NTP’s Proposed Approach to Estimating Gene Set Level Potencies

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

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For a feature to be considered its best model must...

- Have convergent BMD, BMD$_L$ and BMD$_U$ values
  - Indicates model parameters are optimized
  - Ensures complete representation of the uncertainty around the BMD
- Not map to more than one gene
  - Removes features with uncertain gene association
- Not have a BMD$>$ highest dose
  - Avoids model extrapolation
- Have a nominal global goodness of fit p-value $>$0.0001
  - Higher values indicate better fit
  - Ensures a minimum (albeit liberal) fit of the model to the data
- BMD$_U$/BMD$_L$ $<$ 40
  - Removes features with highly uncertain BMDs
Global Goodness of Fit P-value

• EPA Guidance
  – Prior model hypothesis, fit p-value > 0.05
  – No prior hypothesis, multiple models, fit p-value > 0.1

• Justification for the lower threshold fit p-value
  – A number of orthogonal filters for removing non-responsive or noisy data are included in the analysis pipeline
    • Fold change and ANOVA
    • BMDU/BMDL ratio < 40
    • Gene set level filters - 3 genes, 5% populated, Fisher Exact test p < 0.05
  – We use agglomerative estimates of potency
  – Loss of critical information particularly for moderate signal test articles
Identifying Active Gene Sets and Potency

Gene Set 1 (15 genes)

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>BMD</th>
<th>$BMD_L$</th>
<th>$BMD_U$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene 1</td>
<td>10</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Gene 2</td>
<td>50</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td>Gene 3</td>
<td>100</td>
<td>75</td>
<td>120</td>
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<tr>
<td>Gene 4</td>
<td>150</td>
<td>100</td>
<td>175</td>
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<tr>
<td>Gene 5</td>
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<td>210</td>
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<td>Gene 14</td>
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<tr>
<td>Gene 15</td>
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</tr>
</tbody>
</table>

- At least 3 genes
  - Ensure that small gene sets are minimally populated
  - Assuming no prior knowledge, minimum number of genes required to indicate a pathway or gene set is responding to treatment
  - Minimum number of genes from which you can identify a median value
- At least 5% populated
  - Ensure larger gene sets require more than 3 genes
- Fisher Exact Test ($p<0.05$)
  - Nominal Statistical Filter

$\bigcirc$ = Median value = Gene Set BMD, BMD$_L$, BMD$_U$
Points to Consider

- Fit p-value threshold >0.0001
- BMDU/BMDL ratio threshold of <40
- Threshold for “active” gene sets
  - 3 genes, 5% populated and Fisher Exact P-value<0.05
- Determining potency of a gene set
  - Median and Mean BMD

- Other variables to consider
  - GSEA-based approach
  - Bayesian alternative to enrichment
  - Focus only on use of select biomarker genes from AOPNs
  - What if only 2 biomarker genes are active (e.g., p21 and Ccng1)? Ignore?
  - Data supporting use of thresholds of “3 genes, 5% populated and Fisher Exact P-value<0.05”
  - Pathway agnostic agglomerative BMD
    - Median BMD of the 20 most differentially expressed genes