CAP 5510: Introduction to Bioinformatics

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Microarray Data

Gene	Expression Levels	
	Sample A CONTROL	Sample B TREATMENT
Gene1		
Gene2		
Gene3		
...		

Microarray Analysis

- Is Gene X upregulated? Downregulated? Had no change in expression levels?
  - Genes are represented by probes
  - Experiments may have repeats
- **NULL HYPOTHESIS**
  - *There is no change in gene expression levels for Gene X between Control and Treatment*

Accept/Reject H_0 (Null Hypothesis)?

- P-value thresholds
  - P-value is probability of data assuming H_0 holds
  - P-value threshold of 0.05 means probability of error when H_0 is rejected is 5%
- Fold change
  - If no repeats are done
- t-Test
  - Parametric
  - Non-parametric
    - Wilcoxon rank sum

Hypothesis Testing Logic

		Hypothesis Choice	
		H0	H1
Decision	H0	Correctly Accept (TN)	Type II Error (FN) β
	H1	Type I Error (FP) α	Correctly Reject (TP)

- Typical Values:
  - Type I error of 0.05
  - Type II error of 0.2

Problem with Hypothesis Testing

- Not testing just one gene
- If multiple genes are tested, then t-Test assumes each test is independent
- Are the tests independent?
  - No!
- Need Correction
  - P-values need to be adjusted
  - Bonferroni or other correction methods needed
  - Achieved by controlling Type I error

Multiple Testing & Type I Errors

- Type I Error of 0.05 means that there is a 5% error in prediction of FN by t-Test. IMPLICATIONS?
  - If N=1000 genes & d=40 are differentially expressed (DE), then …
    - 960 X 0.05 = 48 FPs
    - There are more FPs than TPs
    - Type I error and correcting for multiple hypothesis testing are connected

Multiple Test Corrections

- Bonferroni correction
  - Use type I error = α / g = FWER = 0.05/1000
    - Family-wise Error (FWER)
    - Too Conservative! Also reduce true positives!
- Other less conservative corrections possible
  - Sidak correction, Westfall-Young correction, …
- Using False Discovery Rate (FDR) [Benjamini & Hochberg '95, Storey '02 & '03]
  - Earlier: 5% of all tests will result in FPs
  - With FDR adjusted p-value (or q-value): 5% of significant tests will result in false positives.

P-value vs Q-value

Ask another question

Rank	Anova (p)	q Value	Power	Cluster
30	0.00436	0.0119	0.993	
77	0.00536	0.0119	0.987	
97	0.00631	0.0119	0.98	
29	0.00655	0.0119	0.979	
43	0.00605	0.0119	0.982	
23	0.0067	0.0119	0.977	
36	0.00632	0.0119	0.98	
28	0.00698	0.0119	0.975	
76	0.00685	0.0119	0.976	
60	0.0067	0.0119	0.977	
10	0.00479	0.0119	0.991	
13	0.00467	0.0119	0.991	
51	0.00432	0.0119	0.993	
91	0.0062	0.0119	0.981	
21	0.00611	0.0119	0.982	
46	0.00414	0.0119	0.994	
45	0.00739	0.0127	0.972	
25	0.00822	0.0137	0.964	
53	0.00903	0.0137	0.956	
6	0.00919	0.0138	0.955	
52	0.01	0.0141	0.946	
2	0.00976	0.0141	0.949	
87	0.0101	0.0141	0.946	
19	0.0109	0.0141	0.938	
96	0.0102	0.0141	0.944	
55	0.011	0.0141	0.937	
50	0.00949	0.0141	0.952	
49	0.0115	0.0144	0.931	
32	0.0127	0.0144	0.918	

Consider example shown. Let N = 839.

Marked item has p-value 0.01 and q-value 0.0141. P-value threshold of 0.01 implies a 1% chance of false positives. Thus, we expect 839*0.01 = 8.39 FPs. Since item has rank 52, we expect to have 8 or 9 of these to be FPs.

Q-value threshold of 0.0141 implies a 1.41% of all spots with q-value less than this to be FPs. Thus, we expect 52*0.0141 = 0.7332 FPs, i.e., less than one FP.