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
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

#### Sample Table 1

<table>
<thead>
<tr>
<th>#</th>
<th>Genomics ID</th>
<th>Sample Type</th>
<th>Sample Site</th>
<th>Pathology/Morphology</th>
<th>Sample Specific Pathologic Type</th>
<th>General Sample Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>10304</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 3+4=7, INVOL... MA</td>
</tr>
<tr>
<td>47</td>
<td>10308</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 3+3=6, INVOL... MA</td>
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<tr>
<td>48</td>
<td>10311</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Normal tissue</td>
<td>Disease type AND/OR category not ass...</td>
<td>NORMAL PROSTATE, FROM PROSTATECTOMY FOR ADENOCARCINOMA. NO</td>
</tr>
<tr>
<td>49</td>
<td>10312</td>
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<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 4+5=9, INVOL... MA</td>
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<tr>
<td>50</td>
<td>10313</td>
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<td>Prostate</td>
<td>Normal tissue</td>
<td>Disease type AND/OR category not ass...</td>
<td>NORMAL PROSTATE, FROM PROSTATECTOMY FOR ADENOCARCINOMA. NO</td>
</tr>
<tr>
<td>51</td>
<td>10314</td>
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<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 3+3=6, INVOL... MA</td>
</tr>
<tr>
<td>52</td>
<td>10315</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 4+5=9, INVOL... MA</td>
</tr>
<tr>
<td>53</td>
<td>10317</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 4+5=9, WITH BIL...</td>
</tr>
<tr>
<td>54</td>
<td>10318</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Normal tissue</td>
<td>Disease type AND/OR category not ass...</td>
<td>NORMAL PROSTATE, FROM PROSTATECTOMY FOR ADENOCARCINOMA. NO</td>
</tr>
<tr>
<td>55</td>
<td>10319</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 5+3=8, EXTENDI... MA</td>
</tr>
<tr>
<td>56</td>
<td>10320</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 3+3=6, INVOL... MA</td>
</tr>
<tr>
<td>57</td>
<td>10322</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 3+4=7, INVOL... MA</td>
</tr>
<tr>
<td>58</td>
<td>10324</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 3+3=6, INVOL... MA</td>
</tr>
<tr>
<td>59</td>
<td>10327</td>
<td>Tissue</td>
<td>Prostate</td>
<td>Adenocarcinoma</td>
<td>Primary malignant neoplasm of prostate</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 3+3=6, INVOL... MA</td>
</tr>
</tbody>
</table>

#### Sample Object Details 1

- **Sample ID**: 324985
- **Sample Type**: Tissue
- **Sample Site**: Prostate
- **Disease of Tissue**: Primary malignant neoplasm of prostate
- **General Pathologic Category**: MALIGNANT
- **Species**: H.sapiens
- **General Sample Description**: RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 4+5, WITH BILATERAL INVOLVEMENT OF SEMINAL VESICLES; REGIONAL NODES NEGATIVE.

#### Sample Profile 2

<table>
<thead>
<tr>
<th>#</th>
<th>Pathology/Morphology</th>
<th>Count</th>
<th>Count %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adenocarcinoma</td>
<td>96</td>
<td>45.07</td>
</tr>
<tr>
<td>2</td>
<td>Normal tissue</td>
<td>65</td>
<td>30.52</td>
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<tr>
<td>3</td>
<td>Nodular hyperplasia</td>
<td>44</td>
<td>20.66</td>
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<tr>
<td>4</td>
<td>Epithelial dysplasia</td>
<td>3</td>
<td>1.41</td>
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<tr>
<td>5</td>
<td>Adenocarcinoma in situ</td>
<td>2</td>
<td>0.94</td>
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<tr>
<td>6</td>
<td>Chronic inflammation</td>
<td>1</td>
<td>0.47</td>
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<tr>
<td>7</td>
<td>Malignant lymphoma</td>
<td>1</td>
<td>0.47</td>
</tr>
<tr>
<td>8</td>
<td>Rhabdomyosarcoma</td>
<td>1</td>
<td>0.47</td>
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**Samples**: 213 (Selected: 1)
## Sample Object Details 1

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>324985</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type</td>
<td>Tissue</td>
</tr>
<tr>
<td>Sample Site</td>
<td>Prostate</td>
</tr>
<tr>
<td>Disease of Tissue</td>
<td>Primary malignant neoplasm of prostate</td>
</tr>
<tr>
<td>General Pathologic Category</td>
<td>MALIGNANT</td>
</tr>
<tr>
<td>Species</td>
<td>H.sapiens</td>
</tr>
<tr>
<td>General Sample Description</td>
<td>RADICAL PROSTATECTOMY: ADENOCARCINOMA, GLEASON SCORE 4+5, WITH BILATERAL INVOLVEMENT OF SEMINAL VESICLES; REGIONAL NODES NEGATIVE.</td>
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### Event

<table>
<thead>
<tr>
<th>Event Order</th>
<th>Timepoint of Event</th>
<th>Type of Event</th>
<th>Event Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 mo</td>
<td>Sample</td>
<td>SAMPLE AT DIAGNOSIS</td>
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### Pathology/Morphology

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Qualifier</th>
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<tbody>
<tr>
<td>Adenocarcinoma</td>
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</tr>
<tr>
<td>ADENOCARCINOMA.</td>
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</tr>
</tbody>
</table>

### Donor ID

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>Species</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>124985</td>
<td>H.sapiens</td>
<td>MALE</td>
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</tbody>
</table>

### Sample Set

<table>
<thead>
<tr>
<th>Sample Set ID</th>
<th>Sample Set Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>179</td>
<td>Prostate, Adenocarcinoma, Primary</td>
</tr>
<tr>
<td>404</td>
<td>Prostate, Adenocarcinoma, Primary, Age 60 and Over</td>
</tr>
<tr>
<td>407</td>
<td>Prostate, Adenocarcinoma, Primary, Elevated PSA</td>
</tr>
</tbody>
</table>

### Extracted RNA (genomics sample)

<table>
<thead>
<tr>
<th>Genomics ID</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>10317</td>
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</tbody>
</table>

### Sample Relationship

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample Type</th>
<th>Relationship</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>324986</td>
<td>Tissue</td>
<td>Normal/Malignant</td>
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</tbody>
</table>

### Autopsy Tissue?

<table>
<thead>
<tr>
<th>Autopsy Tissue?</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
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</table>
### Sample Object Details 1

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>124985</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>H. sapiens</td>
</tr>
<tr>
<td>Gender</td>
<td>MALE</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td>WHITE</td>
</tr>
<tr>
<td>Death Age</td>
<td>72 yr</td>
</tr>
<tr>
<td>Death Cause</td>
<td>PROSTATE CANCER</td>
</tr>
</tbody>
</table>

#### Events

<table>
<thead>
<tr>
<th>Event Order</th>
<th>Timepoint of Event</th>
<th>Type of Event</th>
<th>Event Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 mo</td>
<td>Sample</td>
<td>SAMPLE AT DIAGNOSIS</td>
</tr>
</tbody>
</table>
| 2           | 1 mo               | Medical update | 1ST RECURRENCE: PSA FAILURE, CHEMO: ...
| 3           | 36 mo              | Medical update | PSA RISING, CHEMO: CASODEX ADDED |
| 4           | 48 mo              | Medical update | PSA RISING, CHEMO: LUPRON ADDED |
| 5           | 72 mo              | Medical update | PSA STILL ELEVATED |
| 6           | 84 mo              | Medical update | CASODEX COMPLETED |
| 7           | 96 mo              | Last medical update | DEAD WITH DISEASE S/P CHEMO: MP |

#### Donor's Samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>324985</td>
<td>Tissue</td>
</tr>
<tr>
<td>324986</td>
<td>Tissue</td>
</tr>
</tbody>
</table>
Prostate Cancer Sample Set Selection

Sample Object Details 1

<table>
<thead>
<tr>
<th>Height</th>
<th>Unknown</th>
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<tbody>
<tr>
<td>Medical History Status</td>
<td>NO ADDITIONAL HISTORY REPORTED</td>
</tr>
<tr>
<td>Medication History Status</td>
<td>UNKNOWN</td>
</tr>
<tr>
<td>Family History Status</td>
<td>UNKNOWN</td>
</tr>
<tr>
<td>Donor Other Diseases</td>
<td></td>
</tr>
<tr>
<td>Surgical History</td>
<td>YES</td>
</tr>
<tr>
<td>Surgery for Sample?</td>
<td>YES</td>
</tr>
<tr>
<td>Surgical Procedure</td>
<td>RADICAL PROSTATECTOMY</td>
</tr>
<tr>
<td>Disease</td>
<td>Primary malignant neoplasm of prostate</td>
</tr>
<tr>
<td>Donor Age at Diagnosis</td>
<td>64 yr</td>
</tr>
<tr>
<td>Medical Status</td>
<td>NEW</td>
</tr>
<tr>
<td>Disease Stage</td>
<td>T3BNOM0</td>
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Additional Clinical Data

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<tr>
<th>Category</th>
<th>Area</th>
<th>Panel</th>
<th>Property</th>
<th>Value, reported as String</th>
<th>Qualitative Assessment</th>
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<tbody>
<tr>
<td>Diagnostics</td>
<td>Chemistry</td>
<td>Chemistry</td>
<td>Albumin</td>
<td>4.6 g/dl</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>BUN</td>
<td>13 mg/dl</td>
<td>Normal</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Ca</td>
<td>7.4 mg/dl</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cholesterol</td>
<td>197 mg/dl</td>
<td>Normal</td>
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<td></td>
<td></td>
<td></td>
<td>Cl</td>
<td>109 mEq/L</td>
<td>Normal</td>
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<td></td>
<td></td>
<td></td>
<td>Creatinine</td>
<td>1.4 mg/dl</td>
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<td>Glucose</td>
<td>126 mg/dl</td>
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<td></td>
<td></td>
<td></td>
<td>K</td>
<td>4.4 mEq/L</td>
<td>Normal</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Na</td>
<td>145 mEq/L</td>
<td>Normal</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>PO4</td>
<td>1.6 mg/dl</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum PSA</td>
<td>17.7 ng/ml</td>
<td>High</td>
</tr>
<tr>
<td>Hematology</td>
<td>Hematology</td>
<td></td>
<td>% Eosinophils</td>
<td>5.7 %</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Lymphocytes</td>
<td>17.4 %</td>
<td>Low</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>% Monocytes</td>
<td>7.3 %</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Neutrophils</td>
<td>72.6 %</td>
<td>High</td>
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<tr>
<td></td>
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<td></td>
<td>Hb</td>
<td>15.5 g/dl</td>
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<td></td>
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<td></td>
<td>Hct</td>
<td>43.6 %</td>
<td>Normal</td>
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<td></td>
<td>Platelet count</td>
<td>206 x10^9/L</td>
<td>Normal</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>RBC/blood</td>
<td>4.74 x10^12/L</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>WBC</td>
<td>5.6 x10^9/L</td>
<td>Normal</td>
</tr>
</tbody>
</table>
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

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

Sample PCA 2D Plot 2

Component (2) (linear)
Component (1) (linear)

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

Sample Profile 1

<table>
<thead>
<tr>
<th>#</th>
<th>Pathology/Morphology</th>
<th>Count</th>
<th>Count %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adenocarcinoma</td>
<td>82</td>
<td>47.40</td>
</tr>
<tr>
<td>2</td>
<td>Nodular hyperplasia</td>
<td>34</td>
<td>19.85</td>
</tr>
<tr>
<td>3</td>
<td>Normal tissue</td>
<td>57</td>
<td>32.95</td>
</tr>
</tbody>
</table>
Contrast Analysis: Identifying genes by pattern matching

“Up in Cancer” Pattern
Result Example: Hepsin

List of 205 Genes

<table>
<thead>
<tr>
<th>#</th>
<th>Known Genes: Gene Name</th>
<th>Known Genes: Symbol</th>
<th>Max t-Score</th>
<th>F-Score</th>
<th>Pattern of Max t-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hepsin (transmembrane protease, serine 1)</td>
<td>HPN</td>
<td>13.12</td>
<td>38.77</td>
<td>up in cancer</td>
</tr>
<tr>
<td>2</td>
<td>golgi phosphoprotein 2</td>
<td>GOLPH2</td>
<td>12.78</td>
<td>38.37</td>
<td>up in cancer</td>
</tr>
<tr>
<td>3</td>
<td>tumor-associated calcium signal transducer 1</td>
<td>TACSTD1</td>
<td>11.87</td>
<td>34.05</td>
<td>up in cancer</td>
</tr>
<tr>
<td>4</td>
<td>clusterin (complement lysis inhibitor, SP-40,40, s...)</td>
<td>CLU</td>
<td>12.66</td>
<td>33.47</td>
<td>down in cancer</td>
</tr>
<tr>
<td>5</td>
<td>alpha-methylacyl-CoA racemase</td>
<td>AMACR</td>
<td>10.78</td>
<td>24.64</td>
<td>up in cancer</td>
</tr>
</tbody>
</table>

Principal Components Analysis

Sample PCA 2D Plot 1

r-Pearson = 0, r-Spearman = 0.3955

Genes: 205 (Selected: 1), Samples: 111 (Selected: 27), Normalization: Affymetrix (MAS 5.0), Data Transform: Symmetric Log
Absolute Analysis - E-Northern for Hepsin

“Electronic Northern”

Fragment ID (Chip ID): 235596(28)
Cluster Title: Hepsin (transmembrane protease, serine 1)

Gene: 205 (Selected: 1) Samples: 111 (Selected: 27) Normalization: Affymetrix (MAS 5.0) Data Transf
In the Literature

"...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?

Many Other Normal, Malignant and Diseased Tissues Express Hepsin.
α-Methylacyl-CoA Racemase (AMACR)

Mainly seen in prostate but also in a smaller number of renal samples.
### Gene Table 1

<table>
<thead>
<tr>
<th>#</th>
<th>FragmentID (ChipID)</th>
<th>Fragment Name</th>
<th>Sequence</th>
<th>Known Genes: Gene Symbol</th>
<th>Frequency in Database</th>
<th>% Present (Prostate normal)</th>
<th>% Present (Prostate BPH)</th>
<th>% Present (Prostate malignant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>243326(28)</td>
<td>212805_at</td>
<td>KIAA0367</td>
<td>(KIAA0367, PCA3)</td>
<td>0.84</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>2</td>
<td>243326(28)</td>
<td>212806_at</td>
<td>KIAA0367</td>
<td>(KIAA0367, PCA3)</td>
<td>0.58</td>
<td>98.21</td>
<td>93.94</td>
<td>97.67</td>
</tr>
<tr>
<td>3</td>
<td>263041(29)</td>
<td>232572_at</td>
<td>KIAA0367</td>
<td>(KIAA0367, PCA3)</td>
<td>1.4308E-2</td>
<td>0</td>
<td>0</td>
<td>5.81</td>
</tr>
<tr>
<td>4</td>
<td>263044(29)</td>
<td>232575_at</td>
<td>KIAA0367</td>
<td>(KIAA0367, PCA3)</td>
<td>1.0282E-2</td>
<td>19.64</td>
<td>6.06</td>
<td>86.05</td>
</tr>
</tbody>
</table>

### Sample Table 1

<table>
<thead>
<tr>
<th>#</th>
<th>Genomics ID</th>
<th>Sample Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>196</td>
<td>Tissue</td>
</tr>
<tr>
<td>2</td>
<td>643</td>
<td>Tissue</td>
</tr>
<tr>
<td>3</td>
<td>547</td>
<td>Tissue</td>
</tr>
<tr>
<td>4</td>
<td>549</td>
<td>Tissue</td>
</tr>
<tr>
<td>5</td>
<td>554</td>
<td>Tissue</td>
</tr>
<tr>
<td>6</td>
<td>2368</td>
<td>Tissue</td>
</tr>
<tr>
<td>7</td>
<td>3191</td>
<td>Tissue</td>
</tr>
<tr>
<td>8</td>
<td>3674</td>
<td>Tissue</td>
</tr>
<tr>
<td>9</td>
<td>7979</td>
<td>Tissue</td>
</tr>
<tr>
<td>10</td>
<td>10038</td>
<td>Tissue</td>
</tr>
</tbody>
</table>

#### e-Northern 1

FragmentID (ChipID): 263044(29)
Fragment Name (Chip): 232575_at (HG-U133B)
Cluster Title: KIAA0367

- Prostate normal (20%)
- Prostate BPH (6%)
- Prostate malignant (86%)

Genes: 4 (Selected: 1) Samples: 175 (Selected: 0) Normalization: Affymetrix (MAS 5.0 Statistical) Data Transform: Identity
Absolute Analysis - Disease and Tissue Specificity

Sample Profile 1

<table>
<thead>
<tr>
<th>#</th>
<th>Sample Site</th>
<th>Count</th>
<th>Count %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lymph node</td>
<td>1</td>
<td>1.32</td>
</tr>
<tr>
<td>2</td>
<td>Pancreas</td>
<td>1</td>
<td>1.32</td>
</tr>
<tr>
<td>3</td>
<td>Pelvic lymph node</td>
<td>1</td>
<td>1.32</td>
</tr>
<tr>
<td>4</td>
<td>Prostate</td>
<td>73</td>
<td>96.05</td>
</tr>
</tbody>
</table>

Genes: 4 (Selected: 1) Samples: 8446 (Selected: 76) Normalization: Affymetrix (MAS 5.0 Statistical) Data Transform: Identity
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
DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer.


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 56-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

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

Companies Form Collaboration to Develop Molecular Test for PCA3™ 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™ 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™ 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 APTINA(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

Your Partner for Genomics Outsourcing