepsin



Absolute Analysis - E-Northern for Hepsin

"Electronic Northern"



Genes: 205 (Selected: 1) Samples: 111 (Selected: 27) Normalization: Affymetrix (MAS 5.0) Data Transf

In the Literature

J Urol. 2004 Jan;171(1):187-91.

Related Articles, Links

LWWonline
Lippincott Williams & Wilkins

Hepsin is highly over expressed in and a new candidate for a prognostic indicator in prostate cancer.

Stephan C, Yousef GM, Scorilas A, Jung K, Jung M, Kristiansen G, Hauptmann S, Kishi T, Nakamura T, Loening SA, Diamandis EP.

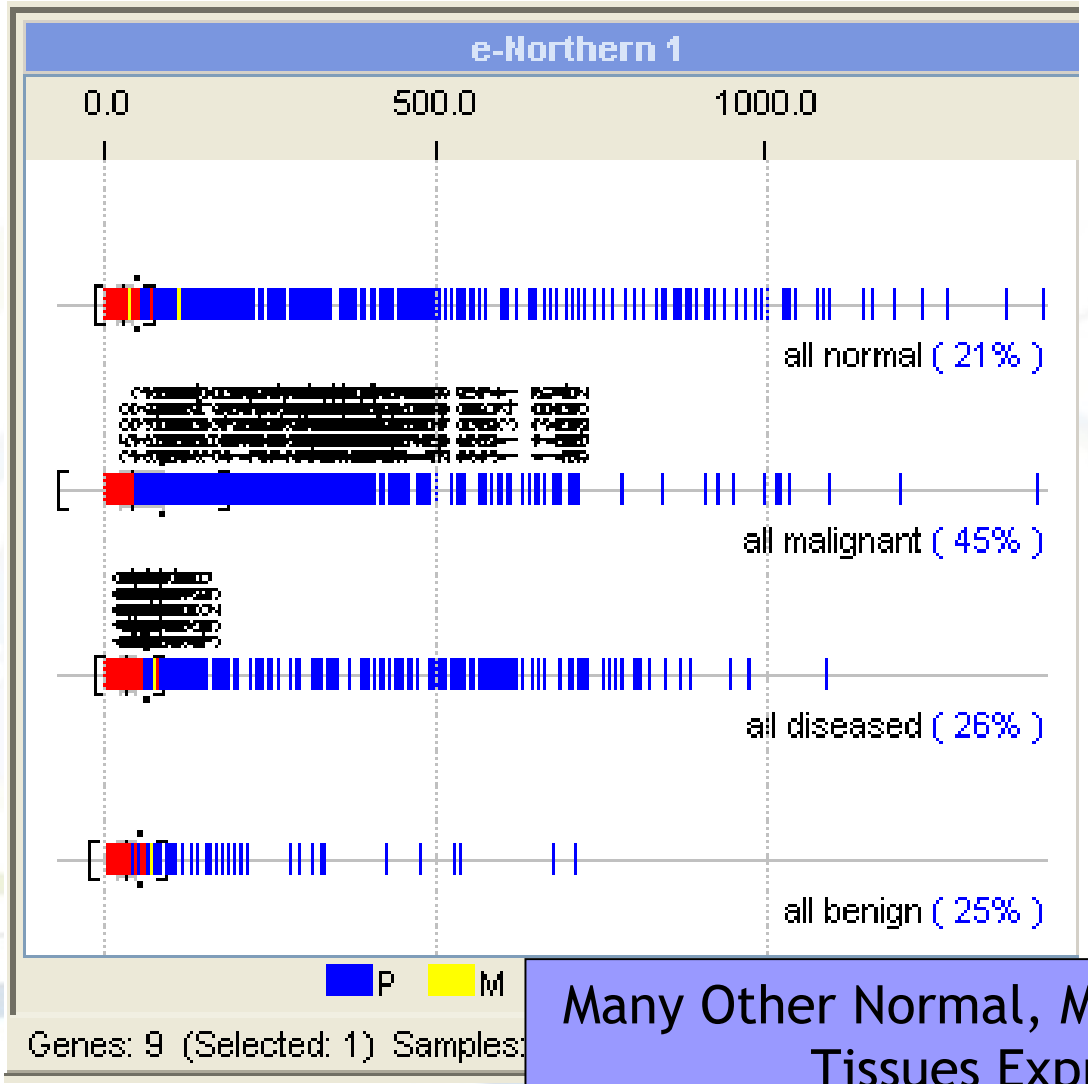
Department of U

PURPOSE: Oth prostate cancer tissue compared e expression with real-time polyme cohort of samples. MATERIALS A of the same prostates were o e reverse transcriptase-pol I on a LightCycler (Roche Diagnost e) was used to normalize data. n 81 of the 90 patient samples (90%, p <0.001). In 48 patients (53%) hepsin over expression was more than 10-fold in cancerous tissue. The ratio of cancerous-to-noncancerous hepsin expression was significantly higher in the 39 patients with grade 3 tumors compared with the 51 with grade 2 tumors (median 15.5 vs 9.6, p = 0.031). For the prognosis a cutoff at the 75th percentile provided a significant difference between patients at lower risk (pT2, G2 and Gleason score less than 7) and higher risk (pT3/4, G3 and Gleason score 7 or greater) for relapse. CONCLUSIONS: This report of the quantitative analysis of hepsin expression, which is the first to our knowledge, shows strong and significant over expression in prostate cancer tissue. Hepsin expression may be a new prognostic marker that could be used for assessing prostate cancer aggressiveness.

"…This report of the quantitative analysis of hepsin expression … shows strong and significant over expression in prostate cancer tissue.

" Hepsin expression may be a new prognostic marker that could be used for assessing prostate cancer aggressiveness."

How Specific is Hepsin to Prostate Cancer?



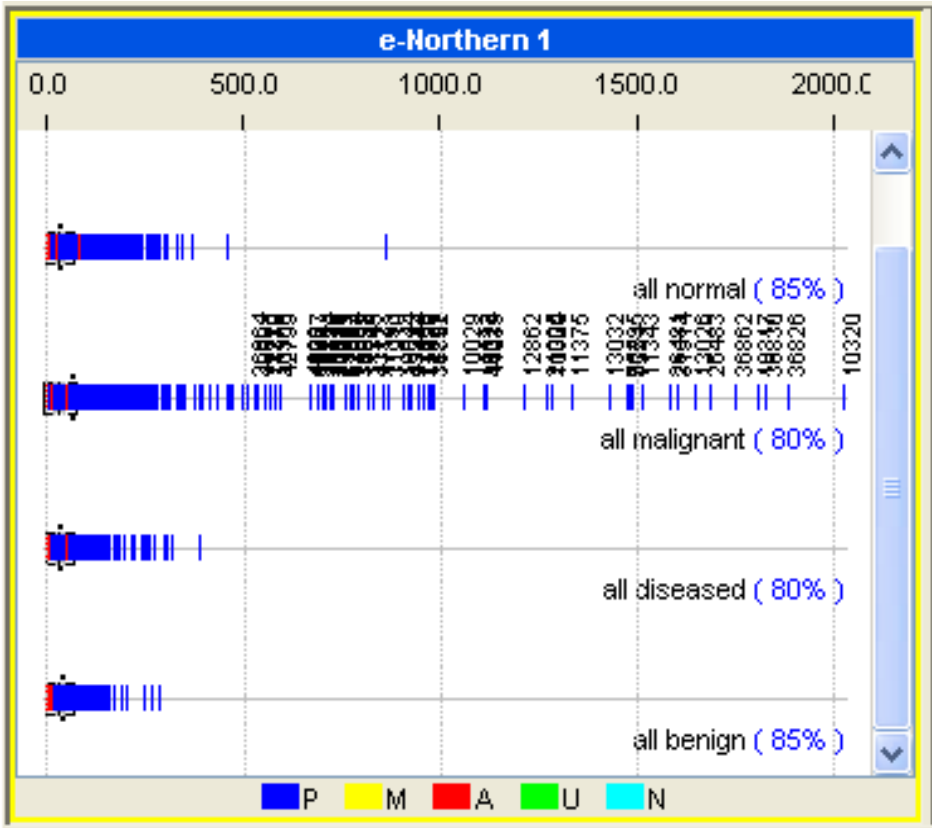
Sample Profile 2

Display: Selected Samples Compare With: None Sort By: Count

#	Sample Site	Count	Count %
1	Prostate	136	100.00

Many Other Normal, Malignant and Diseased Tissues Express Hepsin.

α-Methylacyl-CoA Racemace (AMACR)



Sample Profile 2

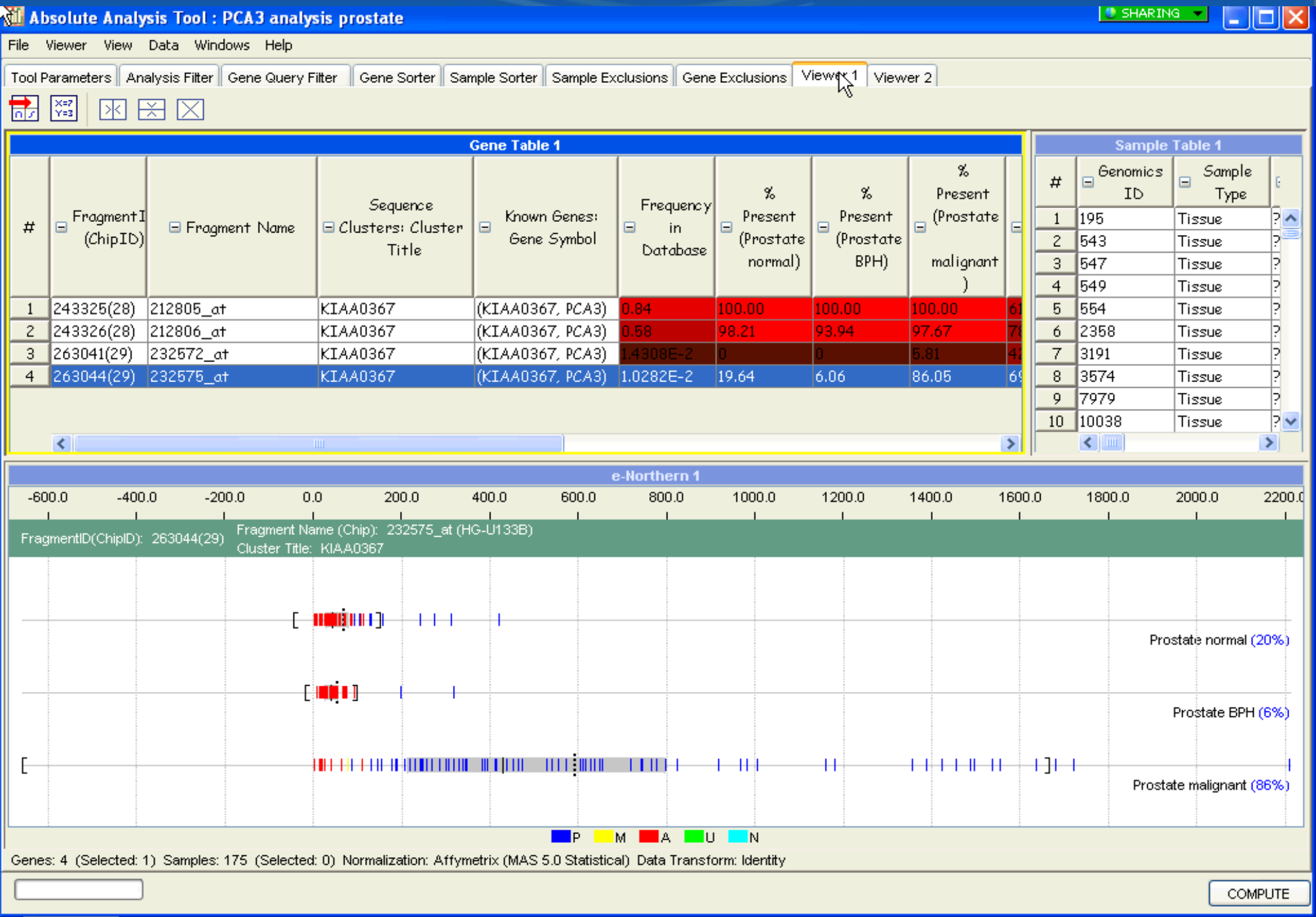
Display: Selected Samples Compare With: None Sort By: Count

#	Sample Site	Count	Count %
1	Prostate	43	78.18
2	Kidney	8	14.55
3	Adrenal cortex	1	1.82
4	Cecum	1	1.82
5	Lymph node	1	1.82
6	Thyroid gland	1	1.82

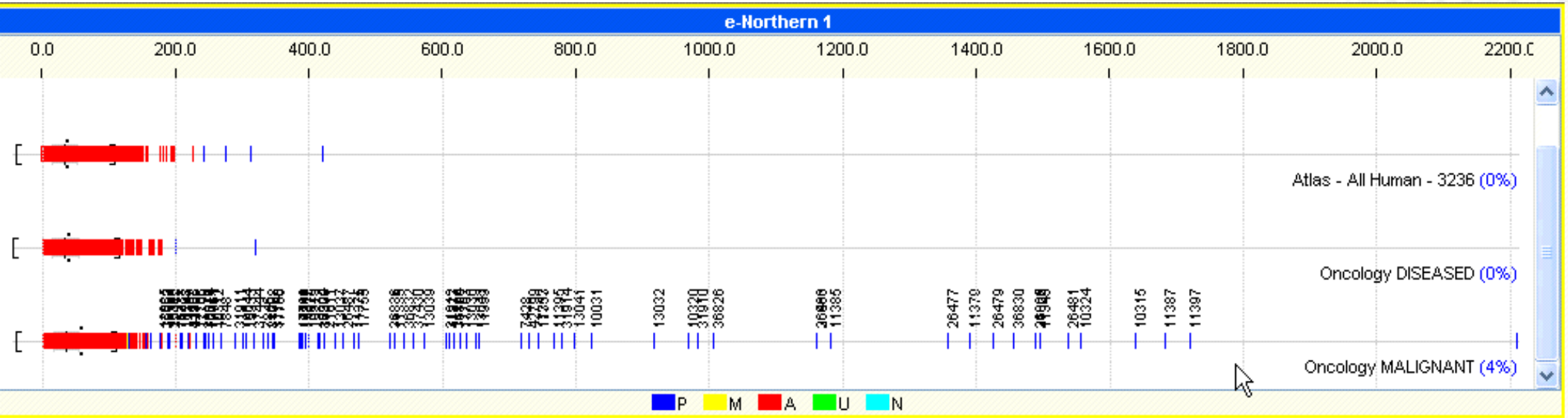
Mainly seen in prostate but also in a smaller number of renal samples.

Genes: 9 (Selected: 1) Samples: 10389 (Selected: 55) Normalization: Affymetrix (MAS 5.0) Data Transform: Identity

Absolute Analysis – Across Prostate Sample Sets



Absolute Analysis – Disease and Tissue Specificity



Sample Profile 1

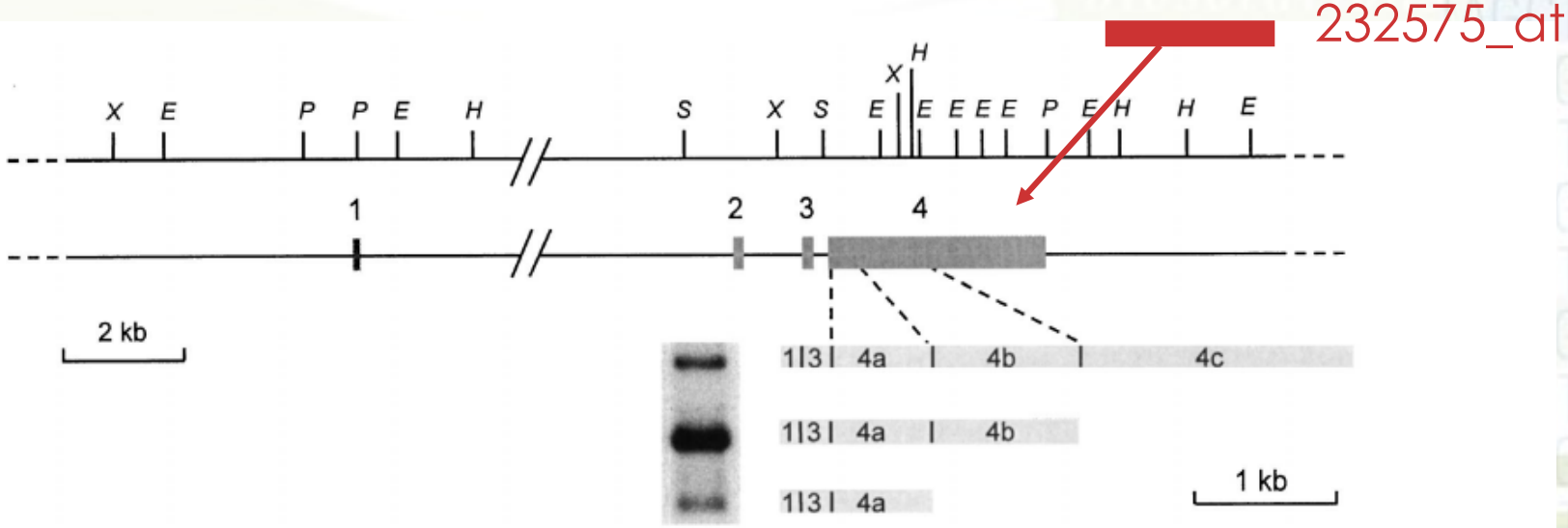
Display: Selected Samples Compare With: None Sort By: Value Groups: 4

#	Sample Site	Count	Count %
1	Lymph node	1	1.32
2	Pancreas	1	1.32
3	Pelvic lymph node	1	1.32
4	Prostate	73	96.05

Genes: 4 (Selected: 1) Samples: 8446 (Selected: 76) Normalization: Affymetrix (MAS 5.0 Statistical) Data Transform: Identity

PCA3 Splice Variants in the Literature

- The PCA3 RNA was described as the most prostate-specific gene and could not be detected in other than prostatic normal and malignant human tissues (*Bussemakers et al., 1999*).



- It has been reported (*Gandini et al., 2003*) that:
  - PCA3 splice variants spanning exons 1-3 can be detected by RT-PCR in a wide variety of normal and malignant human tissues
  - Prostate specific expression was observed only for variants including exon 4

PCA3 in the Literature: A Potential Diagnostic Marker for Prostate Cancer

1: Eur Urol. 2003 Jul;44(1):8-15; discussion 15-6.

Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Comment in:

- Eur Urol. 2004 Aug;46(2):271-2.

DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer.

Hessels D, Klein Gunnewiek JM, van Oort I, Karthaus HF, van Leenders GJ, van Balken B, Kiemeney LA, Witjes JA, Schalken JA.

Department of Experimental Urology, Nijmegen Center for Molecular Life Sciences, P.O Box 9101, 6500 HB Nijmegen, The Netherlands.

BACKGROUND: DD3(PCA3) is the most prostate cancer-specific gene described to date. To assess the clinical utility of DD3(PCA3) a time-resolved fluorescence-based, quantitative RT-PCR analysis for DD3(PCA3) was developed. METHODS: The diagnostic potential of DD3(PCA3) was determined by quantitative measurement of DD3 (PCA3) transcripts in non-malignant and malignant prostate specimens. Moreover, DD3(PCA3) transcripts were determined quantitatively in urine sediments obtained after prostatic massage. A cohort of 108 men, admitted for prostate biopsies based on a PSA of >3ng/ml, was studied. RESULTS: Prostate tumors showed a 66-fold up-regulation of DD3(PCA3) (median 158.4.10(5) copies/microg tissue RNA) when compared to benign prostate tissue (median 2.4.10(5) copies/microg tissue RNA). This up-regulation was found in more than 95% of prostate cancer specimens studied. These data revealed that specimens with less than 10% of cancer cells could be accurately discriminated from non-cancer tissues. Hence, detection of a small fraction of prostate cancer cells in a background of normal cells seemed feasible. Therefore, this DD3(PCA3)-based RT-PCR assay was used for the identification of prostate cancer in urine sediments obtained after prostatic massage. From 108 men with a serum PSA value >3ng/ml, 24 men were shown to have prostate cancer upon biopsy. Of these 24 men, 16 were shown to be positive for DD3(PCA3), indicating a sensitivity of the assay of 67%. Furthermore, a negative predictive value of 90% was calculated. CONCLUSION: The quantitative RT-PCR assay for DD3(PCA3) described, bears great promise as a tool for molecular urine analysis. It has great potential in reducing the number of unnecessary biopsies. A multi-center study using this DD3(PCA3) assay can provide the basis for the utility of molecular diagnostics in clinical urological practice.

PMID: 12814669 [PubMed - indexed for MEDLINE]

PCA3 in the News: A Novel Diagnostic Marker

News

Press Releases
- Financial
- Archive

Industry Events

Media

Gen-Probe Acquires From DiagnoCure Exclusive Worldwide Diagnostic Rights To New Prostate Cancer Gene

Companies Form Collaboration to Develop Molecular Test for PCA3[DD3] That May Offer Advantages Over Traditional PSA Testing

- Agreement Accelerates Gen-Probe's Growth in Oncology -

SAN DIEGO, CA , November 20 -- Gen-Probe (Nasdaq: GPRO) and DiagnoCure (Toronto: CUR) announced today that they have signed a license and collaboration agreement under which they will develop, and Gen-Probe will market, an innovative urine test to detect a new, highly specific genetic marker for prostate cancer.

The diagnostic test will detect a recently described gene called PCA3[DD3] that has been shown by studies to date to be over-expressed only in malignant prostate tissue. The test may offer advantages over prostate specific antigen (PSA) testing, the current standard for initial prostate cancer screening in conjunction with a digital rectal exam.

Under the terms of the agreement, Gen-Probe will pay DiagnoCure an upfront US $3 million fee, and future fees and contract development payments of up to US $7.5 million over the next three years. Gen-Probe will receive exclusive worldwide rights to diagnostic products resulting from the agreement, and will pay DiagnoCure royalties of 8% on cumulative net product sales of up to $50 million, and royalties of 16% on cumulative net sales above $50 million.

"The completion of this license agreement represents a major milestone in our planned and communicated strategy," said Pierre Desy, president and CEO of DiagnoCure. "We expect this test to detect the PCA3[DD3] gene in urine to be the first gene-based, adjunctive screen for this devastating disease. Gen-Probe is the ideal partner to bring this important new test to the market. Their leadership in nucleic acid testing (NAT), their proprietary APTIMA(R) technologies, and their strong desire to become a leader in gene-based testing in oncology are the fundamentals that will realize and optimize all the potential of this marker."

Conclusions: The Power of BioExpress® System

- Identify differentially expressed genes between disease and normal state for many disease indications
- Correlate (or not) expression of chosen gene with relevant clinical parameters from extensive list and/or pathology
- Rapidly confirm expression in wide range of normal tissues
- Access annotation and sequence information for each fragment

Ocimum-Gene Logic

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