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Towards replacing the two-year bioassay with short-term assays: gene expression thresholds can identify rat liver tumorigens

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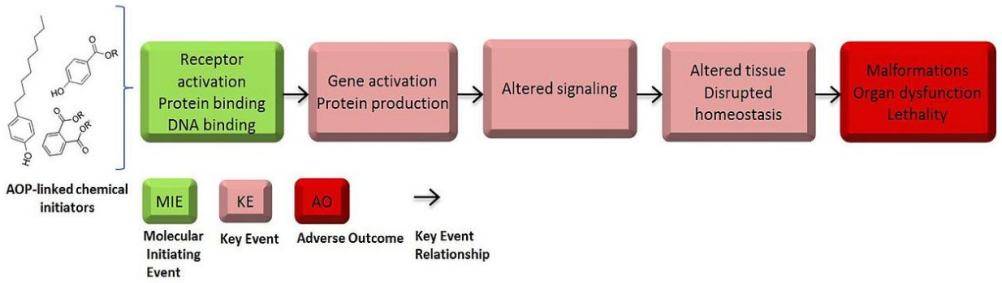
Outline

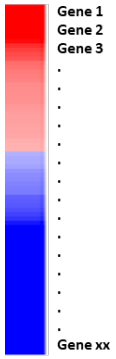
- Methods used to create and determine predictive accuracy of gene expression biomarkers

- Stratification of tumorigenic risk using gene expression biomarkers in short-term animal studies
  - Identification of liver tumorigens

- Identification of biological thresholds predictive of liver cancer
  - Gene expression biomarkers
  - Individual genes
  - Liver weight and clinical chemistry endpoints

Definitions





Treated vs. Control

Adverse Outcome Pathway

- Structured representation of biological events leading to adverse effects; relevant to risk assessment
- A series of causally connected key events (KE) between two points — a molecular initiating event (MIE) and an adverse outcome (AO) that occur at a level of biological organization relevant to risk assessment

Gene Expression Biomarker

- List of genes and associated fold-change values or ranks
- Measures a molecular initiating event or key event in an adverse outcome pathway using transcript profiling

Biological Thresholds

- Empirically-derived by comparing exposure conditions that lead to toxic responses vs. those that do not
- Chemical-independent
- Derived for biomarkers, genes and traditional measures of toxicity

Bioset

- List of statistically-filtered genes derived from a comparison between treated and control groups

Use of biomarkers and thresholds to inform carcinogenic risk and mode of action

Problem: how can we better use 21st century tools in a prospective manner to avoid unnecessary 2-year bioassays?

Can we predict from short-term studies:

- Chemical-dose combinations that will cause tumors?
- Mode of action by which the tumors would arise?
- Whether the mechanism is human-relevant?

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Adverse Outcome Pathways that Lead to Liver Cancer

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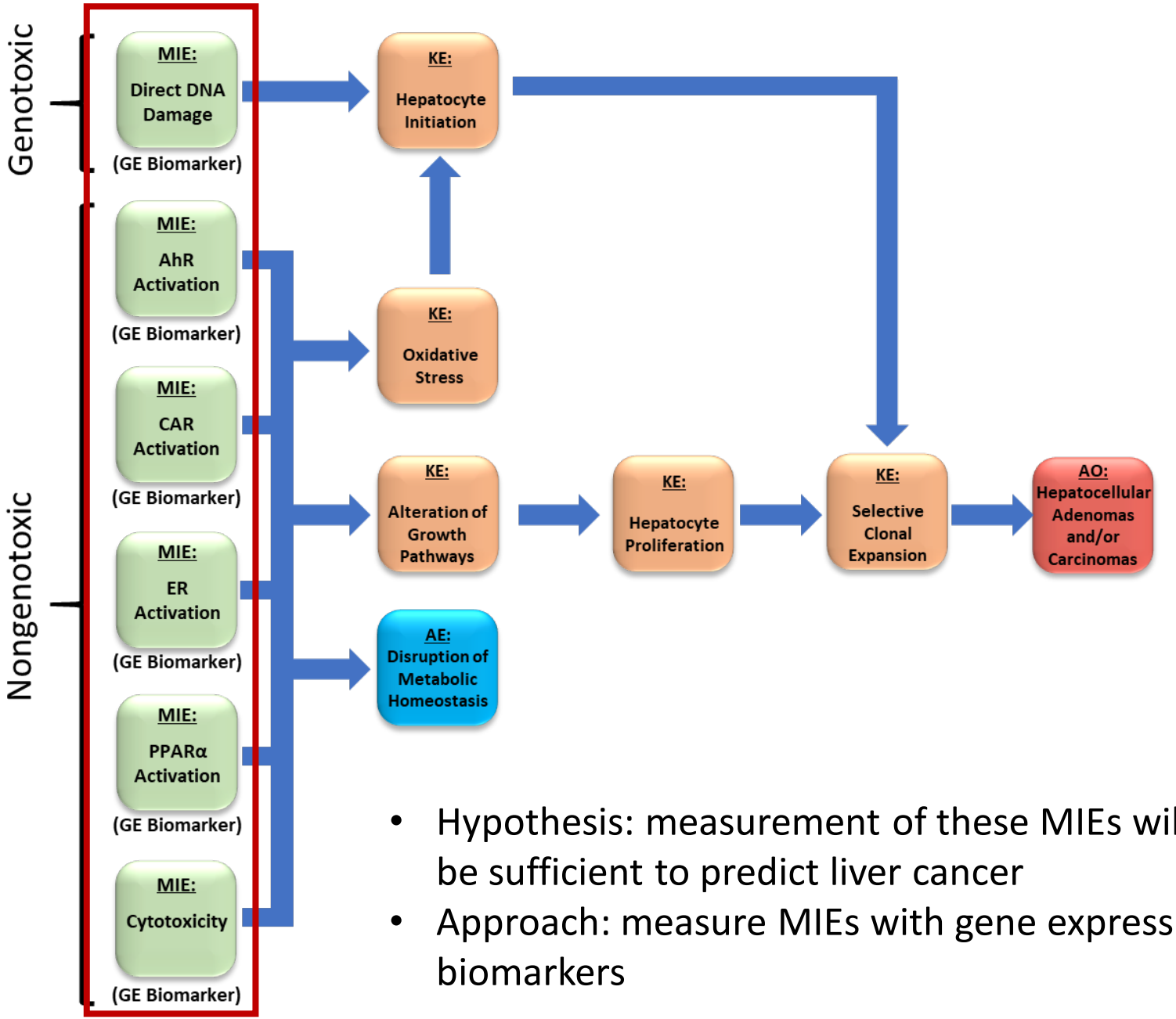
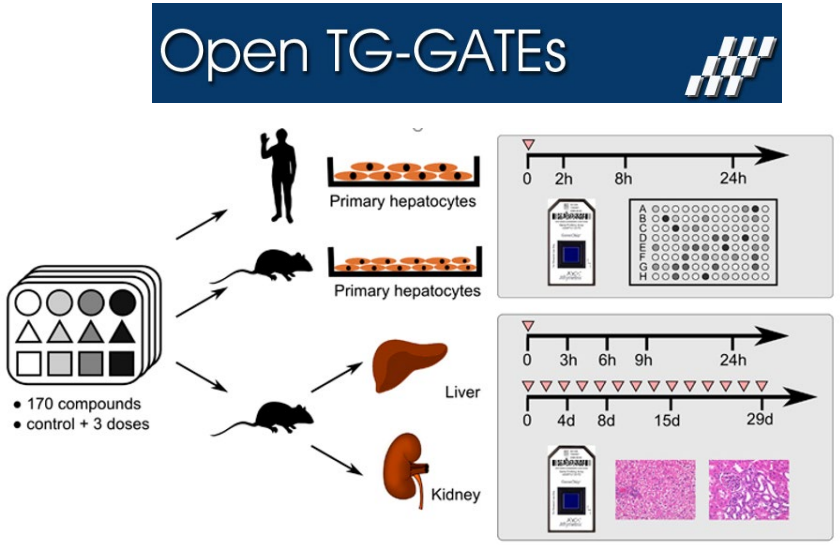
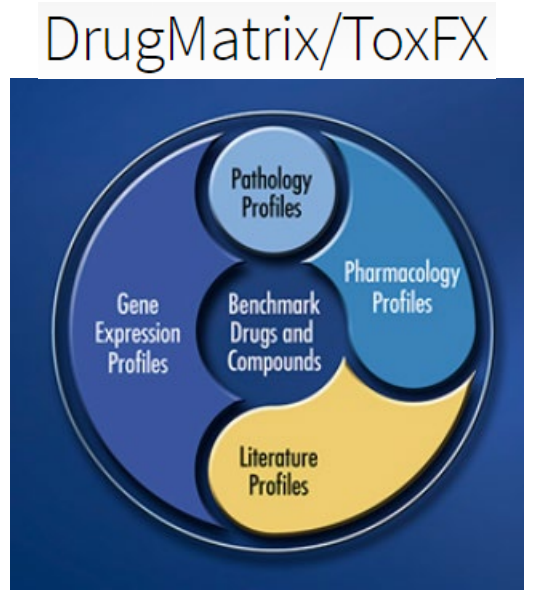


- Hypothesis: measurement of these MIEs will be sufficient to predict liver cancer
- Approach: measure MIEs with gene expression biomarkers

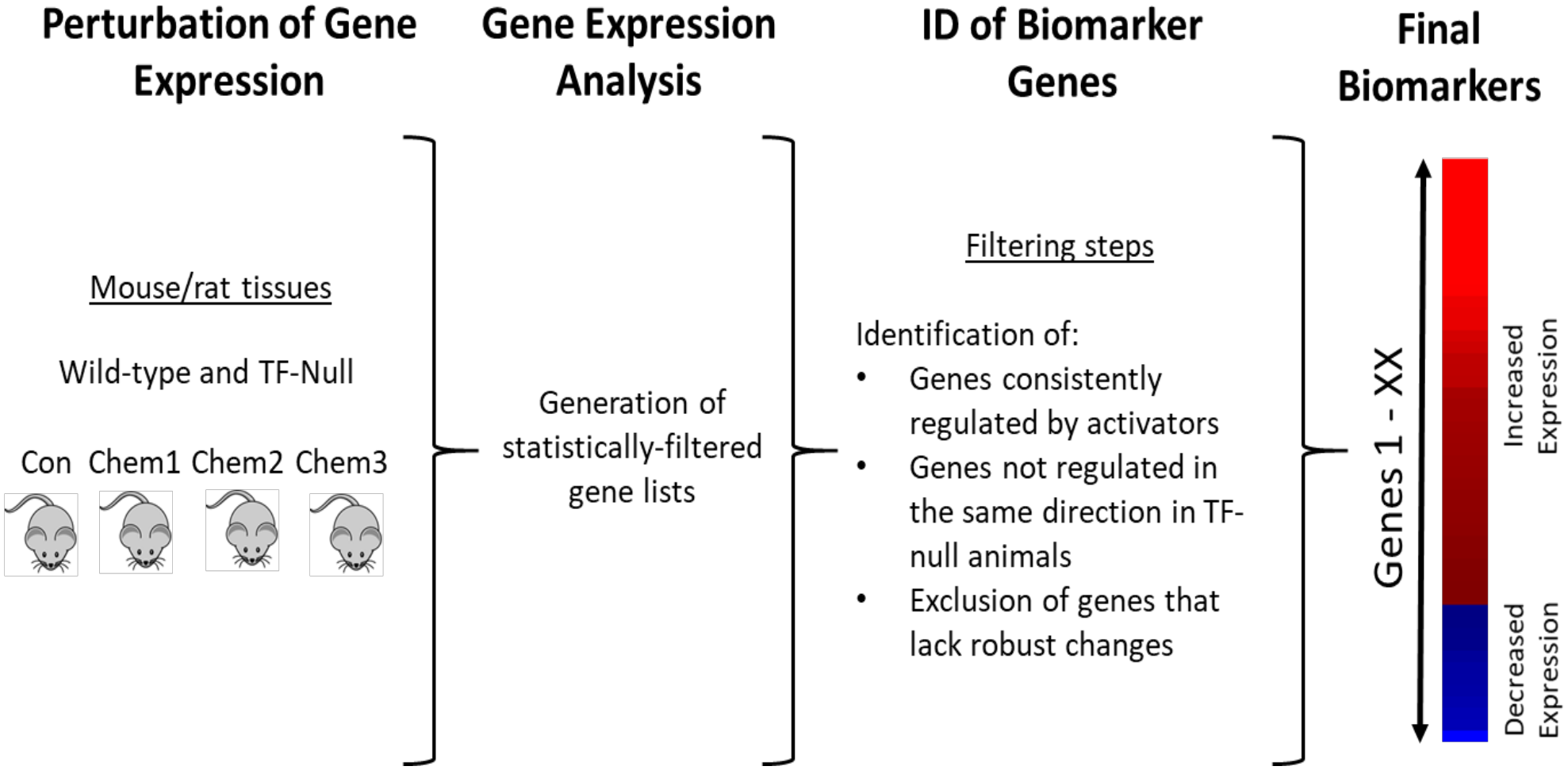
Sources of Rat Liver Tumorigenicity and Microarray Data

- TG-GATES microarray data (rat full genome)
  - ~130 chemicals, 8 time points, 3 doses
- DrugMatrix microarray data (rat full genome)
  - >600 chemicals, 4 time points, 2 doses
- Carcinogenicity Potency Database
  - Carcinogenicity data on >1500 chemicals in rats and mice
  - Used data to categorize the hepatotumorigenic potential of chemical-dose comparisons in TG-GATES and DrugMatrix



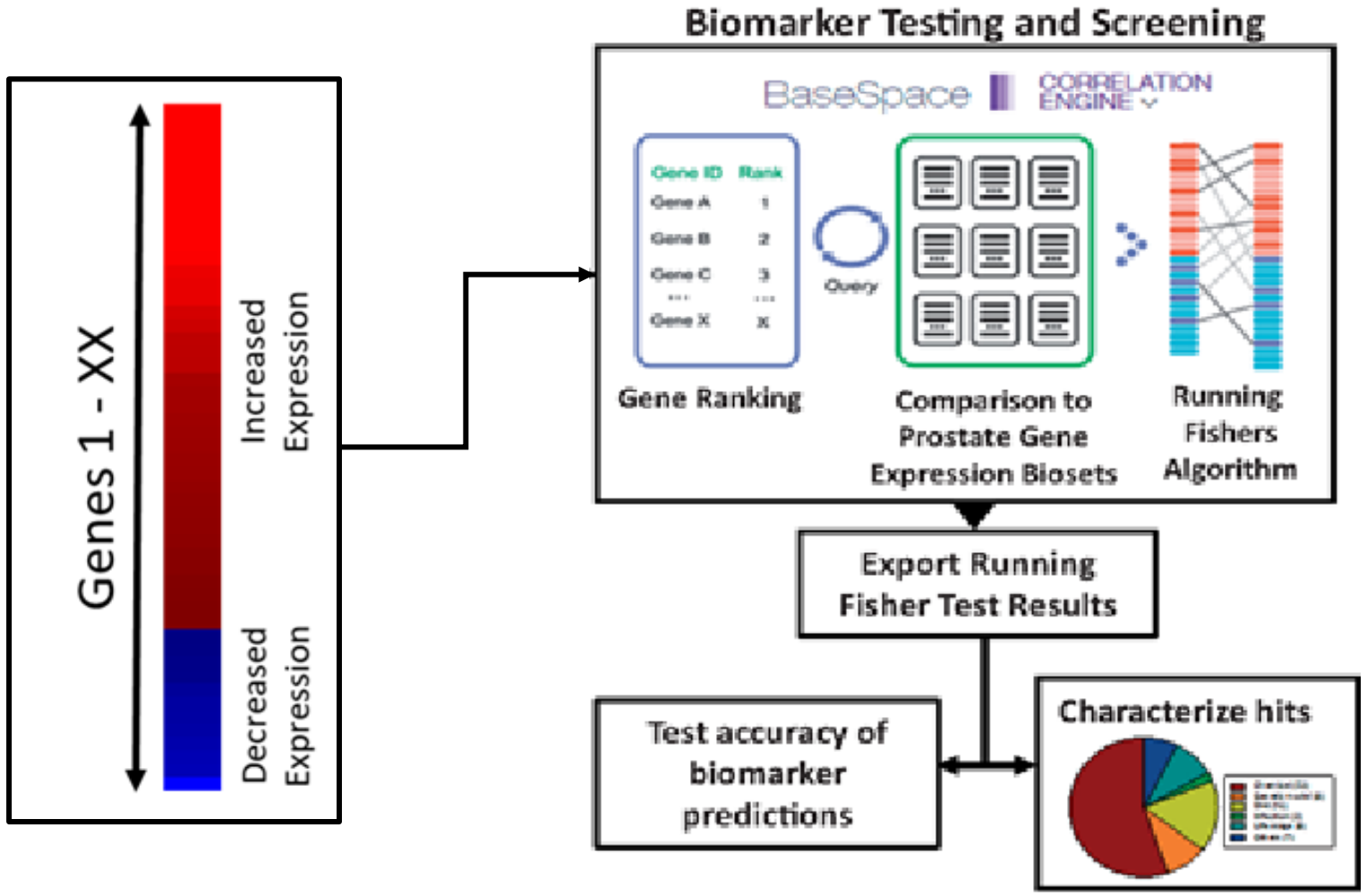


Construction of biomarkers from microarray data generated in animal tissues



From Corton (2019) Current Opinion in Toxicol 18:54

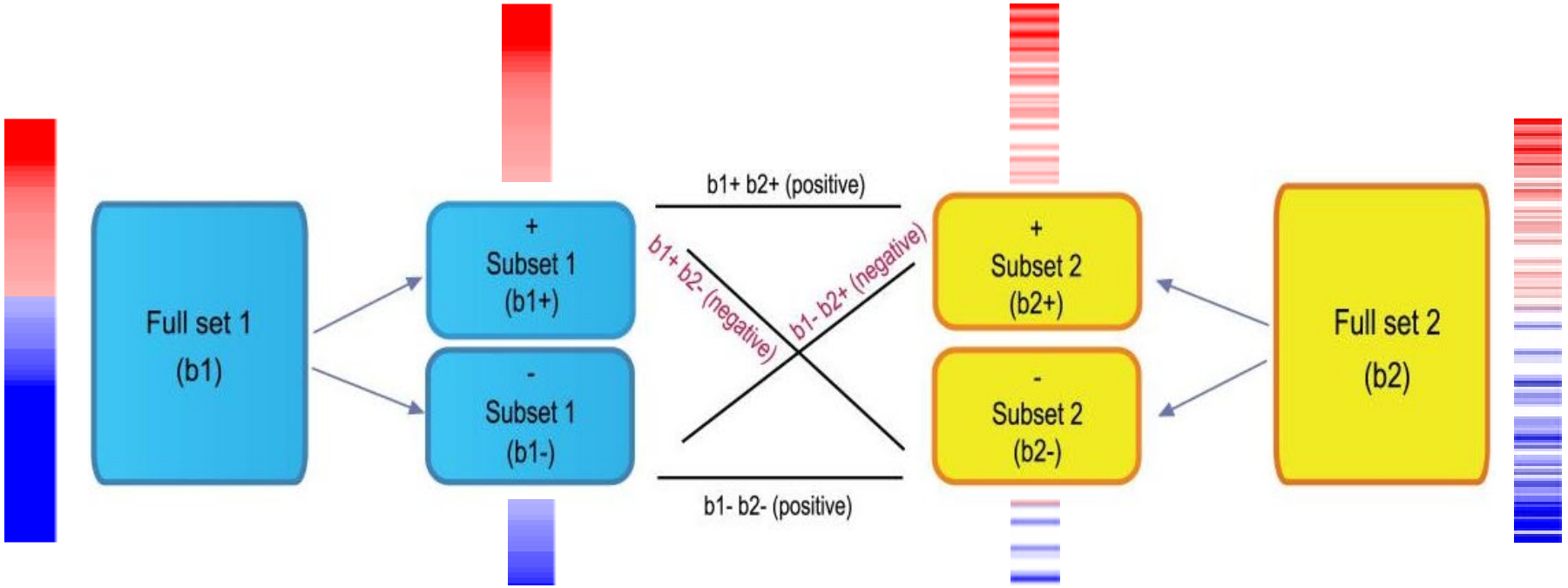
Comparing gene lists in BaseSpace Correlation Engine



>130,000 statistically filtered gene lists from > 25,000 studies

Derived from Rooney et al. Toxicol Sci. 166:146-162

Computing directionality and final correlation scores between two gene lists



- Score(b1, b2) = sum(b1+b2+, b1+b2-, b1-b2+, b1-b2-)
- Running Fisher Test p-value
- Direction of the correlation

- **The Running Fisher test p-value is a useful metric of correlation between gene sets**

Adapted from Kuperschmidt et al. (2010) PLoS One

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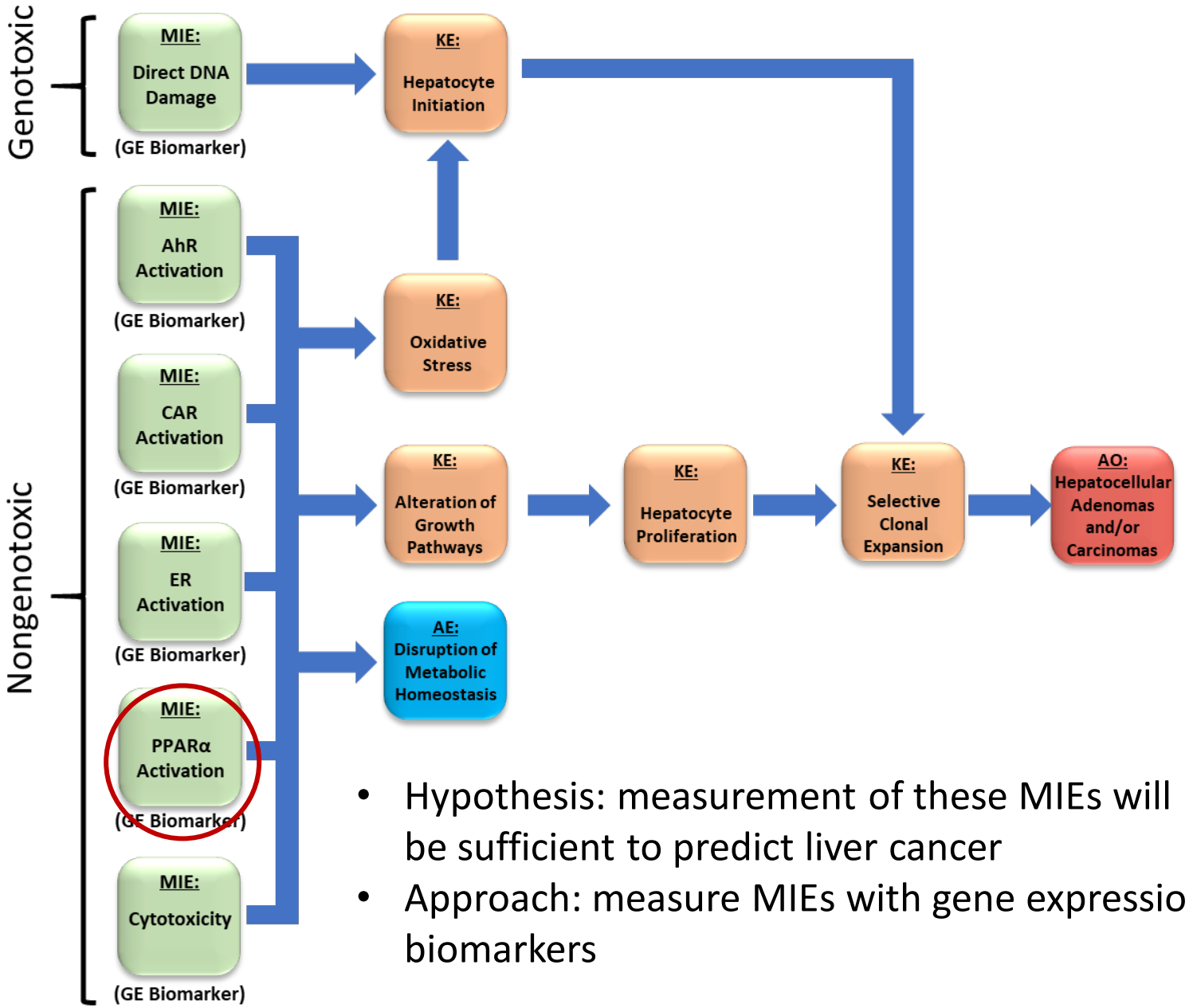

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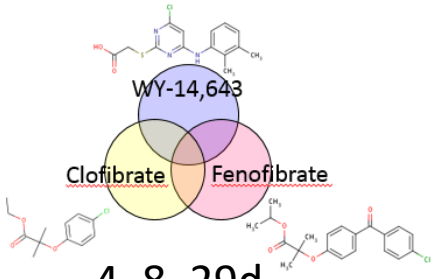
Using weight of evidence to build a rat liver PPARα biomarker

- Microarray data sets from TG-GATES study



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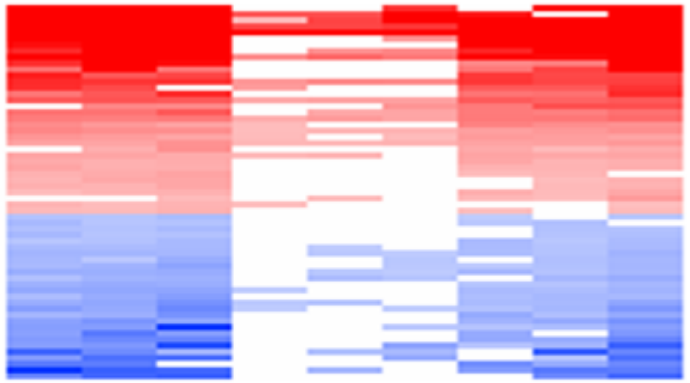
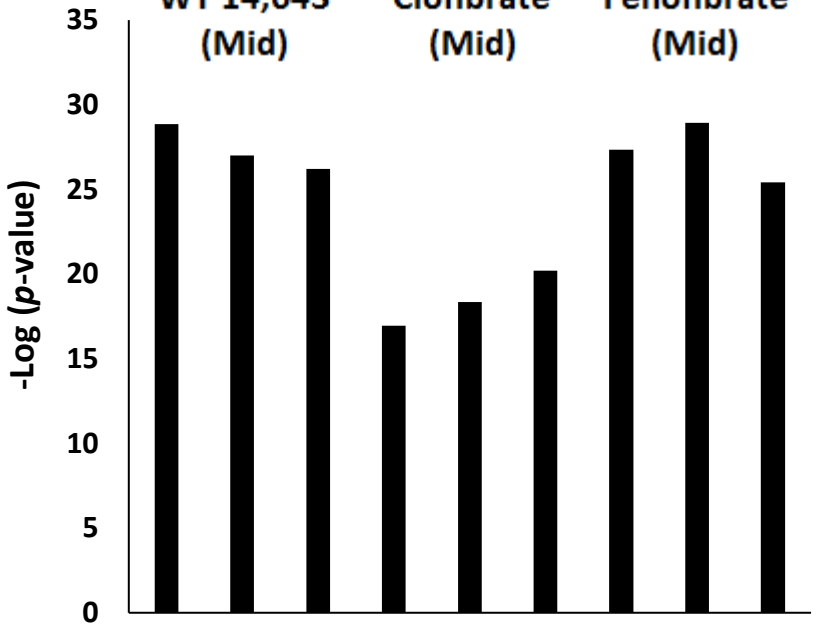
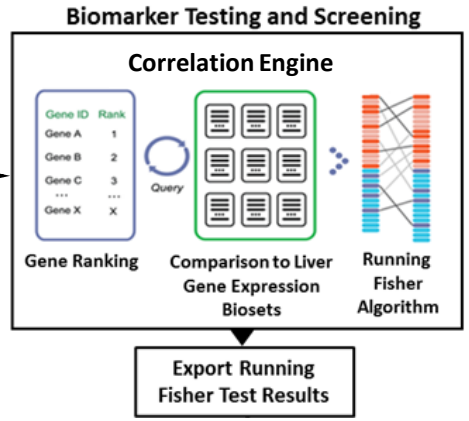




PPARα Biomarker (58 genes)



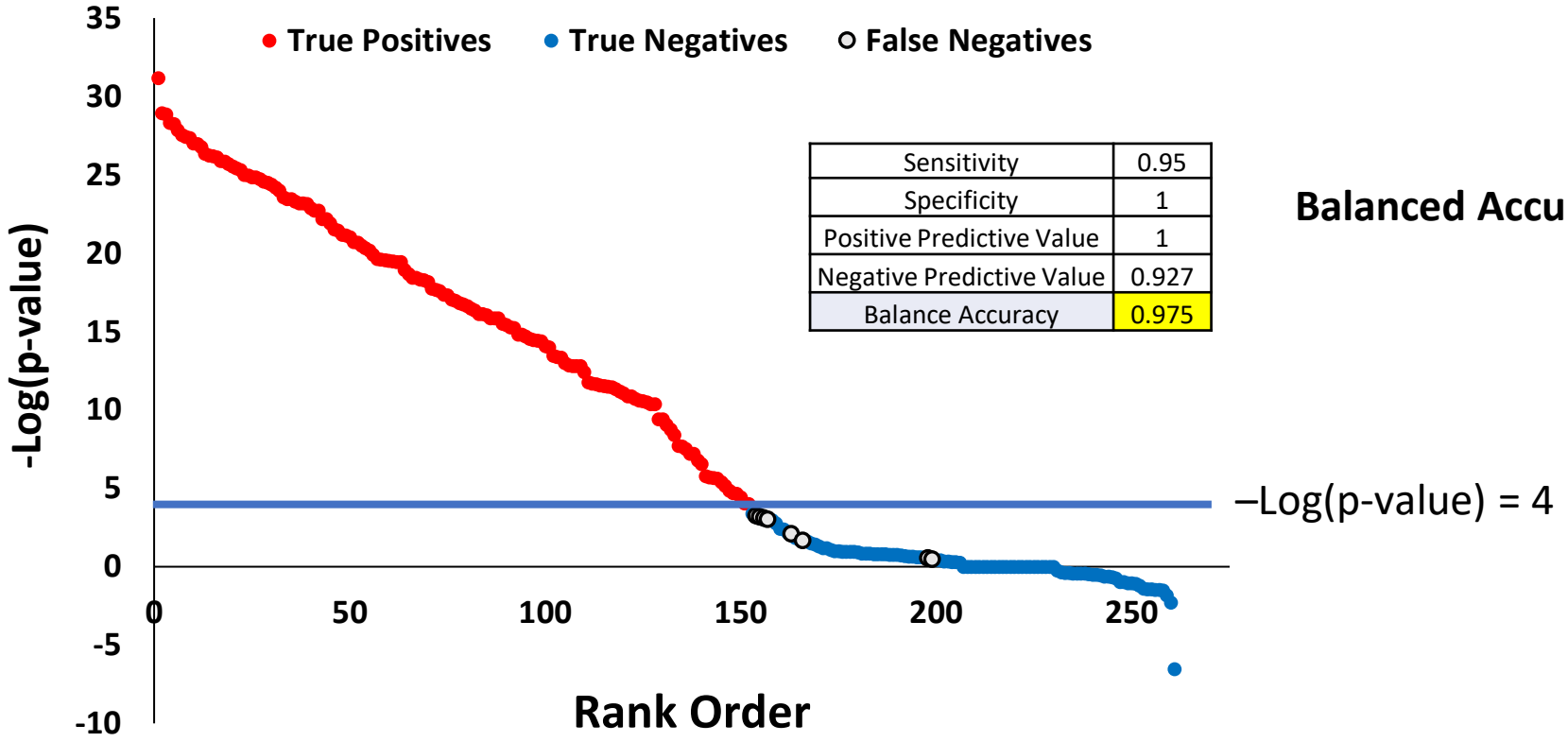
Weight of evidence gene selection

- Same direction of change
- Regulated in the majority of comparisons
- Ave absolute fold-changes ≥ 1.5
- Not found in other biomarkers

Rooney et al. (2018) TAAP

Testing the rat liver PPARα biomarker for predictive accuracy

- Examined 261 comparisons with known PPARα activity in rat liver
- Used a cutoff of –Log(p-value) = 4 as in prior studies
- Excluded comparisons used to create the biomarker



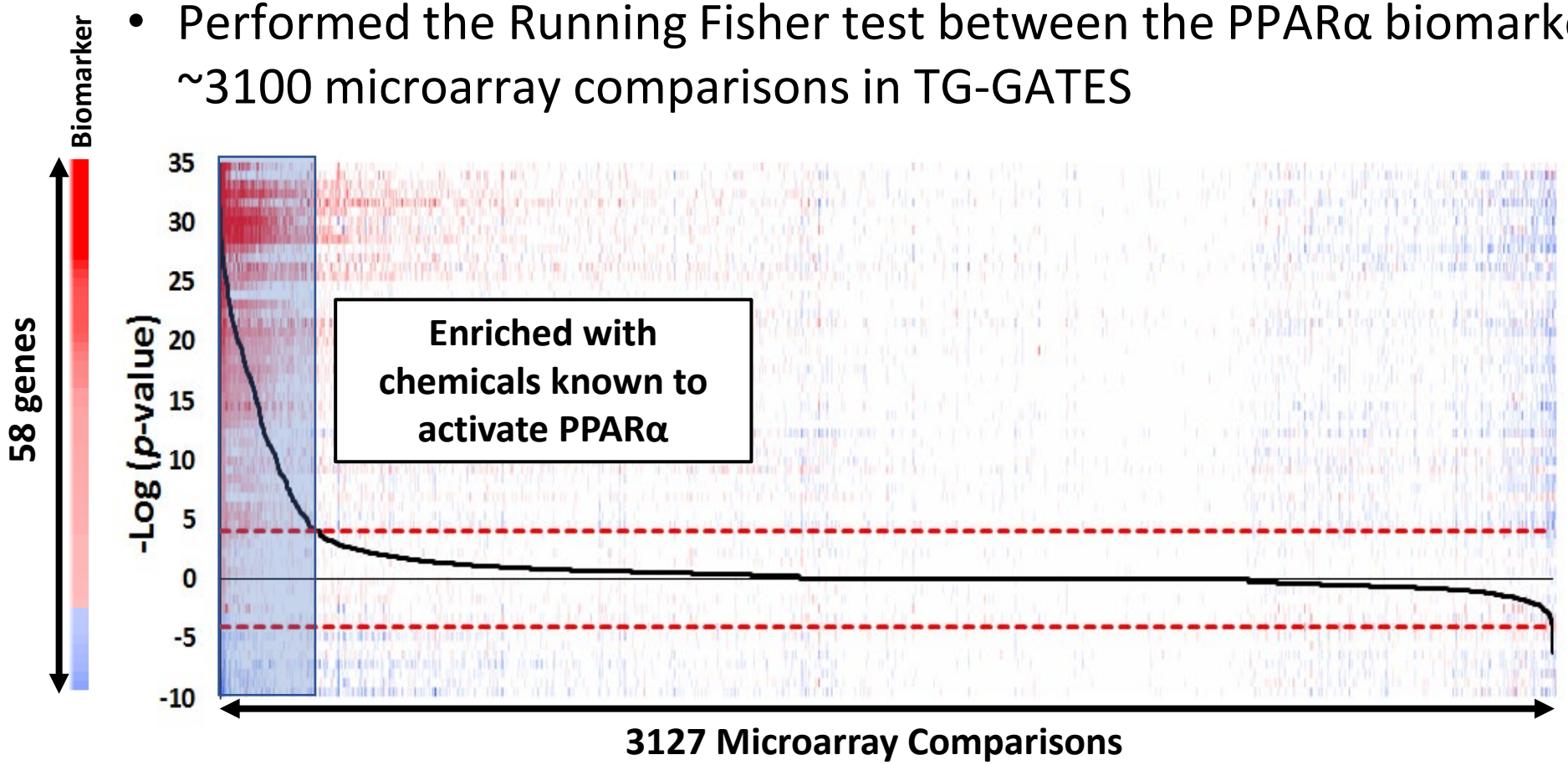
Sensitivity	0.95
Specificity	1
Positive Predictive Value	1
Negative Predictive Value	0.927
Balance Accuracy	0.975

Balanced Accuracy = 98%

Rooney et al. (2018) TAAP

Identification of chemicals with PPARα activity

- Performed the Running Fisher test between the PPARα biomarker and ~3100 microarray comparisons in TG-GATES



- Heat map shows the relationship between expression of biomarker genes and –Log(p-value)s
- Positively correlated comparisons on the left and negatively correlated comparisons on the right

Rooney et al. (2018) TAAP

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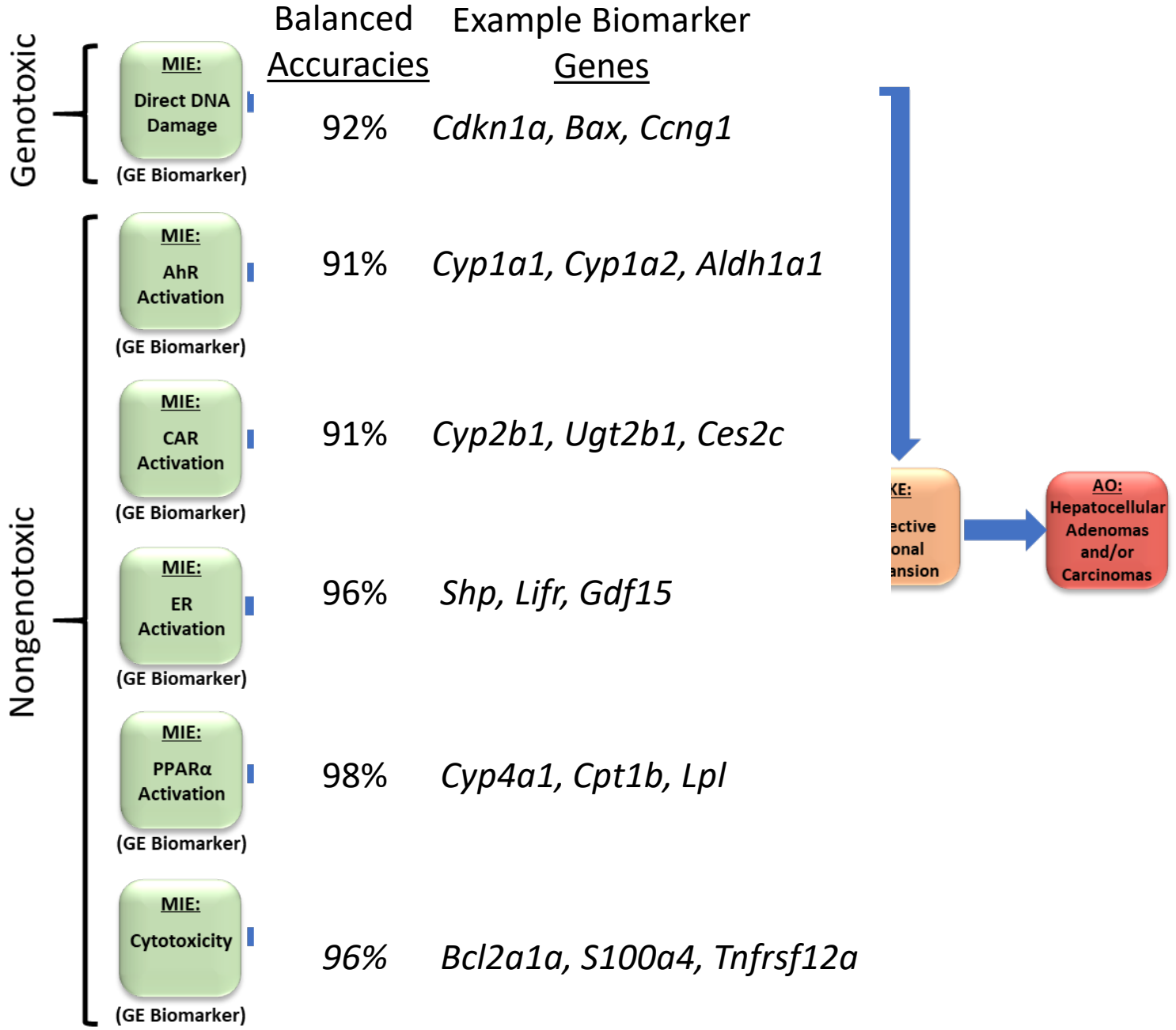

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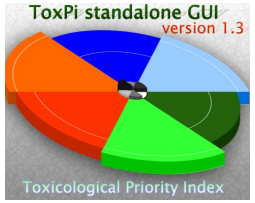
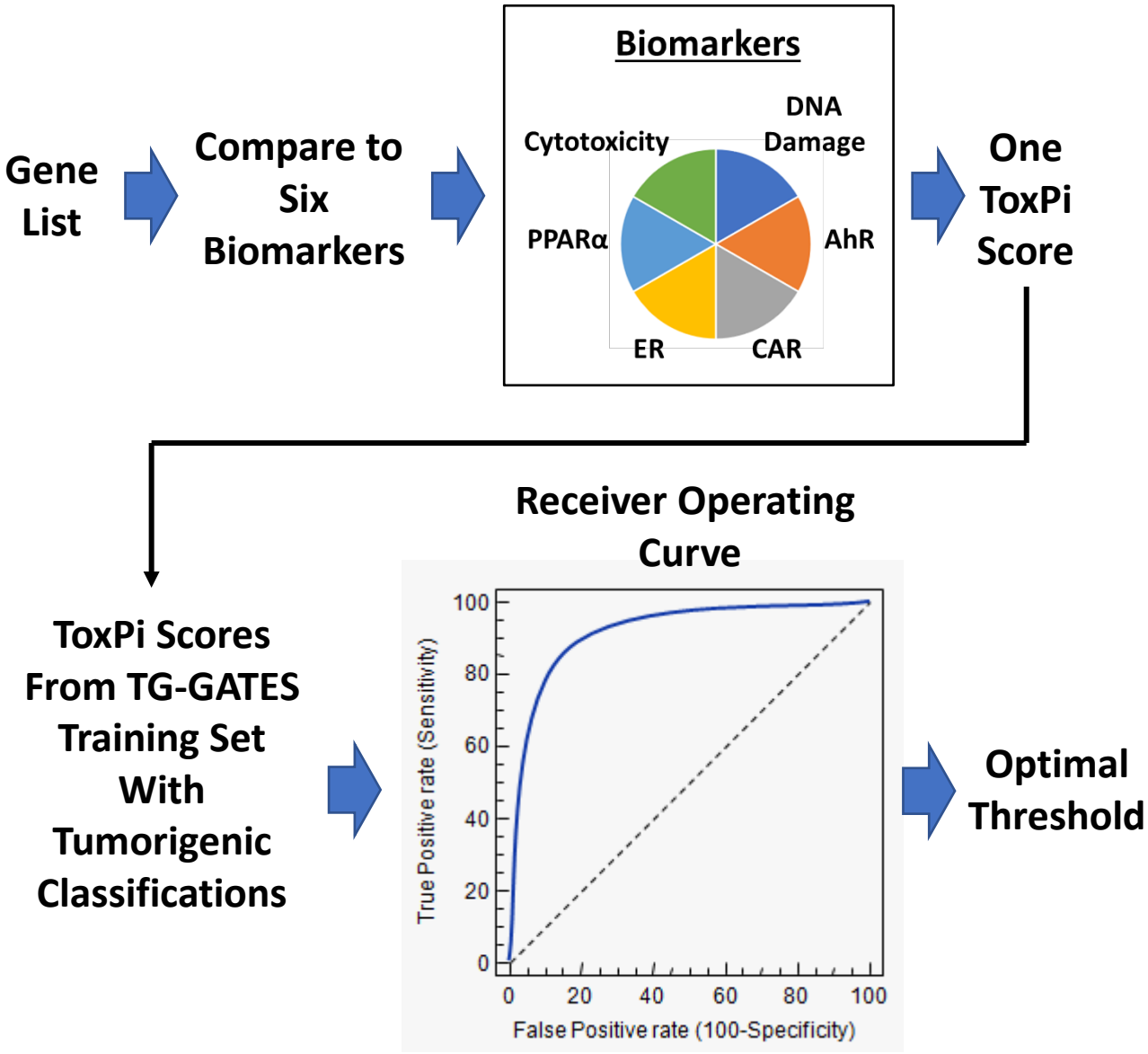
Methods for identification of tumorigenic chemicals

- Compare each chemical-dose-time bioset to each of the 6 biomarkers to get one ToxPi score
  - Using the –Log(p-value)s





- Divided the TG-GATES study into training and test sets

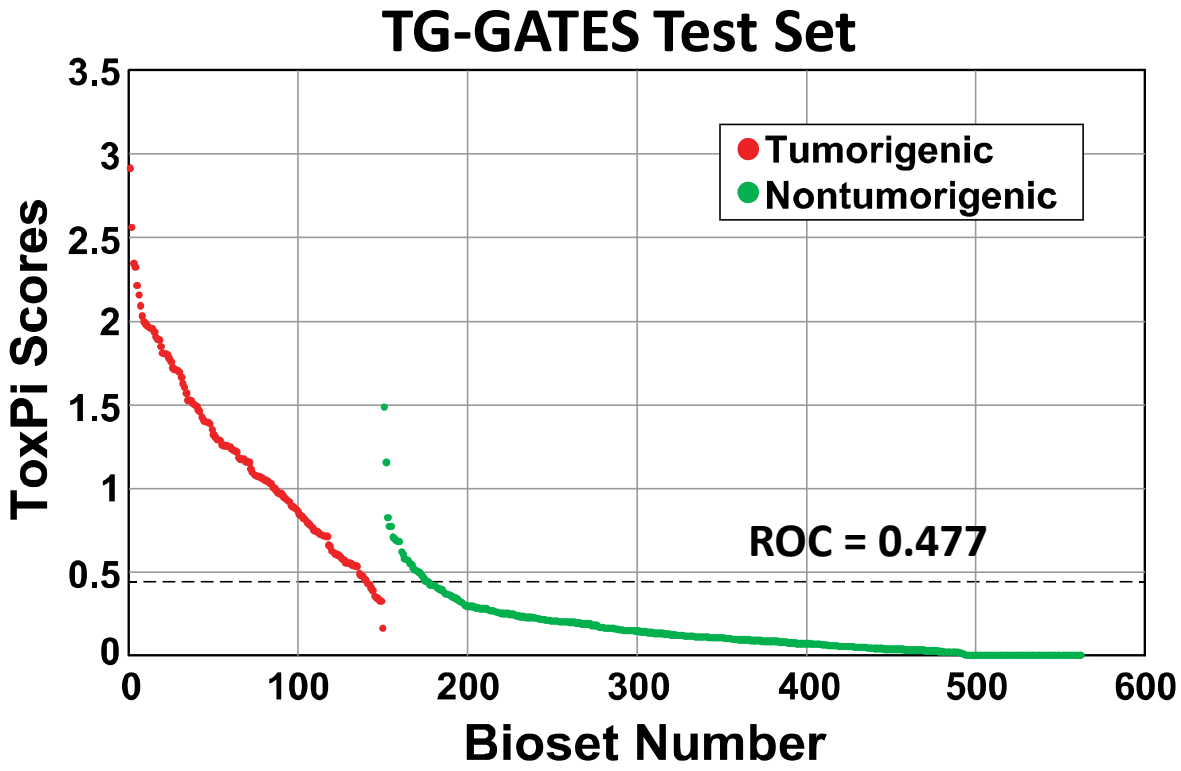
- DeLong, DeLong and Clarke-Pearson receiver operating curve (ROC) analysis to determine the optimal threshold in the training set; ROC=0.477

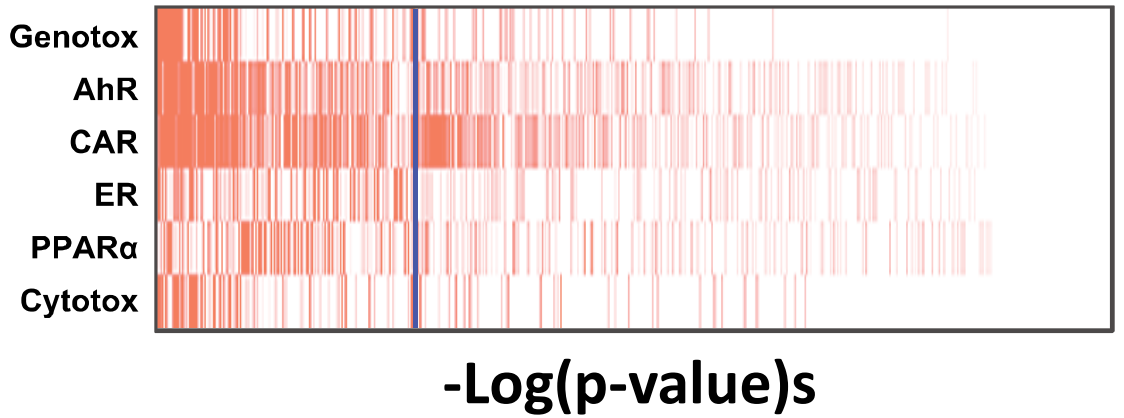
From Corton et al., in preparation

Assessment of the 6 MIEs predicts liver cancer

- Applied the ROC=0.477 to the test set: 90% sensitivity, 97% specificity, and a **balanced accuracy of 93%**

- Out of 44 rat liver tumorigens, only two (5%) were not predicted (acetamide, ethionine)
  - These chemicals may work through different AOPs





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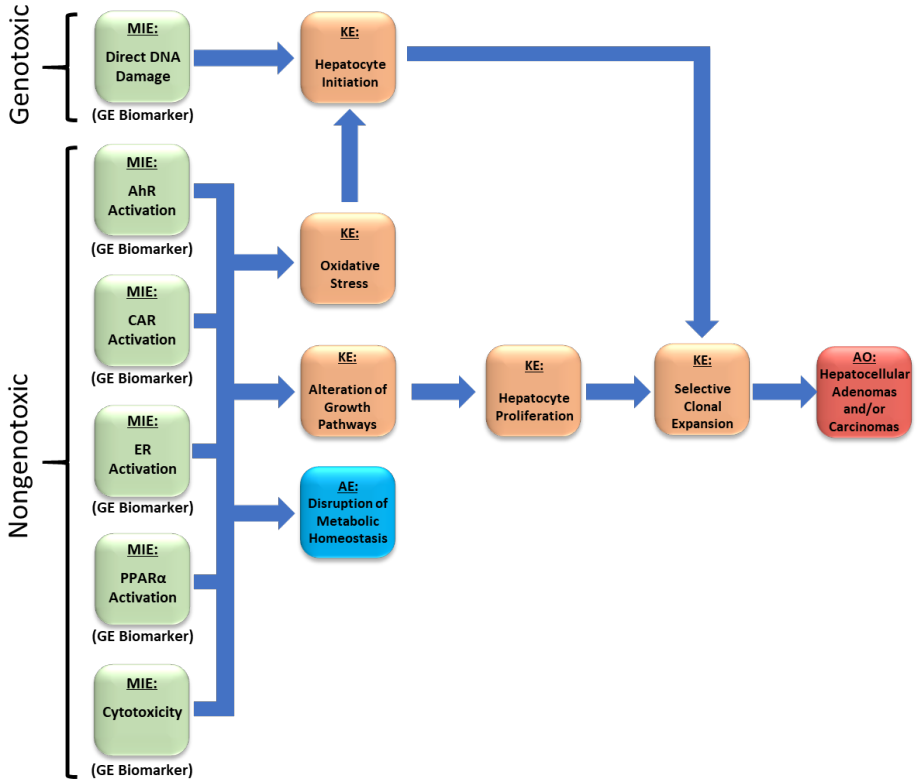
Defining biological thresholds for liver cancer

- Central premise of AOP framework: key events are necessary but not sufficient
  - Depends on the degree or amount of disruption to the particular key event

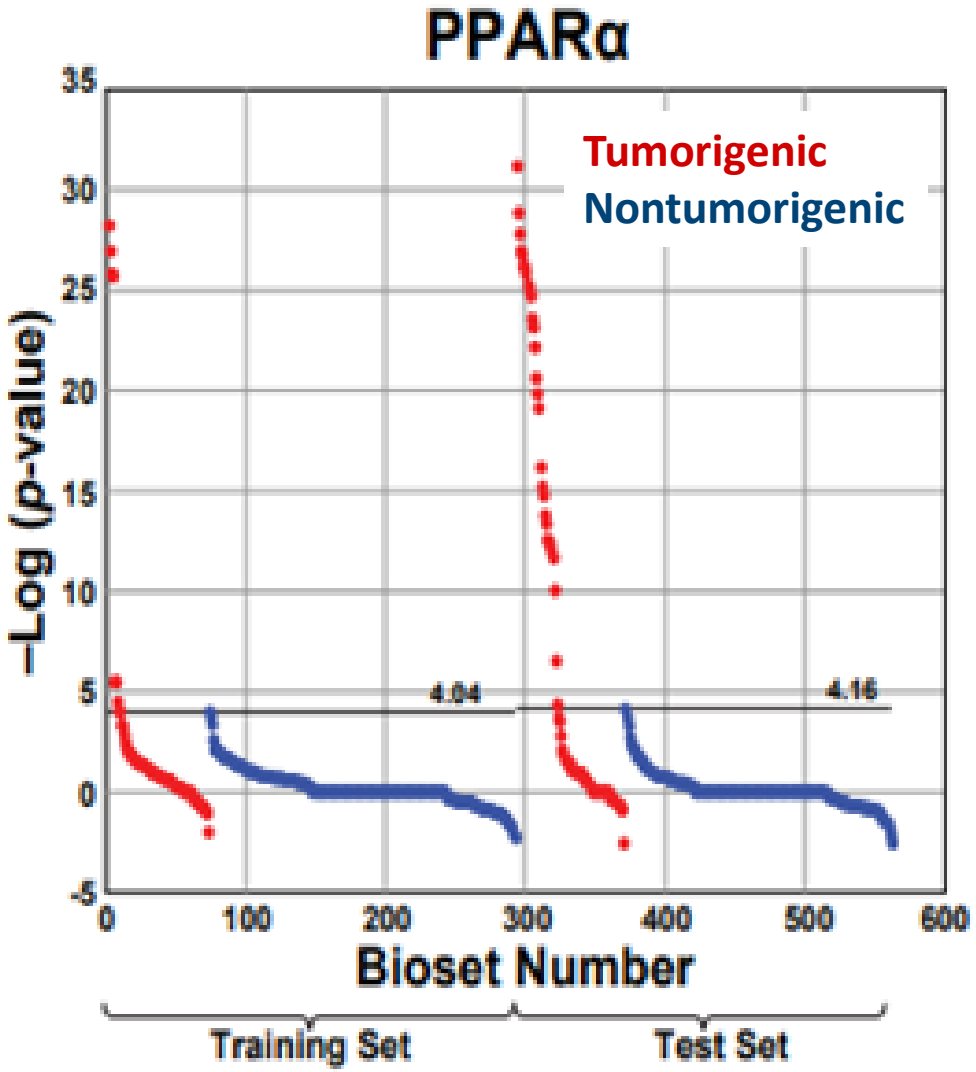
- Can we define thresholds “tipping points” for each of the MIEs?



http://www.silverdoctors.com

Identification of thresholds for gene expression biomarkers

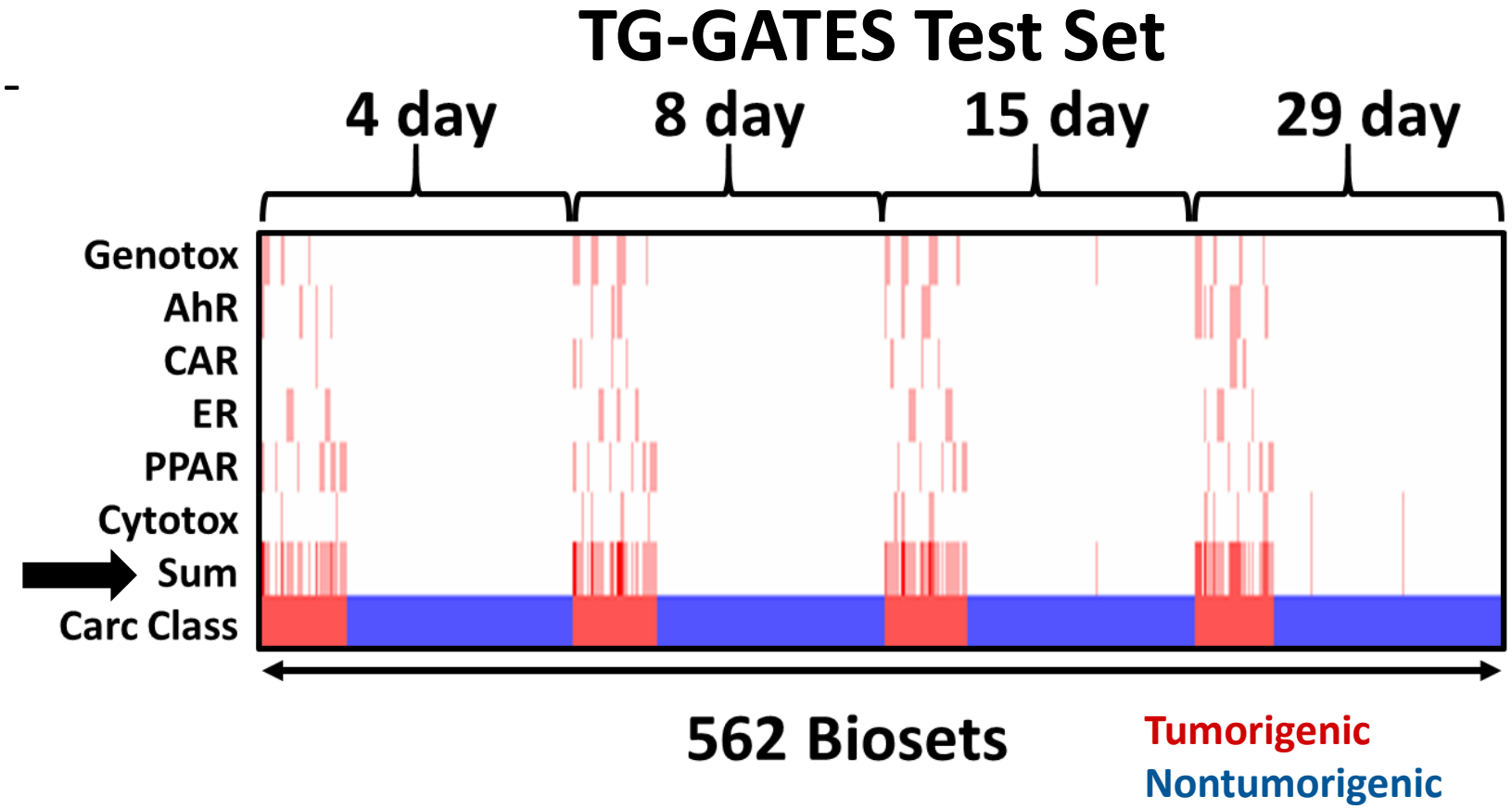
- Divided the chemical-dose conditions into tumorigenic and nontumorigenic groups and training and test sets
- Thresholds defined as the maximum value in the nontumorigenic group
- Thresholds were similar between the training and test sets
- Generated thresholds for all 6 MIEs



From Hill et al., in preparation

A set of biomarker thresholds accurately predict liver cancer

- Derived thresholds from the TG-GATES training set and then applied to the entire dataset
- Each red line is a condition in which the biomarker –Log(p-value) exceeds the threshold
- Most of the tumorigenic conditions exceed one or more of the 6 thresholds
- Thresholds rarely exceeded in any of the nontumorigenic conditions



- **Test set: 100% sensitivity, 94% specificity, and a balanced accuracy of 97%**

From Hill et al., in preparation

Thresholds for individual genes or liver weights and clinical chemistry endpoints are predictive of liver cancer

- Using thresholds for 12 individual genes (2/biomarker)
  - 100% sensitivity, 80% specificity, and a **balanced accuracy of 90%**

- Using thresholds for liver weight to body weight and clinical chemistry endpoints only
  - 88% sensitivity, 100% specificity, and a **balanced accuracy of 94%**

From Hill et al., in preparation and Corton et al. in preparation

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Summary

- An AOP-guided computational approach can be used to identify liver tumorigens in future prospective studies
  - Two sets of tools to apply to toxicogenomic studies
    - Gene expression biomarkers
    - Thresholds

- Identified clear thresholds of response for individual biomarkers, individual genes, and common measures associated with liver cancer
  - Supports the idea that early genomic changes can be used to establish threshold estimates or “tipping points” that are predictive of later-life outcomes

- Approach could be applied to predicting cancer in other tissues dependent on:
  - Knowledge of AOPs that lead to cancer
  - A robust dataset including reference chemicals

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