Multiple testing

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Multiple testing problem

- With thousands of genes on a microarray we're not testing one hypothesis, but many hypotheses – one for each gene
- Analysis of 20,000 genes using commonly accepted significance level $\alpha = 0.05$ will identify 1,000 differentially expressed genes simply by chance
- If probability of making an error in one test is 0.05, probability of making at least one error in ten tests is

$$(1 - (1 - 0.05)^{10}) = 0.40126$$

Multiple Hypothesis Testing for differential expression detection

- The test statistics and hence the p-values are likely correlated due to co-regulation of the genes.
- Would like multiple testing procedures that take into account the dependence structure of the genes.
- This could be accomplished by estimating the joint null distribution of the unadjusted, unknown p-values.

Permutation based methods

Permutation based adjusted p-values

- Under the H_0, the joint distribution of the test statistics can be estimated by permuting the columns of the gene expression matrix
- Permuting entire columns creates a situation in which membership to the groups being compared is independent of gene expression but preserves the dependence structure between genes

Permutation based methods

- Permutation algorithm for the b^{th} permutation, $b = 1, \ldots, B$

1. Permute the n columns of the data matrix X
2. Compute test statistics $t_{j,b}$ for each hypothesis (gene, $j = 1, \ldots, g$)

- The permutation distribution of the test statistic T_j for hypothesis H_j is given by the empirical distribution of $t_{j,1}, \ldots, t_{j,B}$

Permutation based methods

- For two-sided alternative hypotheses, the permutation p-value for hypothesis H_j is

$$p_j^* = \frac{\sum_{b=1}^{B} I(|t_{j,b}| \geq |t_j|)}{B}$$

where $I(*)$ is the indicator function, equaling 1 if the condition in parentheses is true and 0 otherwise.

Permutation based methods

- Permutation method permits estimation of the joint null distribution of the unadjusted unknown p-values.
- Dependency structure between the genes is preserved.
- May suffer from a granularity problem (when two groups, should have 6 arrays in each group to use permutation based method).

$n/n1!n2!$ ways of forming two groups

Results of Multiple hypothesis testing

Assume we are testing $H_1, H_2, \ldots, H_m$. m_0 - # of true null hypotheses

	# false	# true	
	null hypo.	null hypo.	
# non-signif.	U	T	m - R
# significant	V	S	R
	m0	m-m0	

- **U, S** - True negatives/positives *unobservable random variable
- **V** - False positives [Type I errors] *
- **T** - False negatives [Type II errors] *
- **R** - All positives (# of rejected null hypotheses) Observable

Error rates

False Discovery rate (FDR)

$$E\left[\frac{False\ Discoveries}{True\ Discoveries}\right]$$

Family wise error rate (FWER)

$$Pr(Number\ of\ False\ positives \geq 1)$$

Expected number of false positives

$$E[Number\ of\ False\ positives]$$

Interpretation

Suppose 550 out of 10,000 genes are significant at $\alpha = 0.05$

P-value < 0.05

- Expect $0.05 * 10,000 = 500$ false positives

False Discovery Rate < 0.05

- Expect $0.05 * 550 = 27.5$ false positives

Family Wise Error Rate < 0.05

- The probability of at least 1 false positive is ≤ 0.05

Multiple Hypothesis Testing: FWER

- Given p is the probability of error, $1 - p$ is the probability of correct choice in one test
- $1 - (1 - p)^g$ is the probability of one error in g tests

Multiple Hypothesis Testing: FWER

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Sidak single step

- Testing g null hypotheses
- Reject any H_i with $p \leq 1 - \sqrt[g]{1 - \alpha}$
- When testing 22,283 genes for differential expression, use the following cutoff:

$$1 - \sqrt[22,283]{1 - 0.05} = 0.000002302$$

Multiple Hypothesis Testing: FWER

Bonferroni procedure

- Testing g null hypothesis
- Reject any H_i with $p_i \leq \alpha/g$
- 0.05/22,283 = 0.0000022

Multiple Hypothesis Testing: FWER

Bonferroni procedure

- Testing g null hypothesis
- Reject any H_i with $p_i \leq \alpha/g$
- 0.05/22,283 = 0.0000022
- Controls the FWER to be $\leq \alpha$ and to be equal to α if all hypotheses are true.
- As the number of hypotheses increases, the average power for an individual hypothesis decreases
- Very conservative; no attempt to incorporate dependence between tests

Multiple Hypothesis Testing: FWER

Holm step-down procedure

1. Order the p-values and hypotheses $P_1 \geq \ldots \geq P_g$ corresponding to $H_1, \ldots, H_g$
2. Let $i = 1$
3. If $P_{g-i+1} > \alpha/(g-1+1)$ then accept all remaining hypotheses H_{g-i+1} and STOP
4. If $P_{g-i+1} \leq \alpha/(g-1+1)$ then reject H_{g-i+1} and increment i, then return to step 3.

Multiple Hypothesis Testing: FWER

Sidak step down

1. Order the p-values and hypotheses $P_1 \geq \ldots \geq P_g$ corresponding to $H_1, \ldots, H_g$
2. Let $i = 1$
3. If $P_{g-i+1} > 1 - \sqrt[g-1+1]{1-\alpha}$ then accept all remaining hypotheses H_{g-i+1} and STOP
4. If $P_{g-i+1} \leq 1 - \sqrt[g-1+1]{1-\alpha}$ then reject H_{g-i+1} and increment i, then return to step 3.

Multiple Hypothesis Testing: FWER

Hochberg step up

1. Order the p-values and hypotheses $P_1 \geq \ldots \geq P_g$ corresponding to $H_1, \ldots, H_g$
2. Let $i = 1$
3. If $P_i \leq \alpha/i$ then reject all remaining hypotheses $H_i, \ldots, H_g$ and STOP
4. If $P_i > \alpha/i$ then accept H_i and increment i, then return to step 3.

Considerations for controlling the FWER

- Control over FWER is only appropriate in situations where the intent is to identify only a small number of genes that are truly different.
- Otherwise, the severe loss in power in controlling FWER is not justified.

Considerations for controlling the FWER

- Approaches that set out to control the FWER seek to control the probability of at least one false positive regardless of the number of hypotheses being tested.
- When the number of hypotheses N is very large, this may be too strict = too many missed findings.

False discovery rates: FDR

- It may be more appropriate to emphasize the proportion of false positives among the differentially expressed genes.
- The expectation of this proportion is the false discovery rate (FDR) (Benjamini & Hochberg, 1995)

False discovery rate

Benjamini and Hochberg 1995

Definition: FDR is the proportion of false positives among all positives

$$FDR = E\left[\frac{V}{V+S}\right] = E\left[\frac{V}{R}\right]$$

- Select the desired proportion q, e.g., 0.1 (10%)
- Rank the p-values $p_1 \leq p_2 \leq \ldots \leq p_m$.
- Find the largest rank i such that $p_i \leq \frac{i}{m} * q$
- Reject null hypotheses corresponding to $p_1, \ldots, p_i$

False positive vs. False discovery rates

False positive rate is **the rate at which truly null genes are called significant**

$$FPR \approx \frac{false\ positives}{truly\ null} = \frac{V}{m_0}$$

False discovery rate is **the rate at which significant genes are truly null**

$$FDR \approx \frac{false\ positives}{called\ significant} = \frac{V}{R}$$

False Discovery Rates

Two procedures for controlling FDR:

- Fix the acceptable FDR level σ a priori, then find a data-dependent threshold so that the $FDR \geq \sigma$□. (Benjamini & Hochberg)
- Fix the threshold rule and then form an estimate of the FDR whose expectation is $\geq$ the FDR rule over the significance region. (Storey)

Storey's positive FDR (pFDR)

$$BH:\ FDR = E\left[\frac{V}{R}|R>0\right]p(R>0)$$

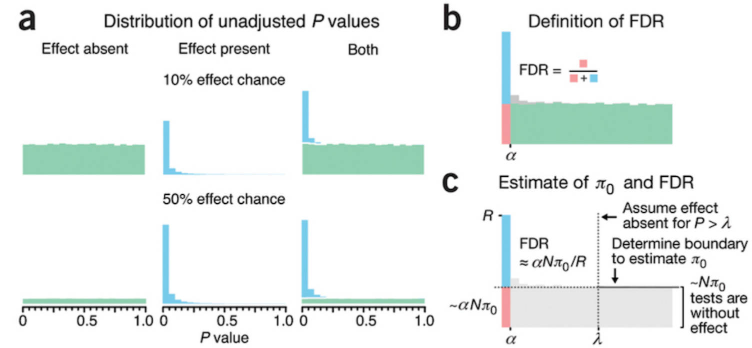
$$Storey:\ pFDR = E\left[\frac{V}{R}|R>0\right]$$

- Since P(R > 0) is ~ 1 in most genomics experiments, FDR and pFDR are very similar
- Omitting P(R > 0) facilitated development of a measure of significance in terms of the FDR for each hypothesis

Q-value

- Storey & Tibshirani, "**Statistical significance for genomewide studies**", PNAS, 2003 http://www.pnas.org/content/100/16/9440.full
- Empirically derived – uses the p-value distribution
- Storey's method first estimates the fraction of comparisons for which the null is true, π_0, counting the number of P values larger than a cutoff λ (such as 0.5) relative to $(1-\lambda) * N$ (such as $N/2$), the count expected when the distribution is uniform
- Multiply the Benjamini & Hochberg FDR by π_0, thus less conservative

Q-value



Martin Krzywinski & Naomi Altman "**Points of significance: Comparing samples—part II**" *Nature Methods* 2016
http://www.nature.com/nmeth/journal/v11/n4/full/nmeth.2900.html

Q-value

- q-value is defined as the minimum FDR that can be attained when calling a "feature" significant (i.e., expected proportion of false positives incurred when calling that feature significant)
- The estimated q-value is a function of the p-value for that test and the distribution of the entire set of p-values from the family of tests being considered
- Thus, in an array study testing for differential expression, if gene X has a q-value of 0.013 it means that 1.3% of genes that show pvalues at least as small as gene X are false positives