# Predicting Developmental Toxicity Using the ToxCast Library and Pluripotent Embryonic Stem Cell Assays

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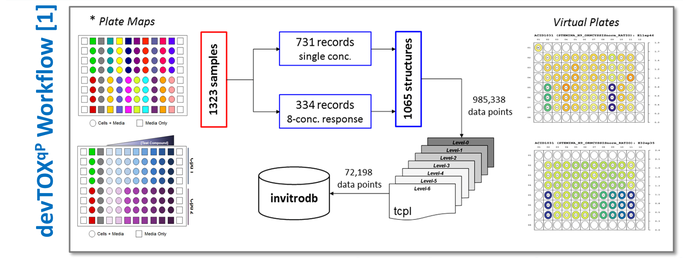
## Introduction

ToxCast chemicals were profiled for developmental toxicity potential in the embryonic stem cell assay platform developed by Stemina Biomarker Discovery and processed in the ToxCast data analysis pipeline (tcpl).

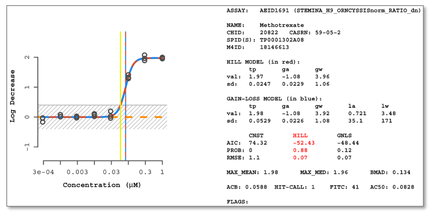
- human pluripotent H9 stem cell-based (hESC) assay monitoring a metabolic biomarker [Palmer et al. 2013, BDRB]

Stemina (STM) assay results were described using ToxCast activity profiles to determine the biological pathways associated with a STM(+) or STM(-) response.

## hESC (pluripotent) assay




### Example: Methotrexate (TI = 0.059 µM)

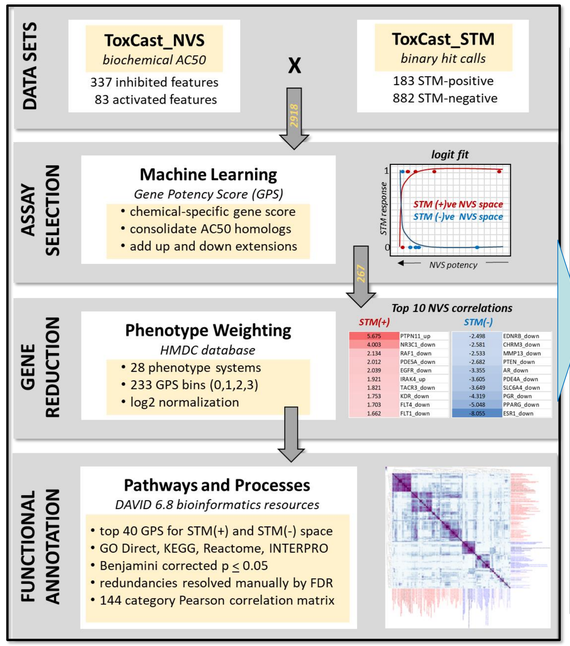



- ↓ ornithine/cysteine in the day 3 secretome predicts µM threshold for teratogenicity (TI) [1];
- point of departure for cell viability equates to 11% reduction in cell number.

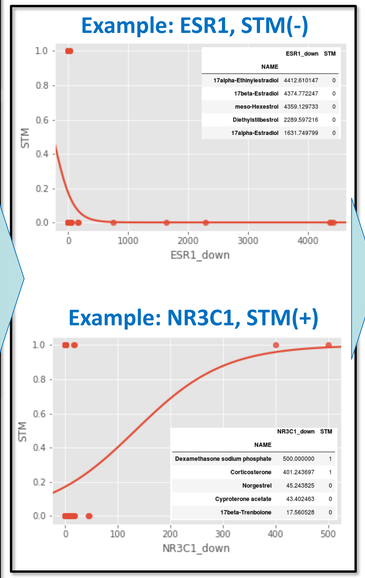
- TI was recorded for 181 chemicals (17% of 1065 tested); model performance used 42 benchmark compounds and ToxRefDB prenatal studies in rats and/or rabbits (dLEL ≤ 200 mg/kg/day) [manuscript in preparation].

## Mining the ToxCast dataset to define assay sensitivity

To gain insight into the biological pathways and targets associated with the STM binary hit call responses, machine-learning was used to mine correlations to 337 enzymatic and receptor signaling assays in the ToxCast NovaScreen dataset (NVS). Each NVS assay was enriched for an AC50 correlation against a hESC-positive or hESC-negative outcome, weighted by an assay-specific logistic regression model, processed through the DAVID Bioinformatics Resource (v6.8) and independently enriched using multiple pathway databases: GO Direct, KEGG, Reactome, INTERPRO (Bonferroni-corrected p ≤ 0.05, minimum 3 genes for a pathway identifier).

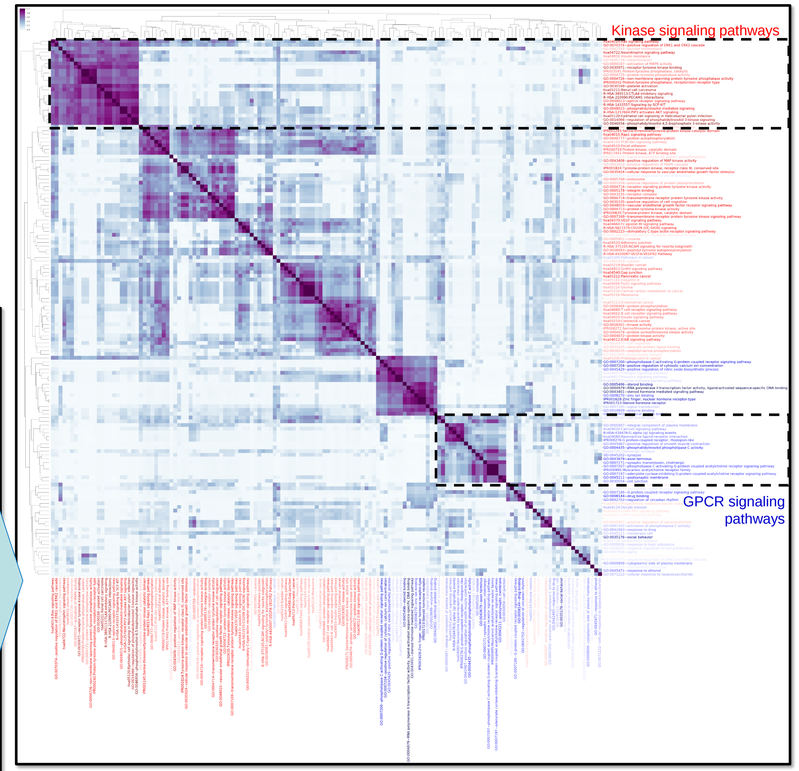



### Example: ESR1, STM(-)




### Example: NR3C1, STM(+)




Pathways in the STM-sensitive domain (red) clustered with the pathways in the STM-insensitive domain (blue) through correlation of genes present in each pathway. Shading represents biological relevance as determined through the Mouse-Human disease connections database.

## Pathway-based DevTox predictions

### hESC model performance

*stringency filter applied to the in vivo anchor*

| | benchmark | none | low | medium | high |
|---|---|---|---|---|---|
| TP | 17 | 85 | 60 | 35 | 19 |
| FP | 0 | 14 | 37 | 23 | 9 |
| FN | 9 | 217 | 127 | 51 | 11 |
| TN | 16 | 116 | 208 | 176 | 88 |
| n | **42** | **432** | **432** | **285** | **127** |
| Sensitivity | 0.654 | 0.281 | 0.321 | 0.407 | 0.633 |
| Specificity | 1.000 | 0.892 | 0.849 | 0.884 | 0.907 |
| Accuracy | 78.6% | 46.5% | 62.0% | 74.0% | 84.3% |
| MCC | 0.647 | 0.190 | 0.202 | 0.332 | 0.554 |

| low | medium | high |
|---|---|---|
| 105 | 50 | 22 |
| 99 | 81 | 38 |
| 82 | 36 | 8 |
| 146 | 118 | 59 |
| 432 | 285 | 127 |
| 0.56 | 0.58 | 0.73 |
| 0.6 | 0.6 | 0.61 |
| 0.58 | 0.59 | 0.64 |
| 0.16 | 0.16 | 0.3 |

### STM+ model performance

- Combine results from STM with pathway-based potency metrics
- Chemical predicted to be teratogen if activity is measured in in the hESC devTOX platform OR in the NVS pathways ( < 1 µM)

## Summary

- ToxCast chemicals were classified for potential developmental toxicity using the hESC devTOX$^{qp}$ platform from Stemina Biomarker Discovery.
- Performance against prenatal animal studies (ToxRefDB) improved from 62% to >84% accuracy as the level of confidence in the *in vivo* anchoring result (dLEL) increased.
- Characterizing the applicability domain at a pathway level sets the stage for new approach methodologies predicting developmental toxicity without vertebrate animal testing.
- Creation of STM+ model (STM + pathways) provided modest gains in DevTox predictivity