Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response

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Diffuse Large B-cell Lymphoma
(DLBCL)

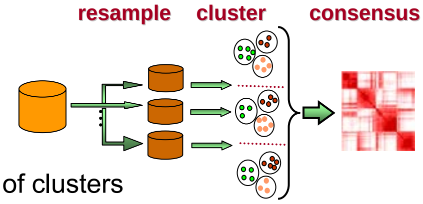
- Most common lymphoid malignancy (~40%)
- Significant clinical & genetic heterogeneity
- Hypothesis:
  - gene expression profiling will reveal disease heterogeneity

DLBCL expression profiling

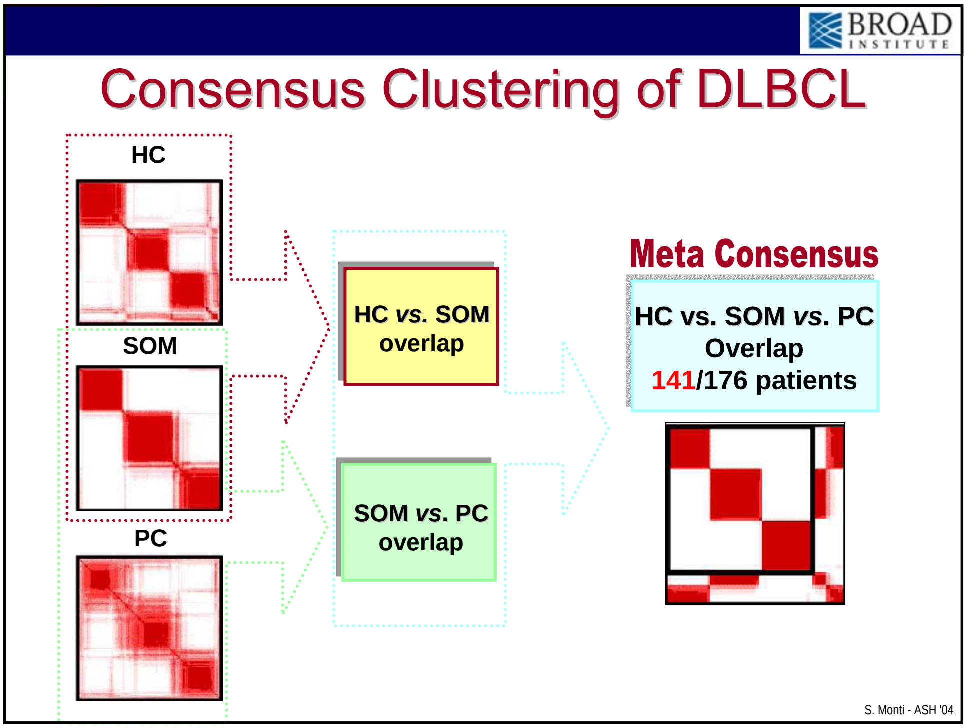
- 176 DLBCL samples.
- Nodal biopsies from untreated patients.
- Affymetrix U133A/B chips (~42K probes)
- Goal: unsupervised analysis to discover novel substructure

Analysis of DLBCL Substructure

by unsupervised analysis



- Consensus clustering
  - identifies robust clusters
  - resampling-based method
  - automatically selects the number of clusters
- Used with 3 different clustering algorithms
  - Hierarchical clustering (HC)
  - Self-organizing Map (SOM)
  - Probabilistic Clustering (PC)



Consensus Clustering of DLBCL

HC

SOM

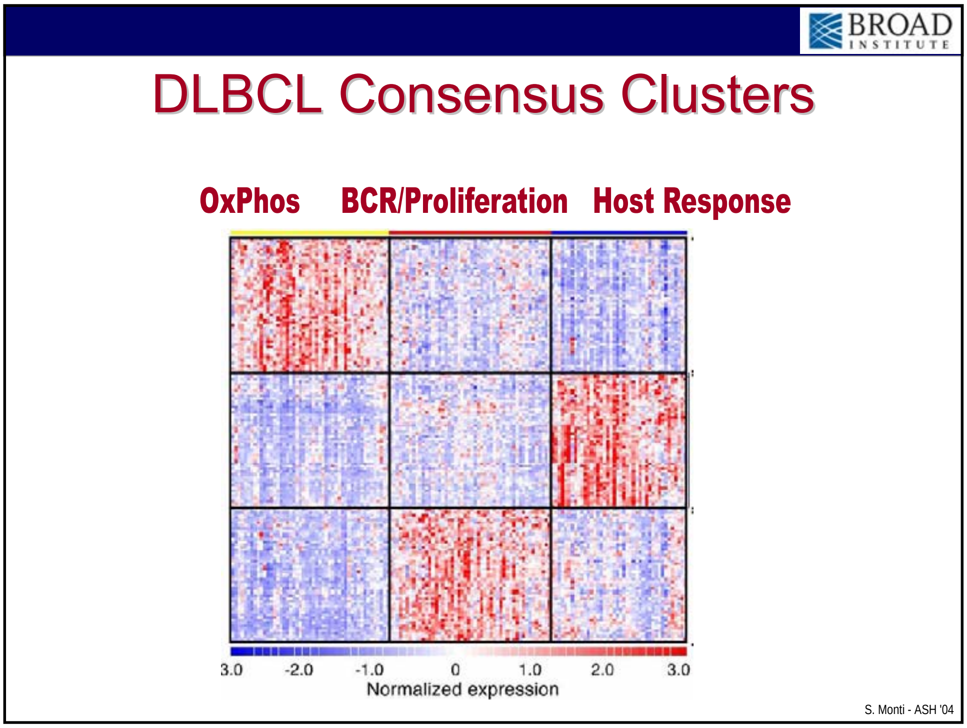
PC

HC *vs.* SOM overlap

SOM *vs.* PC overlap

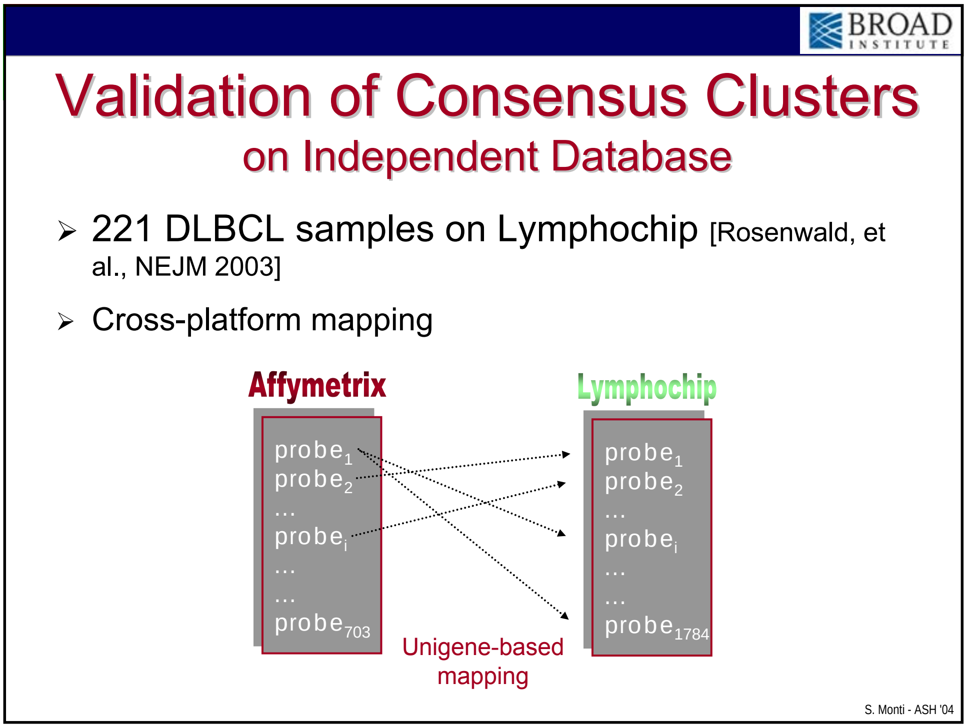
Meta Consensus

HC vs. SOM *vs.* PC
Overlap
141/176 patients



DLBCL Consensus Clusters

OxPhos **BCR/Proliferation** **Host Response**



Validation of Consensus Clusters

on Independent Database

- 221 DLBCL samples on Lymphochip [Rosenwald, et al., NEJM 2003]
- Cross-platform mapping

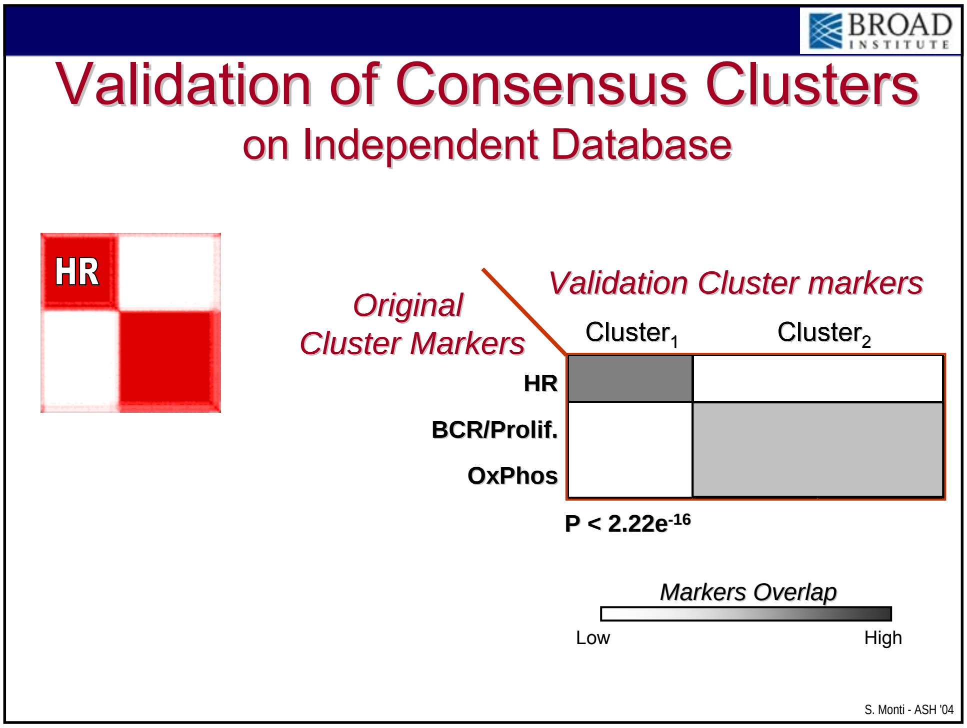
Affymetrix

probe$_1$
probe$_2$
…
probe$_i$
…
…
probe$_{703}$

Lymphochip

probe$_1$
probe$_2$
…
probe$_i$
…
…
probe$_{1784}$

Unigene-based mapping



Validation of Consensus Clusters

on Independent Database

Validation Cluster markers

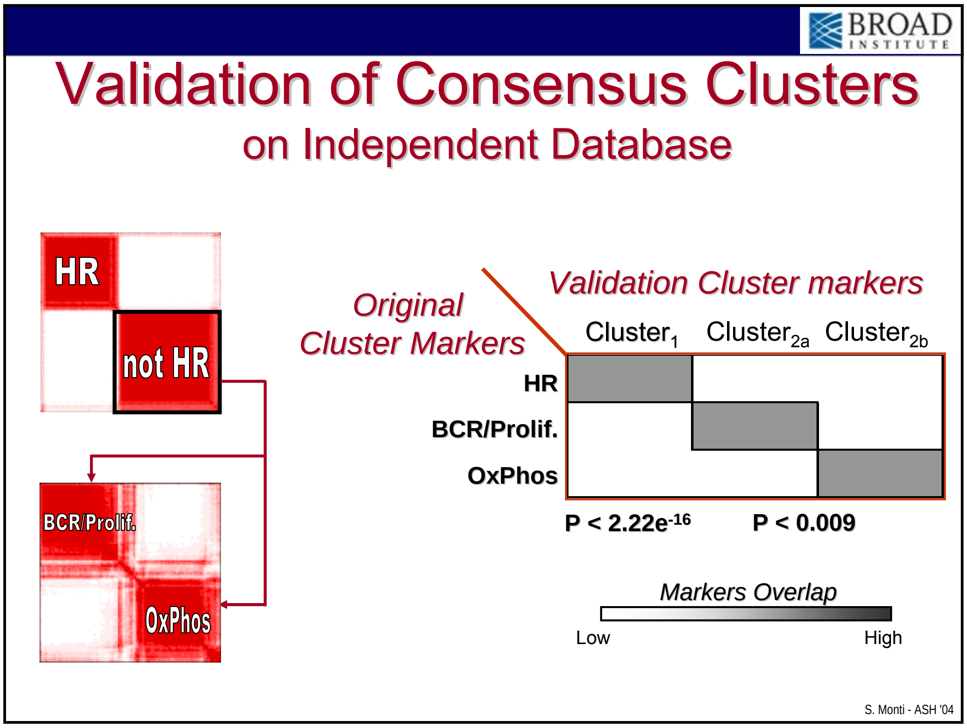
Original Cluster Markers

	Cluster$_1$	Cluster$_2$
HR	▓	
BCR/Prolif.		▒
OxPhos		▒

$P < 2.22e^{-16}$

Markers Overlap

Low High



Validation of Consensus Clusters
on Independent Database

Validation Cluster markers

Original Cluster Markers

	Cluster$_1$	Cluster$_{2a}$	Cluster$_{2b}$
HR	▨		
BCR/Prolif.		▨	
OxPhos			▨

$P < 2.22e^{-16}$ **$P < 0.009$**

Markers Overlap

Low High

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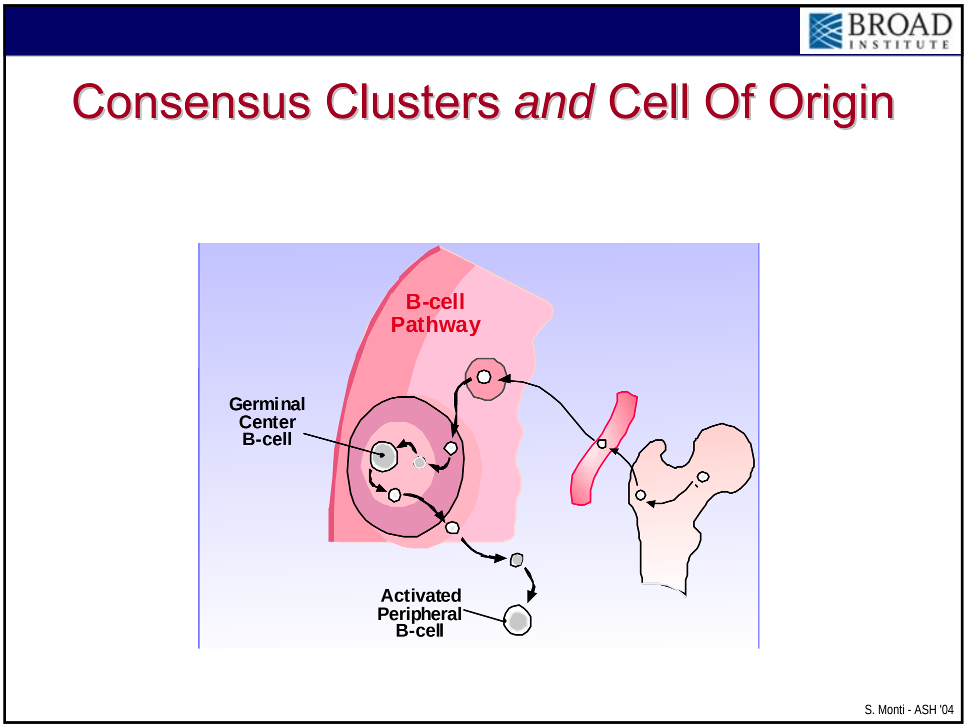

Consensus Clusters *and* Cell Of Origin

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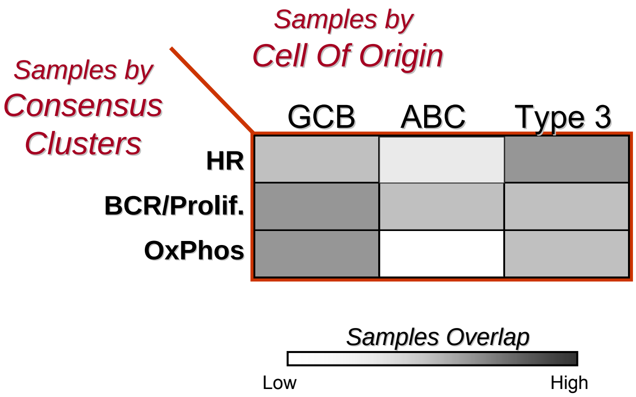
Consensus Clusters *and* Cell Of Origin



Non-overlapping information

COO assignment based on methodology described in [Wright, et al., PNAS 2003].

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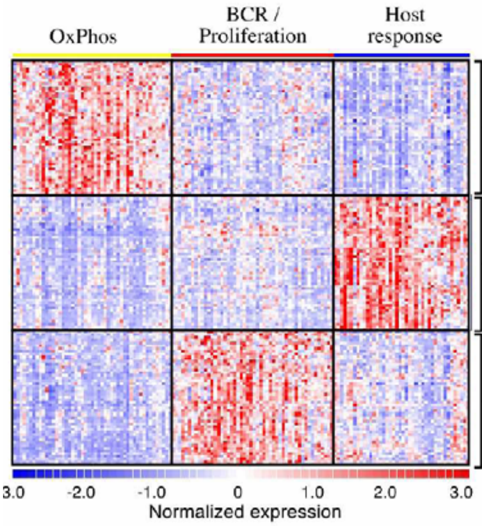
DLBCL Consensus Clusters



NADH dehydrogenase 1 α/β subcomplex 1
Cytochrome c oxidase (COX) 7A2L
ATP binding protein
Proteosome α 5
ATP synthase, mitochon. FO complex, subunit c iso 3
ATP synthase, mitochon. F1 complex, γ polypeptide 1
Proteosome α 2
Proteosome α 6
Mitochondrial ribosomal protein L3
Translocase of inner mitochondrial membrane 8B
NADH dehydrogenase 1 β subcomplex 1

Stromal cell-derived factor 1
TNF-related death ligand 1β (APRIL)
IFN-induced transmembrane protein 2
TNFRSF1β
LAMP1
GATA3
cMAF
CD3ε
Linker for activation of T-cells
CD2
T-cell immune regulator 1
TNFRSF1α
Integrin β2
IFN regulatory factor 1

CD79A
Phospholipase C γ 2
MAP4K1
CD22
CD37
Postmeiotic segreg. increased-2-like 9, 8, 2, 11, 3
Proliferation-associated protein 100
Ki67
Inositol polyphosphate-5-phosphatase
CHL1-related helicase

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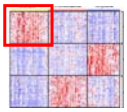
OxPhos cluster

- Genes involved in oxidative phosphorylation (p≤.002) and mitochondrial function (p≤.003)
  - BFL-1/A1 (anti-apoptotic BCL-2 family member)
  - members of NADH dehydrogenase complex
  - members of the COX complex
  - ATP synthase components



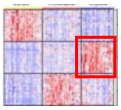
BCR/Proliferation cluster

- BCR signaling components
  - CD19, IG, CD79a, BLK, SYK, PLCgamma2, MAP4K
- B-cell transcription factors
  - PAX5, OBF-1, E2A, BCL6, STAT6, MYC
- Cell cycle regulatory genes
  - CDK2, MCM
- DNA repair genes
  - PMS2, H2AX, PTIP, p53

Host Response (HR) cluster

signature largely defined by the associated host response rather than the tumor itself

- Components of TCR (TCRα/β, CD3), CD2, T/NK cell activation, and complement cascade.
- Co-regulated inflammatory mediators
- More abundant monocyte/macrophage & dendritic cell transcripts
- Interferon-induced genes, TNF ligands/receptors, cytokine receptors.



Tumor Infiltrating Lymphocytes

(TILs)* (morphology)

Consensus clusters	> 20 TILs/HPF
HR	65%
BCR/proliferation	14%
OxPhos	11%

p < .0001

* Morphologically normal (CD2+) lymphocytes with round/oval nuclei and delicately dispersed chromatin.

TILs and Dentritic Cells in HR tumors
(immunostaining)

- Increased # of CD2$^+$/CD3$^+$ T-cells (p≤.005)
- Increased # of GILT$^+$ dendritic cells (DC) (p=.06)
  - Interdigitating DCs (S100$^+$, CD1a$^-$ CDC123$^-$), (p<.009)
  - correlated with TILs (p<.0001)

HR tumors and T-cell/histiocyte-rich LBCLs

- HR tumors appear
  - in younger patients (p=0.04);
  - with higher incidence of splenic (p=0.02) and BM involvement (p=0.03).
- 8/10 patients with T-cell/Histiocyte-rich LBCLs fall in the HR cluster

Genetic abnormalities
in DLBCL consensus clusters

Nearly absent in the HR cluster

Genetic abnormality	OxPhos (n=27)	BCR/ Prolif. (n=50)	HR (n=29)	Total (n=116)
t(14;18)	8 (22%)	5 (10%)	1 (3%)	14 (12%)
t(3; …)	2 (5%)	8 (16%)	1 (3%)	11 (9%)
None	27 (73%)	37 (74%)	27 (93%)	91 (78%)

*

p = .059

* Columns may not add to 100% because of rounding.

Summary

- Identified three robust clusters by transcriptional profiling and confirmed in independent series.
- HR cluster characterized by:
  - inflammatory/immune cell infiltrate
  - fewer known genetic lesions
  - distinct clinical features
- Different mechanisms of transformation?

Participants

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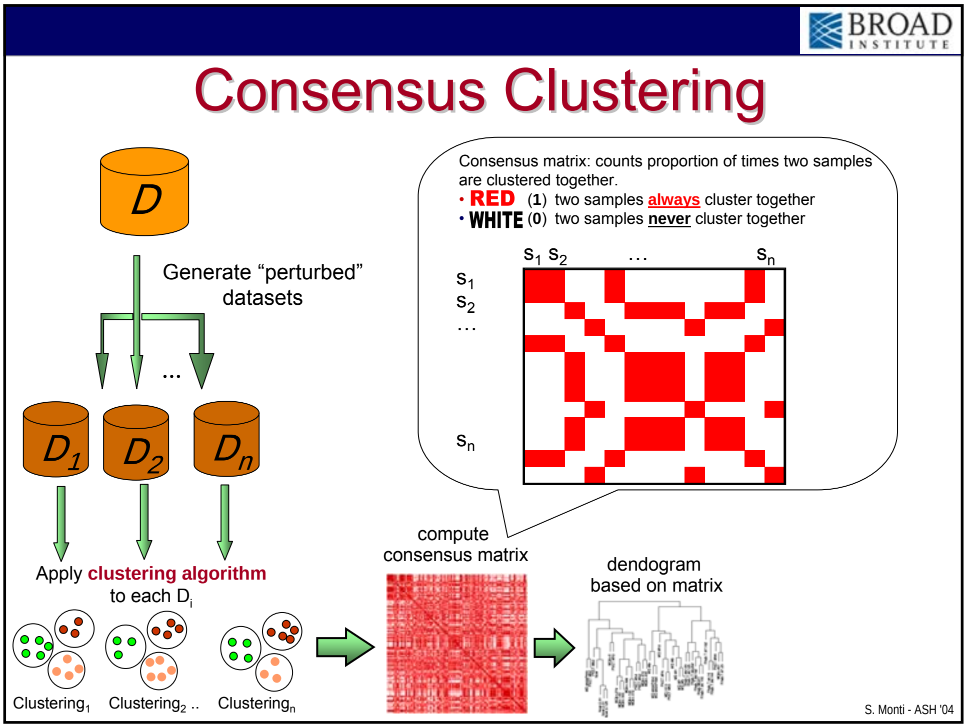
Peter Smith
Erxi Wu
Donna Neuberg
Margaret Shipp

Appendix



Consensus Clustering

Consensus matrix: counts proportion of times two samples are clustered together.

- **RED** (**1**) two samples <u>always</u> cluster together
- **WHITE** (**0**) two samples **<u>never</u>** cluster together

Apply **clustering algorithm** to each D_i

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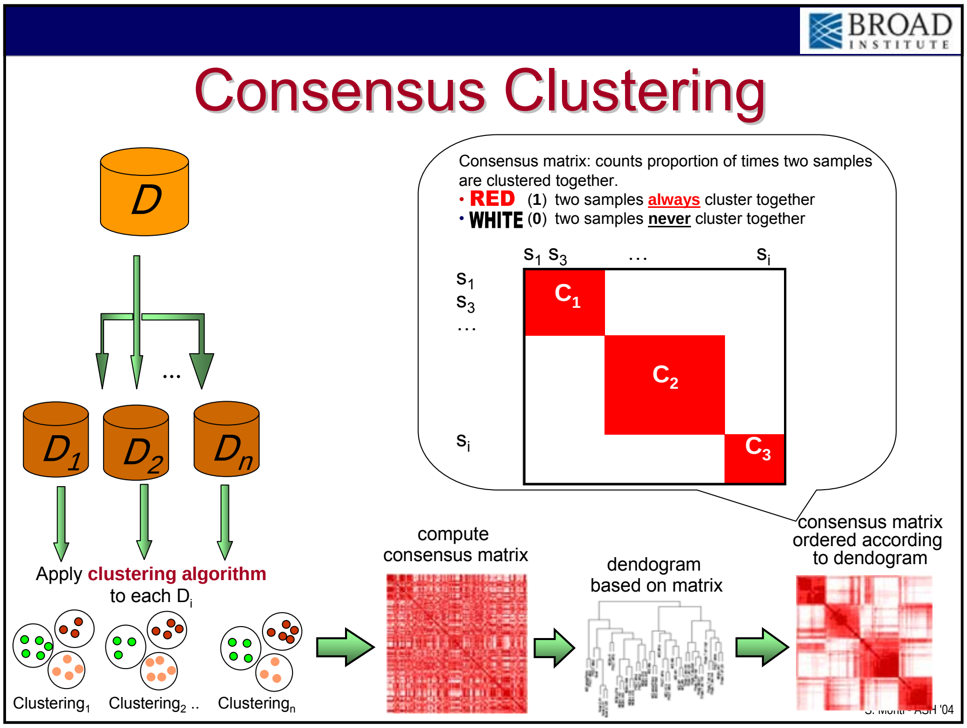

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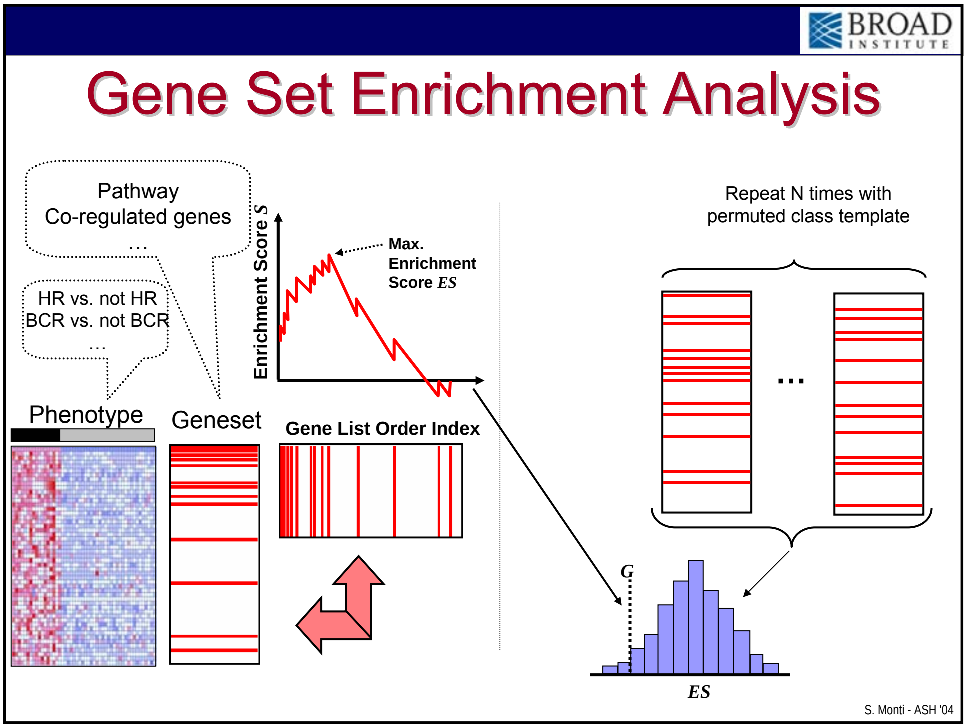

Gene Set Enrichment Analysis

Pathway
Co-regulated genes
…

HR vs. not HR
BCR vs. not BCR
…

Phenotype

Geneset

Enrichment Score *S*

Max.
Enrichment
Score *ES*

Gene List Order Index

Repeat N times with
permuted class template

…

G

ES

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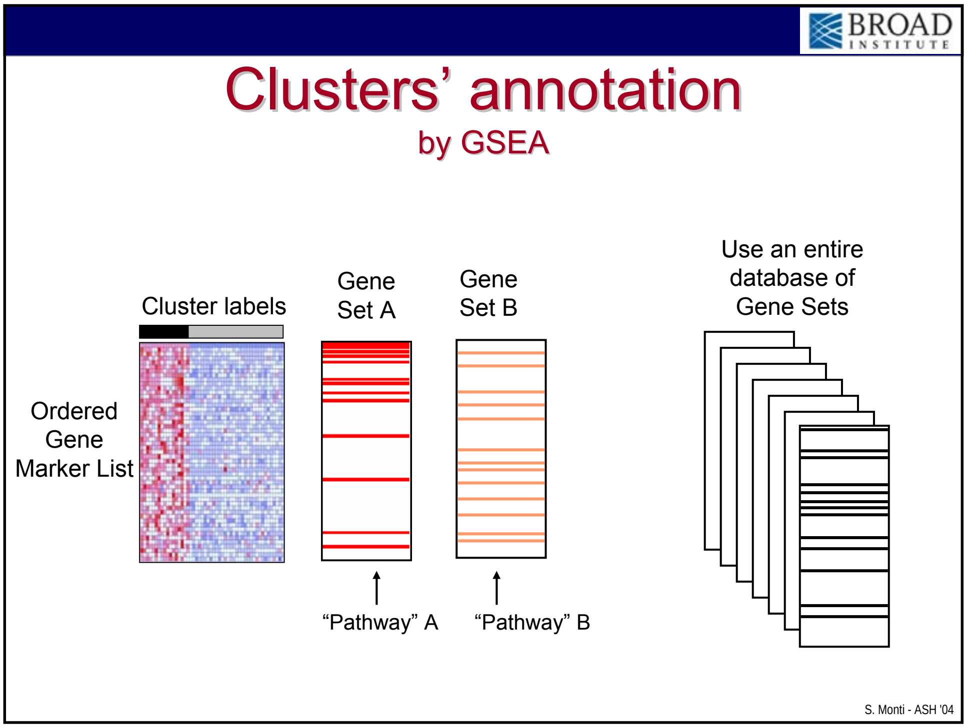

Clusters' annotation

by GSEA

Cluster labels

Ordered
Gene
Marker List

Gene
Set A

Gene
Set B

"Pathway" A

"Pathway" B

Use an entire
database of
Gene Sets

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