

Refining Experimental Training Sets and Classification Algorithms for Optimal Performance of Gene Expression Biomarkers of Pathological Endpoints



Brandon Jeffy¹, Cecelia Pearson¹, Eric Blomme², Yi Yang² and Richard Brennan¹

1. Iconix Biosciences, Mountain View, CA. 2. Abbott Laboratories, Abbott Park, IL

TOXICOGENOMICS

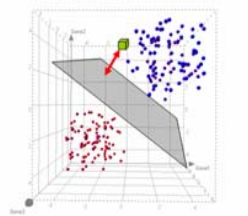


Abstract

DrugMatrix® is a large toxicogenomic database with expression and associated pathology data on over 630 compounds. Comprehensive mining of these data using sparse linear programming (SPLP) supervised classification methods has yielded numerous high-performing biomarkers, termed Drug Signatures®, for a variety of pathological and pharmacological endpoints. Defined rules have been developed to allow automated assignment of experiments to signature training sets, facilitating systematic mining of the data. In order to better understand the factors underlying the performance of Drug Signatures, we evaluated diagnostic and predictive (based on early time point expression data) Drug Signatures for bile duct hyperplasia (BDH). Rats were treated for 1, 5, or 28 days with 10 compounds not used in generating the existing BDH signatures. Liver gene expression from the 1 and 5 day time points was profiled on CodeLink™ RU1 microarrays and liver histopathology assessed for all time points. These data were used to forward validate the two BDH signatures and evaluate alternative strategies for signature definition. By increasing the stringency of the histopathological score defining the positive training set class and using an adaptive SPLP algorithm that allows flexibility in signature specificity and sensitivity tolerances, we were able to dramatically improve the performance of the signatures. Sensitivity increased from 25% and 43% for the diagnostic and predictive signatures respectively to 71% and 86% based on forward validation. Specificity increased from 93% to 100% for the diagnostic signature and slightly decreased from 100% to 83% for the predictive signature. Interestingly, the improved diagnostic signature had excellent predictive properties, identifying 7 out of 11 (64%) 5-day treatments that resulted in BDH only after 28 days. When the data obtained from the new study were incorporated into the training sets, estimated performance was increased even further.

Sparse Linear Programming Algorithm Produces Simple Intuitive Metric for Interpretation

SPLP algorithm attempts to find a linear separation between two classes in n dimensional space



- Log ratios for genes : x_1, x_2, \dots, x_n
- Associated weight : a_1, a_2, \dots, a_n

$$S = \sum a_i x_i - b$$

S = Scalar Product and b = Bias

Interpretation:
If $S > 0$ = True (in class)
If $S < 0$ = False (not in class)

Figure 1: Iconix Signature technology allows prediction of pharmacology and toxicity using gene expression data based on most informative genes on the microarray.

Forward Validation of RU1 SPLP Bile Duct Hyperplasia, Predictive Signature

Compound_Name	Dose (mg/kg)	TreatPt (d)	SP SPLP BILE DUCT HYPERPLASIA, Predictive	BDH at 28d?
1-NAPHTHYL ISOTHIOCYANATE	15	5	1.23	Y
1-NAPHTHYL ISOTHIOCYANATE	15	5	0.57	Y
AFIATOXIN B1	0.3	1	0.71	Y
AFIATOXIN B1	0.3	1	0.26	Y
ALLYL ALCOHOL	16	1	1.03	N
ALLYL ALCOHOL	16	1	0.71	N
CARBON TETRACHLORIDE	3178	1	0.28	Y
CARBON TETRACHLORIDE	3178	1	1.52	Y
ETHANOL	3000	1	1.76	N
ETHANOL	3000	1	1.58	N
ISONIAZID	50	1	1.75	N
ISONIAZID	50	1	1.28	N
METHAPYRILENE	100	1	0.88	Y
METHAPYRILENE	100	1	0.37	Y
N-NITROSODIMETHYLAMINE	10	1	1.02	Y
N-NITROSODIMETHYLAMINE	10	1	0.21	Y
N-NITROSODIMETHYLAMINE	10	1	0.89	Y
N-NITROSODIMETHYLAMINE	10	1	0.38	Y
THIOACETAMIDE	200	1	0.78	Y
THIOACETAMIDE	200	1	0.52	Y

6/14 True Positive (43%)
6/6 True Negative (100%)

Table 2: The SPLP bile duct hyperplasia predictive signature was run against the set of validation compounds. True positive experiments with positive scalar product scores are shown in red.

Forward Validation of Existing RU1 SPLP Bile Duct Hyperplasia, Diagnostic Signature

Compound_Name	Dose (mg/kg)	TreatPt (d)	SP SPLP BILE DUCT HYPERPLASIA, Diagnostic	BDH at 28 or 12
1-NAPHTHYL ISOTHIOCYANATE	15	1	-2.31	N
1-NAPHTHYL ISOTHIOCYANATE	15	1	0.79	Y
AFIATOXIN B1	0.3	1	1.75	Y
AFIATOXIN B1	0.3	1	1.52	Y
ALLYL ALCOHOL	16	1	1.02	N
ALLYL ALCOHOL	16	1	1.05	N
CARBON TETRACHLORIDE	3178	1	0.83	N
CARBON TETRACHLORIDE	3178	1	0.83	N
ETHANOL	3000	1	1.54	N
ETHANOL	3000	1	2.38	N
ISONIAZID	50	1	1.52	N
ISONIAZID	50	1	1.58	N
METHAPYRILENE	100	1	0.71	Y
METHAPYRILENE	100	1	1.02	Y
N-NITROSODIMETHYLAMINE	10	1	1.48	N
N-NITROSODIMETHYLAMINE	10	1	1.53	Y
N-NITROSODIMETHYLAMINE	10	1	0.29	Y
THIOACETAMIDE	200	1	1.96	N
THIOACETAMIDE	200	1	0.73	Y

1/4 True Positive (25%)
15/16 True Negative (94%)

Table 3: The SPLP bile duct hyperplasia diagnostic signature was run against the set of validation compounds. True positive experiments with positive scalar product scores are shown in red.

New ANIT Experiments Do Not Hit Existing RU1 BDH and Fibrosis Signatures

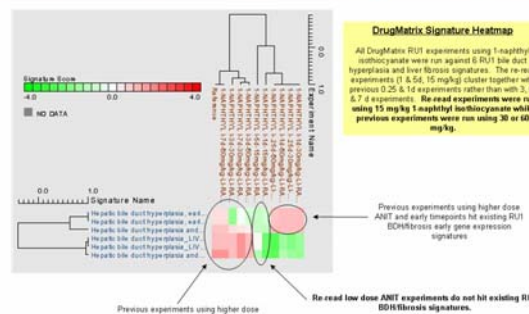


Figure 2: DrugMatrix ANIT experiments were scored against existing BDH signatures

Bile Duct Hyperplasia ASPLP Signatures Rederived Using New Pathology Data

• Diagnostic and predictive signatures

• ASPLP algorithm (adjusted SPLP)

Label Class Definitions for Rederived Signatures

+1 criteria: BDH or periportal fibrosis severity score ≥ 1 in at least 1/3 (or 2/3 in high stringency signature) animals

-1 criteria: BDH or periportal fibrosis severity score = 0 in 3/3 animals

Diagnostic: Time is 3, 5, or 7d

Predictive: Time is 1 or 3d with pathology observation at $\geq 5d$

• Criteria were selected loosely, based on no BDH or fibrosis observed at any time in control animals

• New data suggested 6 new compounds for periportal fibrosis diagnostic signature and 8 new compounds for predictive signature +1 classes

• 8 new compounds in +1 class of diagnostic BDH signature and 10 new compounds in +1 class of predictive BDH signature

Cross-Validation Performance of Rederived RU1 BDH Predictive Signature

Compound_Name	Dose (mg/kg)	TreatPt (d)	ASPLP Predictive SP Scores	BDH at 28d?
1-NAPHTHYL ISOTHIOCYANATE	15	1	0.56	Y
1-NAPHTHYL ISOTHIOCYANATE	15	1	0.47	Y
AFIATOXIN B1	0.3	1	1.95	Y
AFIATOXIN B1	0.3	1	1.18	Y
ALLYL ALCOHOL	16	1	0.88	N
ALLYL ALCOHOL	16	1	0.94	N
CARBON TETRACHLORIDE	3178	1	1.08	Y
CARBON TETRACHLORIDE	3178	1	0.88	Y
ETHANOL	3000	1	0.83	N
ETHANOL	3000	1	0.82	N
ISONIAZID	50	1	0.87	N
ISONIAZID	50	1	0.17	N
METHAPYRILENE	100	1	1.16	Y
METHAPYRILENE	100	1	1.08	Y
N-NITROSODIMETHYLAMINE	10	1	2.19	Y
N-NITROSODIMETHYLAMINE	10	1	0.80	Y
N-NITROSODIMETHYLAMINE	10	1	1.13	Y
N-NITROSODIMETHYLAMINE	10	1	1.25	Y
THIOACETAMIDE	200	1	1.87	Y
THIOACETAMIDE	200	1	1.16	Y

12/14 True Positive (86%)
5/6 True Negative (83%)

Table 4: The rederived ASPLP predictive BDH signature was run against the set of validation compounds. True positive experiments with positive scalar product scores are shown in red.

Effects of Including Reread Data and Increasing Stringency on Estimated Signature Performance

	Average True Positive %	Average True Negative %	Average Log Odds Ratio
Existing BDH diagnostic	65	99.5	5.9
New BDH diagnostic	56.9	98.8	4.6
High stringency BDH diagnostic*	82.1	98.2	5.5
Existing BDH predictive	37	99.7	4.7
New BDH predictive*	49.2	98.3	4.9
High stringency BDH predictive	68.6	98.3	4.8

Table 5: Cross-validation performance metrics for 3 versions of diagnostic and predictive bile duct hyperplasia Drug Signatures®.

New BDH and High stringency BDH signatures include reread experiments

New BDH +1 class definition: BDH severity score ≥ 1 in at least 1/3 animals

High stringency BDH +1 class definition: BDH severity score ≥ 1 in at least 2/3 animals

Conclusion: Inclusion of additional data and increasing stringency of +1 class leads to generation of a higher performance BDH signature (increased avg. true positive % with minimal effect on avg. true negative %)

Existing and Rederived Versions of BDH Signatures Are Comprised of Different Genes

Signature Name	Number of Probes in Existing SPLP Signature	Number of Probes in New ASPLP Signature	Number of Probes Common to Both Signatures
BDH Diagnostic	12	59	3
BDH Predictive	56	38	3

Table 6: Number of RU1 probes in BDH predictive and diagnostic signatures

Overlapping probes in existing and new BDH diagnostic signatures: phosphatidylserine-specific phospholipase A1 (Psp1a1), osteoblast specific factor 2 (fascilin I-like), small inducible cytokine subfamily A20

Overlapping probes in existing and new BDH predictive signatures: Sodium channel voltage-gated type IV alpha polypeptide (Scn4a), Aquaporin 5 (Aqp5), 13 days embryo head cDNA RIKEN full-length enriched library

Inclusion of new compounds and use of ASPLP algorithm changed number of probes as well as identity of probes in each signature

Summary of Bile Duct Hyperplasia Pathology Data

Compound	Time (d)	Dose (mg/kg)	# of Animals	Incidence	Total BDH Score
AFIATOXIN B1	5	0.3	3	1/3	1
AFIATOXIN B1	20	0.3	8	8/8	24
METHAPYRILENE	5	100	3	3/3	5
METHAPYRILENE	20	100	5	5/5	18
1-NAPHTHYL ISOTHIOCYANATE	1	15	3	1/3	1
1-NAPHTHYL ISOTHIOCYANATE	5	15	4	4/4	6
1-NAPHTHYL ISOTHIOCYANATE	20	15	8	8/8	22
ETHANOL	1	3000	3	1/3	3
CARBON TETRACHLORIDE	20	3178	8	8/8	2
N-NITROSODIMETHYLAMINE	1	10	3	3/3	3
N-NITROSODIMETHYLAMINE	5	10	3	3/3	0
N-NITROSODIMETHYLAMINE	20	10	3	3/3	12
THIOACETAMIDE	20	200	8	3/8	5
N-NITROSODIMETHYLAMINE	20	100	8	8/8	25

Table 1: Total BDH score was calculated for each experiment by obtaining the sum of histopathology severity scores for all animals

