Statistical genomics and spatial statistics: Incorporating biological knowledge of genes into analysis of genomic data

Wei Pan
(joint work with Peng Wei)
Division of Biostatistics, SPH
University of Minnesota

CAMDA 2007, Valencia, Spain
Dec 2007
Outline

- Problem
- Standard mixture model
- Stratified mixture model
- Spatially correlated mixture model
- Numerical Results: real and simulated data
- Discussion
Introduction

- Problem: genomic discoveries
  which of the $G$ genes satisfy a specified condition?
- Problem 1: detecting differentially expressed (DE) genes based on microarary expression data
- Problem 2: detecting binding targets of a TF based on ChIP-chip data
- Features:
  - Unsupervised learning/discovery: no or few known cases/controls; e.g. cannot apply logistic regression; use mixture model/clustering.
  - Many genes/subjects: somewhat similar; borrow info.
  - Data: high noise level.
- Statistical problem: testing $H_{0,i}$ vs $H_{1,i}$ for each geen $i$. 
- $H_{0,i}$: gene $i$ is equally expressed for Problem 1;
- $H_{0,i}$: gene $i$ is not a target of the TF for Problem 2;
- $H_{1,i}$: opposite of $H_{0,i}$ (i.e. gene $i$ is DE for Problem 1, is a target for Problem 2).

- Given microarray data $\implies Z_i$'s
  $Z_i$: a summary statistic against $H_{0,i}$ for gene $i$;
e.g. a fold change, t-type statistic, or even p-value.

- We transform $Z_i$ such that the null distribution of $Z_i$'s (i.e. for those genes satisfying $H_{0,i}$) is $N(0, 1)$.
e.g. If $Z_i = P_i$ is a p-value, $z_i = \Phi^{-1}(1 - P_i)$.

- The null distribution may not be exactly $N(0, 1)$, called theoretical null, and hence may need to be estimated as $N(\mu_0, \sigma_0)$, called empirical null (Efron 2004, JASA)
- From now on, we work with $z_i$'s (i.e. transformed $Z_i$'s).
Standard mixture model


- A hierarchical model:

- Prior probability: \( \pi_0 = \text{Prob}(H_{0,i}) \) for any \( i \).
  a constant! common across the genes!

- Null distr: \( f(z_i | H_{0,i}) = f_0(z_i) \);

- Non-null distr: \( f(z_i | H_{1,i}) = f_1(z_i) \);

- Marginally, \( z_i \)'s are iid from
  \[
  f(z_i) = \pi_0 f_0(z_i) + (1 - \pi_0) f_1(z_i),
  \]
a standard mixture model.

- Key:
  all the genes are treated equally and independently \textit{a priori};
  reasonable?
• Inference:

\[ Pr(H_{1,i} | z_i) = \frac{(1-\pi_0)f_1(z_i)}{f(z_i)} = 1 - \frac{\pi_0 f_0(z_i)}{f(z_i)} \propto \frac{f_1(z_i)}{f_0(z_i)} = LRT. \]

Rank the genes based on their \( Pr(H_{1,i} | z_i) \) or LRT.

• False discovery rate (FDR) estimation (Newton 2004, Biostatsitics)

Decision rule: for any given cut-off value \( c \), rejects \( H_{0i} \) if and only if \( Pr(H_{1,i} | z_i) > 1 - c \), then

\[ \widehat{FDR}(c) = \frac{\sum_i [1 - Pr(H_{1,i} | z_i)]1[Pr(H_{1,i} | z_i) > 1 - c]}{\sum_i 1[Pr(H_{1,i} | z_i) > 1 - c]}. \]

\[ FDR = E \left( \frac{\text{#false positives}}{\text{#claimed positives}} \right). \]
Stratified mixture model

• Reference: Pan (2005, Statistical Applications in Genetics and Molecular Biology)

• Known: the genes are annotated in $K > 1$ GO categories or pathways, $G_1, ..., G_K$.
  known: the genes in the same group should be more similar to each other than those from different groups!

• How to take advantage?
  treat the genes in different groups differently a priori.

• Prior probability: $\pi_{0}^{(k)} = \text{Prob}(H_{0,i} | i \in G_k)$.
  NOT a constant; group-dependent!

• Null distr: same as before; $f(z_i | H_{0,i}) = f_1(z_i)$.

• Non-null distr: group-specific; $f(z_i | H_{1,i}, i \in G_k) = f_{1}^{(k)}(z_i)$.

• Marginally, $z_i$’s for those in $G_k$ are iid as
\[ f(z_i | i \in G_k) = \pi_0^{(k)} f_0(z_i) + (1 - \pi_0^{(k)}) f_1^{(k)}(z_i), \]

but the marginal distribution depends on \( k \): genes from different \( G_k \) have different distributions!

\[ \implies \text{treat genes differently } a \text{ priori} \]

- Inference: same as before except working on each \( G_k \) one by one—stratified analysis!

- Efron (in press, AoAS): a general problem; theory.

- A practical problem: depends on the choice of \( G_k \)'s
  - GO: thousands of the groups;
  - GO: DAG; hierarchical: higher level categories are more general, while lower ones more specific
  \[ \implies \text{trade-off: group homogeneity vs group size!} \]

- Hierarchical mixture model (Pan 2006, Applied Statistics)

- Main ideas:
1) each GO category is a stratum;
2) borrowing information: parameters from a category are related to that of its parents; shrinking its sample estimate towards that of its parent!
Spatially correlated mixture model

- A problem with the stratified mixture model: choice of $G_k$’s.
- Some argue that gene functions should be characterized by some categories, rather, by their inter-relationships (Marcotte) $\implies$ gene networks
- gene networks: many types; can be general here.
  undirected graph: genes are nodes; an edge indicates “direct relationship” between the two genes.
  **basic assumption**: any two connected genes in a network are more similar (i.e. more likely to satisfy or not satisfy $H_0$ together) than two random picks.
- Prior probability: $\pi_{i,0} = \text{Prob}(H_{0,i})$ for gene $i$.
  Key: gene-specific!
- Null distr: same; $f(z_i|H_{0,i}) = f_0(z_i)$. 
• Non-null distr: same; \( f(z_i|H_{1,i}) = f_1(z_i) \).

• Marginally \( z_i \) is distributed as
  \[
  f_i(z_i) = \pi_{i,0} f_0(z_i) + \pi_{i,1} f_1(z_i),
  \]

• Too many parameters \( \pi \)'s \( \Rightarrow \) borrowing information!
  have not used information in network yet!

• Assume two latent Markov random fields
  \[
  x_j = \{ x_{i,j}; i = 1, ..., G \},
  \]
  \[
  \pi_{i,j} = \exp(x_{i,j}) / [\exp(x_{i,0}) + \exp(x_{i,1})] \text{ for } j = 0, 1.
  \]

• \( x_j \): intrinsic Gaussian conditional autoregression (CAR) model
  (Besag and Kooperberg 1995, B'ka)
  \[
  x_{i,j} | x_{(-i),j} \sim N \left( \frac{1}{m_i} \sum_{l \in \delta_i} x_{l,j}, \frac{\sigma_{ij}^2}{m_i} \right),
  \]
  where \( \delta_i \): indices for the neighbors of gene \( i \); \( m_i = |\delta_i| \).
  neighborhoods: determined by a gene network!

• A Bayesian implementation ... see Wei and Pan (RR
used MCMC; inference is based on posterior probabilities, e.g. $\hat{Pr}(H_{0,i} | data)$.
a standard mixture model can be similarly implemented.

- Originally proposed by Fernandez and Green (2002, JRSS-B) for spatial statistics; to avoid over-smoothing near “edges”. applied to CGH data by Broet and Richardson (2006, Bioinfo.): 1-dim smoothing over a chromosome to “change point” detection.
**An example**

- **Data**: 3 replicates of ChIP-chip experiments for yeast *S. cerevisiae* by Lee et al (2002, Science); $G \approx 6000$
- **TF**: GCN4; involved in response to amino acid starvation; Used their $p$-values.
- **Positive (negative) control set**: genes believed to be (not to be) the transcriptional targets of GCN4; $n = 80$ (900). compiled by Pokholok et al (2005, Cell); based on 3 sources of data: a newer generation of ChIP-chip; gene expression; DNA motif analysis).
  - two connected genes: functional linkage;
  - based on multiple data sources: gene expression, protein-protein interaction, gene co-citation, gene fusion and
phylogenetic profiles;

- Used their ‘ConfidentNet’: 4681 nodes, 34000 edges. 
  summary of # direct neighbors: min=1, 25%=2, 50%=6, 
  75%=13, max=188.

- Merged the data and network. 
  $G = 4616$ genes/nodes, 33432 edges; 
  positive control set: 66 genes; 
  negative control set: 770 genes;

- Subnetwork with only control genes: Fig 1 
  clustering?

- Evaluation: used only the two control sets to estimate 
  sensitivity and specificity $\implies$ ROC curve.

- Model fitting: Fig 2.
Standard:
\[
\hat{f}(z_i) = 0.91 \phi(z_i; 0, .80^2) + 0.037 \phi(z_i; -1.98, .40^2) + 0.058 \phi(z_i; 1.67, 1.94^2),
\]

Spatial:
\[
\hat{f}(z_i) = \hat{\pi}_{i,0,1} \phi(z_i; 0, .63^2) + \hat{\pi}_{i,0,2} \phi(z_i; -0.38, 1.02^2) + \hat{\pi}_{i,1,1} \phi(z_i; 0.75, 1.53^2)
\]

averages of \( \hat{\pi}_{i,0,1} \), \( \hat{\pi}_{i,0,2} \), \( \hat{\pi}_{i,1,1} \): 0.500, 0.314 and 0.186.

- Statistical power: ROC curves in Fig 3
Figure 1: Subnetwork consisting of positive control genes (dark ones) and negative control genes (black ones).
Figure 2: Fitted mixture models.
Figure 3: ROC curves for the two methods applied to the real data.
Example genes

- **ARG8**: in the positive control set.
  - posterior prob: \( \approx 0.728 \) by the spatial model; \( \approx 0.023 \) by the standard model.
  - data in Lee et al (rich medium): binding ratio=1.02; used here.
  - new data by Harbison et al (2004, Nature) (plus other conditions: amino acid starvation and nutrition deprivation): binding ratio=5.0; p-value=\( 10^{-11} \).
  - **ARG8**: annotated in GO **BP**: *amino acid biosynthetic process*, while GCN4 is a transcriptional activator of amino acid biosynthetic genes in response to amino acid starvation. –a reasonable target.
  - How detected by the spatial model? ARG8 is the direct neighbor of 4 positive control genes but of *none* negative
control genes. borrowing information: its prior prob was estimated to be 0.733 by the spatial model, in contrast to 0.058 by the standard model.

- TRP5: not in either control set.
  - Prior prob: 0.716 by the spatial model vs 0.058 by the standard model;
  - Posterior prob: 0.723 vs 0.032;
  - binding ratio: =1.15 in Lee et al; =1.21 in Harbison et al;
  - Annotated in GO ‘BP: amino acid biosynthetic process’; likely a target!

- ICY2: a positive gene; has 6 neighbors: 2 negative and none positive.
  - Prior prob: 0.668 by the spatial model vs 0.058 by the
standard model;

– Posterior prob: 0.836 vs 0.548. detected!

– its two negative control genes: ADY2 and CRS5,

– 1) ADY2:
Prior prob: 0.08 by the spatial model vs 0.058 by the standard model;
Posterior prob: 0.06 vs 0.02;

– 2) CRS5:
Prior prob: 0.12 by the spatial model vs 0.058 by the standard model;
Posterior prob: 0.09 vs 0.02;
—both negative neighbors are not false positives!
Simulation

• Starting from the same network as in the real data, simulated a binary MRF for the latent states (i.e. whether $H_{0,i}$ holds or not).
  – Note: MRF not for $x_j$ as used in our model; we have a mis-specified spatial model!
  – updated according to the conditional distribution; stopped after 10 iterations, nearly stable;
  – 4609 nodes, 33432 edge; 183 positive genes, and others negatives.
  – accordingly simulated $z_i$ from the fitted model: $\phi(0, 0.63^2)$ for the null distr, $\phi(0.75, 1.53^2)$ for non-null.

• Simulated 5 datasets: ROC curves, Fig 4

• Sensitivity to mis-specified network structures: Fig 5 randomly removed 5% edges;
randomly added 5% edges; randomly removed 5% and then added 5% edges.

- Sensitivity to hyperparameters: Fig 6 prior for the precision of the mixture model; tried to use non-informative priors when possible.
Figure 4: ROC curves for the two methods applied to five simulated data sets. Dashed lines are for the spatial model; solid lines are for the independence model.
Figure 5: ROC curves for misspecified network structures.
Figure 6: ROC curves for sensitivity analysis (two different priors for the precision parameters of the normal mixture components).
Discussion

• A (happy or productive?) marriage of statistical genomics and spatial statistics.

• More comparisons, applications (e.g. to expression data) and extensions.
  – Wei and Li (2007, Bioinformatics): modeling the states of $H_{0,i}$ as a binary MRF; use ICM (Besag, 1986, JRSS-B). give only point estimates; sensitivity to mis-specified network? alternative: fully Bayesian.

• Applicable: clustering genes with expression profiles for gene function discovery.
stratified model: Pan (2006, *Bioinformatics*).
challenge here: computationally too demanding?
penalized methods: connection to Bayesian

- Extensions:
  - variable/gene selection in sample classifications/regression.
  - variable/gene selection in sample clustering.

- My longer-term plan: apply to genome-wide association studies with SNP data.
  - a high-dim problem;
  - are stat genomics and stat genetics converging?
  - E.g., using gene chromosome location, functional groups/pathways or porotein-protein interaction networks...
  - Using linkage analysis as prior for association study (Roeder et al 2006, AJHG) using weighted p-values.
Extending to incorporating network?
Acknowledgement: This research was supported by NIH and a UM AHC Faculty Research Development grant.

You can download our papers from http://www.biostat.umn.edu/rrs.php

Thank you!