CHARACTERIZING DEVELOPMENTAL TOXICITY THROUGH PLURIPOTENT EMBRYONIC STEM CELL ASSAYS AND THE TOXCAST LIBRARY

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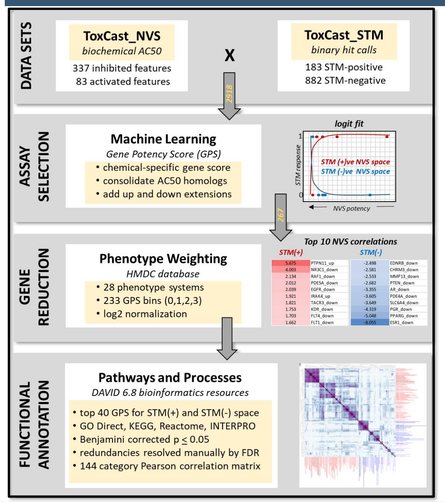
Introduction

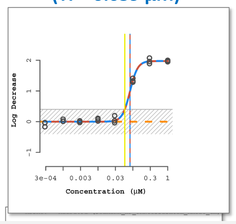
ToxCast chemicals were profiled for developmental toxicity potential in the devTOX qP platform (Stemina Biomarker Discovery) [Palmer et al. 2013, BDRB]. The data (ToxCast_STM) have been processed in the ToxCast data analysis pipeline (tcpl) and include information on the targeted biomarker (µM concentration resulting in point of departure for ↓ ornithine : cystine ratio) in the secretome of pluripotent human embryonic stem cell (H9) line.

In this analysis, we mined ToxCast_STM data against the biochemical activity (AC_{50}) profiles from the ToxCast_NVS dataset [actor.epa.gov/dashboard] to determine biological pathways and processes with an association to the STM(+) and STM(-) domains.

hESC (pluripotent) assay



Example: Methotrexate (TI = 0.059 µM)



- ↓ ornithine/cystine in the day 3 secretome predicts µM point of departure represented as teratogen index (TI) ;
- Point of departure for cell viability equates to 11% reduction in cell number.

- TI was recorded for 183 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) [Zurlinden et al. manuscript in preparation].

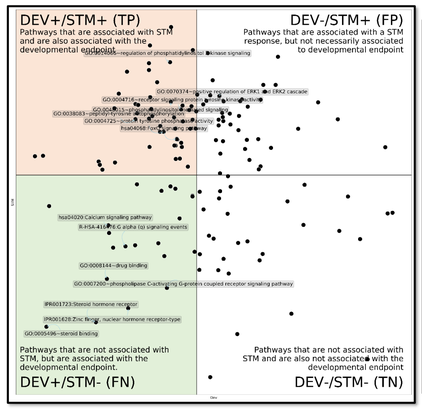
hESC model performance

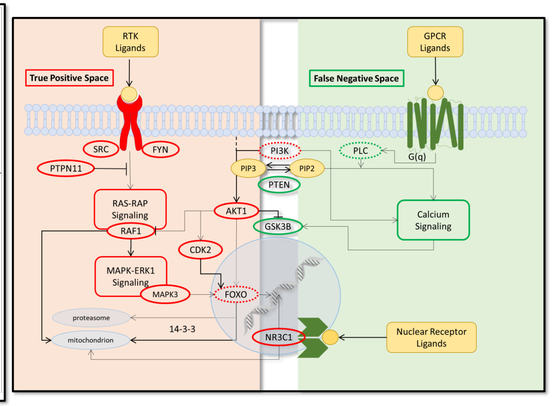
stringency filter applied to the in vivo anchor

	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

Defining the biochemical hESC applicability domain

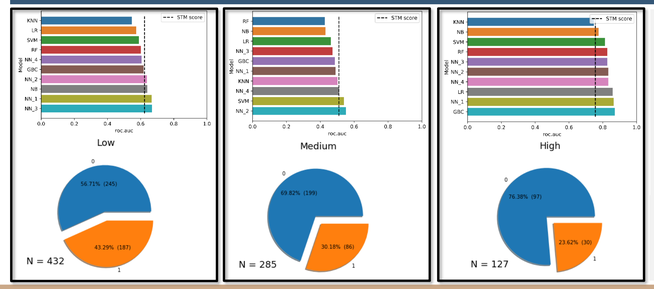
To gain insight into the biological pathways and targets associated with the STM binary hit call responses, machine-learning was used to mine correlations to 337 enzymatic and receptor signaling assays in the ToxCast NovaScreen dataset (NVS). Each NVS assay was enriched for an AC_{50} correlation against 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.





- Pathways were further characterized using chemicals from the medium-stringency ToxRefDB dataset (n=285). Independent of the STM dataset, pathways were fit to a logistic regression model using the binary DevTox hit call (0,1). The coefficient for DevTox was compared to the coefficient for STM to determine which pathways were either represented or not represented as having positive or negative association to STM and DevTox.
- Using DEV (+) pathways, a proposed true positive (TP) and false negative (FN) biochemical space was constructed using known associations from the literature and gene cards (genecards.org). Network constructed using NVS genes present in original STM model.

Defining DevTox space from animal data

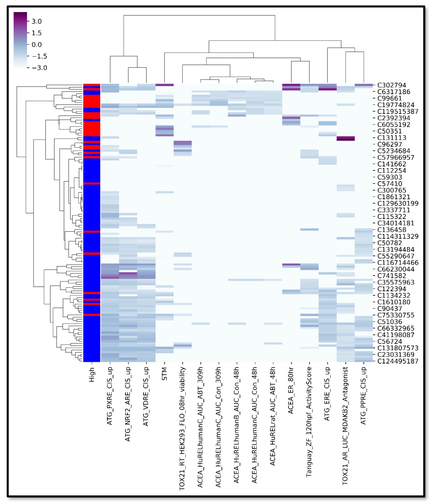


Using the entire ToxCast dataset as features, numerous machine learning algorithms were fit and evaluated using a train/test split of the low, medium, and high stringency ToxRefDB data.

KNN – K Nearest Neighbors
NB – Naive Bayes
SVM – Support Vector Machine
NN_n – Neural Network (n hidden layers)
RF – Random Forest
LR – Logistic Regression

STM score - roc_auc for DevTox prediction using STM hit call only.

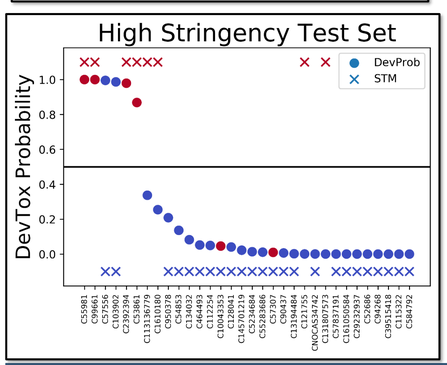
ToxCast-based DevTox predictions



Left: Top weighted features for predicting high stringency dataset. Row side colors indicate DevTox hit call in high stringency model. Clustered values represent chemical AC_{50} potencies in ToxCast.

Bottom Left: Predicted probability of test set data and relationship to STM positive vs STM negative hit call.

Bottom Right: Model performance for high stringency dataset.



Model Performance: test set

	STM only	STM+
TP	5	5
FP	4	1
FN	2	2
TN	21	24
n	32	32
Sensitivity	0.71	0.72
Specificity	0.84	0.96
Accuracy	0.81	0.91
MCC	0.5	0.72

Summary

- Analysis defines RTK versus GPCR (G(q)) and NRs as the developmentally relevant modes of action at the top of the STM positive and STM negative space.
- 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 & ToxCast model provided gains in DevTox predictivity for high stringency anchor, but modest gains for low stringency.