United States Environmental Protection Agency

Accounting for Uncertainty in the Application of High Throughput Datasets

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http://www.epa.gov/ncct/toxcast

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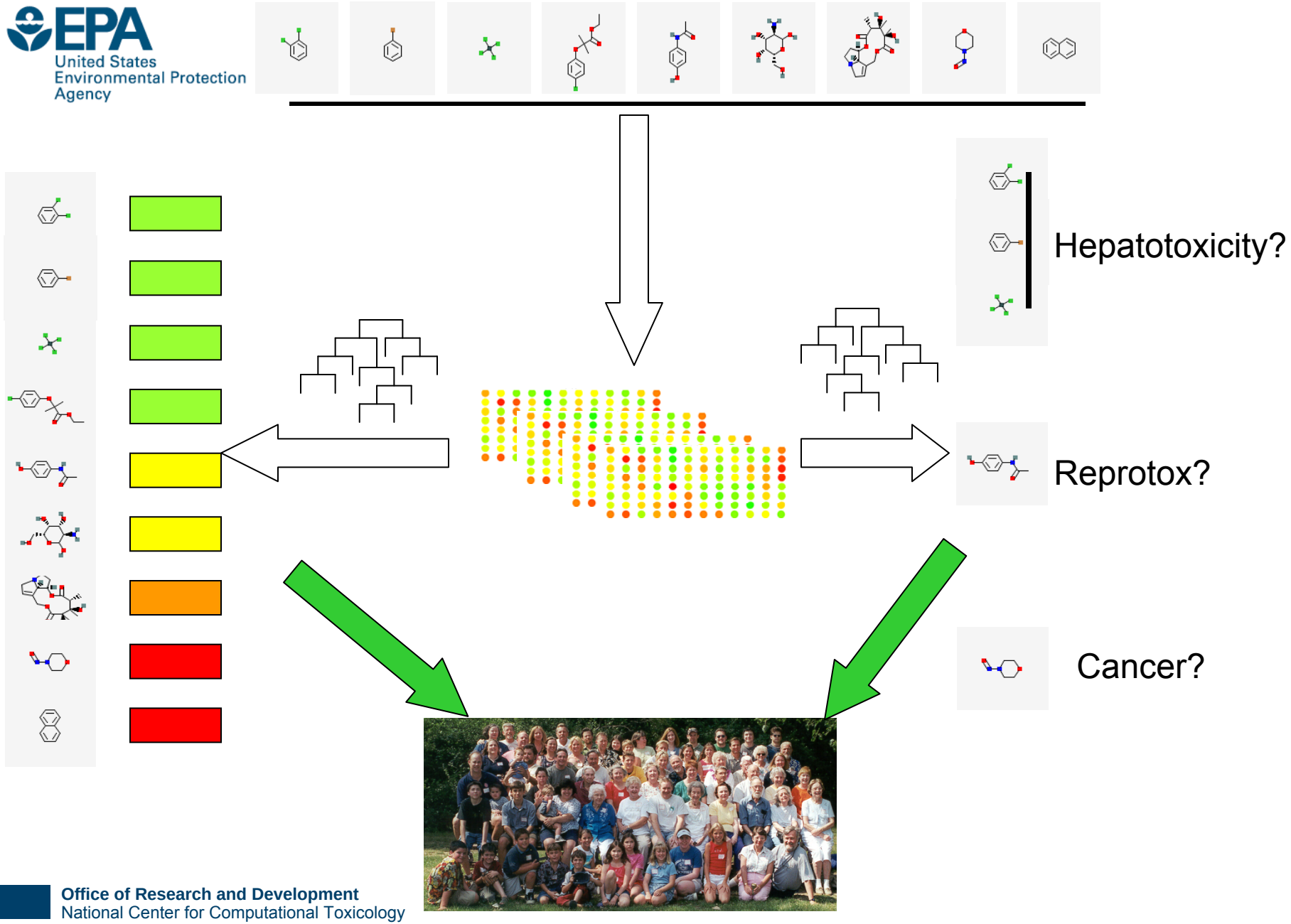
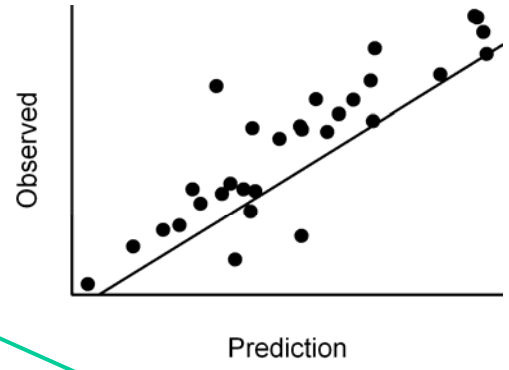
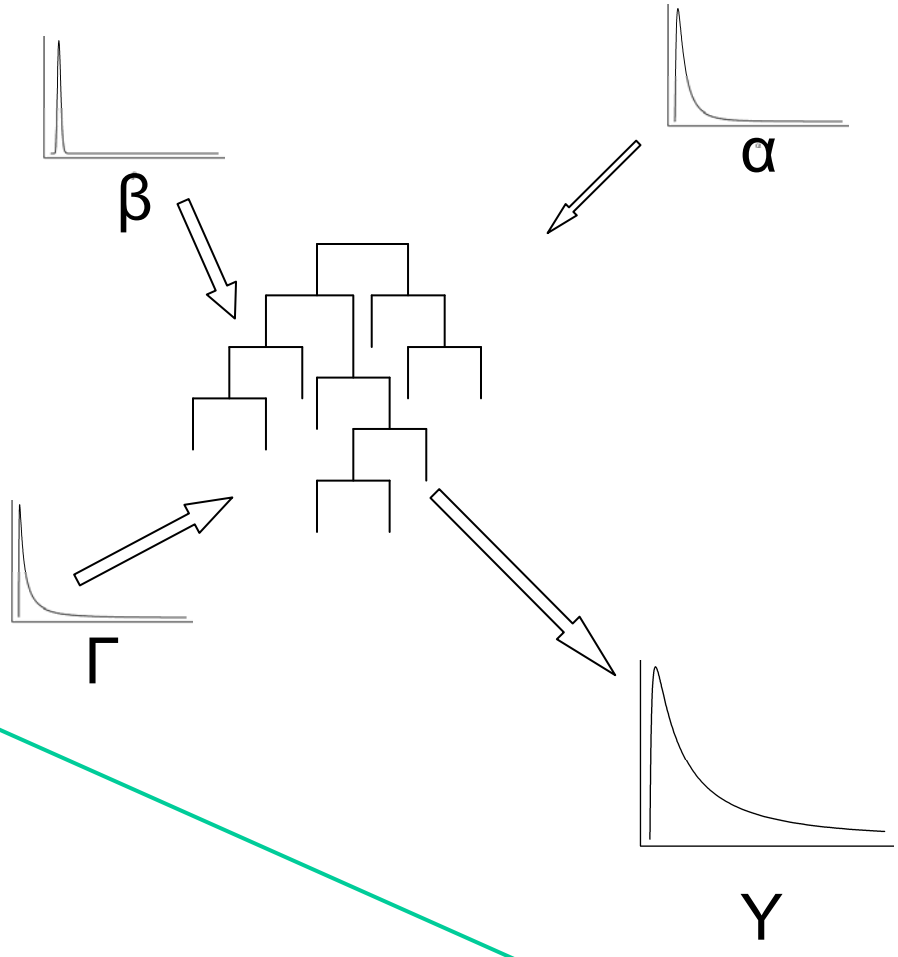
Office of Research and Development
National Center for Computational Toxicology

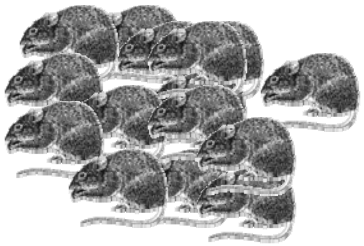

Hepatotoxicity?
Reprotox?
Cancer?

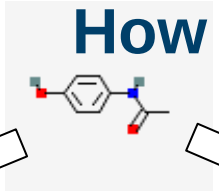
How Do You Quantify the Uncertainty of a Prediction?

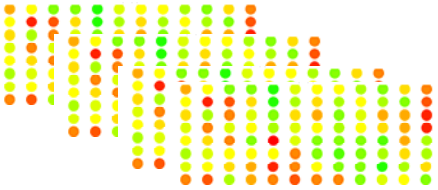


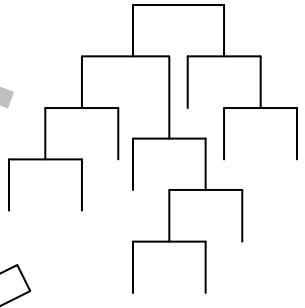


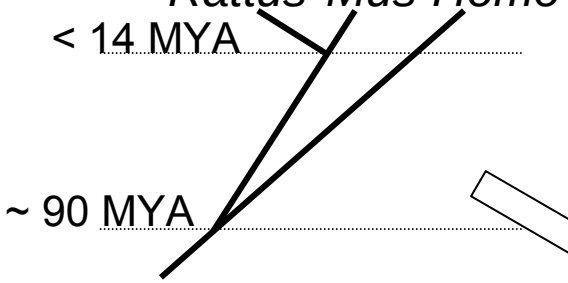
Toxicity Predictions Need Models (Regardless of How They are Done)



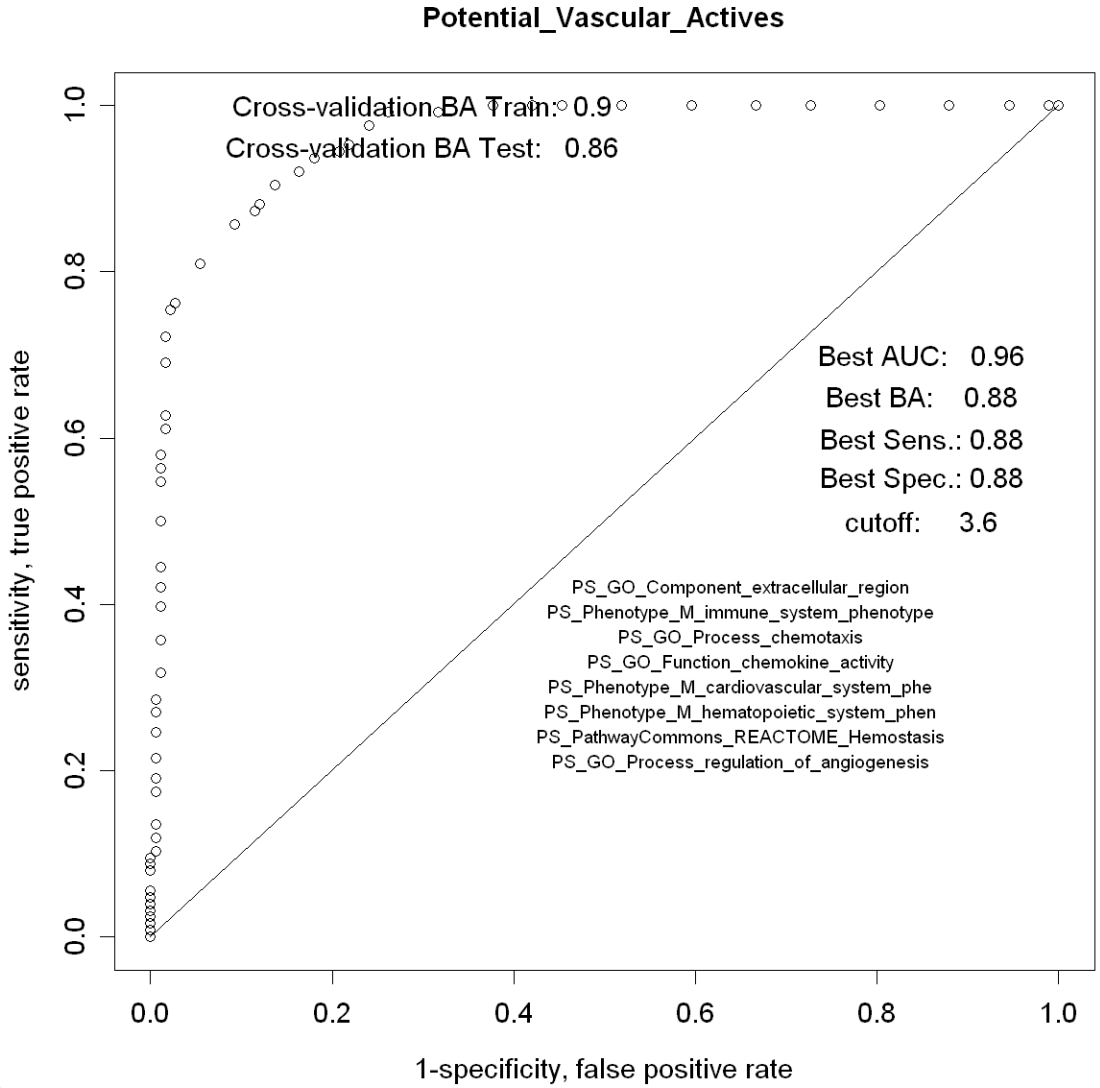




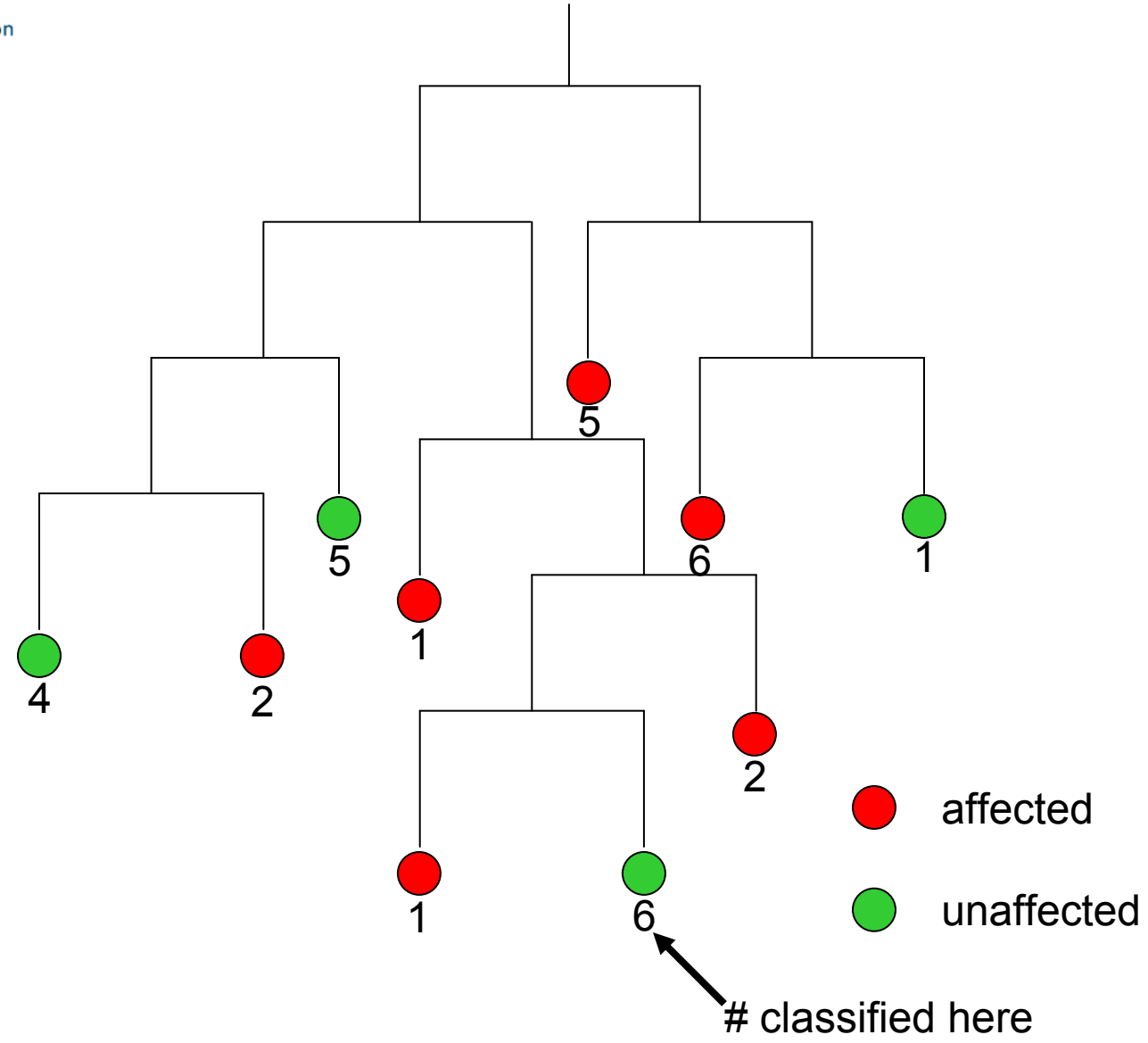


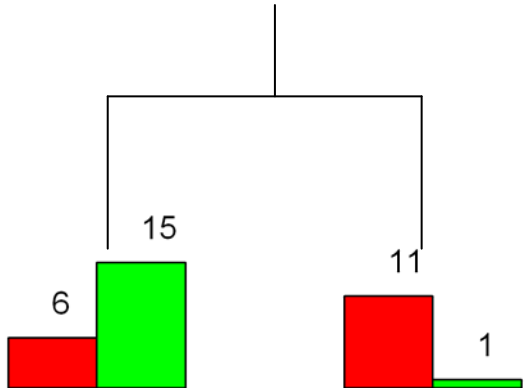


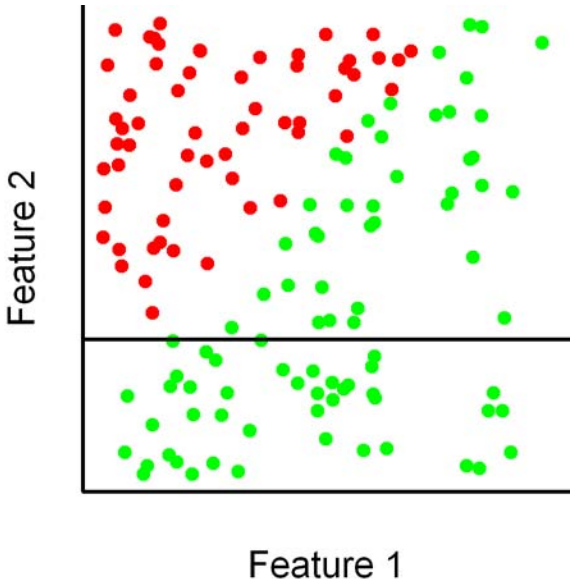
Pathway-level Signature: Toxicity Class Predictor

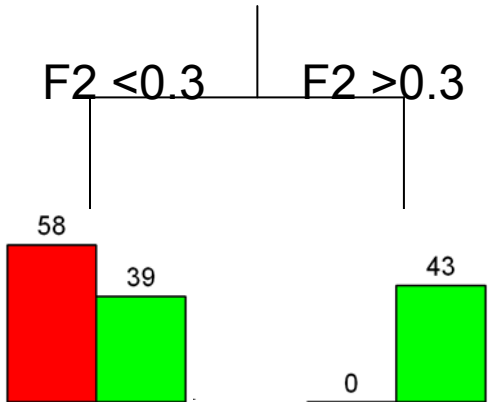


From Kleinstreuer, 2010

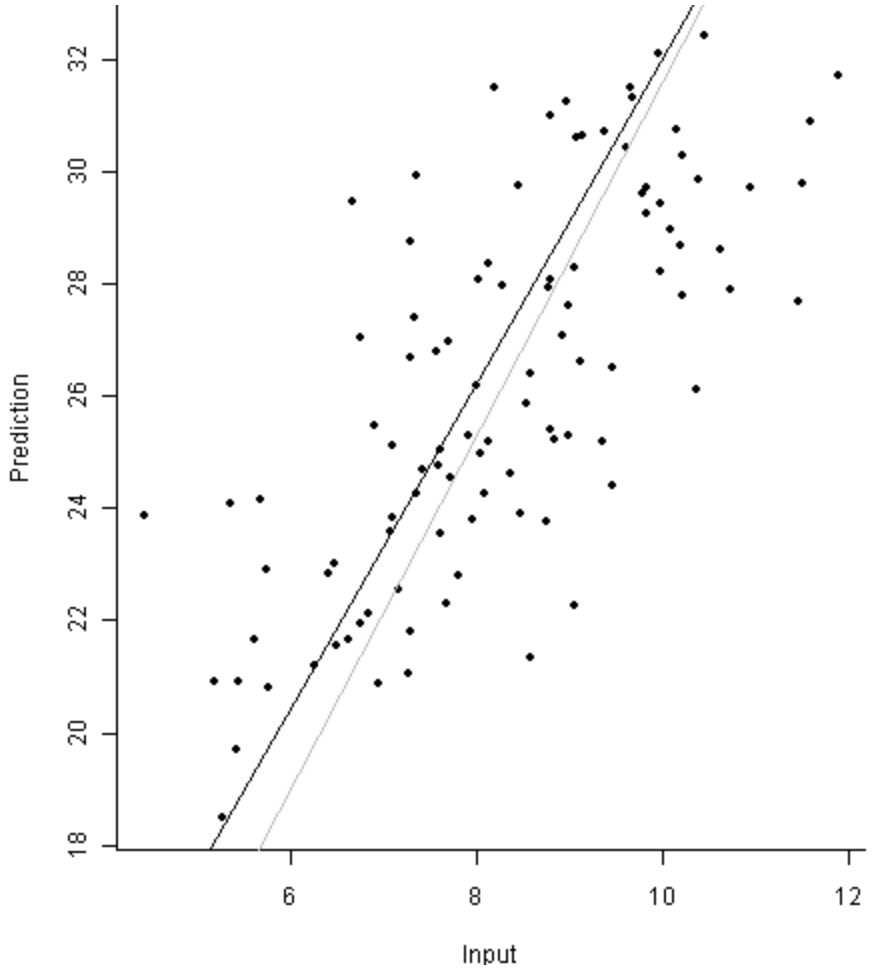

5
5
6
1
1
4
2
2
1
6
affected
unaffected
classified here


15
11
6
1

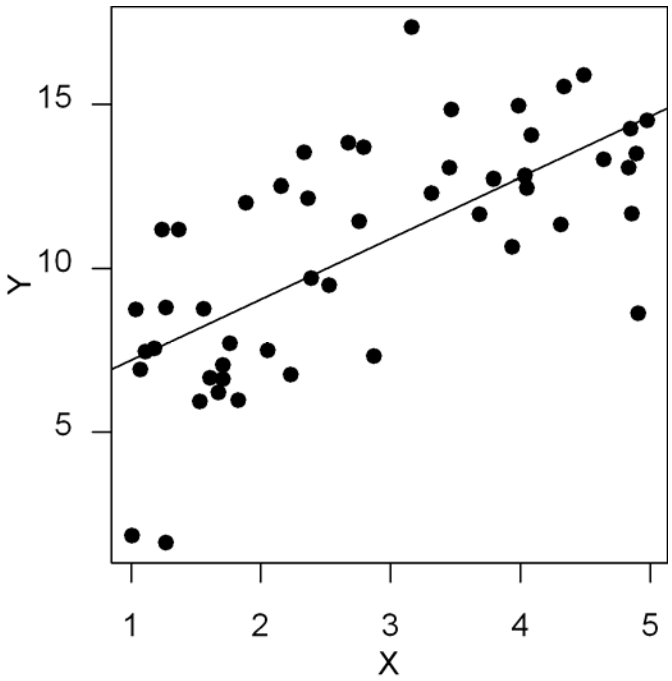

Feature 2
Feature 1

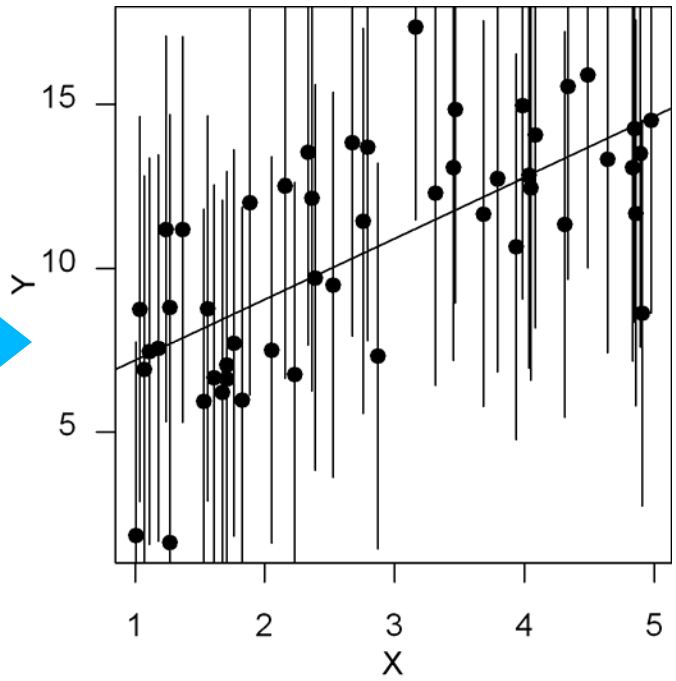

F2 <0.3
F2 >0.3
58
39
0
43

Adjusting for Input Uncertainty



Errors in the Target

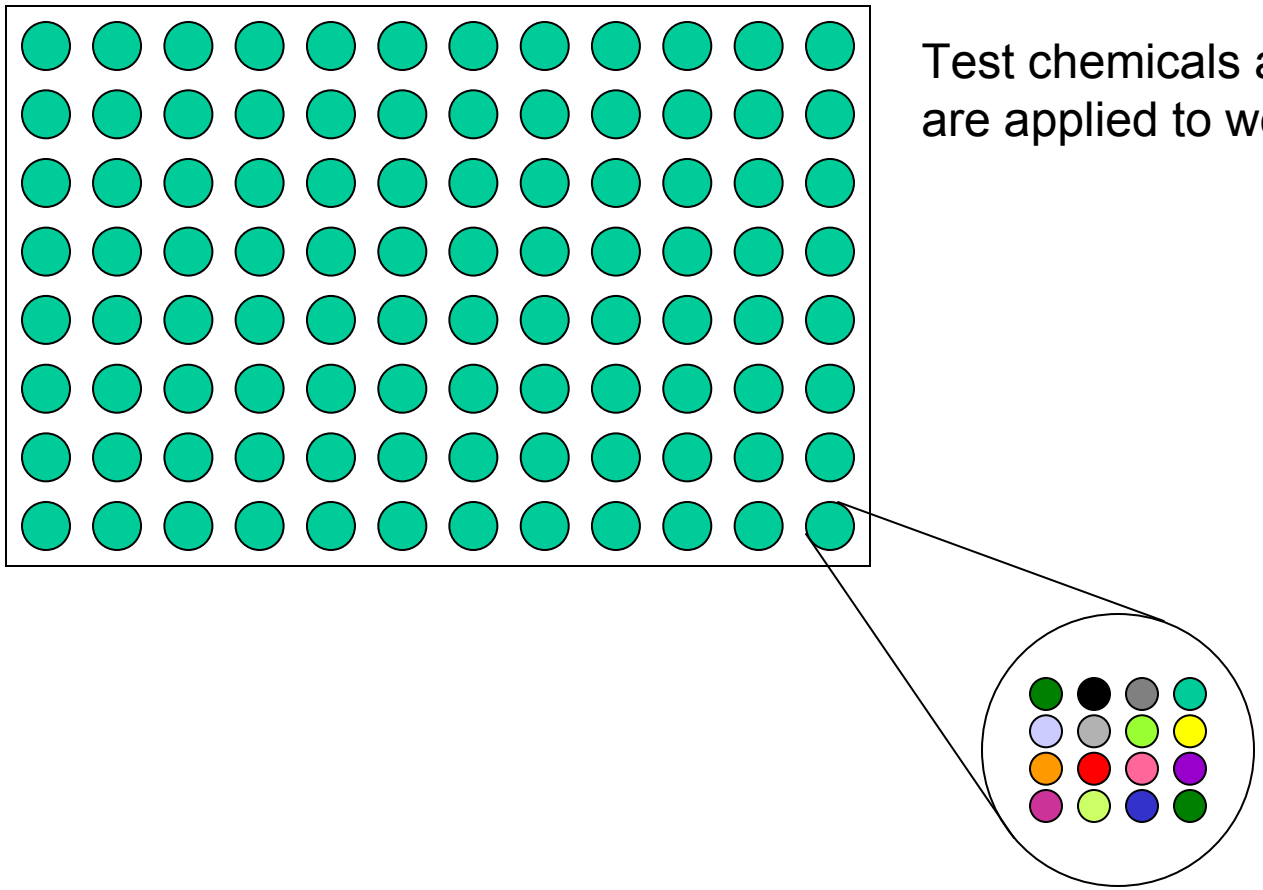




How is our predictor doing?

Maybe not so badly!

Some Statistical Issues in HTS: an Example



Test chemicals and controls are applied to wells

Multiplexed assay, 16 genes/well

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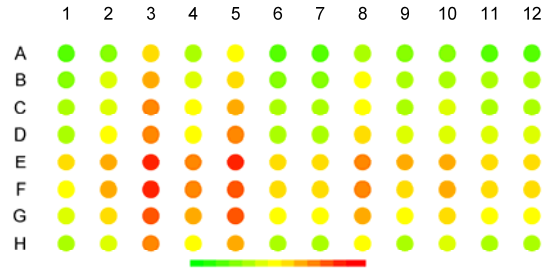
Statistical Models

- Some initial questions stemming from considering spatially arranged data:
  - Are there fixed position effects (persistent from plate to plate)?
  - How large is the variability among plates?
  - After everything else has been accounted for, are there patterns of autocorrelation among wells on the same plate?
  - How well does normalization by the intra-well ‘control’ values work? (Ideally, normalization should remove all the previous effects)
- Statistical methodology
  - Generalized least squares, as implemented in function gls() from package nlme in R
  - Work with log-transformed response – makes distributions of responses more symmetric, and variances more stable.

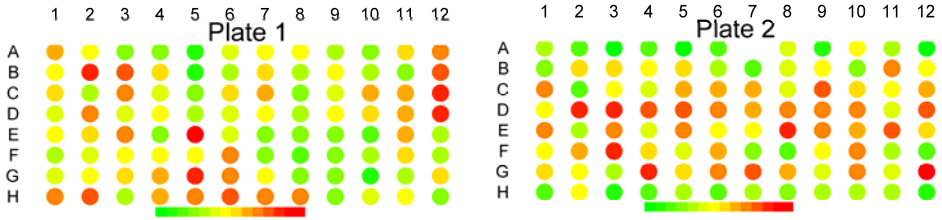
Position Effects, Autocorrelation, and Plate to Plate Variation in Controls

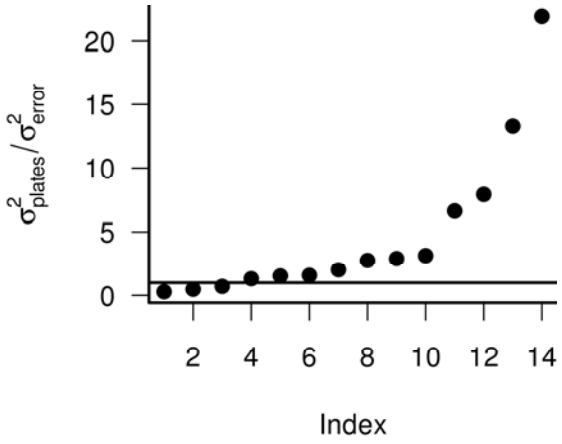
- After normalization: 6 of 14 endpoints have significant row effects and 2 of 14 significant column effects.
- All 14 show significant autocorrelation
- Most have substantial plate to plate variability: for all but 3, inter-plate variance exceeds the error variance.

Position Effects



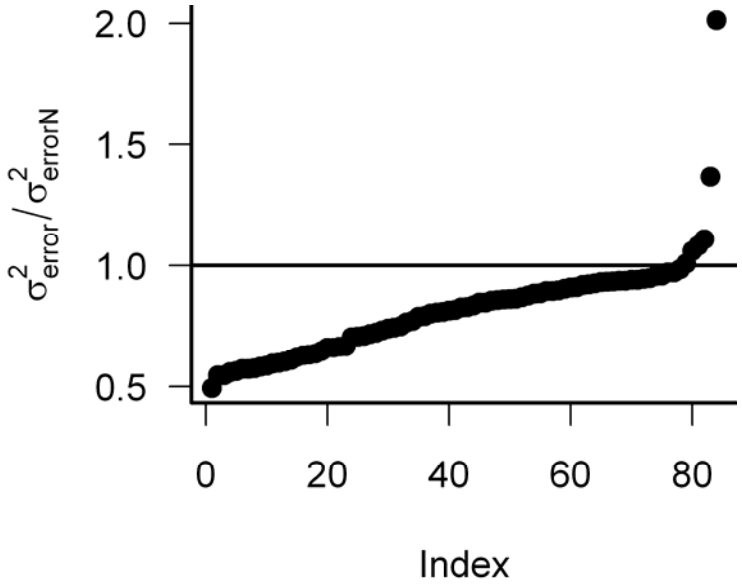
Residuals





Normalization and Sensitivity

- Error variance in normalized data greater than in non-normalized data
- No overall change in ‘sensitivity’, measured by lowest effective dose.



Some Tentative Conclusions

- Just as in agricultural studies, we potentially have to consider effects that stem from location
- The autocorrelation is probably not being modeled very well (and it is not yet clear how important that might be).
- Normalization does not make any of the complicating factors go away.
- Normalization **does** increase the noise (uncertainty) of treatment-specific estimates.

Summary

- Uncertainty in HTS assays considered in the context of their uses in predicting consequences of human exposure.
- Uncertainty in features and targets affect predictor construction differently.
- Predictor construction can be improved with proper quantification of input uncertainty.
- Statistical analysis of HTS data needs to consider the details of the assays, at the level of well and plate, to properly quantify resulting uncertainty.