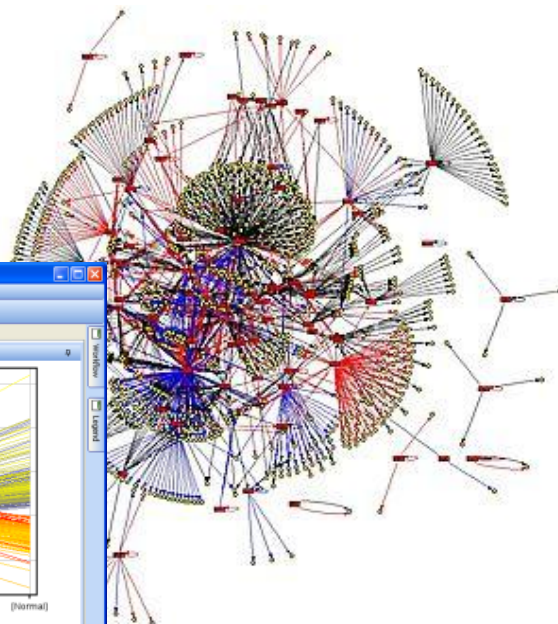


GeneSpring GX 11 and 11.5

Agilent Bioinformatics User Meeting

May 12, 2010



Michael Janis
Product Manager, GeneSpring
Agilent Technologies



Agilent Technologies

We we've been...

GeneSpring GX – Solution for RNA Analysis

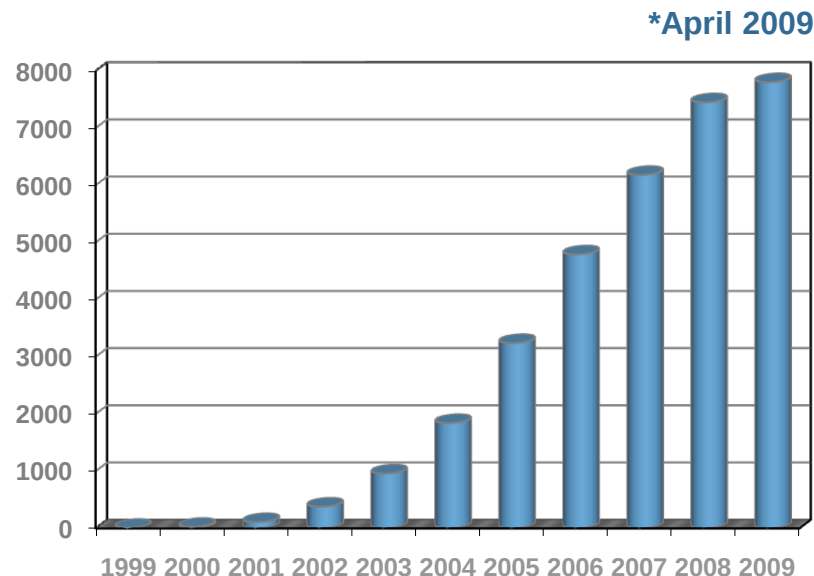
7800 references in Google Scholar and over 1,600 in peer reviewed publications

>10 years of history in RNA-based applications

Open-platform application

GeneSpring strength lies in biological contextualization

- Network building
- NLP
- GO, GSEA, and GSA analysis
- Automated biological entity translation across species or microarray platform



Google Scholar:

“gene expression” and “GeneSpring” = 7,800

“gene expression” and “Partek” = 551

Technical Support: 24 hrs/5 days/wk
Free 1:1 webex sessions



Agilent Technologies

Where we are now

GeneSpring GX 11 Goals

To support genomic research through analysis of all biological entities- genes, variations, microRNAs, and exons - and the combination of such heterogeneous data

Goal 1: Increase usability of application

Goal 2: Expand support to DNA-based applications

- Genome-wide association studies
- Copy number variation analysis

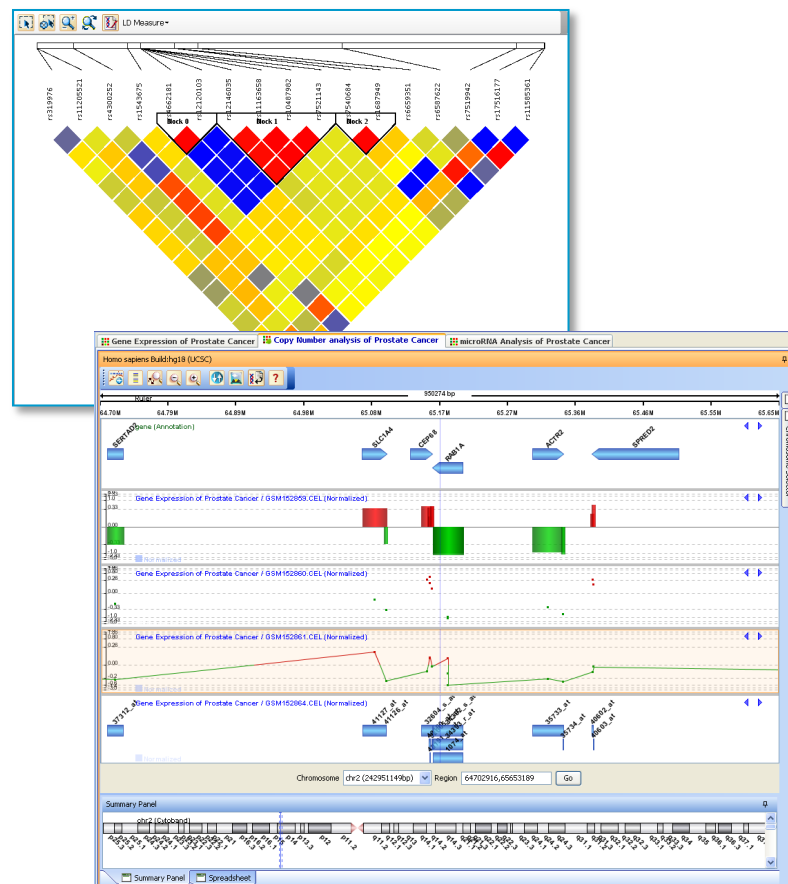
GeneSpring – Release Features v11

GS 11 (2009) – New features

Mac OS X 64-bit support	Genomic Copy Number analysis	Association analysis with genotyping data
Create Gene-level experiment from probe-level data	Circular Binary Segmentation for CN	BRLMM/Birdseed for genotyping
NCBI GEO Importer	HMM for LOH	EIGENSTRAT for genotyping analysis
Baseline transform to Median/Mean	GISTIC for CN	Genomic Control for stratification
Multiple Sample Histogram	Fawkes ASCN	Multiple and linear regression for association
Tabbed Visualization Windows	CN analysis batch correction	Haplotype Trend Regression
Genome Browser	Filter CN by known variant regions	Cochran-Armitage association test
Partial Least Squares class prediction	Find overlapping genes with CN / SNPs	Fisher's Exact association test
Identification of predictor genes		Chi-square association test
MeSH Network Builder		Tag SNP identification
		LD Plot

Highlights:

Genome Browser and DNA applications



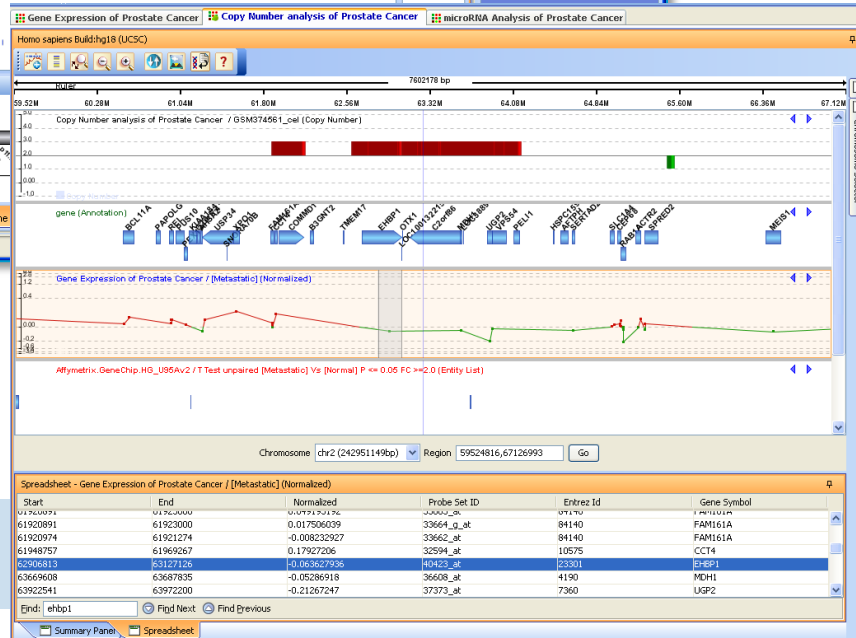
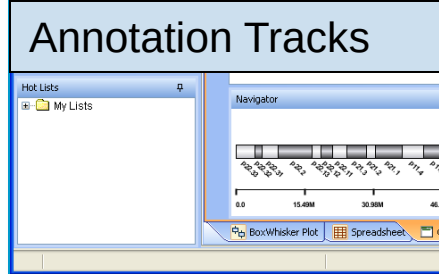
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Flexible and User-friendly Genome Browser



Navigation:

- Intelligent navigation by jumping to next entity of interest (Copy number region or SNP in list etc)
- Zoom in by dragging box in Chromosome Navigator or within the tracks
- Pan around region of interest
- Search for Entity of Interest by annotation (gene symbol/coordinates)



Copy number data

Gene track

Gene expression data

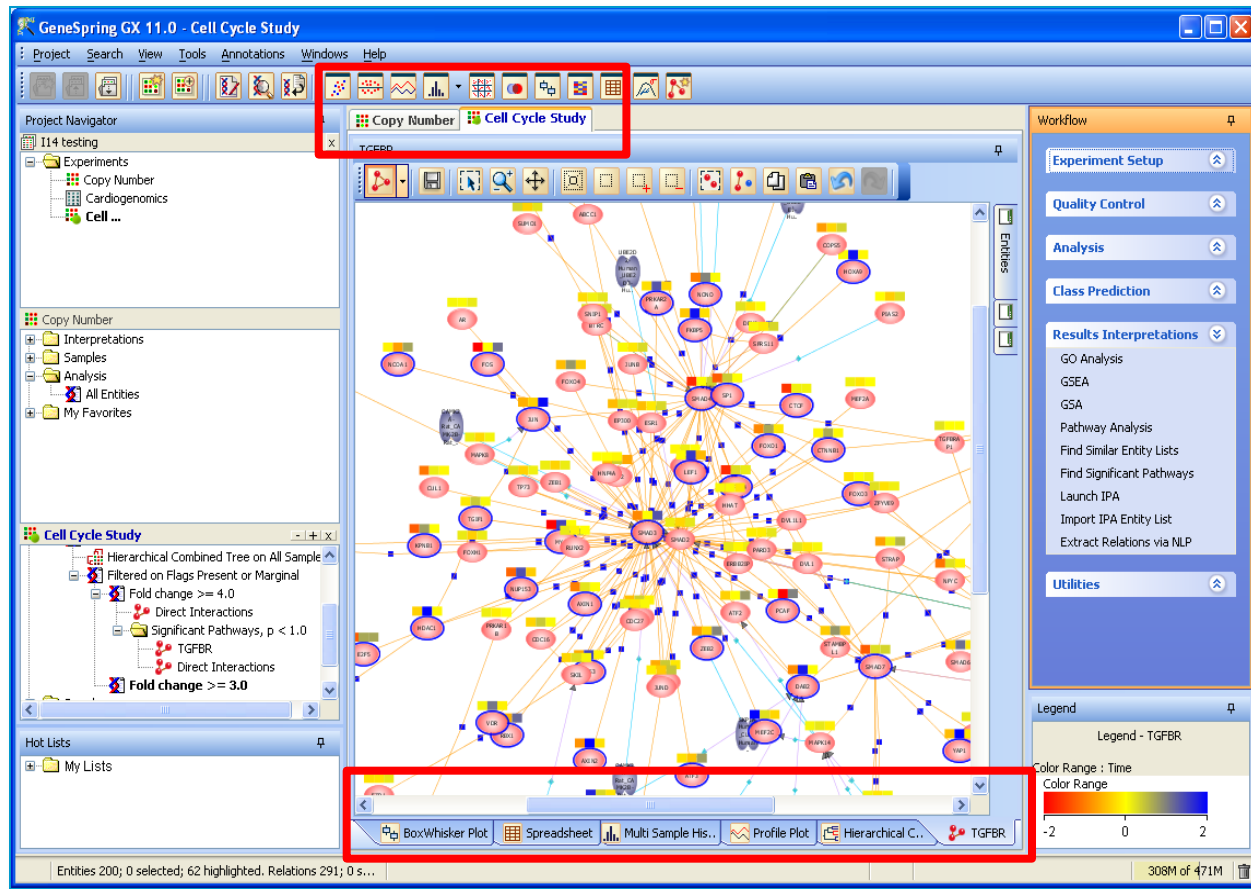
Entity List

Spreadsheet shows data for selected Track



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Tabbed Visualization Windows



Tabbed windows allow easy switching between different visualizations and plots to facilitate interrogation and comparison of data



GeneSpring Analysis

Import GEO Datasets and Create Experiments

Please enter the following:

Please enter the following:

Enter GSE Accession

Temporary download folder

experimentType

OK Cancel



New Experiment

Experiment description

Enter a name for the new experiment, select the appropriate experiment type, and choose the desired workflow. Guided workflow will take you through experiment creation and analysis, while advanced analysis will allow access to full set of analysis tools.

Experiment name

Experiment type

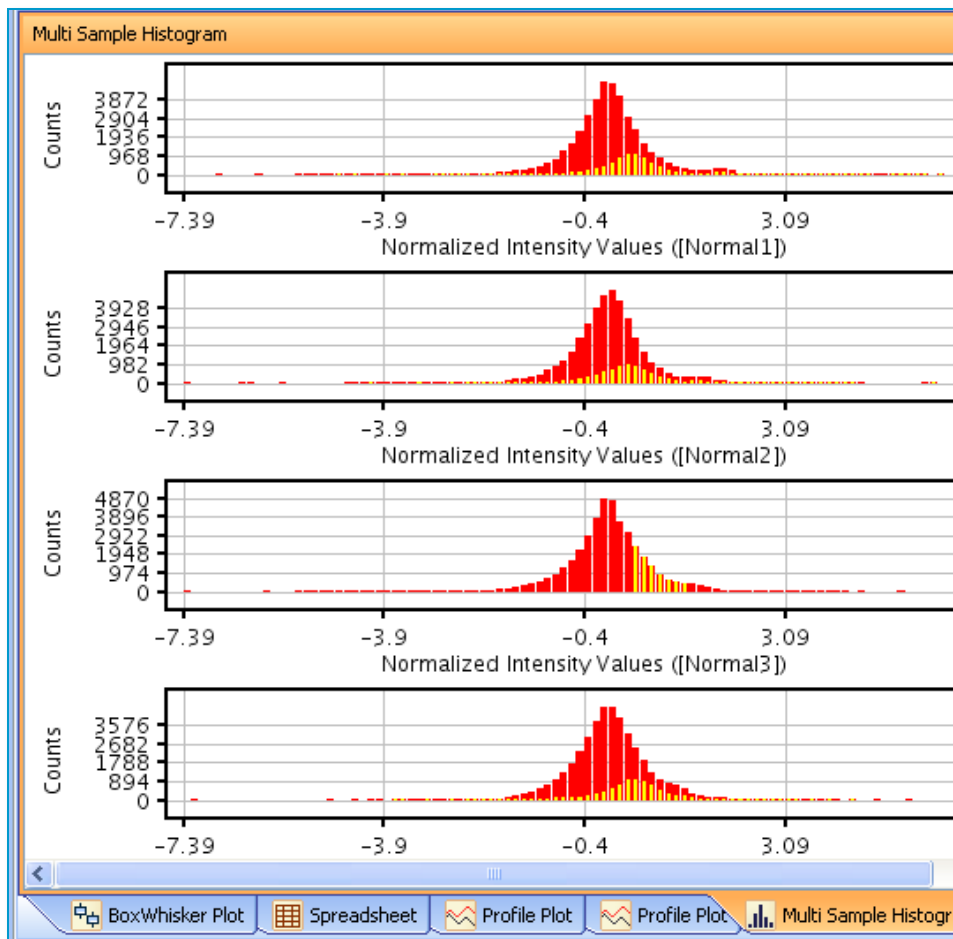
Workflow type

Experiment notes

Help OK Cancel

GeneSpring Analysis - Normalization

Display Multiple Histograms in One View



Compare distributions of intensity values across multiple samples or conditions

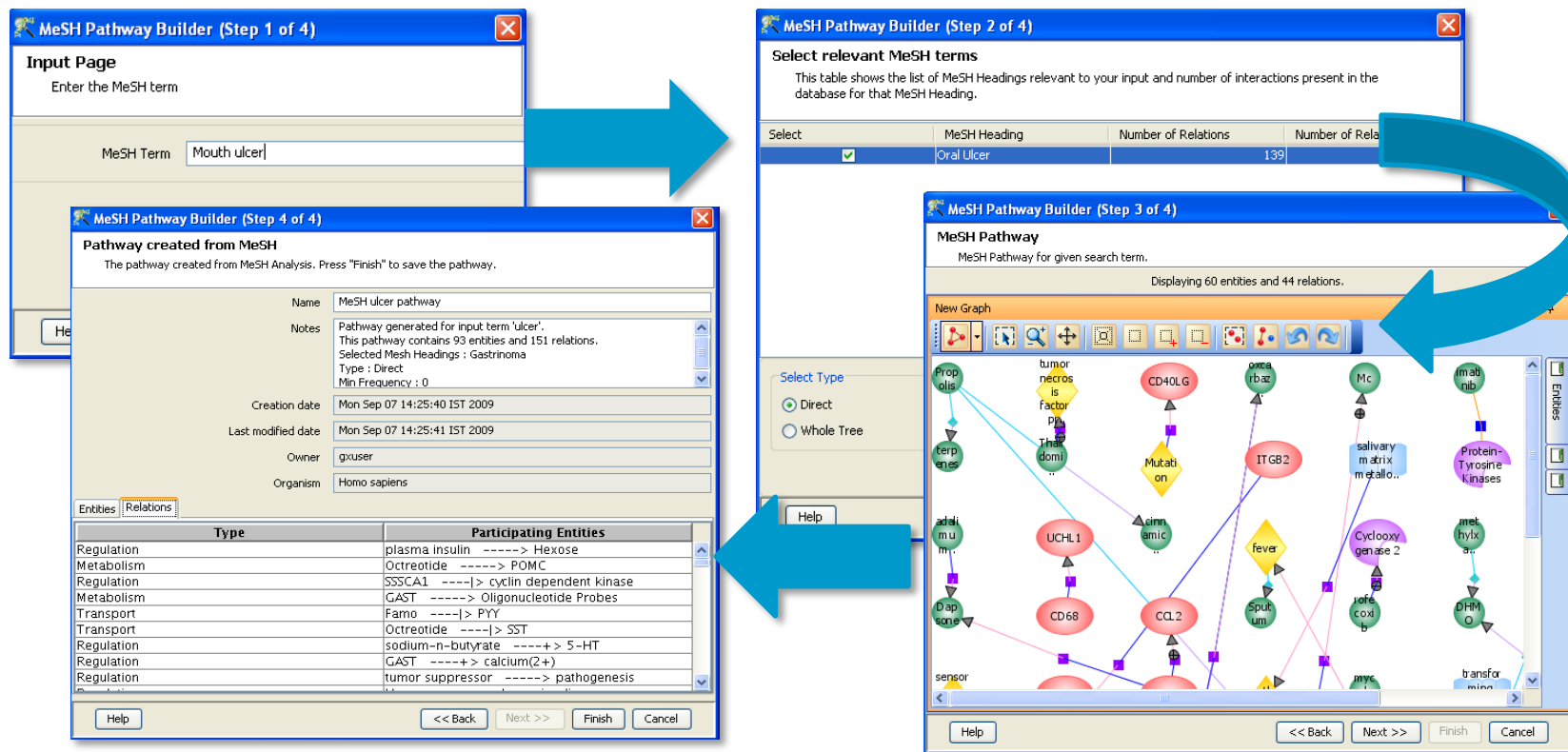
Plot Raw or Normalized values to assess effect of normalization

Selected Entities (yellow) are displayed across all histogram



GeneSpring Analysis

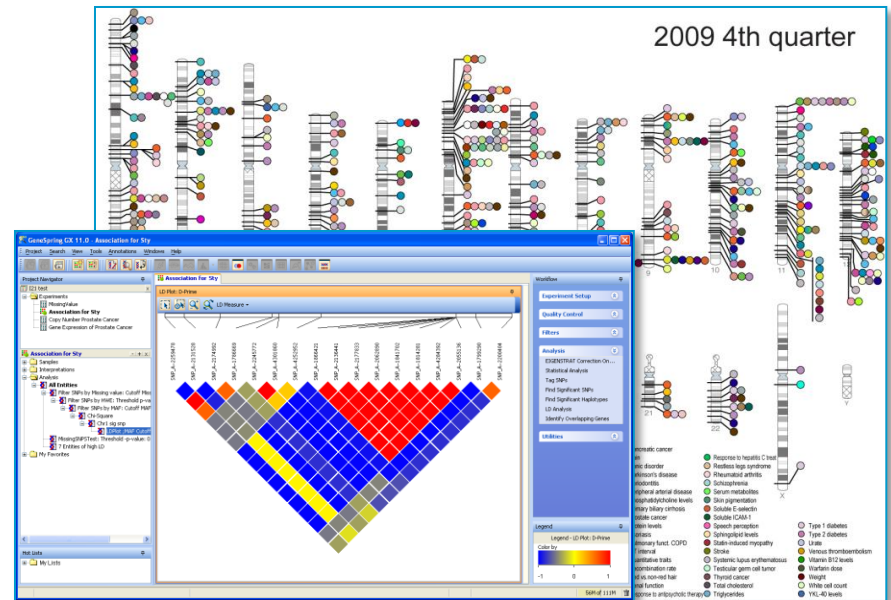
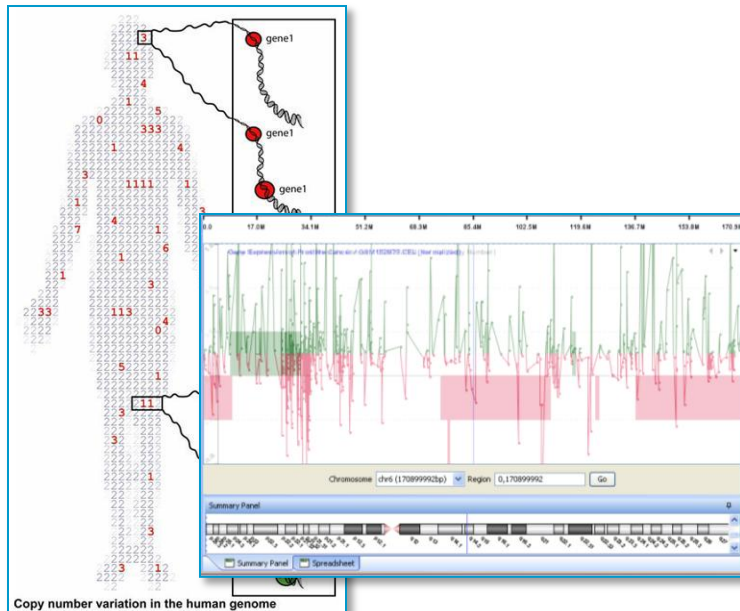
Build Networks Using Medical Subject Heading (MeSH) Terms



- Relations in Pathway Interaction databases are associated with MeSH terms
- Using MeSH term as input, retrieve relations in interaction database and build network using these relations
- This allows you to generate a network that represents molecular interactions associated with the medical concept



DNA Applications in GeneSpring GX11: CNV and GWAS analysis



Support for Affymetrix and Illumina whole-genome genotyping and CNV analysis microarrays

For CNV, Support for paired-normal and reference (standard HapMap or Custom) designs

Support for genome-wide association studies

Supported array technologies

Affymetrix

- 100K (50K Xba, 50K Hind)*
- 500K (250K Nsp, 250K Sty)
- SNP 5.0
- SNP 6.0
- Technology available from server

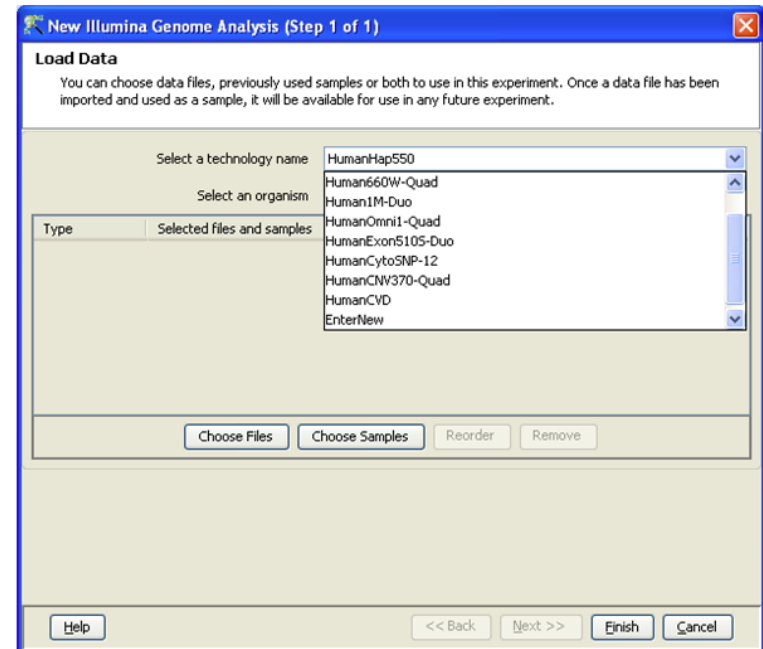
**You need at least 6 distinct cel files for Hind or Xba experiments, and 6 file pairs for combined experiments*

Illumina

- All SNP arrays
- GeneSpring GX plugin for GenomeStudio must be used to export data in the correct format
- Technology created during experiment creation

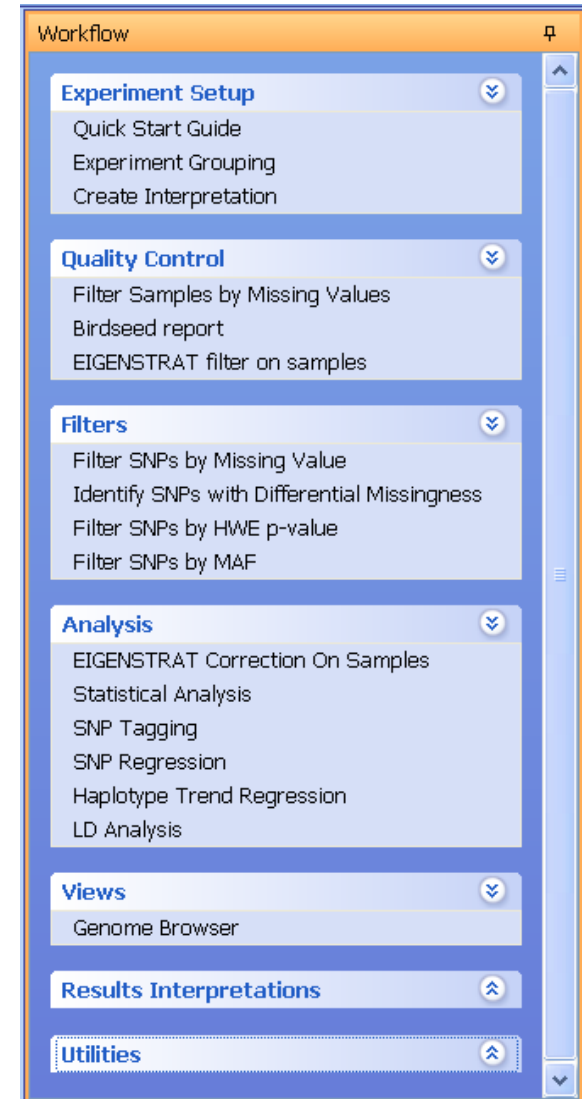
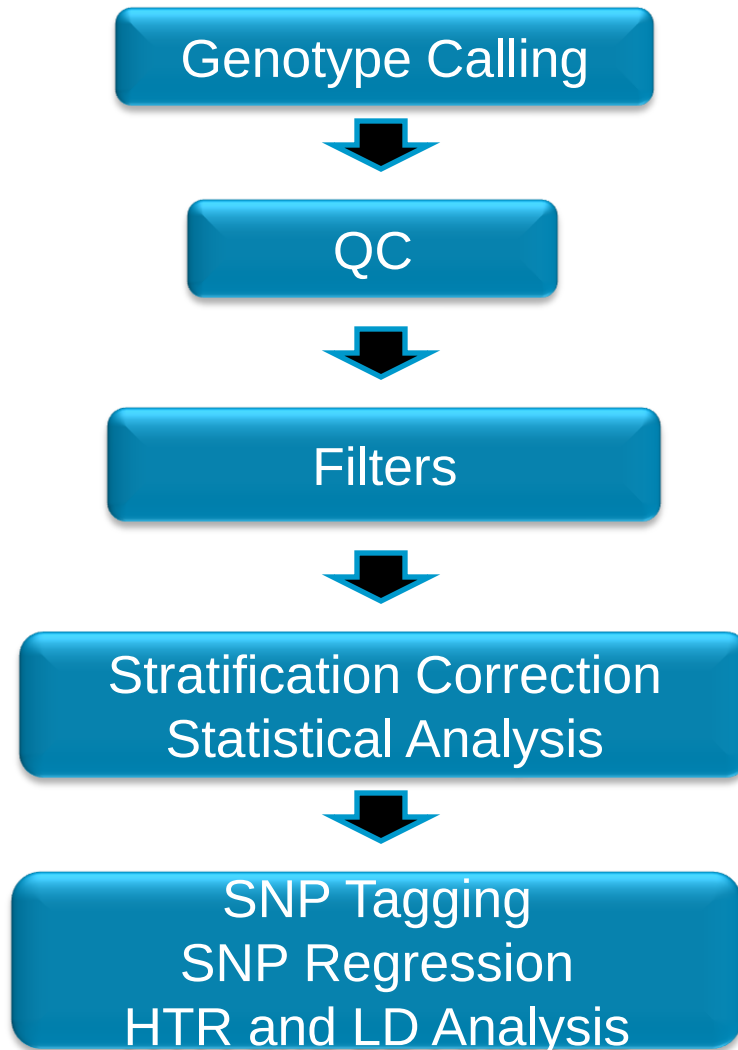
Custom

- Illumina format file input – these could be genotype calls from any other source



Genome-wide association studies

Workflow in GeneSpring GX



GeneSpring Analysis

Test for Association in GWAS Analysis and Linkage Disequilibrium

Statistical Analysis (Step 3 of 5)

Select Inputs

Please select the various options for running the Chi-Square Correlation test. Custom modes of inheritance can be configured using the option Tools->Options->Miscellaneous->Mode of Inheritance after which they would be available in the "Mode of Inheritance" dropdown.

If "Use Corrected Data" option is chosen then please make sure that EIGENSTRAT correction has been run for the chosen mode of inheritance before.

If the chosen Trait has Categorical Phenotype values then you can choose to specify their order by selecting their type as Ordinal.

Mode of Inheritance: Additive

☐ Use Corrected Data

☐ Apply Genomic Control

p-value Computation

☒ Asymptotic

☐ Permutative

Number of Permutations: 100

Multiple Testing Correction

☒ No Correction

☐ Bonferroni FWER

☐ Benjamini Hochberg FDR

☐ Benjamini Yekutieli FDR

☐ Storey FDR

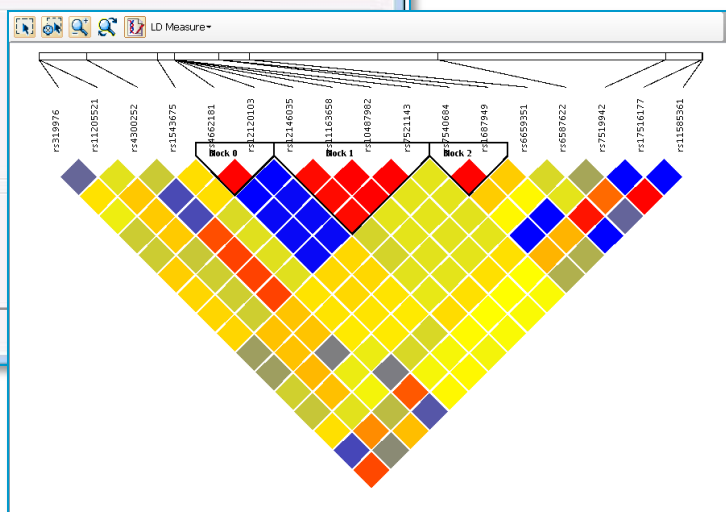
Trait Types

☒ Nominal

☐ Ordinal

Help

- Which SNPs are associated with expression of the phenotype being studied?
- 4 tests can be applied:
- Cochran-Armitage, Chi-square, Fisher's Exact and Correlation Trend for testing Individual SNPs
 - Specify models of inheritance: Additive, Dominant, Recessive, Custom



Visualize LD as R^2 or D'

Select SNPs with high LD in view and save as Entity List



Copy Number Analysis in GeneSpring GX 11

Support for Affymetrix and Illumina whole-genome genotyping and CNV analysis microarrays

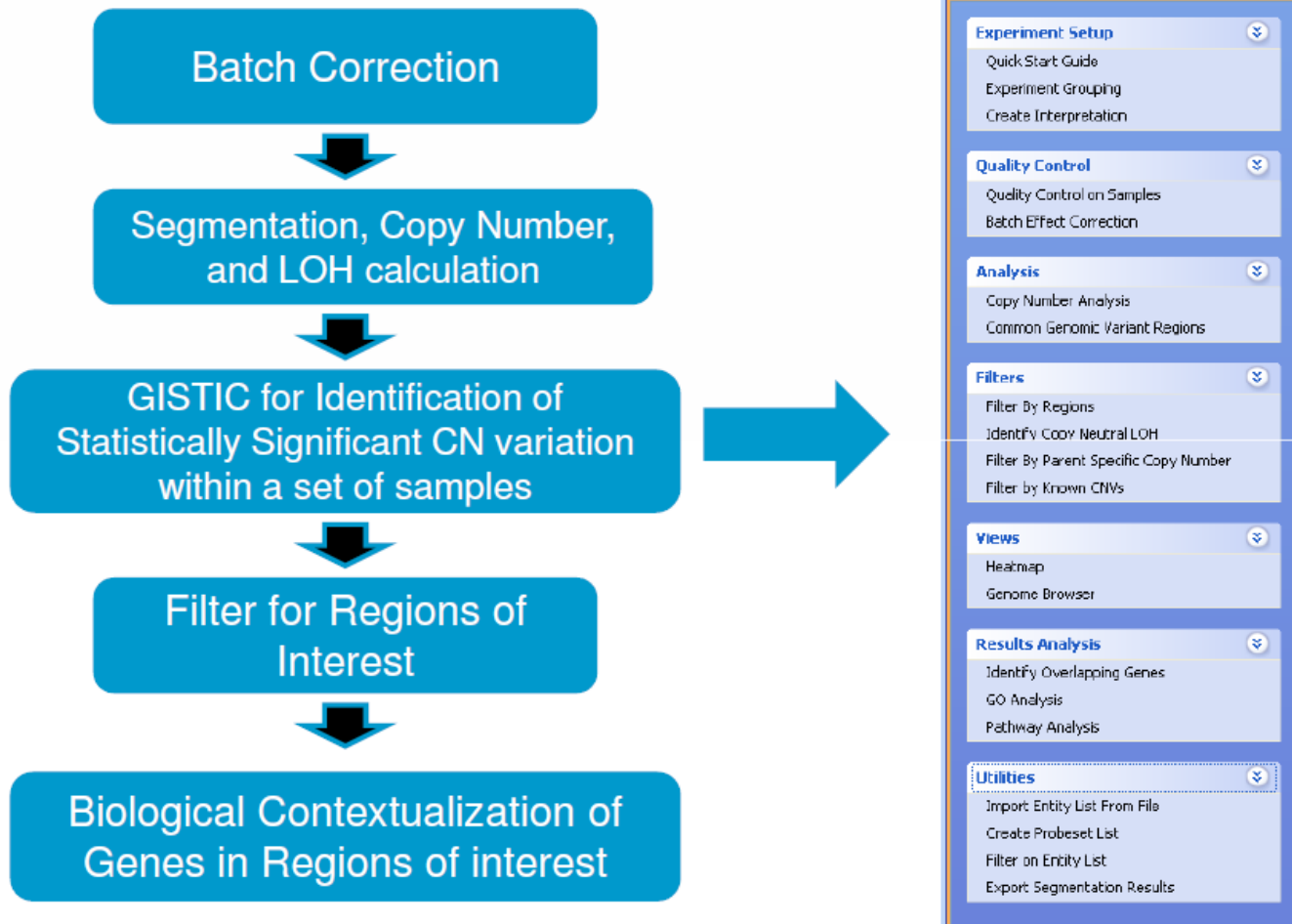
Support for paired-normal and reference (standard HapMap or Custom) designs

Algorithms Used in GeneSpring:

- Affymetrix:
 - Birdseed or BRLMM for generation genotype calls
 - Circular Binary Segmentation for identification of genomic segments of equal copy number
 - HMM for calculation of LOH scores
 - Fawkes for computation of allele-specific copy number
- Illumina:
 - Import genotype calls, copy number and LOH scores from Illumina BeadStudio



Copy Number Analysis Workflow in GeneSpring GX 11



GeneSpring Analysis

Find Common Aberrations in CN Analysis

GISTIC (Step 3 of 4)

Identified Regions

This page shows significant regions which pass the peak q-value cut-off specified below. Regions for Amplification and Deletion are shown separately

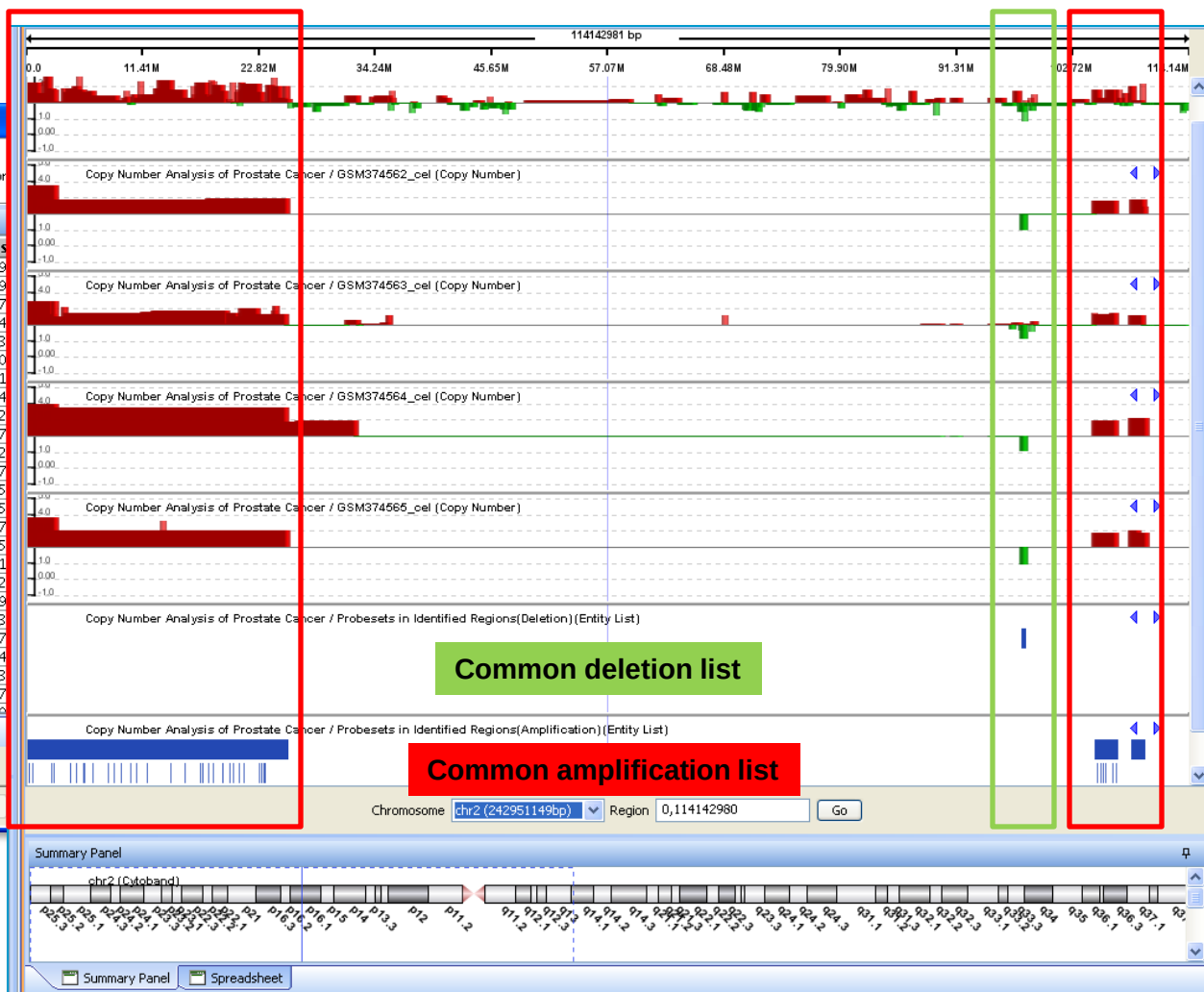
Deletion Regions 63 Rows

Region id	Chromos...	Segment ...	Segment ...	Focal / Br...	Peak Q Va...	Peak Pos
Region1	chr1	77510970	77742241	NA	0.001207...	775109
Region2	chr1	78326475	101366145	NA	4.86149E...	874149
Region3	chr10	120811615	135356694	NA	0.001207...	1248177
Region4	chr11	108846799	113175262	NA	0.001207...	1100104
Region5	chr11	113216154	122350616	NA	0.001207...	1154793
Region6	chr12	6152116	8449755	NA	0.001207...	61530
Region7	chr12	8486161	15630587	NA	0.0	95291
Region8	chr12	131745405	132088232	NA	0.001328...	1317454
Region9	chr12	132101669	132288262	NA	0.001483...	1321242
Region10	chr13	32923577	34539669	NA	0.001207...	332137
Region11	chr13	58291313	95101475	NA	0.0	939752
Region12	chr13	103478818	114126499	NA	6.22661E...	1122737
Region13	chr14	72894548	74298609	NA	0.001207...	729075
Region14	chr16	30918684	31585646	NA	0.001308...	309385
Region15	chr16	57030696	57384010	NA	0.001224...	570317
Region16	chr17	526	16665029	NA	0.001264...	44005
Region17	chr17	37416326	39712771	NA	0.001207...	379081
Region18	chr17	65766285	65854381	NA	0.001264...	657662
Region19	chr19	41910	3787264	NA	0.001207...	419
Region20	chr19	9796678	11243020	NA	0.0175808	102993
Region21	chr19	11314351	15382767	NA	0.0319746	151357
Region22	chr19	23149291	23323630	NA	0.002500...	231564
Region23	chr19	23568228	24177264	NA	0.001526...	235713
Region24	chr19	45870727	48860121	NA	0.001367...	486047
Region25	chr2	6799098	9813006	NA	0.001328...	979946

Amplification Regions 194 Rows Deletion Regions 63 Rows

Default Q-Value cutoff 0.250 Recompute Regions

Help << Back Next >> Finish



Joint and Multi-Omic Analysis & Biological Contextualization in GeneSpring



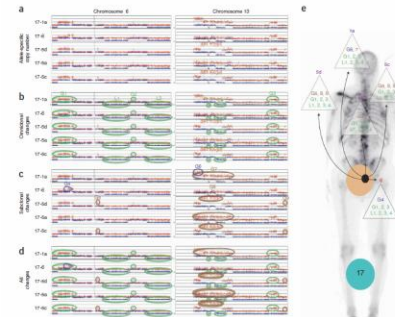
Joint Analysis of Gene Expression and Genomic Copy Number Data in Metastatic Prostate Cancer

Copy Number Analysis in Prostate Cancer Samples

**nature
medicine**

Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer

Wennuan Liu^{1,9}, Sari Laitinen^{2,9}, Sofia Khan³, Mauno Vihinen³, Jeanne Kowalski⁴, Guoqiang Yu⁵, Li Chen⁵, Charles M Ewing⁶, Mario A Eisenberger⁷, Michael A Carducci⁷, William G Nelson⁷, Srinivasan Yegnasubramanian⁷, Jun Luo^{6,7}, Yue Wang⁵, Jianfeng Xu¹, William B Isaacs^{6,7}, Tapio Visakorpi² & G Steven Bova⁶⁻⁸



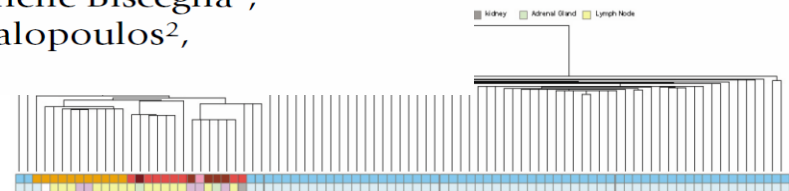
Expression Analysis in Prostate Cancer Samples

BMC Cancer

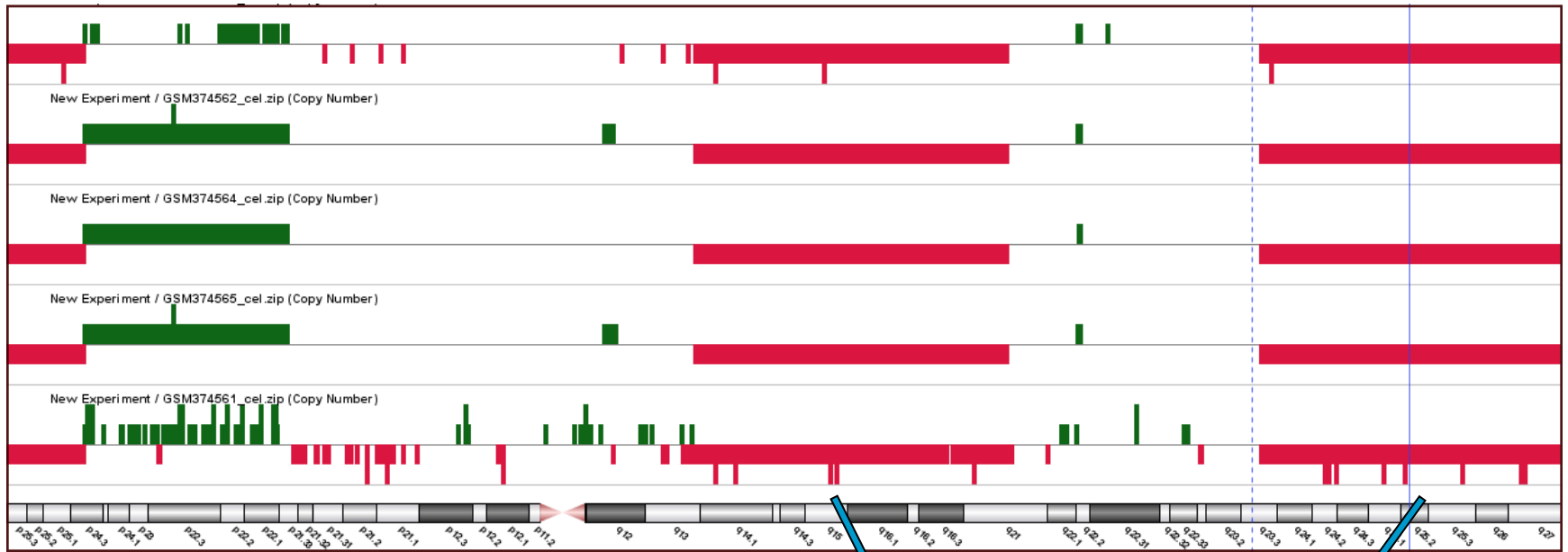
Research article

Gene expression profiles of prostate cancer reveal involvement of multiple molecular pathways in the metastatic process

Uma R Chandran^{*1}, Changqing Ma², Rajiv Dhir², Michelle Bisceglia², Maureen Lyons-Weiler², Wenjing Liang², George Michalopoulos², Michael Becich^{1,2} and Federico A Monzon²



Deletion in q-arm of Chr 6 Detected in all Five Metastatic Locations of Patient #17

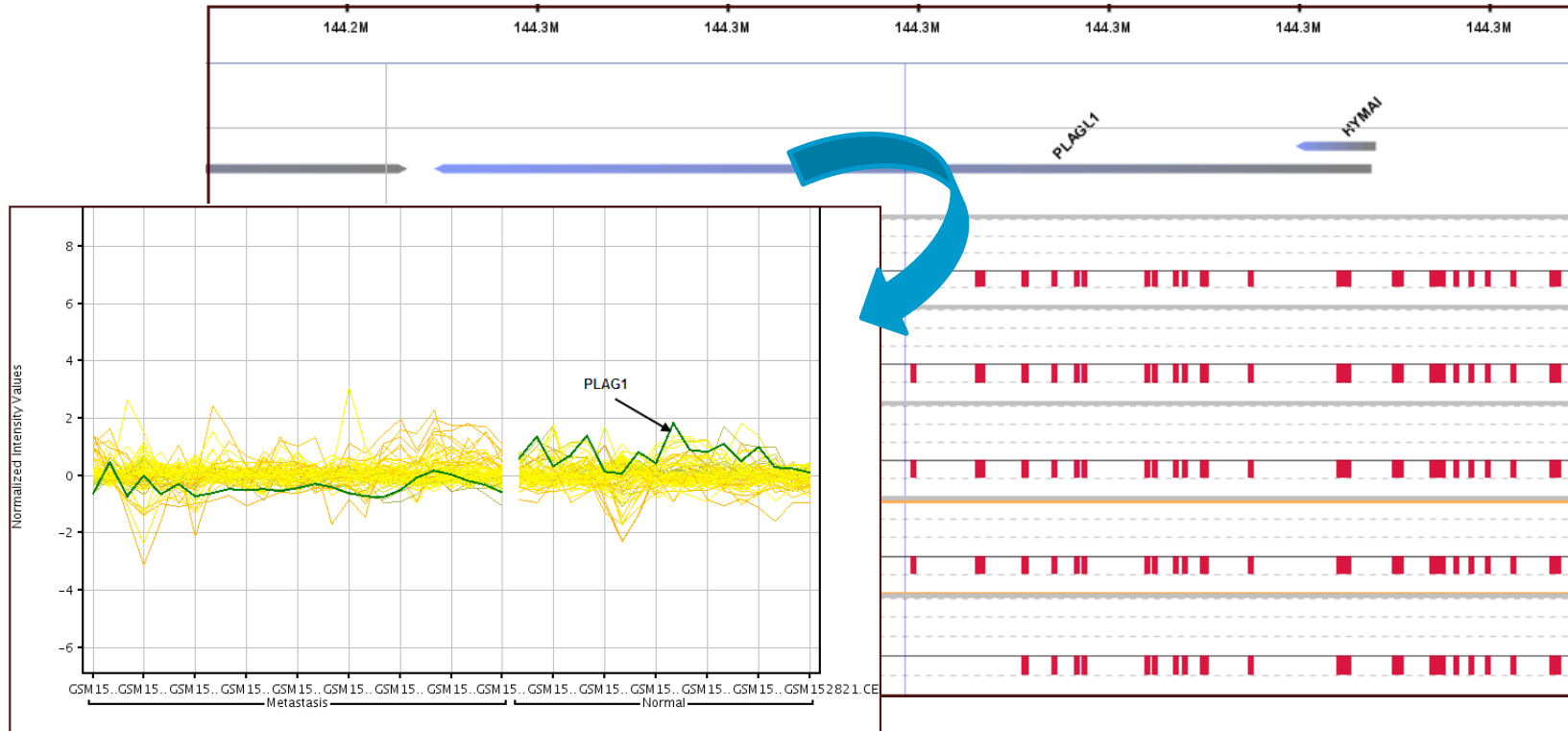


p-arm region amplification

Deletion of 2 large regions across the q-arm observed in all 5 metastatic locations in 1/14 patients

GeneSpring Analysis

Finding Overlapping Genes and Functional Groupings



- In a prostate cancer study, deleted Chr 6 q-arm regions contains enrichment of genes involved in immune response and antigen processing and presentation
- All 5 metastatic tumors from this patient were found in lymph nodes
- PLAGL1 is significantly down-regulated in metastatic prostate cancer

PLAGL1 Gene

- No previous association to prostate cancer reported
- Frequently deleted in many solid tumors such as breast, ovarian, and renal cell carcinomas
- Biological properties:
 - Zinc finger protein with transactivation and DNA binding activity
 - Candidate tumor suppressor gene with anti-proliferative activities

GeneSpring 11 Press Release

Customer quote:

Bruce Aronow

Director of the Center for Computational Medicine
Cincinnati Children's Hospital

"GeneSpring 11 represents a significant advance in data analysis software for life sciences. Here we see for the first time an all-wheel driving machine for multi-omics technologies that shape a new roadmap for integrative systems biology."

The screenshot shows the GeneSpring 11 website. The header features the 'GEN' logo and the text 'Genetic Engineering & Biotechnology News'. A navigation bar includes links for NEWS, BIOBUSINESS, DRUG DISCOVERY, OMICS, BIOPROCESSING, and CLINICAL RES. A search bar is located on the left. The main content area displays a news article titled 'TRADE NEWS: Agilent Technologies Adds Genetic Association and Copy Number Variation Analyses to Bioinformatics Software, Expanding Systems Biology Suite'. The article is dated Dec 7 2009, 11:00 AM EST. The left sidebar contains a 'CURRENT ISSUE' section, a 'SUBSCRIBE' button, an 'AD LINK' button, and a 'Follow GEN On Twitter' button. The bottom of the page has a 'DYNAMIC REPORTS. DYNAMIC CONTENT. DYNAMIC SCIENCE.' banner.

GEN Genetic Engineering & Biotechnology News

NEWS BIOBUSINESS DRUG DISCOVERY OMICS BIOPROCESSING CLINICAL RES

Search site GO

CURRENT ISSUE

TRADE NEWS: Agilent Technologies Adds Genetic Association and Copy Number Variation Analyses to Bioinformatics Software, Expanding Systems Biology Suite

Dec 7 2009, 11:00 AM EST

News source: **Business Wire**

Agilent Technologies, Inc. (NYSE:A) introduced **GeneSpring GX 11**, the latest generation of its popular desktop software for visualizing and analyzing microarray data, and GeneSpring Workgroup 11, the enterprise version of the GeneSpring desktop software.

Designed to support multi-omics research, GeneSpring 11 adds capabilities for genetic association analysis using genotyping data, genomic copy number analysis, and other analytical and visualization tools to facilitate comparison of these heterogeneous data. Agilent's bioinformatics portfolio also includes **Mass Profiler Professional**, built on the same platform as GeneSpring, supporting proteomics and metabolomics data analysis.

"The multi-omics approach to defining mechanism of disease in systems biology research has created a bioinformatics bottleneck, where the ability to extract biological knowledge from the data often lags behind the ability to generate the data," said Chris Grimley, Agilent senior director of marketing, Genomics. "GeneSpring 11 was developed with this bottleneck in mind and addresses some of the challenges of integrative analysis."

"GeneSpring 11 represents a significant advance in data analysis software for life sciences," said Bruce Aronow, Ph.D., scientific director of the Center for Computational Medicine at Cincinnati Children's Hospital Medical Center, and a longtime GeneSpring user. "Here we see for the first time an all-wheel driving machine for multi-omics technologies that shape a new roadmap for integrative systems biology."

In GeneSpring 11, researchers can have multiple experiment types, such as microRNA, gene expression, genotyping, and copy number, open simultaneously within a single window. This allows users to move back and forth between the data as needed without having to load each experiment separately. This functionality also allows researchers to easily combine data within a logical unit and compare results from different experiment types.

GeneSpring 11 also allows researchers to find critical linkages and concordance between them.

While there are other software applications that support multi-omics data analysis, a major differentiator of GeneSpring 11 is the ease with which users can compare

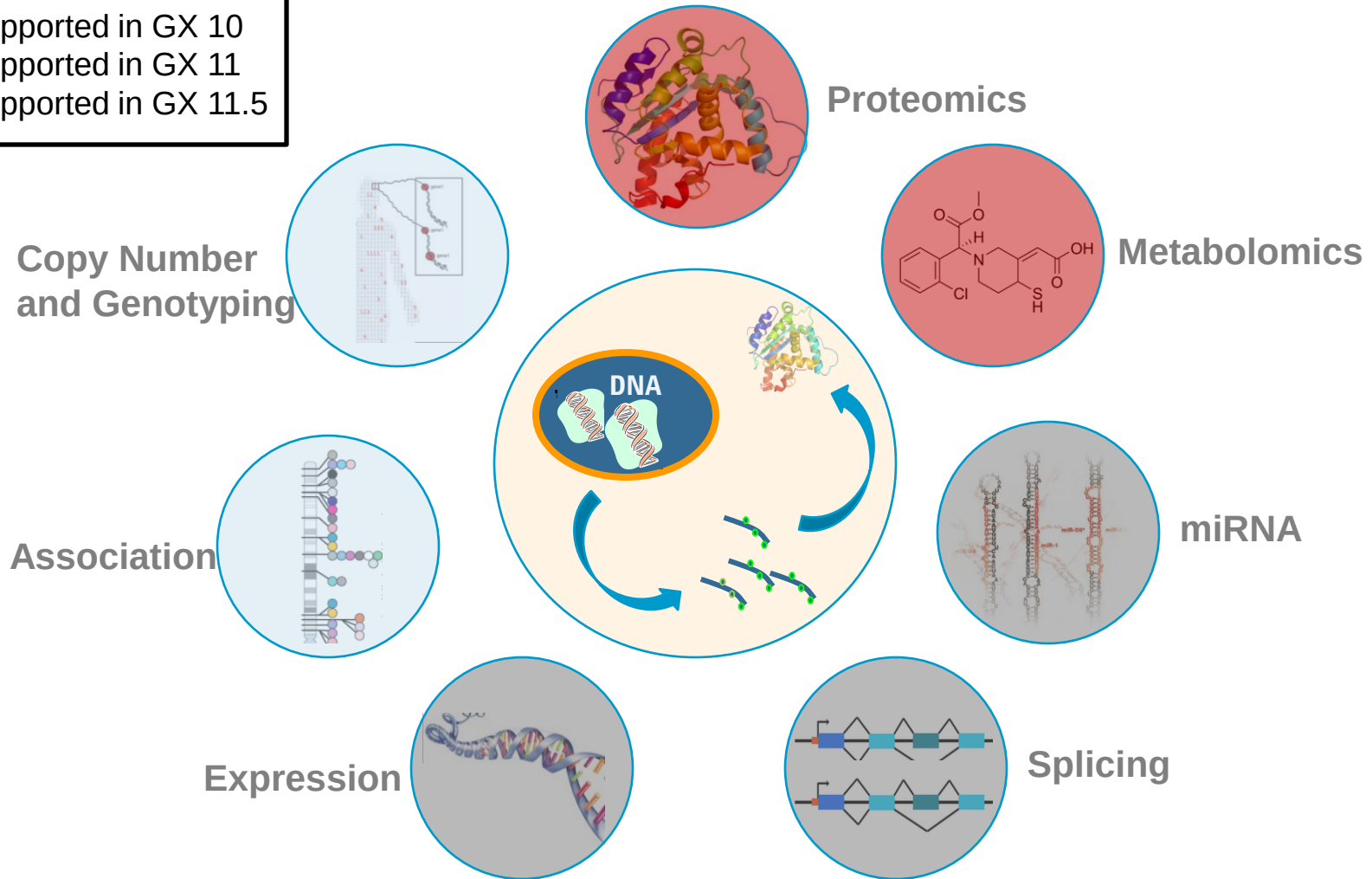
Where we are going: GeneSpring 11.5

2010:

- Integrative Biology
 - Integrated Mass Profiler Pro into GX 11
- Support for Agilent Exon Arrays
- MetaCore Integration
- Useability Enhancements

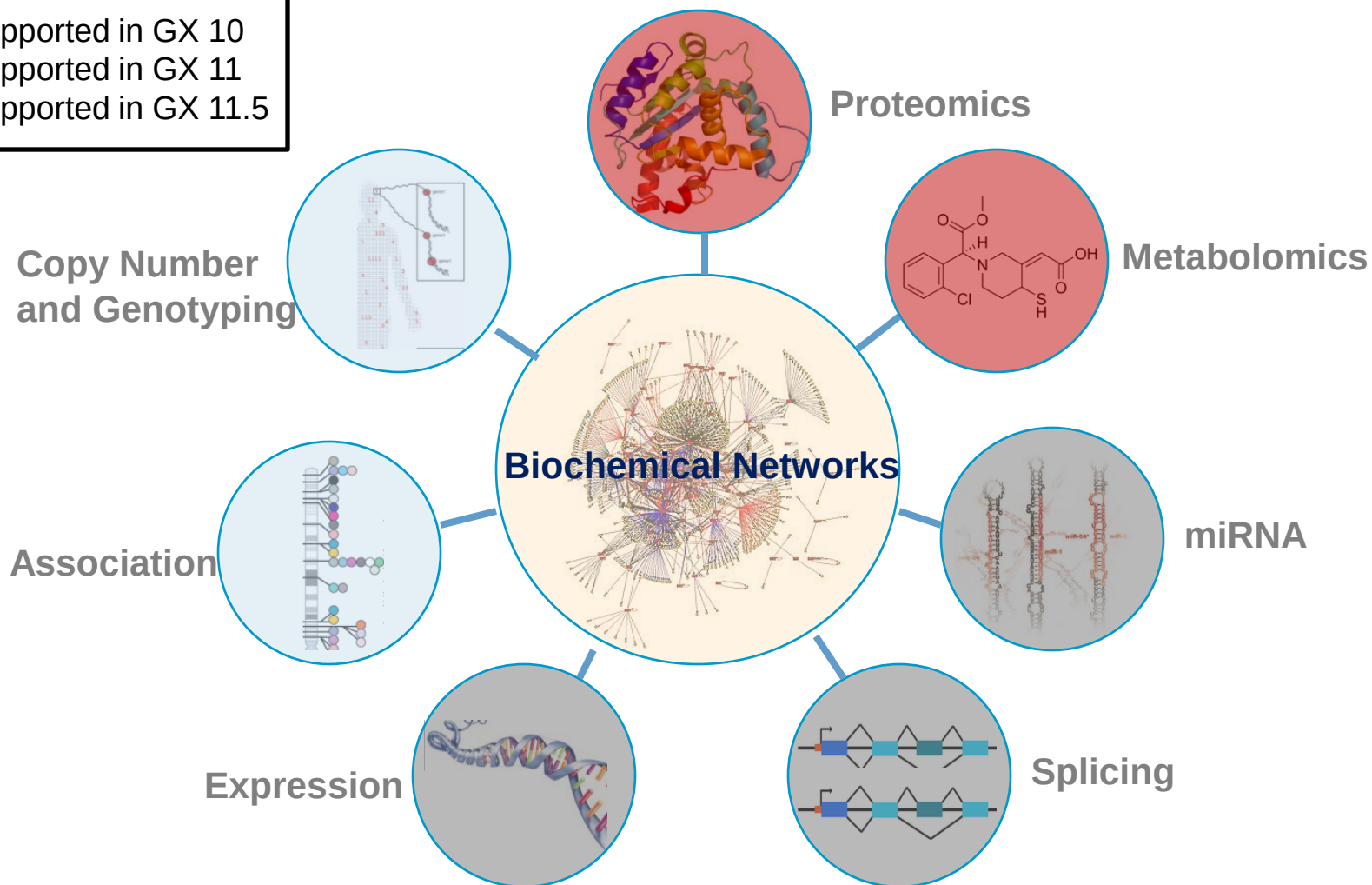
Systems Level Research Leads to a More Complete Understanding of Biological Processes

- Supported in GX 10
- Supported in GX 11
- Supported in GX 11.5



Systems Level Research Leads to a More Complete Understanding of Biological Processes

- Supported in GX 10
- Supported in GX 11
- Supported in GX 11.5



A Case for Multi-Omics Data Analysis

Cross-Technology Panels for Biomarker Research

Gene Symbol	Gene	Gene Symbol	Protein
CHPT1	choline phosphotransferase 1	IGFBP3	insulin-like growth factor binding protein 3 isoform a precursor
RPS26	ribosomal protein S26	MST1	Hepatocyte growth factor-like protein precursor
GBP3	guanylate binding protein 3	CFHR1	Complement factor H-related protein 1 precursor
KLRC1	killer cell lectin-like receptor subfamily C, member 1	CPN1	Carboxypeptidase N catalytic chain precursor
ZCCHC2	zinc finger, CCHC domain containing 2	CDH5	Cadherin-5 precursor
IFIT5	interferon-induced protein with tetratricopeptide repeats 5	APOB	Apolipoprotein B-100 precursor
CLEC2B	C-type lectin domain family 2, member B	HBB	Hemoglobin subunit beta
PDK4	pyruvate dehydrogenase kinase, isozyme 4	C1QB	Complement component 1, q subcomponent, B chain
OSBP2	oxysterol binding protein 2	GC	Vitamin D-binding protein precursor
(242907_at)	Homo sapiens mRNA; cDNA DKFZp451C2311	C9	Complement component C9 precursor

No overlap between 10 genes and 10 proteins for Cardiac Allograft Vasculopathy (CAV)

- Gene-ontology-based analyses revealed a great degree of biological concordance
- Cross-technology panels often outperform corresponding single platform panels
- Long-term survival of cardiac transplant recipients is still a major hurdle
- Cardiac allograft vasculopathy (CAV) is an expression of chronic rejection
- Current standard diagnosis of CAV is expensive and invasive (angiography and intravascular ultrasound)
- Aim is to derive a non-invasive biomarker panel based on whole blood mRNA and plasma proteins

Mass Spectrometry Module to GeneSpring GX

Mass Spectrometry Module to GeneSpring GX has all features of MPP plus...

- Client-server analysis environment (through connection to Workgroup) in addition to desktop
- Ability to combine mass spec experiments with genomics experiments in a GeneSpring Project

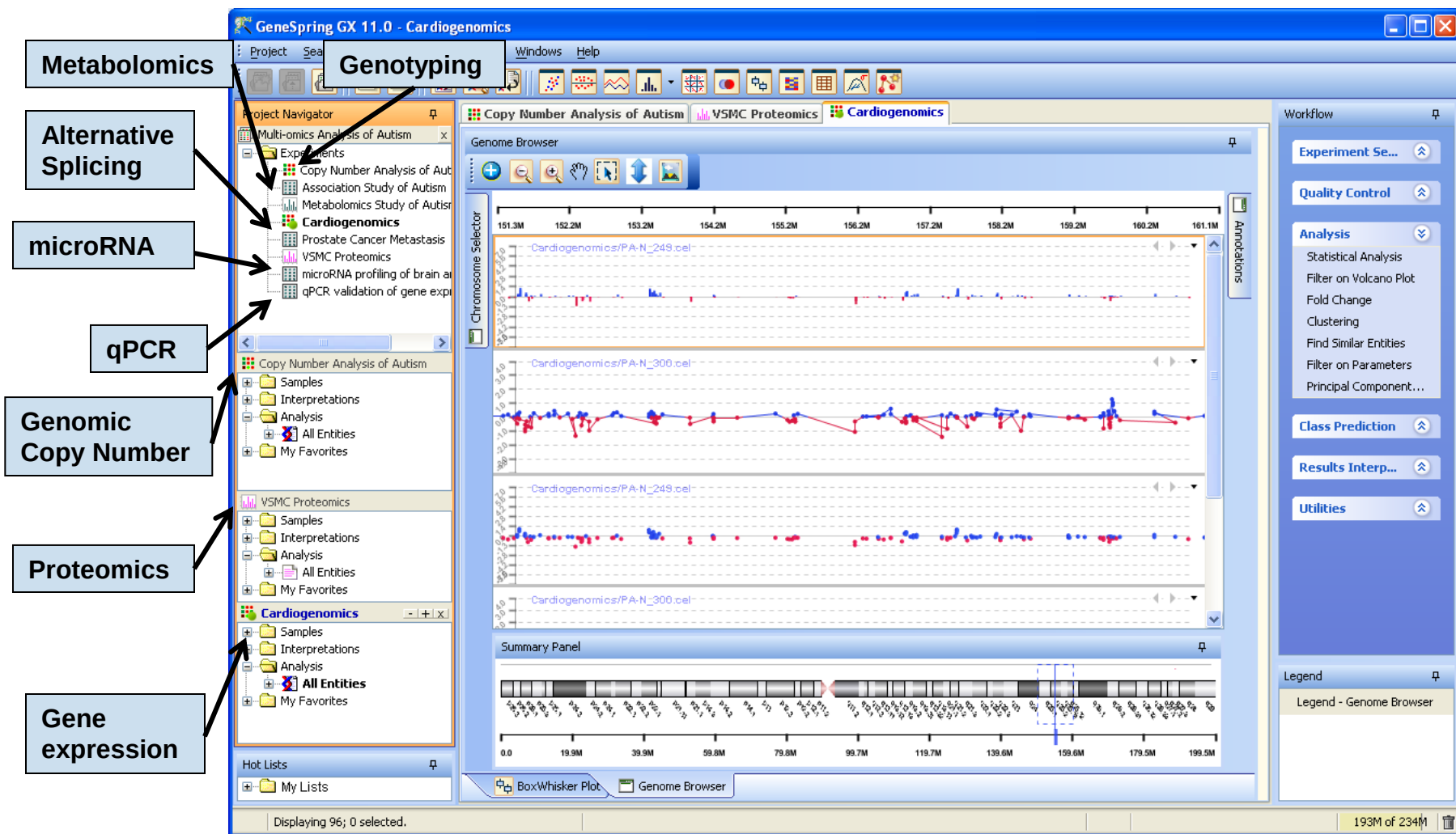
Expected availability date for module- Summer 2010

Module can be purchased as an add-on module to GeneSpring GX



Integrated Analysis in GeneSpring 11.5

(Summer 2010)



Joint and Multi-Omics Analysis

Easily Translate Between Technology Platforms, Organisms, and Data Types

Copy Number **Affymetrix**

Mouse

Agilent

Human

Gene Expression

Copy Number Data



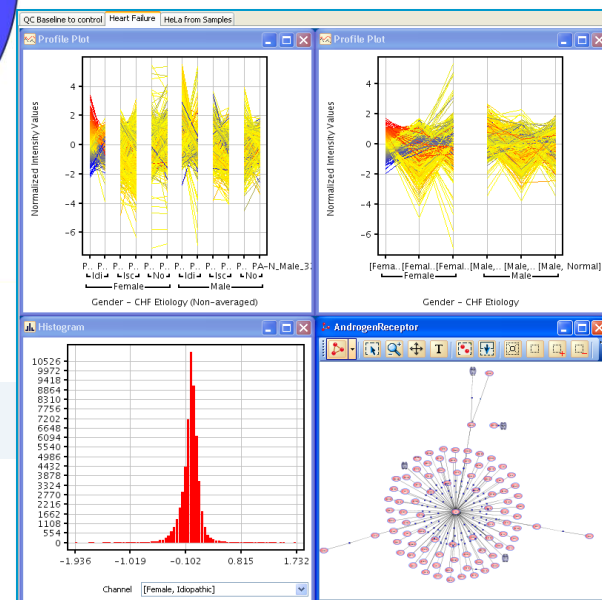
Expression Data



Illumina

Genotyping

Rat

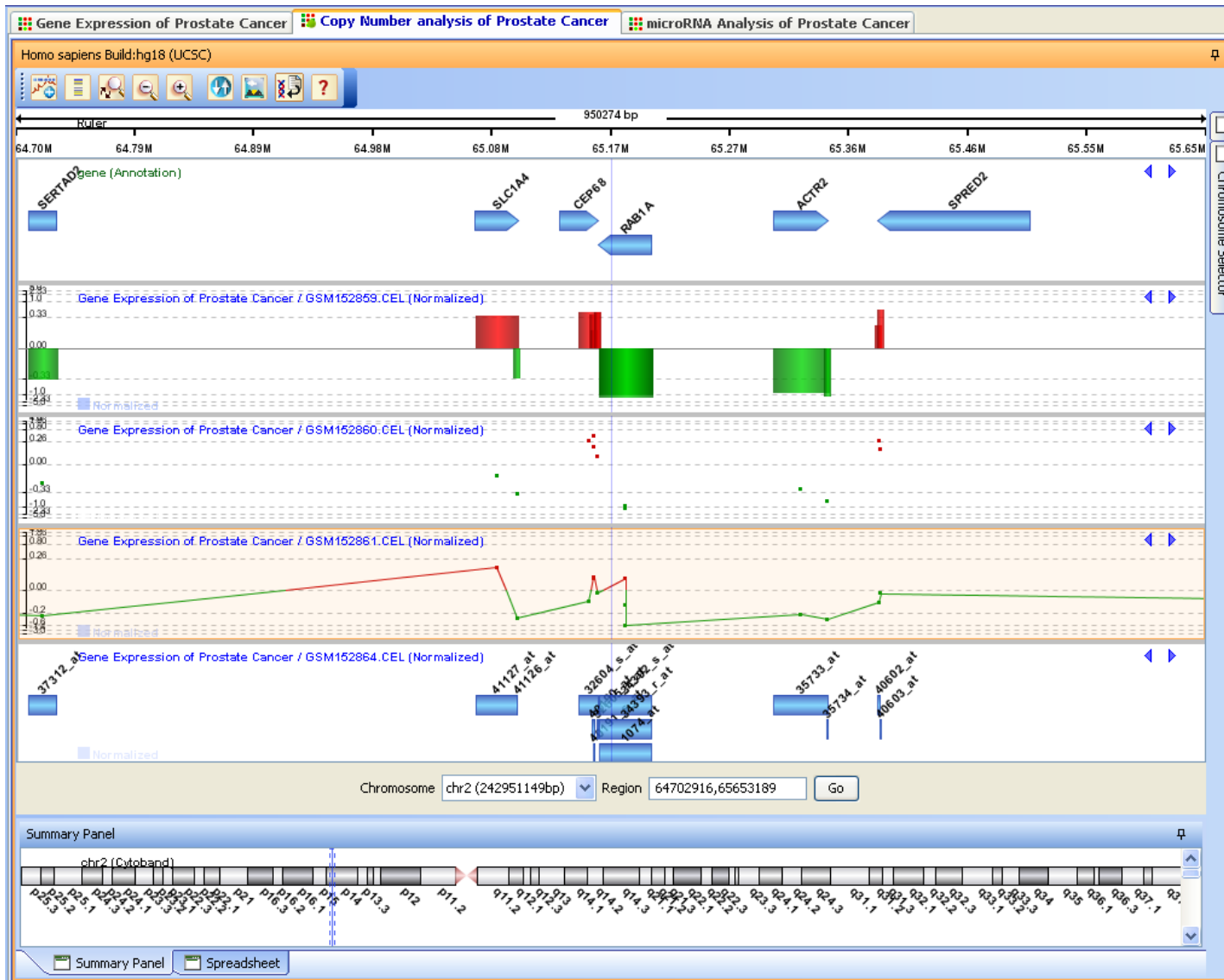


Agilent Technologies

GeneSpring

June 10

Positional Representation - Genome Browser



Annotation Track

Histogram

Scatter Plot

Profile Plot

Region Plot

GeneExpression

SNP

Exon

Agilent CGH/ChIP/CH3



Agilent Technologies

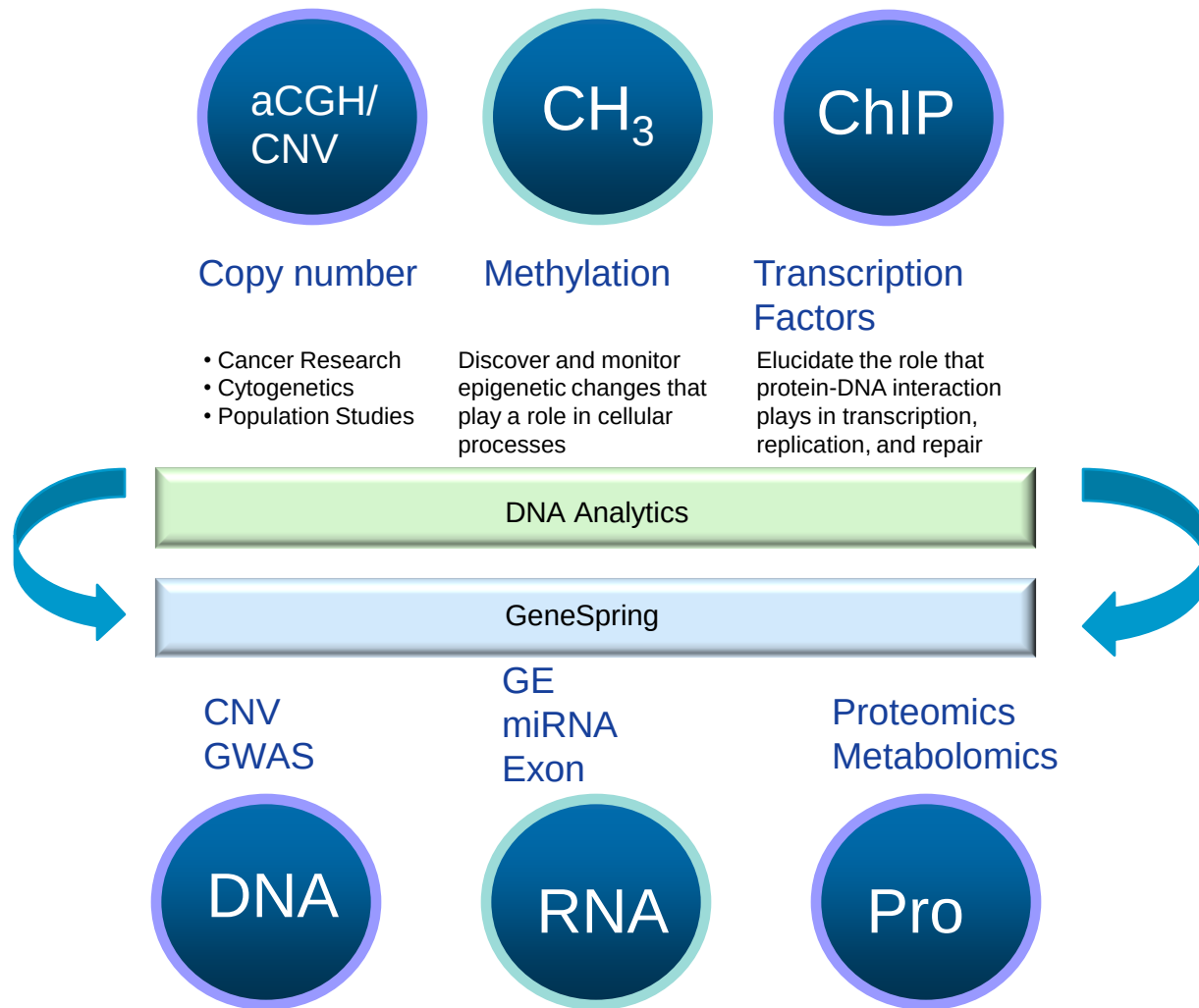
Beyond GeneSpring 11.5

2011:

- Improved Pathway Analysis (additional organisms)
- Integration with DNA Analytics
- Cytoscape connector

Agilent Genomic Workbench – DNA Analytics

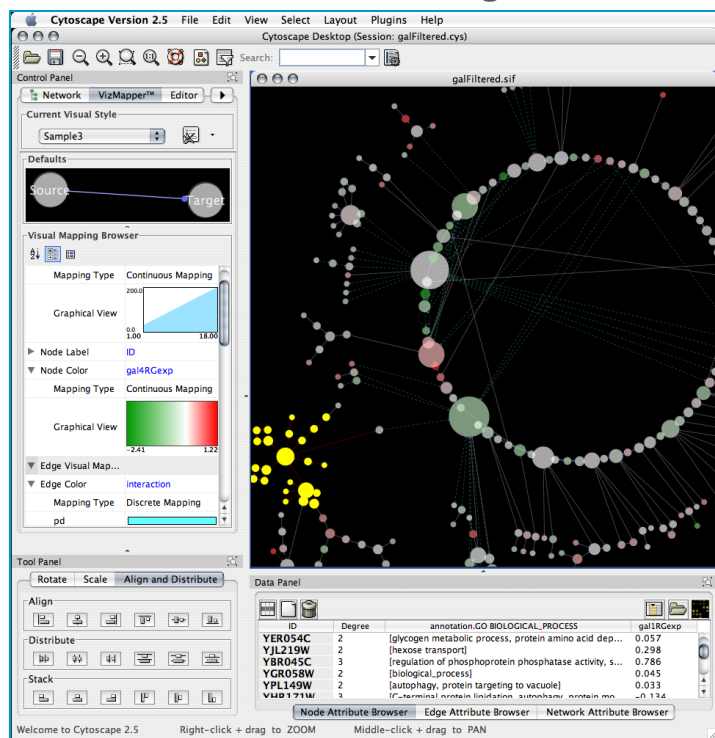
Applications for Agilent Arrays



Multi-omic Analysis

Cytoscape

Cytoscape is an open source software platform for *integrating, analyzing, and visualizing* measurement data in their biological context.



Cytoscape is a collaboration between



University of California, San Diego



Institute for Systems Biology



Memorial Sloan-Kettering Cancer Center



Institute Pasteur



Agilent Technologies



University of Toronto



Gladstone Institute for Cardiovascular Disease



University of California, San Francisco



Unilever



National Center for Integrative
Biomedical Informatics

freely available at <http://www.cytoscape.org>

- 80000+ downloads for 2.x release; 25,000 downloads in 2007 alone; 3500/month
- Currently 100 registered plugins, developed by leading research groups, freely available
- Community development of plugins strongly encouraged and actively supported by core development team.



Agilent Technologies

Web Resources at www.genespring.com

Tutorials

- Data analysis tutorials for Affymetrix gene expression, Agilent gene expression, Exon Splicing, Genomic Copy Number

Viewlets

- Short animated tutorials on various topics such as Quality Control on samples, Updating Pathway Interactions, miRNA Analysis Using TargetScan and more

Recorded and Live eSeminars

- Sign up for live eSeminars on various topics such as Introduction to GeneSpring GX 11, Joint Analysis of Gene Expression and Genomic Copy Number data, and more
- View recorded past eSeminars

GeneSpring GX Workshops

- Register for Levels I, II, and III GeneSpring GX training workshops

The GeneSpring Team

Director of Genomics Software

- Michael Rosenberg

GeneSpring Product Manager

- Michael Janis

GeneSpring Project Manager

- Jayati Ghosh

Workgroup Server Product Manager

- Alexi Zubiria

Global Bioinformatics Support Manager

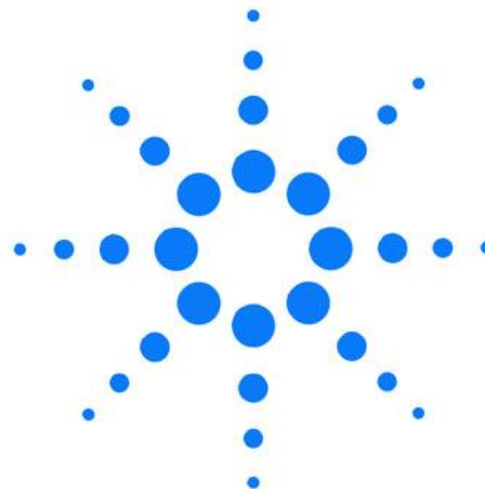
- Mary Jane Van Sant

GeneSpring Support

- Yosuke Konishi

GeneSpring Product Specialist

- Antoni Wandycz



Thank you!



May 12th, 2010



Have you ever tried to find...

the one buffer recipe that produced the best assay results to use today?

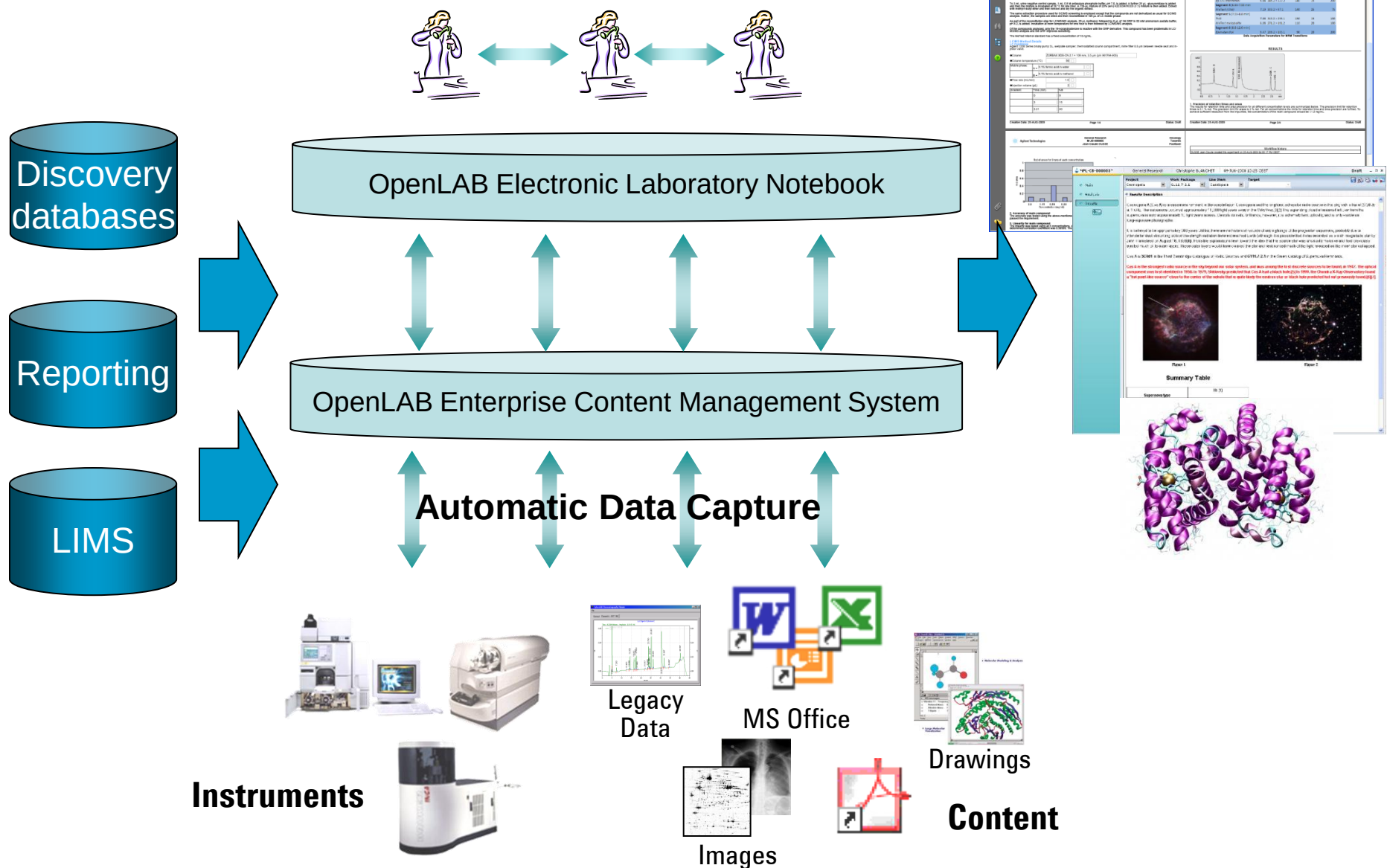
From chaos...

to a single search in seconds...



Agilent Technologies

OpenLAB: Integrated Informatics Portfolio



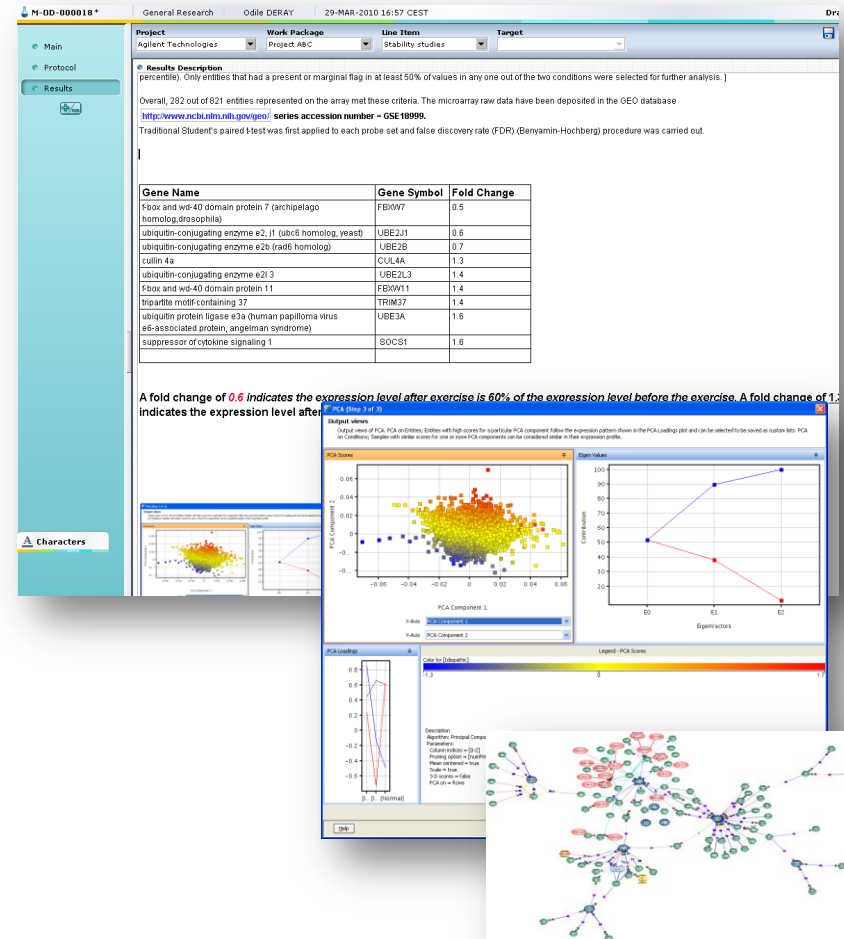
OpenLAB ELN for Genomics

A Web-based ELN which enables you to...

- Document, manage and analyze research across multiple disciplines
- Search and collate disparate data sources into single experiment with high level of traceability
- Reduce cycle times with integrated workflow processing to improve lab efficiency
- Integrate with existing information systems

Resulting in...

- Improved efficiency and data quality
- More secure Intellectual Property protection



OpenLAB ELN: Search and re-use protocols and methods

The screenshot displays the OpenLAB ELN interface. The top bar shows the project name 'M-DD-000018 *', the user 'Odile DERAY', and the date '29-MAR-2010 16:57 CEST'. The left sidebar contains navigation links for 'Main', 'Protocol', and 'Results'. The main workspace is titled 'Project' and 'Work Package', with a 'Line Item' dropdown. Below this, a 'Description' section is visible, containing two protocols: 'Neutrophil Isolation' and 'RNA Extraction'. The 'Neutrophil Isolation' protocol text includes fields for '30' ml EDTA-treated peripheral blood and '98' purification. The 'RNA Extraction' protocol text includes a dropdown for 'TRizol® (Gibco BRL Life Technologies, 141 Rockville, M)'. A red arrow points from a callout box 'Fast data entry with pick lists' to the '30' field. Another red arrow points from a callout box 'Prompt for required values' to the '30' field. A third red arrow points from a callout box 'Quickly add one or more Forms to an experiment' to a menu that is open, showing a list of forms including 'Analysis of Anabolic Agents in Urine by LCMSMS', 'miRNA Microarrays', 'Neutrophil Isolation', 'Passed / Failed', 'Phenol Extraction of rRNA (Rat liver)', 'RNA Extraction', 'Keyword', 'Successful', 'Suspension cell volume (µL)', and 'Manage...'. The menu also includes options like 'Cut', 'Copy', 'Paste', 'Undo', 'Redo', 'Insert Current Date', 'Send to Word', 'Navigate', 'Standard Sentences', 'Dynamic Forms/Fields', 'Link an experiment', and 'Smart Import'.

Project: [Dropdown] Work Package: [Dropdown] Line Item: [Dropdown]

Samples: [Dropdown] Protocol: [Dropdown] +/-

Description:

Neutrophil Isolation

... were isolated from ml EDTA-treated peripheral blood

... Prep® Density Gradient Medium (SIGM [Dropdown]) .

... on from blood draw to stabilization of RNA never exceeded 90 min. Using Wright-Giemsa stain, we determined that this approach to

isolation consistently yielded ≥ purification.

RNA Extraction

Total RNA was extracted using .

RNA pellets were resuspended in diethyl pyrocarbonate-treated water.

RNA integrity was assessed (prior to beginning target processing) by running out a small

amount of each sample (typically 25-250 ng/well) onto a that was evaluated on an

.

Fast data entry with pick lists

Prompt for required values

Quickly add one or more Forms to an experiment

Menu items:

- Cut Ctrl+X
- Copy Ctrl+C
- Paste Ctrl+V
- Undo Ctrl+Z
- Redo Ctrl+Y
- Insert Current Date
- Send to Word Alt+W
- Navigate
- Standard Sentences
- Dynamic Forms/Fields
- Link an experiment
- Smart Import
- Analysis of Anabolic Agents in Urine by LCMSMS
- miRNA Microarrays
- Neutrophil Isolation
- Passed / Failed
- Phenol Extraction of rRNA (Rat liver)
- RNA Extraction
- Keyword
- Successful
- Suspension cell volume (µL)
- Manage...



OpenLAB ELN: Experiment results in context

Project: Agilent Technologies
Work Package: Project ABC
Line Item: Stability studies
Target:

Results Description
percentile). Only entities that had a present or marginal flag in at least 50% of values in any one out of the two conditions were selected for further analysis.]

Overall, 282 out of 821 entities represented on the array met these criteria. The microarray raw data have been deposited in the GEO database
<http://www.ncbi.nlm.nih.gov/geo/> series accession number = GSE18999.

Traditional Student's paired t-test was first applied to each probe set and false discovery rate (FDR) (Benjamin-Horowitz)

Gene Name	Gene Symbol	Fold Change
f-box and wd-40 domain protein 7 (archipelago)	FBXW7	0.5
ubiquitin protein ligase e2, j1 (ubc6 homolog, yeast)	UBE2J1	0.6
ubiquitin protein ligase e2b (rad6 homolog)	UBE2B	0.7
ubiquitin protein ligase e2l 3	CUL4A	1.3
ubiquitin protein ligase e2l 3	UBE2L3	1.4
ubiquitin protein ligase e2l 3	FBXW11	1.4
ubiquitin protein ligase e2l 3	TRIM37	1.4
ubiquitin protein ligase e3a (human papilloma virus E6-associated protein, angelman syndrome)	UBE3A	1.6
ubiquitin protein ligase e3a (human papilloma virus E6-associated protein, angelman syndrome)	SOCS1	1.6

Annotations:

- Add free text, urls and links to external applications:** Points to the "Results Description" section.
- Link to the original data files:** Points to the "Analysis.xls" link.
- Create tables or embed MS Excel spreadsheets:** Points to the gene expression table.
- Insert and annotate images:** Points to the "Key area to watch" box.

Key area to watch: Points to a red circle in a network diagram.

Characters: A small icon in the bottom left corner.



Experiment report contains all data and results

Data traceability and IP protection

ASSAY_PDF_181 - Microsoft Internet Explorer

Print Save a Copy Start Meeting... Previous Page Next Page 1 / 4 Zoom Out Zoom In 61,8% Scrolling Pages One Full Page Find

• β -glucuronidase is purchased from:

To 3 mL urine negative control sample, 1 mL 0.8 M potassium phosphate buffer, pH 7.0, is added. A further 25 μ L β -glucuronidase is added and then the mixture is incubated at 50 °C for one hour. A 750- μ L mixture of 20% (w/v) K₂CO₃/KHCO₃ (1:1) mixture is then added. Extract with methyl-*n*-butyl ether and then remove and dry the organic extract.

The same extraction procedure used for GC/MS screening is employed except that the compounds are not derivatized as usual for GC/MS analysis. Rather, the samples are dried and then reconstituted in 100 μ L of LC mobile phase.

As part of the reconstitution step for LC/MS/MS analysis, 20 μ L methanol, followed by 8 μ L of 1M GRP in 50 mM ammonium acetate buffer, pH 4.2, is added. Incubation at room temperature for one hour is then followed by LC/MS/MS analysis.

Of the compounds analyzed, only the 19-norandrosterone is reactive with the GRP derivative. This compound has been problematic in LC/MS/MS analysis and the GRP improves sensitivity.

The MeTest Internal standard has a fixed concentration of 10 ng/mL.

LC/MS Method Details
LC Conditions
Agilent 1200 Series binary pump SL, wellplate sampler, thermostatted column compartment, inline filter 0.5 μ m between needle seat and injector valve.

● Column ZORBAX XDB-CN 2.1 x 100 mm, 3.5 μ m (pH 961764-905)

● Column temperature (°C) 50

Mobile phase:

A = 0.1% formic acid in water

B = 0.1% formic acid in methanol

● Flow rate (mL/min) 1.0

● Injection volume (μ L) 2

Gradient:

Time (min)	%B
0	5
3	15
3.01	40

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Segment 2 (4.0-6.3 min)
19-norandrosterone 5.82 410.3 > 331.3 130 30 75

Segment 3 (6.3-6.93 min)
4 β -OH-stanozolol 6.84 345.2 > 327.2 140 15 200

Segment 4 (6.93-7.55 min)
MeTest (Sta) 7.19 303.2 > 97.1 140 25 75

Segment 5 (7.55-8.8 min)
THG 7.88 313.2 > 295.1 150 15 100
MeTest metabolite 8.08 271.2 > 161.2 110 20 100

Segment 6 (8.8-12.0 min)
Epimetendiol 9.47 269.2 > 105.1 90 20 200

Data Acquisition Parameters for MRM Transitions

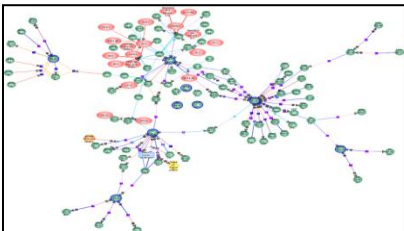
RESULTS

Gene Name	Gene Symbol	Fold Change
F-box and wd-40 domain protein 7 (archipelago homolog, drosophila)	FBXW7	0.5
ubiquitin-conjugating enzyme e2, j1 (ubc8 homolog, yeast)	UBE2J1	0.6
ubiquitin-conjugating enzyme e2b (rad6 homolog)	UBE2B	0.7
cullin 4a	CUL4A	1.3
ubiquitin-conjugating enzyme e2i 3	UBE2L3	1.4
F-box and wd-40 domain protein 11	FBXW11	1.4
tripartite motif-containing 37	TRIM37	1.4
ubiquitin protein ligase e3a (human papilloma virus e6-associated protein, angelman syndrome)	UBE3A	1.6
suppressor of cytokine signaling 1	SOC1	1.6

The results for retention time and area precision for all different concentration levels are summarized below. The precision limit for retention times is 0.1 % rsd. The precision limit for areas is 2 % rsd. For all concentrations the limits for retention time and area precision are fulfilled. To achieve sufficient resolution from the impurities, the concentration of the main compound should be <1.2 mg/mL.

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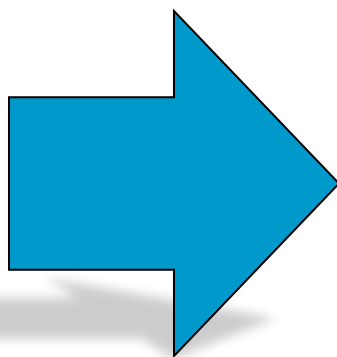
Agilent Technologies General Research M-JD-000005 Jean-Claude DUSSE Oncology Taxanes Paclitaxel



A fold change of 0.6 indicates the expression level after exercise is 60% of the expression level before the exercise. A fold change of 1.3 indicates the expression level after the exercise is 130% of the expression level before the exercise.



Providing a Collaboration Platform



Discovery
databases

Reporting

LIMS

OpenLAB Electronic Laboratory Notebook

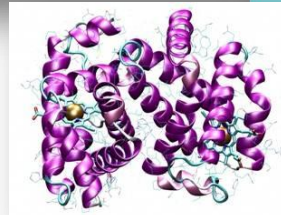
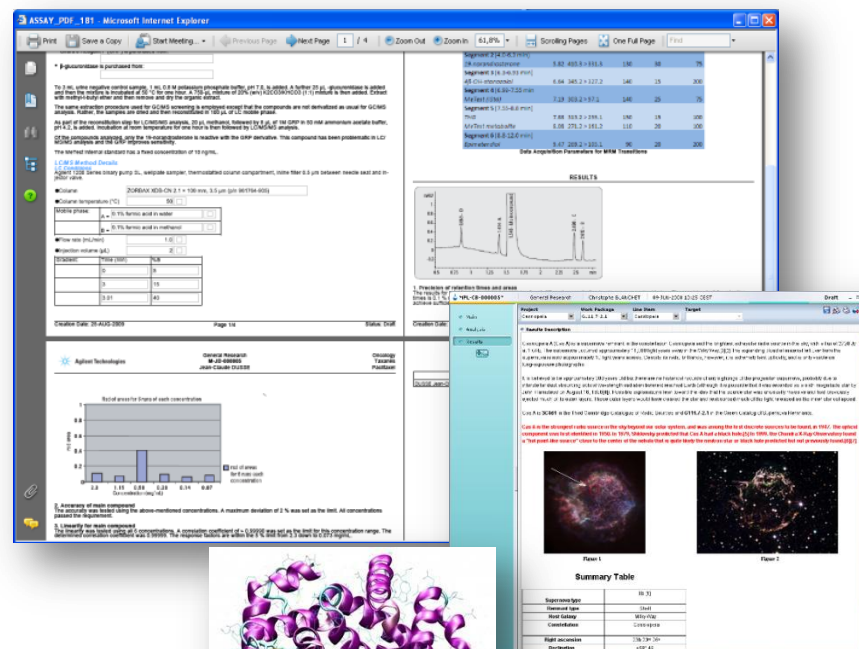
OpenLAB Enterprise Content Management System

Automatic Data Capture

Data generators

Instruments

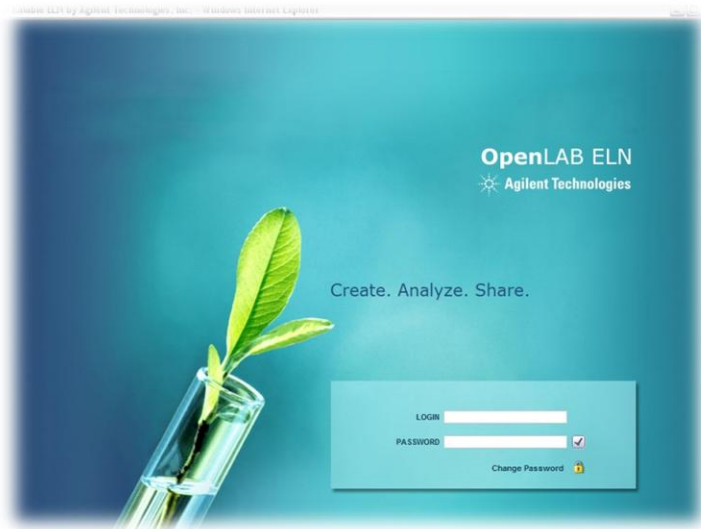
Office Applications



- Enable knowledge management
- Improve IP protection
- Improve regulatory compliance
- Increase productivity



Agilent Technologies



Questions?

Demonstrations available at lunch or after the sessions

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