

NTP's Proposed Approach to Filtering Unresponsive Genes

Scott S. Auerbach Ph.D., DABT
Biomolecular Screening Branch
National Institute of Environmental Health Science

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Approach to Genomic Dose-Response Modeling
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Filtering Measured Features

- One way ANOVA / Fold Change
 - Parameter thresholds are technology dependent
 - ANOVA allows for inclusion on non-monotonic responses
 - Fold change incorporates a biologically meaningful effect size
- Using the orthogonal filters (statistic and fold change) is in alignment with the MAQC recommendations
- Maximizes reproducibility, minimizes false discovery

One Way ANOVA

One Way Anova

Expression Data: DMPT Liver RMA Normalized

P-Value Cutoff: 0.05

Multiple Testing Correction: ☐ Benjamini & Hochberg (FDR)

Filter Out Control Genes: ☒ (probes starting with AFFX...)

Fold Change

☒ Use Fold Change Filter

☒ Log Transformation ☒ 2 ☐ 10 ☐ Natural

Fold Change Value: 2

Start Cancel Done



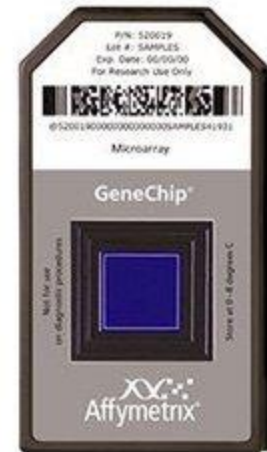
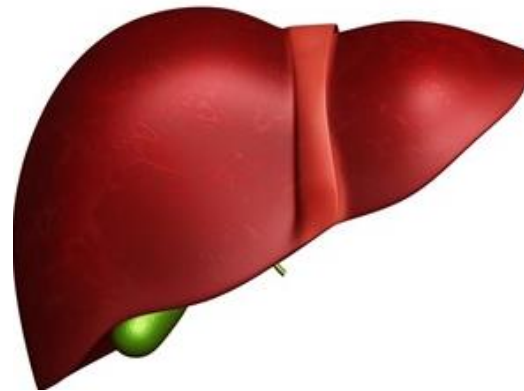
Determining Thresholds

- Identify the most permissive filter threshold that adequately eliminates noise and maximizes reproducibility

- Data

TG-Gates (<http://toxico.nibiohn.go.jp/english/>)

- Rat Liver
- Affymetrix 230 2.0 microarrays





Permissiveness and Noise Elimination

- Select ~100 control expression samples with no batch or quality control problems
- 5 null dose-response data sets by random sampling
- 20 different feature filters coupled to a complete BMDExpress Analysis
 - Statistical Threshold (with or without multiple testing correction)
 - Fold change
- Ranked the filtering methods based on lowest number of “active” gene sets with BMDs
 - Gene sets = GO Biological Processes
- If multiple methods reported “0” active GO Biological Processes they were ranked with the more permissive method receiving a higher rank
- Permissiveness determined by the total number of probe sets allowed through by each filter on multiple independent data sets with varying amounts of signal



Permissiveness and Noise Elimination

GO Biological Processes

ANOVA (P-Value)	Multiple Testing Correction	Fold Change Filter	Number of “Active” GO Biological Processes (N~12,000)	Permissivity Rank (High to Low)	Overall Noise Elimination Rank
0.1	MTC	none	0	1	1
0.01	NOMTC	1.2	0	2	2
0.1	MTC	1.2	0	3	3
0.05	MTC	none	0	4	4
0.05	MTC	1.2	0	6	5
0.05	NOMTC	1.5	0	9	6
0.01	NOMTC	1.5	0	10	7
0.1	MTC	1.5	0	11	8
0.05	MTC	1.5	0	12	9
0.01	MTC	none	0	13	10
0.1	NOMTC	2	0	14	11
0.05	NOMTC	2	0	15	12
0.01	MTC	1.2	0	16	13
0.1	MTC	2	0	17	14
0.01	NOMTC	2	0	18	15
0.05	MTC	2	0	19	16
0.01	MTC	2	0	20	17
0.05	NOMTC	1.2	>1	5	18
0.1	NOMTC	1.2	>1	7	19
0.1	NOMTC	1.5	>1	8	20



- Chemicals (same mechanism of action – PPARA)
 - WY-14,643, Fenofibrate, Gemfibrozil
- 3, 7, 14 and 28 day liver studies from TG-Gates
 - 3 dose levels and control
- 20 different gene filters with a complete BMD Analysis
 - Statistical threshold; Multiple testing correction; Fold change
- Reproducibility Metric
 - Percent of overlapping “Active” GO Biological Processes with a BMD for all 3 chemicals at the 4 different time points



GO Biological Processes

ANOVA (P-value)	Multiple Testing Correction	Fold Change Filter	Percentage of Overlapping GO Biological Processes	Reproducibility Rank
0.1	NOMTC	1.5	17.705	1
0.05	NOMTC	1.5	17.16	2
0.1	NOMTC	2	17.06	3
0.01	NOMTC	1.5	16.8	4
0.05	NOMTC	2	16.605	5
0.05	NOMTC	1.2	15.69	6
0.01	NOMTC	1.2	14.69	7
0.01	NOMTC	2	14.295	8
0.1	NOMTC	1.2	14.25	9
0.1	MTC	2	12.345	10
0.1	MTC	1.5	12.305	11
0.1	MTC	none	9.955	12
0.1	MTC	1.2	9.845	13
0.01	MTC	2	8.615	14
0.05	MTC	2	8.33	15
0.05	MTC	1.5	8.255	16
0.05	MTC	1.2	6.275	17
0.05	MTC	none	6.045	18
0.01	MTC	none	4.06	19
0.01	MTC	1.2	4	20



GO Biological Processes

ANOVA (p-value)	Multiple Testing Correction	Fold Change	Noise Elimination Rank	Reproducibility Rank	Combined Rank
0.05	No	1.5	6	2	1



- Combined statistical and effect size threshold filter for responsive genes
- Empirical method for deriving statistical and effect size thresholds that considers noise reduction, permissiveness and reproducibility
- Reviewer suggestions:
 - Rank regression
 - Trend test
 - More rigorous statistical filter (e.g., $FDR < 0.05$)