Significance Analysis of Microarrays (SAM)

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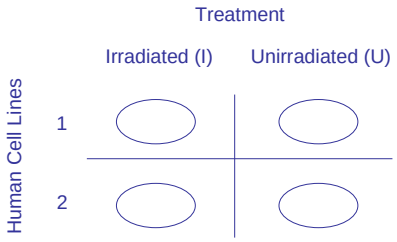
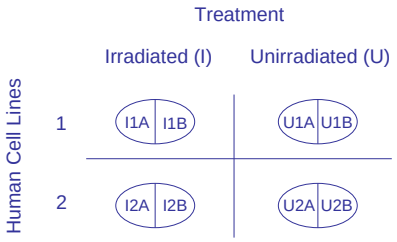
- Tusher, V. G., Tibshirani, R., and Chu, G. (2001). Significance analysis of microarrays applied to the ionizing radiation response. *Proceedings of the National Academy of Sciences* **98(9)**, 5116-5121.
- See http://www-stat.stanford.edu/~tibs/SAM/ for more recent developments.

Motivating Experiment



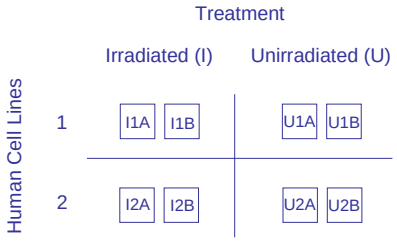
One RNA sample for each combination of cell line and treatment

Motivating Experiment



After labeling, each RNA sample was split into two aliquots denoted A and B.

Motivating Experiment



8 GeneChips, one for each sample, were used to obtain measures of expression.

The Measure of Expression

- The Tusher et al. paper appeared before much work had been done on computing expression measures from PM and MM intensities.
- It appears that they used something similar to an average of PM-MM intensities over the probe pairs in a probe set as their expression measure.
- They conducted a regression-based normalization in which each GeneChip was normalized to the average over all GeneChips.
- Furthermore they worked with normalized data on the original rather than the log scale.

Test Statistic for the i^{th} Gene

Average of 4 normalized measures from irradiated samples

Average of 4 normalized measures from unirradiated samples

$$d(i) = \frac{\bar{x}_I(i) - \bar{x}_U(i)}{s(i)+s_0}$$

The usual standard error in the denominator of a two-sample t-stat

A constant common to all genes that is added to make variation in d(i) similar across genes of all intensity levels

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Selecting the constant s_0

- At low expression levels, variance in d(i) can be high because of small values of s(i).
- To stabilize the variance of d(i) across genes, a small positive constant s_0 was used in the denominator of the test statistic.
- "The coefficient of variation of d(i) was computed as a function of s(i) in moving windows across the data. The value for s_0 was chosen to minimize the coefficient of variation."
- s_0 was chosen to be 3.3 for the ionizing radiation data.

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More Detail on Selecting s_0

- The d(i) are separated into approximately 100 groups. The 1% of the d(i) values with the smallest s(i) values are placed in the first group, the 1% of the d(i) values with the next smallest s(i) are placed in the second group, etc.
- The median absolute deviation (MAD) of the d(i) values is computed separately for each group.
- The coefficient of variation (CV) of these 100 MAD values is computed.

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More Detail on Selecting s_0 (continued)

- This process is repeated for values of s_0 equal to the minimum of s(i) over i, the 5^{th} percentile of the s(i) values, the 10^{th} percentile of the s(i) values,..., the 95^{th} percentile of the s(i) values, and the maximum of the s(i) values.
- The value of s_0 that minimizes the CV of the 100 MAD values over candidate s_0 described above is selected as the constant s_0.

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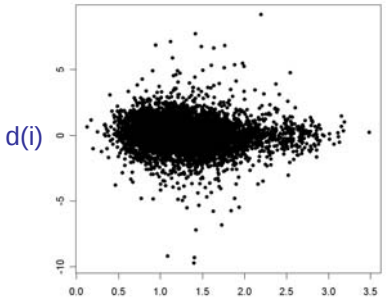
Plot of d(i) vs. $\log_{10}$s(i) for the Ionizing Radiation Data



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A Permutation Procedure for Assessing Significance

1. The irradiated and unirradiated GeneChips were shuffled within each cell line.
2. The d(i) statistic was computed for each gene and ordered across genes from smallest to largest to obtain $d_1(1)<d_1(2)<\cdots<d_1(g)$ where g denotes the number of genes.
3. Steps 1 and 2 were repeated for all possible data permutations described in step 1 to obtain $d_p(1)<d_p(2)<\cdots<d_p(g)$ for p=1,...,36. $\binom{4}{2}\binom{4}{2}$

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Example Permutations

Treatment

Human Cell Lines	Irradiated (I)	Unirradiated (U)
1	I1A, I1B	U1A, U1B
2	I2A, I2B	U2A, U2B

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A Permutation Procedure for Assessing Significance (continued)

4. For each i, $d_1(i),\ldots,d_{36}(i)$ were averaged to obtain $d_E(i)$, the "expected relative difference."
5. The original d(i) statistics were also sorted so that $d(1)<d(2)<\cdots<d(g)$.
6. Genes for which $| d(i) - d_E(i) | > \Delta$ were declared significant, where Δ is a user specified cutoff for significance.

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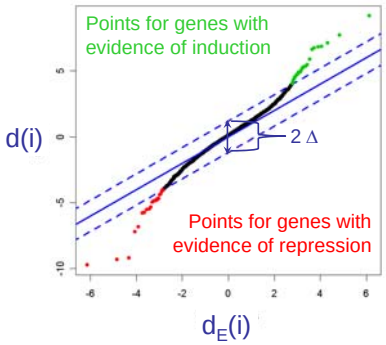
Plot of Observed vs. "Expected" Test Statistics



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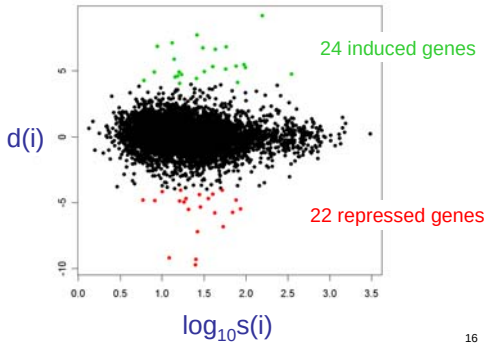
Plot of d(i) vs. $\log_{10}$s(i) for the Ionizing Radiation Data



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Estimating FDR for a Selected Δ

1. Find the smallest d(i) among those d(i) for which $d(i) - d_E(i) > \Delta$ and call it d_{up}.
2. Find the largest d(i) among those d(i) for which $d(i) - d_E(i) < -\Delta$ and call it d_{down}.
3. For each permuted data set, find the number of genes with $d(i) >= d_{up}$ or $d(i) <= d_{down}$ and denote these counts by $n_1,\ldots,n_{36}$.
4. FDR is estimated by $\bar{n} / n$ where $\bar{n}$ is the average of $n_1,\ldots,n_{36}$ and n is the number of genes identified as significant in the original data.

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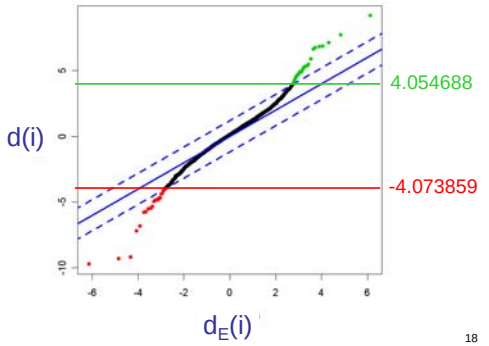
Plot of Observed vs. "Expected" Test Statistics



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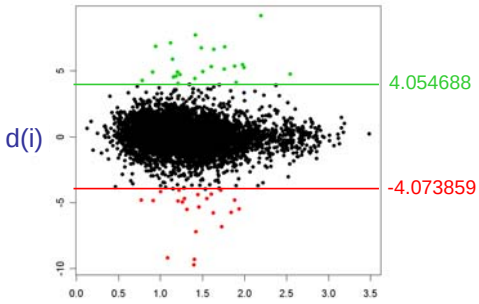
Plot of d(i) vs. $\log_{10}$s(i) for the Ionizing Radiation Data



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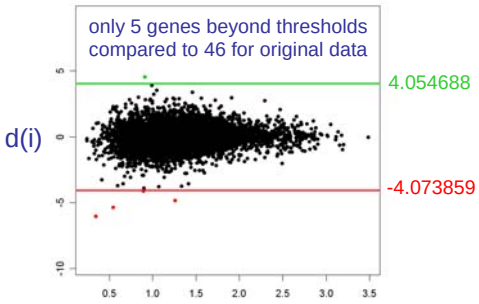
Same Plot for One of the Permuted Data Sets



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Counts of Genes beyond the Threshold for Each Permutation

Perm	Count	Perm	Count	Perm	Count
1	45	13	4	25	4
2	5	14	1	26	2
3	2	15	3	27	1
4	3	16	9	28	1
5	4	17	12	29	5
6	11	18	31	30	9
7	8	19	31	31	11
8	5	20	12	32	4
9	1	21	9	33	3
10	1	22	3	34	2
11	3	23	1	35	5
12	4	24	4	36	46

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Mean Count = 8.472 FDR Estimate = 8.472/46 = 18.4%

Perm	Count	Perm	Count	Perm	Count
1	45	13	4	25	4
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4	3	16	9	28	1
5	4	17	12	29	5
6	11	18	31	30	9
7	8	19	31	31	11
8	5	20	12	32	4
9	1	21	9	33	3
10	1	22	3	34	2
11	3	23	1	35	5
12	4	24	4	36	46

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Concluding Remarks

- Ideally the method would be applied in situations with more true biological replications than were available in the ionizing radiation data.
- Can be extended in many ways.
- Other choices for test statistics are possible.
- Should permute in such a way that all permutations are equally likely under the null hypothesis of interest.
- The properties of SAM regarding error rate control are not well known.

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