For written notes on this lecture, please read chapter 14 of *The Practical Bioinformatician*,

CS2220: Introduction to Computational Biology

Lecture 5: Gene Expression and Proteome Analysis

Limsoon Wong

22 February 2008

Plan

- **Microarray background**
- **Gene expression profile classification**
- **Gene expression profile clustering**
- **Extreme sample selection**
- **Intersection Analysis**

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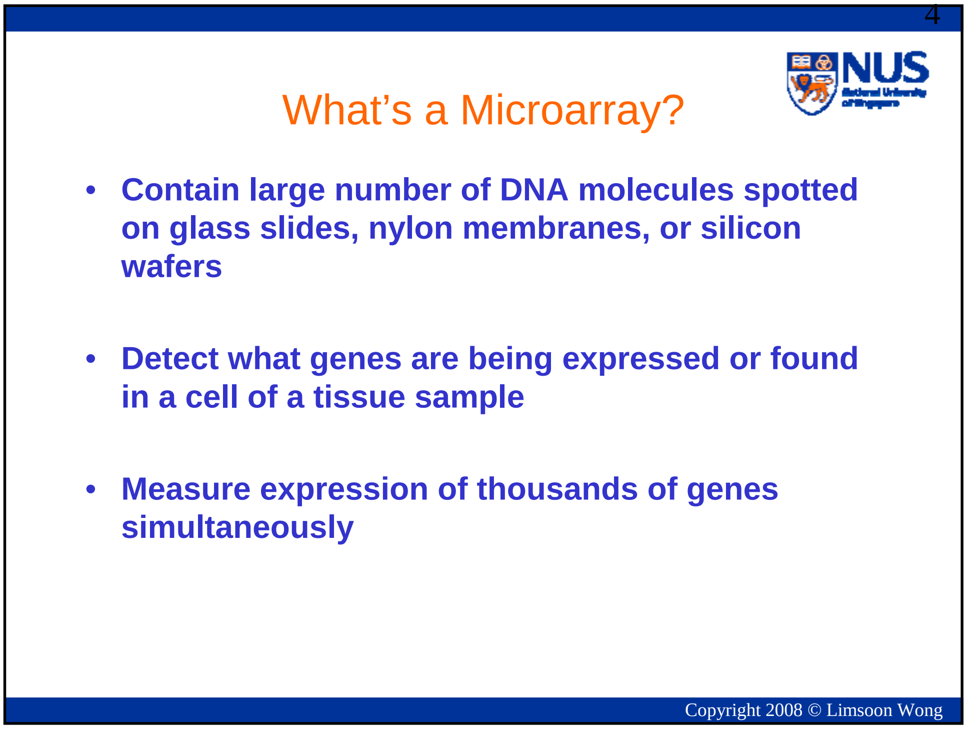
Background on Microarrays



What's a Microarray?

- **Contain large number of DNA molecules spotted on glass slides, nylon membranes, or silicon wafers**
- **Detect what genes are being expressed or found in a cell of a tissue sample**
- **Measure expression of thousands of genes simultaneously**

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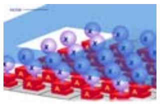
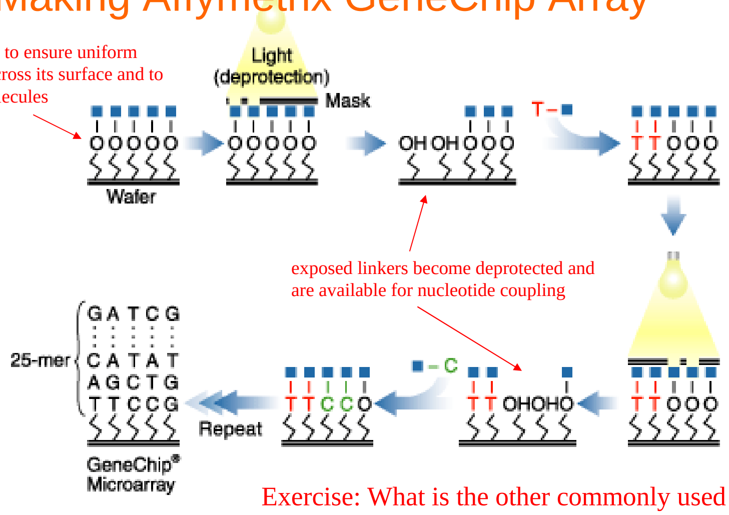


Affymetrix GeneChip Array

Making Affymetrix GeneChip Array

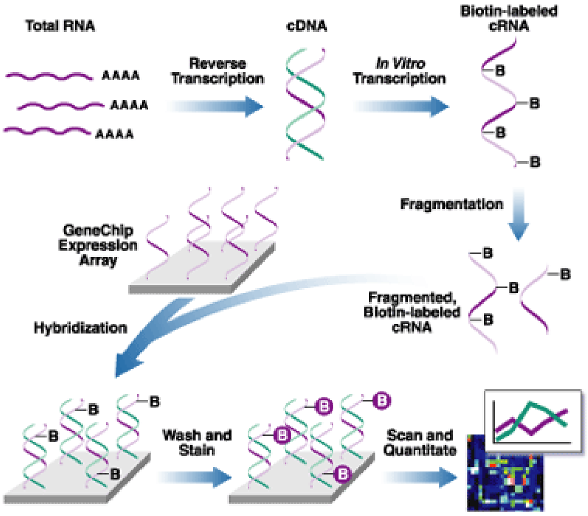
quartz is washed to ensure uniform hydroxylation across its surface and to attach linker molecules



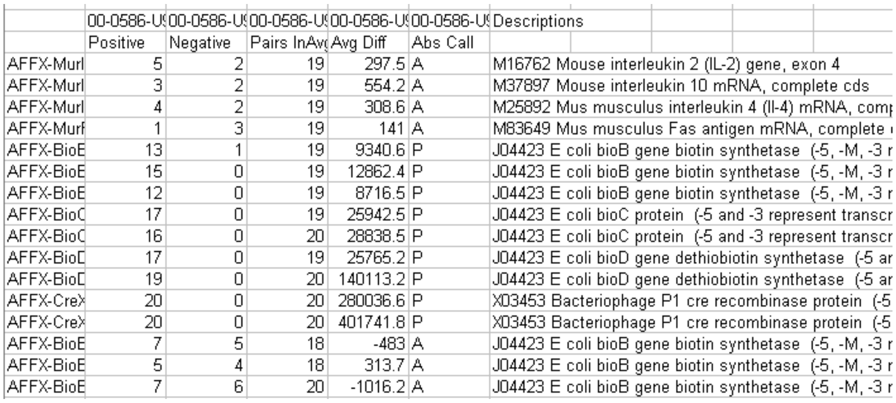
exposed linkers become deprotected and are available for nucleotide coupling

Exercise: What is the other commonly used type of microarray? How is that one different from Affymetrix's?

Gene Expression Measurement by Affymetrix GeneChip Array



A Sample Affymetrix GeneChip Data File (U95A)



	00-0586-U	00-0586-U	00-0586-U	00-0586-U	00-0586-U	Descriptions
	Positive	Negative	Pairs InAvg	Avg Diff	Abs Call	
AFFX-MurI	5	2	19	297.5	A	M16762 Mouse interleukin 2 (IL-2) gene, exon 4
AFFX-MurI	3	2	19	554.2	A	M37897 Mouse interleukin 10 mRNA, complete cds
AFFX-MurI	4	2	19	308.6	A	M25892 Mus musculus interleukin 4 (Il-4) mRNA, comp
AFFX-MurF	1	3	19	141	A	M83649 Mus musculus Fas antigen mRNA, complete
AFFX-BioE	13	1	19	9340.6	P	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioE	15	0	19	12862.4	P	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioE	12	0	19	8716.5	P	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioC	17	0	19	25942.5	P	J04423 E coli bioC protein (-5 and -3 represent transcr
AFFX-BioC	16	0	20	28838.5	P	J04423 E coli bioC protein (-5 and -3 represent transcr
AFFX-BioD	17	0	19	25765.2	P	J04423 E coli bioD gene dethiobiotin synthetase (-5 ar
AFFX-BioD	19	0	20	140113.2	P	J04423 E coli bioD gene dethiobiotin synthetase (-5 ar
AFFX-CreX	20	0	20	280036.6	P	X03453 Bacteriophage P1 cre recombinase protein (-5
AFFX-CreX	20	0	20	401741.8	P	X03453 Bacteriophage P1 cre recombinase protein (-5
AFFX-BioE	7	5	18	-483	A	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioE	5	4	18	313.7	A	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r
AFFX-BioE	7	6	20	-1016.2	A	J04423 E coli bioB gene biotin synthetase (-5, -M, -3 r

Some Advice on Affymetrix Gene Chip Data

- **Ignore AFFX genes**
  - These genes are control genes
- **Ignore genes with "Abs Call" equal to "A" or "M"**
  - Measurement quality is suspect
- **Upperbound 40000, lowerbound 100**
  - Accuracy of laser scanner
- **Deal with missing values**

Exercise: Suggest 2 ways to deal with missing value

Type of Gene Expression Datasets

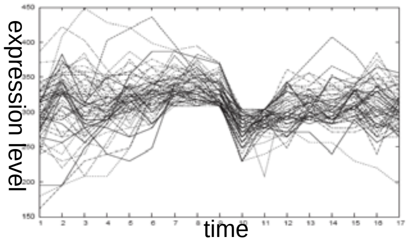
- Gene-Conditions or **Gene-Sample** (**numeric** or discretized)

1000 - 100,000 columns

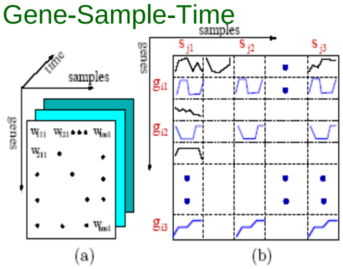
100-500 rows

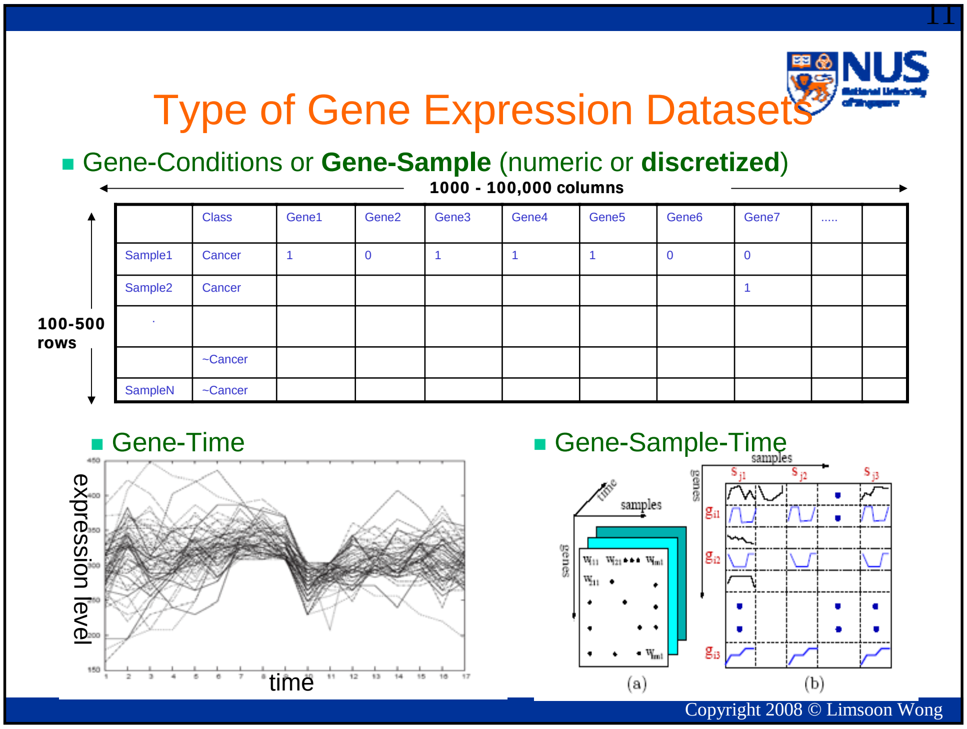
	Class	Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Gene7	.....	
Sample1	Cancer	0.12	-1.3	1.7	1.0	-3.2	0.78	-0.12		
Sample2	Cancer							1.3		
.										
	~Cancer									
SampleN	~Cancer									

- Gene-Time



- Gene-Sample-Time





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Type of Gene Expression Datasets

- Gene-Conditions or **Gene-Sample** (numeric or **discretized**)

1000 - 100,000 columns

100-500 rows

	Class	Gene1	Gene2	Gene3	Gene4	Gene5	Gene6	Gene7	.....	
Sample1	Cancer	1	0	1	1	1	0	0		
Sample2	Cancer							1		
.										
	~Cancer									
SampleN	~Cancer									

- Gene-Time
- Gene-Sample-Time

expression level
time
450
400
350
300
250
200
150
1
2
3
4
5
6
7
11
12
13
14
15
16
17
time
samples
genes
w_{111}
w_{211}
w_{m11}
w_{n11}
w_{nm1}
samples
genes
s_{j1}
s_{j2}
s_{j3}
g_{i1}
g_{i2}
g_{i3}
(a)
(b)

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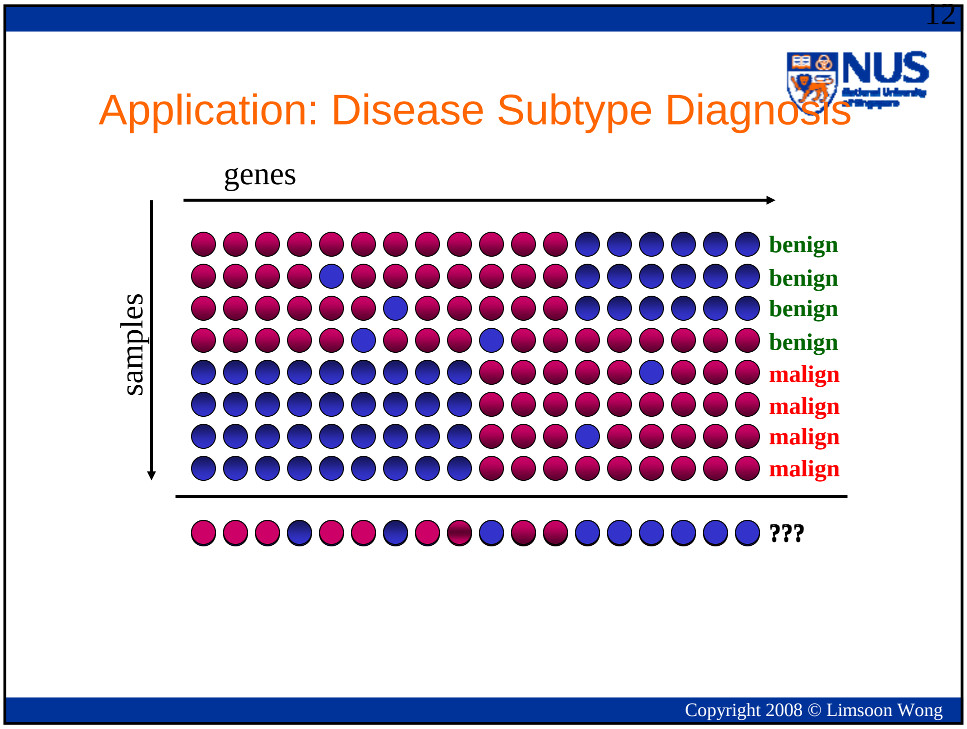

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Application: Disease Subtype Diagnosis

genes
samples
benign
benign
benign
benign
malign
malign
malign
malign
???

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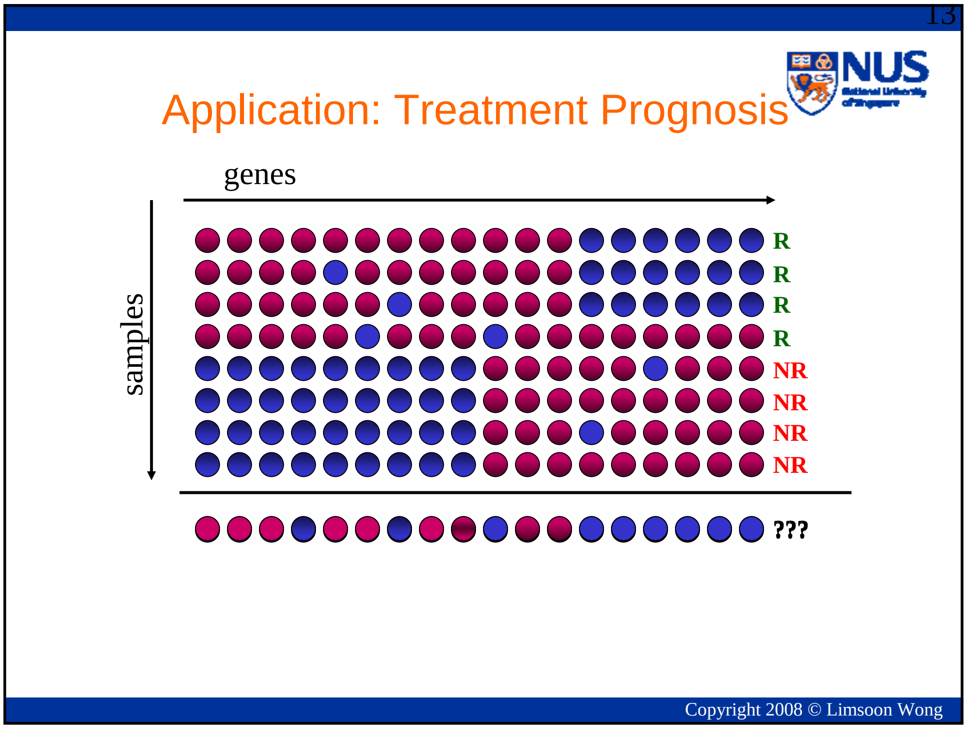

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Application: Treatment Prognosis

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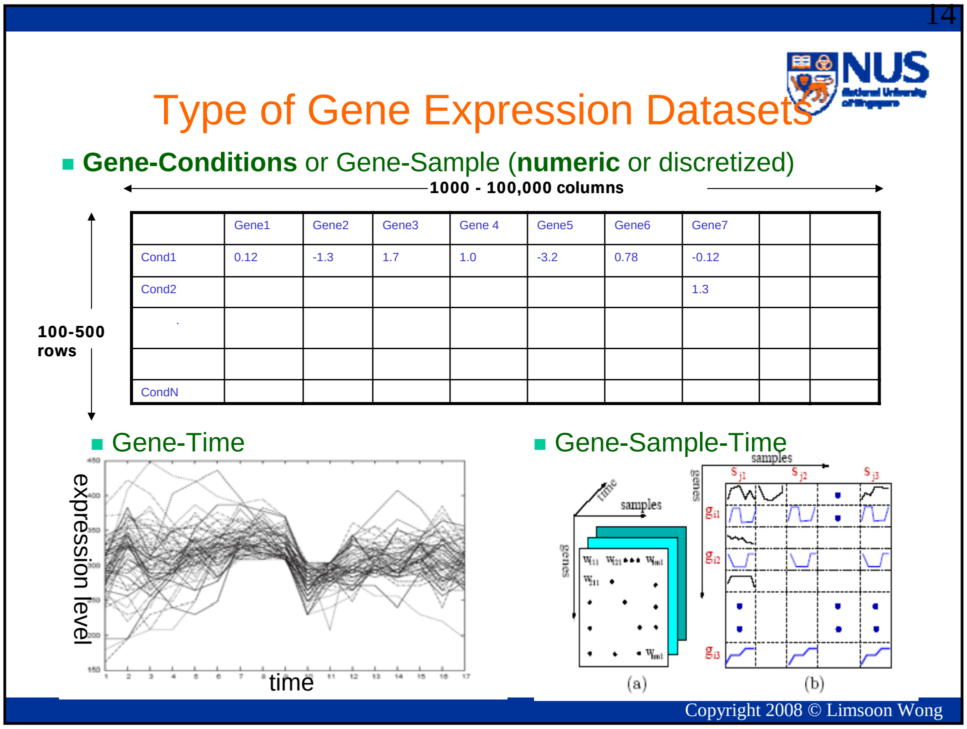

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Type of Gene Expression Datasets

- **Gene-Conditions** or Gene-Sample (**numeric** or discretized)

1000 - 100,000 columns

100-500 rows

	Gene1	Gene2	Gene3	Gene 4	Gene5	Gene6	Gene7		
Cond1	0.12	-1.3	1.7	1.0	-3.2	0.78	-0.12		
Cond2							1.3		
.									
CondN									

- Gene-Time
- Gene-Sample-Time

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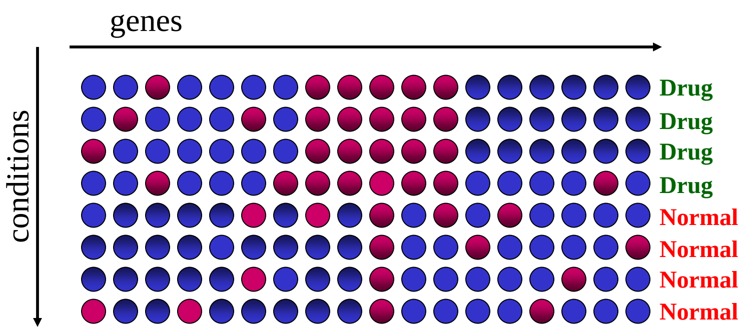
Application: Drug Action Detection



Which group of genes are the drug affecting on?

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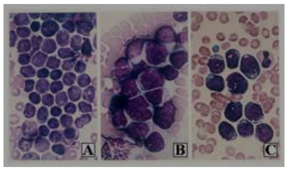
Gene Expression Profile Classification

Diagnosis of Childhood Acute Lymphoblastic Leukemia and Optimization of Risk-Benefit Ratio of Therapy

Childhood ALL

- **Major subtypes: T-ALL, E2A-PBX, TEL-AML, BCR-ABL, MLL genome rearrangements, Hyperdiploid>50**
- **Diff subtypes respond differently to same Tx**
- **Over-intensive Tx**
  - Development of secondary cancers
  - Reduction of IQ
- **Under-intensiveTx**
  - Relapse

- **The subtypes look similar**



- **Conventional diagnosis**
  - Immunophenotyping
  - Cytogenetics
  - Molecular diagnostics
- **Unavailable in most ASEAN countries**

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Mission

- **Conventional risk assignment procedure requires difficult expensive tests and collective judgement of multiple specialists**

- **Generally available only in major advanced hospitals**

⇒ **Can we have a single-test easy-to-use platform instead?**

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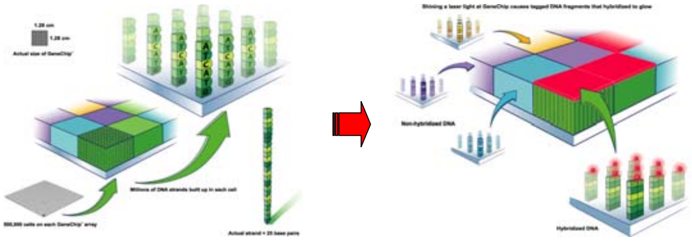
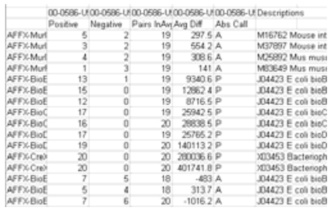
Single-Test Platform of Microarray & Machine Learning


1.28 cm
1.28 cm
Actual size of GeneChip
500,000 cells on each GeneChip array
Millions of DNA strands built up in each cell
Actual strand = 25 base pairs
Shining a laser light at GeneChip causes tagged DNA fragments that hybridized to glow
Non-hybridized DNA
Hybridized DNA



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AFFX-BioE	12	0	19	8716.5	P	J04423 E coli bioB
AFFX-BioC	17	0	19	25942.5	P	J04423 E coli bioC
AFFX-BioC	16	0	20	28838.5	P	J04423 E coli bioC
AFFX-BioD	17	0	19	25765.2	P	J04423 E coli bioD
AFFX-BioD	19	0	20	140113.2	P	J04423 E coli bioD
AFFX-Cre>	20	0	20	280036.6	P	X03453 Bacterioph
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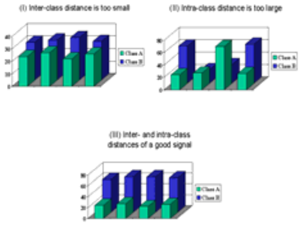
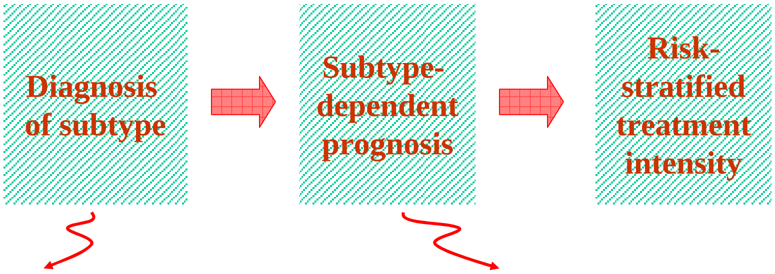


Overall Strategy



Diagnosis of subtype

Subtype-dependent prognosis

Risk-stratified treatment intensity

- For each subtype, select genes to develop classification model for diagnosing that subtype
- For each subtype, select genes to develop prediction model for prognosis of that subtype

Subtype Diagnosis by PCL

- Gene expression data collection
- Gene selection by $\chi 2$
- Classifier training by emerging pattern
- ~~Classifier tuning (optional for some machine learning methods)~~
- Apply classifier for diagnosis of future cases by PCL

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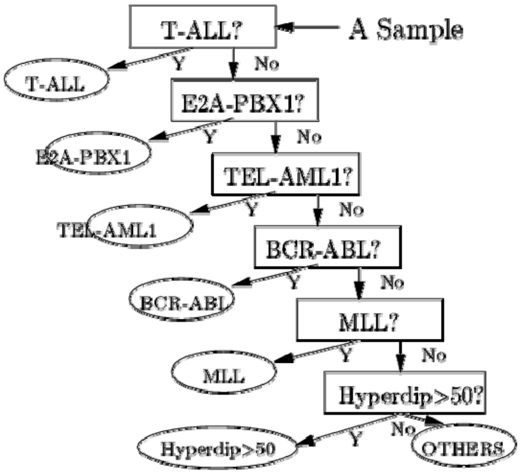
Childhood ALL Subtype Diagnosis Workflow

A tree-structured diagnostic workflow was recommended by our doctor collaborator



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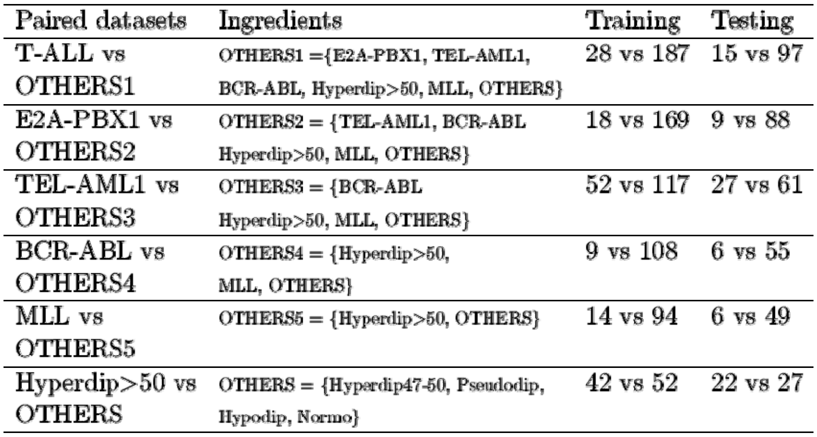
Training and Testing Sets



Paired datasets	Ingredients	Training	Testing
T-ALL vs OTHERS1	OTHERS1 ={E2A-PBX1, TEL-AML1, BCR-ABL, Hyperdip>50, MLL, OTHERS}	28 vs 187	15 vs 97
E2A-PBX1 vs OTHERS2	OTHERS2 = {TEL-AML1, BCR-ABL Hyperdip>50, MLL, OTHERS}	18 vs 169	9 vs 88
TEL-AML1 vs OTHERS3	OTHERS3 = {BCR-ABL Hyperdip>50, MLL, OTHERS}	52 vs 117	27 vs 61
BCR-ABL vs OTHERS4	OTHERS4 = {Hyperdip>50, MLL, OTHERS}	9 vs 108	6 vs 55
MLL vs OTHERS5	OTHERS5 = {Hyperdip>50, OTHERS}	14 vs 94	6 vs 49
Hyperdip>50 vs OTHERS	OTHERS = {Hyperdip47-50, Pseudodip, Hypodip, Normo}	42 vs 52	22 vs 27

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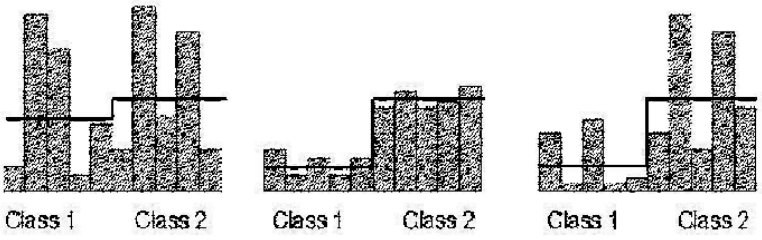
Signal Selection Basic Idea

- Choose a signal w/ low intra-class distance
- Choose a signal w/ high inter-class distance



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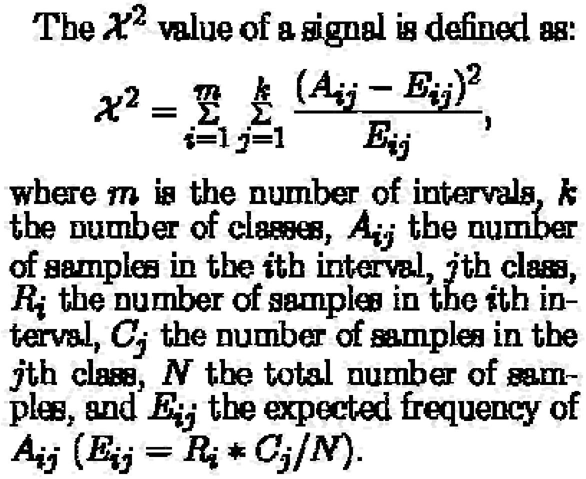
Signal Selection by χ2



The χ^2 value of a signal is defined as:

$$\chi^2 = \sum_{i=1}^{m} \sum_{j=1}^{k} \frac{(A_{ij} - E_{ij})^2}{E_{ij}},$$

where m is the number of intervals, k the number of classes, A_{ij} the number of samples in the ith interval, jth class, R_i the number of samples in the ith interval, C_j the number of samples in the jth class, N the total number of samples, and E_{ij} the expected frequency of A_{ij} $(E_{ij} = R_i * C_j / N)$.

Emerging Patterns

- **An emerging pattern is a set of conditions**
  - usually involving several features
  - that most members of a class satisfy
  - but none or few of the other class satisfy
- **A jumping emerging pattern is an emerging pattern that**
  - some members of a class satisfy
  - but no members of the other class satisfy
- **We use only jumping emerging patterns**

Examples

Patterns	Frequency (P)	Frequency(N)
{9, 36}	38 instances	0
{9, 23}	38	0
{4, 9}	38	0
{9, 14}	38	0
{6, 9}	38	0
{7, 21}	0	36
{7, 11}	0	35
{7, 43}	0	35
{7, 39}	0	34
{24, 29}	0	34

Easy interpretation

Reference number 9: the expression of gene 37720_at > 215
Reference number 36: the expression of gene 38028_at ≤ 12

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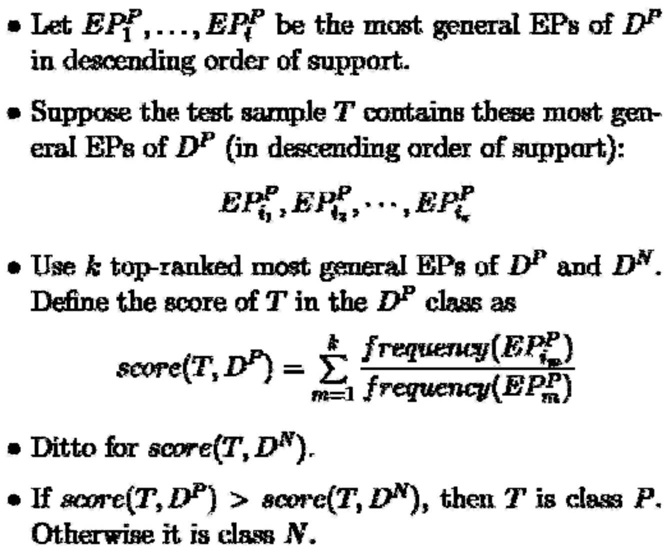
PCL: Prediction by Collective Likelihood



- Let $EP_1^P, \ldots, EP_i^P$ be the most general EPs of D^P in descending order of support.
- Suppose the test sample T contains these most general EPs of D^P (in descending order of support):

$$EP_{i_1}^P, EP_{i_2}^P, \cdots, EP_{i_x}^P$$

- Use k top-ranked most general EPs of D^P and D^N. Define the score of T in the D^P class as

$$score(T, D^P) = \sum_{m=1}^{k} \frac{frequency(EP_{i_m}^P)}{frequency(EP_m^P)}$$

- Ditto for $score(T, D^N)$.
- If $score(T, D^P) > score(T, D^N)$, then T is class P. Otherwise it is class N.

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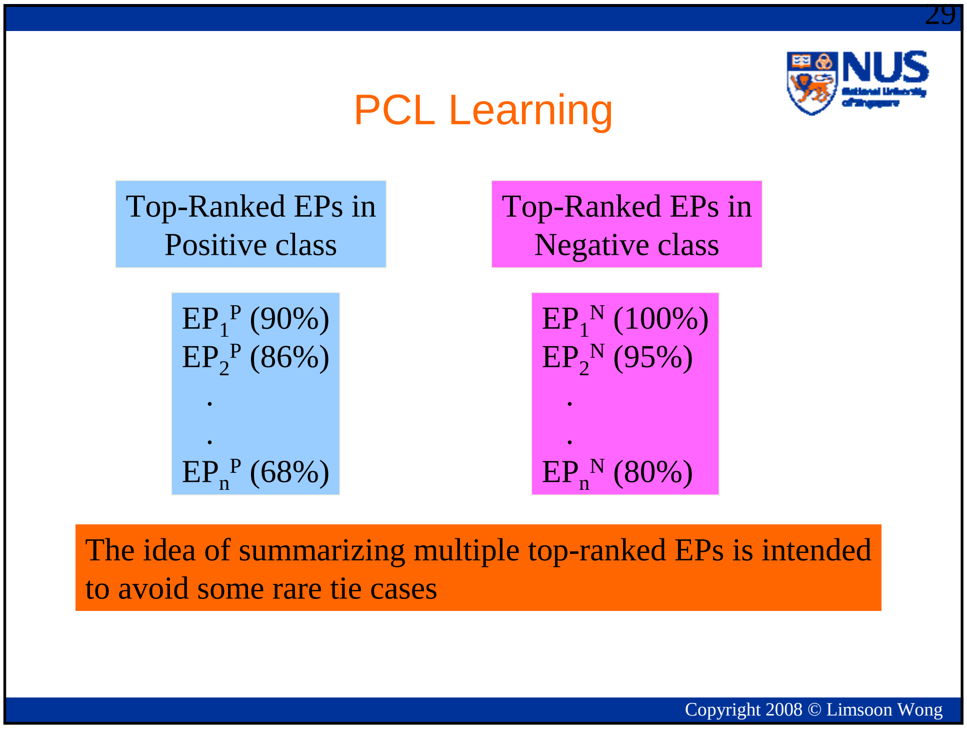


PCL Learning

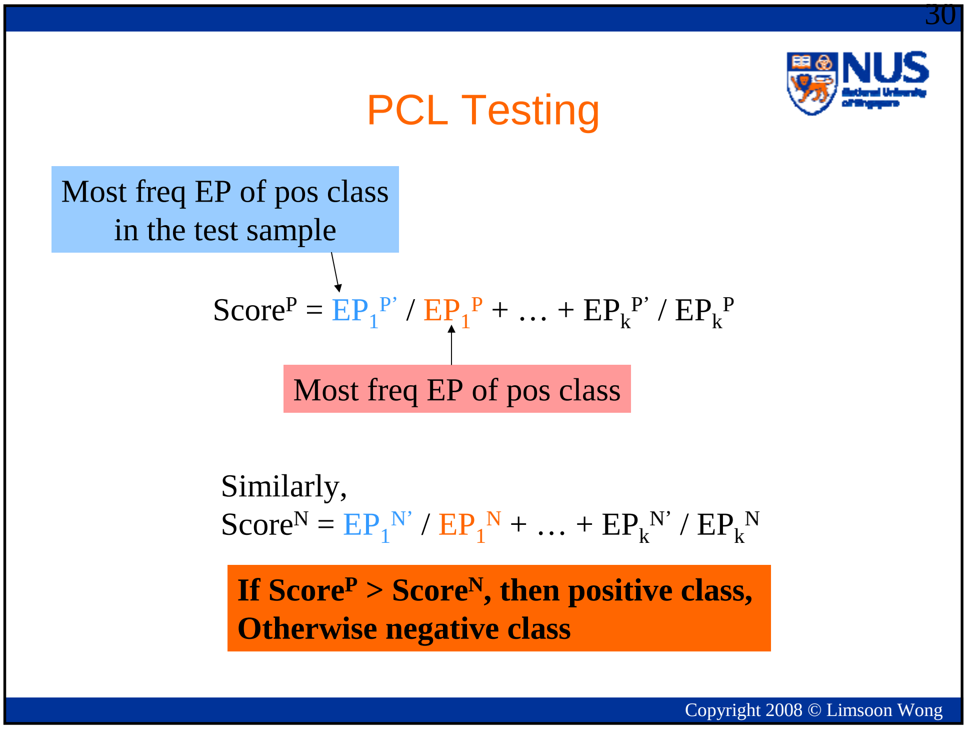
Top-Ranked EPs in Positive class

EP_1^P (90%)
EP_2^P (86%)
.
.
EP_n^P (68%)

Top-Ranked EPs in Negative class

EP_1^N (100%)
EP_2^N (95%)
.
.
EP_n^N (80%)

The idea of summarizing multiple top-ranked EPs is intended to avoid some rare tie cases



PCL Testing

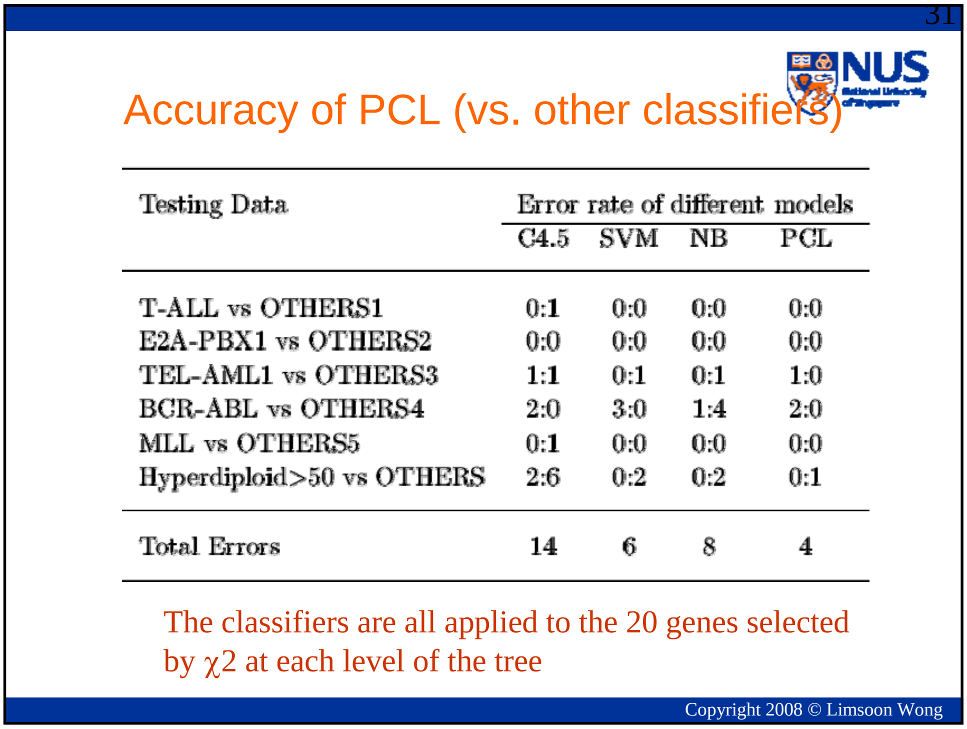
Most freq EP of pos class in the test sample

$$Score^P = EP_1^{P'} / EP_1^P + \ldots + EP_k^{P'} / EP_k^P$$

Most freq EP of pos class

Similarly,
$$Score^N = EP_1^{N'} / EP_1^N + \ldots + EP_k^{N'} / EP_k^N$$

If $Score^P > Score^N$, then positive class, Otherwise negative class



Accuracy of PCL (vs. other classifiers)

Testing Data	Error rate of different models			
	C4.5	SVM	NB	PCL
T-ALL vs OTHERS1	0:1	0:0	0:0	0:0
E2A-PBX1 vs OTHERS2	0:0	0:0	0:0	0:0
TEL-AML1 vs OTHERS3	1:1	0:1	0:1	1:0
BCR-ABL vs OTHERS4	2:0	3:0	1:4	2:0
MLL vs OTHERS5	0:1	0:0	0:0	0:0
Hyperdiploid>50 vs OTHERS	2:6	0:2	0:2	0:1
Total Errors	**14**	**6**	**8**	**4**

The classifiers are all applied to the 20 genes selected by χ2 at each level of the tree

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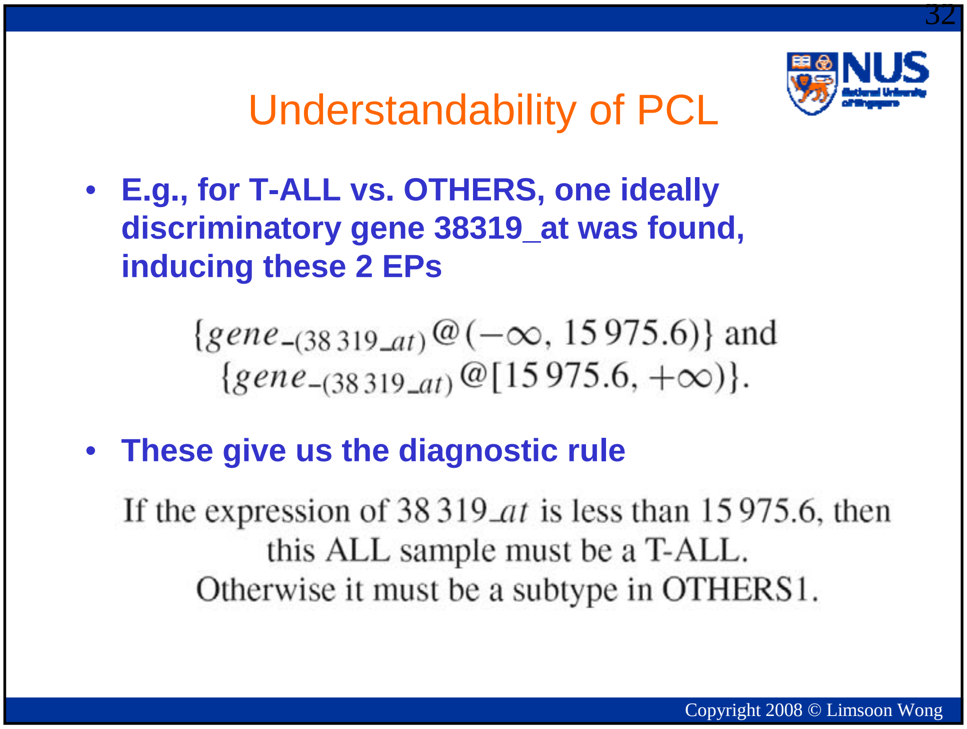

Understandability of PCL

- **E.g., for T-ALL vs. OTHERS, one ideally discriminatory gene 38319_at was found, inducing these 2 EPs**

$$\{gene_{(38\,319\_at)}@(-\infty, 15\,975.6)\} \text{ and }$$
$$\{gene_{(38\,319\_at)}@[15\,975.6, +\infty)\}.$$

- **These give us the diagnostic rule**

If the expression of 38 319_*at* is less than 15 975.6, then this ALL sample must be a T-ALL. Otherwise it must be a subtype in OTHERS1.

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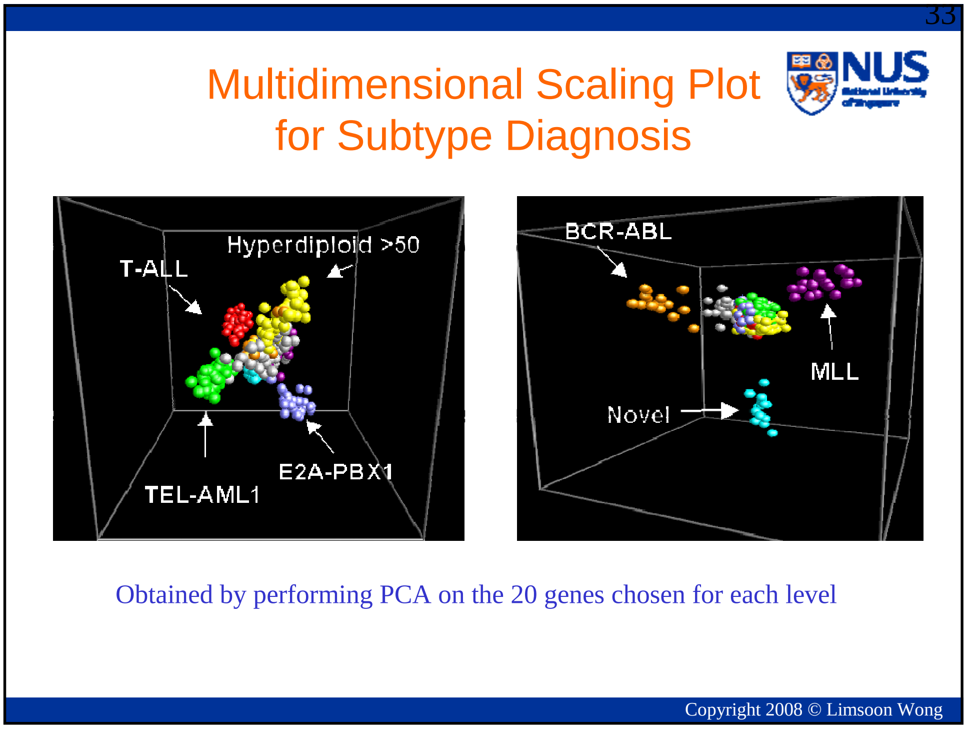

Multidimensional Scaling Plot for Subtype Diagnosis

Obtained by performing PCA on the 20 genes chosen for each level

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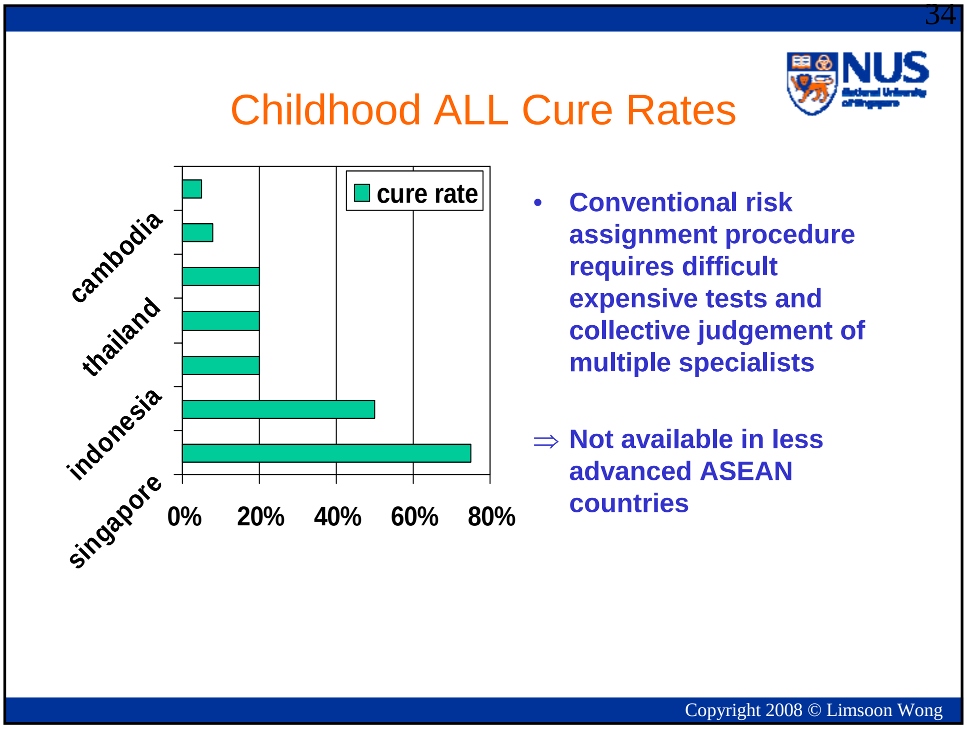

Childhood ALL Cure Rates

- Conventional risk assignment procedure requires difficult expensive tests and collective judgement of multiple specialists

⇒ Not available in less advanced ASEAN countries

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Childhood ALL Treatment Cost

- **Treatment for childhood ALL over 2 yrs**
  - Intermediate intensity: US$60k
  - Low intensity: US$36k
  - High intensity: US$72k
- **Treatment for relapse: US$150k**
- **Cost for side-effects: Unquantified**

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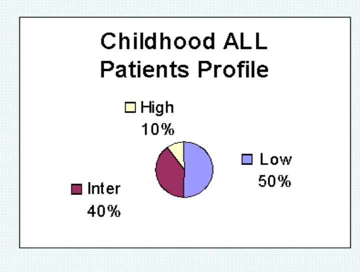
Current Situation (2000 new cases/yr in ASEAN)



- **Intermediate intensity conventionally applied in less advanced ASEAN countries**

- **Over intensive for 50% of patients, thus more side effects**
- **Under intensive for 10% of patients, thus more relapse**
- **US$120m (US$60k * 2000) for intermediate intensity tx**
- **US$30m (US$150k * 2000 * 10%) for relapse tx**
- **Total US$150m/yr plus un-quantified costs for dealing with side effects**

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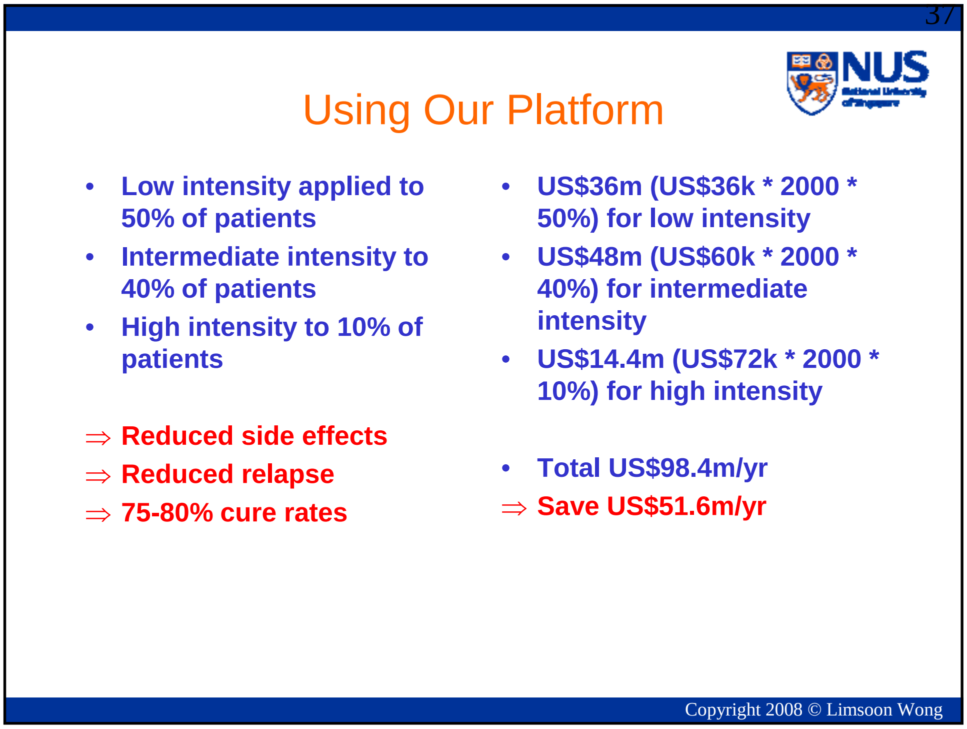

Using Our Platform

- Low intensity applied to 50% of patients
- Intermediate intensity to 40% of patients
- High intensity to 10% of patients

⇒ Reduced side effects
⇒ Reduced relapse
⇒ 75-80% cure rates

- US$36m (US$36k * 2000 * 50%) for low intensity
- US$48m (US$60k * 2000 * 40%) for intermediate intensity
- US$14.4m (US$72k * 2000 * 10%) for high intensity

- Total US$98.4m/yr

⇒ Save US$51.6m/yr

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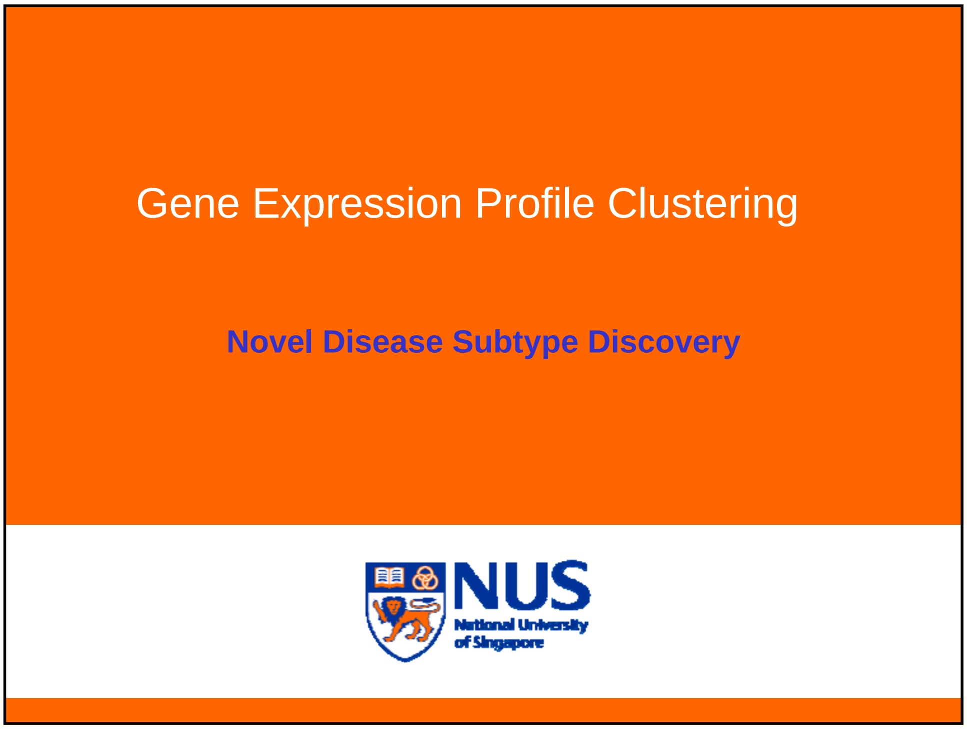
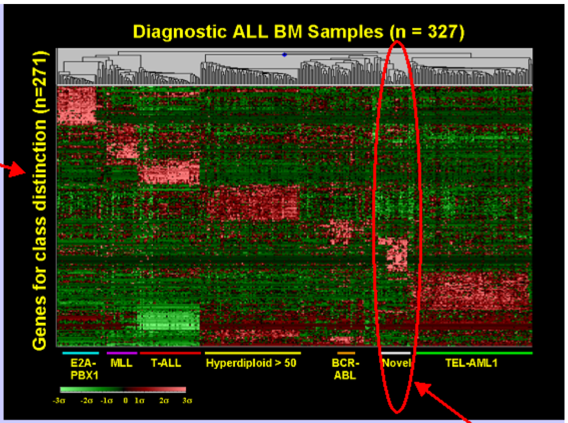


Gene Expression Profile Clustering

Novel Disease Subtype Discovery

Is there a new subtype?



Genes selected by χ2

New subtype discovered

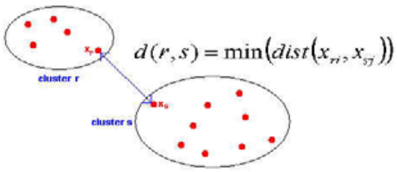
- **Hierarchical clustering of gene expression profiles reveals a novel subtype of childhood ALL**

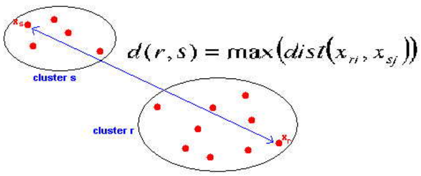
Exercise: Name and describe one bi-clustering method

Hierarchical Clustering

- **Assign each item to its own cluster**
  – If there are N items initially, we get N clusters, each containing just one item
- **Find the “most similar” pair of clusters, merge them into a single cluster, so we now have one less cluster**
  – “Similarity” is often defined using
    - **Single linkage**
    - **Complete linkage**
    - **Average linkage**
- **Repeat previous step until all items are clustered into a single cluster of size N**

Single, Complete, & Average Linkage





Single linkage defines distance betw two clusters as min distance betw them

Complete linkage defines distance betw two clusters as max distance betw them

Exercise: Give definition of “average linkage”

Image source: UCL Microcore Website

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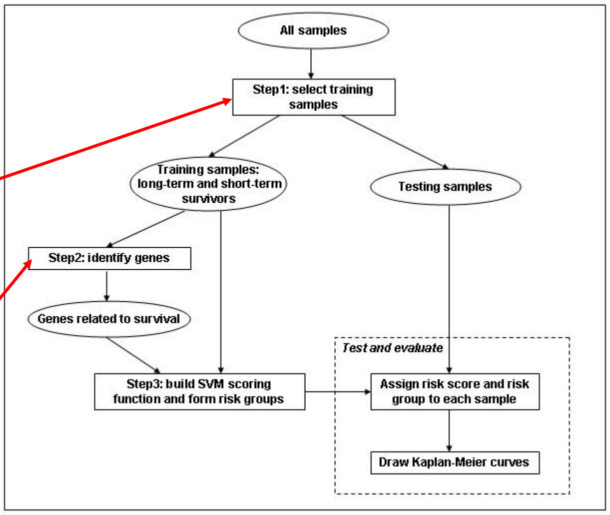
Selection of Patient Samples and Genes for Disease Prognosis

Gene Expression Profile + Clinical Data ⇒ Outcome Prediction

- **Univariate & multivariate Cox survival analysis** (Beer et al 2002, Rosenwald et al 2002)
- **Fuzzy neural network** (Ando et al 2002)
- **Partial least squares regression** (Park et al 2002)
- **Weighted voting algorithm** (Shipp et al 2002)
- **Gene index and “reference gene”** (LeBlanc et al 2003)
- ……

Our Approach



All samples

Step1: select training samples

Training samples: long-term and short-term survivors

Testing samples

Step2: identify genes

Genes related to survival

Step3: build SVM scoring function and form risk groups

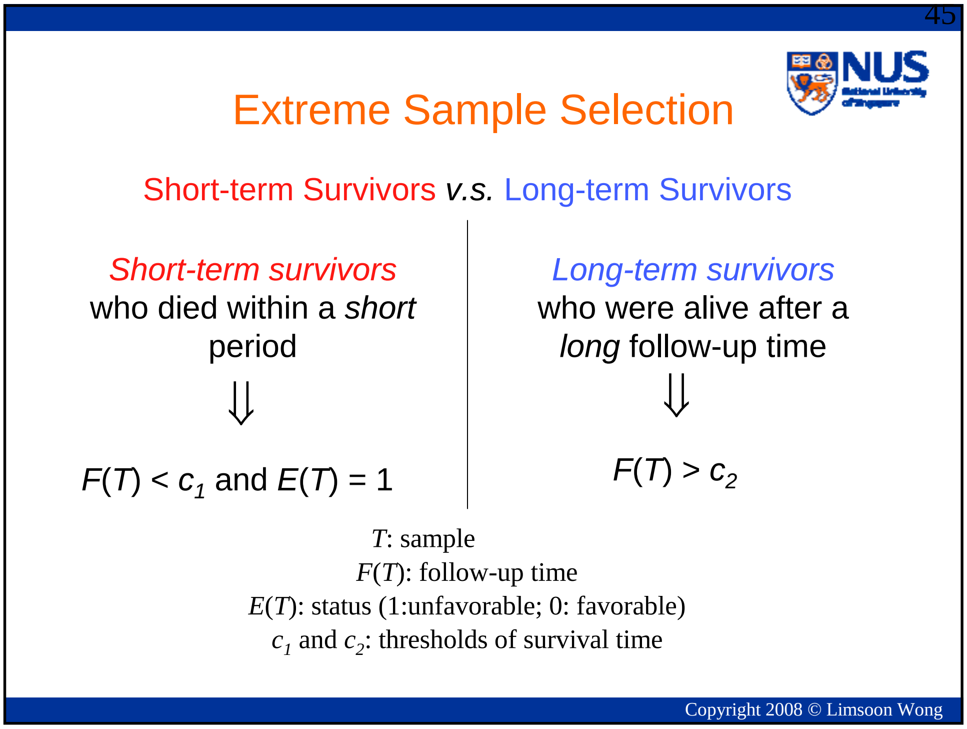
Test and evaluate

Assign risk score and risk group to each sample

Draw Kaplan-Meier curves

“extreme” sample selection

ERCOF



Extreme Sample Selection

Short-term Survivors *v.s.* Long-term Survivors

Short-term survivors who died within a *short* period

⇓

$F(T) < c_1$ and $E(T) = 1$

Long-term survivors who were alive after a *long* follow-up time

⇓

$F(T) > c_2$

T: sample
$F(T)$: follow-up time
$E(T)$: status (1:unfavorable; 0: favorable)
c_1 and c_2: thresholds of survival time

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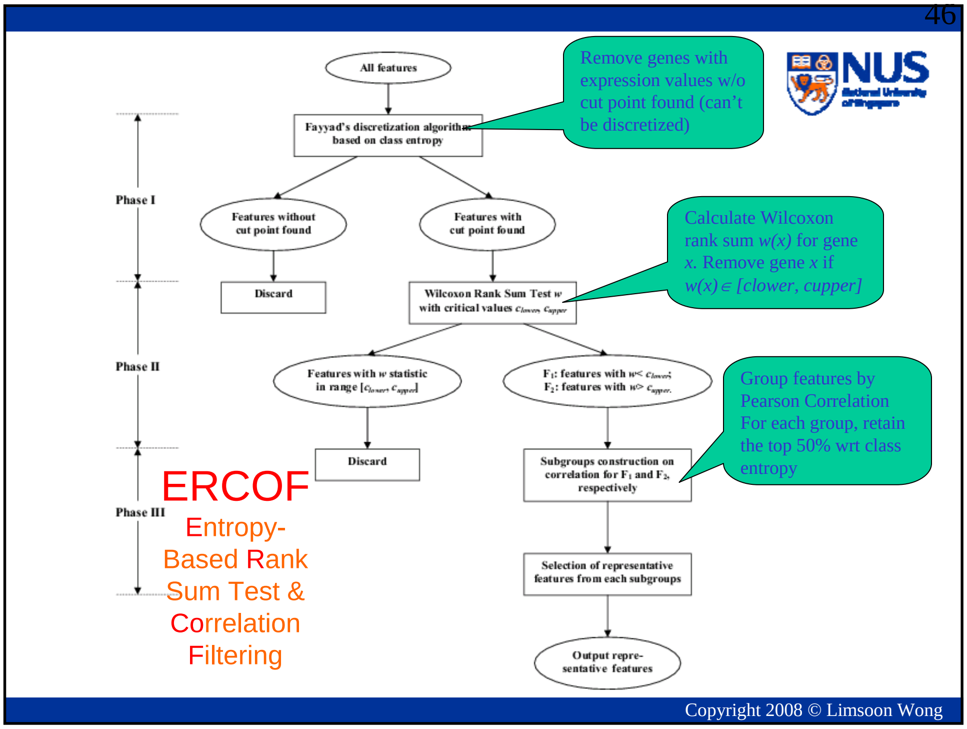
All features
Fayyad's discretization algorithm based on class entropy
Phase I
Features without cut point found
Features with cut point found
Discard
Wilcoxon Rank Sum Test w with critical values c_{lower}, c_{upper}
Phase II
Features with w statistic in range $[c_{lower}, c_{upper}]$
F_1: features with $w < c_{lower}$; F_2: features with $w > c_{upper}$.
Discard
Subgroups construction on correlation for F_1 and F_2, respectively
Phase III
Selection of representative features from each subgroups
Output representative features

Remove genes with expression values w/o cut point found (can't be discretized)

Calculate Wilcoxon rank sum *w(x)* for gene *x*. Remove gene *x* if *w(x) ∈ [clower, cupper]*

Group features by Pearson Correlation For each group, retain the top 50% wrt class entropy

ERCOF

Entropy-Based Rank Sum Test & Correlation Filtering

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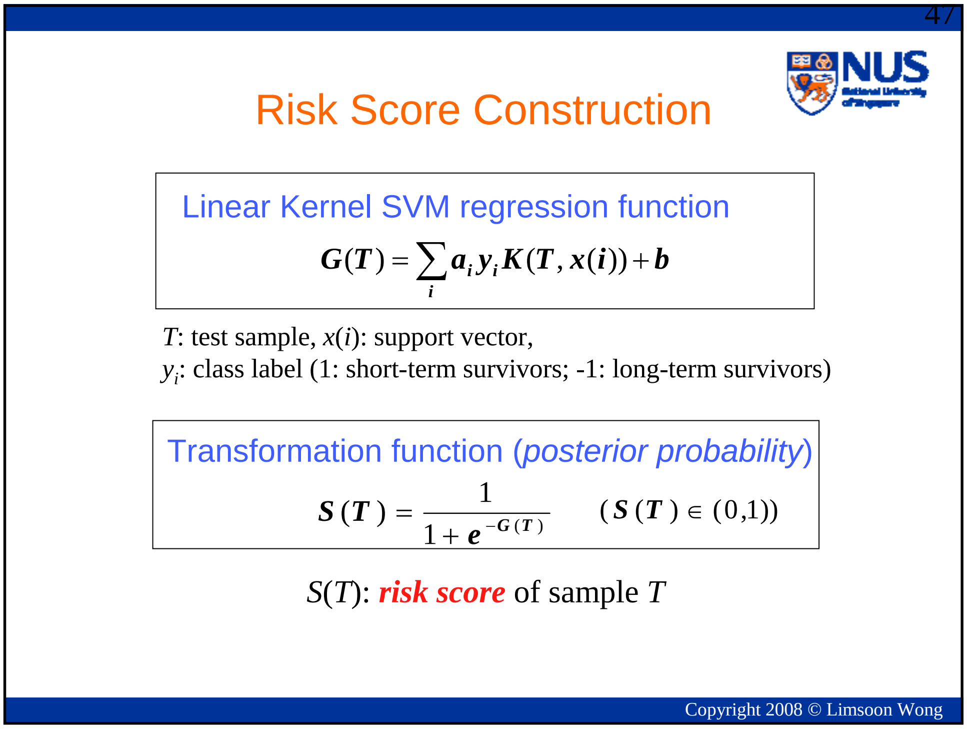

Risk Score Construction

Linear Kernel SVM regression function

$$G(T) = \sum_i a_i y_i K(T, x(i)) + b$$

T: test sample, *x*(*i*): support vector,
y_i: class label (1: short-term survivors; -1: long-term survivors)

Transformation function (*posterior probability*)

$$S(T) = \frac{1}{1 + e^{-G(T)}} \qquad (S(T) \in (0,1))$$

S(*T*): ***risk score*** of sample *T*

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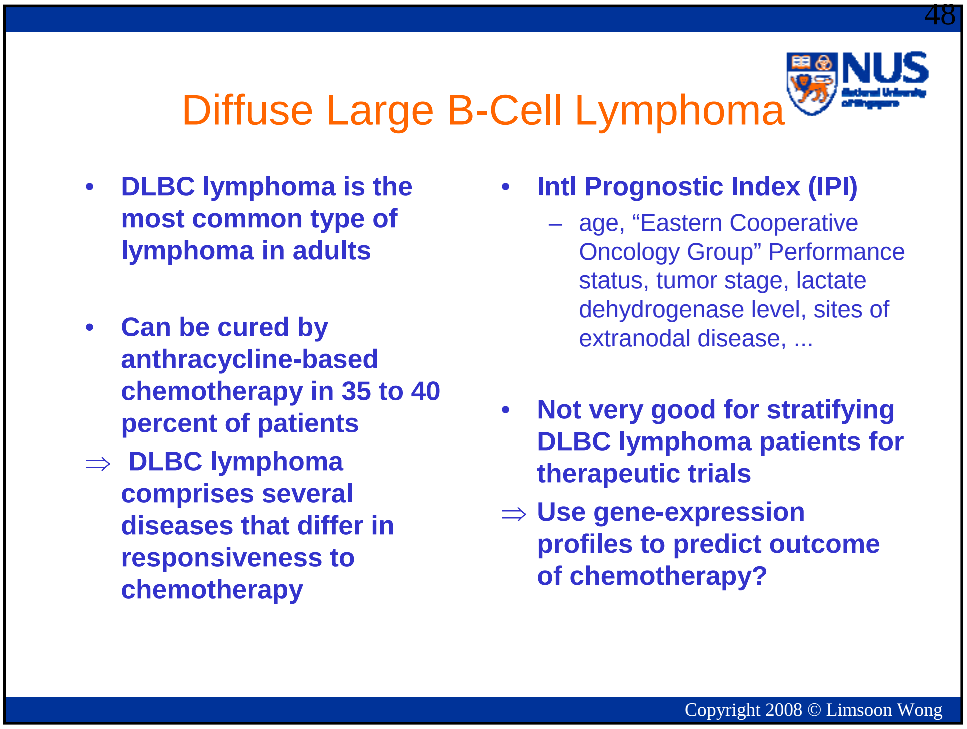

Diffuse Large B-Cell Lymphoma

- **DLBC lymphoma is the most common type of lymphoma in adults**

- **Can be cured by anthracycline-based chemotherapy in 35 to 40 percent of patients**

⇒ **DLBC lymphoma comprises several diseases that differ in responsiveness to chemotherapy**

- **Intl Prognostic Index (IPI)**
  - age, “Eastern Cooperative Oncology Group” Performance status, tumor stage, lactate dehydrogenase level, sites of extranodal disease, ...

- **Not very good for stratifying DLBC lymphoma patients for therapeutic trials**

⇒ **Use gene-expression profiles to predict outcome of chemotherapy?**

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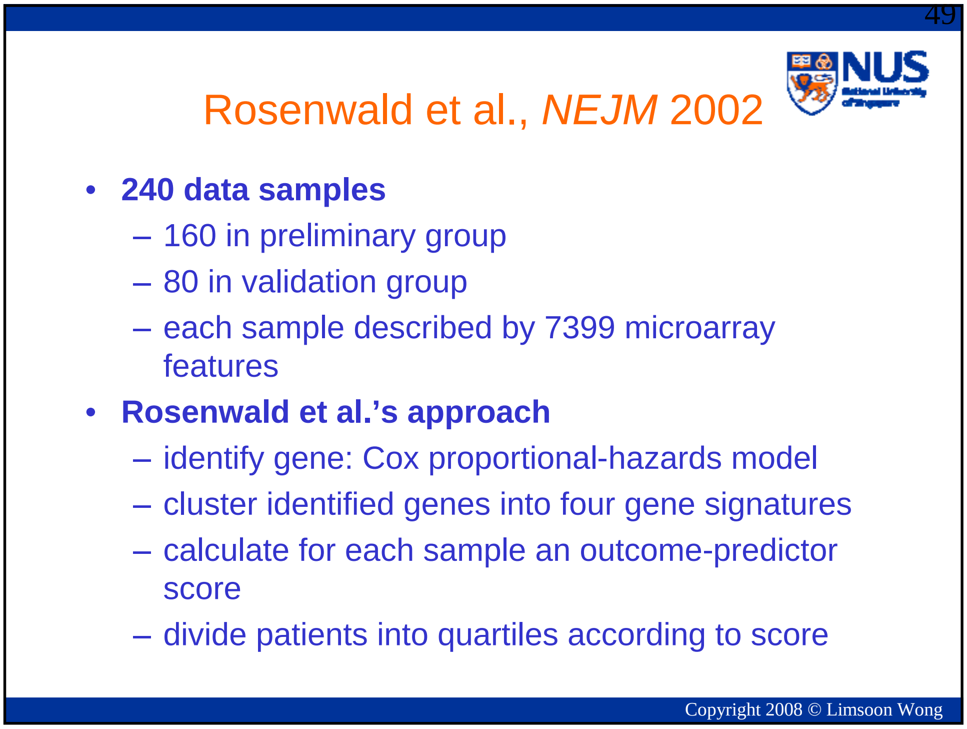

Rosenwald et al., *NEJM* 2002

- **240 data samples**
  - 160 in preliminary group
  - 80 in validation group
  - each sample described by 7399 microarray features
- **Rosenwald et al.'s approach**
  - identify gene: Cox proportional-hazards model
  - cluster identified genes into four gene signatures
  - calculate for each sample an outcome-predictor score
  - divide patients into quartiles according to score

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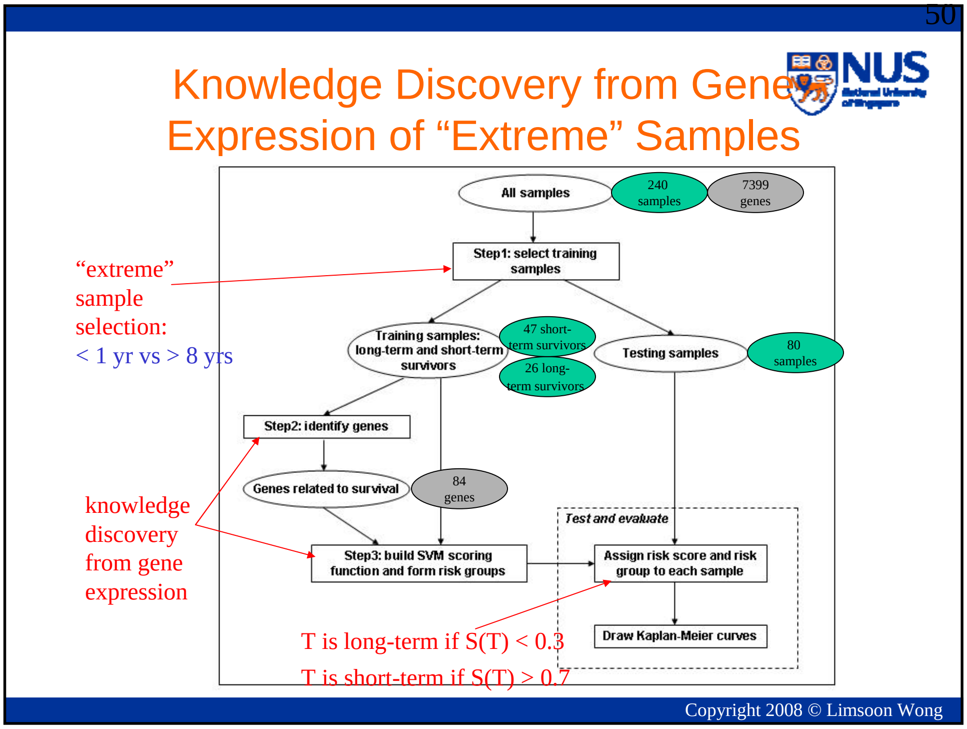

Knowledge Discovery from Gene Expression of "Extreme" Samples

All samples
240 samples
7399 genes
Step1: select training samples
Training samples: long-term and short-term survivors
47 short-term survivors
26 long-term survivors
Testing samples
80 samples
Step2: identify genes
Genes related to survival
84 genes
Test and evaluate
Step3: build SVM scoring function and form risk groups
Assign risk score and risk group to each sample
Draw Kaplan-Meier curves

"extreme" sample selection:
< 1 yr vs > 8 yrs

knowledge discovery from gene expression

T is long-term if S(T) < 0.3
T is short-term if S(T) > 0.7

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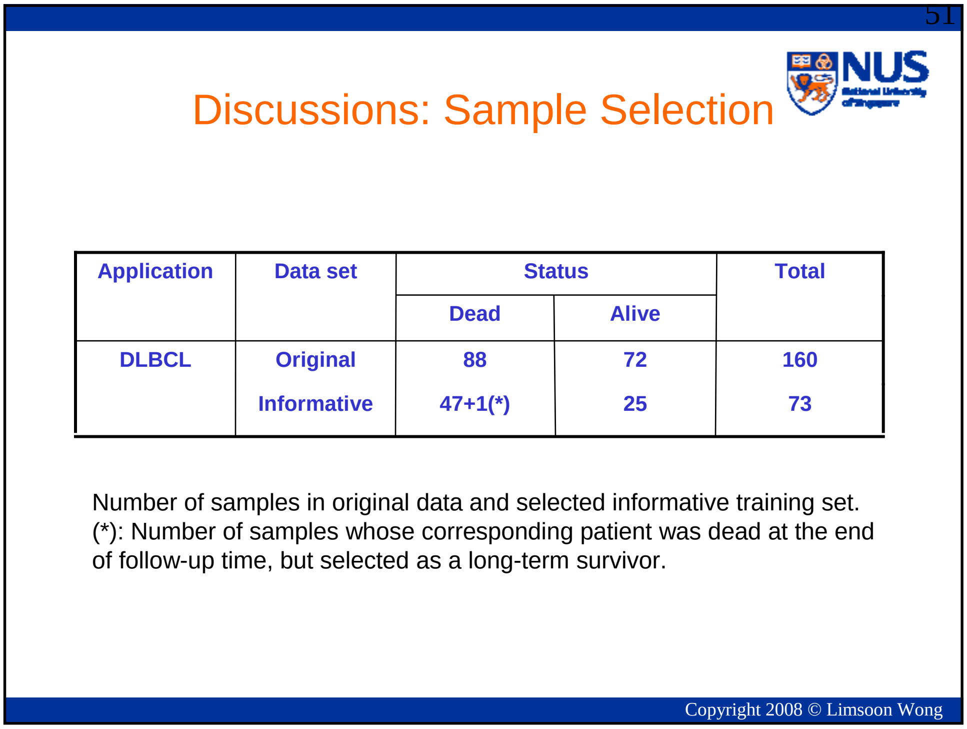

Discussions: Sample Selection

Application	Data set	Status		Total
		Dead	Alive	
DLBCL	Original	88	72	160
	Informative	47+1(*)	25	73

Number of samples in original data and selected informative training set. (*): Number of samples whose corresponding patient was dead at the end of follow-up time, but selected as a long-term survivor.

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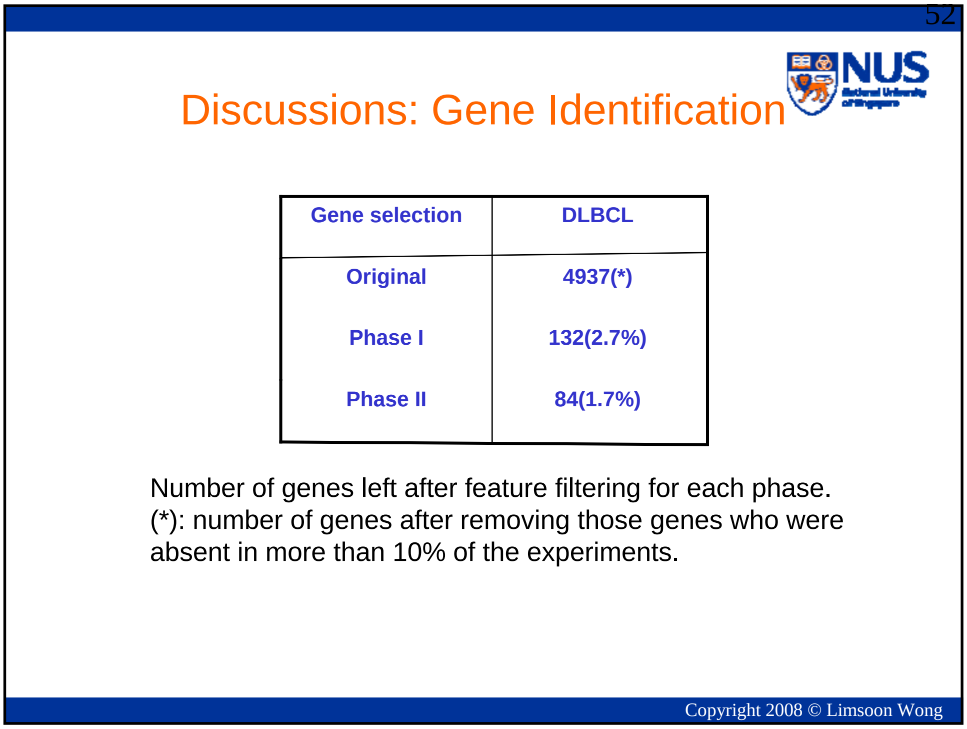

Discussions: Gene Identification

Gene selection	DLBCL
Original	4937(*)
Phase I	132(2.7%)
Phase II	84(1.7%)

Number of genes left after feature filtering for each phase. (*): number of genes after removing those genes who were absent in more than 10% of the experiments.

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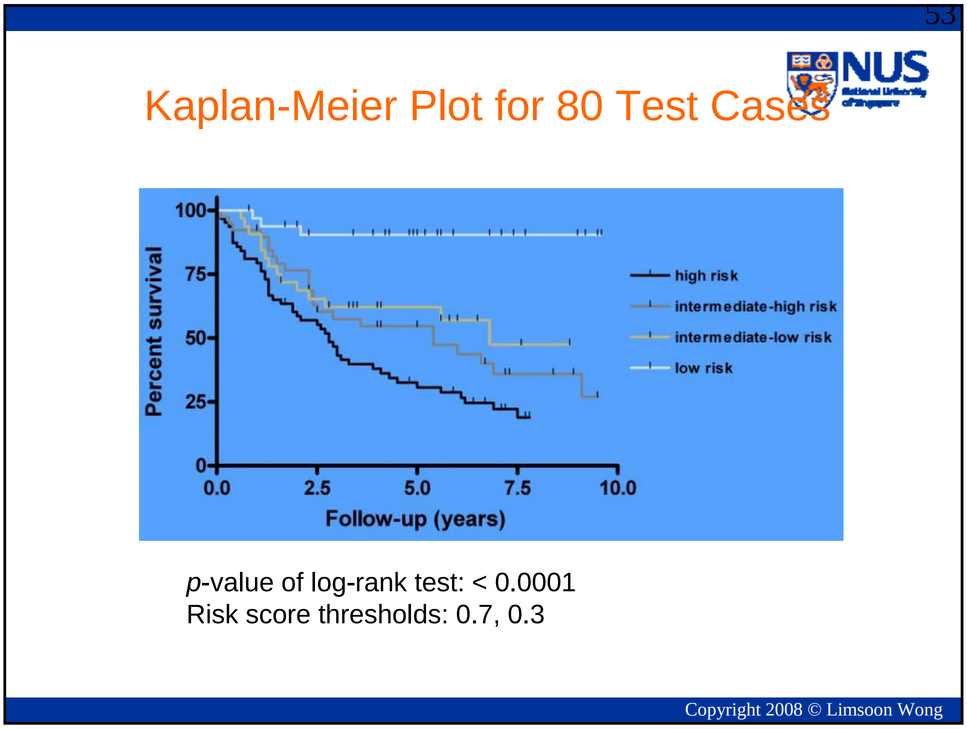

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Kaplan-Meier Plot for 80 Test Cases

p-value of log-rank test: < 0.0001
Risk score thresholds: 0.7, 0.3

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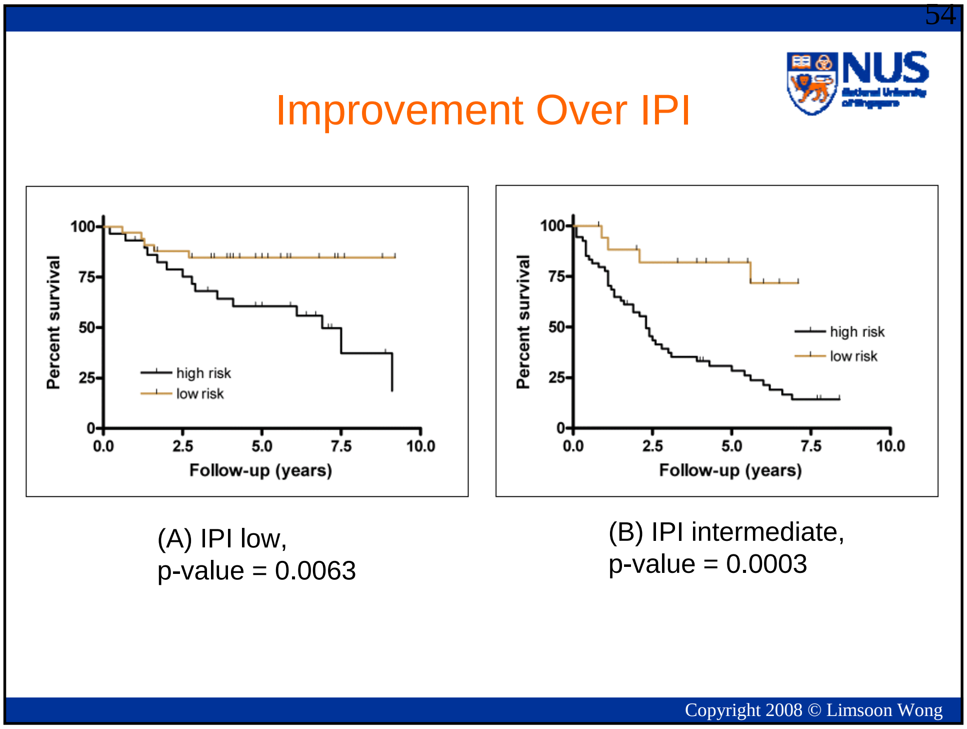

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Improvement Over IPI

(A) IPI low,
p-value = 0.0063

(B) IPI intermediate,
p-value = 0.0003

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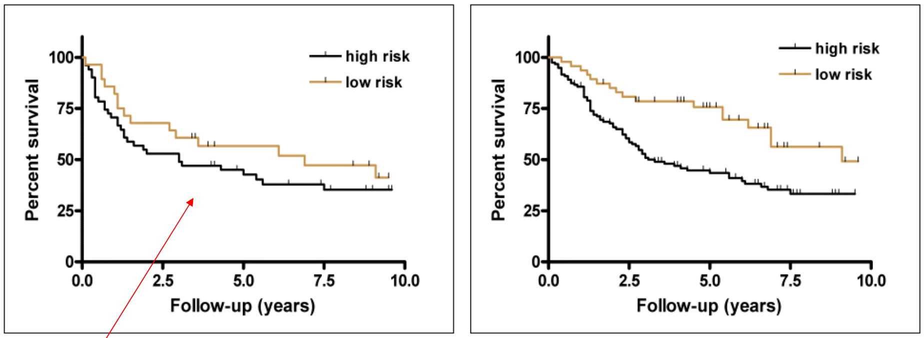
Merit of “Extreme” Samples



(A) W/o sample selection (*p* =0.38)

(B) With sample selection (p=0.009)

No clear difference on the overall survival of the 80 samples in the validation group of DLBCL study, if **no training sample selection conducted**

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Is ERCOF Useful? Observations from 1000+ Expts

- **Feature selection methods considered**
  - All use all features
  - All-entropy select features whose value range can be partitioned by Fayyad & Irani’s entropy method
  - Mean-entropy select features whose entropy is better than the mean entropy
  - Top-number-entropy select the top 20, 50, 100, 200 genes by their entropy
  - ERCOF at 5% significant level for Wilcoxon rank sum test and 0.99 Pearson correlation coeff threshold
- **Data sets considered**
  - Colon tumor
  - Prostate cancer
  - Lung cancer
  - Ovarian cancer
  - DLBC lymphoma
  - ALL-AML
  - Childhood ALL
- **Learning methods considered**
  - C4.5
  - Bagging, Boosting, CS4
  - SVM, 3-NN

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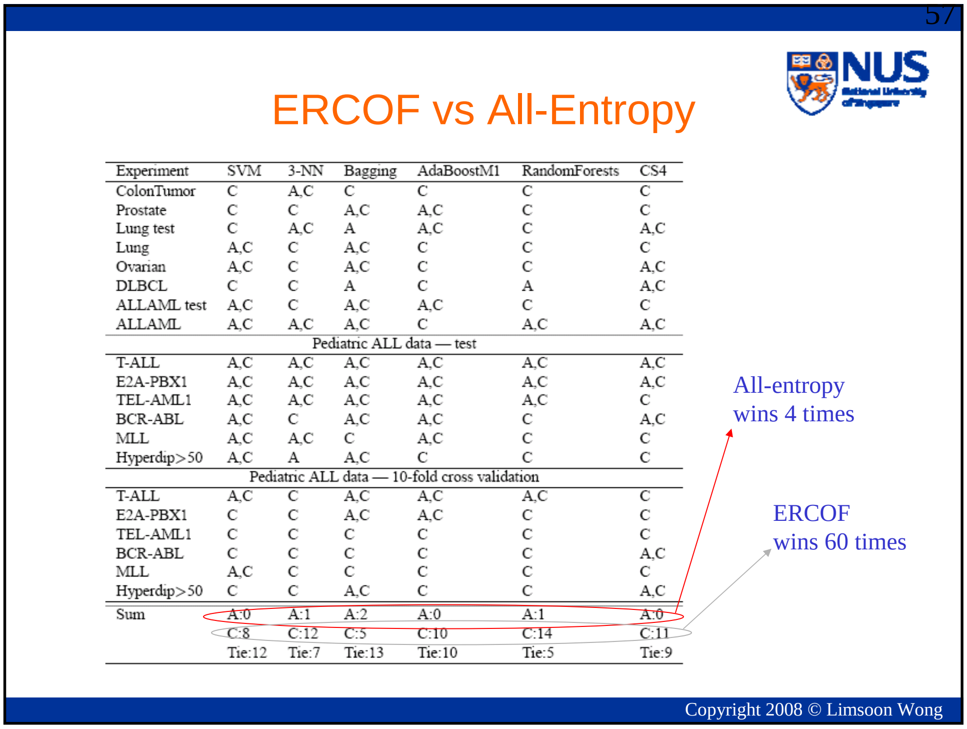

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ERCOF vs All-Entropy

Experiment	SVM	3-NN	Bagging	AdaBoostM1	RandomForests	CS4
ColonTumor	C	A,C	C	C	C	C
Prostate	C	C	A,C	A,C	C	C
Lung test	C	A,C	A	A,C	C	A,C
Lung	A,C	C	A,C	C	C	C
Ovarian	A,C	C	A,C	C	C	A,C
DLBCL	C	C	A	C	A	A,C
ALLAML test	A,C	C	A,C	A,C	C	C
ALLAML	A,C	A,C	A,C	C	A,C	A,C
Pediatric ALL data — test						
T-ALL	A,C	A,C	A,C	A,C	A,C	A,C
E2A-PBX1	A,C	A,C	A,C	A,C	A,C	A,C
TEL-AML1	A,C	A,C	A,C	A,C	A,C	C
BCR-ABL	A,C	C	A,C	A,C	C	A,C
MLL	A,C	A,C	C	A,C	C	C
Hyperdip>50	A,C	A	A,C	C	C	C
Pediatric ALL data — 10-fold cross validation						
T-ALL	A,C	C	A,C	A,C	A,C	C
E2A-PBX1	C	C	A,C	A,C	C	C
TEL-AML1	C	C	C	C	C	C
BCR-ABL	C	C	C	C	C	A,C
MLL	A,C	C	C	C	C	C
Hyperdip>50	C	C	A,C	C	C	A,C
Sum	A:0	A:1	A:2	A:0	A:1	A:0
	C:8	C:12	C:5	C:10	C:14	C:11
	Tie:12	Tie:7	Tie:13	Tie:10	Tie:5	Tie:9

All-entropy wins 4 times

ERCOF wins 60 times

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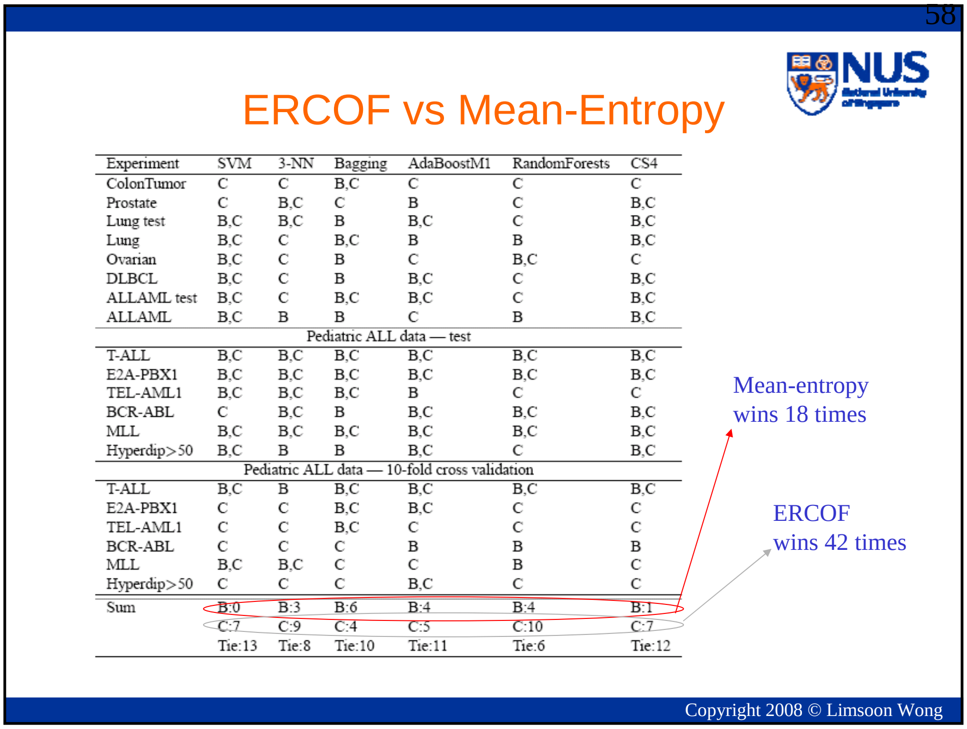

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ERCOF vs Mean-Entropy

Experiment	SVM	3-NN	Bagging	AdaBoostM1	RandomForests	CS4
ColonTumor	C	C	B,C	C	C	C
Prostate	C	B,C	C	B	C	B,C
Lung test	B,C	B,C	B	B,C	C	B,C
Lung	B,C	C	B,C	B	B	B,C
Ovarian	B,C	C	B	C	B,C	C
DLBCL	B,C	C	B	B,C	C	B,C
ALLAML test	B,C	C	B,C	B,C	C	B,C
ALLAML	B,C	B	B	C	B	B,C
Pediatric ALL data — test						
T-ALL	B,C	B,C	B,C	B,C	B,C	B,C
E2A-PBX1	B,C	B,C	B,C	B,C	B,C	B,C
TEL-AML1	B,C	B,C	B,C	B	C	C
BCR-ABL	C	B,C	B	B,C	B,C	B,C
MLL	B,C	B,C	B,C	B,C	B,C	B,C
Hyperdip>50	B,C	B	B	B,C	C	B,C
Pediatric ALL data — 10-fold cross validation						
T-ALL	B,C	B	B,C	B,C	B,C	B,C
E2A-PBX1	C	C	B,C	B,C	C	C
TEL-AML1	C	C	B,C	C	C	C
BCR-ABL	C	C	C	B	B	B
MLL	B,C	B,C	C	C	B	C
Hyperdip>50	C	C	C	B,C	C	C
Sum	B:0	B:3	B:6	B:4	B:4	B:1
	C:7	C:9	C:4	C:5	C:10	C:7
	Tie:13	Tie:8	Tie:10	Tie:11	Tie:6	Tie:12

Mean-entropy wins 18 times

ERCOF wins 42 times

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Effectiveness of ERCOF

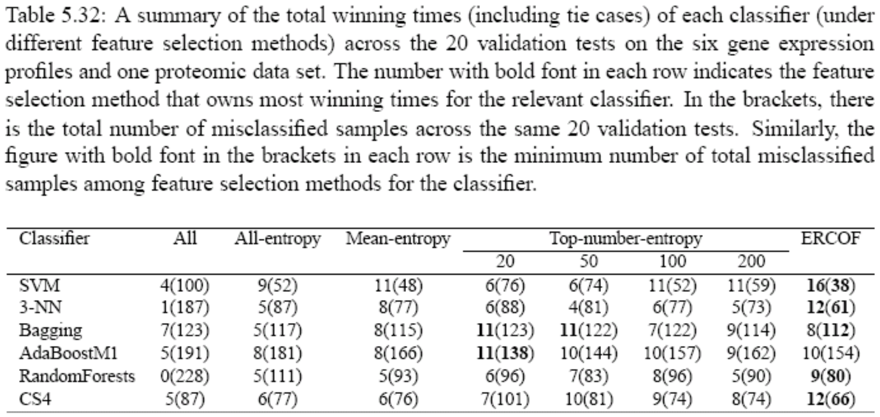

Table 5.32: A summary of the total winning times (including tie cases) of each classifier (under different feature selection methods) across the 20 validation tests on the six gene expression profiles and one proteomic data set. The number with bold font in each row indicates the feature selection method that owns most winning times for the relevant classifier. In the brackets, there is the total number of misclassified samples across the same 20 validation tests. Similarly, the figure with bold font in the brackets in each row is the minimum number of total misclassified samples among feature selection methods for the classifier.

Classifier	All	All-entropy	Mean-entropy	Top-number-entropy				ERCOF
				20	50	100	200	
SVM	4(100)	9(52)	11(48)	6(76)	6(74)	11(52)	11(59)	**16**(**38**)
3-NN	1(187)	5(87)	8(77)	6(88)	4(81)	6(77)	5(73)	**12**(**61**)
Bagging	7(123)	5(117)	8(115)	**11**(123)	**11**(122)	7(122)	9(114)	8(**112**)
AdaBoostM1	5(191)	8(181)	8(166)	**11**(**138**)	10(144)	10(157)	9(162)	10(154)
RandomForests	0(228)	5(111)	5(93)	6(96)	7(83)	8(96)	5(90)	**9**(**80**)
CS4	5(87)	6(77)	6(76)	7(101)	10(81)	9(74)	8(74)	**12**(**66**)
Total wins	22	38	46	47	48	51	47	67

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Conclusions

- Selecting extreme cases as training samples is an effective way to improve patient outcome prediction based on gene expression profiles and clinical information
- ERCOF is very suitable for SVM, 3-NN, CS4, Random Forest, as it gives these learning algos highest no. of wins
- ERCOF is suitable for Bagging also, as it gives this classifier the lowest no. of errors

⇒ ERCOF is a systematic feature selection method that is very useful

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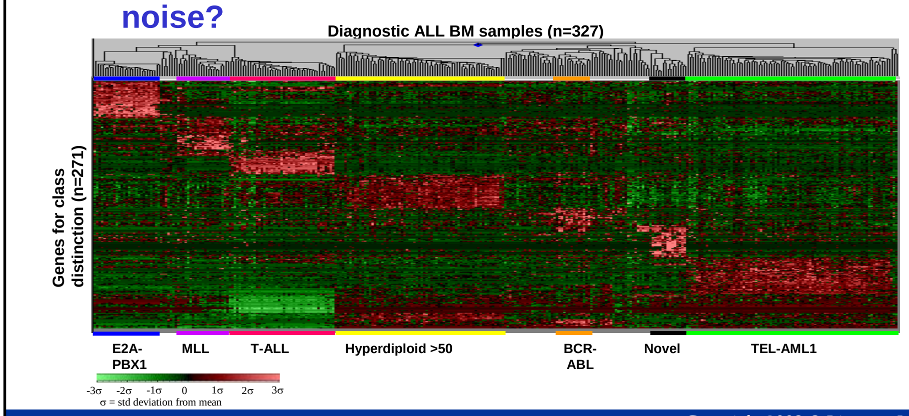
Beyond Disease Diagnosis & Prognosis

Beyond Classification of Gene Expression Profiles

- After identifying the candidate genes by feature selection, do we know which ones are causal genes, which ones are surrogates, and which are noise?



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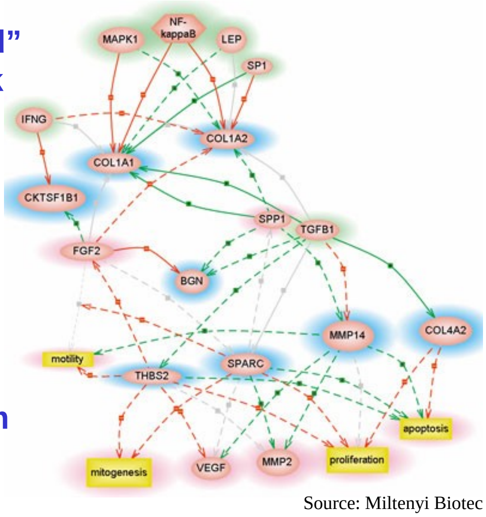
Gene Regulatory Circuits

- Genes are “connected” in “circuit” or network
- Expr of a gene in a network depends on expr of some other genes in the network
- Can we “reconstruct” the gene network from gene expression and other data?



Source: Miltenyi Biotec

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Hints to extend reach of prediction

- Each disease subtype has underlying cause

⇒ There is a unifying biological theme for genes that are truly associated with a disease subtype.

- Uncertainty in reliability of selected genes can be reduced by considering molecular functions and biological processes associated with the genes
- The unifying biological theme is basis for inferring the underlying cause of disease subtype

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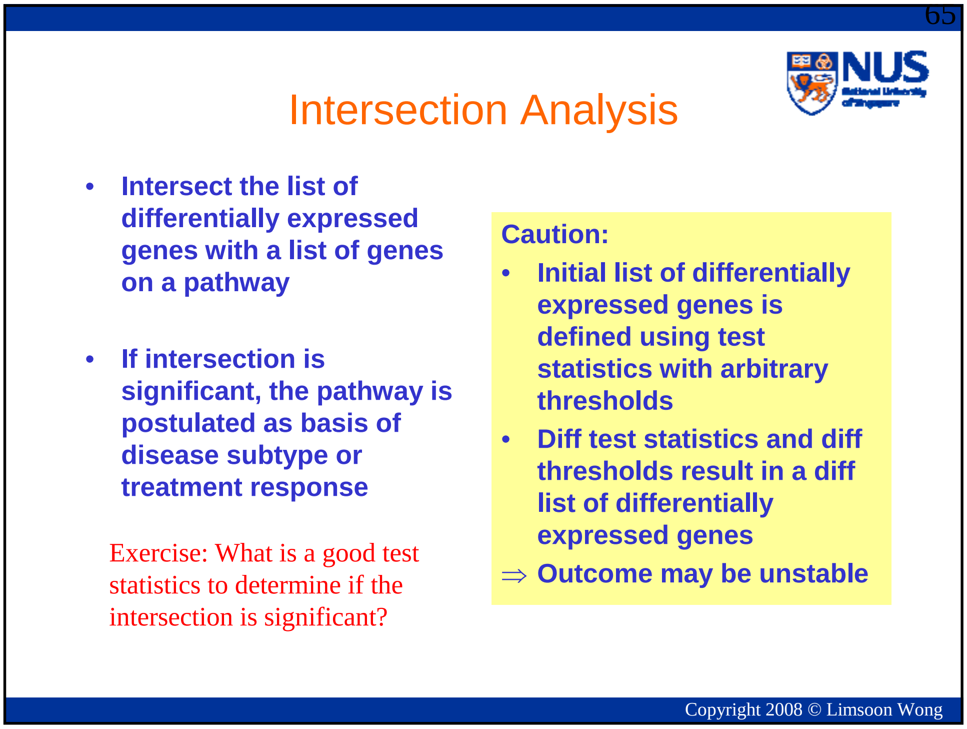

Intersection Analysis

- Intersect the list of differentially expressed genes with a list of genes on a pathway
- If intersection is significant, the pathway is postulated as basis of disease subtype or treatment response

Exercise: What is a good test statistics to determine if the intersection is significant?

Caution:

- Initial list of differentially expressed genes is defined using test statistics with arbitrary thresholds
- Diff test statistics and diff thresholds result in a diff list of differentially expressed genes

⇒ Outcome may be unstable

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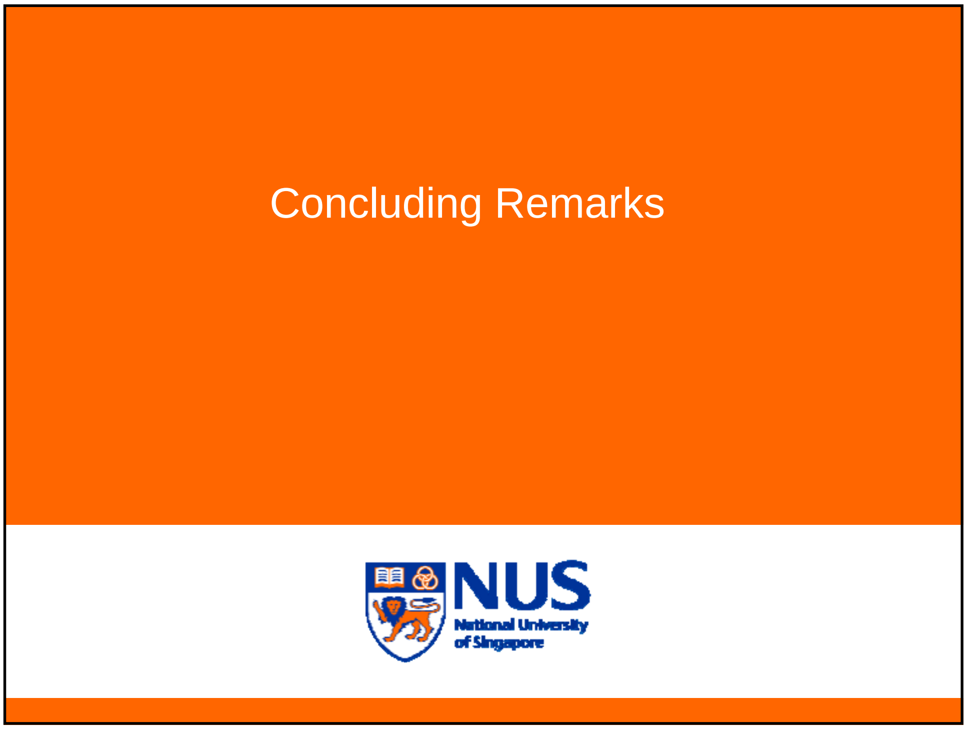

Concluding Remarks

What have we learned?

- **Technologies**
  - Microarray
  - PCL, ERCOF
- **Microarray applications**
  - Disease diagnosis by supervised learning
  - Subtype discovery by unsupervised learning
- **Important tactics**
  - Extreme sample selection
  - Intersection analysis

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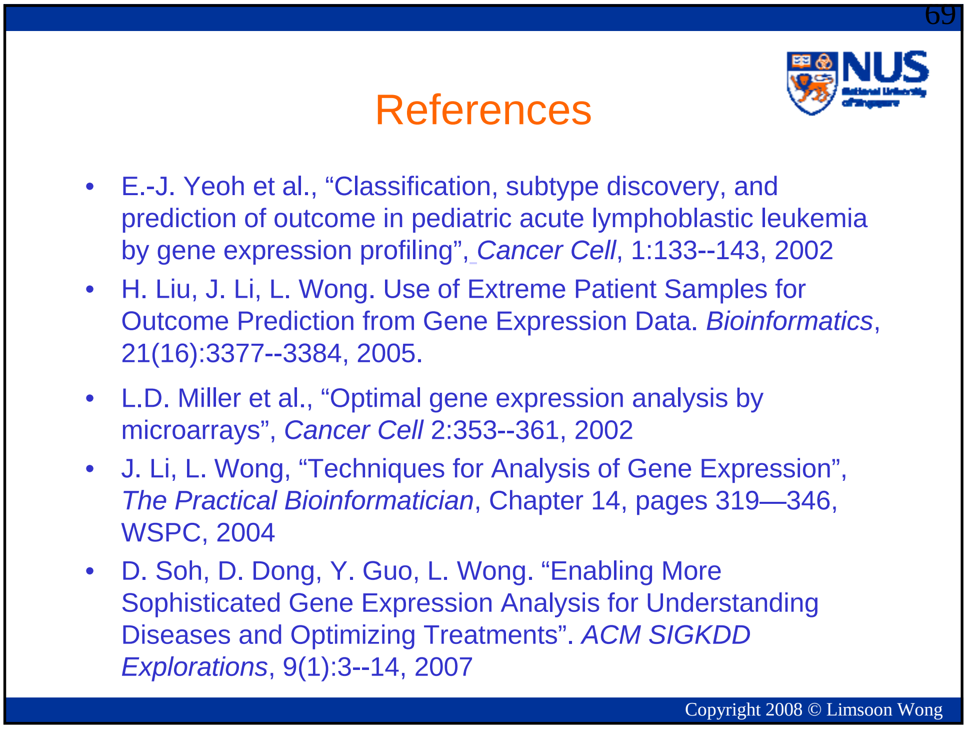
Any Question?



References

- E.-J. Yeoh et al., “Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling”, *Cancer Cell*, 1:133--143, 2002
- H. Liu, J. Li, L. Wong. Use of Extreme Patient Samples for Outcome Prediction from Gene Expression Data. *Bioinformatics*, 21(16):3377--3384, 2005.
- L.D. Miller et al., “Optimal gene expression analysis by microarrays”, *Cancer Cell* 2:353--361, 2002
- J. Li, L. Wong, “Techniques for Analysis of Gene Expression”, *The Practical Bioinformatician*, Chapter 14, pages 319—346, WSPC, 2004
- D. Soh, D. Dong, Y. Guo, L. Wong. “Enabling More Sophisticated Gene Expression Analysis for Understanding Diseases and Optimizing Treatments”. *ACM SIGKDD Explorations*, 9(1):3--14, 2007

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