# Evaluating adaptive stress consensus gene signatures using transcriptomics

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*The views expressed in this presentation are those of the author[s] and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.*

# Building a transcriptomic basis for adaptive stress response

- Thousands of untested chemicals with little or no health effects data

- New approach methods (NAMs) can inform chemical hazard/risk without animal testing *e.g.:-*
  - High throughput screening (HTS)
  - High-throughput transcriptomics (HTTr)

- Half of the 1063 chemicals examined in an early ToxCast study do not act via specific mechanisms
  - Did activate stress response systems

- Overwhelming adaptive stress response systems beyond “tipping point” can lead to adverse outcomes (Shah et al. 2016)

# Stress response (SR) pathways provide a “systems” basis for categorizing perturbagen action

- SR pathways maintain cellular homeostasis
- SR pathway activation

Sensor(SENS) -> TF -> Transducer (TRD) -> Response Element (RE) -> Gene expression (EFF)

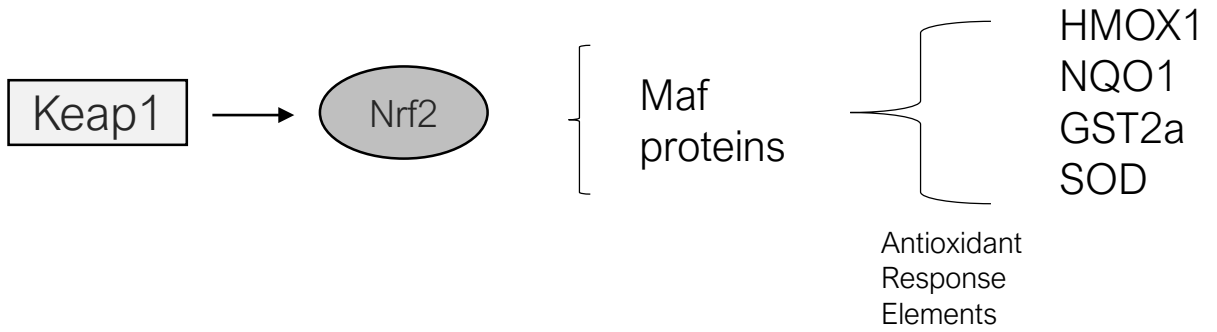
- Key questions:
  - How to develop signature sets?
  - Can we uniquely classify SRPs?
  - Can SR gene sets be used to evaluate tipping points and quantify cell stress?

**Outcome: A NAM to classify non-specific chemicals using HTTr-SR activity**

**TABLE 1**
**The Major Adaptive Stress response pathways**

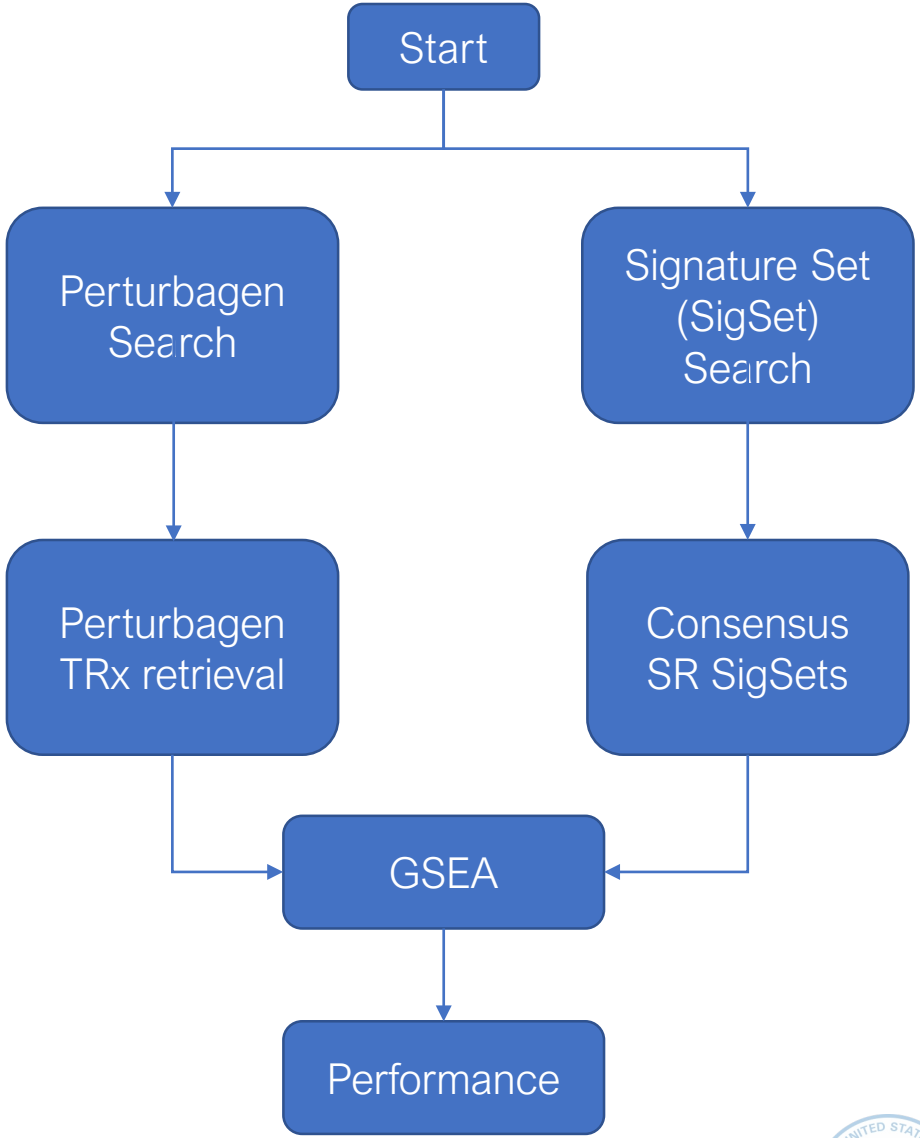
| Stress response pathway | Chemical inducers | TF | Activated gene promoters |
|---|---|---|---|
| Oxidative stress | Quinones, hydroperoxides, heavy metals, trivalent arsenicals | Nrf2 | *HMOX1*, *NQO1*, *GST2A* |
| Heat shock response | Heat, Heavy Metals | HSF-1 | *HSPA6* |
| DNA damage response | Etoposide, Methyl Methanesulfonate, N-Dimethylnitrosamine, Cyclophosphamide, UV radiation | p53 | *CDKNIA*, *GADD45A*, *MDM2*, *BCL2*, *TP53I3* |
| Hypoxia | Hypoxia, Cobalt, Desferriozamine, Quercetin, Dimethyloxalylglycine | HIF-1 | *VEGF*, *TF*, *EPO* |
| ER stress | Tunicamycin, Thapsigargin, Caplain, Brefeldin A | XBP-1, ATF6, ATF4 | *HSP90B1*, *HSPA5*, *DNAJB9* |
| Metal stress | Heavy Metals | MTF-1 | *MT1E*, *MT2A* |
| Inflammation | Metal, PCBs, Exhaust Particles, Smoke Particles | NF-κB | *IL1A*, *TNFA* |
| Osmotic stress | High salt, polyethylene glycol, mannitol | NFAT5 | *AKR1B1*, *SLC6A12*, *SLC5A3* |

Simmons, S. O.; Fan, C.-Y.; Ramabhadran, R. Cellular Stress Response Pathway System as a Sentinel Ensemble in Toxicological Screening. Toxicol. Sci. 2009, 111 (2), 202–225. https://doi.org/10.1093/toxsci/kfp140.



# Approach to evaluate SR pathway activity

1. Find reference chemicals
2. Reference chemical transcriptomics dataset (TRx)
3. Construct consensus SR signature sets
4. Evaluate performance of signature sets for characterizing reference chemicals



# Three search methods used to compile hallmark perturbagen profiles

Three methods to search

- Literature

  Known Chemicals identified by searching against stress response system induction

- Comparative Toxicogenomic Database (CTD)

  Confirmed by reference association

- Library of Integrated Network-Based Cellular Signatures (LINCS)

  Confirmed stress system activity by querying TF/SENS/TRD/EFF gene lists against for identified chemicals within the

Transcriptomic profiles sourced from public datasets on GEO

| DNA | ERS | HTS | HYP | MTL | OXD |
|---|---|---|---|---|---|
| MDM2 | HSPA5, BiP, Grp78 | HSF1 | VHL | MTF1 | NFE2L2 |
| ATM | PERK, CREB2 | HSF2 | PHD* | MT1E | MAPK |
| JNK | IRE1alph | HSF3 | PSMA7 | MT2A | ERK |
| Chk1 | ATF4 | HSF4 | HIF1alph | PRNP | p38 |
| Chk2 | ATF6 | HSPA6 | HIF1bet | Zip10 | PKC |
| p53 | XBP1 | DNAJB1, DNAJ1, HDJ1, HSPF1 | p300, CBP | MT-I: MT1A, MT1B, MT1E, MT1F, MT1G1, MT1G2, MT1H, MT1HL1, MT1M, MT1X, | Keap1 |
| GADD45a | DDIT3 | HSP90AA1 HSP 90A, HSPC1, HSPCA, HSP90AB1 HSP90B, HSPC2, HSPCB | | MT-II: MT2A, CES1, MT2 | HMOX1 |
| BRCA1 | IGFBP1 | CK2 | VEGF | AFP | MAF * |
| STRAP | IL8 | CaMK2 | EDN* | BiP | Roc1 |
| DNA2 | ATF3 | HSPA4* | TFR* | CZBP | CUL*, CUL3 |
| APE | HERPUD1, CHOP, GADD153 | HSPA1A | ALDO* | Sepw1 | SOD3, SOD* |
| RPA | HSP90 | HSPA1B | GTR* | ZnT-1 | CAT |
| LIG4 | DNAJB9, | HSPA1L | VLP | yGCS | PRDX1 PAGA, PAGB, TDPX2 |
| MRE1 | ERdj4, ERdj5 | HSPA2 | VDU2 | P1GF | GPX* |
| RAD51 | p58 | HSPA6 | MT1 | C/EBPbeta | TXN* TRDX, TRX, TRX1 |
| PARP | PDI, MPD1, EUG1, MPD2, EPS1 | HSPA7 | HMOX* | PI3K | GSR* 1 |
| EXO1 | ERO1 | HSPA8 | ENO* | GSK-3 | MT1, MT2 |
| WRN | ERp57 | HSP12A | HOGA* | HAMP | GST* |
| OGG1 | Sec61 | HSP12B | | Fer2LCH | NQO1 |

# Reference Perturbagen Dataset

| Perturbagen | Profiles |
|---|---|
| Hypoxia | |
| 0.1% Oxygen | 2 |
| VU-0418946-1 VU-0418946-2 | 2 |
| | |
| ERS/Unfolded Protein Response | |
| Bredfeldin A | 2 |
| Tunicamycin | 3 |
| Thapsigargin | 3 |
| | |
| Metal Stress | |
| AgNO3 | 1 |
| ZnO | 3 |
| | |
| Heat Shock | |
| Heat and Heat w/ recovery | 1 |
| Geldanimycin | 3 |
| Radcicol | 2 |
| | |
| DNA Repair Stress | |
| Lasicoarpine | 1 |
| Methylmethanesulfonate | 1 |
| Gylcidamide | 1 |
| Benzopyrene | 1 |
| | |
| Oxidative Stress | |
| hydrogen peroxide | 3 |
| tertbuytlhydroperoxide | 1 |

# Process to find consensus SR Signature Sets

- SR systems are not formally defined
  - Incorporate many features

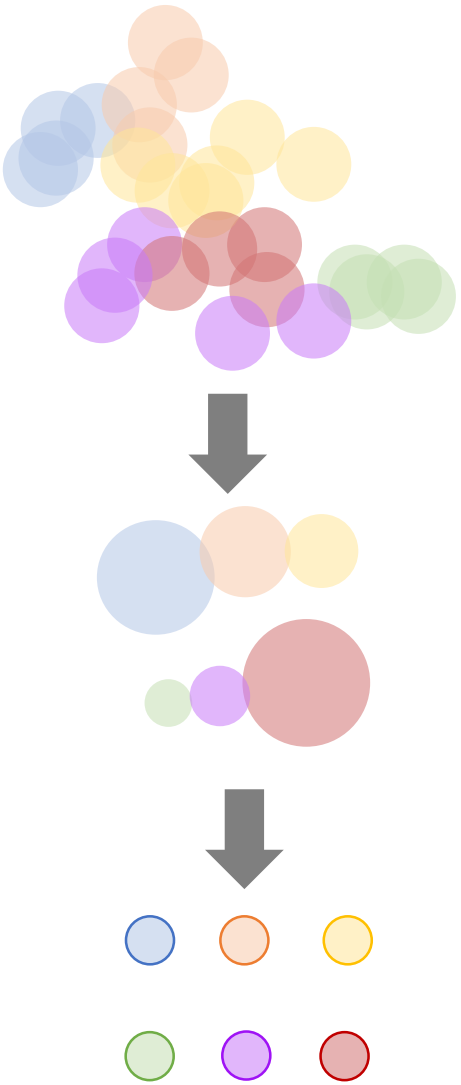
- Find existing signature sets
  - Use key SENS/TF/TRD/EFF genes from initial characterization to identify
  - Choose only those that are well associated with the SR in question

  - Significant cross-talk expected

- Prepare consensus signature sets

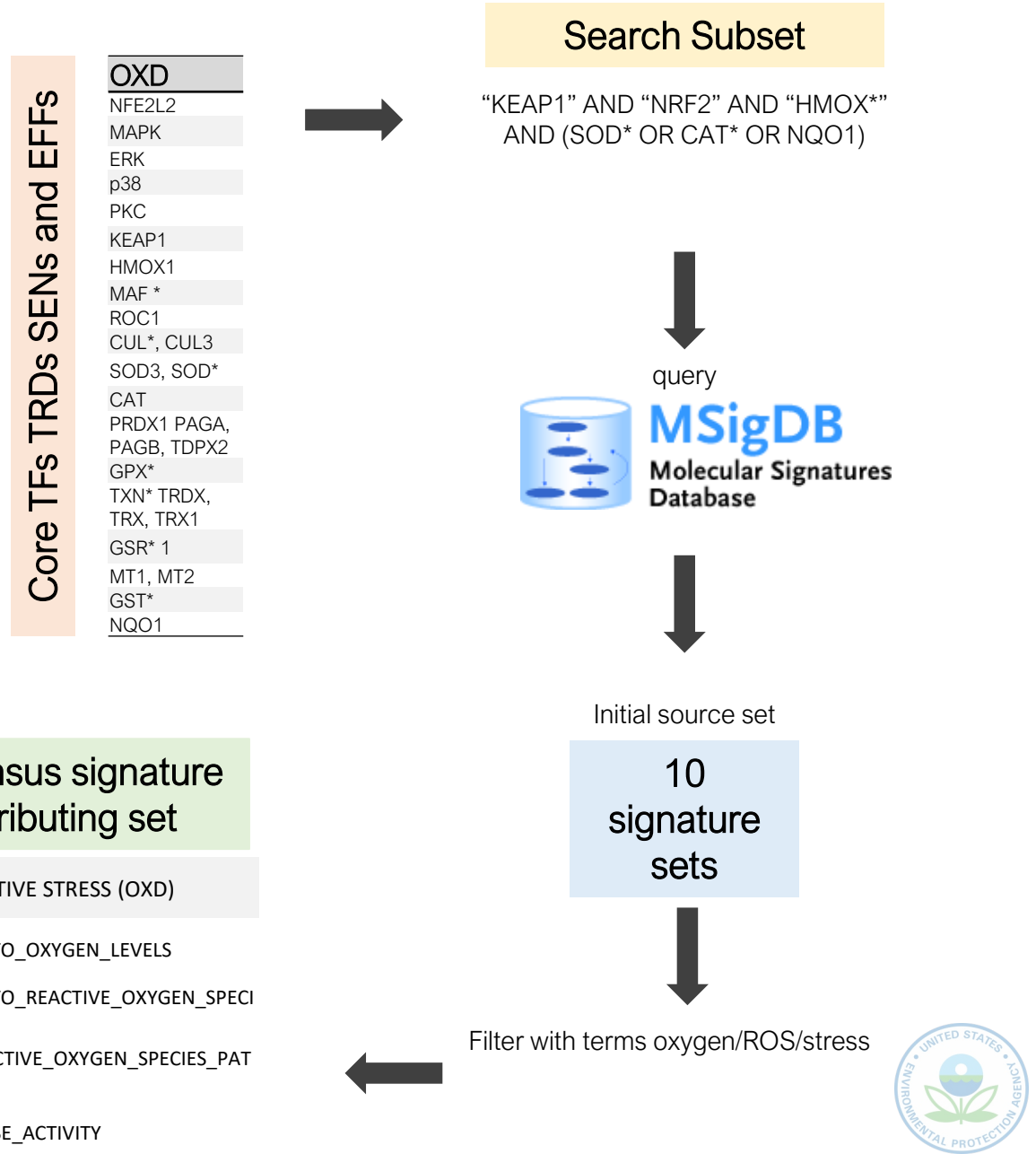
- Cut size to limit overlap while applying a filter to ensure only most central genes are included

Consensus SigSet approach



# Identifying relevant signatures with SPR central elements

- Search for signature sets in MSigDB with combination of SR system TF, TRD, SENS and subset of key EFF
  - Similar to approach used to identify hallmark perturbagens
- Find EFF combination returning maximum and use keyword filter to return SigSets of interest
  - *i.e.,* "hypoxia", "oxidative", or "damage"
- Construct consensus signature sets



# Consensus signature source sets vary in size and abundance

| DNA DAMAGE (DNA) | ER STRESS (ERS) | HEAT SHOCK (HTS) | HYPOXIA (HYP) | METAL STRESS (MTL) | OXIDATIVE STRESS (OXD) |
|---|---|---|---|---|---|
| BIOCARTA_P53_PATHWAY | GO_CELLULAR_RESPONSE_TO_TOPOLOGICALLY_INCORRECT_PROTEIN | GO_CELLULAR_RESPONSE_TO_HEAT | GROSS_HYPOXIA_VIA_ELK3_AND_HIF1A_UP | GO_METAL_ION_TRANSMEMBRANE_TRANSPORTER_ACTIVITY | GO_RESPONSE_TO_OXYGEN_LEVELS |
| GO_BASE_EXCISION_REPAIR | GO_CHAPERONE_COFACTOR_DEPENDENT_PROTEIN_REFOLDING | GO_CHAPERONE_COFACTOR_DEPENDENT_PROTEIN_REFOLDING | GROSS_HYPOXIA_VIA_HIF1A_DN | GO_RESPONSE_TO_CADMIUM_ION | GO_RESPONSE_TO_REACTIVE_OXYGEN_SPECIES |
| GO_CELLULAR_RESPONSE_TO_DNA_DAMAGE_STIMULUS | GO_CHAPERONE_MEDIATED_PROTEIN_FOLDING | GO_CHAPERONE_MEDIATED_PROTEIN_FOLDING | HARRIS_HYPOXIA | GO_RESPONSE_TO_METAL_ION | HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY |
| GO_DNA_REPAIR | GO_DE_NOVO_PROTEIN_FOLDING | GO_PROTEIN_FOLDING | JIANG_HYPOXIA_NORMAL | GO_TRANSITION_METAL_ION_HOMEOSTASIS | OXIDOREDUCTASE_ACTIVITY |
| GO_DOUBLE_STRAND_BREAK_REPAIR | GO_PROTEIN_FOLDING | | | ON_METAL_ION_TRANSME NSPORTER_ACTIVITY | |
| GO_G1_DNA_DAMAGE_CHECKPOINT | GO_RESPONSE_TO_TO NCORRECT_PROTEIN | | | ESPONSE_TO_METAL_ION | |
| GO_NUCLEASE_ACTIVITY | GO_UNFOLDED_PROTE | | | | |
| GO_REGULATION_OF_DNA_DAMAGE_RESPONSE_SIGNAL_TRANSDUCTION_BY_P53_CLASS_MEDIATOR | HALLMARK_MTORC1_S | | | | |
| GO_REGULATION_OF_RESPONSE_TO_DNA_DAMAGE_STIMULUS | HALLMARK_UNFOLDED ONSE | | | | |
| GO_SIGNAL_TRANSDUCTION_IN_RESPONSE_TO_DNA_DAMAGE | | | | | |
| HALLMARK_DNA_REPAIR | | | | | |
| KEGG_MISMATCH_REPAIR | | | | | |
| KEGG_NUCLEOTIDE_EXCISION_REPAIR | | | | | |
| KEGG_P53_SIGNALING_PATHWAY | | | | | |
| REACTOME_DNA_REPAIR | | | | | |
| REACTOME_G1_S_DNA_DAMAGE_CHECKPOINTS | | | | | |
| REACTOME_TRANSCRIPTION_COUPLED_NER_TC_NER | | | | | |

- Abundance of DNA is significantly greater than OXD in MSigDB
- Overlap in available sets for ERS and HTS

# Frequency of occurrence determines consensus signature set

- Merge gene sets into consensus SR sig set by filtering genes based on…
- Occurrence Frequency factor (OFF)

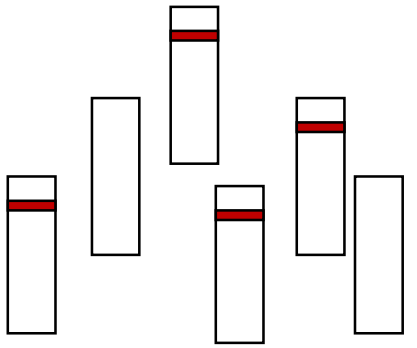
$$OFF = \frac{f_{in\ group} - f_{out\ group}}{f_{in\ group} + f_{out\ group}}$$

Where *f* = frequency (count/#groups sampled)

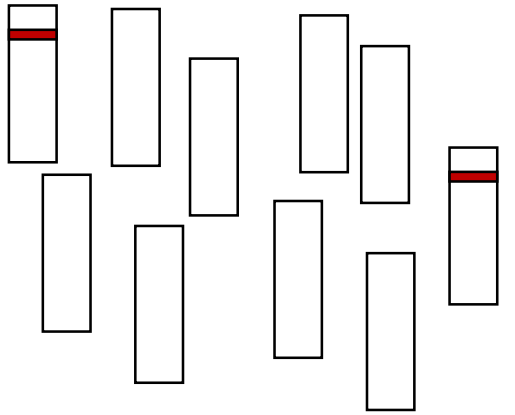
- All genes in set ranked by OFF and thresholded by SigSet sizes:

TH50 = 50 top genes
TH100 = 100 top genes,
TH200 = 200 top genes
…
FULL = all genes

*In group*
*(e.g., all HYP sig sets)*

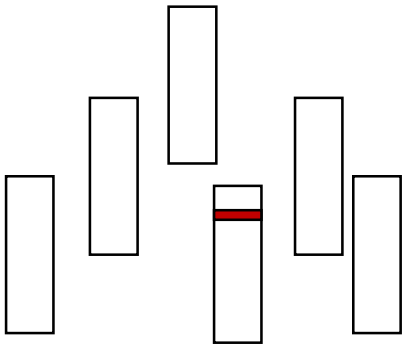


*Out group*
*(e.g., all **non-**HYP sig sets)*

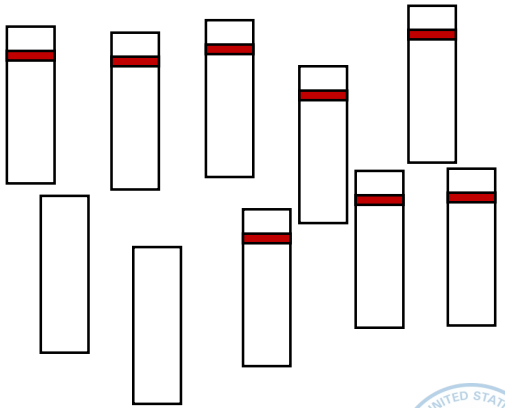


$f_{in\ group}$ = 4/6 $f_{out\ group}$ = 2/10
*occurrence frequency factor = (.667 - .2)/(0.867) = 0.54*

*In group*
*(e.g., all HYP sig sets)*



*Out group*
*(e.g., all **non-**HYP sig sets)*



$f_{in\ group}$ = 1/6 $f_{out\ group}$ = 8/10
*occurrence frequency factor = (.167 - .8)/(0.967) = -0.66*

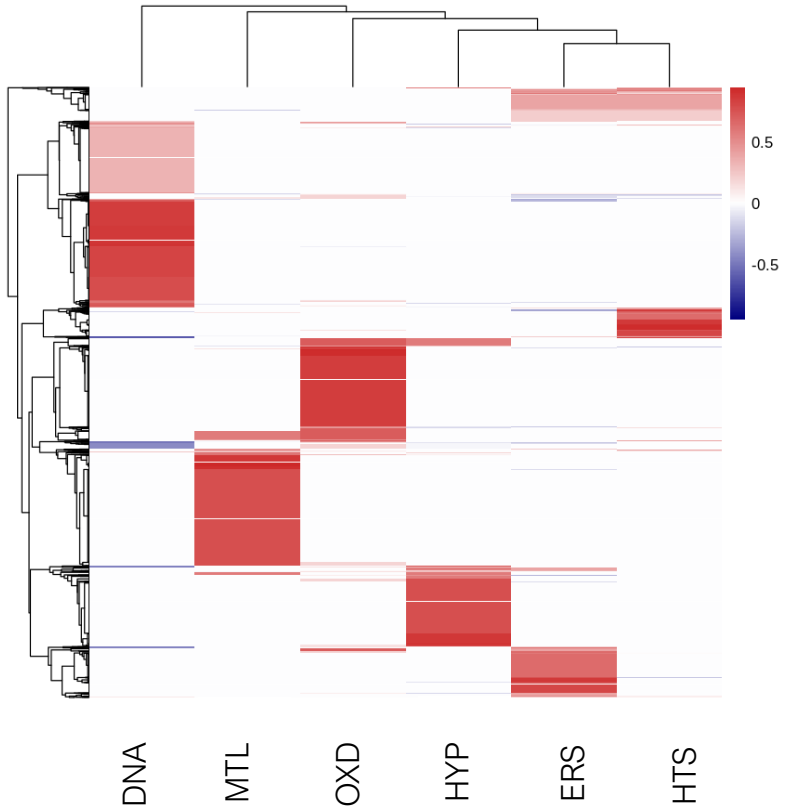
# Consensus Signature construction reduces gene space overlap

All signature sets share approximately 50 or more genes

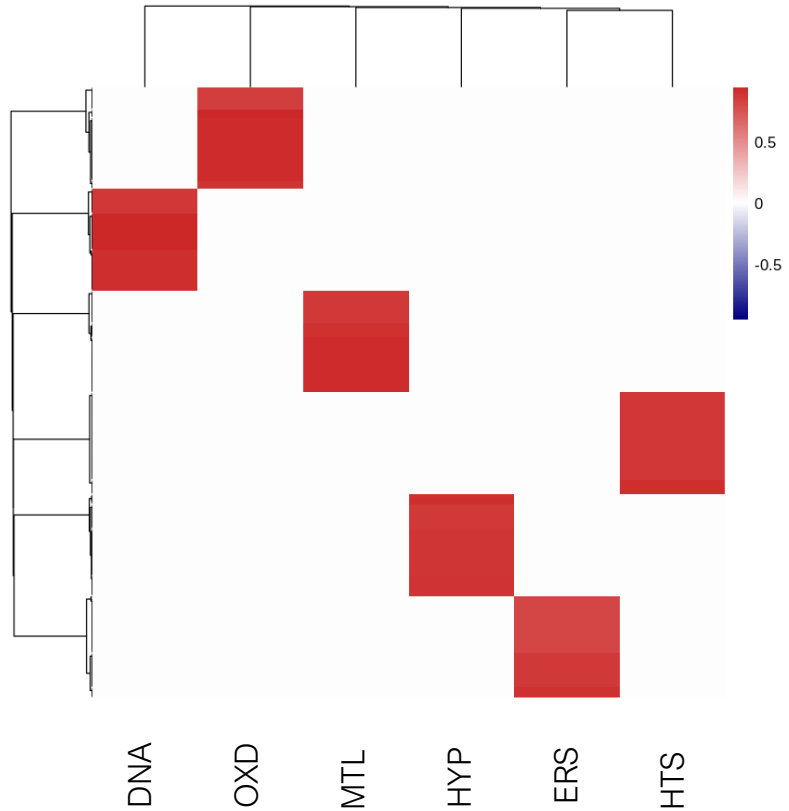
Similar signatures like ER and HS share many more

Thresholding reduces Signature set overlap by only 4 genes at a size of 200 total genes and overlap coefficients increase after this thresholding

Source Overlap




Consensus overlap top 200




Szymkiewicz-Simpson Overlap Coefficient overlap

$(overlap = \frac{|X \cap Y|}{\min(|X|,|Y|)})$

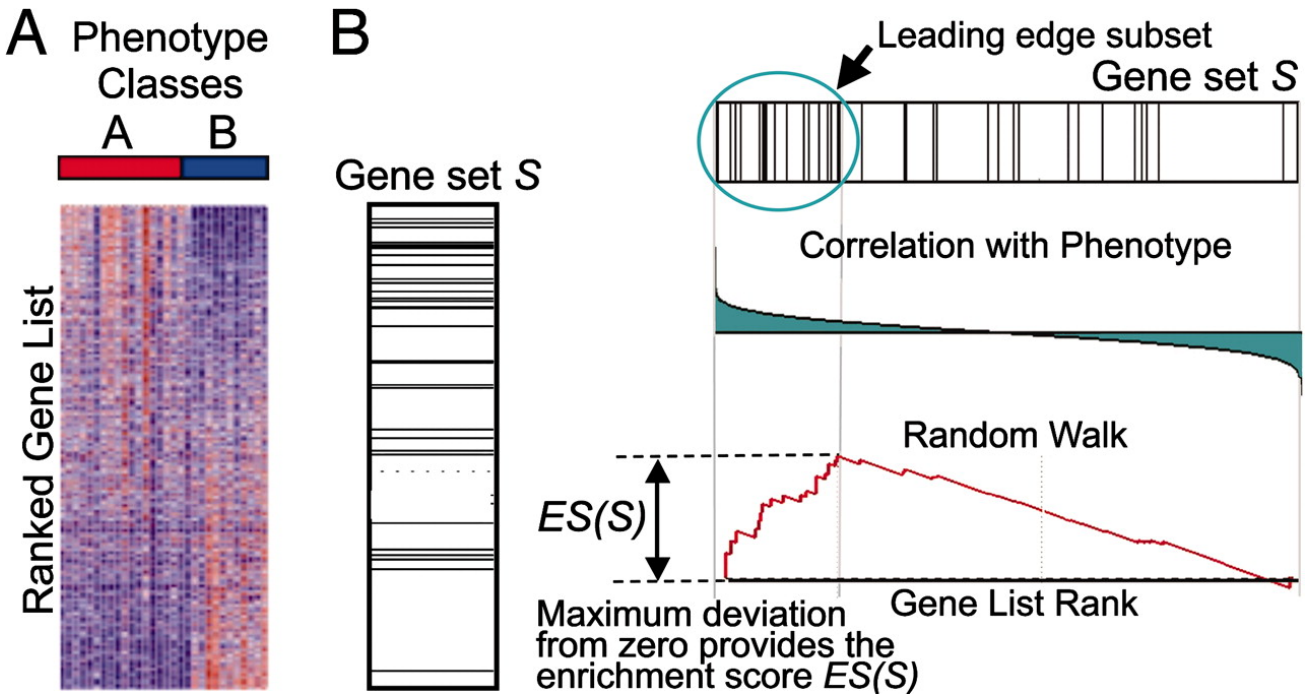
~ 100 x less overlap by thresholding ranked OFF signatures sets

| | DNA | ERS | HTS | HYP | MTL | OXD |
|---|---|---|---|---|---|---|
| DNA | 1 | 0.13 | 0.10 | 0.16 | 0.06 | 0.17 |
| ERS | 0.13 | 1 | 0.58 | 0.13 | 0.05 | 0.13 |
| HTS | 0.10 | 0.58 | 1 | 0.07 | 0.10 | 0.13 |
| HYP | 0.16 | 0.13 | 0.07 | 1 | 0.08 | 0.28 |
| MTL | 0.06 | 0.05 | 0.10 | 0.08 | 1 | 0.17 |
| OXD | 0.17 | 0.13 | 0.13 | 0.28 | 0.17 | 1 |

| | DNA | ERS | HTS | HYP | MTL | OXD |
|---|---|---|---|---|---|---|
| DNA | 1 | 0 | 0 | 0 | 0 | 0 |
| ERS | 0 | 1 | 0.005 | 0 | 0 | 0 |
| HTS | 0 | 0.005 | 1 | 0.005 | 0.005 | 0 |
| HYP | 0 | 0 | 0.005 | 1 | 0.005 | 0 |
| MTL | 0 | 0 | 0.005 | 0.005 | 1 | 0 |
| OXD | 0 | 0 | 0 | 0 | 0 | 1 |

# GSEA scores activity of a pathway in transcriptomic data

- Gene signature enrichment analysis
  1. Rank order genes by expression
  2. KS random walk through SigSet
  3. Count genes in SigSet with up score
  4. Count genes in SigSet with down score
  5. Calculate total
- Calculate GSEA NES
  - Used myGSEA implementation of vGSEA (Richard Judson at the EPA)
  - No Up/Down characteristic included

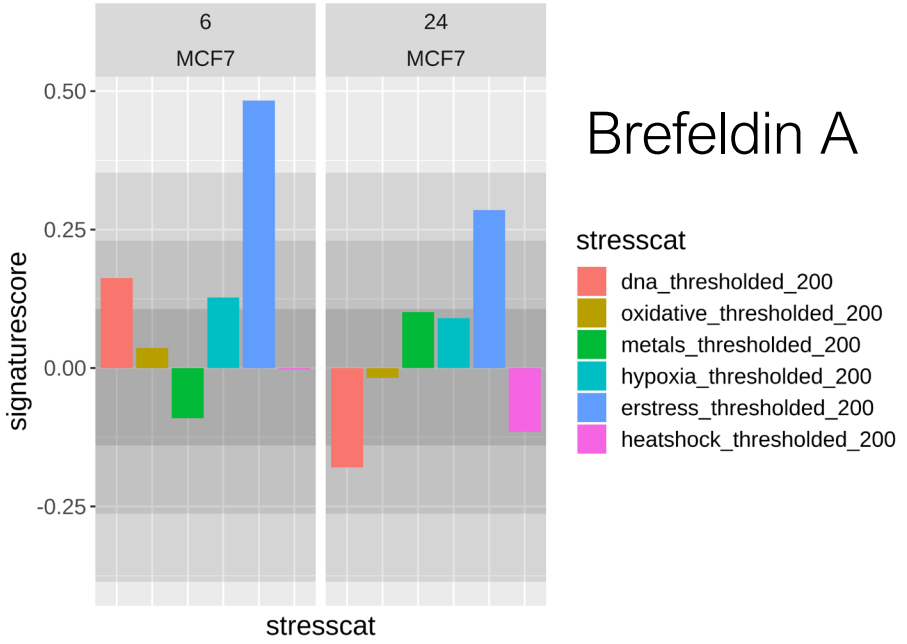



Aravind Subramanian *et al.* PNAS Oct 2005, 102 (43) 15545-15550; DOI: 10.1073/pnas.0506580102

# GSEA scores to assess SR activity and assign stress class to perturbagens

preliminary run scored with TH200 SigSet

- *Brefeldin as ERS case study*
- *Decrease in DNA at 24 may indicate cell death*




"Accuracy by" is the depth into the ranked GSEA scores at which the reference categorical assignment is met

- *e.g.:*

  1/4 correctly classified by GSEA score at top ranked score depth is 25% Accuracy by 1st

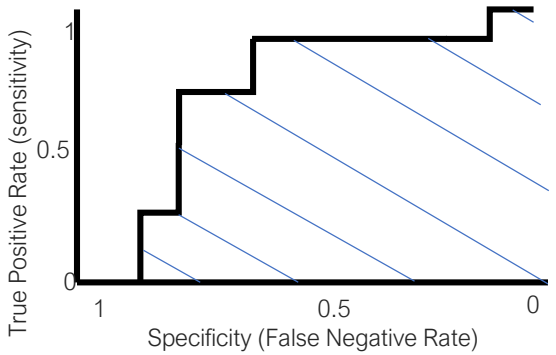
  3/4 correctly classified by GSEA score at the second highest score rank depth is 75% accuracy by 2nd

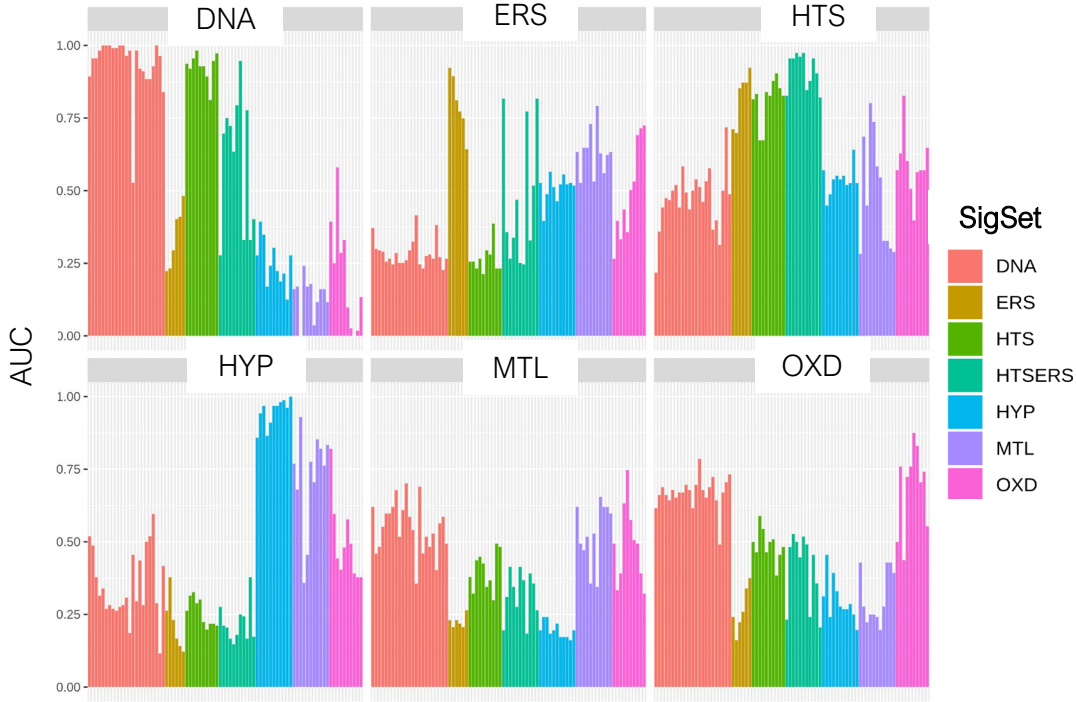
| SR pathway | Accuracy by 1st | Accuracy by 2nd | Accuracy by 3rd |
|---|---|---|---|
| DNA | 50% | 100% | 100% |
| ERS | 74% | 100% | 100% |
| HTS | 0% | 50% | 89% |
| HYP | 100% | 100% | 100% |
| MTL | 25% | 25% | 25% |
| OXD | 25% | 50% | 100% |
| *Mean* | **46%** | **71%** | **86%** |

Accuracy by score depth for TH200 Consensus Set

# Assess diagnostic ability of all consensus signature sets with receiver operating characteristics(ROC)/Area Under Curve (AUC) analysis

- Calculate the receiver operating characteristic (ROC) area under the curve (AUC) as a measure of performance for each SR category
- Assess the ability of all signature sets to diagnose each SR pathway
- The winning set was chosen by selecting on two parameters:
  - Maximized $AUC_{in\ group}$ /(Total $AUC_{out\ group}$)
  - Signatures set Size
- A group of the 6 most diagnostic sig sets were chosen
  - MTL has some overlap with HYP
  - OXD is not very selective and is overpowered by DNA



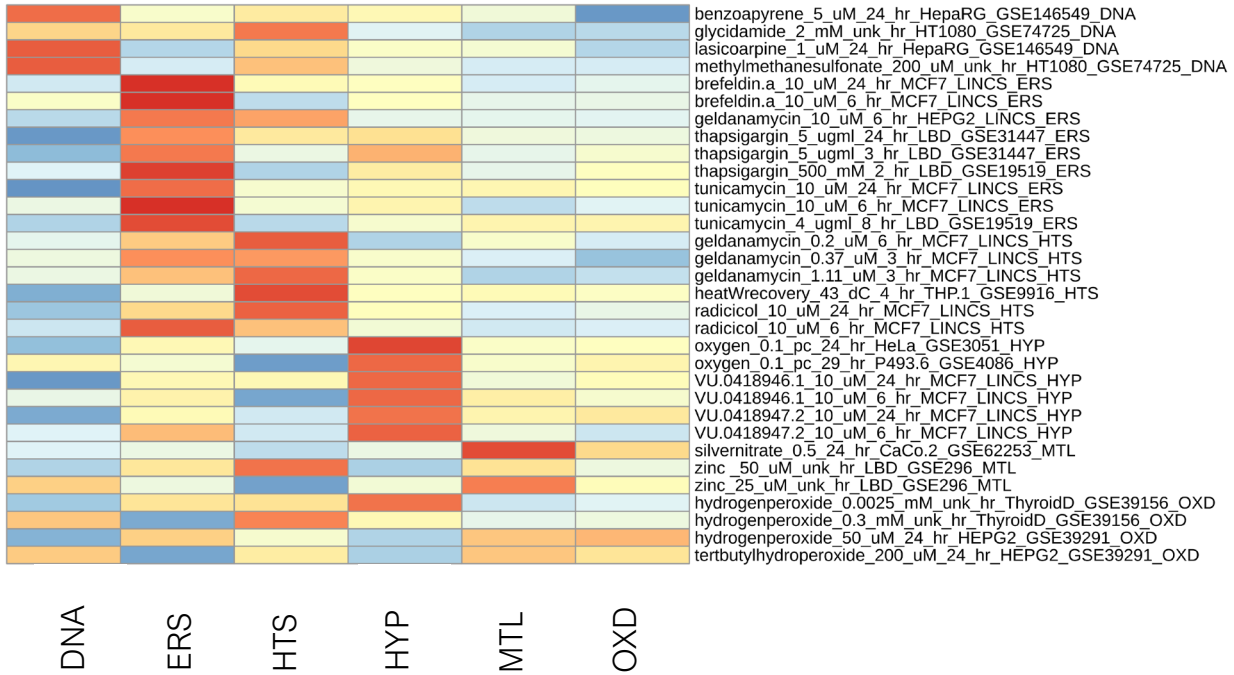




| names | DNA | ERS | HTS | HYP | MTL | OXD |
|---|---|---|---|---|---|---|
| DNA_thresholded_300 | 100 | 25.60 | 46.79 | 33.97 | 59.77 | 64.28 |
| DNA_thresholded_400 | 100 | 26.57 | 50 | 26.92 | 59.77 | 67.85 |
| DNA_thresholded_477 | 100 | 24.63 | 51.92 | 28.20 | 62.06 | 65.17 |
| GO_DNA_REPAIR | 100 | 25.12 | 49.35 | 27.56 | 60.91 | 69.64 |
| GO_DOUBLE_STRAND_BREAK_REPAIR | 100 | 26.08 | 43.58 | 28.20 | 70.11 | 67.85 |
| REACTOME_DNA_REPAIR | 100 | 27.05 | 50 | 28.84 | 56.32 | 66.96 |
| GO_BASE_EXCISION_REPAIR | 99.10 | 28.50 | 44.23 | 26.92 | 67.81 | 66.96 |
| GO_CELLULAR_RESPONSE_TO_DNA_DAMAGE_STIMULUS | 99.10 | 25.12 | 58.33 | 26.28 | 51.72 | 66.96 |

# SR consensus SigSet mean accuracy improved by selecting top AUC

- The six most diagnostic sig sets were primarily defined by consensus SigSets
- Improvement to 78% using new sigsets at top GSEA score and 91% by second
- OXD is least diagnostic – perhaps due to centrality of system

| Signatures Set | DNA | ERS | HTS | HYP | MTL | OXD |
|---|---|---|---|---|---|---|
| DNA_thresholded_400 (DNA) | 100 | 26.6 | 50 | 26.9 | 59.8 | 67.9 |
| ERS_thresholded_200 (ERS) | 23.2 | 89.4 | 69.9 | 37.8 | 20.7 | 16.1 |
| GO_DE_NOVO_PROTEIN_FOLDING (HTS) | 72.3 | 33.8 | 97.4 | 16.7 | 34.5 | 50 |
| HYP_thresholded_400 (HYP) | 27.7 | 51.7 | 52.6 | 100 | 19.5 | 19.6 |
| MTL_thresholded_200 (MTL) | 11.6 | 62.8 | 32.7 | 85.3 | 65.5 | 27.7 |
| OXD_thresholded_200 (OXD) | 9.82 | 50.2 | 39.7 | 57.7 | 57.5 | 87.5 |




| Category | Accuracy in 1st | Accuracy by 2nd | Accuracy by 3rd |
|---|---|---|---|
| DNA | 75 | 100 | 100 |
| ERS | 100 | 100 | 100 |
| HTS | 66.7 | 100 | 100 |
| HYP | 100 | 100 | 100 |
| MTL | 66.7 | 100 | 100 |
| OXD | 25 | 25 | 50 |
| Mean Accuracy | 78% | 91% | 93% |

# Null targets and negative scores do not overlap with positive scores

Find chemicals and TRx profiles from LINCS for chemicals acting on targets (based on Tau scores):-

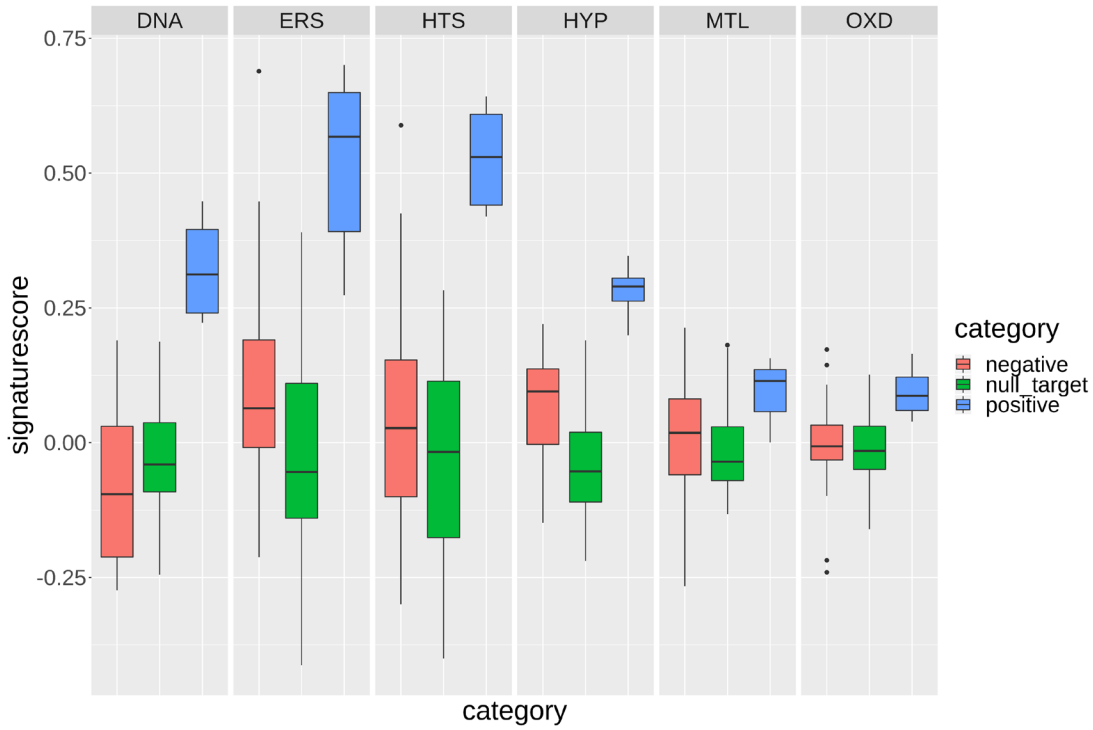
Estrogen
Dopamine/Serotonin
Antipsychotics
Etc.

- 31 Chemicals / 64 profiles
- Defined as null targets

| Name | Description | Median Tau |
|---|---|---|
| azaperone | dopamine receptor antagonist | 2.62 |
| tetrahydropalmatine | serotonin release inhibitor | 2.55 |
| elvitegravir | HIV integrase inhibitor, HIV inhibitor | 2.51 |
| midazolam | benzodiazepine receptor agonist, GABA benzodiazepine site receptor agonist | 2.47 |
| androstanol | constitutive androstane receptor (CAR) inhibitor | 2.3 |
| bicalutamide | androgen receptor antagonist | 2.26 |
| tibolone | estrogen receptor agonist, androgen receptor agonist, progesterone receptor agonist, selective estrogen receptor modulator (SERM), steryl sulfatase inhibitor | 2.22 |
| GBR-13069 | dopamine uptake inhibitor | 2.18 |
| estriol | estrogen receptor agonist, estrogen receptor antagonist | -1.36 |

Categories of comparison

- Positive: hallmark assignment and pathway assignment should match
- Negative: hallmark assignment and pathway assignment should not match
- Null target: chemical should not have any pathway activity




- ERS shows some broader activation but this is expected
- While OXD and MTL are less sensitive they still exhibit good separation from negative and null scores in their class

# Key Outcomes

- We can build gene sets to represent adaptive stress response pathways
  - While existing gene sets did exist, consensus SigSets were more accurate

- These stress response gene sets can accurately classify the 18 hallmark perturbagens
  - Based on ROC AUC analysis of GSEA scores
  - ERS tends to be less specific with null targets

- Limitations of this approach
  - The transcriptional fingerprint only as good as the starting gene sets
  - Evaluation performance as good as the set of perturbagen set – need more profiles

- SR gene set analysis can potentially characterize non-specific stress responses and enable identification of hazard and potency (using concentration response modeling)
  - Not shown: Failed Pharmaceutical, Concentration response mapping

# Finding additional SR pathway inducers – a taste of what is to come

Identify list of chemicals perturbagens in LINCS (~20,547 chemicals)

- Remove chemical only identified by Broad Institute ID (e.g., BRD-)
- Reduced to 4671 chemicals

Search PubMed with all chemicals & a set of stress response pathways (SRPs)

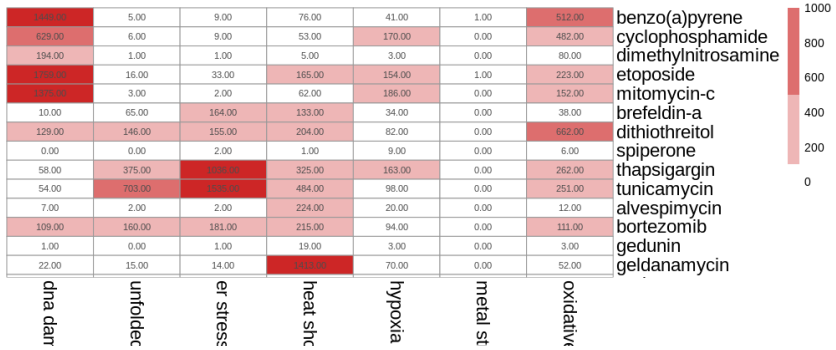
- 7 terms (e.g., 'dna damage', 'er stress', unfolded protein response'
- Totaled 32,679 searches

PMI calculated for all returned chemicals exceeding threshold abstracts to guide perturbagen selection and assignment

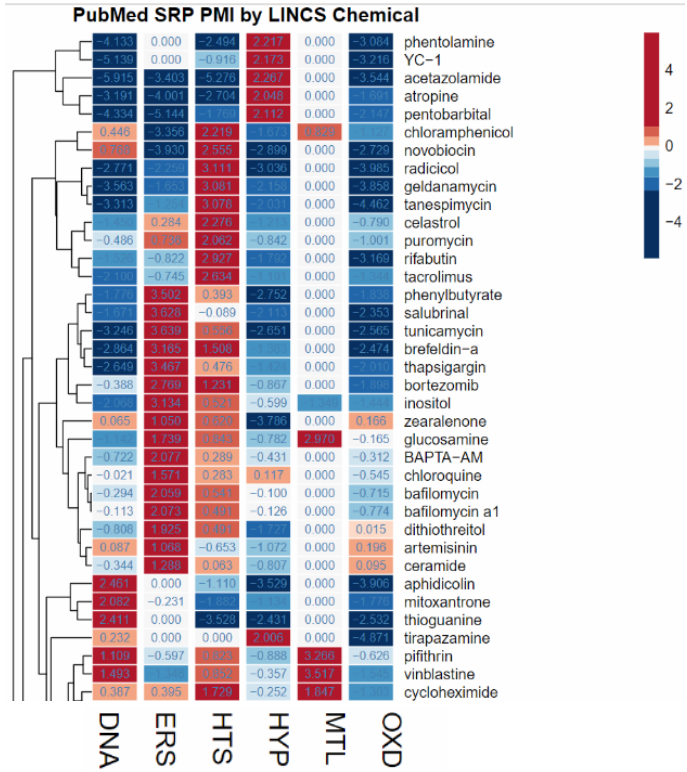
$$PMI(chemical, stress) = \log \frac{F(chemical, stress)}{F\ (chem)F(stress)}$$

Pulled first 500 abstracts for each selected perturbagen and validated assignment by hand for 97 chemicals

Inclusion of genetic perturbations (TR/SENS/EFF gene KD and OEs) currently in progress




Example of reference counts for a common set of DNA damaging and ER stressing chemicals




Clustering of PMI scored LINCS perturbagens

# Breakdown of chemical SR pathway inducing perturbagens TRx profiles

Total result is 11,364 transcriptomic profiles characterized by:

- 6 SRPs (DNA, ERS, HTS, HYP, MTL, and OXD)
- 83 cells types
- 26 doses
- 5 time points

| stresscat | nchems | n_cell_types | n_time_points | time_points | n_doses | number_of_profiles |
|---|---|---|---|---|---|---|
| DNA | 24 | 81 | 4 | 3, 6, 24, 48 | 17 | 4162 |
| ERS | 8 | 61 | 3 | 6, 24, 144 | 13 | 485 |
| HTS | 11 | 79 | 4 | 3, 6, 24, 48 | 16 | 5135 |
| HYP | 7 | 17 | 2 | 6, 24 | 8 | 168 |
| MTL | 2 | 4 | 2 | 6, 24 | 3 | 21 |
| OXD | 17 | 62 | 2 | 6, 24 | 19 | 1405 |

| stresscat | pert_iname | n_cell_types | cell_types | n_time_points | time_points | n_doses | dosing_conc | mber_of_profi | id | description | direction | PMID | Linked PMI | NOTES | additional_PMIDS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DNA | azacitidine | 22 | A375, A549, . | 2 | 6, 24 | 9 | 0.04, 0.12, 0. | 181 | BRD-K03406 | DNA methyltr | positive | 32676814 | link | some potentia | 31208284, 29167115, 2885 |
| DNA | benzo(a)pyre | 4 | HA1E, HCC5 | 2 | 6, 24 | 1 | 10 | 8 | BRD-K09668 | pro carcinoge | positive | 32866872 | link | NA | 32609278, 32470682, 3223 |
| DNA | camptothecir | 14 | A375, A549, . | 2 | 6, 24 | 12 | 0.001, 0.01, ( | 129 | BRD-A30437 | topoisomeras | positive | 32846134 | link | some potentia | 32753484 |
| DNA | cyclophospha | 8 | A375, A549, | 2 | 6, 24 | 2 | 10, 20 | 29 | BRD-A09722 | alkylating agr | positive | 32717509 | link | some potentia | 32609954, 32534006, 3234 |
| DNA | cytarabine | 12 | A375, A549, | 2 | 6, 24 | 6 | 0.04, 0.12, 0. | 61 | BRD-K33106 | antimetabolite | positive | 32474729 | link | NA | 32460231 |
| DNA | dacarbazine | 7 | A375, HA1E, | 2 | 6, 24 | 6 | 0.04, 0.12, 0. | 46 | NA | NA | positive | 25697728 | link | NA | |
| DNA | daunorubicin | 21 | A375, A549, . | 2 | 6, 24 | 12 | 0.001, 0.01, ( | 171 | BRD-K43389 | RNA synthesi | positive | 32554494 | link | NA | |
| DNA | dimethylnitros | 1 | HA1E | 2 | 6, 24 | 1 | 10 | 3 | NA | NA | positive | 31092975 | link | NA | 27482301, 25410580, 2426 |
| DNA | DMBA | 1 | HA1E | 2 | 6, 24 | 1 | 10 | 3 | NA | NA | positive | 32159784 | link | NA | 31070092, 31037472, 3031 |
| DNA | doxorubicin | 14 | A375, A549, . | 2 | 6, 24 | 12 | 0.001, 0.01, ( | 192 | BRD-K92093 | topoisomeras | positive | 27852227 | link | NA | 32866497, 32855734, 3282 |
| DNA | etoposide | 14 | A375, A549, | 2 | 6, 24 | 9 | 0.04, 0.12, 0. | 100 | BRD-K37798 | topoisomeras | positive | 32866497 | link | some potentia | 32846134, 32786121, 3252 |
| DNA | gemcitabine | 53 | A375, A549, . | 2 | 6, 24 | 12 | 0.001, 0.01, ( | 195 | BRD-K15108 | cell cycle inhi | positive | 32688248 | link | some potential ER stress | |
| DNA | hydroxyurea | 7 | A375, HA1E, | 1 | 24 | 6 | 0.04, 0.12, 0. | 42 | NA | NA | positive | 32541066 | link | NA | |
| DNA | irinotecan | 15 | A375, A549, . | 2 | 6, 24 | 6 | 0.04, 0.12, 0. | 76 | BRD-K08547 | topoisomeras | positive | 27852227 | link | NA | |
| DNA | melphalan | 1 | HA1E | 2 | 6, 24 | 1 | 10 | 3 | NA | NA | positive | 32717133 | link | NA | |
| DNA | mitomycin-c | 15 | A375, A549, . | 2 | 6, 24 | 6 | 0.04, 0.12, 0. | 76 | BRD-A48237 | DNA alkylatin | positive | 32087850 | link | NA | |
| DNA | mitoxantrone | 32 | A375, A549, . | 4 | 3, 6, 24, 48 | 13 | 0.001, 0.01, ( | 439 | BRD-K21680 | topoisomeras | positive | 27852227 | link | NA | |
| DNA | nocodazole | 15 | A375, A549, . | 2 | 6, 24 | 4 | 0.5, 1, 3, 10 | 40 | BRD-K12539 | tubulin inhibit | positive | 30311985 | link | NA | |

# Questions & Comments?

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