NTP
National Toxicology Program

Overview of the NTP Proposed Approach to Genomic Dose-Response Modeling

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Overview of Approach GDRS

Study Design

Many dose levels, limited biological replication, select target organs/cell types

BMDExpress 2.0

Filtering Measured Features

Statistical and effect size filter

Fitting Features to Dose-Response Models

Fit multiple models, determine potency using best fit model

Determination of Gene Set Level Potencies

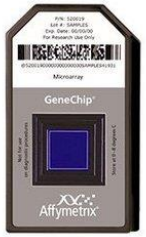
Identify “active” gene sets, report potency for active gene set

Biological Interpretation

Use detailed curation of gene sets to provide toxicological associations/contextualization

NOEL/LOEL vs BMD Design

X 30 X



Benchmark Dose

- **Goal:** Accurately determine a point of departure
- **Example:** 9 Dose Levels and Control
  - 3 Biological Replicates
- Derives power from using all of the data and a fitted model to identify a minimum effect level (point of departure)
- More effective at capturing a diversity of dose-response patterns and potencies in the same study
- **Shortcoming**: Limited ability to detect hazards

NOEL/LOEL

- **Goal:** Identify hazards
- **Example:** 4 Dose Levels and Control
  - 6 Biological Replicates
- Derives power from high biological replication which allows for robust pairwise comparisons (treated vs control)
- More effective at identifying low incidence hazards
- **Shortcoming**: Due to limited number of dose groups often does not support dose-response analysis and accurate estimation on minimum effect levels

Conclusion

Our goal with GRDS to accurately estimate the minimum biological potency, not to detect hazards. In addition, we will me fitting a diversity of features from a single study with an array of potencies with the hope of accurately estimating their POD. For this reason we have chosen the Benchmark Dose Approach.

Feature Filtering: To do it or not?

- Identified ~100 microarrays from vehicle treated rat liver (TG-Gates, 7 day) with no batch effect
  - http://toxico.nibio.go.jp/english/index.html
- Randomly sample arrays to create 5 null data sets of 30 microarrays
  - Dose levels 0, 0.1, 1, 10, 100, 1000
  - 5 samples per dose group
- Ran null sets through BMDExpress using no statistical/effect size filter
  - Instead a global goodness of fit statistic (p>0.1) and BMDL/BMD ratio<20 to the dose-response model was used to remove features

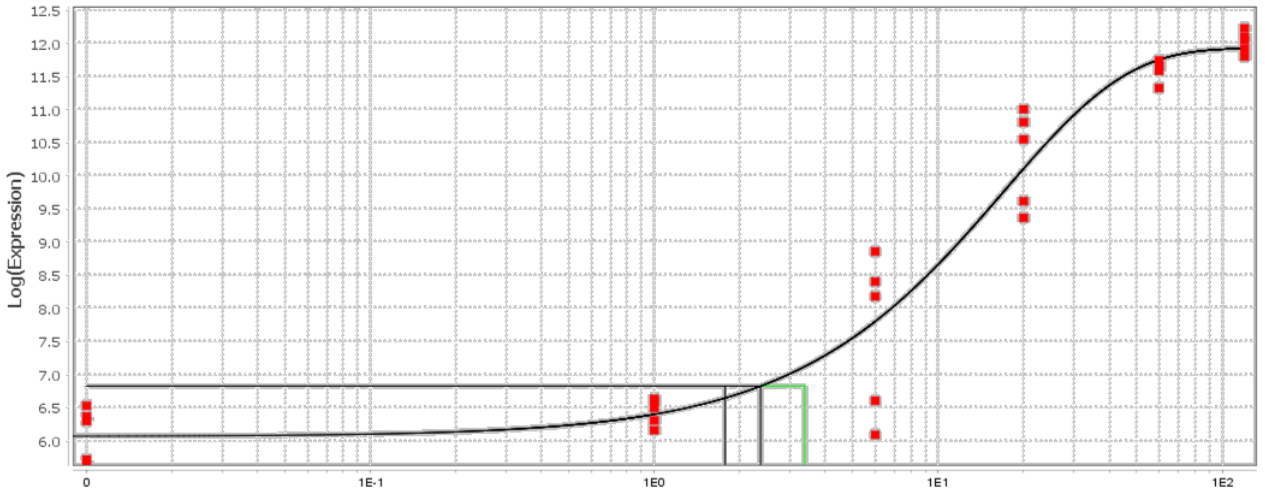
Null Set	ANOVA (FDR<0.05) (31,000)	Individual Genes (n=14073)	GO BP (n=12355)	MSigDB Pathways (n=4725)
1	0	2672	3108	2881
2	0	4956	4110	3805
3	0	4670	4031	3691
4	0	3635	3647	3401
5	0	5116	4451	3910

Conclusion

We need some sort of filter other than the global goodness of fit filter and the BMD/BMDL ratio

Dose-Response Modeling: Type and How many?

- Why use the US EPA models?
  - They are validated
  - They are used broadly in risk assessment to derive potencies (BMD)
    - Our target audience is comfortable with the BMD metric and the modeling process
  - Diversity of models allows for adequate fitting of variety of dose response patterns
  - Great documentation and guidance on how to use the models



Potency Reporting: Gene sets or Genes?

- Why use gene sets?
  - Better coverage of biological space
    - Less blind spots in uncharacterized biological settings
  - Gene sets better represent the underlying totality of the emergent properties at the cellular and tissue level
  - Gene sets give a better representation of the uncertainty in biological potency

Biological Interpretation: To do it or not?

- Why attempt biological interpretation?
  - Facilitate uptake by the toxicology community
  - A degree of consensus exists as to interpretation of subset of gene signatures and biological processes
  - Start the process of building some consensus in regard ranking levels of toxicological “distress” when a gene set is altered by treatment
    - P53 vs. Xenobiotic Metabolism
  - Importantly, interpretation will not be used for traditional hazard labeling at this time

Points to Consider

- Overall Analysis Approach and Design: NOEL/LOEL approach vs. BMD
- Statistical and effect size filtering: To do it or not?
- Dose-response modeling: Type and how many?
- Potency reporting: Gene sets vs. Genes
- Biological interpretation: Do it or not?

- Variables to consider?
  - Better established define how data will inform screening level risk assessment?
  - Greater detail biological interpretation and engagement of the toxicology community?