High-Throughput Transcriptomics Platform for Screening Environmental Chemicals

Supplemental Material

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Running title: High-Throughput Transcriptomics

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Table S1. Further information about chemicals tested in this study. The table shows the known molecular targets or mechanisms of each chemical tested in this study and associated references

Table S2. Cell viability assay results. The table provides the mean increase in % of non-viable cells (CV_Mean_rval) as measured by propidium iodide dye exclusion and mean increase in % of apoptotic cells (Apop_Mean_rval) using caspase 3/7 activation for each concentration of each test chemical. The final column indicates the specific concentrations which were masked from further analysis due to cytotoxicity.

Table S3. Sample-level read depth and QC metrics. The treatment information, read depth, and count-based QC metrics for each sample are shown.

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Figure S1. Technical reproducibility of high-throughput transcriptomics on reference RNA mixtures. Pairwise correlations of log2 CPM values were calculated between samples of the same reference RNA treatment type (UHRR and HBRR groups) and between samples of different reference RNA types (HBRR:UHRR group).

Figure S2. Accuracy of reference chemical treatments for multiple analysis parameters. Distributions of absolute ssGSEA signature scores for specific molecular target signatures across each treatment group, based on 4 different combinations of DESeq2 analysis parameters: +/- plate effect and +/- L2FC shrinkage. PE = Plate Effect; GEN = Genistein; SIRO = Sirolimus; TSA = Trichostatin A; NULL = 1000 simulated chemicals derived from the null distribution (see methods).

List of Data Files on FigShare

Additional supplemental data files can be found at DOI: 10.23645/epacomptox.13368914

- hWTv1_mcf7_probes.csv: TempO-Seq human transcriptome version 1 (hWTv1) assay. The identifier, gene name, quality flag, and MCF-7-specific attenuation factor for each probe is shown.
- mcf7_probe_counts.csv: Raw probe counts for each sample in CSV format. Each row corresponds to a sample. The first 6 columns contain sample IDs and treatment information, each subsequent column corresponds to a TempO-seq probe.
- mcf7_gene_l2fc_results.csv: Differential expression data by gene. Each row corresponds to a distinct treatment (chemical x concentration). The first 7 columns contain treatment information, including chemical identifiers and concentration. Subsequent columns correspond to gene symbols, each data value is a log2 fold-change estimated using DESeq2, with plate effects included in the model, and shrinkage applied.
- signatureDB_master_catalog_pilot.xlsx: Description of gene sets used in the analysis (Excel Format).
- signatureDB_genelists.RData: Gene Signature Sets (RData Format).
- mcf7_bpac_values.xlsx: The point of departure concentrations for each test chemicals based on ToxCast assays, BMD Express and HTTr signature analysis.

List of Data Files on Gene Expression Omnibus

Raw and processed data is available through Gene Expression Omnibus (GEO) under accession GSE162855

- Raw read data for all samples (FASTQ Format)
- Probe Set Manifest (CSV Format): Supplementary file “GSE162855_hWTv1_mcf7_probes.csv.gz
- Probe counts for all samples (CSV format): Supplementary file “GSE162855_httr_mcf7_pilot_count_data.csv.gz”

Table S1. Further information about chemicals tested in this study. The table shows the known molecular targets or mechanisms of each chemical tested in this study and associated references.

DTXSID [1]	Name	CASRN	Target annotation	Reference for target annotation	% of ToxCast assays Active (# of ToxCast assays)	potential mammalian effect
DTXSID8023216	3,5,3'-Triiodothyronine	6893-02-3	Thyroid hormone receptor agonist	https://www.drugbank.ca/drugs/DB00279	100 / 739 (13.5 %)	alters thyroid pathway activity
DTXSID3022536	4-Cumylphenol	599-64-4	ER agonist	https://pubs.acs.org/doi/pdf/10.1021/acs.est.5b02641	389 / 739 (52.6 %)	alters ER pathway activity
DTXSID3037094	4-Hydroxytamoxifen	68392-35-8	ER antagonist	https://www.drugbank.ca/drugs/DB04468	345 / 756 (45.6 %)	alters ER pathway activity
DTXSID5029055	4-Nonylphenol, branched	84852-15-3	ER agonist	https://pubs.acs.org/doi/pdf/10.1021/acs.est.5b02641	479 / 804 (59.5 %)	alters ER pathway activity
DTXSID7037185	Amiodarone hydrochloride	19774-82-4	Blocks myocardial calcium, potassium and sodium channels	https://www.drugbank.ca/drugs/DB01118	253 / 749 (33.8 %)	Block ion channel activity
DTXSID9020112	Atrazine	1912-24-9	Herbicide, photosystem II inhibitor	https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/43.htm	29 / 866 (3.3 %)	alters mitochondrial activity
DTXSID9020160	Bifenthrin	82657-04-3	Sodium channel modulator	https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/78.htm	73 / 844 (8.6 %)	alters ion channel activity
DTXSID7020182	Bisphenol A	80-05-7	ER agonist	https://pubs.acs.org/doi/pdf/10.1021/acs.est.5b02641	455 / 989 (46.0 %)	alters ER pathway activity
DTXSID4022442	Bisphenol B	77-40-7	ER agonist	https://pubs.acs.org/doi/pdf/10.1021/acs.est.5b02641	340 / 714 (47.6 %)	alters ER pathway activity
DTXSID9034365	Butafenacil	134605-64-4	Herbicide, protoporphyrinogen oxidase (PPO) inhibition	https://sitem.herts.ac.uk/aeru/iupac/Reports/1163.htm	54 / 793 (6.8 %)	unknown
DTXSID8022828	Cladribine	4291-63-8	DNA synthesis inhibitor	https://www.drugbank.ca/drugs/DB00242	85 / 722 (11.8 %)	general cell stress

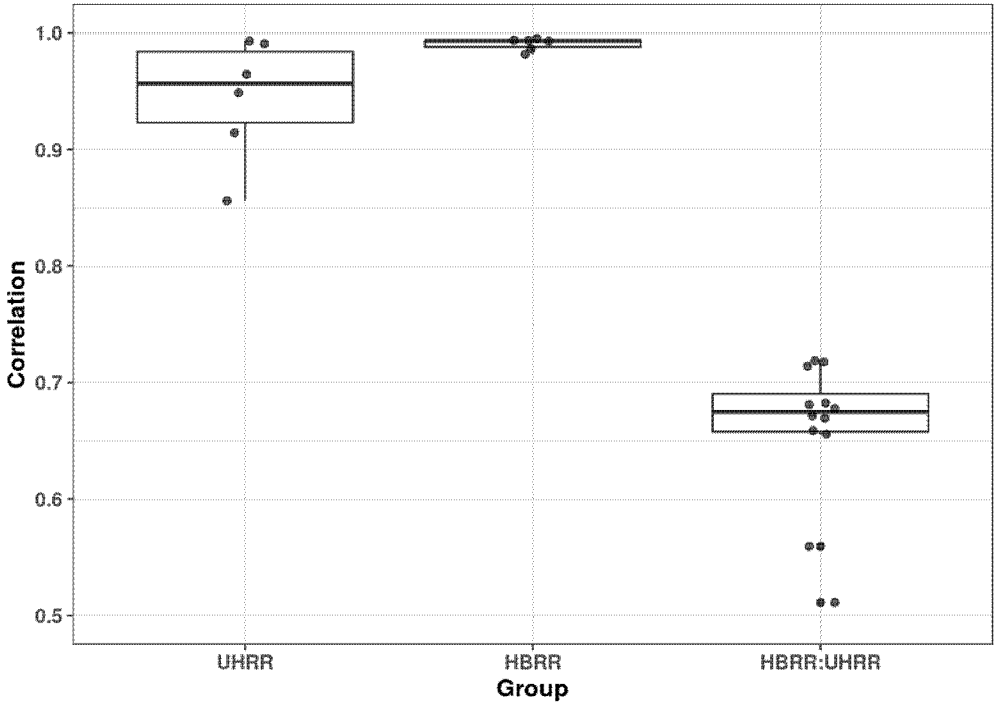
DTXSID3020336	Clofibrate	637-07-0	PPARa agonist, upregulates extrahepatic lipoprotein lipase	https://www.drugbank.ca/drugs/DB00636	17 / 736 (2.3 %)	alters PPAR pathway activity
DTXSID8020337	Clomiphene citrate (1:1)	50-41-9	ER antagonist	https://www.drugbank.ca/drugs/DB00882	343 / 828 (41.4 %)	alters ER pathway activity
DTXSID1023990	Cyanazine	21725-46-2	Herbicide, photosystem II inhibitor	https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/185.htm	25 / 826 (3.0 %)	alters mitochondrial activity
DTXSID6024882	Cycloheximide	66-81-9	Protein synthesis inhibitor	https://en.wikipedia.org/wiki/Cycloheximide	337 / 703 (47.9 %)	general cell stress
DTXSID1023998	Cypermethrin	52315-07-8	Sodium channel modulator	https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/197.htm	59 / 872 (6.8 %)	alters ion chanel activity
DTXSID0032601	Cyproconazole	94361-06-5	Ergosterol-biosynthesis inhibitor. Pan-cyp inhibitor	https://www.ncbi.nlm.nih.gov/pubmed/17178690	47 / 862 (5.4 %)	alters CYP activity
DTXSID5020366	Cyproterone acetate	427-51-0	AR antagonist	https://www.drugbank.ca/drugs/DB04839	298 / 720 (41.4 %)	alters AR pathway activity
DTXSID1047310	Farglitazar	196808-45-4	PPARg agonist	https://adisinsight.springer.com/drugs/800008802	222 / 750 (29.6 %)	alters PPAR pathway activity
DTXSID2029874	Fenofibrate	49562-28-9	PPARa agonist, upregulates extrahepatic lipoprotein lipase	https://www.drugbank.ca/drugs/DB01039	139 / 678 (20.5 %)	alters PPAR pathway activity
DTXSID2032550	Fenpyroximate (Z,E)	111812-58-9	Mitochondrial electron transport inhibitor	https://sitem.herts.ac.uk/aeru/iupac/Reports/309.htm	153 / 812 (18.8 %)	alters mitochondrial activity
DTXSID7032004	Flutamide	13311-84-7	AR antagonist	https://pubs.acs.org/doi/abs/10.1021/acs.chemrestox.6b00347	176 / 712 (24.7 %)	alters AR pathway activity
DTXSID7024112	Fomesafen	72178-02-0	Herbicide, protoporphyrinogen oxidase (PPO) inhibition	https://sitem.herts.ac.uk/aeru/iupac/Reports/355.htm	14 / 676 (2.1 %)	unknown

DTXSID4022369	Fulvestrant	129453-61-8	ER antagonist	https://www.drugbank.ca/drugs/DB00947	186 / 730 (25.5 %)	alters ER pathway activity
DTXSID8024151	Imazalil	35554-44-0	Ergosterol-biosynthesis inhibitor. Pan-cyp inhibitor	https://www.ncbi.nlm.nih.gov/pubmed/17178690	252 / 920 (27.4 %)	alters CYP activity
DTXSID7024160	Lactofen	77501-63-4	Herbicide, protoporphyrinogen oxidase (PPO) inhibition	https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/1160.htm	97 / 858 (11.3 %)	unknown
DTXSID5020784	Lovastatin	75330-75-5	HMGCR inhibitor	https://www.drugbank.ca/drugs/DB00227	270 / 750 (36 %)	alters cholesterol biosynthesis
DTXSID9020794	Maneb	12427-38-2	Inhibition of metal-dependent and sulfhydryl enzyme systems	https://archive.epa.gov/scipoly/sap/meetings/web/pdf/dithiofinal_aug17.pdf	57 / 1019 (5.6 %)	general cell stress
DTXSID3034165	Nilutamide	63612-50-0	AR antagonist	https://www.drugbank.ca/drugs/DB00665	73 / 684 (10.6 %)	alters AR pathway activity
DTXSID8031865	PFOA	335-67-1	PPARg, PPARa agonist	https://www.ncbi.nlm.nih.gov/pubmed/17047030	32 / 918 (3.5 %)	alters PPAR pathway activity
DTXSID3031864	PFOS	1763-23-1	PPARg, PPARa agonist	https://www.ncbi.nlm.nih.gov/pubmed/17047030	326 / 1007 (32.3 %)	alters PPAR pathway activity
DTXSID4024270	Prochloraz	67747-09-5	Ergosterol-biosynthesis inhibitor. Pan-cyp inhibitor	https://www.ncbi.nlm.nih.gov/pubmed/17178690	186 / 942 (19.7 %)	alters CYP activity
DTXSID8024280	Propiconazole	60207-90-1	Ergosterol-biosynthesis inhibitor. Pan-cyp inhibitor	https://www.ncbi.nlm.nih.gov/pubmed/17178690	141 / 883 (16.0 %)	alters CYP activity
DTXSID7032638	Pyraclostrobin	175013-18-0	Mitochondria (complex III inhibitor)	PMid:17474024	178 / 856 (20.8 %)	alters mitochondrial activity
DTXSID7021237	Reserpine	50-55-5	inhibition of the ATP/Mg2+ pump	https://www.drugbank.ca/drugs/DB00206	258 / 700 (36.9 %)	altera adrenergic signaling

DTXSID6021248	Rotenone	83-79-4	Mitochondria (complex I inhibitor)	https://en.wikipedia.org/wiki/Rotenone	361 / 865 (41.7 %)	alters mitochondrial activity
DTXSID4021268	Simazine	122-34-9	Herbicide, photosystem II inhibitor	https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/592.htm	22 / 847 (2.6 %)	alters mitochondrial activity
DTXSID0023581	Simvastatin	79902-63-9	HMGCR inhibitor	https://www.drugbank.ca/drugs/DB00641	198 / 728 (27.2 %)	alters cholesterol biosynthesis
DTXSID5048186	Tetrac	67-30-1	T4 synthesis inhibitor	https://www.ncbi.nlm.nih.gov/pubmed/26307023	188 / 798 (23.6 %)	alters thyroid pathway activity
DTXSID5021332	Thiram	137-26-8	Inhibition of metal-dependent and sulfhydryl enzyme systems	https://archive.epa.gov/scipoly/sap/meetings/web/pdf/dithiofinal_aug17.pdf	415 / 875 (47.4 %)	general cell stress
DTXSID4032580	Trifloxystrobin	141517-21-7	Mitochondria (complex III inhibitor)	PMid:17474024	155 / 822 (18.8 %)	alters mitochondrial activity
DTXSID8023719	Troglitazone	97322-87-7	PPARg, PPARa agonist	https://www.drugbank.ca/drugs/DB00197	237 / 713 (33.2 %)	alters PPAR pathway activity
DTXSID4022361	Vinclozolin	50471-44-8	AR antagonist	https://pubs.acs.org/doi/abs/10.1021/acs.chemrestox.6b00347	75 / 861 (8.7 %)	alters AR pathway activity
DTXSID0021464	Ziram	137-30-4	Inhibition of metal-dependent and sulfhydryl enzyme systems	https://archive.epa.gov/scipoly/sap/meetings/web/pdf/dithiofinal_aug17.pdf	402 / 690 (58.3 %)	general cell stress
DTXSID5022308	Genistein *	446-72-0	ER agonist	https://www.drugbank.ca/drugs/DB01645	334 / 732 (45.6 %)	alters ER pathway activation
DTXSID5023582	Sirolimus *	53123-88-9	mTOR inhibitor	https://en.wikipedia.org/wiki/Sirolimus	69 / 97 (71.1 %)	alters mTOR activity
DTXSID6037063	Trichostatin A *	58880-19-6	Class I & II HDAC inhibitor	https://en.wikipedia.org/wiki/Trichostatin_A	NA	alters HDAC activity

[1] DTXSID is the DSSTox Generic Substance ID which provides a unique identifier for the substance and references the EPA CompTox Chemicals Dashboard: https://comptox.epa.gov/dashboard/ . ToxCast activity information accessed via this dashboard on 06-05-2020. (*) Used as transcriptomic reference treatment.

Supplemental Figure 1 (Related to Figure 3)



Supplemental Figure 2 (Related to Figure 4)