Microarray Analysis Strategy

辛致煒

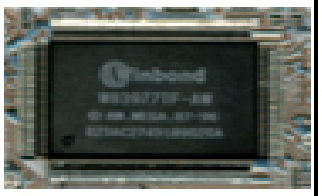
國立成功大學醫學院生物晶片中心

Microarray Center, National Cheng Kung University

改變二十世紀的大事

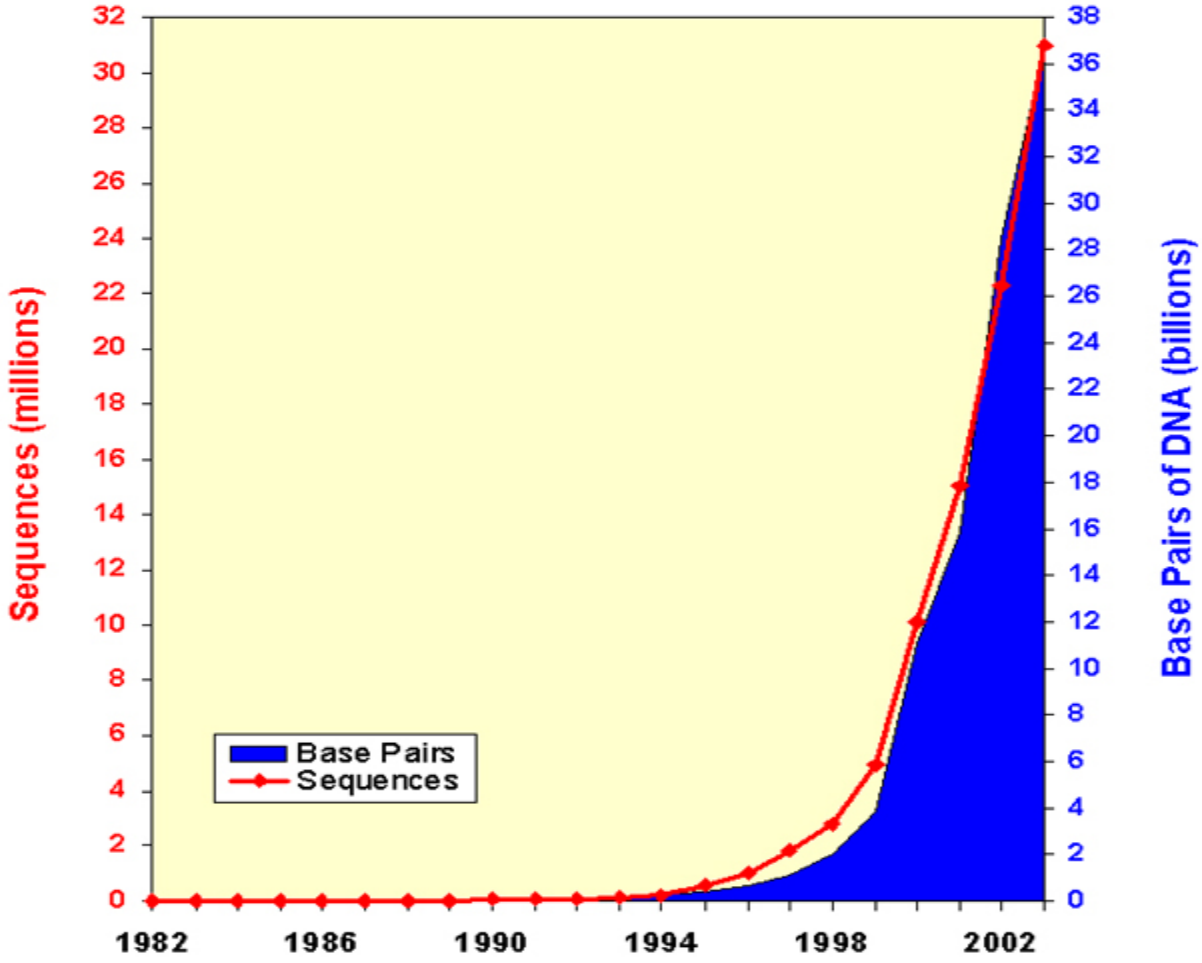


Four Generations of Computer

	起止年代	主要元件	主要元件图例	速度（次/秒）	特点与应用领域
第一代	40 年代末至 50 年代末	电子管		5 千~1 万次	计算机发展的初级阶段，体积巨大，运算速度较低，耗电量大，存储容量小。主要用来进行科学计算。
第二代	50 年代末至 60 年代末	晶体管		几万~几十万次	体积减少，耗电较少，运算速度较高，价格下降，不仅用于科学计算，还用于数据处理和事务管理，并逐渐用于工业控制。
第三代	60 年代中期开始	中、小规模集成电路		几十万~几百万次	体积、功耗进一步减少，可靠性及速度进一步提高。应用领域进一步拓展到文字处理、企业管理、自动控制、城市交通管理等方面。
第四代	70 年代初开始	大规模和超大规模集成电路		几千万~千百亿次	性能大幅度提高，价格大幅度下降，广泛应用于社会生活的各个领域，进入办公室和家庭。在办公室自动化、电子编辑排版、数据库管理、图像识别、语音识别、专家系统等领域中大显身手。

New Technologies Have Produced Explosion of Data

http://www.ncbi.nlm.nih.gov/Genbank/genbankstats.html



Complex Biological Systems



Overview
People
Research
Academics
Seminars
Admissions
Contact
CBS Home

Systems biology is in fashion right now, and everyone is jumping on the bandwagon.

Andreas Wagner
University of New Mexico

http://cbs.montana.edu/

A systems biology view...

PERSPECTIVES: SYSTEMS BIOLOGY

Life's Complexity Pyramid

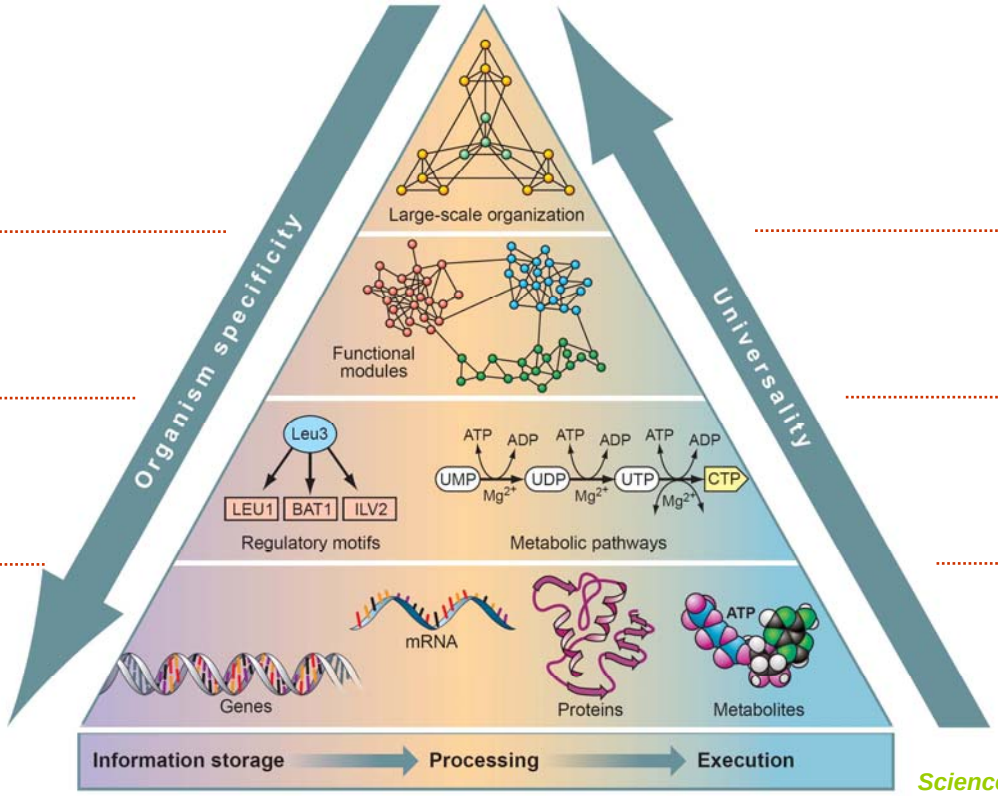
Zoltán N. Oltvai and Albert-László Barabási

System

Functional Modules

Building Blocks

Components



System

Functional Modules

Building Blocks

Components

Science, 298, 763-764 (2002)

news feature

Are you ready for the revolution?

If biologists do not adapt to the powerful computational tools needed to exploit huge data sets, says Declan Butler, they could find themselves floundering in the wake of advances in genomics.

Nature 409, (2001)

SI Home | About the SI | Activities @ SI | Lodging & Logistics | Leaders | Registration Information | PKAL Home



Biology as an Interdisciplinary Science

PKAL 2002 Summer Institute - Cluster I
May 29 - June 1, 2002

Session: Quantitative Life Science Education: Preparing Fearless Biologists

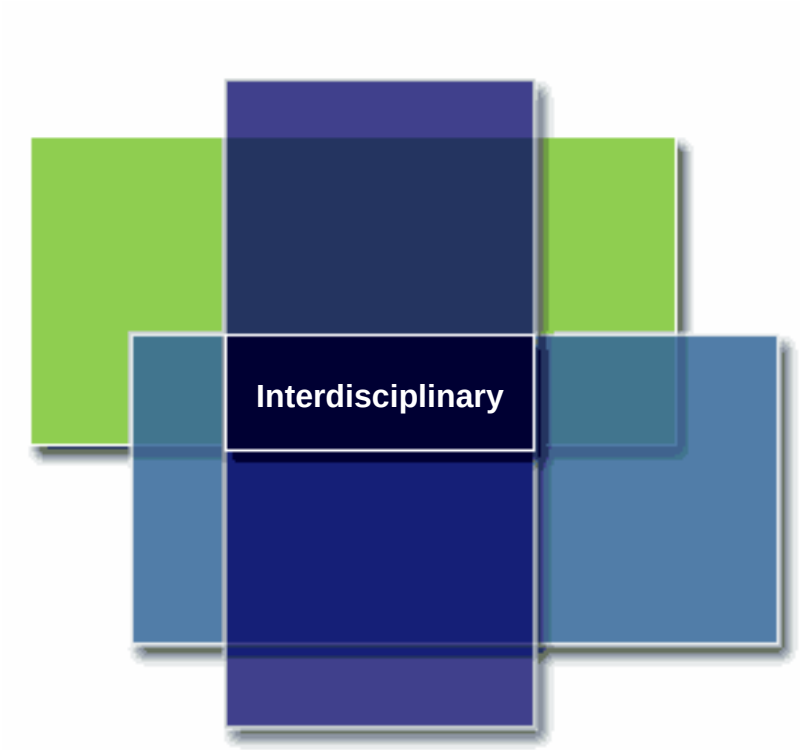
Presenter(s):
Louis Gross, University of Tennessee at Knoxville

http://lists.pkal.org/si/1-2.html

Interdisciplinary

adjective

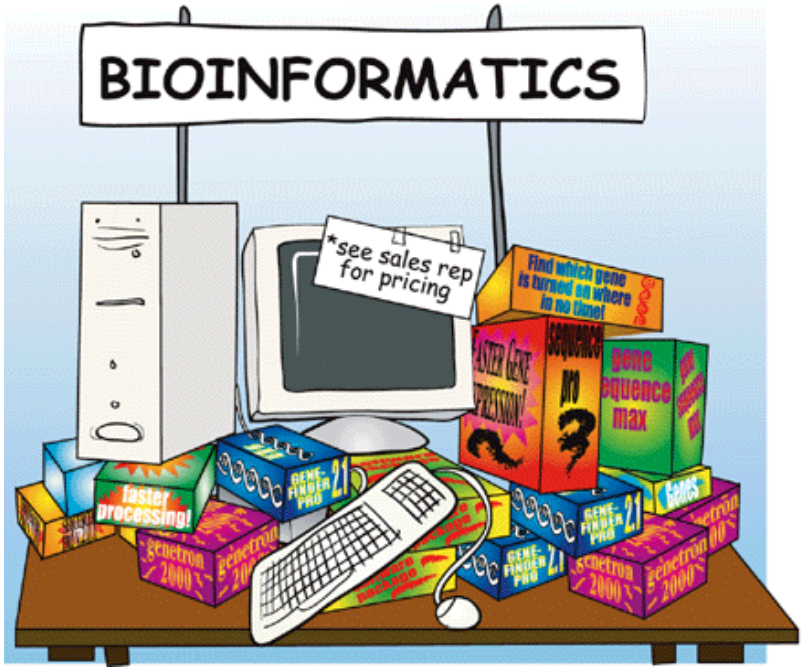
relating to more than one branch of knowledge



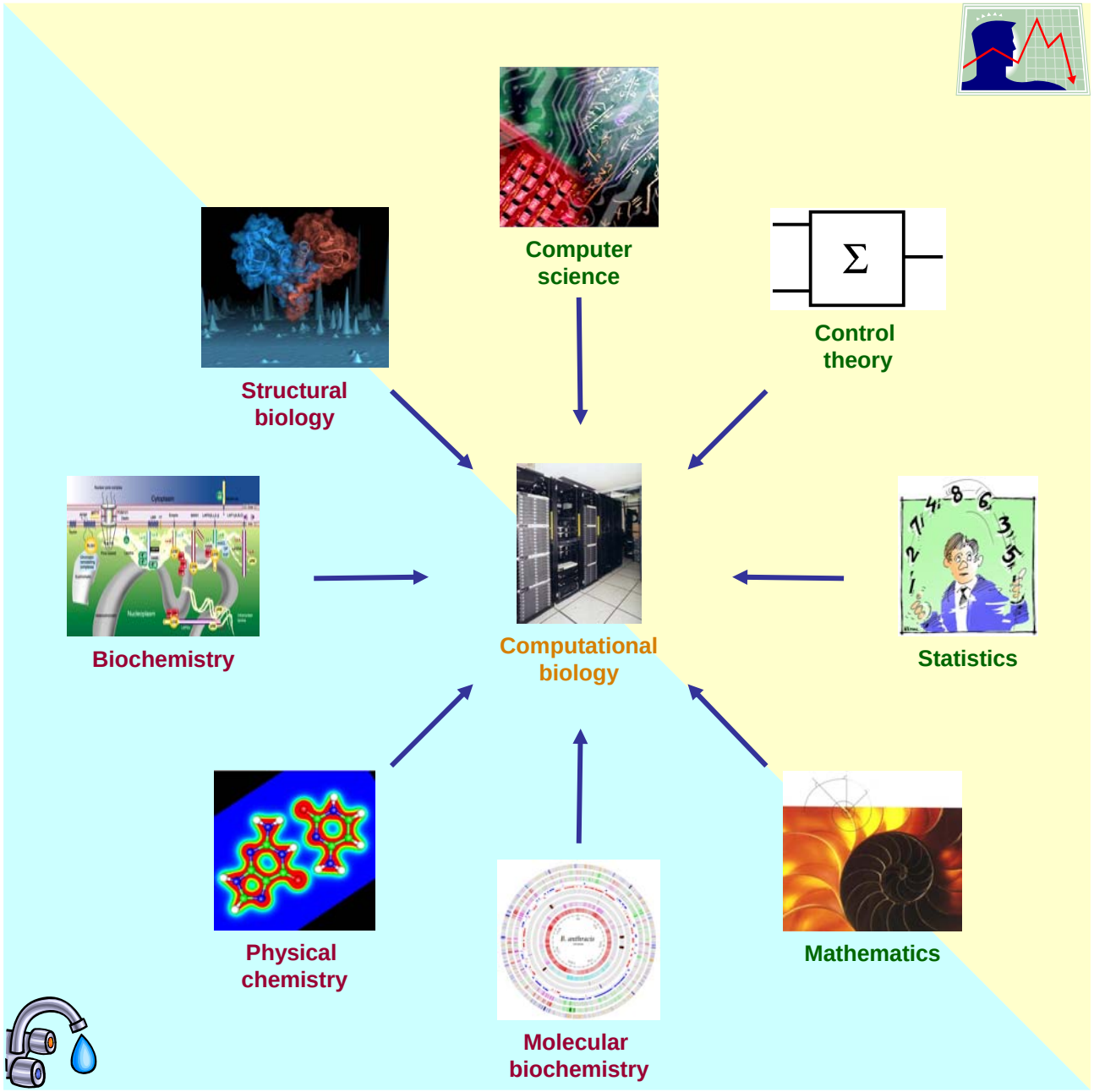
Bioinformatics

plural noun

the science of collecting and analyzing complex biological data such as genetic codes.



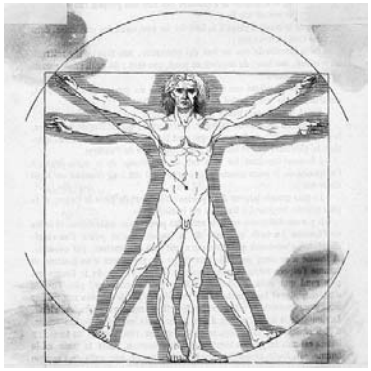
http://www.mit.edu/people/geneyeo/bioinformatics.gif


Computer science
Control theory
Structural biology
Biochemistry
Computational biology
Statistics
Physical chemistry
Molecular biochemistry
Mathematics

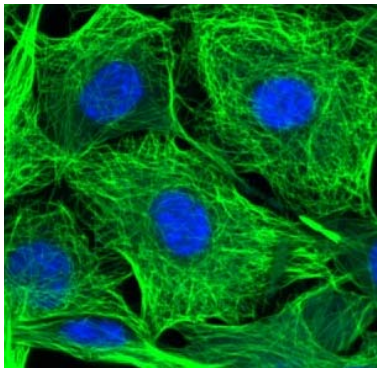


Molecular Biology Overview

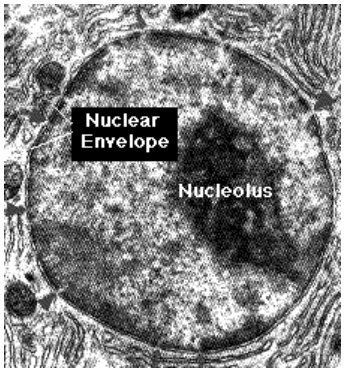
Human



Cell

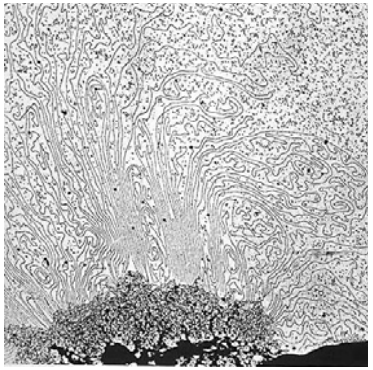


Nucleus

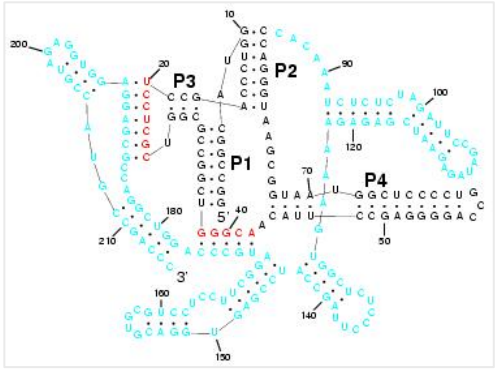


Chromosome

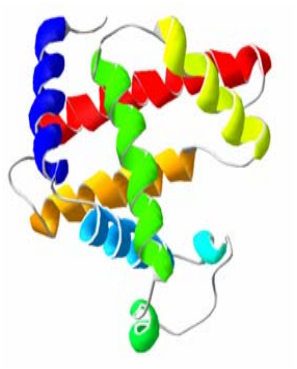




Gene (DNA)



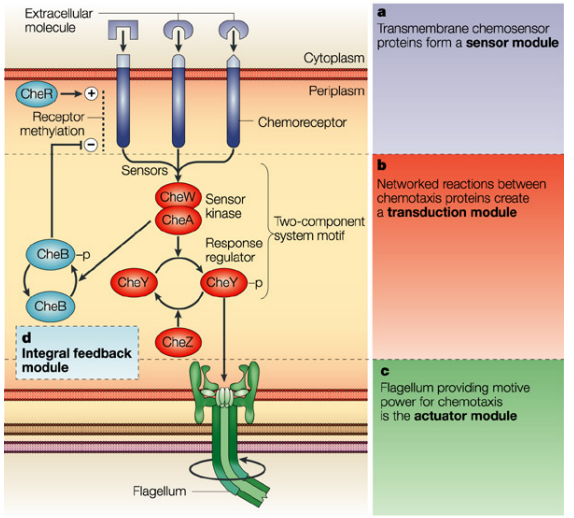
Gene (mRNA)

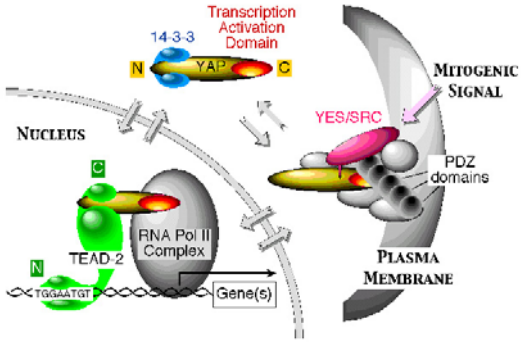


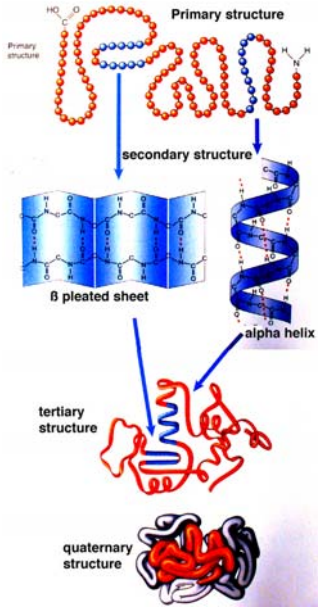
Protein

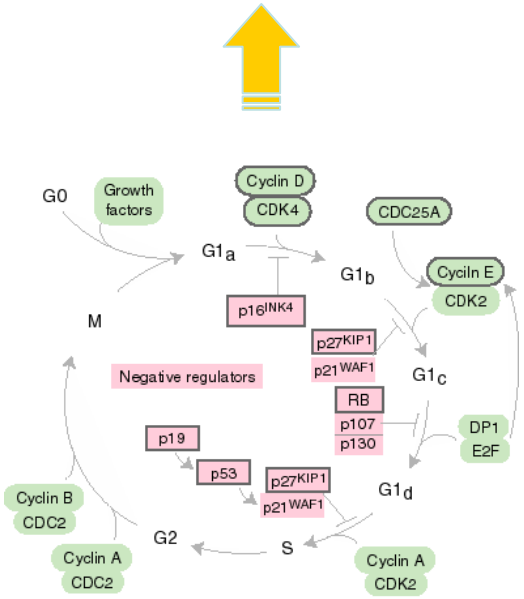
The role of module in cell biology

1. Genes / proteins
2. Protein complexes
3. Modules (Functions / pathways)
4. Regulatory circuit









Some Current Research Focus Areas

Array and EST Informatics

- Clustering and classification
- EST Software Pipeline
- Common regulatory element finding

Comparative Genomics

- whole genome alignment
- SNP and Haplotype detection
- dictionary-based genome analysis
- meta-genome analysis
- Protein function identification
- visualization
- Gene finding

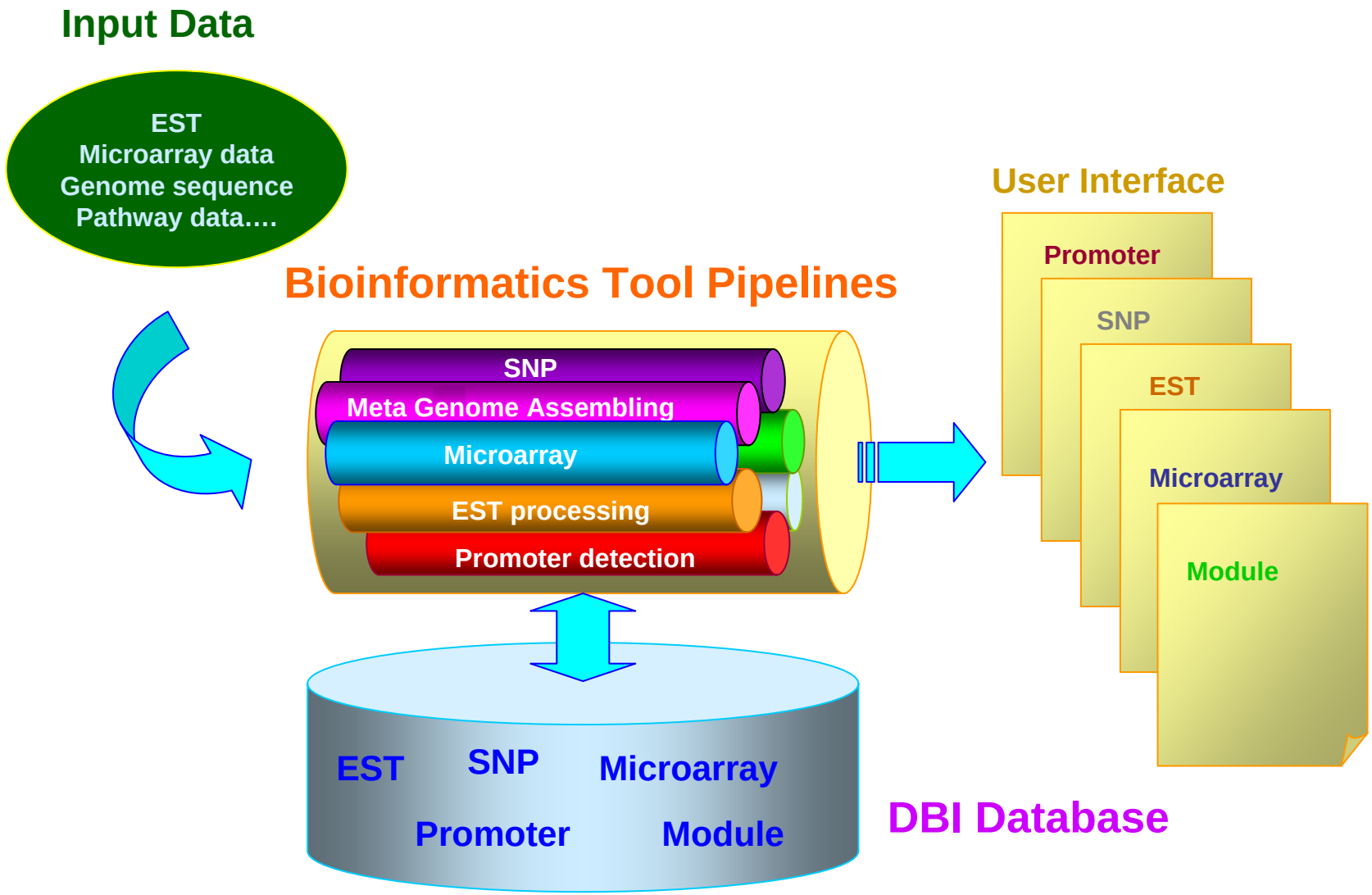
High-performance bioinformatics instrumentation

- Parallelization of key bioinformatics algorithms on high-performance machines
- Cellular multiprocessor system–on-a-chip (SOC) for biological pattern recognition

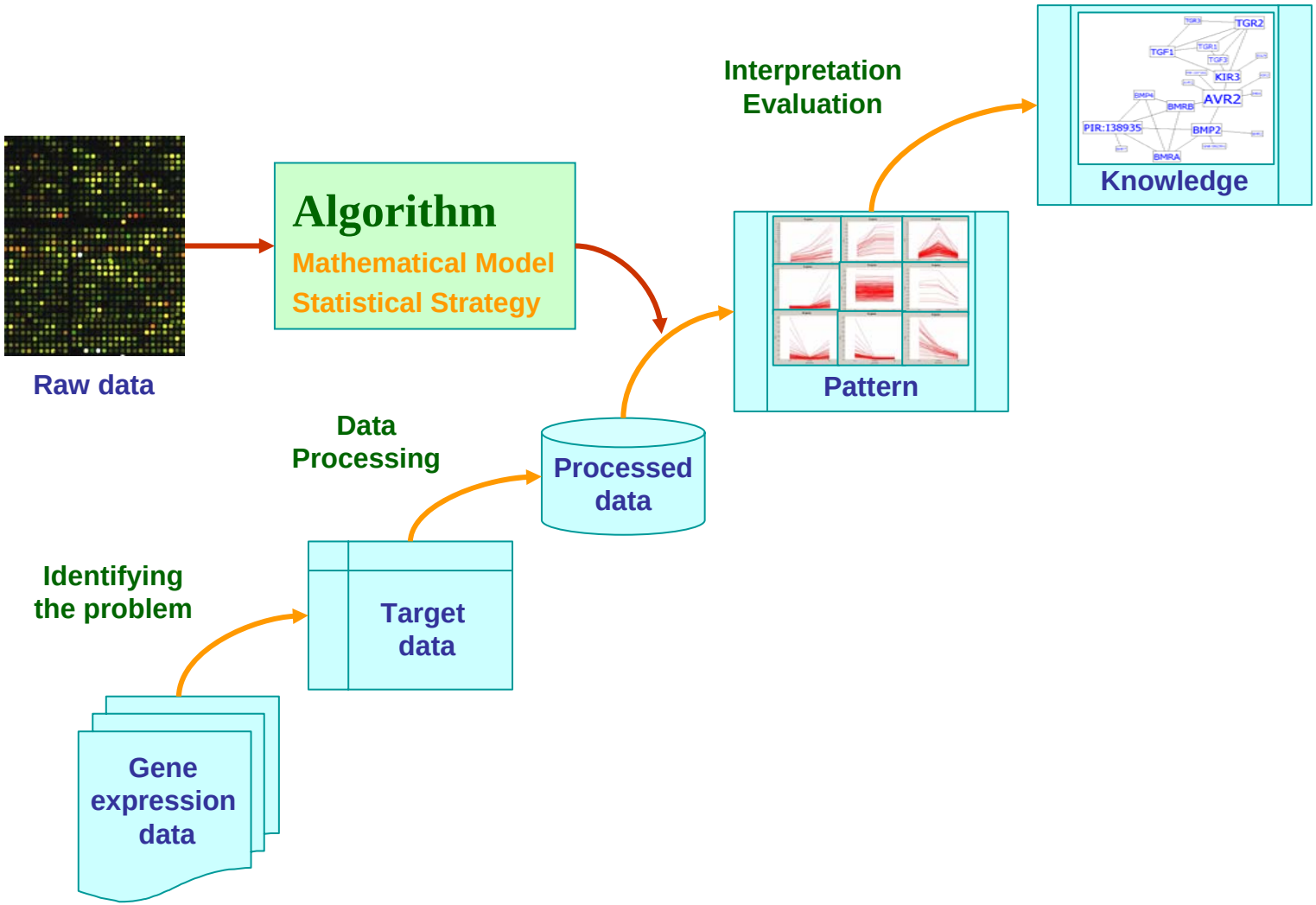
In-Silicon Biological Structure and Process Modeling / Simulation

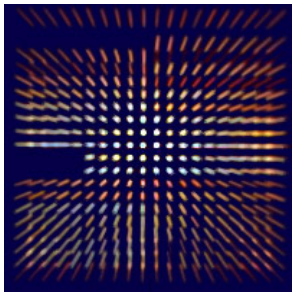
- regulatory pathway modeling
- protein structure dynamics and simulation
- pathway database for modeling/simulation

Bioinformatics Tool and Data Network



An Overview of Data Mining Process

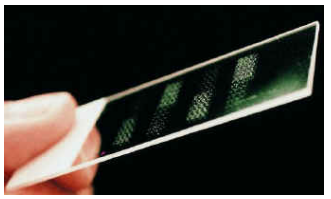


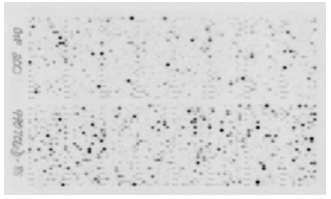


Microarray

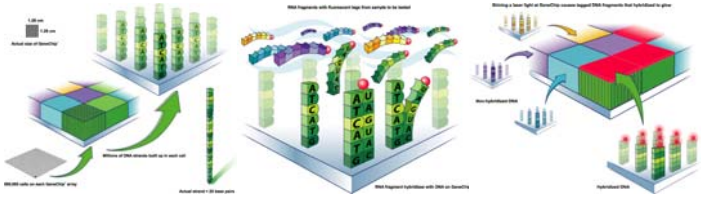
Small + place in an orderly arrangement

DNA chip / cDNA (glass and membrane)

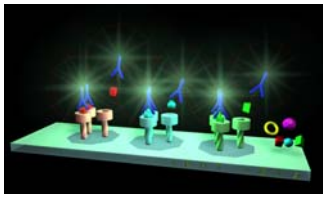




oligo chip / 50~70 mer nucleotides



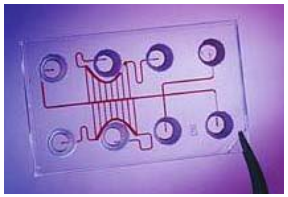
Protein chip / SELDI-TOF-MS
receptor - ligand



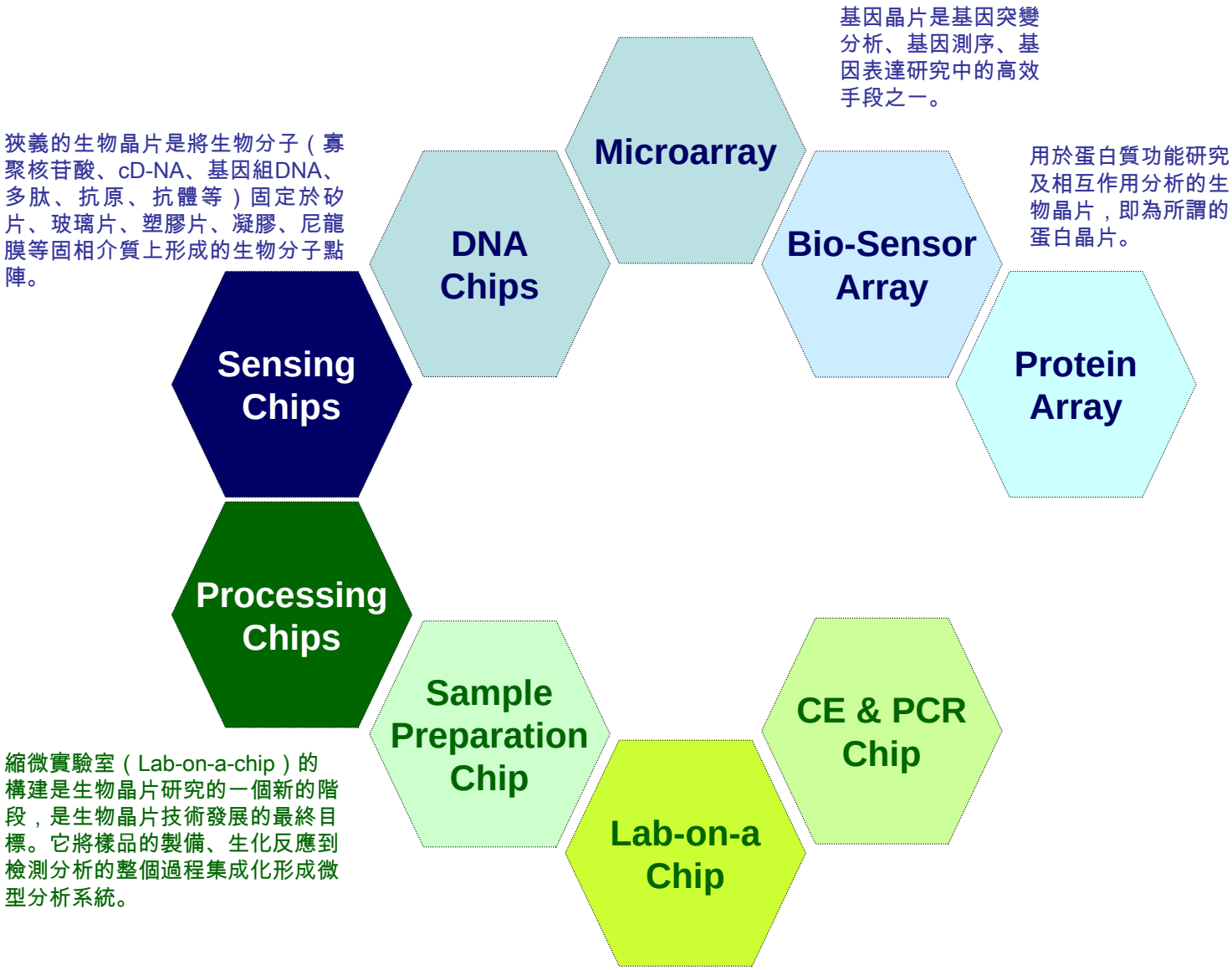
Tissue array / tissue section



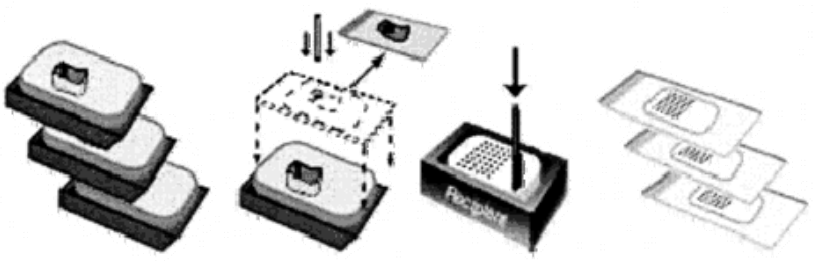
Biochip / Lab-on-a-chip



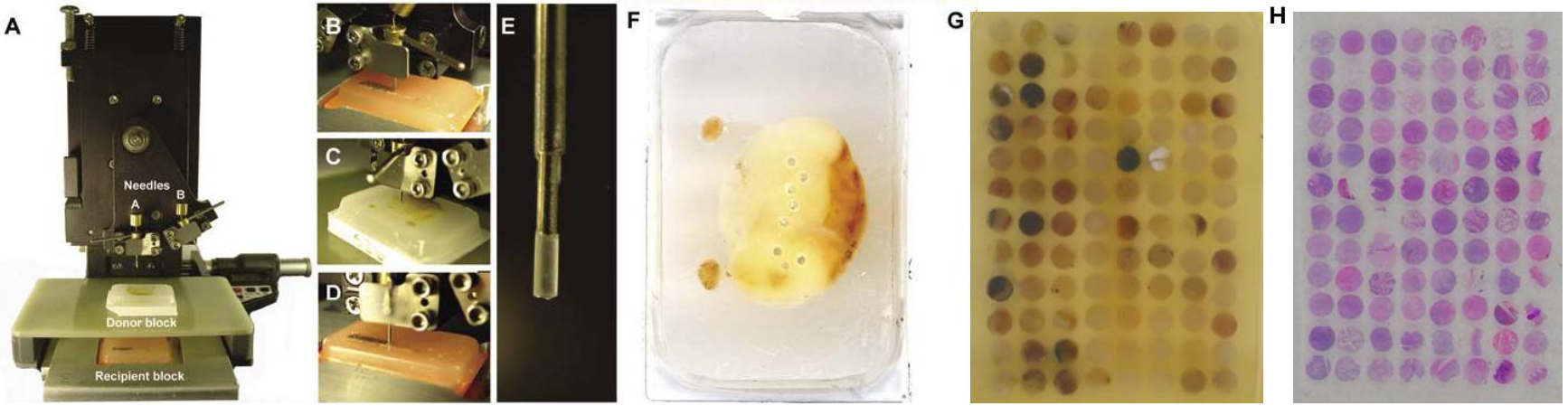
Type of Biochips



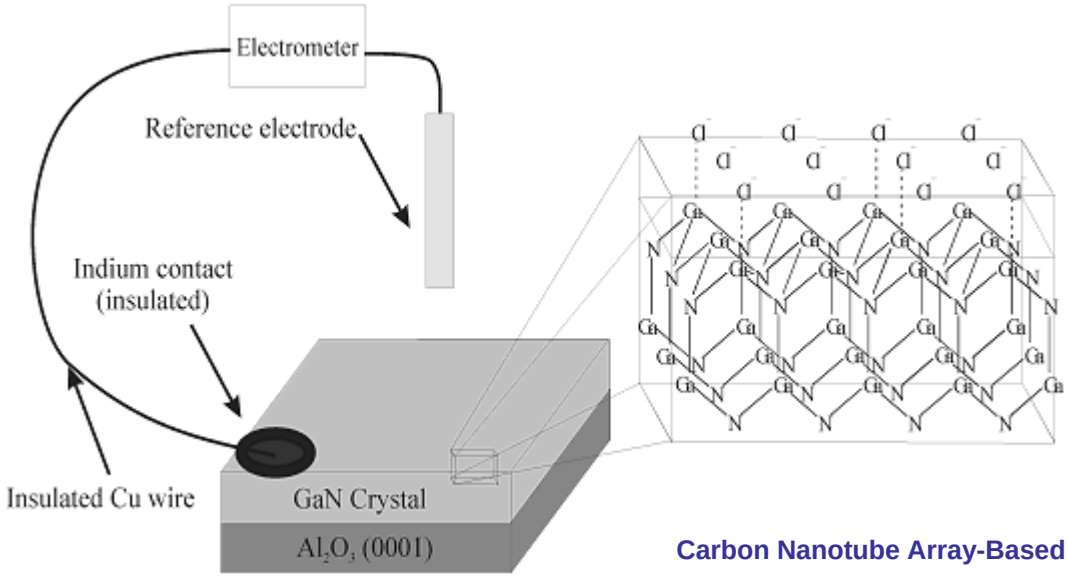
Multi Tissue Array



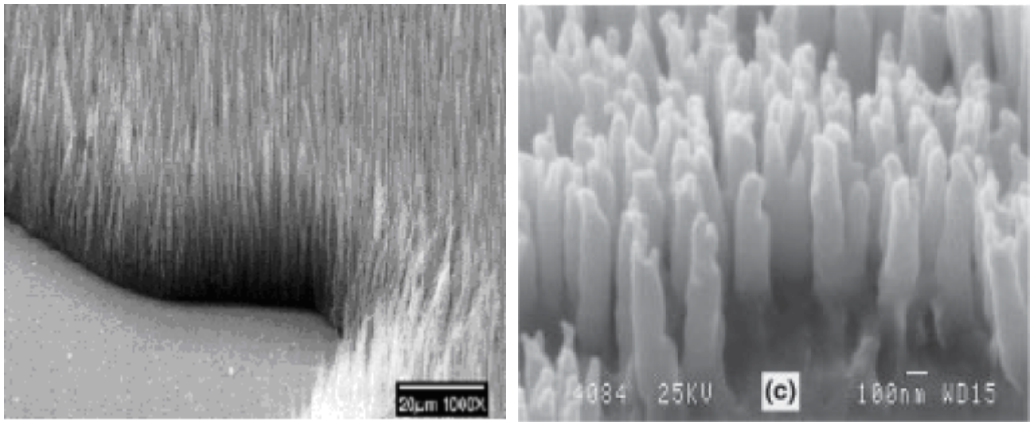
Donor blocks | Cores | Recipient block array | Multi-Tissue array



Bio-Sensor Array



Carbon Nanotube Array-Based Biosensor

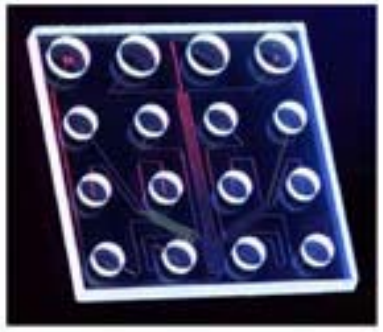


scanning electron micrograph of columnar gold nanowire structure.

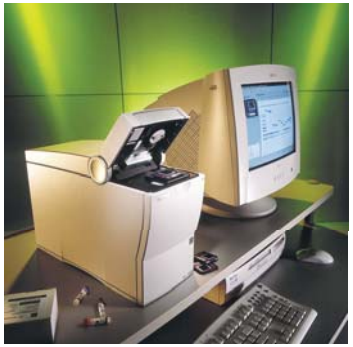
Lab-on-a Chip



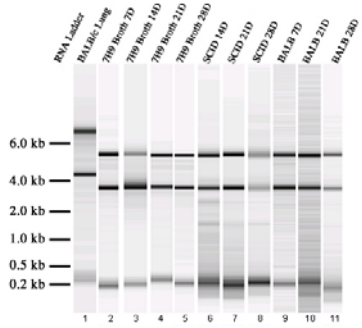
Size selection



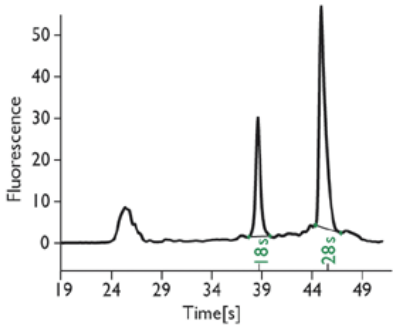
Sample loading



Sample application



Data report 1

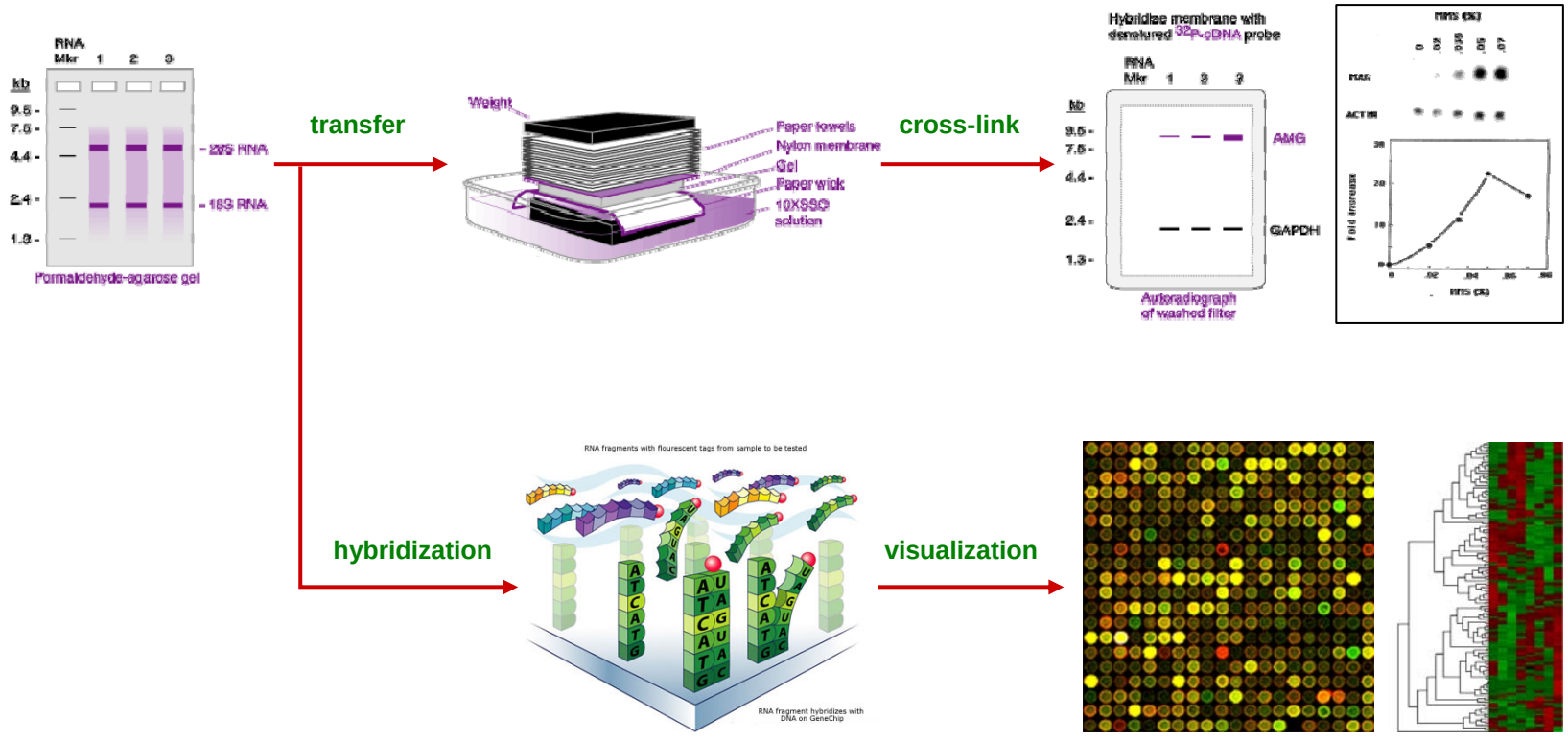


Data report 2

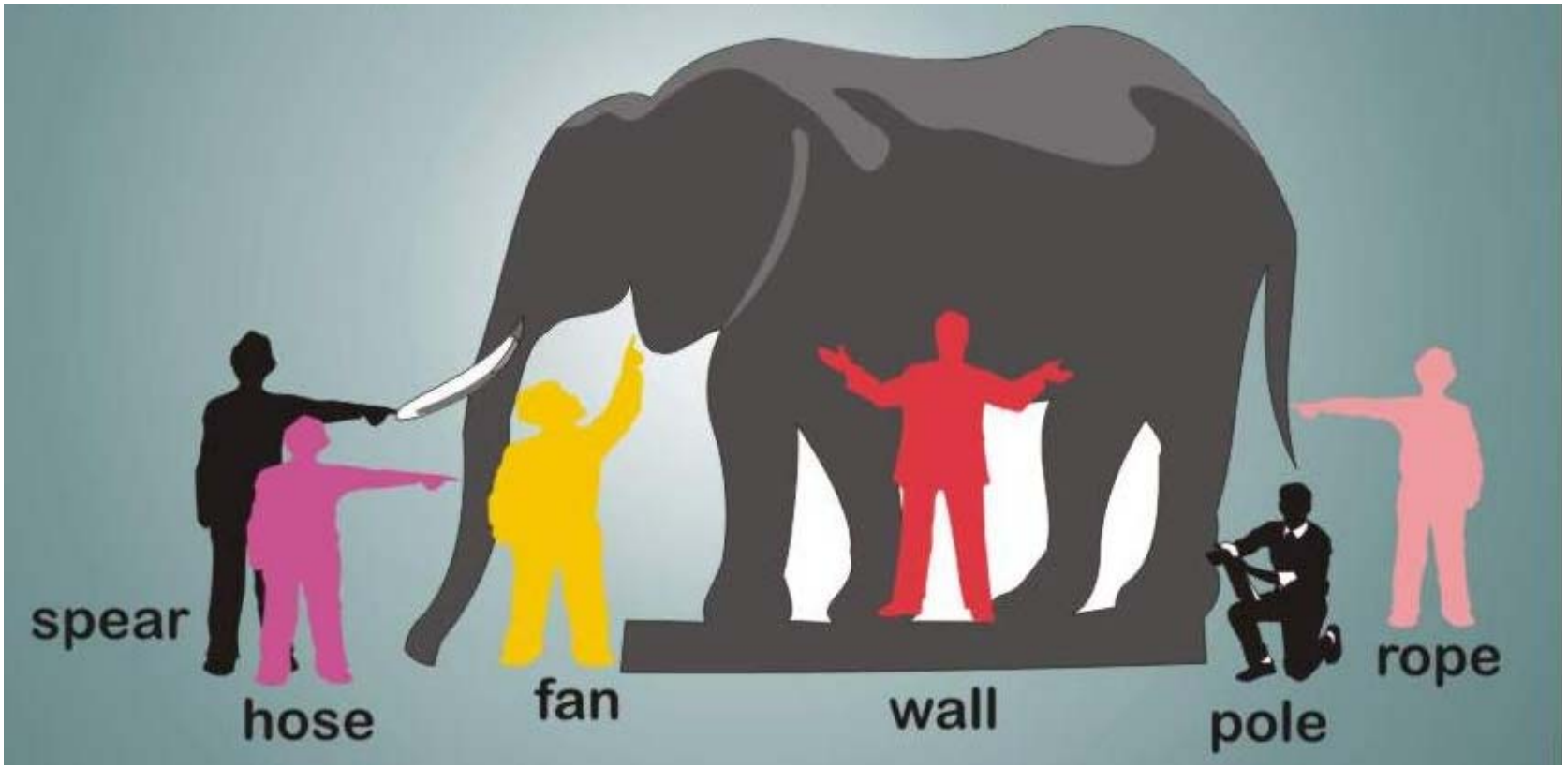
LabChip® Devices used with the Agilent 2100 Bioanalyzer

Northern blotting *versa* microarray

One gene (clone)!



We could just 35,000 Northern to monitor expression of all genes!!!



blind men study the elephant

Microarray Potential Applications

- **Biological discovery**
  - **new and better molecular diagnostics**
  - **new molecular targets for therapy**
  - **finding and refining biological pathways**
- **Recent examples**
  - **molecular diagnosis of leukemia, breast cancer, …**
  - **appropriate treatment for genetic signature**
  - **potential new drug targets**

DNA Microarray Applications

- **Human disease diagnostics and treatment**
  - determination of predisposition and risk factors especially for certain diseases
  - prediction of risk factors involved using certain treatment schemes
  - monitor disease stage and treatment progress
- **Agricultural diagnostics and development**
  - identify plant pathogens to allow suitable plant protection to be improved
  - efficiency and economy in plant biotechnology
- **Analysis of food and genetically modified organisms (GMO)**
  - determine the integrity of food
  - detect alterations and contaminations
  - quantify GMOs
- **Drug discovery and drug development**

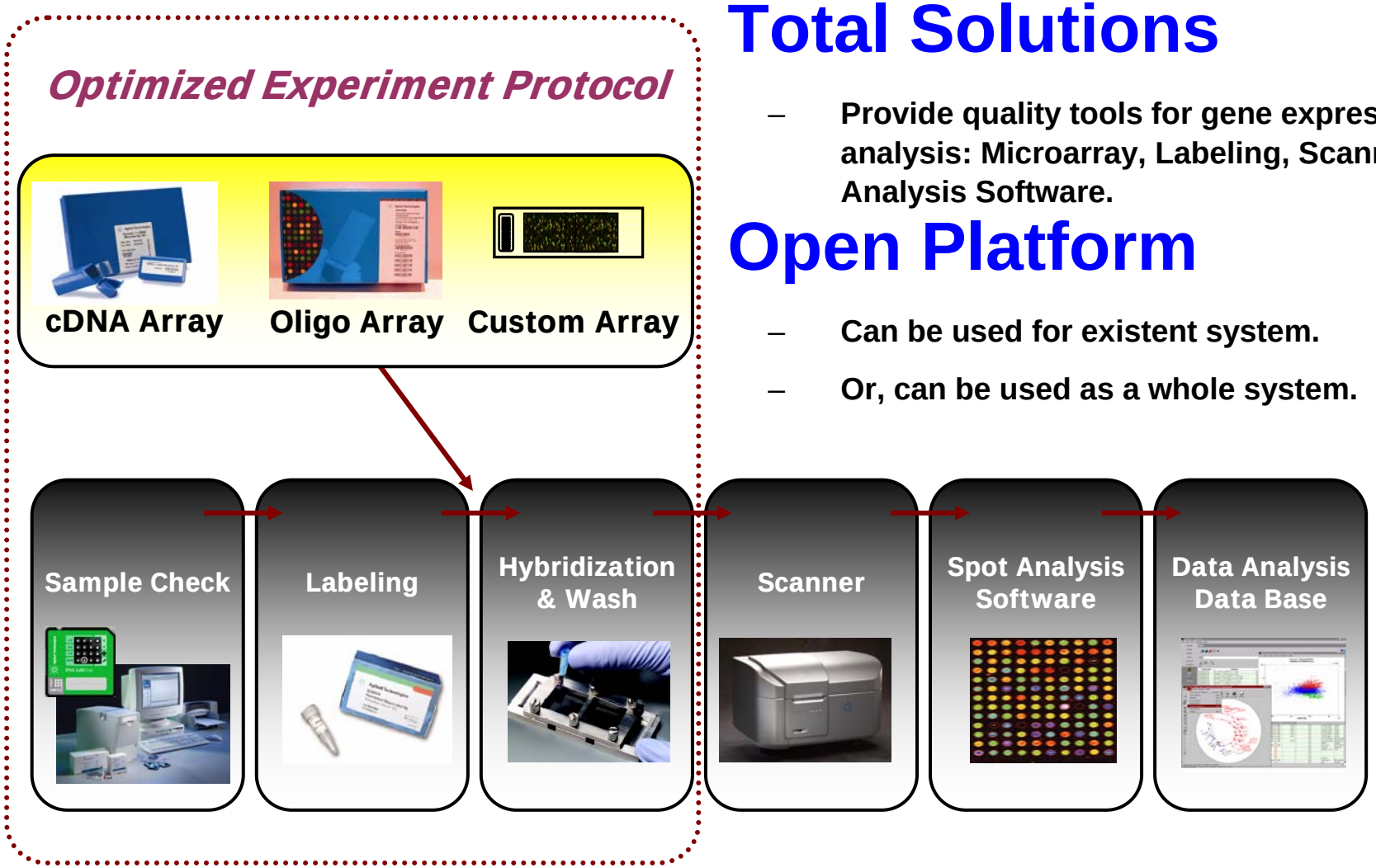
Microarray and Recent Development

- **Chip fabrications**
  - cDNA, oligonucleotide, PNA et al.,
  - Glass, membrane, other polymers
  - Spotting vs. in situ synthesis
  - 2D, 3D configurations
  - Institutional vs. industrial
- **Signals and detection system**
  - Fluorescence (spectrum analysis, quantom-dot)
  - SPR and other optical methods
  - MassSpec-based detection
- **Data analysis**
  - Instrument and protocols control, common references
  - Data consortium

Bioinformatics Issues in Microarray Experiment

1. How to manage data ?
2. Which data should be stored ?
3. Validated database or just data storage ?
4. How to integrate in-house data with public repositories ?
5. Which databases ?
6. Local mirror DBs versus web link ?
7. How to analyze and visualize data ?
8. Which analysis should be standardized ?
9. Buy versus develop ?
10. How to integrated microarray data with other omics data ?

Total Solutions for Gene Expression

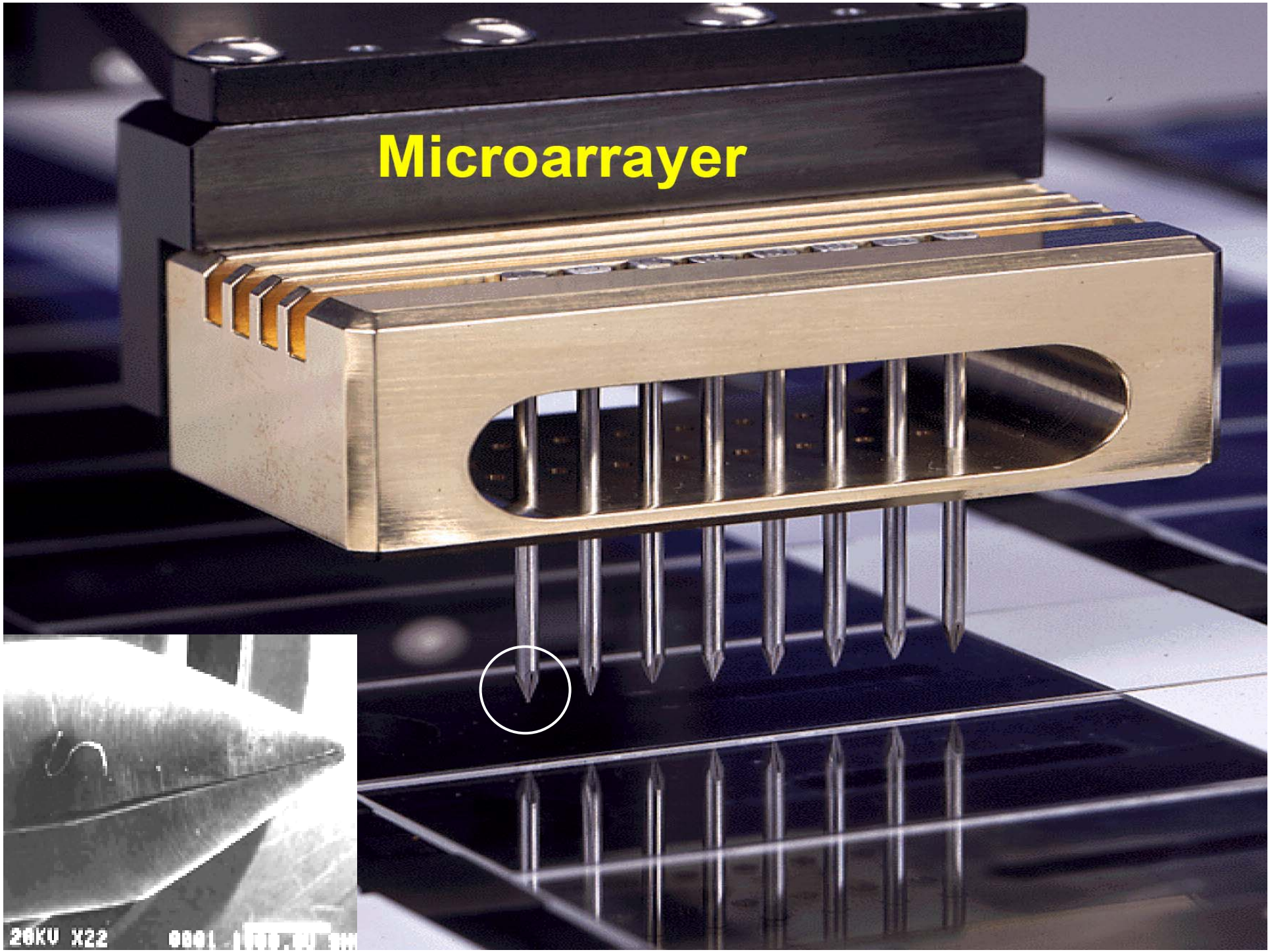


Total Solutions

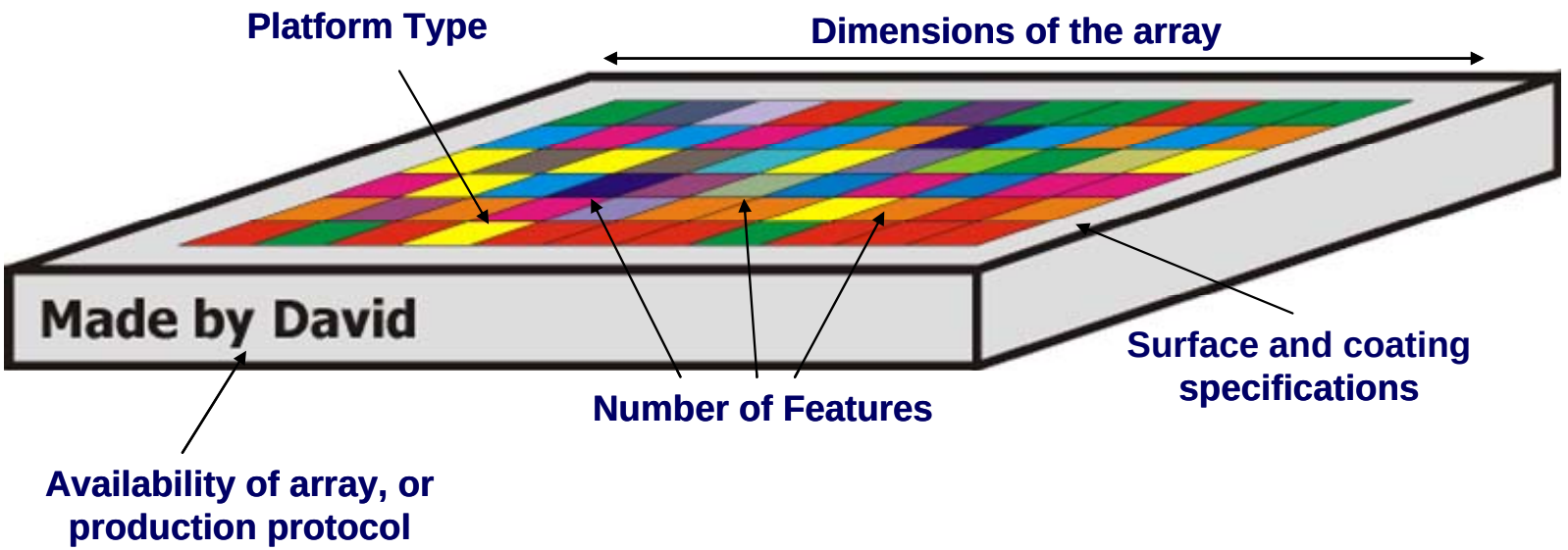
- Provide quality tools for gene expression analysis: Microarray, Labeling, Scanner, Analysis Software.

Open Platform

- Can be used for existent system.
- Or, can be used as a whole system.


Microarrayer
20KV X22

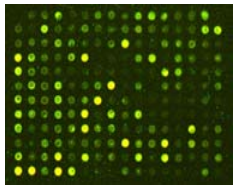
Array: Array as a whole



Unigene	Symbol	Locus	Cytoband	Accession
Hs.446504	ABL1	25	9q34.1	NM_005157
Hs.410104	ACVRL1	94	12q11-q14	NM_000020
Hs.83341	AXL	558	19q13.1	H15718
Hs.83341	AXL	558	19q13.1	H15718
Hs.748	BFGF	2260	8p11.2-p11.1	NP_000595
Hs.159494	BTK	695	xq21.33-q22	N75948
Hs.7542	BTK	283489	13q34	AI005099
Hs.77793	CSK	1445	15q23-q25	H90752
Hs.77793	CSK	1445	15q23-q25	NM_004383
Hs.440905	DDR2	4921	1q12-q23	H04823
Hs.77432	EGFR	1956	7p12	T84221
Hs.77432	EGFR	1956	7p12	T84221
Hs.89839	EPH	2041	7q34	W16661
Hs.123642	EPHA3	2042	3p11.2	H38363
Hs.73964	EPHA4	2043	2q36.1	NM_004438
Hs.73962	EPHA7	2045	6q16.1	R15219
Hs.73962	EPHA7	2045	6q16.1	AW421430
Hs.2913	EPHB3	2049	3q21-qter	BC052968
Hs.2913	EPHB3	2049	3q21-qter	AA455591
Hs.437008	EPHB4	2050	7q22	W94076
Hs.446352	ERBB2	2064	17q11.2-q12	R80150
Hs.306251	ERBB3	2065	12q13	BC002706
Hs.74137	ERBB3	10972	14q24.3	AW572218
Hs.1939	ERBB4	2066	2q33.3-q34	NM_005235
Hs.132942	FAK	23092	5q31	NP_055886
Hs.748	FGFR1	2260	8p11.2-p11.1	N99235
Hs.748	FGFR1	2260	8p11.2-p11.1	AY585209
Hs.748	FGFR1	2260	8p11.2-p11.1	N99235
Hs.404081	FGFR2	2263	10q26	AA190616
Hs.1420	FGFR3	2261	4p16.3	R84974
Hs.1420	FGFR3	2261	4p16.3	R84974
Hs.1422	FGR	2268	1p36.2-p36.1	W81586
Hs.1422	FGR	2268	1p36.2-p36.1	W81586
Hs.347713	FLT1	2321	13q12	X51602
Hs.347713	FLT1	2321	13q12	BC039007
Hs.347713	FLT1	2321	13q12	BC039007
Hs.415048	FLT4	2324	5q34-q35	NP_002011
Hs.390567	FYN	2534	6q21	R56124
Hs.390567	FYN	2534	6q21	H96234
Hs.390567	FYN	2534	6q21	R56124
Hs.169476	GAPD	2597	12p13	J04038
Hs.258561	GTF2B	2959	1p22-p21	H23978
Hs.89555	HCK	3055	20q11-q12	AA035407
Hs.118625	HK1	3098	10q22	AK095086
	HTG	695	Xq21.33-q22	Z73639
Hs.239176	IGF1R	3480	15q26.3	H13300
Hs.239176	IGF1R	3480	15q26.3	H13300
Hs.438669	INSR	3643	19p13.3-p13.2	H03917
Hs.211576	ITK	3702	5q31-q32	R00673
Hs.12337	KDR	3791	4q11-q12	NM_002253
Hs.1765	LCK	3932	1p34.3	AA040732
Hs.434481	LTK	4058	15q15.1-q21.1	BC045607
Hs.437808	MATK	4145	19p13.3	BC000114
Hs.2942	MST1R	4486	3p21.3	AA129089
Hs.2942	MST1R	4486	3p21.3	AA129089
Hs.439109	NTRK2	4915	9q22.1	AF410902
Hs.26776	NTRK3	4916	5q25	BC013693
Hs.440905	NTRKR3	4921	1q12-q23	AK095975
Hs.2853	OR5AX1	81453	1	AC026038
Hs.74615	PDGFRA	5156	4q11-q13	H02958
Hs.307783	PDGFRB	5159	5q31-q32	P09619
Hs.307783	PDGFRB	5159	5q31-q32	R56211
Hs.307783	PDGFRB	5159	5q31-q32	R56211
Hs.349845	PRKCB1	5579	16p11.2	BC036472
Hs.90572	PTK7	5754	6p21.1-p12.2	AF447176
Hs.405474	PYK2	2185	8p21.1	2119367A
Hs.2942	RON	4486	3p21.3	AA129088
Hs.1041	ROS	6098	6q22	W24195
Hs.1041	ROS1	6098	6q22	W24195
Hs.1041	ROS1	6098	6q22	W24195
Hs.356371	RPL28	6158	19q13.4	AA064618
Hs.430207	RPL29	6159	3p21.3-p21.2	R97429
Hs.469653	RPL5	6125	1p22.1	AB061848
Hs.337307	RPS27	6232	1q21	AA186639
Hs.285346	RYK	6259	3q22	S59184
Hs.75367	SLA	6503	8q22.3-qter	R52378
Hs.75367	SLA	6503	8q22.3-qter	R52378
Hs.390567	SLK	2534	6q21	M14676
Hs.436015	SRC	6714	20q12-q13	AA402622
Hs.192182	SYK	6850	9q22	AK075020
Hs.89640	TEK	7010	9p21	AB086825
Hs.89640	TEK	7010	9p21	H02848
Hs.78824	TIE	7075	1p34-p33	R60297
Hs.78824	TIE1	7075	1p34-p33	R40609
Hs.89640	TIE2	7010	9p21	W46173
Hs.203420	TNK1	8711	17p13.1	AA480582
Hs.29877	TXK	7294	4p12	R79606
Hs.75516	TYK2	7297	19p13.2	BC014243
Ssc.16085	TYK2	397363		AF100454
Hs.381282	TYRO3	7301	15q15.1-q21.1	AI090723
Hs.381282	TYRO3	7301	15q15.1-q21.1	W69497
Hs.15951	UPA	118471	10q26.3	AF421885
Hs.179526	VDUP1	10628	1q21.2	AF333001
Cfa3778	YES	403917		P07947
Hs.194148	YES1	7525	18p11.31-p11.21	R25397

Kinase gene list





slide serial number

Corning Microarray UltraGAPS™ Coated Slides

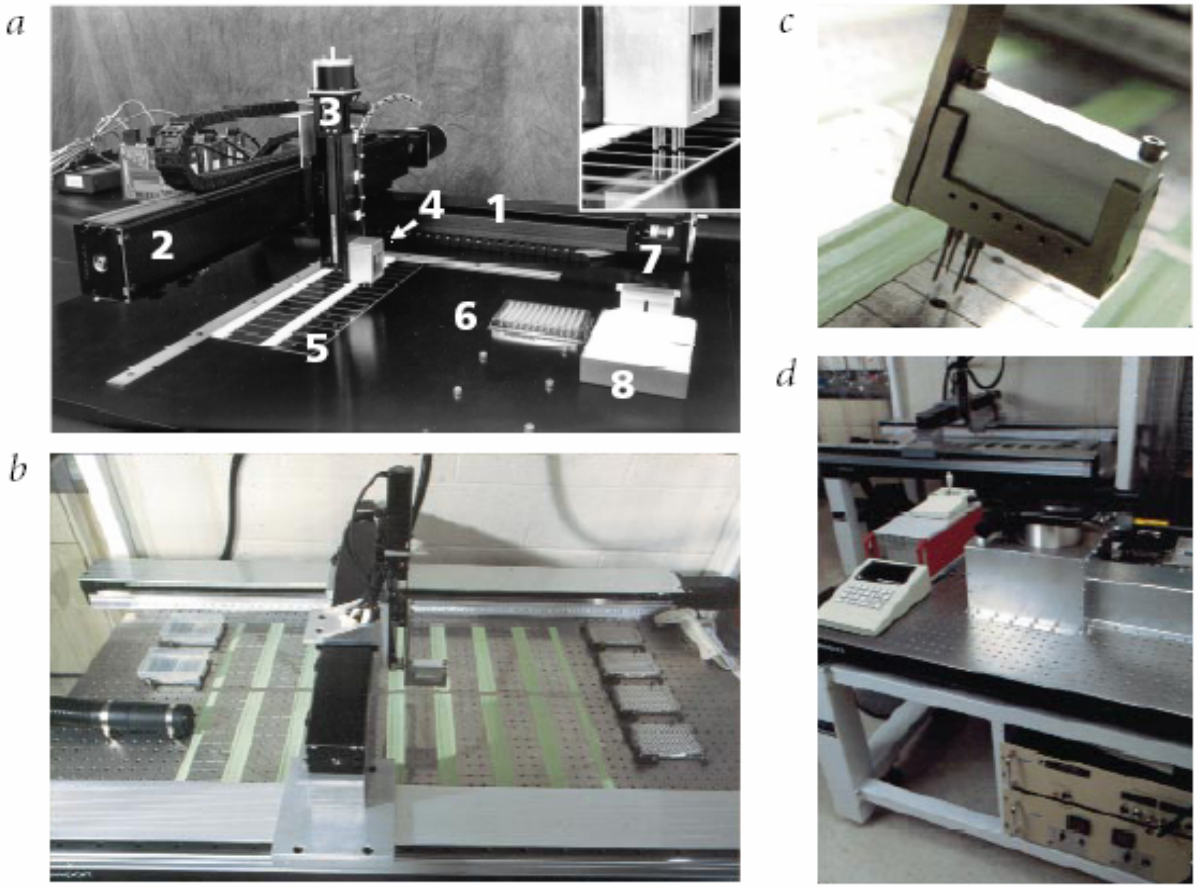
microarray chip type number

Oncogene list

Unigene	Symbol	Locus	Cytoband	Accession	Unigene	Symbol	Locus	Cytoband	Accession	Unigene	Symbol	Locus	Cytoband	Accession
Hs.446504	ABL1	25	9q34.1	NM_007313	Hs.2132	EPS8	2059	12q23-q24	AA291354	Hs.408312	P53	7157	17p13.1	AF135121
Hs.1288	ACTA1	58	1q42.13-q42.2	P02568	Hs.446352	ERBB2	2064	17q11.2-q12; 17q21.1	R80150	Hs.408312	P53	7157	17p13.1	AF135121
Hs.515706	AKIP	54998	1p36.33	NM_003600	Hs.446352	ERBB2	2064	17q21.1	NM_004448	Hs.498910	PCNA	5111	20pter-p12	P46087
Hs.106674	BAP1	8314	3p21.31-p21.2	NM_004656	Hs.45514	ERG	2078	21q22.3	BC040168	Hs.78996	PCNA	5111	20pter-p12	AF527838
Hs.79241	BCL2	596	18q21.33; 18q21.3	NM_000633	Hs.104349	FGD1	2245	xp11.21	H83405	Hs.75323	PHB	5245	17q21	I52690
Hs.380403	BMI1	648	10p11.23	NM_005180	Hs.390567	FYN	2534	6q21	H96234	Hs.151536	RAB13	5872	1q21.2	NM_002870
Hs.1274	BMP1	649	8p21	H95151.1	X1.28453	GADD153	398615		AY302440	Hs.78305	RAB2	5862	8q12.1	AF070629
Hs.79428	BNIP3	664	10q26.3	NM_004052	Hs.79000	GAP43	2596	3q13.1-q13.2	H05445	Hs.758	RASA1	5921	5q13.3	BC020761
Hs.194143	BRCA1	672	17q21	NM_007294	Hs.169476	GAPD	2597	12p13	J04038	Hs.408528	RB1	5925	3p21.31	NP_000312
Hs.34012	BRCA2	675	13q12.3	NP_000050	Hs.118625	HK1	3098	10q22	AK095086	Hs.408528	RB1	5925	3p21.31	NP_000312
Hs.159494	BTK	695	Xq21.33-q22	U01923.1	Hs.37003	HRAS	3265	11p15.5	NM_005343	Hs.406078	RBBP7	5931	Xp22.22	NM_002893
Hs.284245	C6orf204	387119	6q22	NM_206921	Hs.77695	HURP	5787	14q22.2	NM_014750	Hs.2942	RON	4486	3p21.3	NM_002447
Hs.417050	CCNA1	8900	13q12.3-q13	NM_003914	Hs.78465	JUN	3725	1p32-p31	P05412	Hs.356371	RPL28	6158	19q13.4	AA064618
Hs.417050	CCNA1	8900	13q12.3-q13	NM_003914	Hs.380850	KAL1	3730	Xp22.32	NM_000216	Hs.430207	RPL29	6159	3p21.3-p21.2	R97429
Hs.23960	CCNB1	891	5q12	AA113936	Hs.81665	KIT	3815	4q11-q12	NM_000222	Hs.469653	RPL5	6125	1p22.1	AB061848
Hs.244723	CCNE1	898	19q12	NM_001238	Hs.183428	KRAS	8082	11p11.2	AF016028	Hs.337307	RPS27	6232	1q21	AA186639
Hs.244723	CCNE1	898	19q12	NP_001229	Hs.276238	KSR	8844	17q11.2	BC042106	Hs.30868	RTN4R	65078	22q11.21	BC011787
Hs.79101	CCNG1	900	5q32-q34	R14996	Hs.155048	LU	4059	19q13.2	P50895	Hs.50984	SAS	6302	12q13.3	S78432
Hs.347990	CD34	947	1q32	BC039146	Hs.75862	MADH4	4089	18q21.1	BC002379	Hs.55279	SERPINB5	5268	18q21.3	W30956
Hs.355832	CDC42	998	1p36.1	AL121734	Hs.153863	MADH6	4091	15q21-q22	AF035528	Hs.433795	SHC	6464	1q21	BC014158
Rn.103750	CFOS	314322	6q31	AF126534	Hs.212217	MDM2	4193	12q14.3-q15	H13638	Hs.421342	STAT3	6774	17q21.31	BC014482
Hs.202453	CMYC	4609	8q24.12-q24.13	R43857	Hs.419124	MET	4233	7q31	NM_000245	Hs.442707	TERF1	7013	8q13	NP_003209
Hs.183650	CRABP2	1382	1q21.3	H44233	Hs.419124	MET	4233	7q31	NM_000245	Hs.174312	TLR4	7099	9q32	AB020878
Hs.173894	CSF1	1435	1p21-p13	M11038	Hs.433618	MLH1	4292	3p21.3	NM_000249	Hs.116237	VAV1	7409	19p13.2	AI369096
Hs.172562	DCC	1630	18q21.5	P43146	Hs.279843	MLH3	27030	14q24.3	L40399	Hs.116237	VAV1	7409	19p13.2	AI369096
Hs.77091	DNASE1L1	1774	xq28	P49184	Hs.153837	MNDA	4332	1q22	R77431	Hs.436803	VBP1	7411	Xq28	NM_003372
Hs.5120	DNCL1	8655	12q24.23	R18848	Hs.407830	MYB	4602	6q22-q23	NM_005375	Hs.179526	VDUP1	10628	1q21.2	AF333001
Hs.79306	EIF4E	1977	4q21-q25	BC012611	Hs.81687	NME3	4832	16q13	AA129463	Hs.421597	VHL	7428	3p26-p25	NM_198156
Hs.181128	EIK1	2002	xq11.2	W40449	Hs.25960	NMYC	4613	2p24.1	P04198	Hs.512715	WRN	7486	8p12-p11.2	AA287923
Hs.356835	EMP2	2013	16p13.2	NM_001424	Hs.25960	NMYC	4613	2 p24.1	BC002712	Hs.78919	XK	7504	xp21.1	H18932
Hs.356835	EMP2	2013	16p13.2	NM_001424	Hs.260523	NRAS	4893	1p13.2	NM_002524	Hs.98493	XRCC1	7515	19q13.2	AC018758

How do we manufacture a microarray ?

Fig. 1 ***a,*** Penn microarray robot. The X-, Y-, Z- axes are labesled 1, 2, and 3, respectively. The key component of the arrayer is the print-head, containing pens (4). Microscope glass slides are placed on the slide station (5). Samples are prepared and arrayed from 96-well sample plates (6). The pins are cleaned between sample acquisitions at the washing (7) and drying (8) stations. ***b,*** AECOM microarray robot. The table configuration shown contains 160 slides with four microtitre plates, two wash stations and the dryer. The print-head (*c*) shows four of the possible twelve pen tips in use. ***d,*** AECOM laser scanner. Visible are the optical table, power supplies for lasers and PMT cooling, the Ludl stage, and lasers. The 20× microscope objective is inside the ludl stage while lenses, mirrors and other optics are enclosed in the metal casing. PMTs are to the right and outside the photo.



Microarray Scale

Customized Scale Inkjet Printer

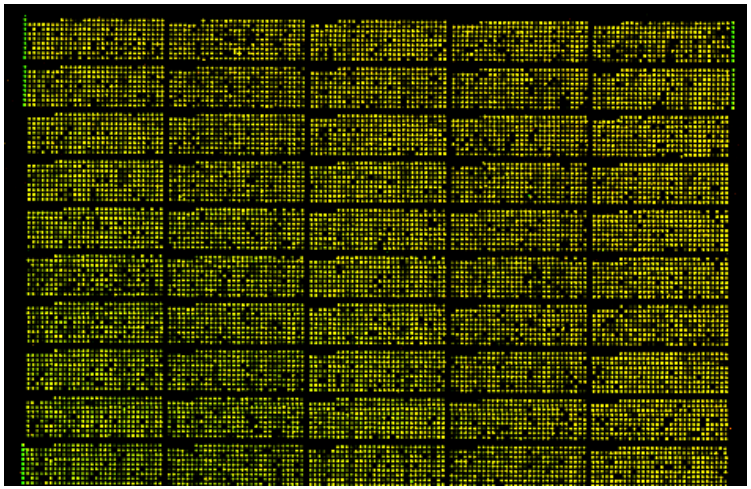


Industrial Scale Inkjet Printer



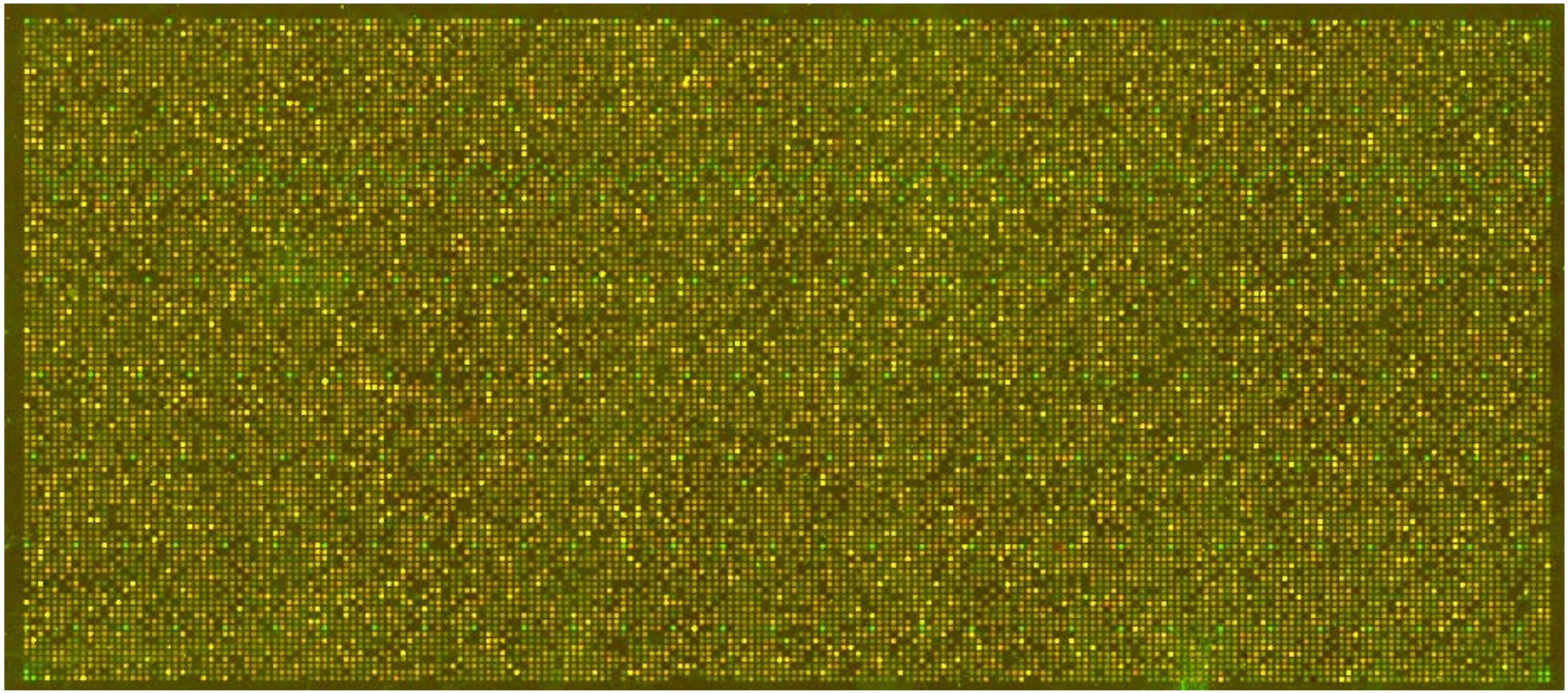
Array Quality Control





Process	QC Test
Glass	Test 1. Flatness / thickness 2. Low fluorescence
Substrate	Batch Test 1. Surface Integrity 2. Attachment Chemistry
Array	Writer QC 1. Vision System 2. Sensitivity 3. Specificity 4. Microarray Uniformity
Quarantine	Array Lot QC 1. Synthesis 2. Functional Test 3. Defects
Package	

In Situ Microarray: Microarrays & Formats



***In situ* Oligonucleotide - 60 mer**

Array Contents 1:
Agilent Catalog Oligo DNA Microarray

Human1A V2 Oligo DNA Microarray G4110B



- **Human gene: 17,803**
- **22K array format**

60mer oligo probe design: Based on full-length cDNA of Incyte Human Foundation clone set
Annotation: Description based on Incyte LifeSeq Foundation.

Human1B Oligo DNA Microarray G4111A



- **Over 20K genes and transcripts.**
- **22K array format**

60mer oligo probe design: Based on Incyte LifeSeq Foundation.
Less well annotated genes/transcripts.

Yeast Oligo Microarray G4120A



- **Yeast gene: 6,256**
- **11K array format**

60mer oligo probe design: Based on Saccharomayces Genome Database (SGD) May 25, 2002 ORF
Annotation: 100% based on Public DB.

Array Contents 2:
Agilent Catalog Oligo DNA Microarray

Rat Oligo DNA Microarray G4130A



- **Rat gene: 20K**
- **22K array format**

60mer oligo probe design: Mainly based on public Database (RefSeq, GB). Colaboration with Amgen, Incyte, Paradigm and NIEHS. Includes toxio marker genes.
Annotation: Public DB or Incyte ZooSeq annotation.

Mouse Development Oligo Microarray G4120A



- **Mouse gene: 20,371**
- **22K array format**

60mer oligo probe design: Based on NIA Ko's cDNA clone set, focused on development stage.
Annotation: >99% based on Public DB.

Mouse Oligo Microarray G4121A



- **Over 20K of well characterized genes and transcripts.**
- **22K array format**

60mer oligo probe design: from Public DB (including 9,836 RefSeq). Toxicology markers from NIEHS, TRC, and Paradigm.

Whole Mouse Oligo Microarray G4122A



- **Over 40K of well characterized genes and transcripts.**
- **44K array format**

60mer oligo probe design : from Public DB. Toxicology markers from NIEHS, TRC, and Paradigm.

Array Contents 3:
Agilent Catalog Oligo DNA Microarray

Magnaporthe Oligo Microarray G4137A



- Rice blast disease gene: 13,600
- Rice gene: 7,100
- 22K array format

Based on International Rice Blast Disease Genome consortium data.
97% Magnaporthe genome coverage.

Arabidopsis2 Oligo Microarray G4136A



- Arab genes: 21,500
- 22K array format

14,400 genes from Arabidopsis 1 + additional 7,000 genes.

Rice Oligo DNA Microarray G4138A



- Rice gene: 21,500
- 22K array format

60mer oligo probe design & Annotation :
Japan - National Institute of Agrobiological Sciences, Dr. Shoshi Kikuchi

Microarray Analysis Strategy

- **Microarray data processing**
  - Normalization
  - Visualization
  - QA/QC
- **Data analysis**
  - Contrast comparison
  - Series experiment
- **Database**
  - Storage and maintenance
  - Data integration and transform
  - User friendly interface
- **Data mining and function prediction**
  - Gene annotation
  - Gene function prediction

Journals are Adopting the MGED Standards



nature

26 September 2002 Volume 419 Issue no 6905

Microarray standards at last

Not a moment too soon, the microarray community has issued guidelines that will make their data much more useful and accessible. *Nature* and the Nature research journals will respond accordingly.

Use of Minimal Information About Microarray Experiment (MIAME)

MIAME Checklist

Experiment Design:

- Type of experiment: for example, is it a comparison of normal vs. diseased tissue, a time course, or is it designed to study the effects of a gene knock-out?
- Experimental factors: the parameters or conditions tested, such as time, dose, or genetic variation.
- The number of hybridizations performed in the experiment.
- The type of reference used for the hybridizations, if any.
- Hybridization design: if applicable, a description of the comparisons made in each hybridization, whether to a standard reference sample, or between experimental samples. An accompanying diagram or table may be useful.
- Quality control steps taken: for example, replicates or dye swaps.
- URL of any supplemental websites or database accession numbers

Samples used, extract preparation and labeling:

- The origin of the biological sample (for instance, name of the organism, the provider of the sample) and its characteristics: for example, gender, age, developmental stage, strain, or disease state.
- Manipulation of biological samples and protocols used: for example, growth conditions, treatments, separation techniques.
- Protocol for preparing the hybridization extract: for example, the RNA or DNA extraction and purification protocol.
- Labeling protocol(s).
- External controls (spikes).

Hybridization procedures and parameters:

- The protocol and conditions used during hybridization, blocking and washing.

Measurement data and specifications:

- The quantitations based on the images.
- The set of quantitations from several arrays upon which the authors base their conclusions. While access to images of raw data is not required (although its value is unquestionable), authors should make every effort to provide

Available from:
http://www.mged.org/Workgroups/MIAME/miame_checklist.html

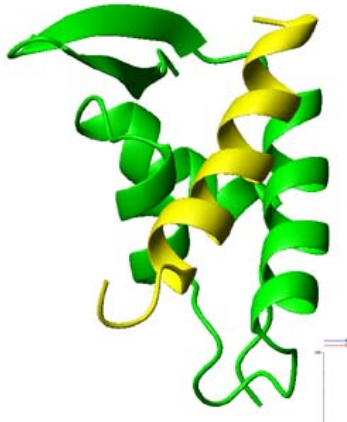
Microarray Data Analysis Types

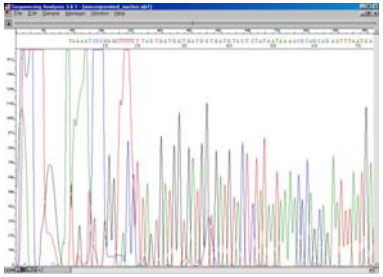
- **Gene Selection**
  - find genes for therapeutic targets
  - avoid false positives (FDA approval ?)
- **Classification (Supervised)**
  - identify disease
  - predict outcome / select best treatment
- **Clustering (Unsupervised)**
  - find new biological classes / refine existing ones
  - exploration
- ...

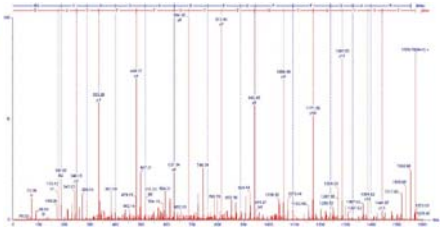


Taxonomic Classification

Kingdom Animalia
Phylum Chordata
Class Mammalia
Order Primate
Family Hominidae
Genus *Homo*
Species *sapien*



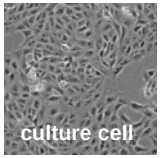




Molecule Identification

TITLE	BIOCARTA
UNIGENE	KEGG
SYMBOL	GO
LOCUSID	MOTIF
CYTOBAND	HOMOLOG
OMIM	ALIAS
SEQUENCE	MGC_CLONE
	SNP
	SV_CLONE

Microarray experiment procedure

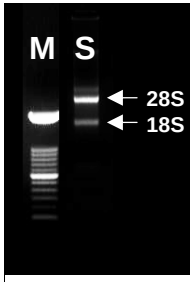






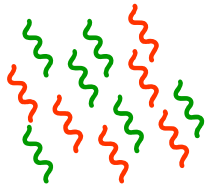


RNA extraction





Quality and
Quantity check



RT labeling
Cy3 and Cy5

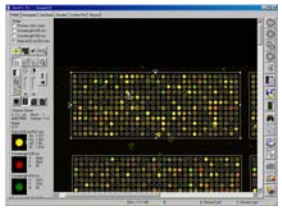


hybridization





Scanning



Raw data

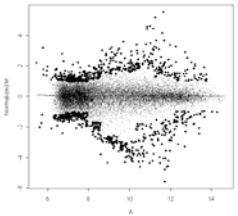


NCKUMC_chip4.gal
gene ANNOTATION



20041010_boss.gpr
image RESULT

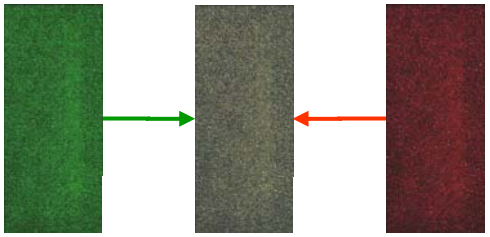




Normalization
Data process

Cy3 image

Cy5 image



Merged image

Image process



Chip report



Experiment report

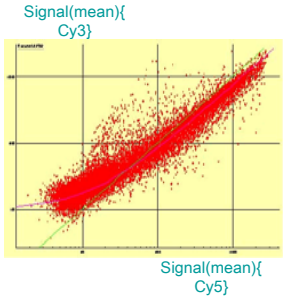


Further analysis

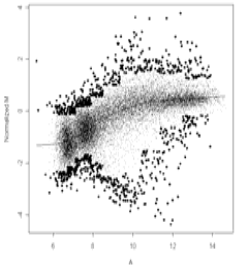
Microarray data process flowchart



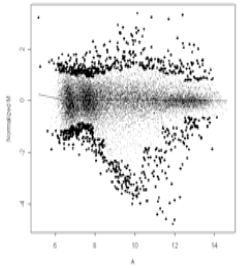
Microarray



Unit intensity



Raw data



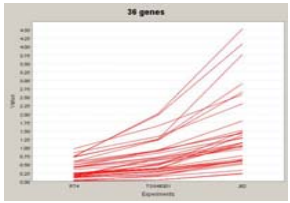
Normalized data



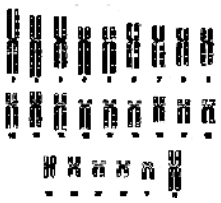
Data, data, and data … ….



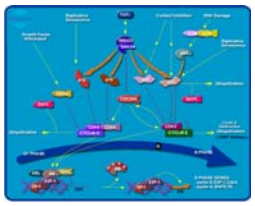
Data classifier



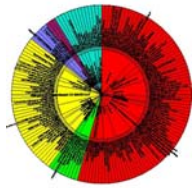
Expression



Cytoband

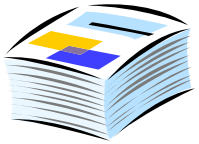


Pathway



GO term

Gene list



Candidate gene

- Unified the database query and storage system
- Consummated the text mining system
- Standardized microarray hybridization protocol
- Consummated the normalization method
- Gene Ontology term alignment system
- Visualization protein-protein interaction
- Renew GeneFilter™ version
- Core laboratory service
- Gene expression analysis interface build up













Clustering and filtering methods

***Principal approaches*:**

- **Hierarchical clustering (kdb trees, CART, gene shaving)**
- **K-means clustering**
- **Self organizing (Kohonen) maps**
- **Vector support machines**
- **Gene Filtering via Multiobjective Optimization**
- **Independent Component Analysis (ICA)**

***Validation approaches*:**

- **Significance analysis of microarrays (SAM)**
- **Bootstrapping cluster analysis**
- **Leave-one-out cross-validation**
- **Replication (additional gene chip experiments, quantitative PCR)**

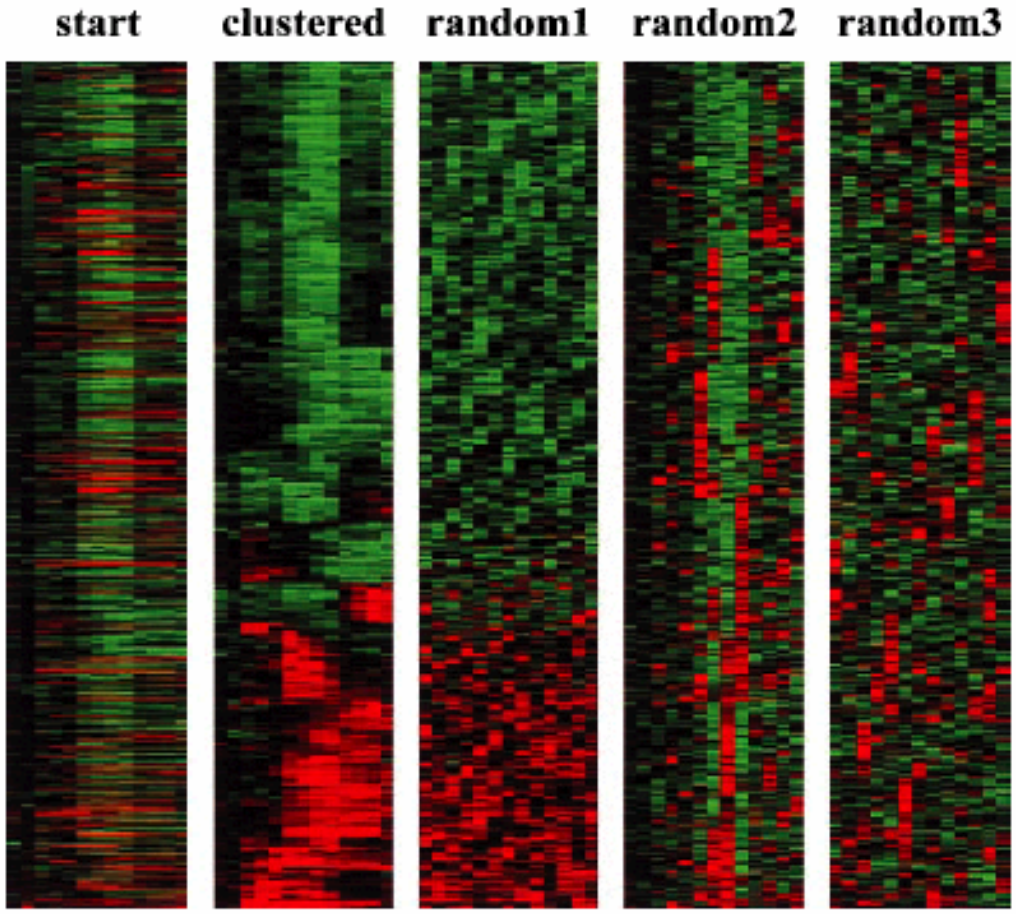
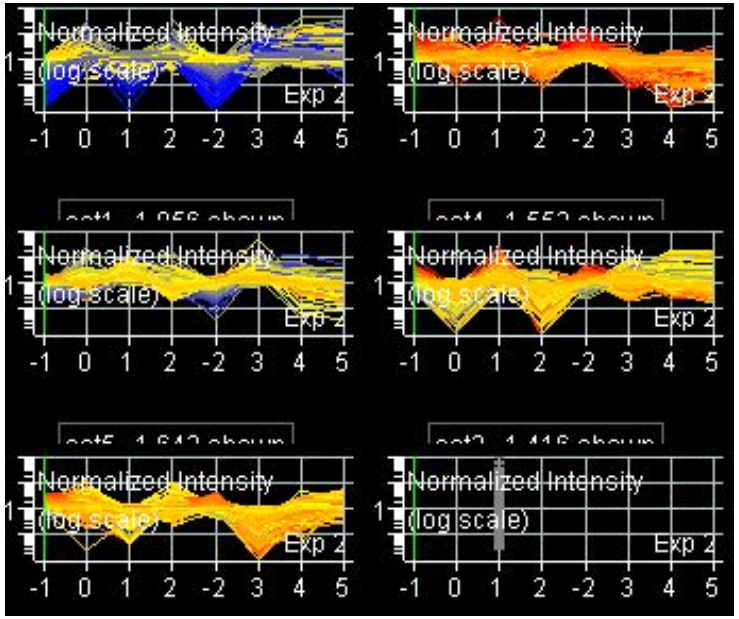
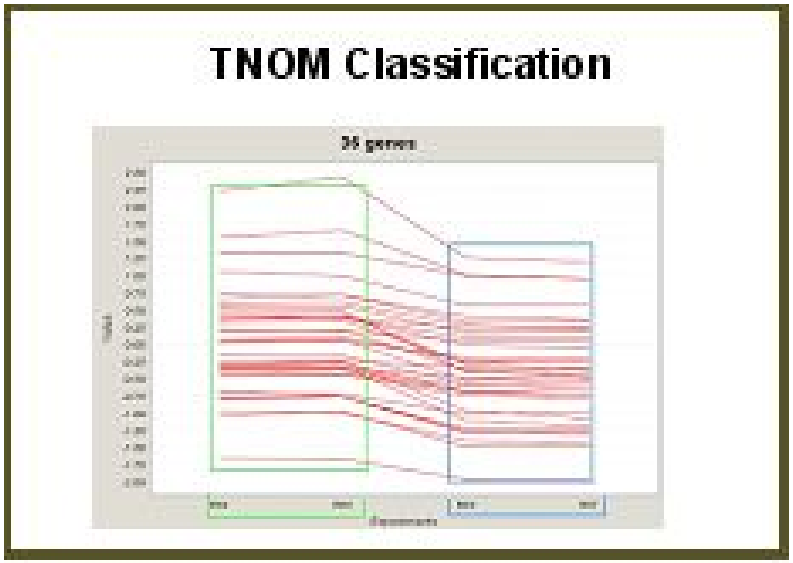


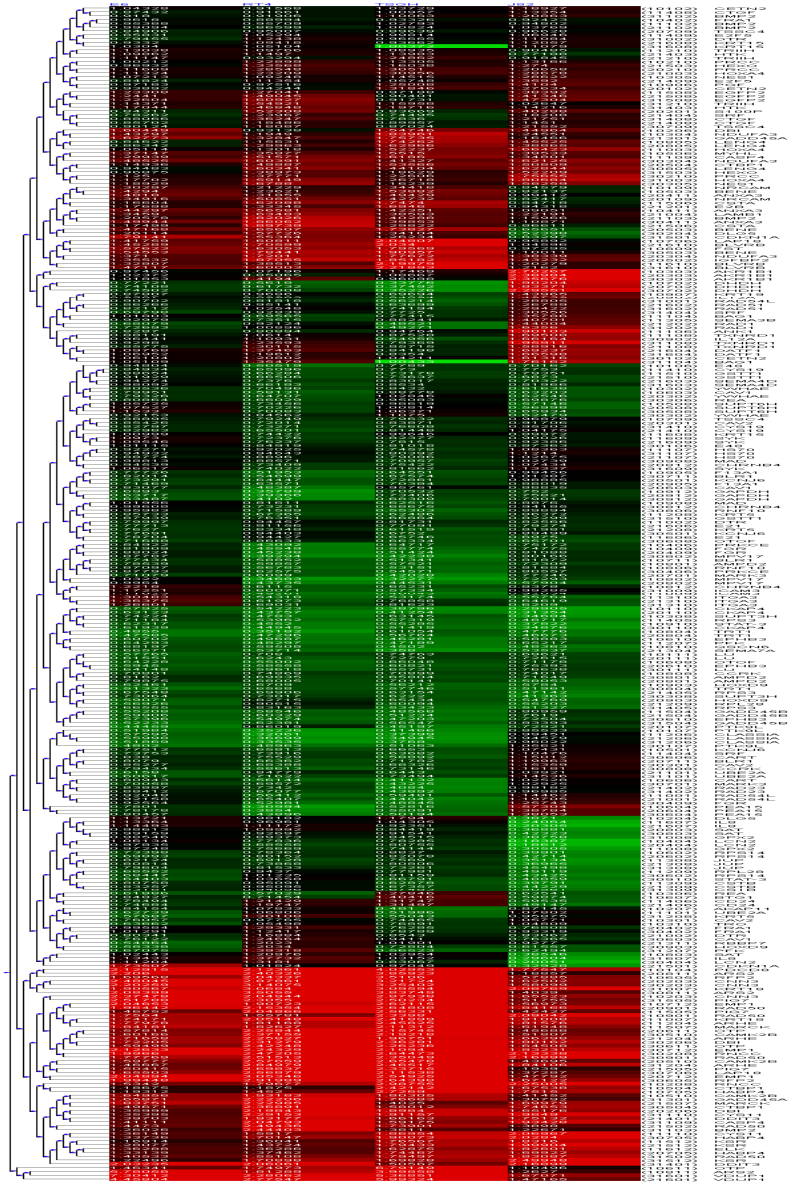
FIG. 3. To demonstrate the biological origins of patterns seen in Figs. 1 and 2, data from Fig. 1 were clustered by using methods described here before and after random permutation within rows (random 1), within columns (random 2), and both (random 3).

Eisen et al.
Proc. Natl. Acad. Sci. USA 95 (1998)

Clustering Analysis







Clustering: follow-up

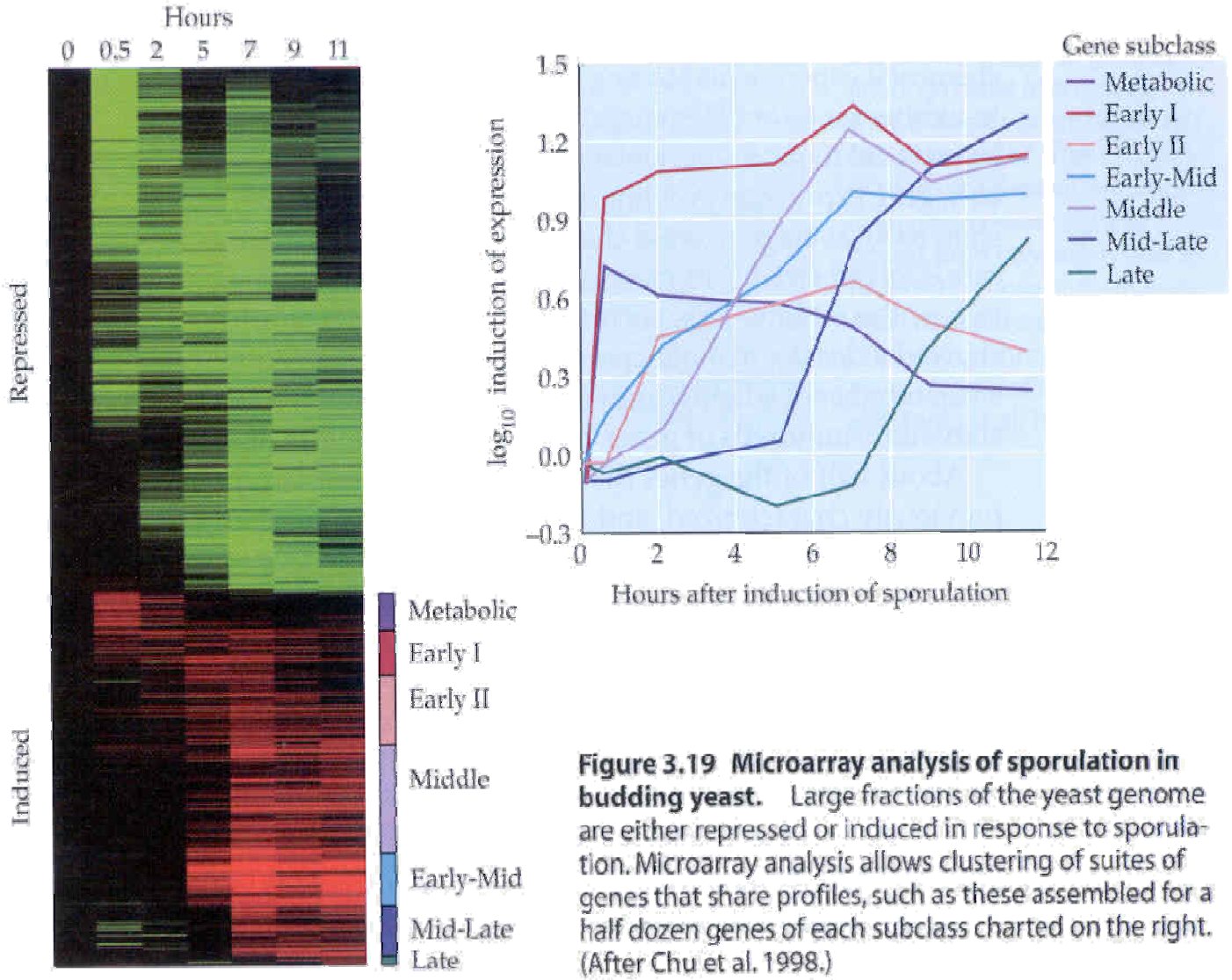
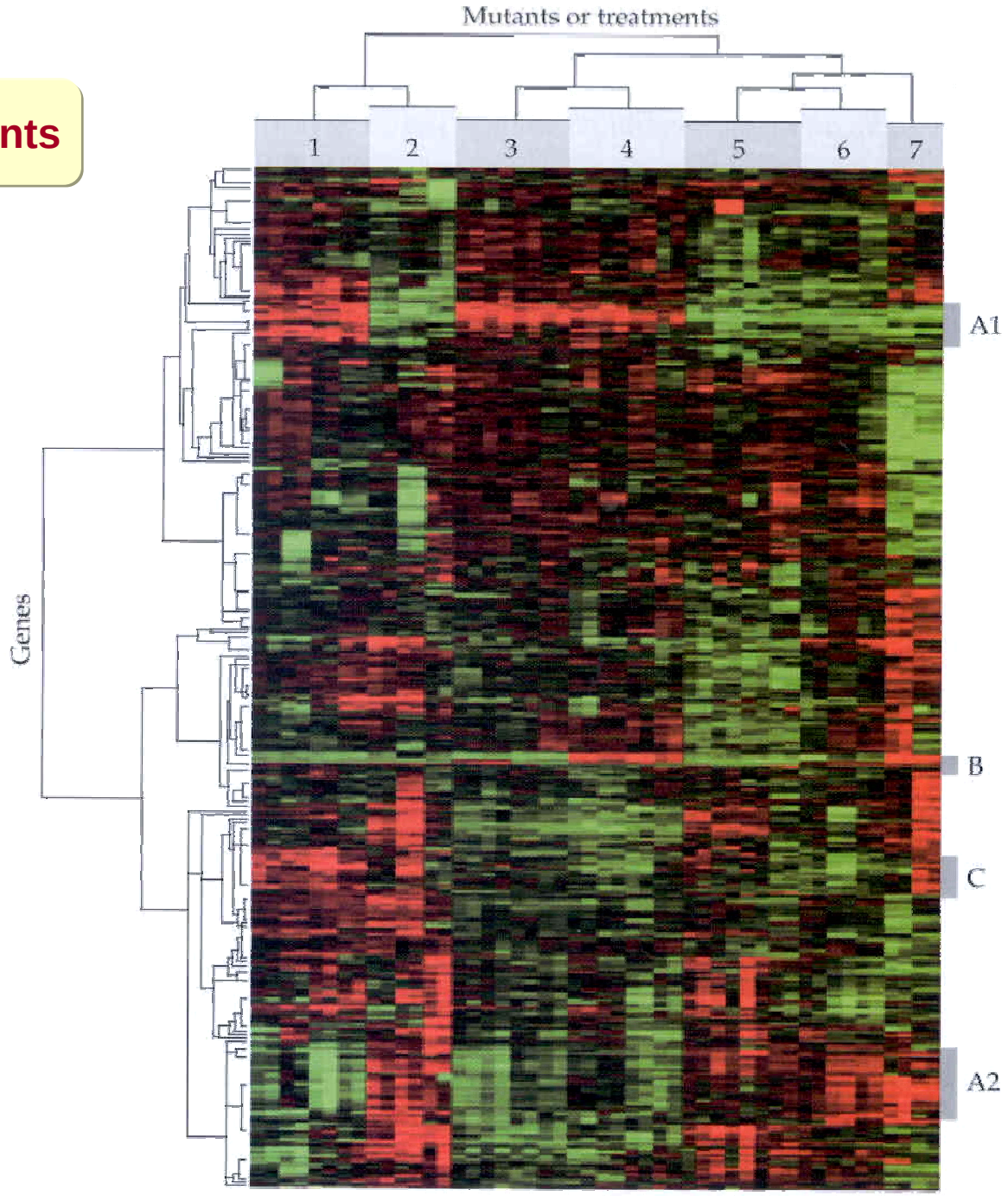
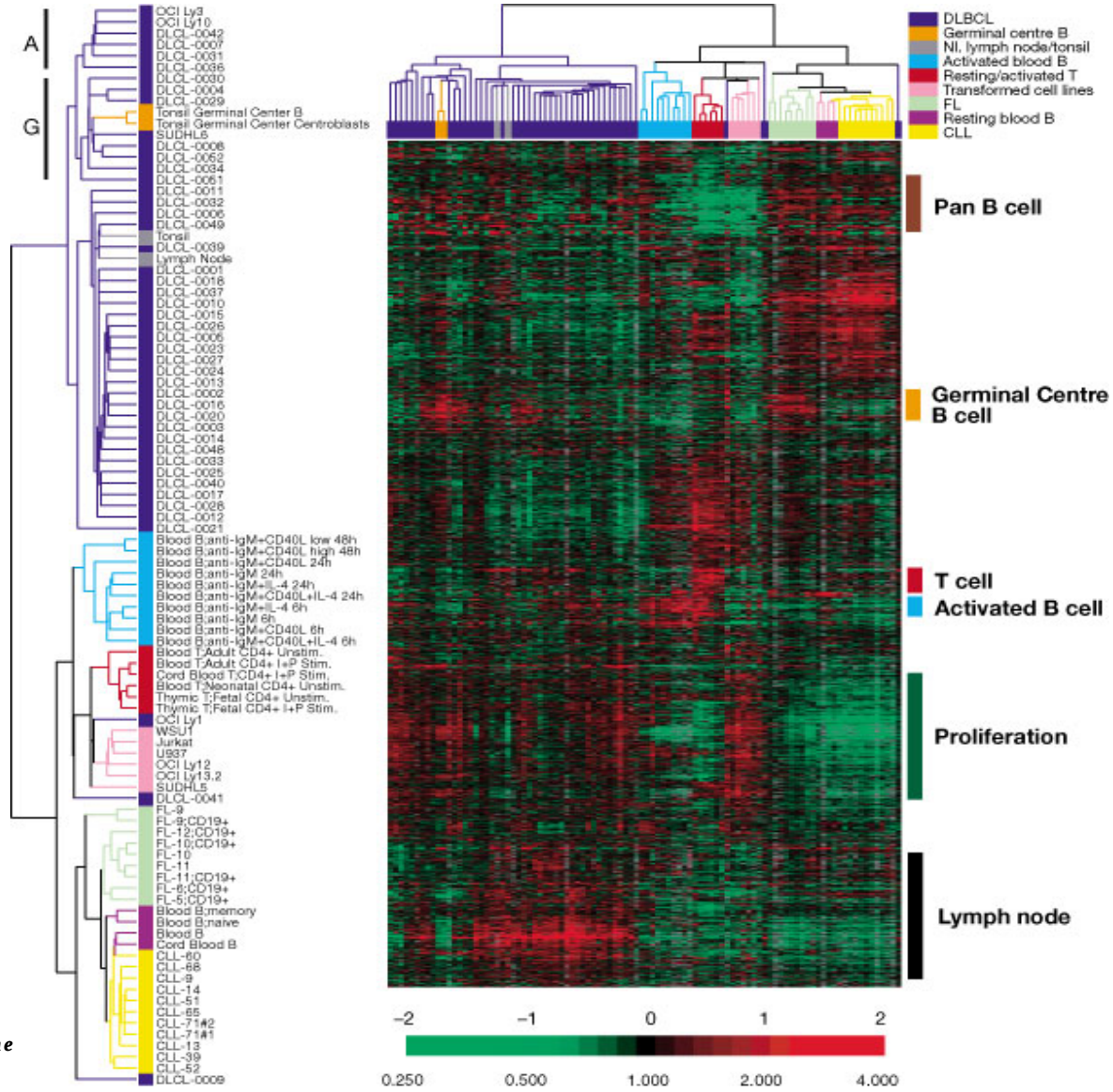


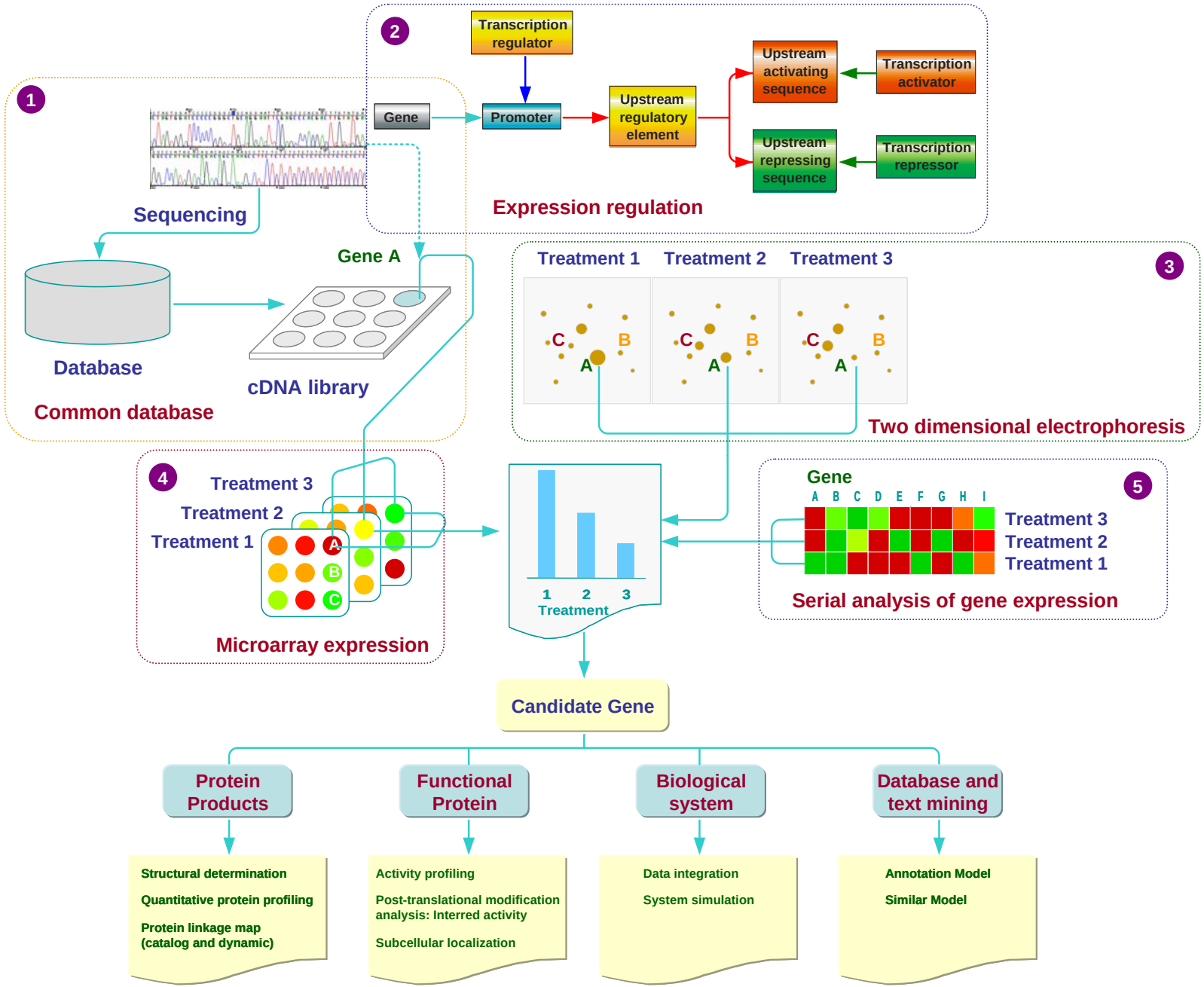
Figure 3.19 Microarray analysis of sporulation in budding yeast. Large fractions of the yeast genome are either repressed or induced in response to sporulation. Microarray analysis allows clustering of suites of genes that share profiles, such as these assembled for a half dozen genes of each subclass charted on the right. (After Chu et al. 1998.)

Clustering: treatments

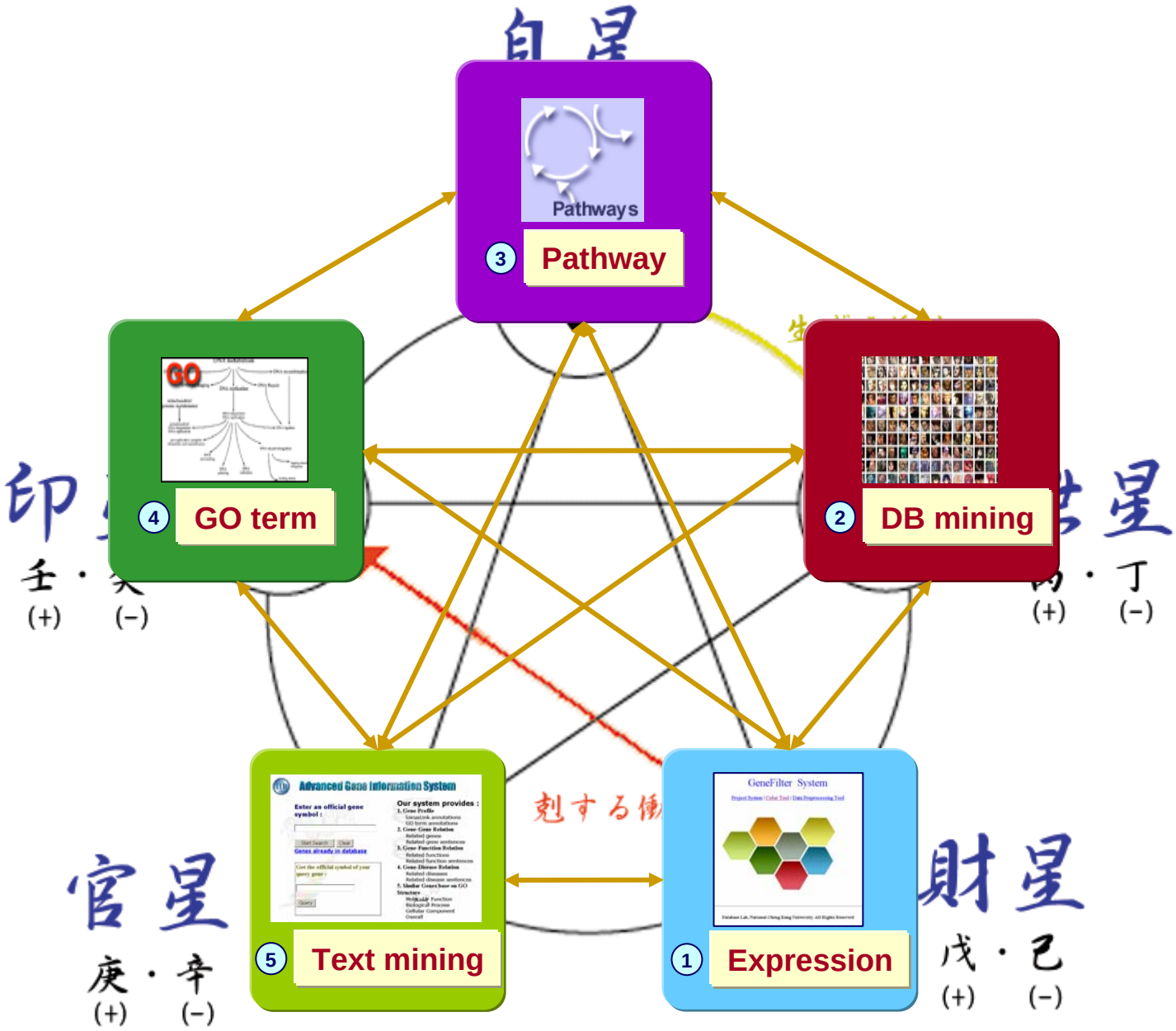




Nature Feb, 2000
Paper by Allzadeh. A *et al*
Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling


1
2
3
4
5
Transcription regulator
Promoter
Gene
Upstream regulatory element
Upstream activating sequence
Transcription activator
Upstream repressing sequence
Transcription repressor
Expression regulation
Sequencing
Gene A
Database
cDNA library
Common database
Treatment 1
Treatment 2
Treatment 3
C
B
A
Two dimensional electrophoresis
Treatment 3
Treatment 2
Treatment 1
Microarray expression
1 2 3
Treatment
Gene
A B C D E F G H I
Treatment 3
Treatment 2
Treatment 1
Serial analysis of gene expression
Candidate Gene
Protein Products
Functional Protein
Biological system
Database and text mining
Structural determination
Quantitative protein profiling
Protein linkage map (catalog and dynamic)
Activity profiling
Post-translational modification analysis: Interred activity
Subcellular localization
Data integration
System simulation
Annotation Model
Similar Model


實戰
THE
FIFTH ELEMENT


Pathways
3 Pathway
GO
4 GO term
2 DB mining
Advanced Gene Information System
5 Text mining
GeneFilter System
1 Expression

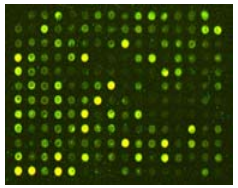

Gene Expression Base Analysis

NCKUMC Chip IV

Kinase gene list

Unigene	Symbol	Locus	Cytoband	Accession
Hs.446504	ABL1	25	9q34.1	NM_005157
Hs.410104	ACVRL1	94	12q11-q14	NM_000020
Hs.83341	AXL	558	19q13.1	H15718
Hs.83341	AXL	558	19q13.1	H15718
Hs.748	BFGF	2260	8p11.2-p11.1	NP_000595
Hs.159494	BTK	695	xq21.33-q22	N75948
Hs.7542	BTK	283489	13q34	AI005099
Hs.77793	CSK	1445	15q23-q25	H90752
Hs.77793	CSK	1445	15q23-q25	NM_004383
Hs.440905	DDR2	4921	1q12-q23	H04823
Hs.77432	EGFR	1956	7p12	T84221
Hs.77432	EGFR	1956	7p12	T84221
Hs.89839	EPH	2041	7q34	W16661
Hs.123642	EPHA3	2042	3p11.2	H38363
Hs.73964	EPHA4	2043	2q36.1	NM_004438
Hs.73962	EPHA7	2045	6q16.1	R15219
Hs.73962	EPHA7	2045	6q16.1	AW421430
Hs.2913	EPHB3	2049	3q21-qter	BC052968
Hs.2913	EPHB3	2049	3q21-qter	AA455591
Hs.437008	EPHB4	2050	7q22	W94076
Hs.446352	ERBB2	2064	17q11.2-q12	R80150
Hs.306251	ERBB3	2065	12q13	BC002706
Hs.74137	ERBB3	10972	14q24.3	AW572218
Hs.1939	ERBB4	2066	2q33.3-q34	NM_005235
Hs.132942	FAK	23092	5q31	NP_055886
Hs.748	FGFR1	2260	8p11.2-p11.1	N99235
Hs.748	FGFR1	2260	8p11.2-p11.1	AY585209
Hs.748	FGFR1	2260	8p11.2-p11.1	N99235
Hs.404081	FGFR2	2263	10q26	AA190616
Hs.1420	FGFR3	2261	4p16.3	R84974
Hs.1420	FGFR3	2261	4p16.3	R84974
Hs.1422	FGR	2268	1p36.2-p36.1	W81586
Hs.1422	FGR	2268	1p36.2-p36.1	W81586
Hs.347713	FLT1	2321	13q12	X51602
Hs.347713	FLT1	2321	13q12	BC039007
Hs.347713	FLT1	2321	13q12	BC039007
Hs.415048	FLT4	2324	5q34-q35	NP_002011
Hs.390567	FYN	2534	6q21	R56124
Hs.390567	FYN	2534	6q21	H96234
Hs.390567	FYN	2534	6q21	R56124
Hs.169476	GAPD	2597	12p13	J04038
Hs.258561	GTF2B	2959	1p22-p21	H23978
Hs.89555	HCK	3055	20q11-q12	AA035407
Hs.118625	HK1	3098	10q22	AK095086
	HTG	695	Xq21.33-q22	Z73639
Hs.239176	IGF1R	3480	15q26.3	H13300
Hs.239176	IGF1R	3480	15q26.3	H13300
Hs.438669	INSR	3643	19p13.3-p13.2	H03917
Hs.211576	ITK	3702	5q31-q32	R00673
Hs.12337	KDR	3791	4q11-q12	NM_002253
Hs.1765	LCK	3932	1p34.3	AA040732
Hs.434481	LTK	4058	15q15.1-q21.1	BC045607
Hs.437808	MATK	4145	19p13.3	BC000114
Hs.2942	MST1R	4486	3p21.3	AA129089
Hs.2942	MST1R	4486	3p21.3	AA129089
Hs.439109	NTRK2	4915	9q22.1	AF410902
Hs.26776	NTRK3	4916	5q25	BC013693
Hs.440905	NTRKR3	4921	1q12-q23	AK095975
Hs.2853	OR5AX1	81453	1	AC026038
Hs.74615	PDGFRA	5156	4q11-q13	H02958
Hs.307783	PDGFRB	5159	5q31-q32	P09619
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Hs.307783	PDGFRB	5159	5q31-q32	R56211
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Hs.405474	PYK2	2185	8p21.1	2119367A
Hs.2942	RON	4486	3p21.3	AA129088
Hs.1041	ROS	6098	6q22	W24195
Hs.1041	ROS1	6098	6q22	W24195
Hs.1041	ROS1	6098	6q22	W24195
Hs.356371	RPL28	6158	19q13.4	AA064618
Hs.430207	RPL29	6159	3p21.3-p21.2	R97429
Hs.469653	RPL5	6125	1p22.1	AB061848
Hs.337307	RPS27	6232	1q21	AA186639
Hs.285346	RYK	6259	3q22	S59184
Hs.75367	SLA	6503	8q22.3-qter	R52378
Hs.75367	SLA	6503	8q22.3-qter	R52378
Hs.390567	SLK	2534	6q21	M14676
Hs.436015	SRC	6714	20q12-q13	AA402622
Hs.192182	SYK	6850	9q22	AK075020
Hs.89640	TEK	7010	9p21	AB086825
Hs.89640	TEK	7010	9p21	H02848
Hs.78824	TIE	7075	1p34-p33	R60297
Hs.78824	TIE1	7075	1p34-p33	R40609
Hs.89640	TIE2	7010	9p21	W46173
Hs.203420	TNK1	8711	17p13.1	AA480582
Hs.29877	TXK	7294	4p12	R79606
Hs.75516	TYK2	7297	19p13.2	BC014243
Ssc.16085	TYK2	397363		AF100454
Hs.381282	TYRO3	7301	15q15.1-q21.1	AI090723
Hs.381282	TYRO3	7301	15q15.1-q21.1	W69497
Hs.15951	UPA	118471	10q26.3	AF421885
Hs.179526	VDUP1	10628	1q21.2	AF333001
Cfa3778	YES	403917		P07947
Hs.194148	YES1	7525	18p11.31-p11.21	R25397





slide serial number

microarray chip type number

Corning Microarray UltraGAPS™ Coated Slides

Oncogene list

Unigene	Symbol	Locus	Cytoband	Accession	Unigene	Symbol	Locus	Cytoband	Accession	Unigene	Symbol	Locus	Cytoband	Accession
Hs.446504	ABL1	25	9q34.1	NM_007313	Hs.2132	EPS8	2059	12q23-q24	AA291354	Hs.408312	P53	7157	17p13.1	AF135121
Hs.1288	ACTA1	58	1q42.13-q42.2	P02568	Hs.446352	ERBB2	2064	17q11.2-q12; 17q21.1	R80150	Hs.408312	P53	7157	17p13.1	AF135121
Hs.515706	AKIP	54998	1p36.33	NM_003600	Hs.446352	ERBB2	2064	17q21.1	NM_004448	Hs.498910	PCNA	5111	20pter-p12	P46087
Hs.106674	BAP1	8314	3p21.31-p21.2	NM_004656	Hs.45514	ERG	2078	21q22.3	BC040168	Hs.78996	PCNA	5111	20pter-p12	AF527838
Hs.79241	BCL2	596	18q21.33; 18q21.3	NM_000633	Hs.104349	FGD1	2245	xp11.21	H83405	Hs.75323	PHB	5245	17q21	I52690
Hs.380403	BMI1	648	10p11.23	NM_005180	Hs.390567	FYN	2534	6q21	H96234	Hs.151536	RAB13	5872	1q21.2	NM_002870
Hs.1274	BMP1	649	8p21	H95151.1	X1.28453	GADD153	398615		AY302440	Hs.78305	RAB2	5862	8q12.1	AF070629
Hs.79428	BNIP3	664	10q26.3	NM_004052	Hs.79000	GAP43	2596	3q13.1-q13.2	H05445	Hs.758	RASA1	5921	5q13.3	BC020761
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Hs.159494	BTK	695	Xq21.33-q22	U01923.1	Hs.37003	HRAS	3265	11p15.5	NM_005343	Hs.406078	RBBP7	5931	Xp22.22	NM_002893
Hs.284245	C6orf204	387119	6q22	NM_206921	Hs.77695	HURP	5787	14q22.2	NM_014750	Hs.2942	RON	4486	3p21.3	NM_002447
Hs.417050	CCNA1	8900	13q12.3-q13	NM_003914	Hs.78465	JUN	3725	1p32-p31	P05412	Hs.356371	RPL28	6158	19q13.4	AA064618
Hs.417050	CCNA1	8900	13q12.3-q13	NM_003914	Hs.380850	KAL1	3730	Xp22.32	NM_000216	Hs.430207	RPL29	6159	3p21.3-p21.2	R97429
Hs.23960	CCNB1	891	5q12	AA113936	Hs.81665	KIT	3815	4q11-q12	NM_000222	Hs.469653	RPL5	6125	1p22.1	AB061848
Hs.244723	CCNE1	898	19q12	NM_001238	Hs.183428	KRAS	8082	11p11.2	AF016028	Hs.337307	RPS27	6232	1q21	AA186639
Hs.244723	CCNE1	898	19q12	NP_001229	Hs.276238	KSR	8844	17q11.2	BC042106	Hs.30868	RTN4R	65078	22q11.21	BC011787
Hs.79101	CCNG1	900	5q32-q34	R14996	Hs.155048	LU	4059	19q13.2	P50895	Hs.50984	SAS	6302	12q13.3	S78432
Hs.347990	CD34	947	1q32	BC039146	Hs.75862	MADH4	4089	18q21.1	BC002379	Hs.55279	SERPINB5	5268	18q21.3	W30956
Hs.355832	CDC42	998	1p36.1	AL121734	Hs.153863	MADH6	4091	15q21-q22	AF035528	Hs.433795	SHC	6464	1q21	BC014158
Rn.103750	CFOS	314322	6q31	AF126534	Hs.212217	MDM2	4193	12q14.3-q15	H13638	Hs.421342	STAT3	6774	17q21.31	BC014482
Hs.202453	CMYC	4609	8q24.12-q24.13	R43857	Hs.419124	MET	4233	7q31	NM_000245	Hs.442707	TERF1	7013	8q13	NP_003209
Hs.183650	CRABP2	1382	1q21.3	H44233	Hs.419124	MET	4233	7q31	NM_000245	Hs.174312	TLR4	7099	9q32	AB020878
Hs.173894	CSF1	1435	1p21-p13	M11038	Hs.433618	MLH1	4292	3p21.3	NM_000249	Hs.116237	VAV1	7409	19p13.2	AI369096
Hs.172562	DCC	1630	18q21.5	P43146	Hs.279843	MLH3	27030	14q24.3	L40399	Hs.116237	VAV1	7409	19p13.2	AI369096
Hs.77091	DNASE1L1	1774	xq28	P49184	Hs.153837	MNDA	4332	1q22	R77431	Hs.436803	VBP1	7411	Xq28	NM_003372
Hs.5120	DNCL1	8655	12q24.23	R18848	Hs.407830	MYB	4602	6q22-q23	NM_005375	Hs.179526	VDUP1	10628	1q21.2	AF333001
Hs.79306	EIF4E	1977	4q21-q25	BC012611	Hs.81687	NME3	4832	16q13	AA129463	Hs.421597	VHL	7428	3p26-p25	NM_198156
Hs.181128	EIK1	2002	xq11.2	W40449	Hs.25960	NMYC	4613	2p24.1	P04198	Hs.512715	WRN	7486	8p12-p11.2	AA287923
Hs.356835	EMP2	2013	16p13.2	NM_001424	Hs.25960	NMYC	4613	2 p24.1	BC002712	Hs.78919	XK	7504	xp21.1	H18932
Hs.356835	EMP2	2013	16p13.2	NM_001424	Hs.260523	NRAS	4893	1p13.2	NM_002524	Hs.98493	XRCC1	7515	19q13.2	AC018758

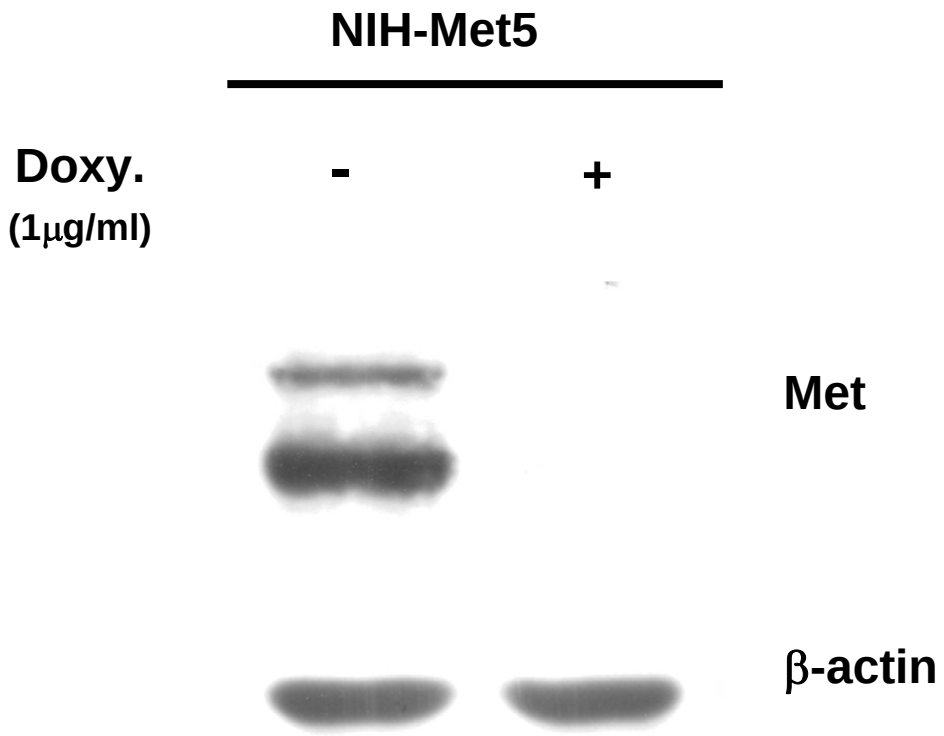
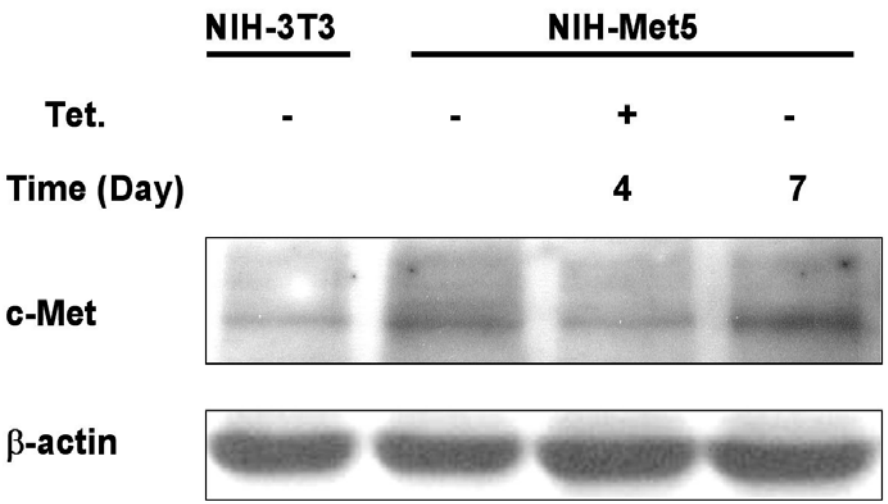
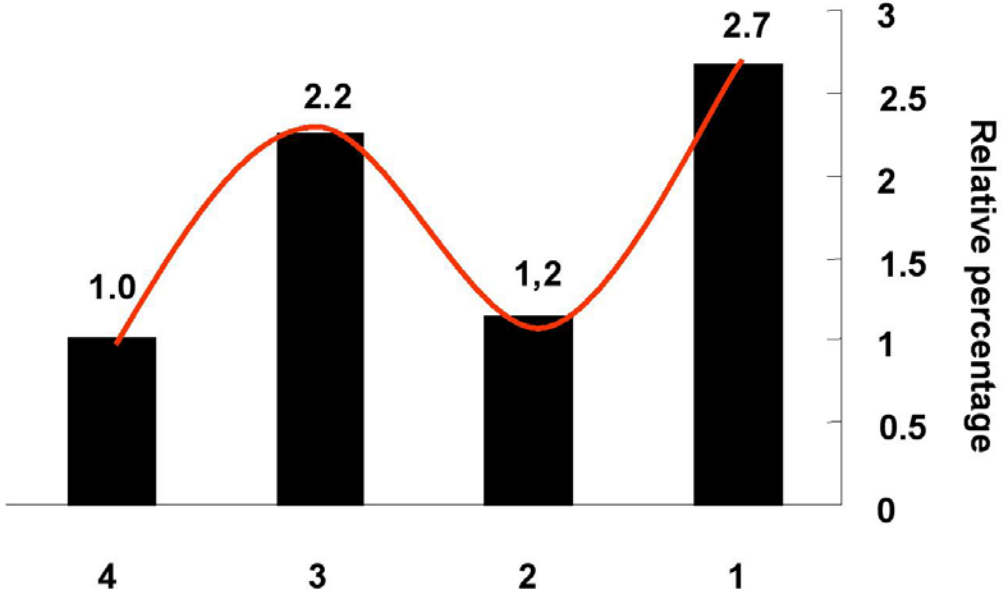


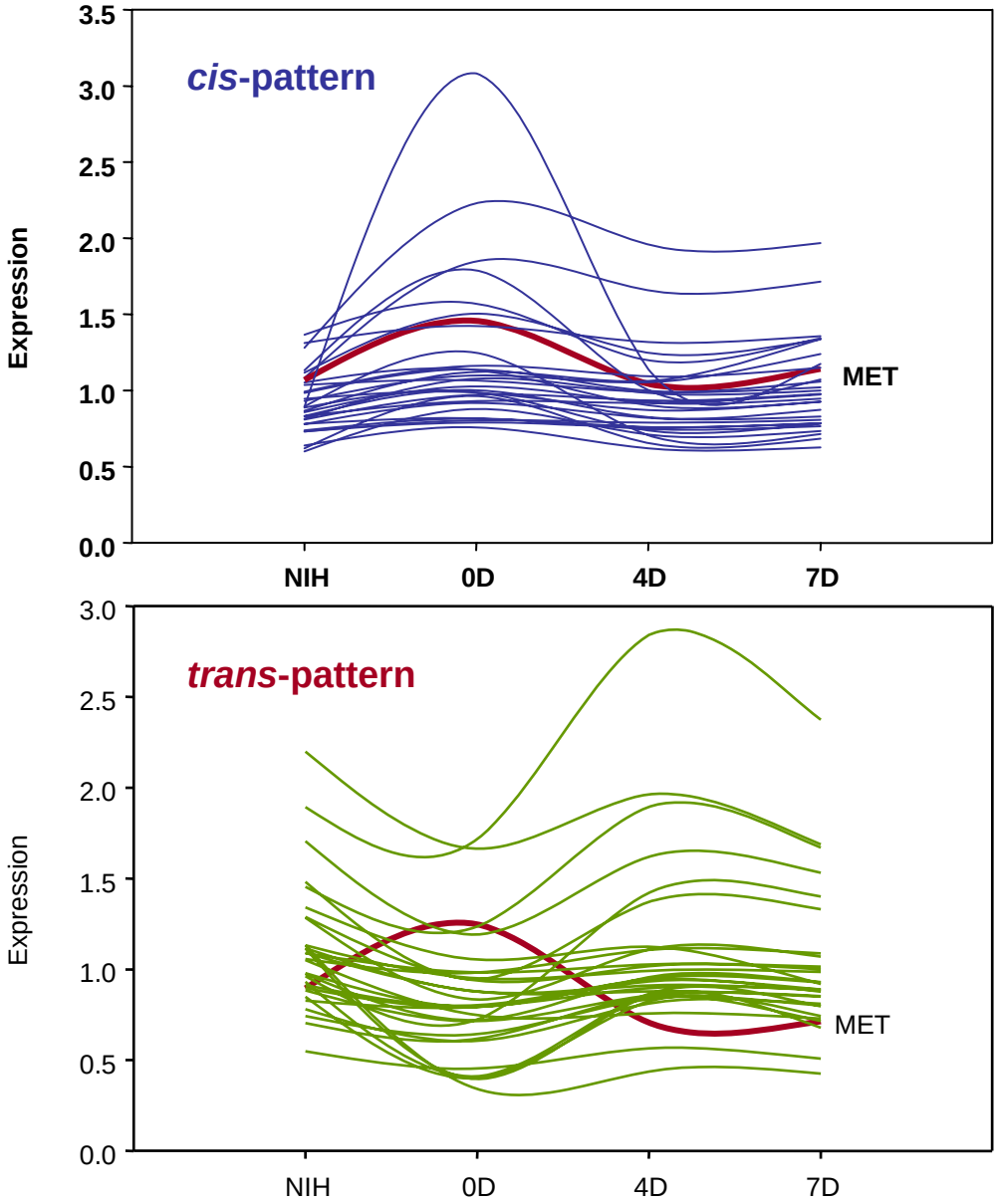
Fig. 1 Establishment of a inducible stable cell line expressing exogenous c-Met gene.

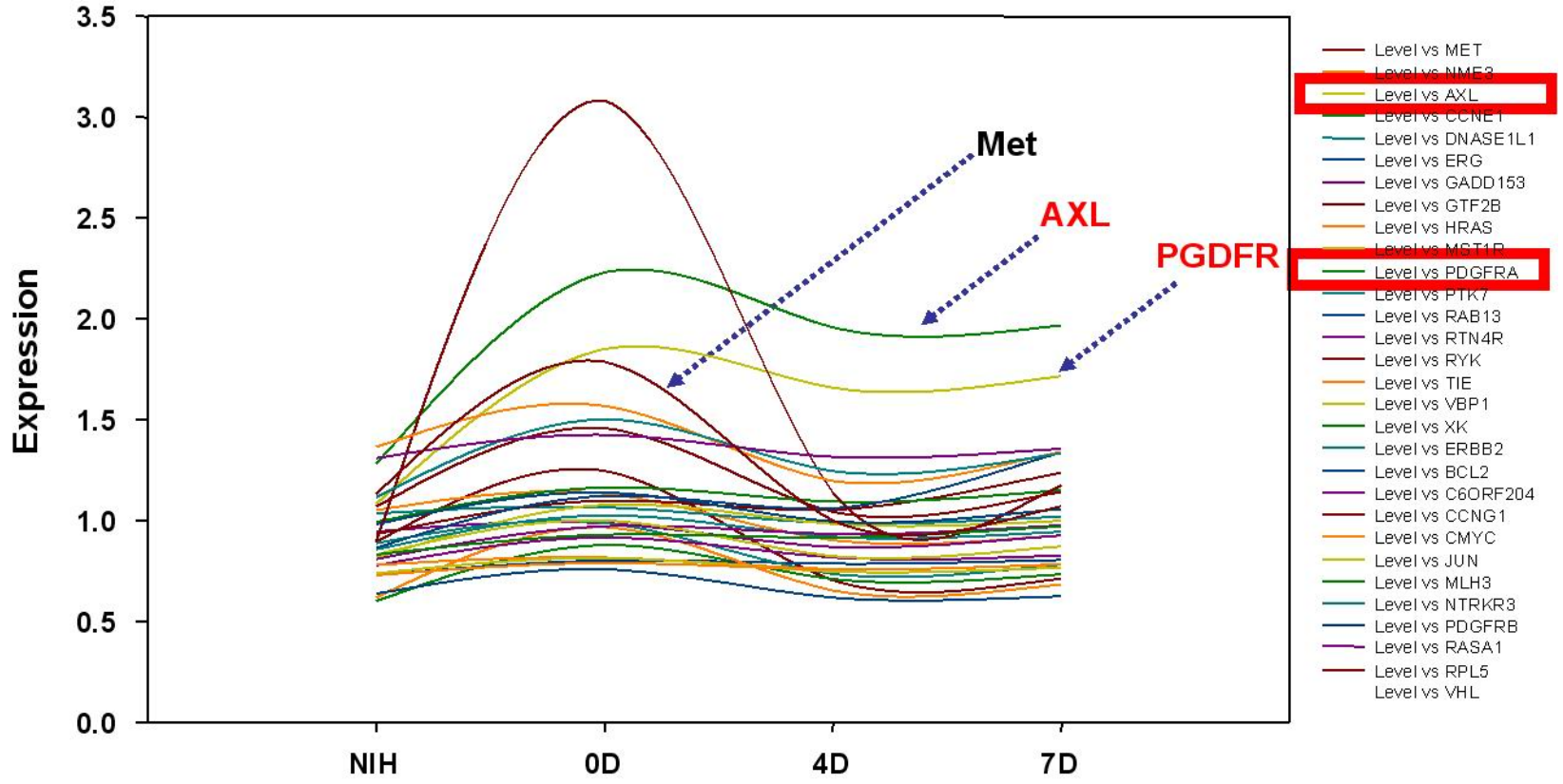
The pTRE-Met and pTet-Lac-Hyg plasmid DNA were transfected into NIH/3T3 cells by lipofectamine, and the stable cell line was selected using hygromycine. The inducible stable cell line, NIH-Met5, was established, and c-Met expression was detected using western blotting.



Lane 2: Tet. added and removed next day. Cells harvested on day 4.
Lane 3: cells harvested on day 7.




cis-pattern
Expression
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MET
NIH
0D
4D
7D
trans-pattern
Expression
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MET
NIH
0D
4D
7D



The gene expression of microarray in inducible c-Met system

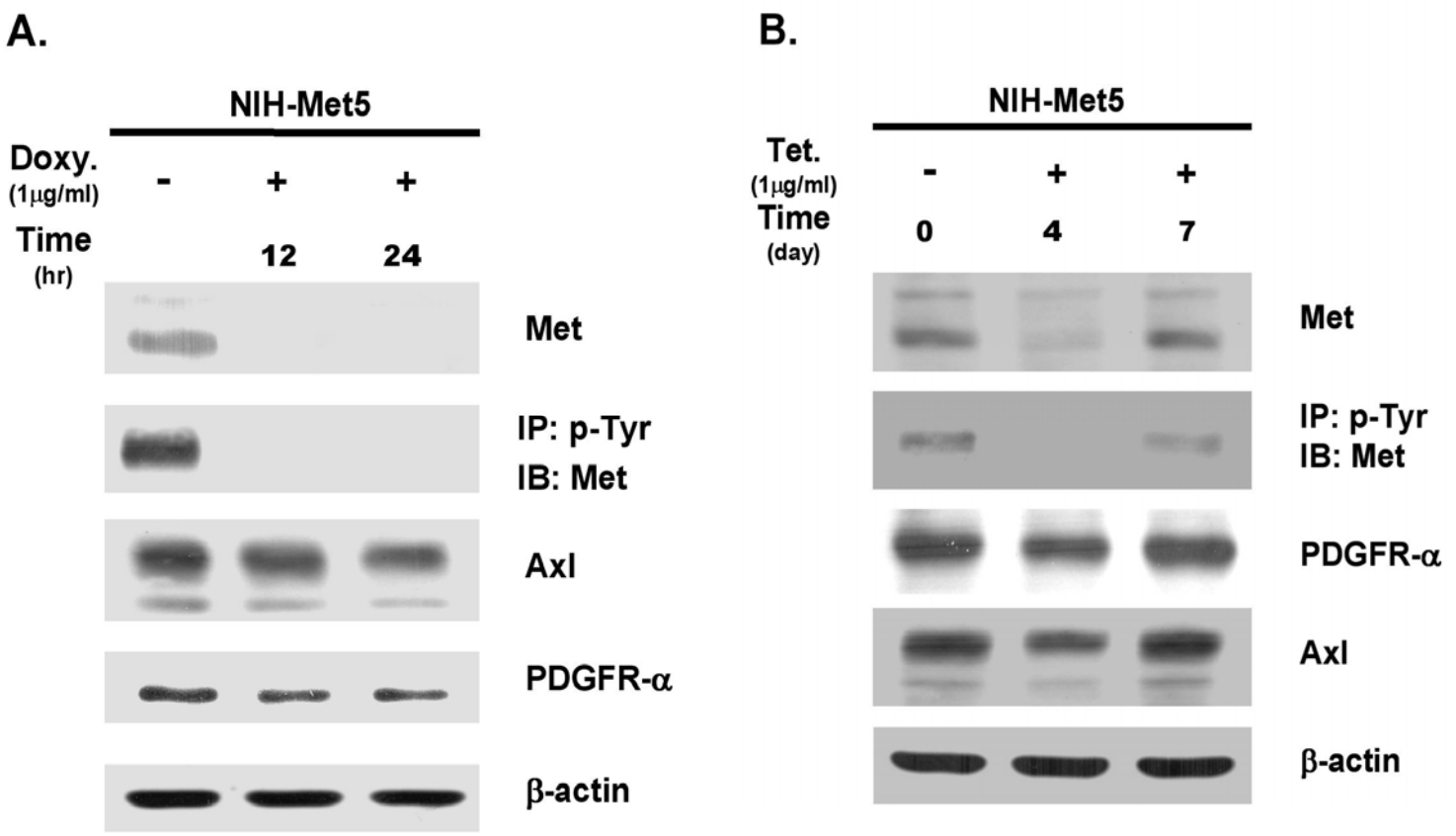
We used the tyrosine kinase receptor chip and selected the related gene with c-Met. The expression of Axl and PDGFR-α was highly increased with up-regulation of c-Met. We would detect the relation of Axl, PDGFR-α and c-Met.

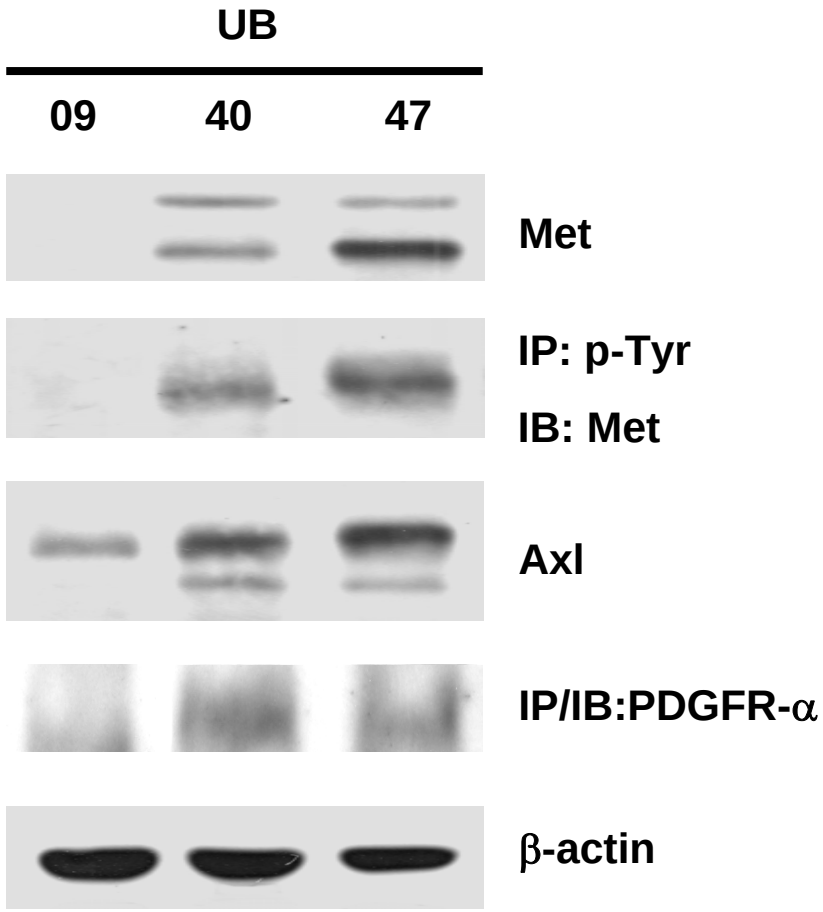
Axl (UFO, Ark)

- A receptor tyrosine kinase
- Contains immunoglobulin-like and fibronectin III-like domain.
- Axl-mediated signaling has been shown related with cell proliferation and survival.

PDGFR-α

- A receptor tyrosine kinase.
- The receptor of PDGF AA, AB, BB, CC
- Regulates the cell Migration, proliferation, transformation, anti-apoptosis and embryo differentiation





The expression of Axl and PDGFR-α in bladder cancer cell lines

The expression and phosphorylation of c-Met was detected in UB40 and UB47, but UB09 was not. The expression of Axl and PDGFR-a in UB40 and UB47 was also higher than in UB09.

Multi-dimensional Analysis



Colorectal cancer cell gene expression

—DNA microarray analysis strategy base on chromosome banding analysis

大腸直腸癌細胞基因表達分析

—利用核酸微陣列分析探討染色體色帶變化



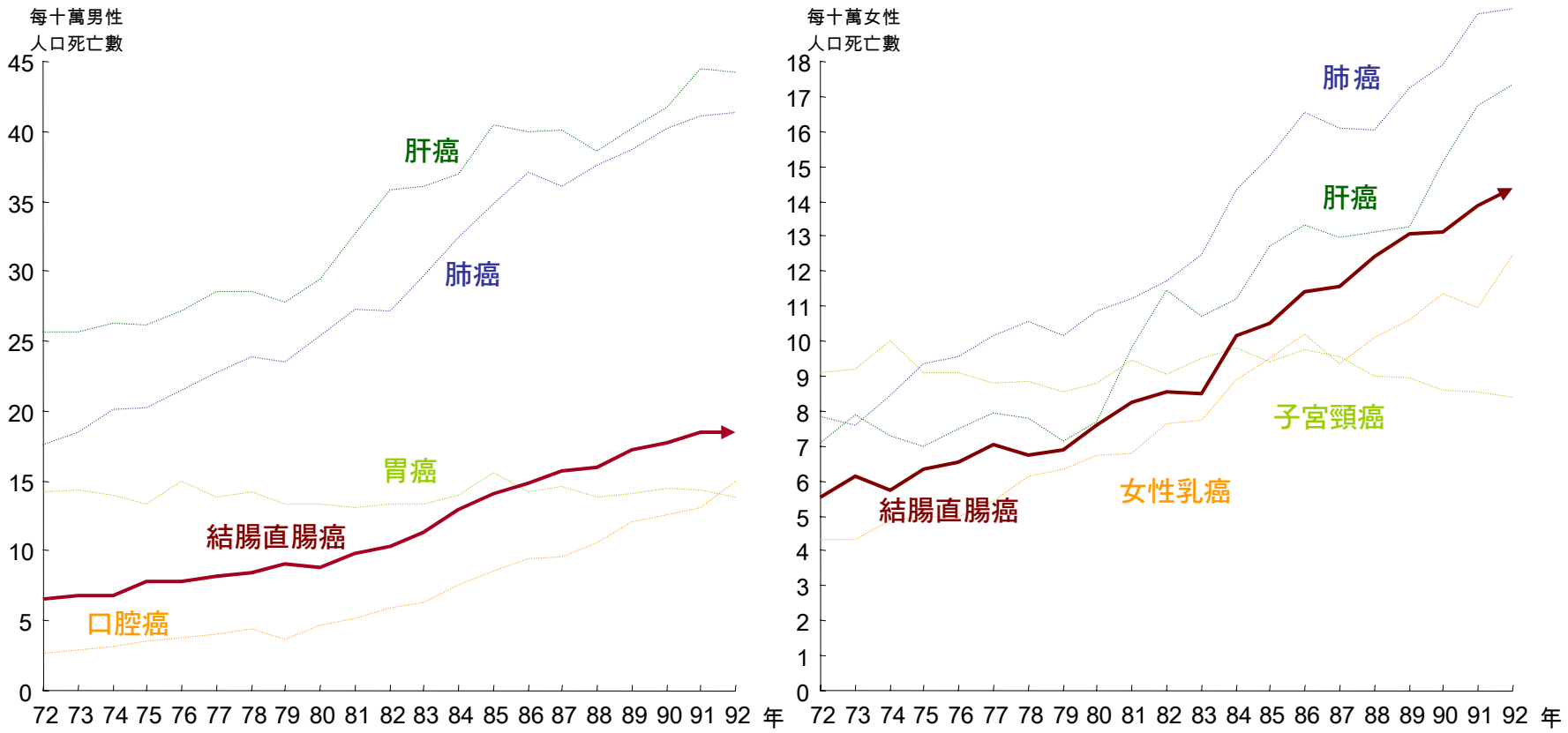
Shin, Jyh-wei *hippo@mail.ncku.edu.tw*

Systems Parasitology Laboratory

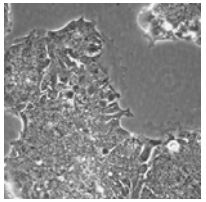
Microarray Center and Departement of Parasitology

College of Medicine, National Chung Kung UNiversity

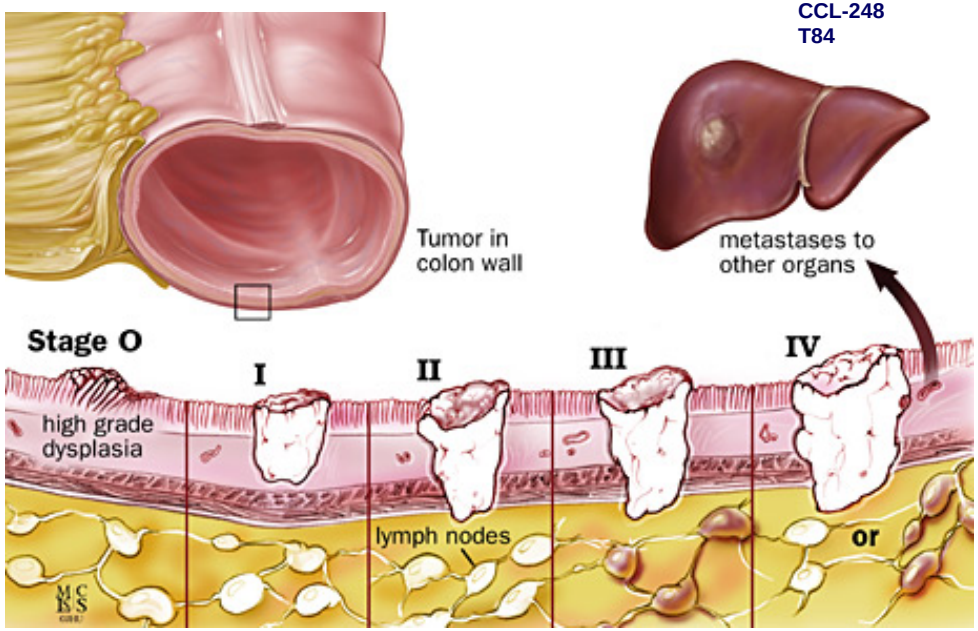
臺灣地區主要癌症死亡率趨勢圖

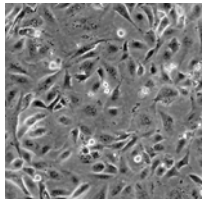


Colorectal Cancer Cell Lines

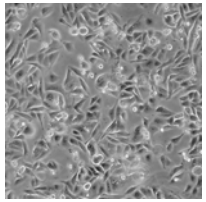


CCL-248
T84

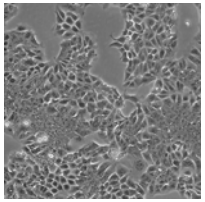




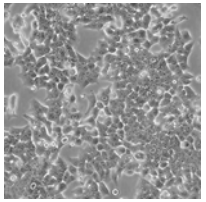
CCL-233
SW1116



CCL-228
SW480



CCL-227
SW620



CCL-222
COLO205

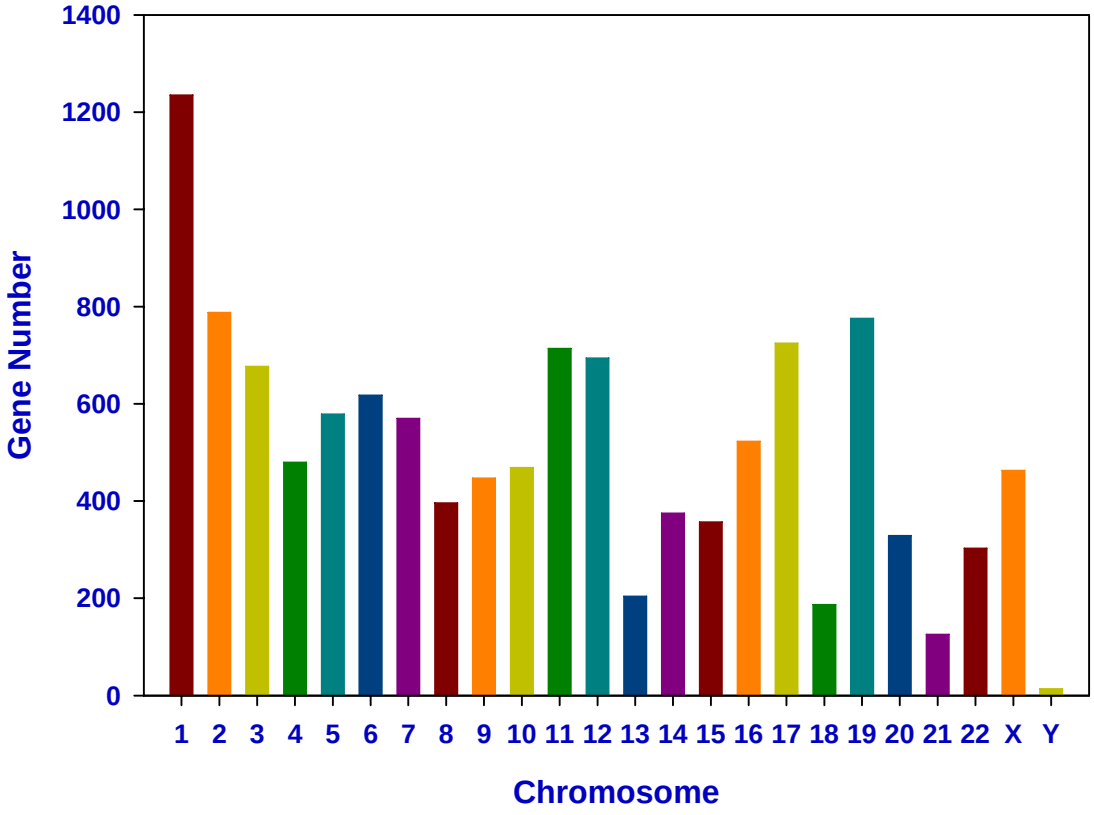
Dukes' stage classification

ATCC	CCL-233	CCL-228	CCL-227	CCL-222	CCL-248
Cell	SW1116	SW480	SW620	COLO205	T84
Organ	colon	colon	colon	colon	colon
Disease	colorectal adeno-carcinoma	colorectal adeno-carcinoma	colorectal adeno-carcinoma	colorectal adeno-carcinoma	colorectal carcinoma
Stage	Dukes' type A (III)	Dukes' type B	Dukes' type C	Dukes' type D	
Derived Tissue			lymph node	ascites	Lung
Oncogene	myc+; myb+; ras+; fos+; sis+; p53+; abl-; ros-; src-	myc+; myb+; ras+; fos+; sis+; p53+; abl-; ros-; src-	myc+; myb+; ras+; fos+; sis+; p53+; abl-; ros-; src-	myc+; ras+ ; myb+; fos+; sis+; p53+; abl-; ros-; src-	
Cellular Products	CEA; keratin	CEA; keratin; TGFβ	CEA; TGFα; keratin; matrilysin	CEA; keratin; IL-10	CEA; Keratin
Culture medium	Leibovitz's L-15	Leibovitz's L-15	Leibovitz's L-15	RPMI 1640	DMEM / Ham's F12

Metastases

http://www.hopkins-gi.org
http://www.atcc.org

Genes located on chromosome

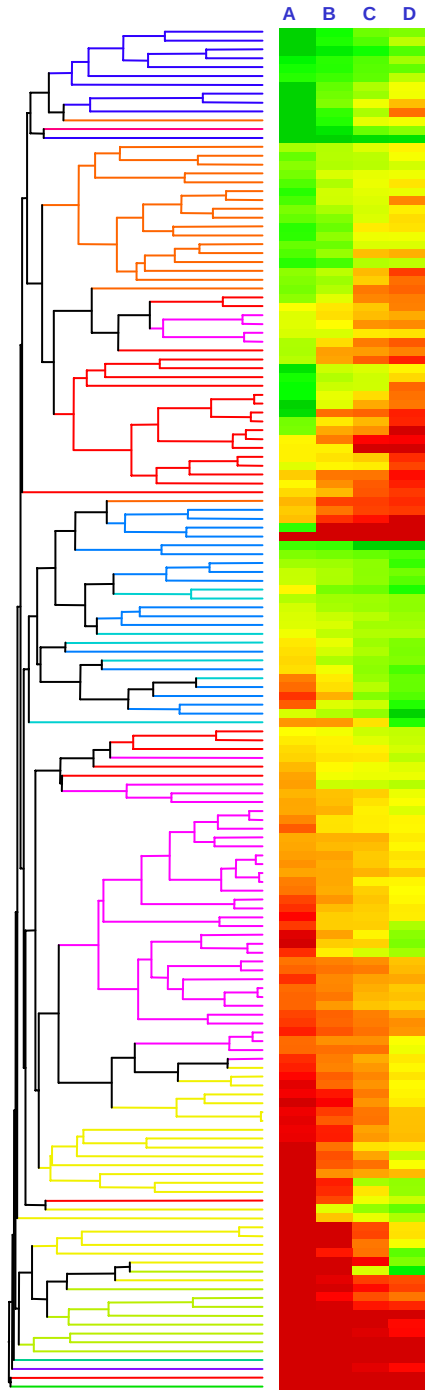


Chromosome		Total	Location
	1	1235	190
	2	788	147
	3	677	138
	4	480	105
	5	579	112
	6	618	127
	7	570	109
	8	396	89
	9	447	87
	10	469	103
	11	714	101
	12	694	111
	13	204	47
	14	375	61
	15	357	70
	16	523	74
	17	725	83
	18	187	47
	19	776	52
	20	329	64
	21	126	17
	22	303	33
	X	463	90
	Y	14	7



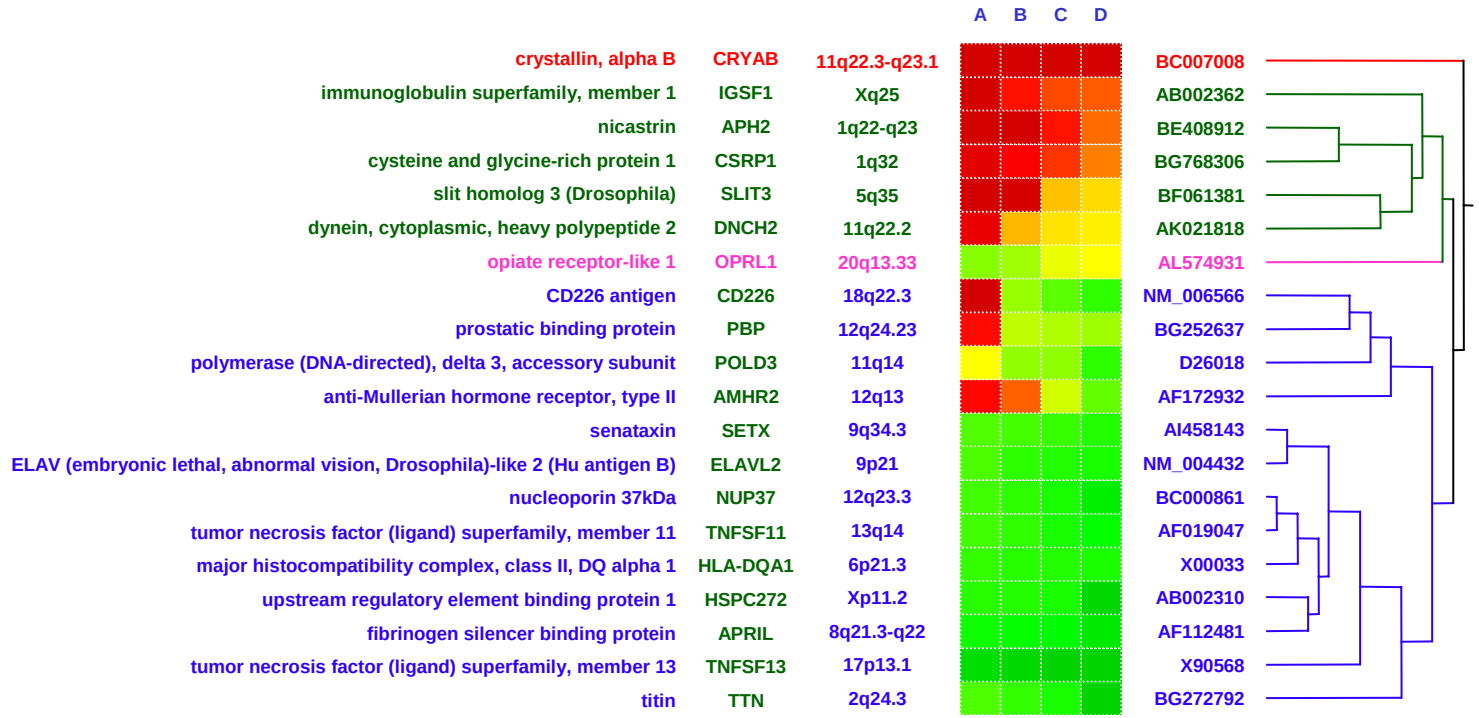
Expression analysis

Up regulation		Down regulation	
Chromosome	Count	Chromosome	Count
1	5	1	14
2	4	2	5
3	5	3	4
4	2	4	3
5	6	5	2
6	1	6	4
7	3	7	2
8	2	8	6
9	1	9	3
10	2	10	3
11	1	11	4
12	3	12	6
14	2	13	1
15	3	14	2
16	2	16	6
17	7	17	8
18	1	18	4
19	4	19	7
20	2	20	1
21	0	21	0
22	2	22	4
X	0	X	7
Y	0	Y	0
Total	58	Total	96



expression (up and down join stage specific (>90%)

expression (up and down join stage specific (<10%)



Hierarchical Clustering, Method = Average

K Means Clustering



1

Accession	BC007008
Chromosome	11
Position	11q22.3-q23.1
Name	crystallin, alpha B
Locus	1410
OMIM	123590
Gene Card	CRYAB
Unigene	Hs.408767

Biological process

muscle contraction , protein folding , visual perception

Cellular Component

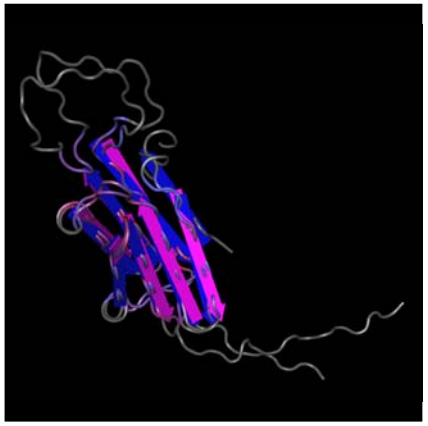
cytoplasm , nucleus

Molecular Function

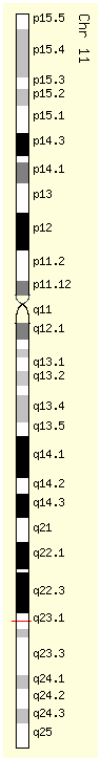
structural constituent of eye lens , unfolded protein binding

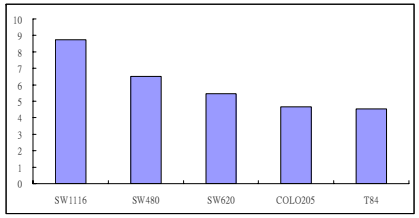
No Pathway available

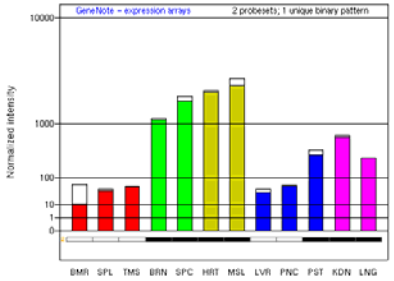
Diseases: desmin related myopathy, characterized by adult onset, proximal and distal limb muscle weakness often associated with neck, trunk and velopharynx muscle involvement, cardiomyopathy, cataract in many cases and delayed accumulation of aggregates of desmin in sarcoplasma of skeletal and cardiac muscle

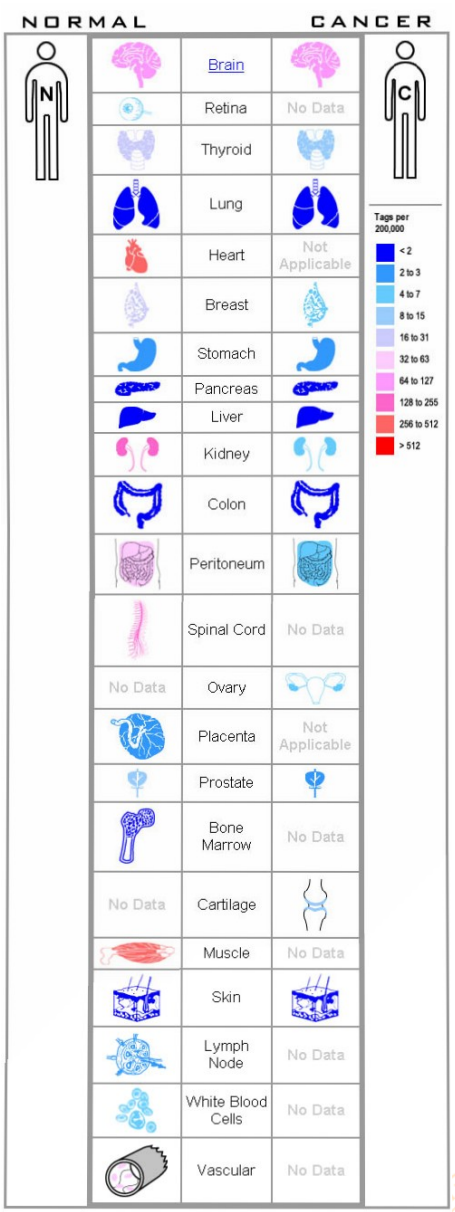


alpha-crystallin-type heat shock proteins (Hsps), family of small stress induced proteins ranging from 12 -43 kDa, whose common feature is the alpha-crystallin domain. They are generally active as large oligomers consisting of multiple subunits. They are believed to be ATP-independent chaperones that prevent aggregation, and are important in refolding in combination with other Hsps. They are present in all kingdoms, but not in all organisms.











Agilent
Human 1 cDNA Microarray

18564 dots

Microarray image

Raw data

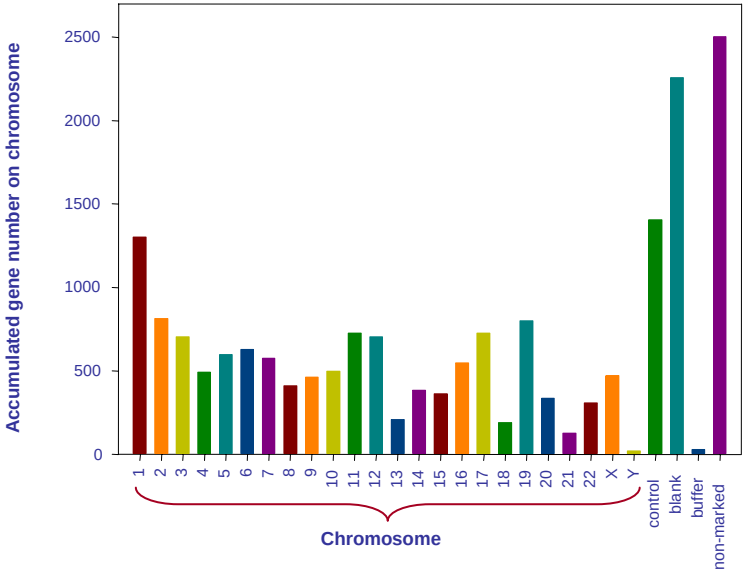


*.gpr

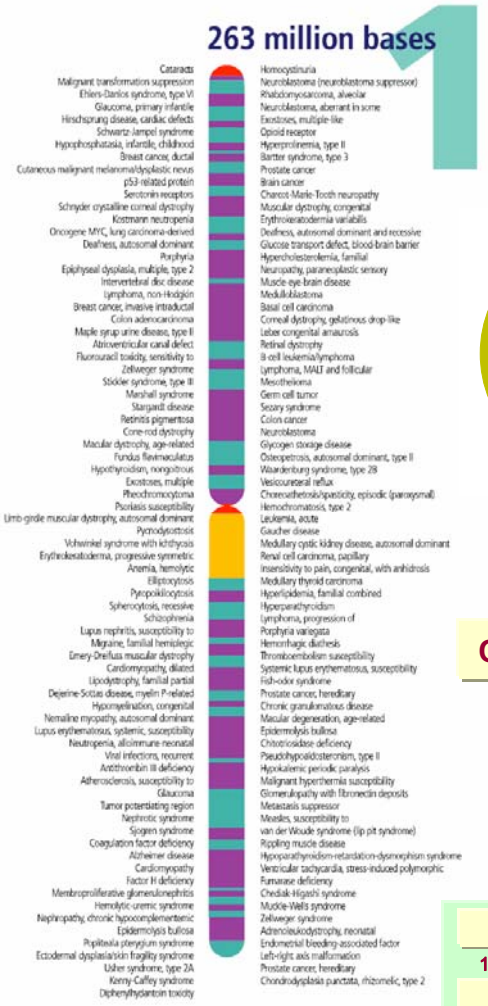
Normalized data

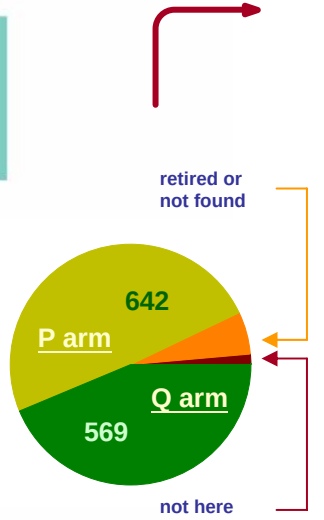


*.xls

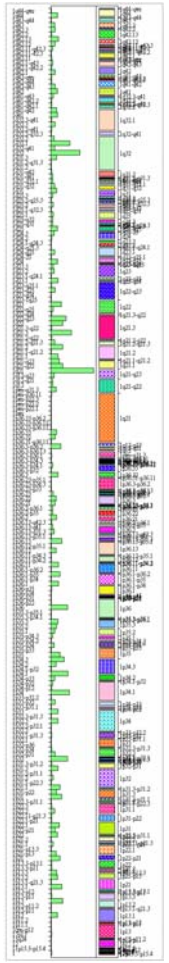


Gene on chromosome

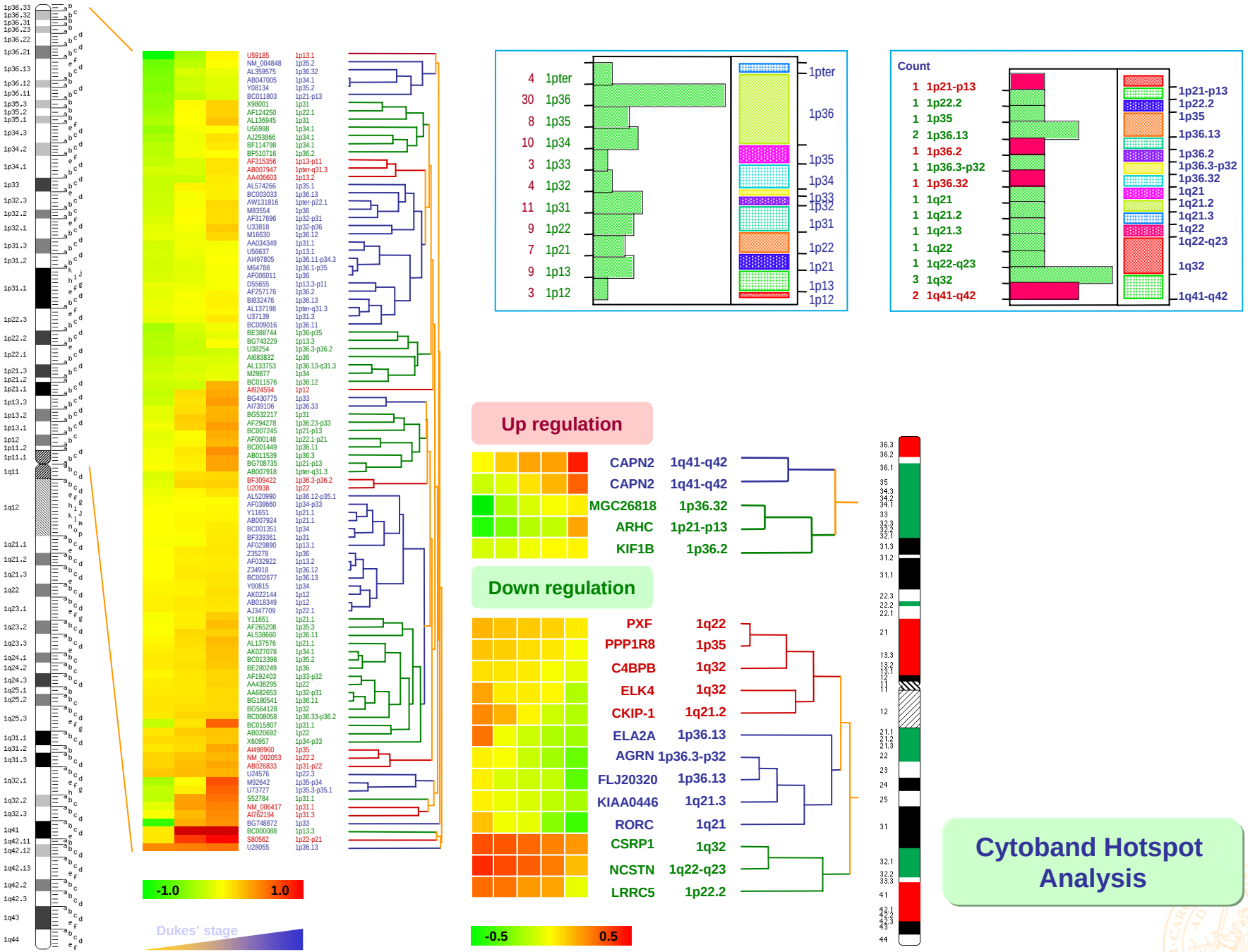




Cytoband analysis

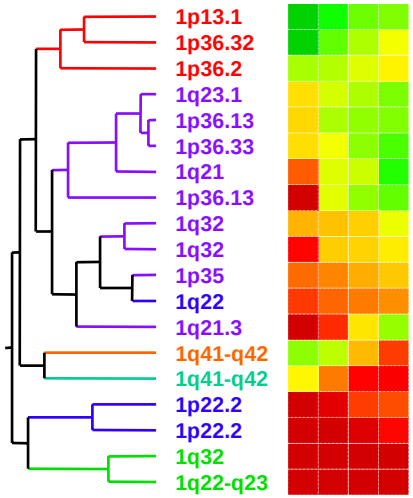


1	2	3	4	5	6	7	8
1303	812	702	490	597	626	576	411
9	10	11	12	13	14	15	16
462	497	726	703	208	384	361	547
17	18	19	20	21	22	X	Y
726	188	798	336	126	308	470	18

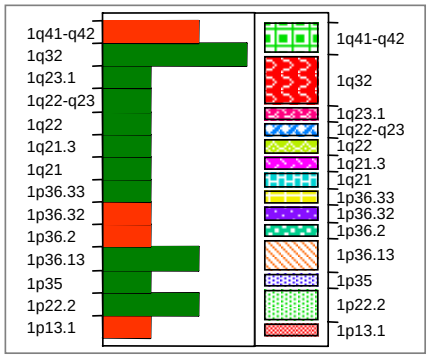

-1.0
1.0
Dukes' stage
4 1pter
30 1p36
8 1p35
10 1p34
3 1p33
4 1p32
11 1p31
9 1p22
7 1p21
9 1p13
3 1p12
Count
1 1p21-p13
1 1p22.2
1 1p35
2 1p36.13
1 1p36.2
1 1p36.3-p32
1 1p36.32
1 1q21
1 1q21.2
1 1q21.3
1 1q22
1 1q22-q23
3 1q32
2 1q41-q42
Up regulation
CAPN2 1q41-q42
CAPN2 1q41-q42
MGC26818 1p36.32
ARHC 1p21-p13
KIF1B 1p36.2
Down regulation
PXF 1q22
PPP1R8 1p35
C4BPB 1q32
ELK4 1q32
CKIP-1 1q21.2
ELA2A 1p36.13
AGRN 1p36.3-p32
FLJ20320 1p36.13
KIAA0446 1q21.3
RORC 1q21
CSRP1 1q32
NCSTN 1q22-q23
LRRC5 1p22.2
-0.5
0.5
Cytoband Hotspot Analysis

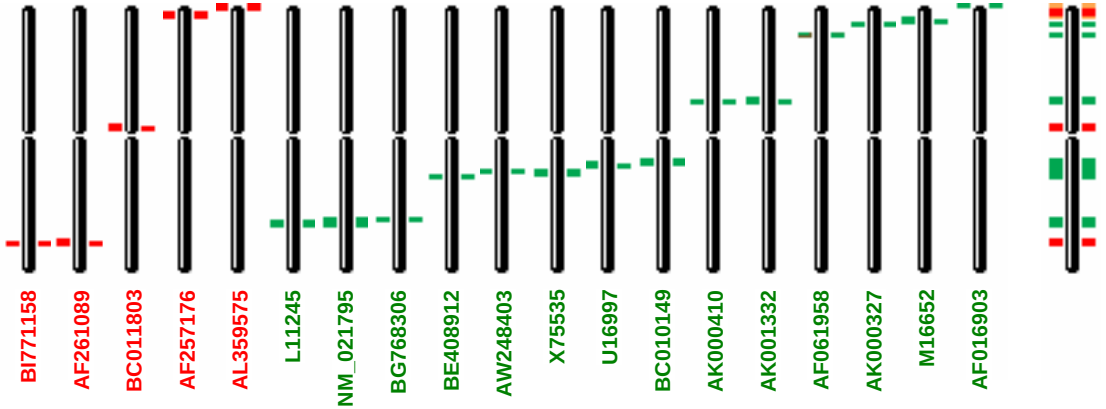
Chromosome 1

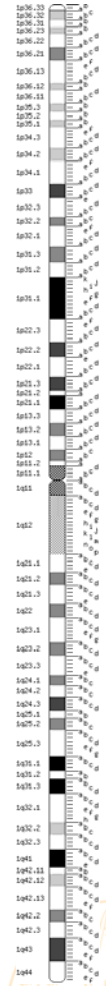




1p13.1	BC011803	Hs.179750	ARHC	Homo sapiens transcribed sequences	389	165380
1p36.32	AL359575	Hs.23765	MGC26818	hypothetical protein MGC26818	127281	603787
1p36.2	AF257176	Hs.444757	KIF1B	kinesin family member 1B	23095	605995
1q23.1	AW248403	Hs.512854	KIAA0446	KIAA0446 gene product	9673	607185
1p36.13	AK000327	Hs.263081	FLJ20320	hypothetical protein FLJ20320	54896	603651
1p36.33	AF016903	Hs.273330	AGRN	agrin	375790	103320
1q21	U16997	Hs.528667	RORC	RAR-related orphan receptor C	6097	602943
1p36.13	M16652	Hs.21	ELA2A	elastase 2A	63036	103720
1q32	L11245	Hs.99886	C4BPB	complement component 4 binding protein, beta	725	120831
1q32	NM_021795	Hs.129969	ELK4	ELK4, ETS-domain protein (SRF accessory protein 1)	2005	600246
1p35	AF061958	Hs.356590	PPP1R8	protein phosphatase 1, regulatory (inhibitor) subunit 8	5511	602636
1q22	X75535	Hs.517232	PEX19	peroxisomal biogenesis factor 19	5824	600279
1q21.3	BC010149	Hs.173380	CKIP-1	CK2 interacting protein 1; HQ0024c protein	51177	608335
1q41-q42	BI771158	Hs.350899	CAPN2	calpain 2, (m/II) large subunit	824	114230
1q41-q42	AF261089	Hs.350899	CAPN2	calpain 2, (m/II) large subunit	824	114230
1p22.2	AK000410	Hs.306221	FLJ20403	hypothetical protein FLJ20403	284695	606534
1p22.2	AK001332	Hs.199364	LRRC5	leucine rich repeat containing 5	55144	606421
1q32	BG768306	Hs.108080	CSRP1	cysteine and glycine-rich protein 1	1465	123876
1q22-q23	BE408912	Hs.4788	NCSTN	nicastrin	23385	605254



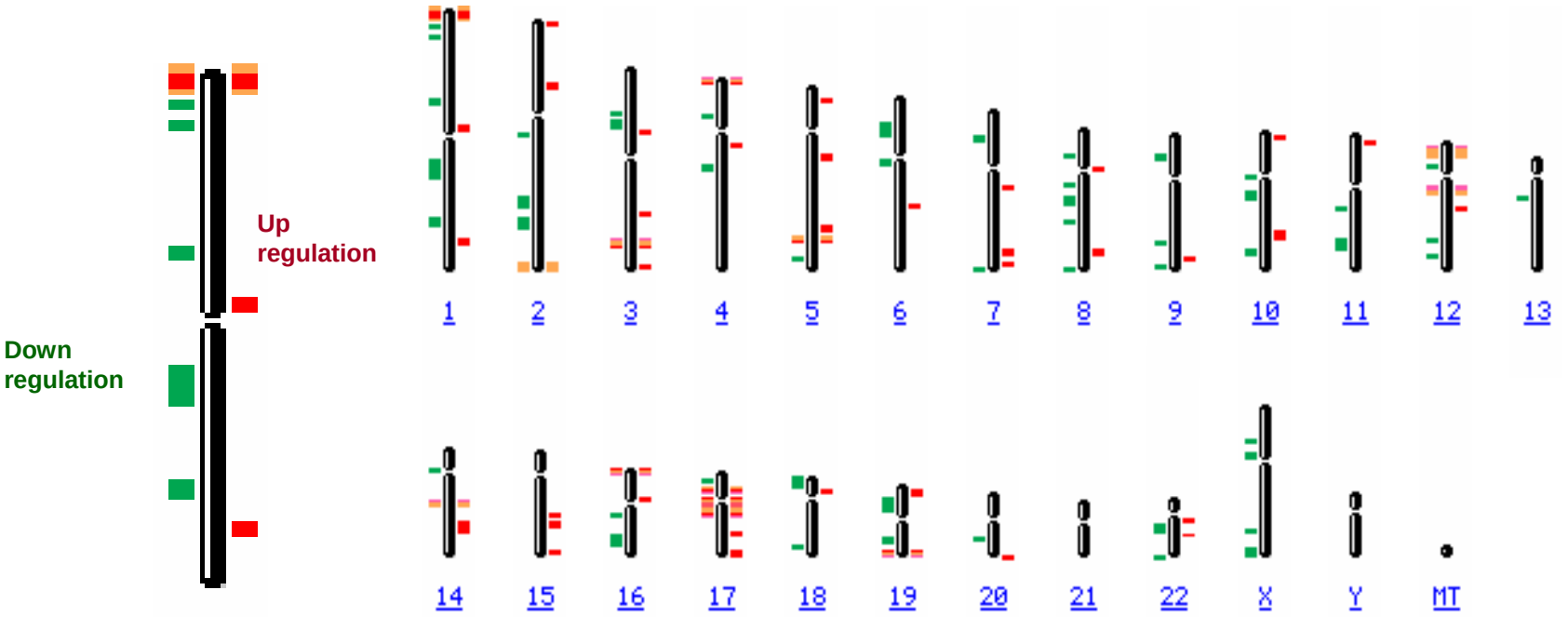




http://gai.nci.nih.gov/html-snp/imagemaps.html

http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=9606&query=&qchr=&strain=All&advsrch=off&neighb=off

Hot and Cold spots

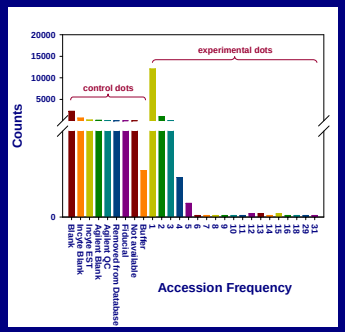


Aglient Human cDNA1 Array

18,564 Dots

Trimming

Blank (2,256)
Incyte Blank (699)
Incyte EST (261)
Agilent Blank (180)
Agilent QC (120)
Removed from Database (82)
Fiducial (81)
Not available (62)
Buffer (27)

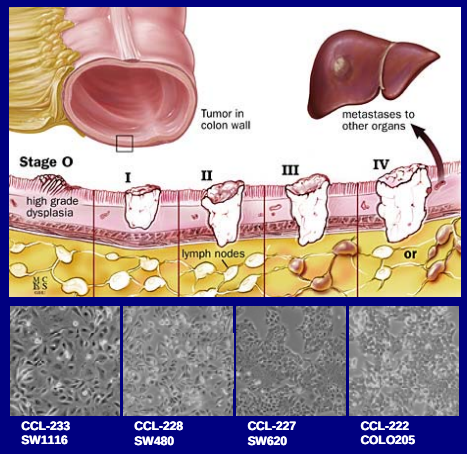


14,796 Dots

13,258 Accessions

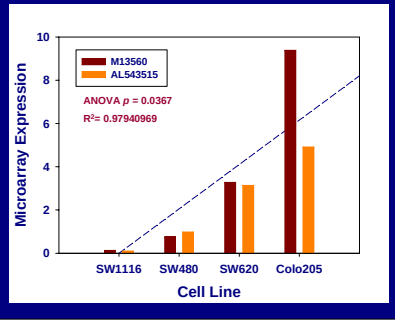
12,004 Symbols

Colorectal Cancer Cell Lines



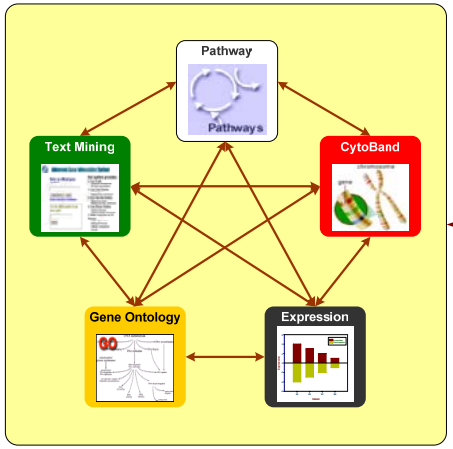
CCL-233 SW1116 | CCL-228 SW480 | CCL-227 SW620 | CCL-222 COLO205

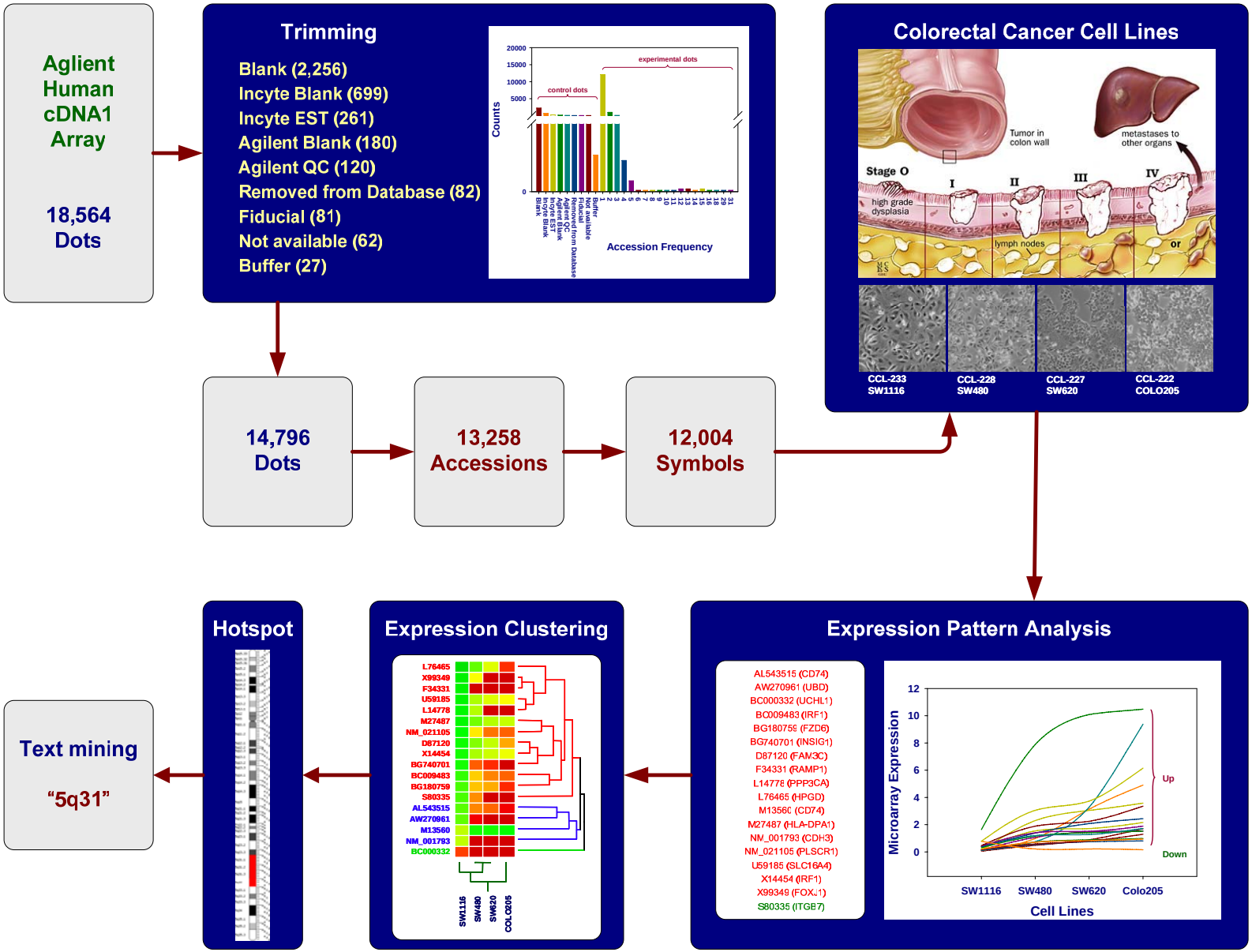
Expression Pattern Analysis "CD74" (up-regulation; fold>2)



Hs.436568 CD74 antigen 5q32

1. protein folding
2. immune response
3. integral to membrane
4. MHC class II receptor activity
5. unfolded protein binding
6. chaperone activity



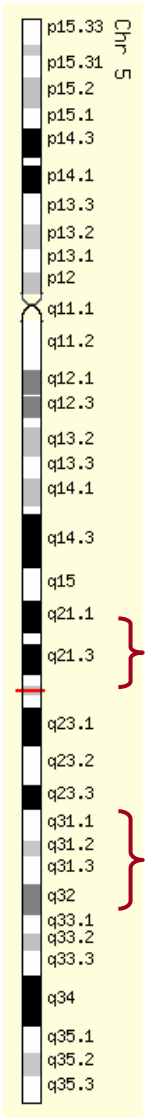

Aglient Human cDNA1 Array
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Trimming
Blank (2,256)
Incyte Blank (699)
Incyte EST (261)
Agilent Blank (180)
Agilent QC (120)
Removed from Database (82)
Fiducial (81)
Not available (62)
Buffer (27)
control dots
experimental dots
Counts
Accession Frequency
Colorectal Cancer Cell Lines
Tumor in colon wall
metastases to other organs
Stage 0
I
II
III
IV
high grade dysplasia
lymph nodes
or
CCL-233 SW1116
CCL-228 SW480
CCL-227 SW620
CCL-222 COLO205
14,796 Dots
13,258 Accessions
12,004 Symbols
Expression Pattern Analysis
AL543515 (CD74)
AW270961 (UBD)
BC000332 (UCHL1)
BC009483 (IRF1)
BG180759 (FZD6)
BG740701 (INSIG1)
D87120 (FAM3C)
F34331 (RAMP1)
L14778 (PPP3CA)
L76465 (HPGD)
M13560 (CD74)
M27487 (HLA-DPA1)
NM_001793 (CDH3)
NM_021105 (PLSCR1)
U59185 (SLC16A4)
X14454 (IRF1)
X99349 (FOXJ1)
S80335 (ITGB7)
Microarray Expression
Up
Down
SW1116
SW480
SW620
Colo205
Cell Lines
Expression Clustering
L76465
X99349
F34331
U59185
L14778
M27487
NM_021105
D87120
X14454
BG740701
BC009483
BG180759
S80335
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SW620
COLO205
Hotspot
Text mining
“5q31”



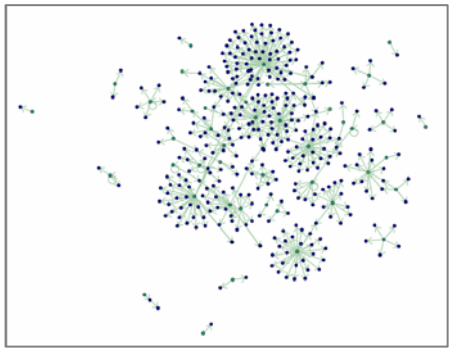
Anticancer Res. 1991 11(6):2047-50.
Gope R. et al.

Genetic changes at the beta-2-adrenergic receptor locus on chromosome 5 in human colorectal carcinomas.

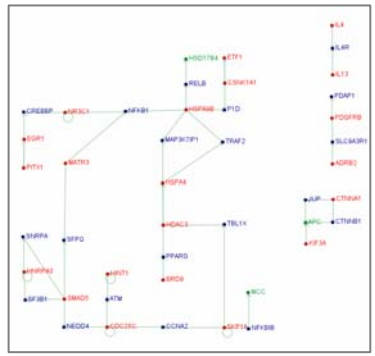
Matched normal/tumor DNA pairs from 44 colorectal carcinoma patients were examined for tumor-specific genetic changes using a probe for the beta-2-adrenergic receptor (ADRB2R) gene on chromosome 5. **This locus (5q31-q32) maps close to the site of chromosomal deletions recently reported to occur in colorectal carcinomas and distal to the chromosomal location of the familial adenomatous polyposis (FAP) gene (5q21-q22).** Our investigation shows tumor-specific allele loss or allelic rearrangement of at least 29% at the AdRb2R locus on chromosome 5 in informative cases. These results suggest that the mechanism by which colorectal carcinomas lose genetic material on chromosome 5 can affect this functional gene located distally to the FAP gene. The possible functional significance that ADRB2R gene changes may have in neoplastic progression is discussed.



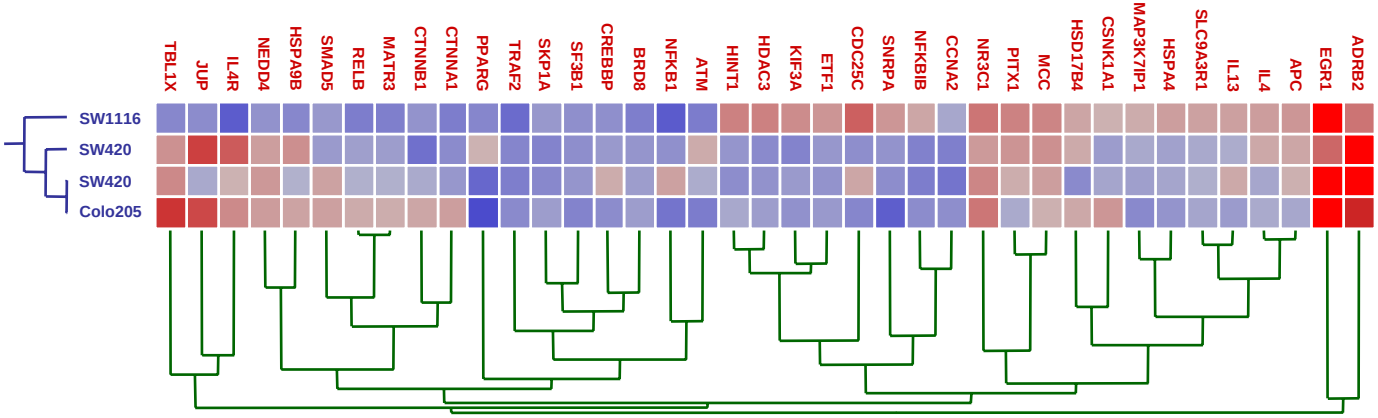
5q21-22 and 5q31-32
PPI visualization



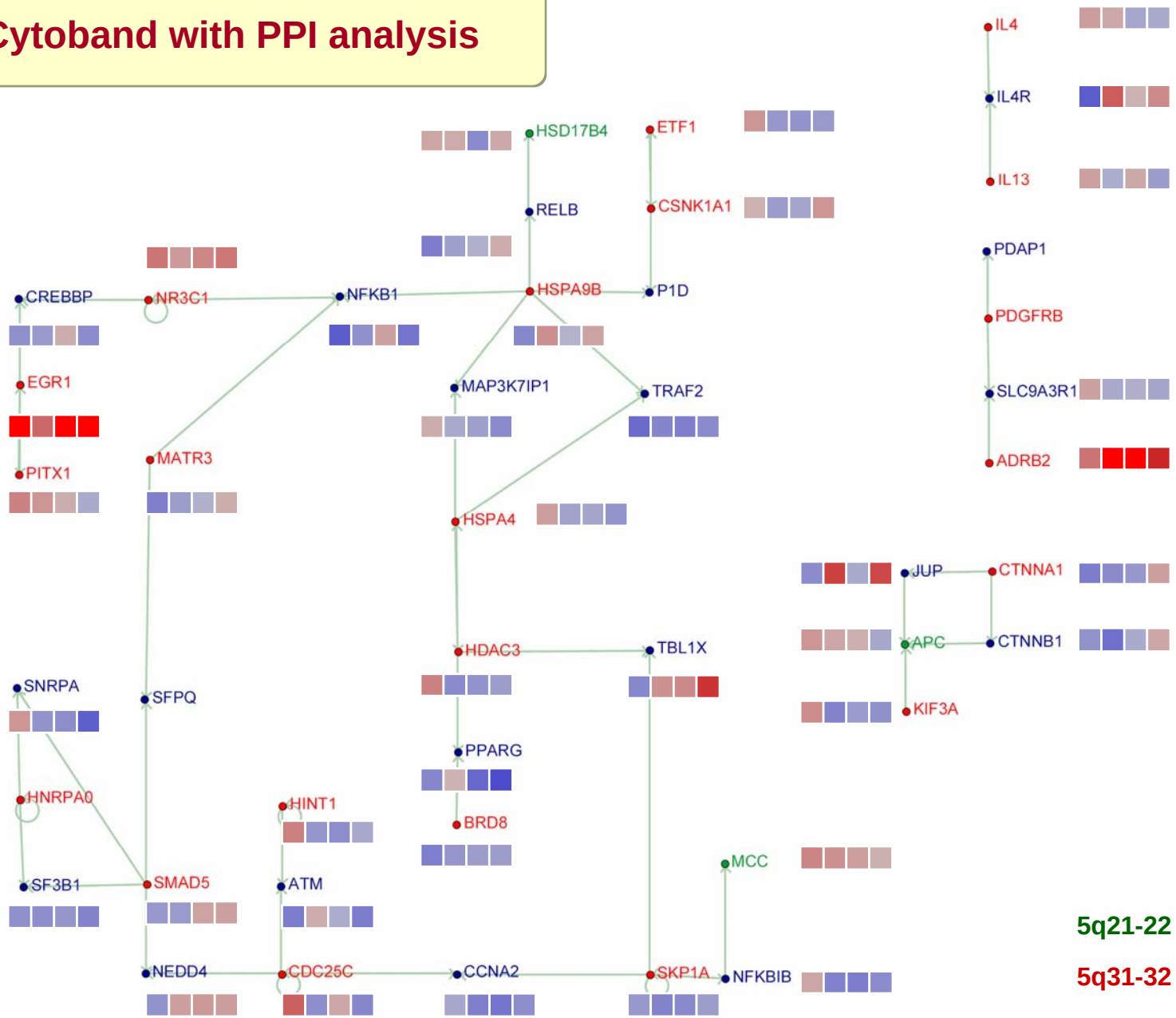
Trimming



Microarray gene list

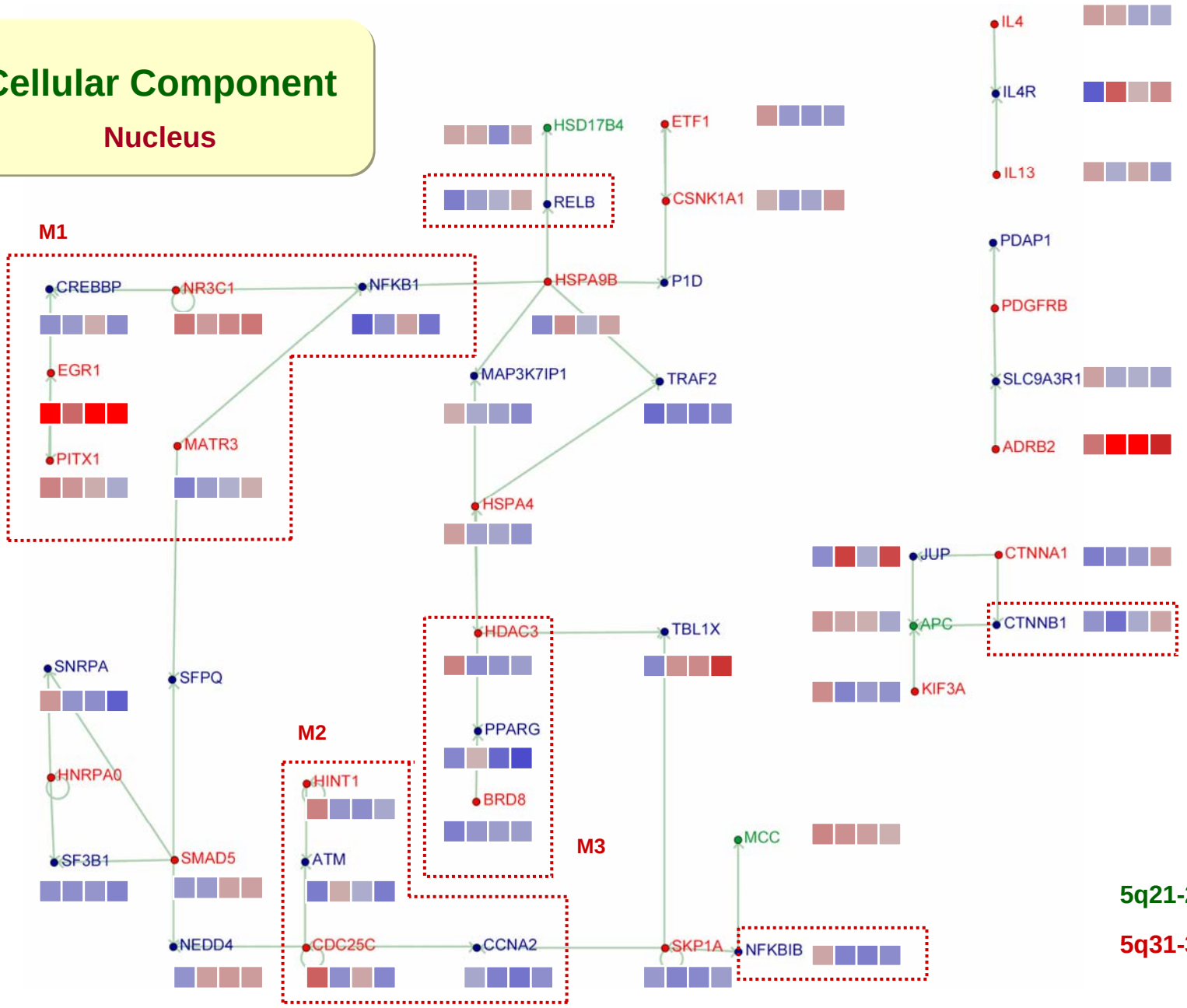


Cytoband with PPI analysis



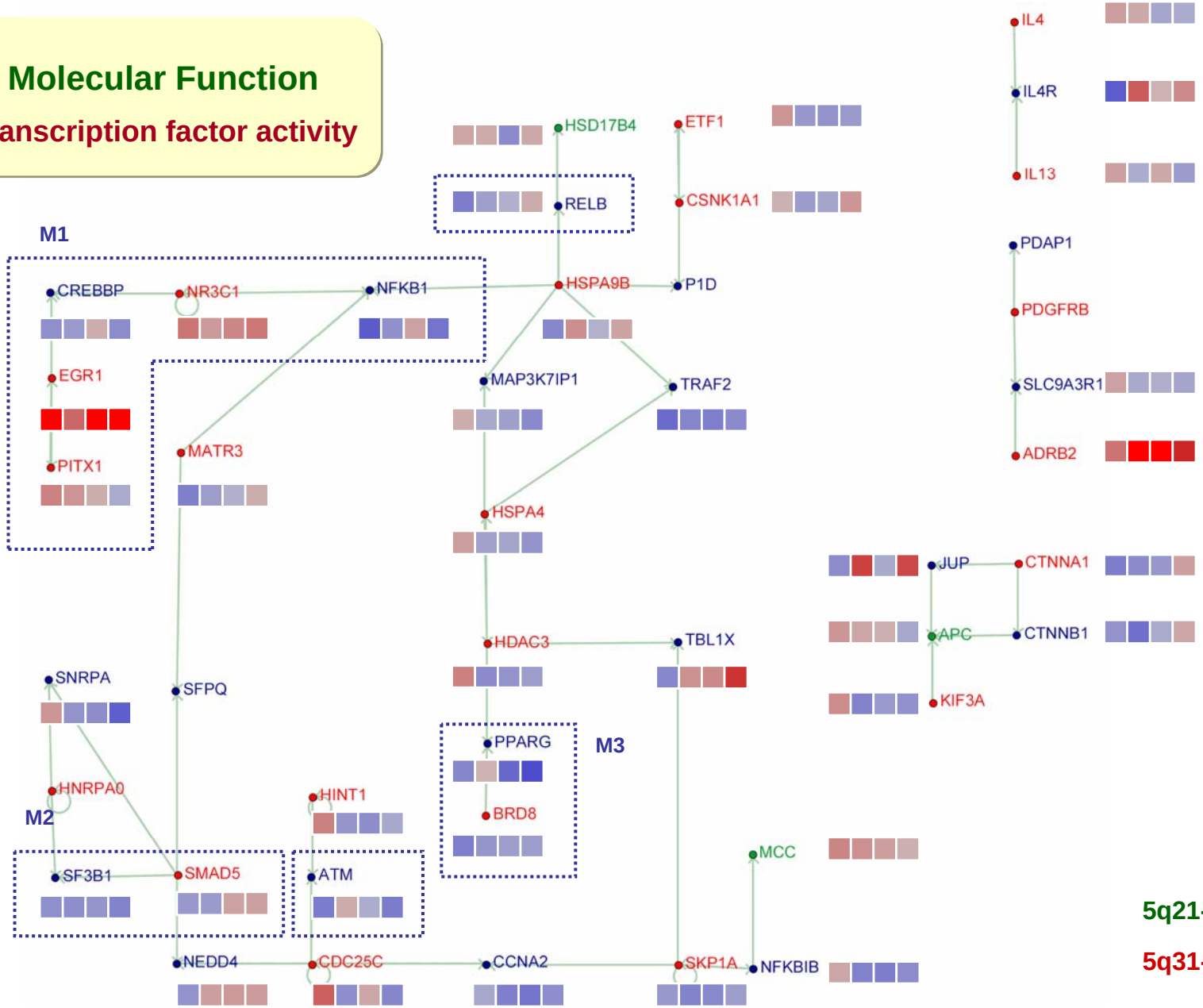
Cellular Component

Nucleus



Molecular Function

Transcription factor activity

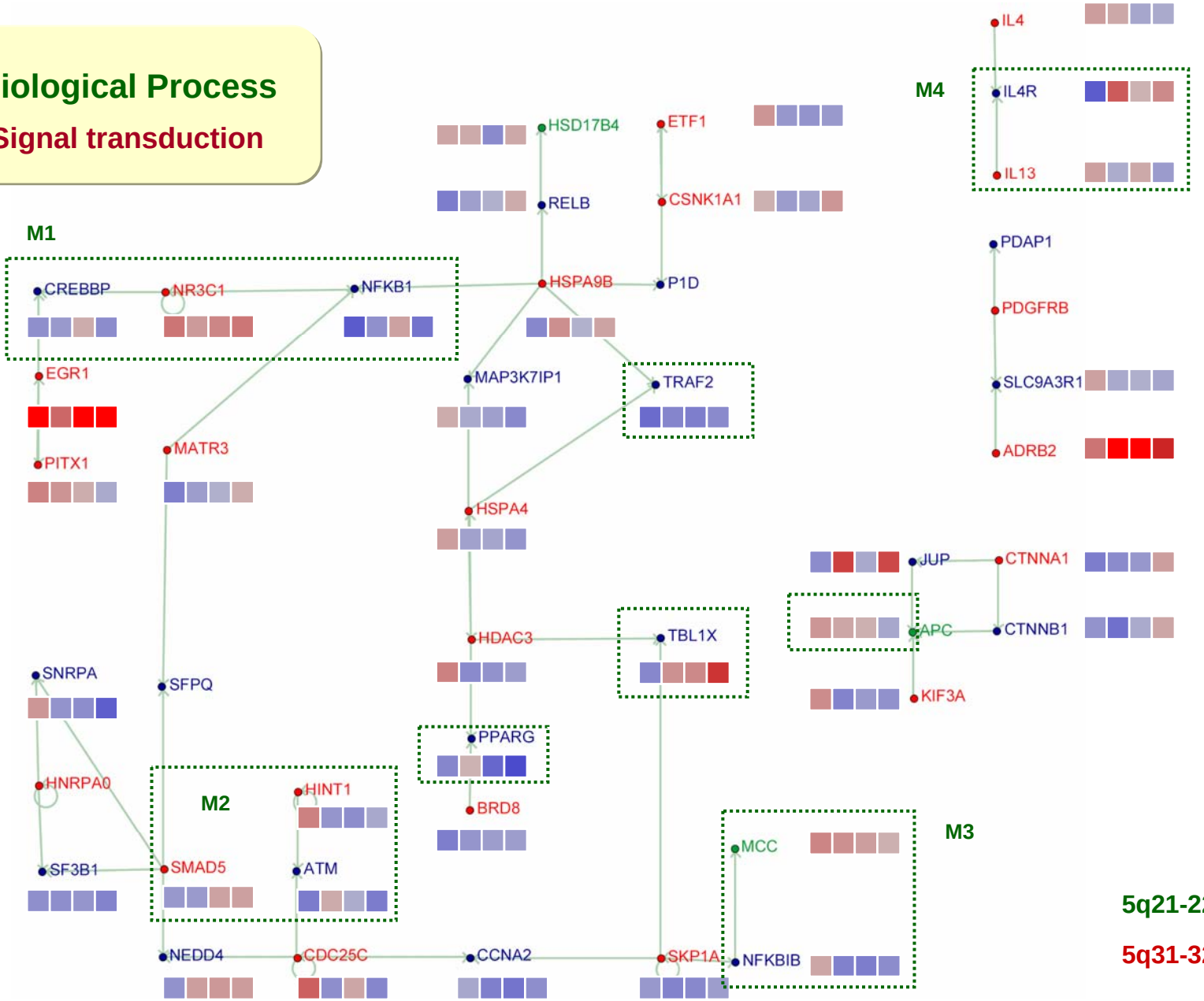


5q21-22

5q31-32

Biological Process

Signal transduction

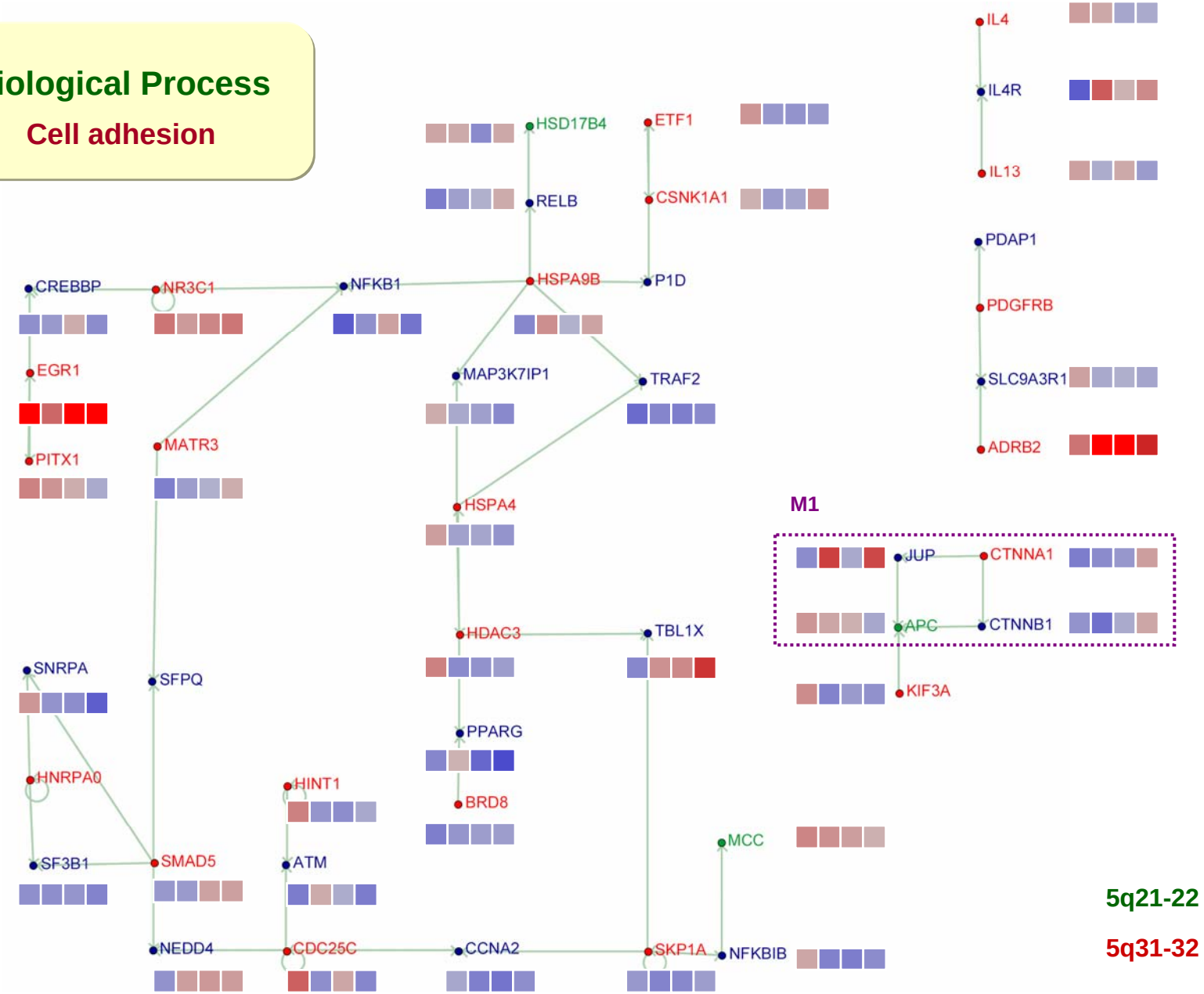


5q21-22

5q31-32

Biological Process

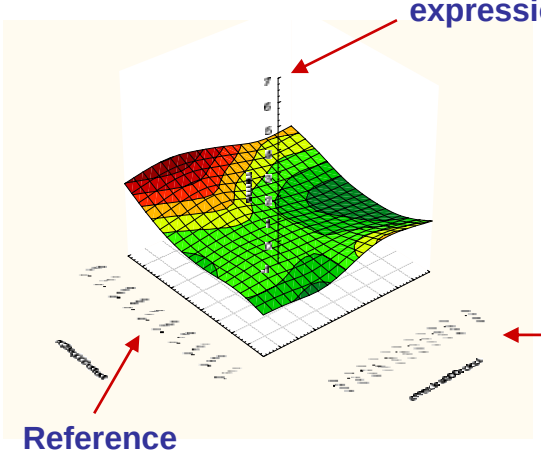
Cell adhesion

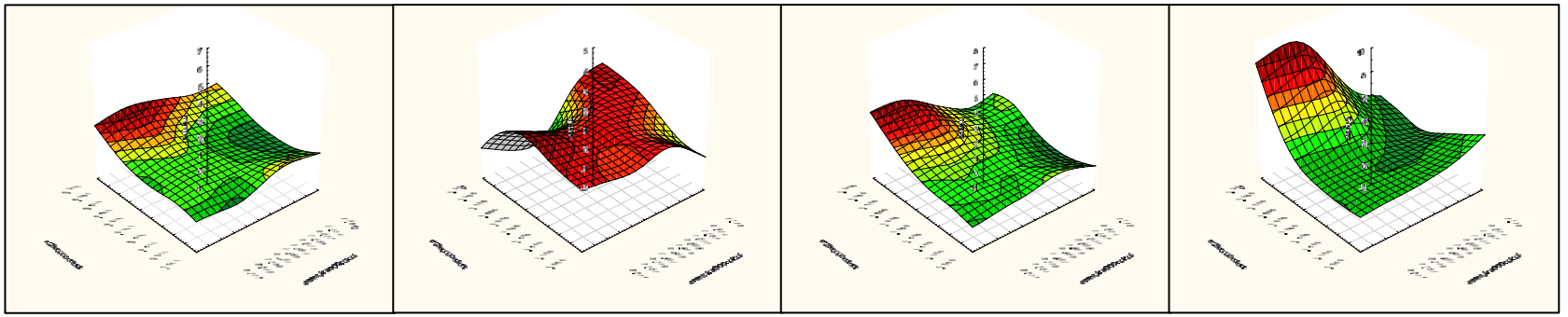


5q21-22

5q31-32

Expression relationship among microarray value, cDNA and reference sequences G+C content





SW1116 SW480 SW620 Colo205


Thanks for your attention
2004.12.17
The Introduction of Microarray Analysis Platform in NCKUMC