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Bioinformatics

Common Tools & Tricks of the Trade



Welcome to Day 3 bioteach.ubc.ca/bioinfo2008

Workshop Schedule

2

- Laptops, available here for your use 9am - 4:30pm
- wireless login

mslguest

4myguest

• Vancouver guide books available



Today's Topics

- **PRACTICAL EXERCISES CONTINUED** Three different views of the BRCAI gene
- **Discovering GEO**, the Gene Expression Omnibus.
- **Pathway Resources** for Systems Biology
- Bioinformatics Links Directory, Conducting Research on the Web
- Let's go to the Pub! ... and chat more informally about what you'd like to do next with bioinformatics



Two tasks

- What genes are on either side of BRCA1 on chr 17?
- Can you figure out how to download the genomic sequence for the BRCA1 region?



http://www.ensembl.org/





Two tasks

- Using GeneView, can you figure out how many different alternatively spliced isoforms exist for BRCAI?
- Using ContigView, can you figure out how to download the genomic sequence for the BRCAI region?



http://www.ncbi.nlm.nih.gov/mapview/





NCBI Map Viewer

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Two tasks

- Can you figure out how to LinkOut to the OMIM and/or Homologene entries for BRCAI?
- Can you figure out how to download the genomic sequence for the BRCA1 region?



Credits

- UCSC Genome Browser
 <u>http://genome.cse.ucsc.edu</u>/
- Ensembl Genome Browser
 <u>http://www.ensembl.org/index.html</u>
- NCBI MapViewer

http://www.ncbi.nlm.nih.gov/mapview/ index.html

Bioinformatics

Session 3.1 - Discovering GEO, the Gene Expression Omnibus.



Functional Genomics

- What kinds of questions can you ask with microarray data?
 - \checkmark basic research
 - \checkmark drug target discovery
 - \checkmark biomarker discovery
 - pharmacology & toxico genomics

- clinical diagnosis prognosis, diagnosis, &
 disease classification
- \checkmark gene regulatory networks
- \checkmark protein-DNA binding
- 🗸 + more



scanned into computer

cDNA spotted on glass slide or oligonucleotides built on slide

Figure I | **Schematized experimental process using a microarray.** Although the specific protocols differ, the microarray approach first involves isolating RNA or messenger RNA from appropriate biological samples, making the RNA (or a copy of it) fluorescent, hybridizing it to the microarray, washing off the excess and scanning the microarray under laser light.

Different Platforms

in situ oligonucleotide

single sample, absolute levels

spotted DNA/cDNA

two samples, relative levels



Microarray Experiment

- Design
- Collect
- Pre-Process Example = Normalization
- Analyze Examples = Distance measures, data
- Interpret
- Examples = Distance measures, data classification, clustering, + more

- Submit
- Publish

Rate Limiting Step = What do these results actually mean?

Public Microarray Data

- The Gene Expression Omnibus (GEO)
 repository/archive gene expression data
- data submitted by the research community in fulfillment of journal requirements
- this public data represents an untapped resource; potential discovery from existing data sets is at your fingertips

GEO Database

<u>Organized by:</u> Platform Sample Series/DataSet Profile



http://www.ncbi.nlm.nih.gov/geo/

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Searching GEO

• Are you interested in a particular type of expt?

✓ GEO DataSets

- Are you looking for your favorite gene?
- ✓ GEO Profiles



Data in GEO

>120,000 samples >3.2 billion measurement 200+ organisms from >2000 labs

freely available online ftp downloads

Total holdings

	Public	Unreleased	Total
Platforms	4407	355	4762
Samples	201401	45428	246829
Series	7883	1623	9506

Browse public holdings

 All contacts All platforms in situ oligonucleotide (1260) spotted oligonucleotide (1099) spotted DNA/cDNA (1850) antibody (5) tissue (0) MS (10) SARST (1) MPSS (12) RT-PCR (7) oligonucleotide beads (50) mixed spotted oligonucleotide/cDNA (6) spotted protein (4) SAGE (54) All samples RNA (167588) genomic (30043) protein (651) SAGE (993) mixed (913) All series



An Example

• Find microarray experiments that look at the expression of genes in cancer



You can use these GEO data mining tools for quick and easy identification of relevant & noteworthy data sets. For serious analyses, you should download the data and use a microarray data analysis software suite.

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- thumbnail image represents the abundance profile for an individual gene across each Sample in a DataSet
- bars at the bottom of the chart represent experimental subsets within the DataSet.
- Red bar: measured level of abundance
- Blue square: indication of where the expression of that gene falls with respect to all other genes on that array

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Annotation:	LIPC: Lipase, hepatic	
Reporter:	N68256	أماعك ومبابع والمنابلة المرابية والمساحدة والمسابقة والمسابقة والمسابقة والمسابقة والمسابقة والمسابقة والمسابعة
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□ 4: GDS2415	record GPL3558 19172 [Homo sapiens]	59 samples Chromosome Neighbors, Links
Annotation:	SMARCA1: SWI/SNF related, matrix associated, actin dependent regulator of chromatin,	in file and a sin
Reporter:	AA496809	وليفاريه دعانيه وطناوه فالمورون والمعالية
Experiment:	Breast carcinomas and local recurrence, gene expre4sion array-based, log2 ratio	

A vs B Query Tool

Take home message: GEO data analysis tools are great for quick identification of interesting leads; you download the data to carry out more robust statistical analyses

- **Purpose:** To help identify gene profiles that display marked differences in expression level between two subsets of experimental factors (e.g. tissue, strain, time, dose, etc).
- **Caveats:** The "mean group A vs B" is perhaps the most rudimentary means of filtering data; t-test is well established but comes with a set of basic assumptions.

A Simple Test

- Student's t-test
 - Assumptions: Normality, equal variance



Using GEO for differential expression

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Assession	Lone 2446	Lon Mary Evenenia	- /CEO				
Title:	Breast co	reinomas and local	recurrence	6)			
DataSet type:	Dieast Ce	ression array hased	(RNA / spotte	d DNA/cDNA)			
Summary:	Analysis	of primary breast ca	rcinoma tum	ve from 50 nationts wi	on received brea	st-conserving t	herany (BC)
ounnary.	19 patien	ts subsequently dev	eloped a loca	I recurrence of the car	cinoma. 9 recur	rent tumors also	examined.
	Compare	d to mastectomy, BI	CT is associa	ted with a higher rate	of local recurren	ce.	
Platform:	GPL3558	: NKI-AVL Homo sa	piens 18K cD	NA microarray			
Citations:	Kreike B, patients a 1;12(19):	Halfwerk H, Kristel at high risk for local 5705-12. PMID: 170	P, Glas A et recurrence af 20974	al. Gene expression p er breast-conserving t	rofiles of primar, herapy. Clin Ca	y breast carcino ncer Res 2006 (omas from Oct
Sample organism	: Homo sa	piens	Platform	organism:	Ho	mo sapiens	
Feature count:	19200		Value typ	D:	log	g2 ratio	
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http://www.ncbi.nlm.nih.gov/projects/geo/gds/gds_browse.cgi?gds=2415

GEO limitations

- Differential expression can only be done for "Datasets" (GDS****)
- T-tests only
- Very little control over parameters
- Output is not that easy to use

Be careful with p < 0.05

- In the example, 2912 genes met p<0.05
- This is 11% of the genes on the array
- Expect 5% by chance (this is what p < 0.05 means)
- Probably ~1/2 of the selected 2912 are false positives
- This is the "multiple testing" problem

Download Data Types

SOFT - text based MiNiML* - xml based



*MIAME Notation in Markup Language

all GEO data are available for bulk download: ftp://ftp.ncbi.nih.gov/pub/geo/DATA

More Serious Tools

• Free

...

...

- R + Bioconductor
- TIGR MultiExperimentViewer (MeV)

- Commerical
 - Genespring–ArrayAssist
 - Rosetta Resolver

Gene Expression Profiles of Primary Breast Carcinomas from Patients at High Risk for Local Recurrence after Breast-Conserving Therapy

Bas Kreike,^{1,3} Hans Halfwerk,^{2,3} Petra Kristel,^{2,3} Annuska Glas,² Hans Peterse,² Harry Bartelink,¹ and Marc J, van de Viiver²

gene of interest FABP3

Abstract Purpose: Several risk factors for local recurrence of breast cancer after breast-conserving therapy (BCT) have been identified. The identification of additional risk factors would be very useful in guiding optimal therapy and also in improving understanding of the mechanisms underlying local recurrence. We used cDNA microarray analysis to identify gene expression profiles associated with local recurrence.

> Experimental Design: Using 18K cDNA microarrays, gene expression profiles were obtained from 50 patients who underwent BCT. Of these 50 patients, 19 developed a local recurrence; the remaining 31 patients were selected as controls as they were free of local recurrence at least 11 years after treatment. For 9 of 19 patients, the local recurrence was also available for gene expression profiling. Unsupervised and supervised methods of classification were used to separate patients in groups corresponding to disease outcome and to study the overall gene expression pattern of primary tumors and their recurrences.

> Results: Hierarchical clustering of patients did not show any grouping reflecting local recurrence status. Supervised analysis revealed no significant set of genes that was able to distinguish recurring tumors from nonrecurring tumors. Paired-data analysis of primary tumors and local recurrences showed a remarkable similarity in gene expression profile between primary tumors and their recurrences.

> Conclusions: No significant differences in gene expression between primary breast cancer tumors in patients with or without local recurrence after BCT were identified. Furthermore, analyses of primary tumors and local recurrences show a preservation of the overall gene expression pattern in the local recurrence, even after radiotherapy.

Breast-conserving therapy (BCT) has become the therapy of choice for a large proportion of breast cancer patients. Several randomized controlled trials have shown no difference in survival rates after BCT or mastectomy for stage I and II breast cancer (1-4). Studies comparing the psychological effects of BCT with mastectomy have shown that patients treated with RCT had a batter body image, and some studies reported lass

recurrence compared with mastectomy. A local recurrence rate of 10% in 10 years follow-up is generally considered as clinically acceptable for T1-2N0-1 breast cancers. However, local recurrence up to 30% have been reported in young patients (7, 8).

Several risk factors for local recurrence after BCT have been identified, involvement of the surgical manning by investor

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✓ (50)	specimen	primary tumor			\leftrightarrow		Γ
🗹 (9)	specimen	recurrent tumor			\leftrightarrow		
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1: GDS2415	record GPL3558 1925 [Homo sapiens] 59 samples	Profile Neighbors, Chromosome Neighbors, Links
Annotation:	FABP3: Fatty acid binding protein 3, muscle and heart (mammary-derived growth inhib	
Reporter:	AA044307	հղետոնդերությ
Experiment:	Breast carcinomas and local recurrence, gene expression array-based, log2 ratio	
C 2: GDS2415	record GPL3558 11434 [Homo sapiens]	59 samples Chromosome Neighbors, Links
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T1: GDS2415	record GPL3558 1925 [Homo	sapiens]		3	59 samples Profile Neighb	ors, Chromosome Neighbors, Links
Annotation:	FABP3: Fatty acid binding p	rotein 3, muscle an	d heart (mammary-der	ived growth inhib.		
Reporter:	AA044307				נותנה הארק העולם הארק	ւսնակերություն
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C 2: GDS2415	record GPL3558 13888 [Hom	o sapiens]			59 si	amples Profile Neighbors , Links
Annotation:	Transcribed locus, strongly	similar to NP_0664	07.1 histone family, mo	ember B		· · · · · · · · · · · · · · · · · · ·
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3: GDS2415	record GPL3558 2942 [Homo	sapiens]			59 samples Profile Neighb	ors, Chromosome Neighbors, Links
Annotation:	HIST2H2BE: Histone cluste	r 2, H2be			6	
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Experiment:	Breast carcinomas and loca	al recurrence, gene	expression array-based	d, log2 ratio	R and the faile note	Chambert 1. 1.

Profile Neighbors

Take home message: GEO data analysis tools are great for quick identification of interesting leads; you download the data to carry out more robust statistical analyses

- Connects groups of genes that have similar expression profiles within a DataSet
- pre-computed
- calculated by Pearson correlation coefficients

Other Features

- Cluster Heat Maps
 - precomputed sample and gene hierarchical cluster heat maps provided
 - different methods available;
 can select, expand, download

• GEO BLAST

 retrieve gene expression profiles by sequence similarity



GEO, the gene expression omnibus

- public repository of expression data from many different experimental platforms
- Main uses
 - \checkmark search for experiments of interest
 - search for expression information about
 gene of interest
- submit, search, analyses tools available
- data standards required MIAME, MiNiML

Credits & References

- NCBI GEO: mining tens of millions of expression profiles—database and tools update. Barrett T, et al. Nucleic Acids Res. 35 (2007) D760-5. [PMID: 17099226]
- GEO: the Gene Expression Omnibus

http://www.ncbi.nlm.nih.gov/projects/geo/info/ GEOHandoutFinal.pdf

• Dr. Paul Pavlidis, UBC Bioinformatics Centre

Bioinformatics

Session 3.2 - Pathway Resources for Systems Biology



Proteomics

• How large is the human proteome, anyway?

Class	Size	Description
Non Redundant Proteins	20,000-25,000	representative protein from every gene locus
Variants	50,000-500,000	different proteins obtained by splicing or proteolysis
Combinatorial Variants	>10,000,000	different proteins generated by somatic DNA rearrangements
Protein Species	>100,000	proteins that differ in chemical composition due to PTM
Protein Alleles	75,000-150,000	proteins that differ by genetic variation (coding SNPs)

source: Uhlen M et al, Molecular & Cellular Proteomics (2005)

Cellular Pathways



 A striking similarity between intracellular signaling pathways and the Tokyo subway system

Pathway Information

- Databases
 - Fully electronic
 - Easily computer readable
- Literature
 - Increasingly electronic
 - Human readable
- Biologist's brains
 - Richest data source
 - Limited bandwidth access
- Experiments
 - Basis for models



http://www.pathguide.org/

Home BloPAX CBIO MSK

Many new search options are

Detailed Pathquide resource

Please cite the Pathouide Publication

statistics now available

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News

available

Find it Easier

Get the Stats

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Navigation

Protein-Protein Interactions Metabolic Pathways Signaling Pathways Pathway Diagrams Transcription Factors / Gene Regulatory Networks Protein-Compound Interactions Genetic Interaction Networks **Protein Sequence** Focused Other Search Organiama

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Comments, Questions, Suggestions are Always

Complete Listing of All Pathguide Resources

Pathguide contains information about 240 biological pathway resources. Click on a link to go to the resource home page or 'Details' for a description page. Databases that are free and those supporting BioPAX, CelIML, PSI-MI or SBML standards are respectively indicated.

If you know of a pathway resource that is not listed here, or have other questions or comments, please send us an e-mail.

Protein-Protein Interactions

Database Name (Order: alphabetically by web popularity o)	Full Record	Availability	Standards
3DID - 3D interacting domains	Details	Free	
ABCdb - Archaea and Bacteria ABC transporter database	Details	Free	
AfCS - Alliance for Cellular Signaling Molecule Pages Database	Details	Free	
AllFuse - Functional Associations of Proteins in Complete Genomes	Details	Free	
ASEdb - Alanine Scanning Energetics Database	Details	Free	
ASPD - Artificial Selected Proteins/Peptides Database	Details	?	
BID - Binding Interface Database	Details	Free	
BIND - Biomolecular Interaction Network Database	Details	Free	PSI-MI
BioGRID - General Repository for Interaction Datasets	Details	ED.	PSI-MI
BRITE - Biomolecular Relations in Information Transmission and Expression	Details	Free	
CA1Neuron - Pathways of the hippocampal CA1 neuron	Details	Free	
Cancer Cell Map - The Cancer Cell Map	Details	Free	BIOPAX
CellCircuits - CellCircuits	Details	Free	
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Using Pathway Information



Aim: Convenient Access to Pathway Information



Using Pathway Information



Cytoscape - Network Visualization and Analysis



http://cytoscape.org



- Freely-available (open-source, java) software
- Visualizing biological networks (e.g. molecular interaction networks)
- Analyzing networks with gene expression profiles and other cell state data

UCSD, ISB, Agilent, MSKCC, Pasteur, UCSF, UToronto Other software: Osprey, BioLayout, VisANT, Navigator, PIMWalker, ProViz





 In addition to describing the network topology, nodes and edges can each have their own attributes

Visual Style

- Customized views of experimental data in a network context
- Network has node and edge attributes
 - · E.g. expression data, interaction type, GO function
- Mapped to visual attributes
 - E.g. node/edge size, shape, colour...
- E.g. Visualize gene expression data as node colour gradient on the network






Systems Biology

• Goals:

 \checkmark integrating diverse data types, pathways \checkmark cellular simulations

- Community approaches:
 - pathguide, pathway commons, cytoscape
- Open data exchange key to success

Credits & References

• Dr. Gary Bader, DCCBR, UofT

slides/images used with permission

 Cary MP, Bader GD, Sander C "Pathway Information for Systems Biology", FEBS Letters (2005)

Bioinformatics Links Directory

Finding online tools & resources for Life Sciences research



http://bioinformatics.ca/links_directory/

Bioinformatics Links Directory

The Bioinformatics Links Directory features curated links to molecular resources, tools and databases. The links listed in this directory are selected on the basis of recommendations from bioinformatics experts in the field. We also rely on input from our community of bioinformatics users for suggestions. Starting in 2003, we have also started listing all links contained in the NAR Webserver issue.

Search Directory

Computer Related (64)

This category contains links to resources relating to programming languages often used in bioinformatics. Other tools of the trade, such as web development and database resources, are also included here.

Education (75)

Links to information about the techniques, materials, people, places, and events of the greater bioinformatics community. Included are current news headlines, literature sources, educational material and links to bioinformatics courses and workshops.

Human Genome (128)

This section contains links to draft annotations of the human genome in addition to resources for sequence polymorphisms and genomics. Also included are links related to ethical discussions surrounding the study of the human genome.

Model Organisms (204)

Included in this category are links to resources for various model organisms ranging from mammals to

DNA (441)

This category contains links to useful resources for DNA sequence analyses such as tools for comparative sequence analysis and sequence assembly. Links to programs for sequence manipulation, primer design, and sequence retrieval and submission are also listed here.

Expression (272)

Links to tools for predicting the expression, alternative splicing, and regulation of a gene sequence are found here. This section also contains links to databases, methods, and analysis tools for protein expression, SAGE, EST, and microarray data.

Literature (35)

Links to resources related to published literature, including tools to search for articles and through literature abstracts. Additional text mining resources, open access resources, and literature goldmines are also listed.

Other Molecules (15)

Bioinformatics tools related to molecules other than DNA, RNA, and protein. This category will include resources.

Main Page

Citations

Acknowledgements

News

Suggest URL

NAR Collaboration

RSS Feeds

Nucleic Acids Research



Conducting Research on the Web: 2007 Update for the Bioinformatics Links Directory

Joanne A. Fox, Scott McMillan and B. F. Francis Ouellette*

UBC Bioinformatics Centre (http://bioinformatics.ubc.ca/), University of British Columbia, 2185 East Mall, Vancouver, British Columbia, Canada, V6T 1Z4

Received May 18, 2007; Accepted May 22, 2007

ABSTRACT

Bioinformatics Links Directory, http:// The bioinformatics.ca/links_directory, is an actively maintained compilation of servers published in this and previous issues of Nucleic Acids Research issues together with many other useful tools, databases and resources for life sciences research. The 2007 update includes the 130 websites highlighted in the July 2007 Web Server issue of Nucleic Acids Research and brings the total number of servers listed in the Bioinformatics Links Directory to just under 1200 links. In addition to the updated content, the 2007 update of the Bioinformatics Links Directory includes new features for improved navigation, accessibility and open data exchange. A complete listing of all links listed in this Nucleic Acids Research 2007 Web Server issue can be accessed online at, http://bioinformatics.ca/ links_directory/narweb2007. The 2007 update of the Bioinformatics Links Directory, which includes the Web Server list and summaries is also available online, at the Nucleic Acids Research web site, http://nar.oupjournals.org.

COMMENTARY

With the publication of the 2007 Nucleic Acids Research Web Server issue, we have a chance to reflect on how the web has transformed the way we conduct scientific trusted directories, portals and useful search engines to keep up with the latest science. The Bioinformatics Links Directory is one such directory that includes a compilation of molecular biology servers, bioinformatics tools and online resources for life sciences research.

The Bioinformatics Links Directory, http:// bioinformatics.ca/links directory, is a listing of specialized servers and general purpose resources that aims to help scientists navigate the rapidly changing landscape of online research tools. Researchers can find relevant servers using the straight forward keyword search or by navigating to the biological categories, where tools that do similar tasks are grouped together. Each entry in the Bioinformatics Links Directory is highlighted by providing a short description for each link, listing relevant PubMed citations, and identifying links as servers from the Nucleic Acids Research Web Server Issue. This directory is designed to make it easy to connect information together. Any researcher can assemble a comprehensive listing of resources by downloading and importing the RSS feeds, a format used to publish frequently updated content (http://en.wikipedia.org/wiki/RSS). For example, a researcher can import the RSS feed for a search of the Bioinformatics Links Directory for 'transcription factor binding site' and publish it on her wiki alongside the feeds from the 'RNA. Structure Prediction, Visualization, and Design' and the 'Protein, Interactions, Pathways and Enzymes' subcategories as a resource for the whole research laboratory interested in transcriptional regulatory mechanisms.

The 2007 update of the Bioinformatics Links Directory

Your Feedback is Important!

navigate to: bioteach.ubc.ca/bioinfo2008





Thanks for attending the AMBL Bioinformatics Workshop

• Questions? Please contact:

Dr. Joanne Fox Michael Smith Laboratories joanne@msl.ubc.ca

