Transcriptomic and metabolomic approaches to investigate molecular responses of human exposure to flame retardants

Progress to date

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Stockholm, Sweden 15-11-2013

Outline

1) Previous results of omics study of cells molecular response to HBCD – manuscript in preparation

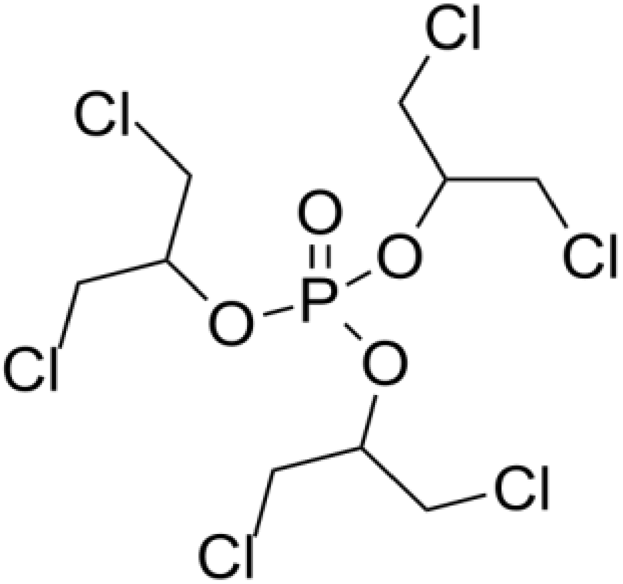
2) Recent progress: omics study of cells molecular response to TDCPP

3) Next 5 months plan

Summary of HBCD study

Cell type	Methodology	HBCD (0-2μM or 0-4μM)		Positive control	
A549	Transcriptomics	24h; n=6 $_{\text{rep.}}$ *5 $_{\text{group}}$ ✗			confirmed by Real time PCR
	Metabolomics (MS & NMR _polar)	24h; n=8$_{\text{rep.}}$ *5$_{\text{group}}$ ✗			
HepG2/C3A	Metabolomic s (MS_polar)	24h; n=6$_{\text{rep.}}$ *2 $_{\text{group}}$ ✗	72h; n=6 ≈		
	Transcriptomics	24h; n=6$_{\text{rep.}}$ *2 $_{\text{group}}$ ✗		10μM DMBA 24h; n=6 ✓	confirmed by Real time PCR
A549 **HepG2/C3A**		HBCD levels has been measured after exposure experiments . (~100,000ng/g cell pellets(wet weight))			
Conclusion		Two cell lines have few molecular response after exposure to HBCD (at up to 4μM) in this study.			

TDCPP



Chemical Structure of Tris (1,3-dichloro-2-propyl) phosphate (TDCPP)

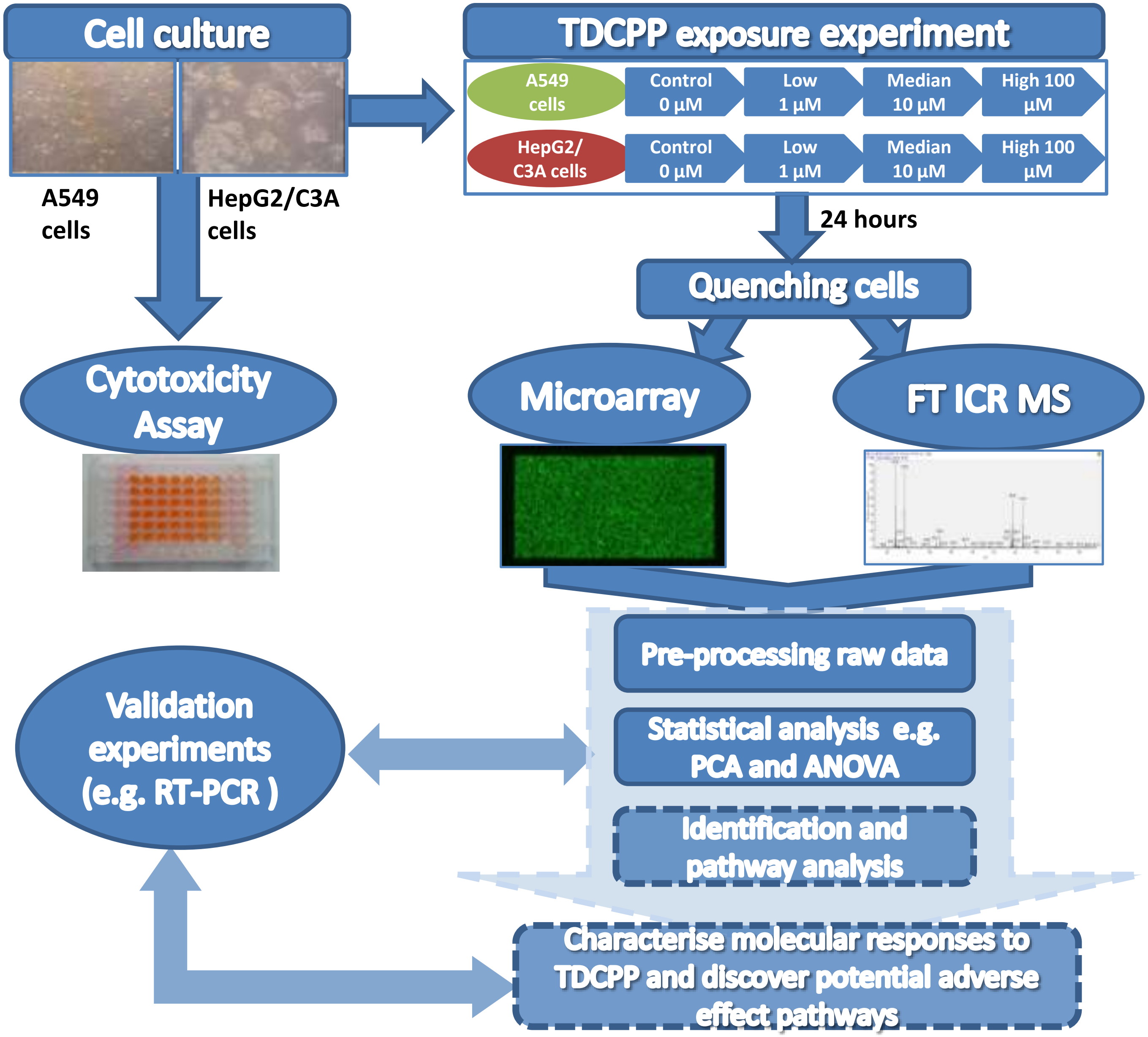
- TDCPP is mainly used as **additive flame retardants** adding into polyurethane foam.

- TDCPP has been detected in different samples including **indoor air** and **house dust** (van der Veen and de Boer 2012, Brommer et al., 2012, Allen et al., 2013) which may be an important source of human exposure to TDCPP (Meeker et al., 2013).

- **Limited studies** suggest that TDCPP may be **carcinogenic, neurotoxic**, **endocrine-disruptive** and/or **reproductive toxicants** (Andresen et al., 2004; Dishaw et al. 2011; Liu et al. 2012; Liu et al. 2013; van der Veen and de Boer 2012).

- **More research** is necessary to determine potential health risks and understand mechanisms of potential adverse effects resulting from exposure to TDCPP.

Experimental Design (1)



Cell culture

A549 cells

HepG2/C3A cells

TDCPP exposure experiment

A549 cells	Control 0 µM	Low 1 µM	Median 10 µM	High 100 µM
HepG2/ C3A cells	Control 0 µM	Low 1 µM	Median 10 µM	High 100 µM

24 hours

Quenching cells

Cytotoxicity Assay

Microarray

FT ICR MS

Pre-processing raw data

Statistical analysis e.g. PCA and ANOVA

Identification and pathway analysis

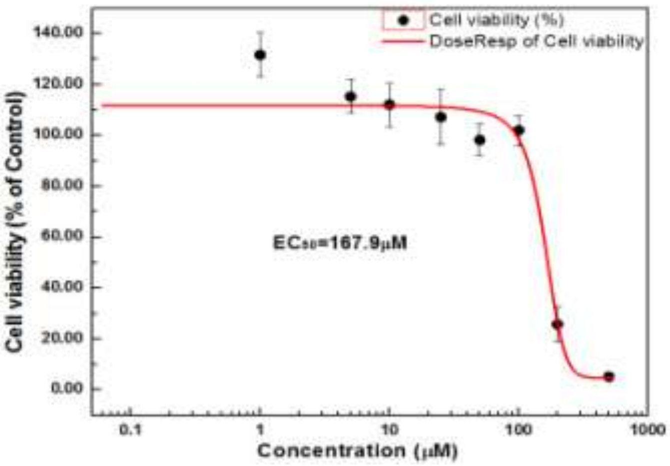
Validation experiments (e.g. RT-PCR)

Characterise molecular responses to TDCPP and discover potential adverse effect pathways

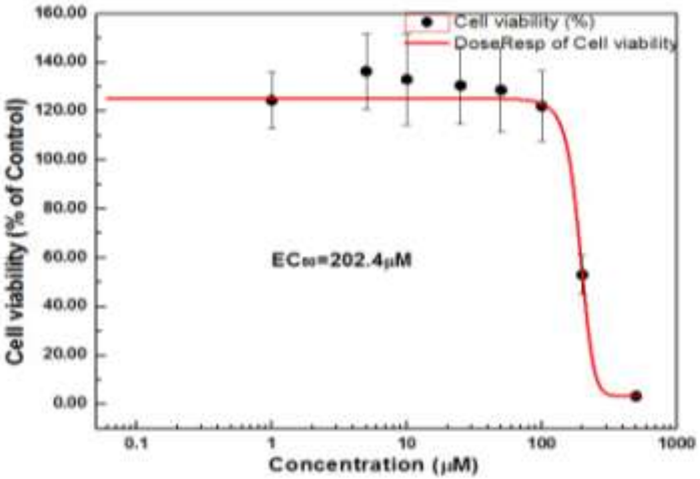
Experimental Design (2)

Group **(Media type/exposure time/Cell lines)**		**High Dose (100 µM)**	**Medium Dose (10 µM)**	**Low Dose (1 µM)**	**Solvent Control**
A	+ FBS; 24h; HepG2/C3A	8 + 4 replicates	8 + 4	8	8 + 4
B	**-FBS**; 24h; HepG2/C3A	8	8	8	8
C	+ FBS; **72h**; HepG2/C3A	8	8	8	8
D	+ FBS; 24h; **A549**	8+ 4	8 + 4	8	8 + 4

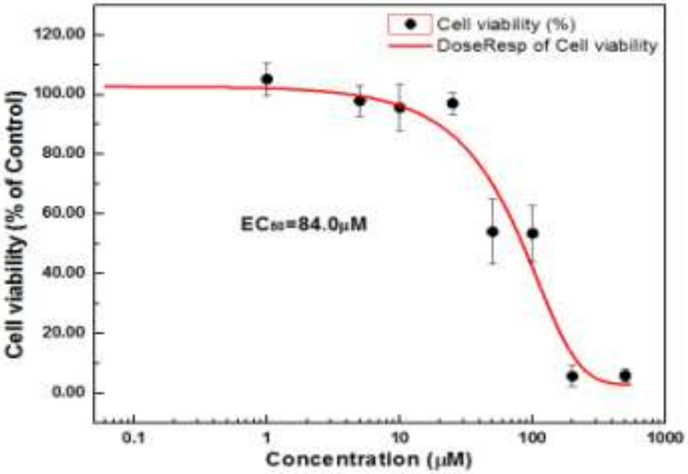
Cytotoxicity Screening (CCK-8 Assay)



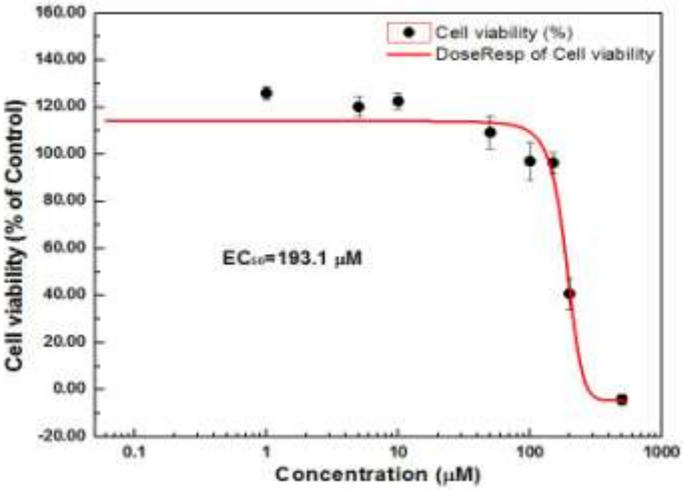
HepG2/C3A+FBS_24h



HepG2/C3A-FBS_24h

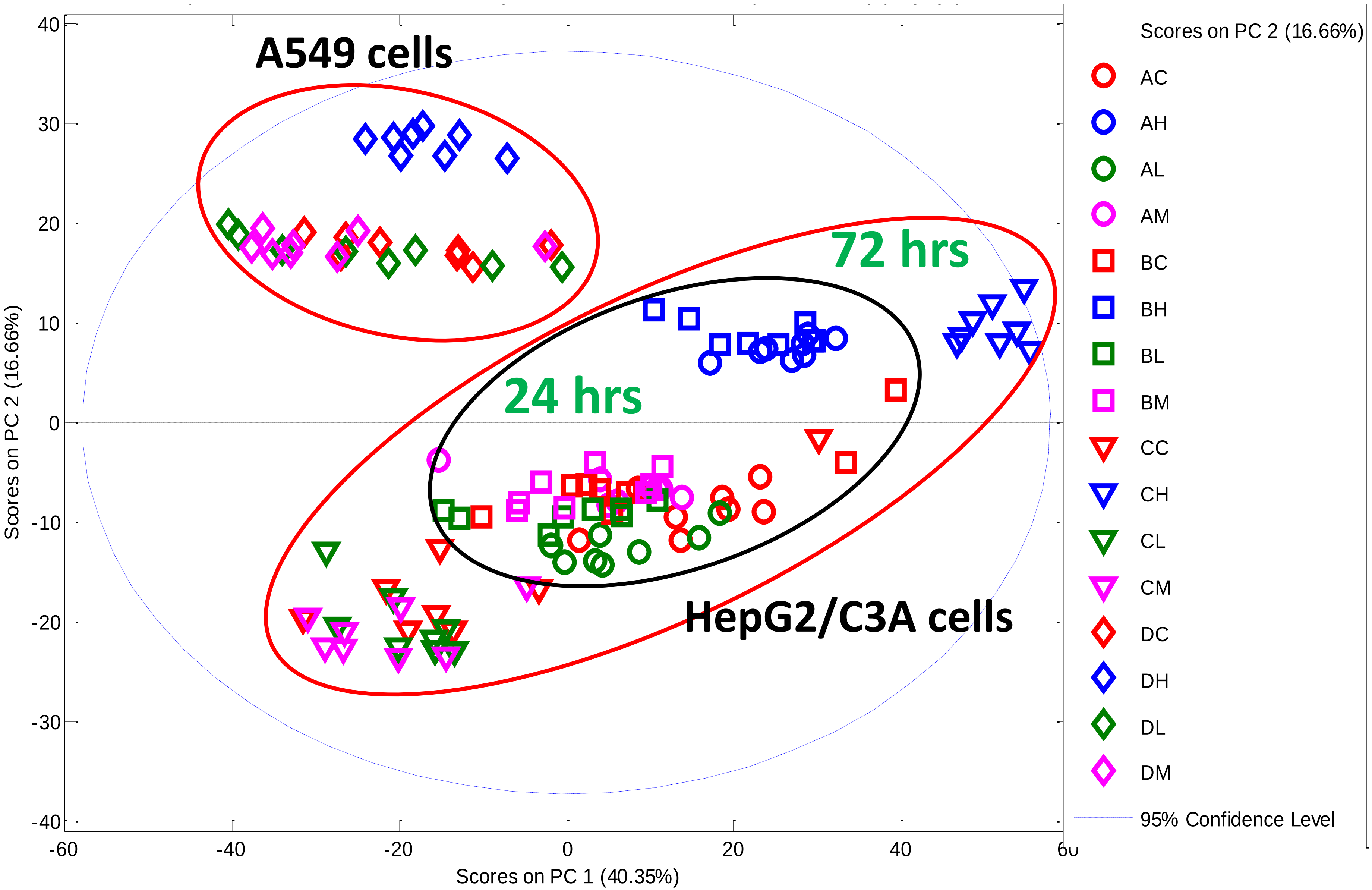


HepG2/C3A+FBS_72h

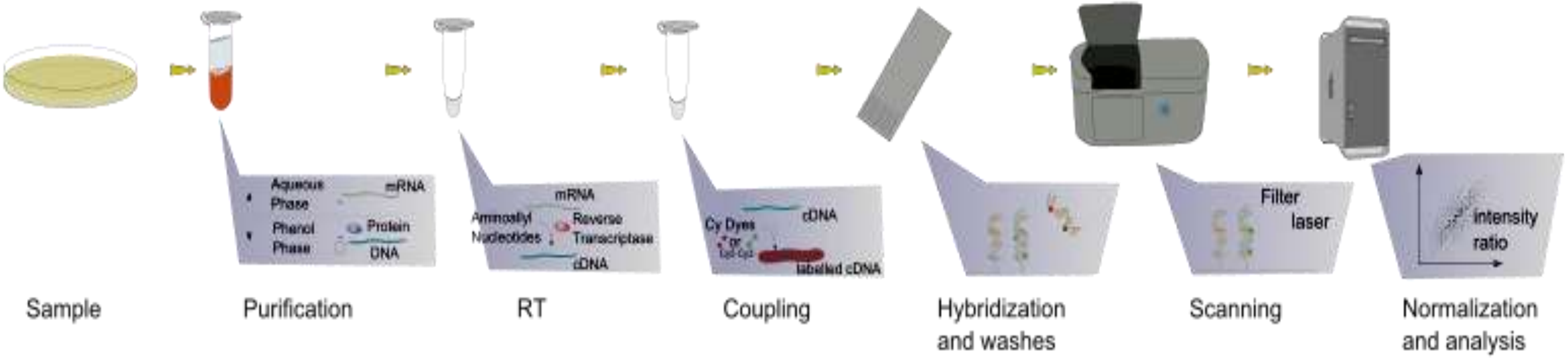


A549+FBS_24h

Principal component analysis (PCA) of positive MS peaks dataset

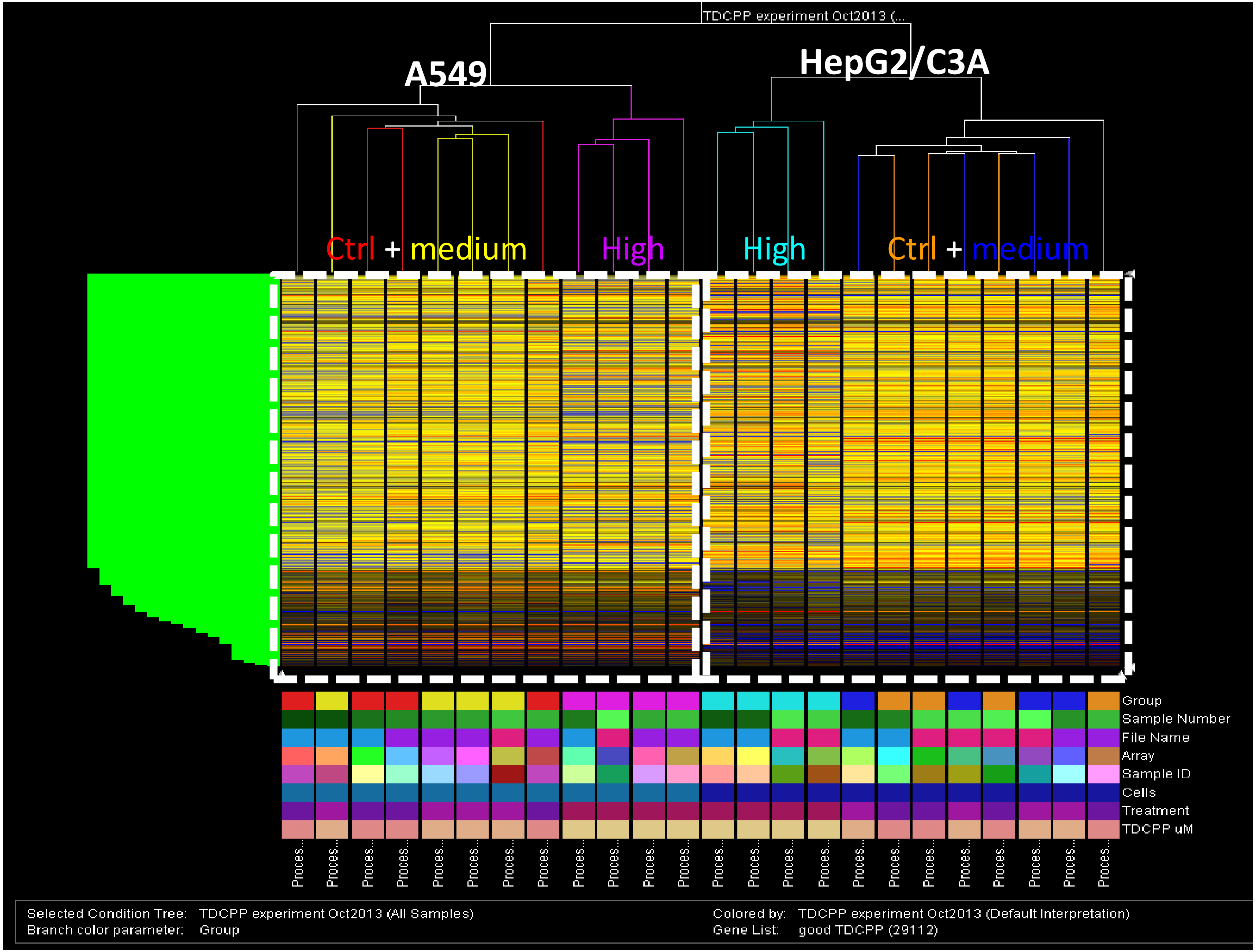


Microarray



Agilent SurePrint G3 Human Gene Expression 8x60K v2

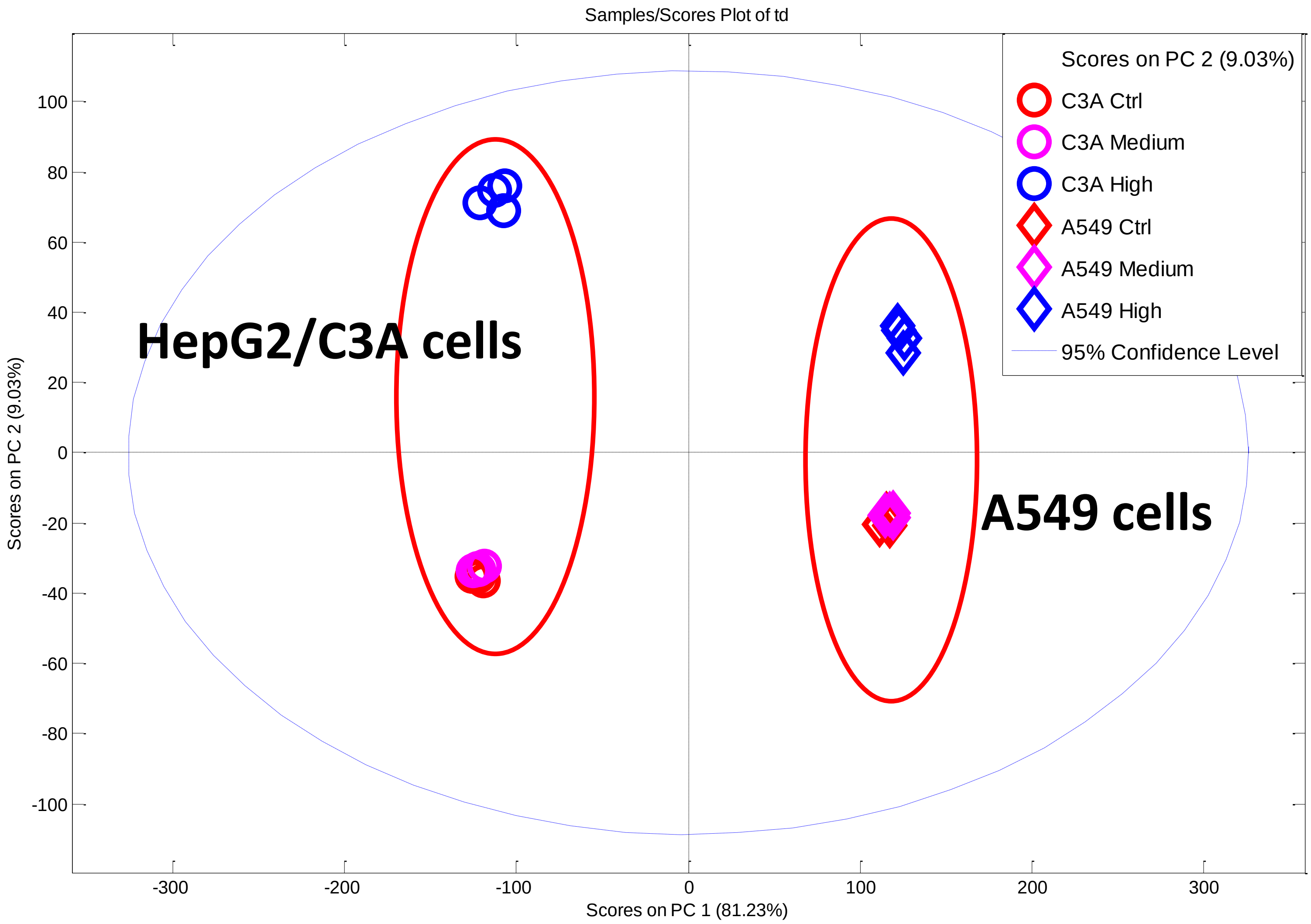
Cluster Analysis of all microarray data



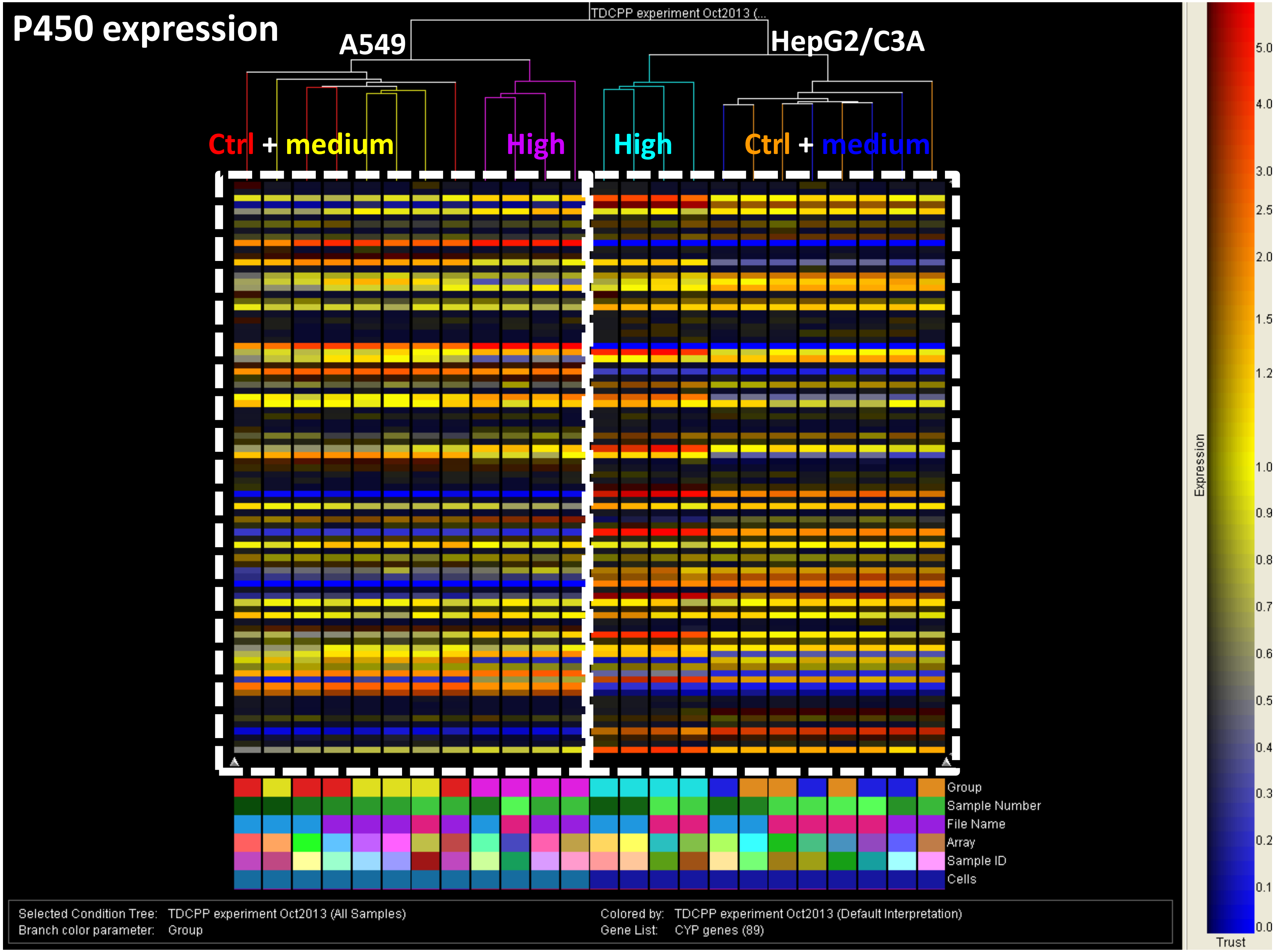
Selected Condition Tree: TDCPP experiment Oct2013 (All Samples)
Branch color parameter: Group

Colored by: TDCPP experiment Oct2013 (Default Interpretation)
Gene List: good TDCPP (29112)

PCA of all normalised microarray dataset (1)



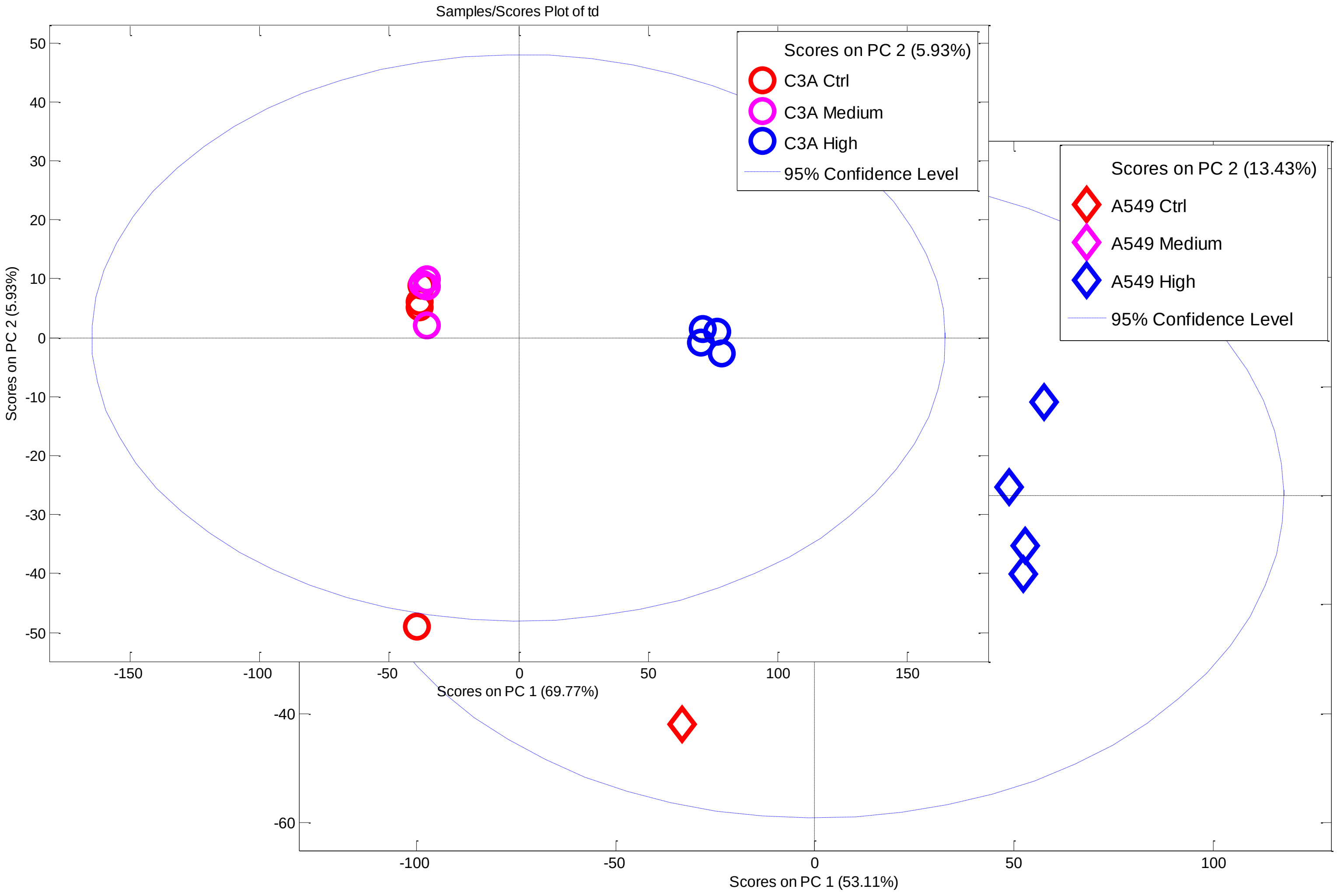
Cluster Analysis of cytochrome P450 genes expression



Selected Condition Tree: TDCPP experiment Oct2013 (All Samples)
Branch color parameter: Group

Colored by: TDCPP experiment Oct2013 (Default Interpretation)
Gene List: CYP genes (89)

PCA of normalised microarray dataset (2)



Significance Analysis for Microarray (SAM) (in MeV)

http://www.tm4.org/mev.html

pick out significant genes based on differential expression between sets of samples. (Chu et al. 2002)

SAM	Significant genes	False Discovery Rate (FDR) %	Delta value
A549	4319	< 0.5	0.321
	2462	< 0.1	0.434
C3A	5551	< 0.5	0.156
	3462	< 0.1	0.208

Statistical analysis

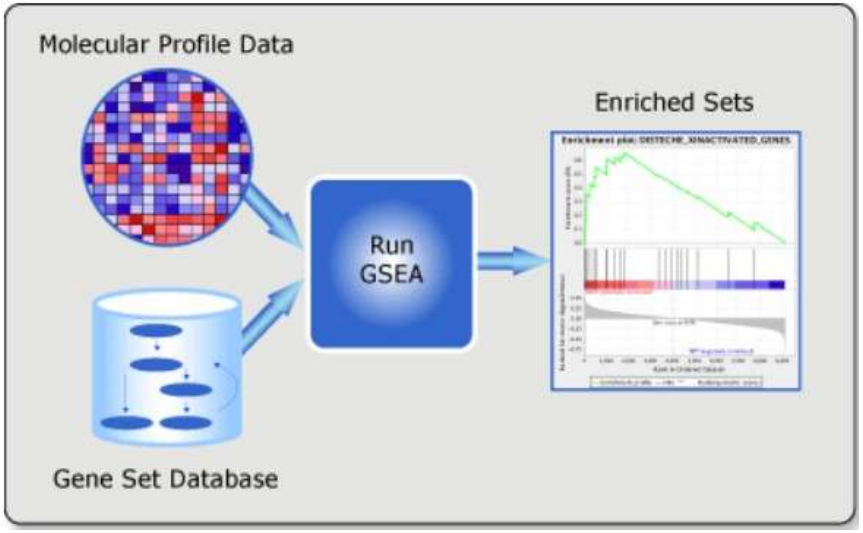
One way ANOVA (following adjusted Bonferroni correction)	significant genes	
A549	445	
C3A	3472	

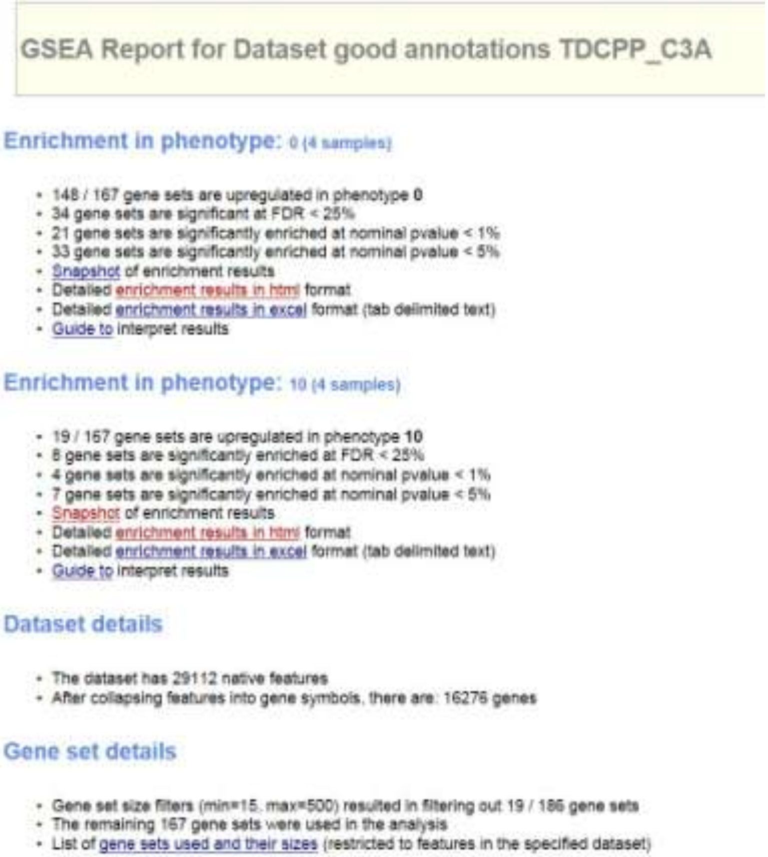
t- test	Significant genes		
	Ctrl vs. Medium	Ctrl vs. High	Medium vs. High
A549	0	67	132
C3A	0	922	871

Gene set enrichment analysis (GSEA)

http://www.broadinstitute.org/gsea/index.jsp

is a computational method that determines whether an *a priori* defined set of genes shows statistically significant, concordant differences between two biological states (e.g. phenotypes).(Jiang and Gentleman 2007)





GSEA Report for Dataset good annotations TDCPP_C3A

Enrichment in phenotype: 0 (4 samples)

- 148 / 167 gene sets are upregulated in phenotype **0**
- 34 gene sets are significant at FDR < 25%
- 21 gene sets are significantly enriched at nominal pvalue < 1%
- 33 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

Enrichment in phenotype: 10 (4 samples)

- 19 / 167 gene sets are upregulated in phenotype **10**
- 8 gene sets are significantly enriched at FDR < 25%
- 4 gene sets are significantly enriched at nominal pvalue < 1%
- 7 gene sets are significantly enriched at nominal pvalue < 5%
- Snapshot of enrichment results
- Detailed enrichment results in html format
- Detailed enrichment results in excel format (tab delimited text)
- Guide to interpret results

Dataset details

- The dataset has 29112 native features
- After collapsing features into gene symbols, there are: 16276 genes

Gene set details

- Gene set size filters (min=15, max=500) resulted in filtering out 19 / 186 gene sets
- The remaining 167 gene sets were used in the analysis
- List of gene sets used and their sizes (restricted to features in the specified dataset)

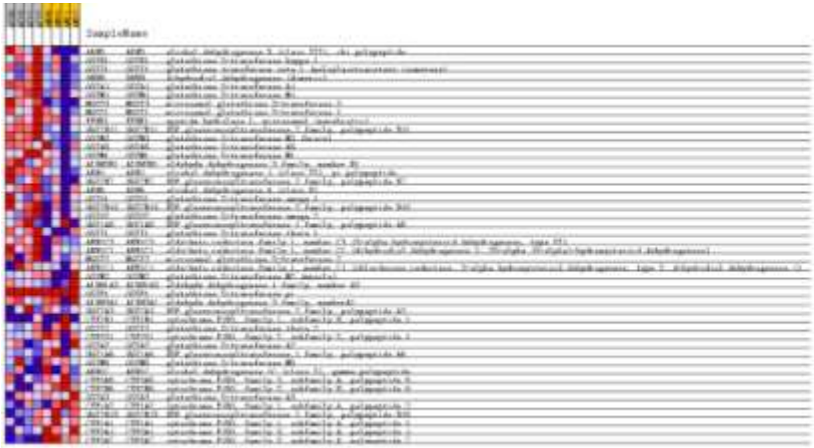


Fig 3: KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450
Blue-Pink O' Gram in the Space of the Analyzed GeneSet

Number of gene sets significantly enriched in A549 and HepG2/C3A cells

GSEA_A549_TDCPP_Kegg_2k permutation

	gene sets are up regulated in first phenotype	gene sets are significant at FDR < 25%	gene sets are significantly enriched at nominal p value < 1%	gene sets are significantly enriched at nominal p value < 5%	gene sets are up regulated in second phenotype	gene sets are significant at FDR < 25%	gene sets are significantly enriched at nominal p value < 1%	gene sets are significantly enriched at nominal p value < 5%
Ctrl vs. Medium(10 uM)	**133 / 167**	**21**	**18**	**22**	**34 / 167**	**4**	**3**	**4**
Ctrl vs. High(100uM)	**97 / 167**	**31**	**18**	**29**	**70 / 167**	**0**	**0**	**4**
High vs. Medium	**77 / 167**	**2**	**2**	**6**	**90 / 167**	**36**	**17**	**31**

GSEA_C3A_TDCPP_Kegg_2k permutation

	gene sets are up regulated in first phenotype	gene sets are significant at FDR < 25%	gene sets are significantly enriched at nominal p value < 1%	gene sets are significantly enriched at nominal p value < 5%	gene sets are up regulated in second phenotype	gene sets are significant at FDR < 25%	gene sets are significantly enriched at nominal p value < 1%	gene sets are significantly enriched at nominal p value < 5%
Ctrl vs. Medium(10uM)	**148 / 167**	**34**	**21**	**33**	**19 / 167**	**8**	**4**	**7**
Ctrl vs. High(100uM)	**100 / 167**	**55**	**27**	**43**	**67/ 167**	**13**	**6**	**14**
High vs. Medium	**75 / 167**	**9**	**7**	**12**	**92 / 167**	**55**	**24**	**37**

C3A cells_Medium vs. Ctrl

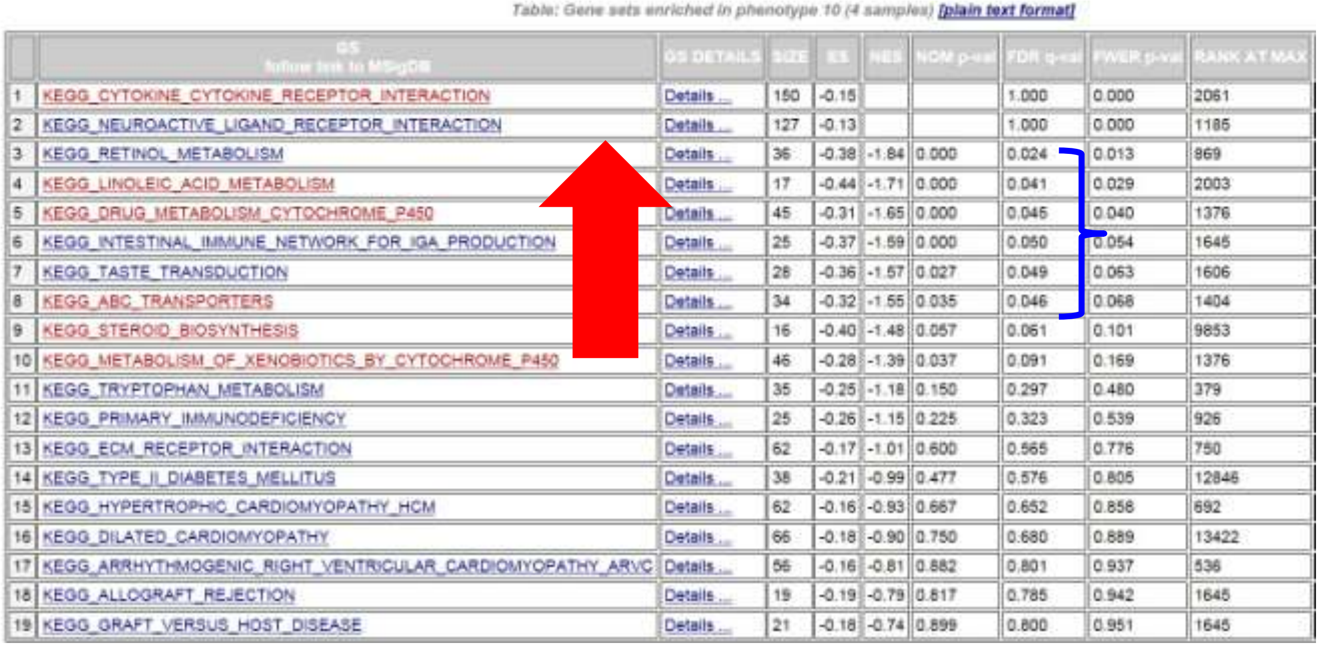
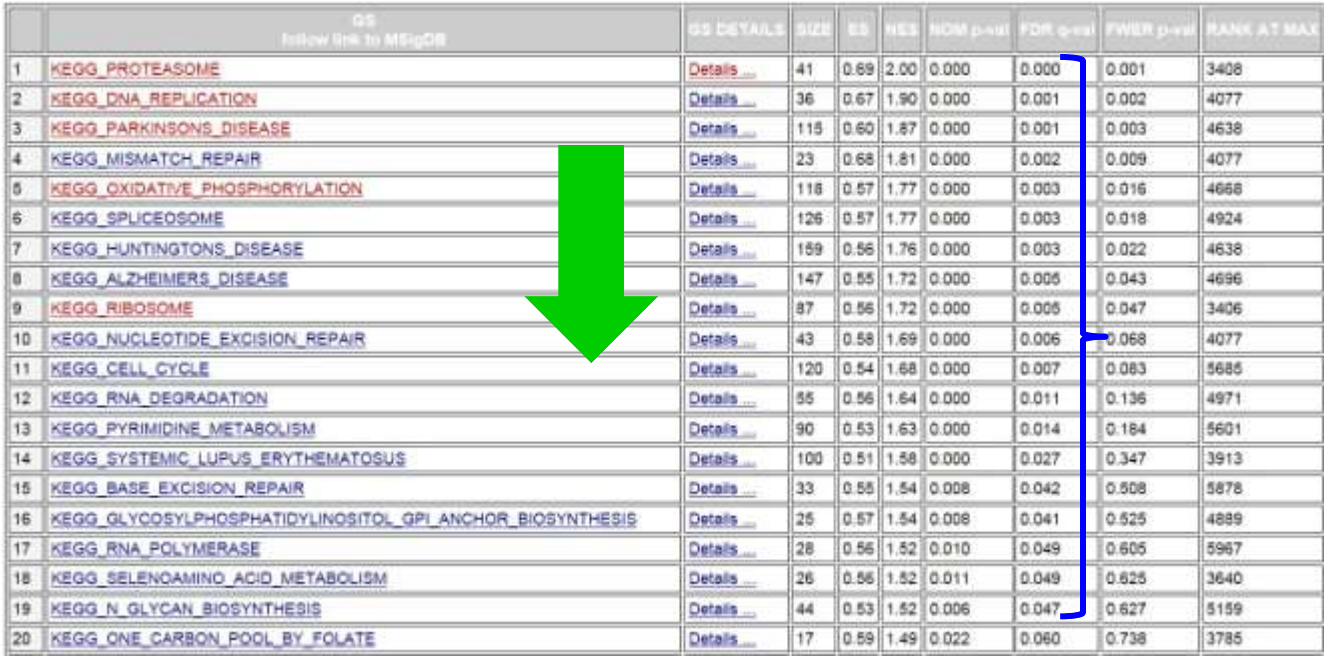


Table: Gene sets enriched in phenotype 10 (4 samples) [plain text format]

	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	Details ...	150	-0.15			1.000	0.000	2061
2	KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	Details ...	127	-0.13			1.000	0.000	1185
3	KEGG_RETINOL_METABOLISM	Details ...	36	-0.38	-1.84	0.000	0.024	0.013	869
4	KEGG_LINOLEIC_ACID_METABOLISM	Details ...	17	-0.44	-1.71	0.000	0.041	0.029	2003
5	KEGG_DRUG_METABOLISM_CYTOCHROME_P450	Details ...	45	-0.31	-1.65	0.000	0.045	0.040	1376
6	KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	Details ...	25	-0.37	-1.59	0.000	0.050	0.054	1645
7	KEGG_TASTE_TRANSDUCTION	Details ...	28	-0.36	-1.57	0.027	0.049	0.063	1606
8	KEGG_ABC_TRANSPORTERS	Details ...	34	-0.32	-1.55	0.035	0.046	0.068	1404
9	KEGG_STEROID_BIOSYNTHESIS	Details ...	16	-0.40	-1.48	0.057	0.061	0.101	9853
10	KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	Details ...	46	-0.28	-1.39	0.037	0.091	0.169	1376
11	KEGG_TRYPTOPHAN_METABOLISM	Details ...	35	-0.25	-1.18	0.150	0.297	0.480	379
12	KEGG_PRIMARY_IMMUNODEFICIENCY	Details ...	25	-0.26	-1.15	0.225	0.323	0.539	926
13	KEGG_ECM_RECEPTOR_INTERACTION	Details ...	62	-0.17	-1.01	0.600	0.565	0.776	750
14	KEGG_TYPE_II_DIABETES_MELLITUS	Details ...	38	-0.21	-0.99	0.477	0.576	0.805	12846
15	KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	Details ...	62	-0.16	-0.93	0.667	0.652	0.858	692
16	KEGG_DILATED_CARDIOMYOPATHY	Details ...	66	-0.18	-0.90	0.750	0.680	0.889	13422
17	KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	Details ...	56	-0.16	-0.81	0.882	0.801	0.937	536
18	KEGG_ALLOGRAFT_REJECTION	Details ...	19	-0.19	-0.79	0.817	0.785	0.942	1645
19	KEGG_GRAFT_VERSUS_HOST_DISEASE	Details ...	21	-0.18	-0.74	0.899	0.800	0.951	1645

Xenobiotic metabolism by CYPs



	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	KEGG_PROTEASOME	Details ...	41	0.69	2.00	0.000	0.000	0.001	3408
2	KEGG_DNA_REPLICATION	Details ...	36	0.67	1.90	0.000	0.001	0.002	4077
3	KEGG_PARKINSONS_DISEASE	Details ...	115	0.60	1.87	0.000	0.001	0.003	4638
4	KEGG_MISMATCH_REPAIR	Details ...	23	0.68	1.81	0.000	0.002	0.009	4077
5	KEGG_OXIDATIVE_PHOSPHORYLATION	Details ...	118	0.57	1.77	0.000	0.003	0.016	4668
6	KEGG_SPLICEOSOME	Details ...	126	0.57	1.77	0.000	0.003	0.018	4924
7	KEGG_HUNTINGTONS_DISEASE	Details ...	159	0.56	1.76	0.000	0.003	0.022	4638
8	KEGG_ALZHEIMERS_DISEASE	Details ...	147	0.55	1.72	0.000	0.005	0.043	4696
9	KEGG_RIBOSOME	Details ...	87	0.56	1.72	0.000	0.005	0.047	3406
10	KEGG_NUCLEOTIDE_EXCISION_REPAIR	Details ...	43	0.58	1.69	0.000	0.006	0.068	4077
11	KEGG_CELL_CYCLE	Details ...	120	0.54	1.68	0.000	0.007	0.083	5685
12	KEGG_RNA_DEGRADATION	Details ...	55	0.56	1.64	0.000	0.011	0.136	4971
13	KEGG_PYRIMIDINE_METABOLISM	Details ...	90	0.53	1.63	0.000	0.014	0.184	5601
14	KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	Details ...	100	0.51	1.58	0.000	0.027	0.347	3913
15	KEGG_BASE_EXCISION_REPAIR	Details ...	33	0.55	1.54	0.008	0.042	0.508	5878
16	KEGG_GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR_BIOSYNTHESIS	Details ...	25	0.57	1.54	0.008	0.041	0.525	4889
17	KEGG_RNA_POLYMERASE	Details ...	28	0.56	1.52	0.010	0.049	0.605	5967
18	KEGG_SELENOAMINO_ACID_METABOLISM	Details ...	26	0.56	1.52	0.011	0.049	0.625	3640
19	KEGG_N_GLYCAN_BIOSYNTHESIS	Details ...	44	0.53	1.52	0.006	0.047	0.627	5159
20	KEGG_ONE_CARBON_POOL_BY_FOLATE	Details ...	17	0.59	1.49	0.022	0.060	0.738	3785

Oxidative phosphorylation; transcription

C3A cells_High vs. Ctrl

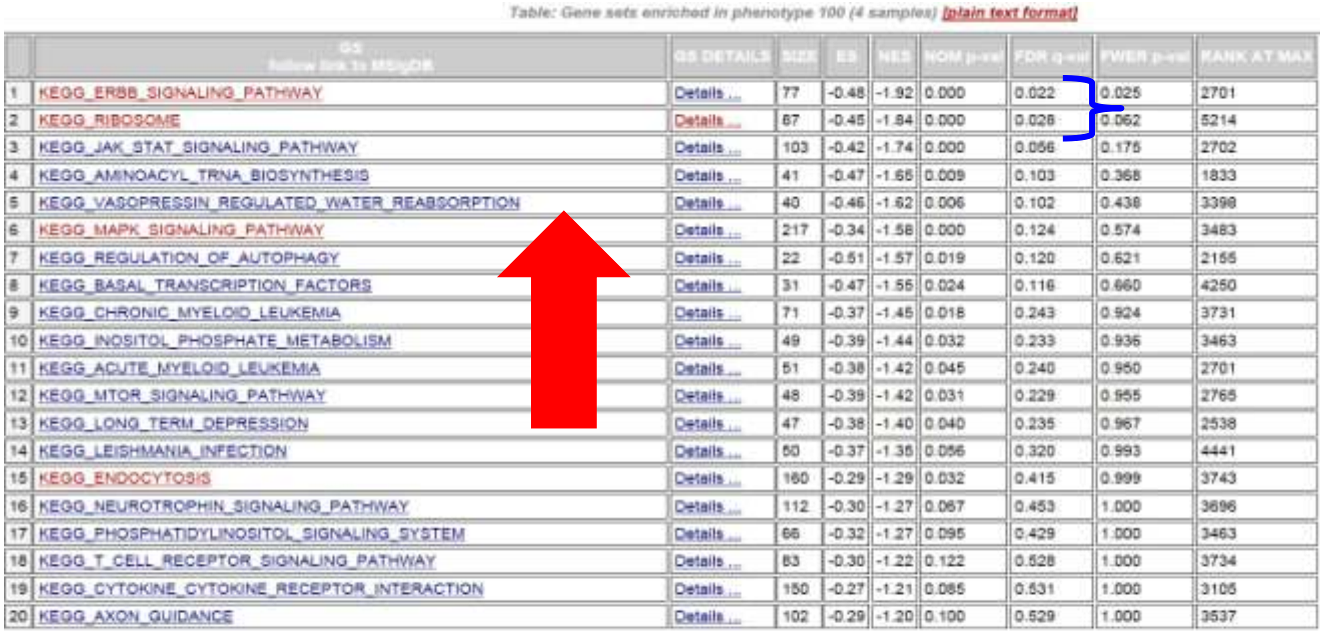
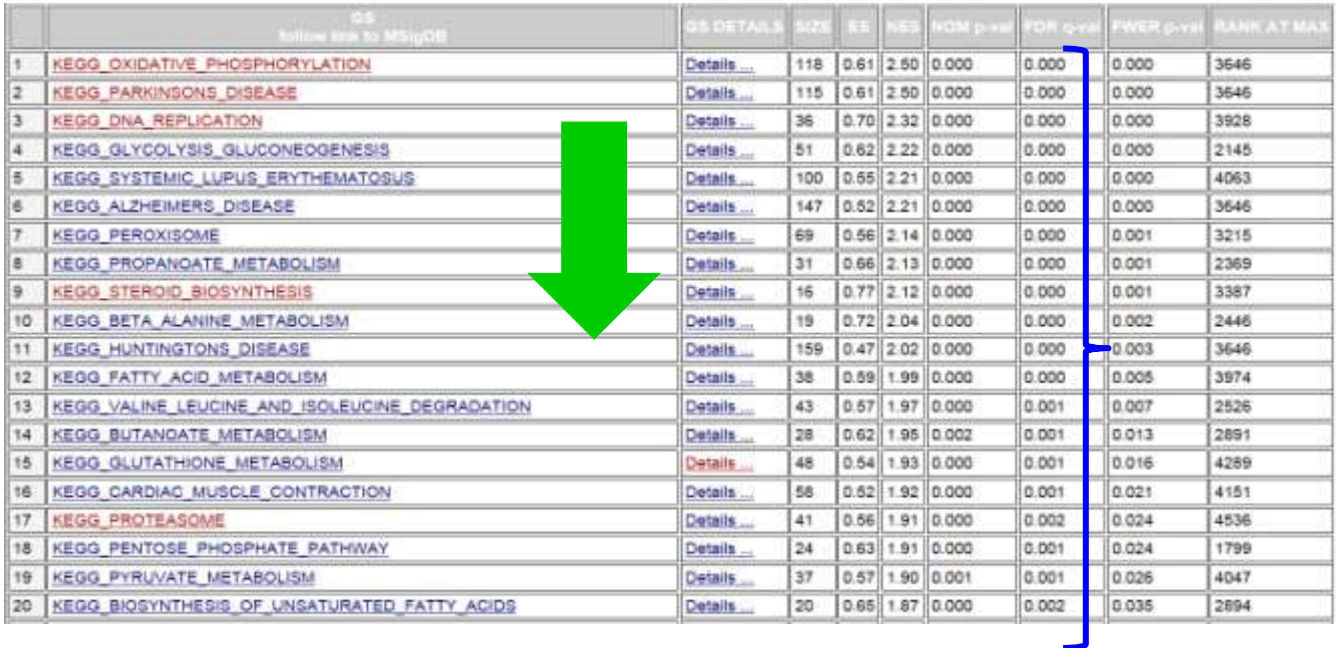


Table: Gene sets enriched in phenotype 100 (4 samples) [plain text format]

	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	KEGG_ERBB_SIGNALING_PATHWAY	Details ...	77	-0.48	-1.92	0.000	0.022	0.025	2701
2	KEGG_RIBOSOME	Details ...	87	-0.45	-1.84	0.000	0.028	0.062	5214
3	KEGG_JAK_STAT_SIGNALING_PATHWAY	Details ...	103	-0.42	-1.74	0.000	0.056	0.175	2702
4	KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	Details ...	41	-0.47	-1.65	0.009	0.103	0.368	1833
5	KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION	Details ...	40	-0.46	-1.62	0.006	0.102	0.438	3398
6	KEGG_MAPK_SIGNALING_PATHWAY	Details ...	217	-0.34	-1.58	0.000	0.124	0.574	3483
7	KEGG_REGULATION_OF_AUTOPHAGY	Details ...	22	-0.51	-1.57	0.019	0.120	0.621	2155
8	KEGG_BASAL_TRANSCRIPTION_FACTORS	Details ...	31	-0.47	-1.55	0.024	0.116	0.660	4250
9	KEGG_CHRONIC_MYELOID_LEUKEMIA	Details ...	71	-0.37	-1.45	0.018	0.243	0.924	3731
10	KEGG_INOSITOL_PHOSPHATE_METABOLISM	Details ...	49	-0.39	-1.44	0.032	0.233	0.936	3463
11	KEGG_ACUTE_MYELOID_LEUKEMIA	Details ...	51	-0.38	-1.42	0.045	0.240	0.950	2701
12	KEGG_MTOR_SIGNALING_PATHWAY	Details ...	48	-0.39	-1.42	0.031	0.229	0.955	2765
13	KEGG_LONG_TERM_DEPRESSION	Details ...	47	-0.38	-1.40	0.040	0.235	0.967	2538
14	KEGG_LEISHMANIA_INFECTION	Details ...	50	-0.37	-1.36	0.056	0.320	0.993	4441
15	KEGG_ENDOCYTOSIS	Details ...	160	-0.29	-1.29	0.032	0.415	0.999	3743
16	KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	Details ...	112	-0.30	-1.27	0.067	0.453	1.000	3696
17	KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	Details ...	66	-0.32	-1.27	0.095	0.429	1.000	3463
18	KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	Details ...	83	-0.30	-1.22	0.122	0.528	1.000	3734
19	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	Details ...	150	-0.27	-1.21	0.085	0.531	1.000	3105
20	KEGG_AXON_GUIDANCE	Details ...	102	-0.29	-1.20	0.100	0.529	1.000	3537

Transcription;
ERBB signalling
pathway



	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	KEGG_OXIDATIVE_PHOSPHORYLATION	Details ...	118	0.61	2.50	0.000	0.000	0.000	3646
2	KEGG_PARKINSONS_DISEASE	Details ...	115	0.61	2.50	0.000	0.000	0.000	3646
3	KEGG_DNA_REPLICATION	Details ...	36	0.70	2.32	0.000	0.000	0.000	3928
4	KEGG_GLYCOLYSIS_GLUCONEOGENESIS	Details ...	51	0.62	2.22	0.000	0.000	0.000	2145
5	KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	Details ...	100	0.55	2.21	0.000	0.000	0.000	4063
6	KEGG_ALZHEIMERS_DISEASE	Details ...	147	0.52	2.21	0.000	0.000	0.000	3646
7	KEGG_PEROXISOME	Details ...	69	0.56	2.14	0.000	0.000	0.001	3215
8	KEGG_PROPANOATE_METABOLISM	Details ...	31	0.66	2.13	0.000	0.000	0.001	2369
9	KEGG_STEROID_BIOSYNTHESIS	Details ...	16	0.77	2.12	0.000	0.000	0.001	3387
10	KEGG_BETA_ALANINE_METABOLISM	Details ...	19	0.72	2.04	0.000	0.000	0.002	2446
11	KEGG_HUNTINGTONS_DISEASE	Details ...	159	0.47	2.02	0.000	0.000	0.003	3646
12	KEGG_FATTY_ACID_METABOLISM	Details ...	38	0.59	1.99	0.000	0.000	0.005	3974
13	KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	Details ...	43	0.57	1.97	0.000	0.001	0.007	2526
14	KEGG_BUTANOATE_METABOLISM	Details ...	28	0.62	1.95	0.002	0.001	0.013	2891
15	KEGG_GLUTATHIONE_METABOLISM	Details ...	48	0.54	1.93	0.000	0.001	0.016	4289
16	KEGG_CARDIAC_MUSCLE_CONTRACTION	Details ...	58	0.52	1.92	0.000	0.001	0.021	4151
17	KEGG_PROTEASOME	Details ...	41	0.56	1.91	0.000	0.002	0.024	4536
18	KEGG_PENTOSE_PHOSPHATE_PATHWAY	Details ...	24	0.63	1.91	0.000	0.001	0.024	1799
19	KEGG_PYRUVATE_METABOLISM	Details ...	37	0.57	1.90	0.001	0.001	0.026	4047
20	KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	Details ...	20	0.65	1.87	0.000	0.002	0.035	2894

Oxidative
phosphorylation;
Energy metabolism
related pathway ;
Glutathione
metabolism.

A549 cells_Medium vs. Ctrl

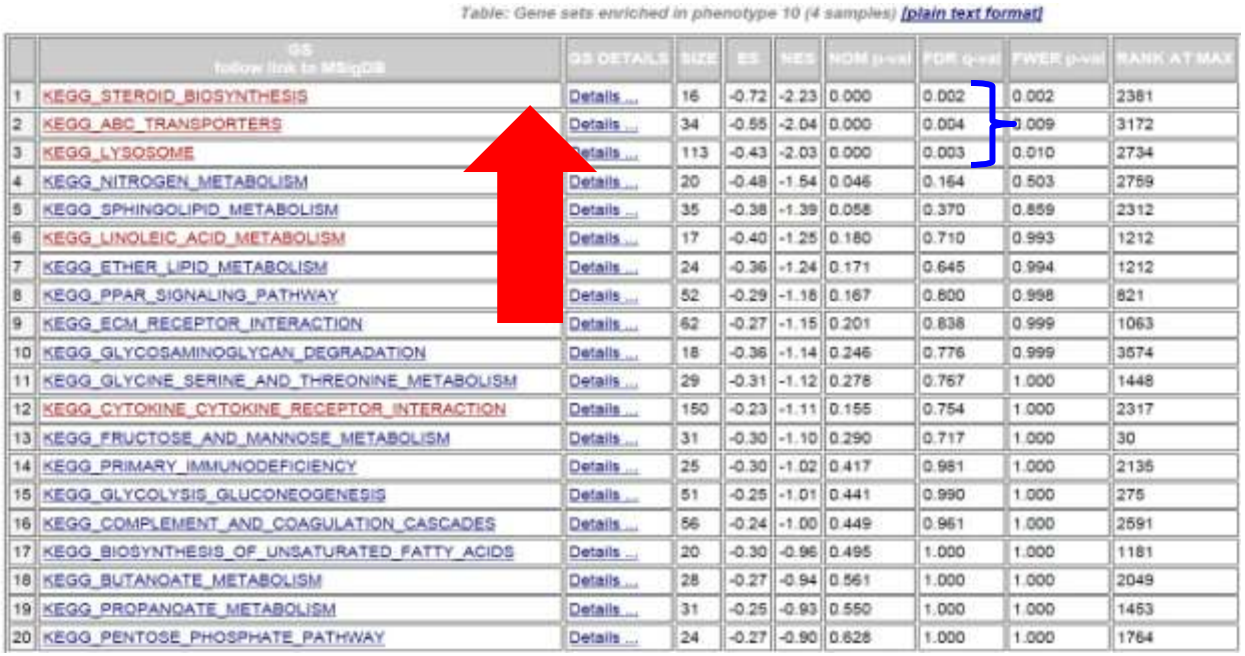
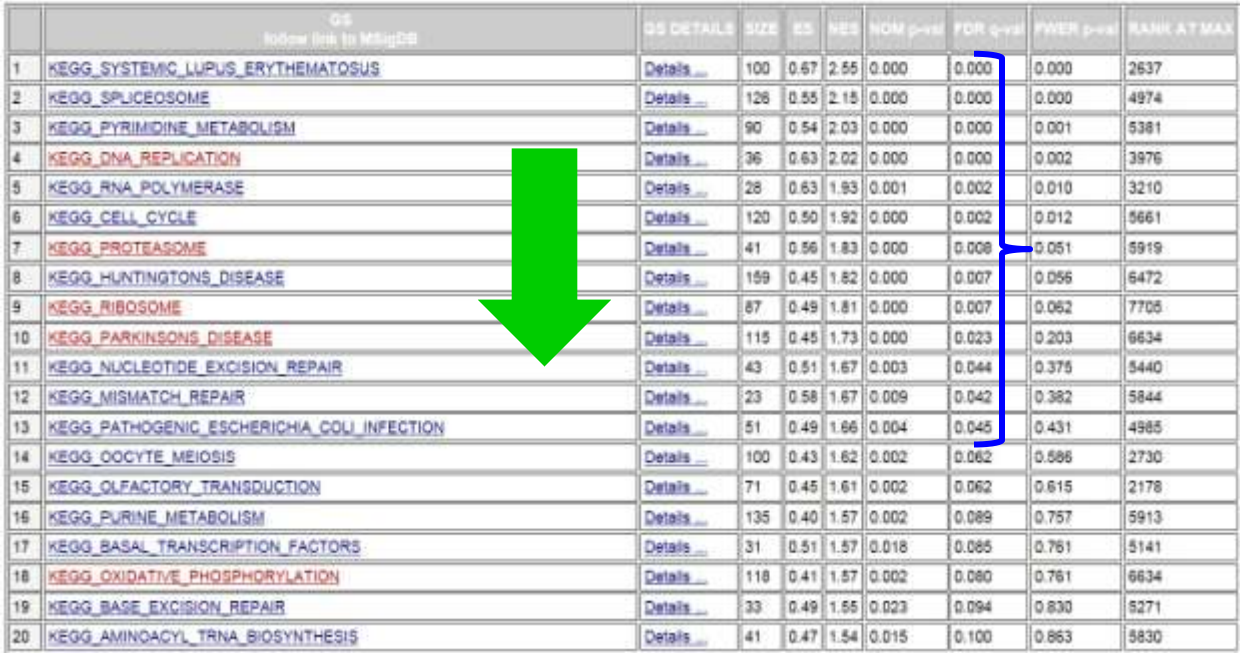


Table: Gene sets enriched in phenotype 10 (4 samples) [plain text format]

	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	KEGG_STEROID_BIOSYNTHESIS	Details ...	16	-0.72	-2.23	0.000	0.002	0.002	2381
2	KEGG_ABC_TRANSPORTERS	Details ...	34	-0.55	-2.04	0.000	0.004	0.009	3172
3	KEGG_LYSOSOME	Details ...	113	-0.43	-2.03	0.000	0.003	0.010	2734
4	KEGG_NITROGEN_METABOLISM	Details ...	20	-0.48	-1.54	0.046	0.164	0.503	2769
5	KEGG_SPHINGOLIPID_METABOLISM	Details ...	35	-0.38	-1.39	0.058	0.370	0.859	2312
6	KEGG_LINOLEIC_ACID_METABOLISM	Details ...	17	-0.40	-1.25	0.180	0.710	0.993	1212
7	KEGG_ETHER_LIPID_METABOLISM	Details ...	24	-0.36	-1.24	0.171	0.645	0.994	1212
8	KEGG_PPAR_SIGNALING_PATHWAY	Details ...	52	-0.29	-1.18	0.167	0.800	0.998	821
9	KEGG_ECM_RECEPTOR_INTERACTION	Details ...	62	-0.27	-1.15	0.201	0.838	0.999	1063
10	KEGG_GLYCOSAMINOGLYCAN_DEGRADATION	Details ...	18	-0.36	-1.14	0.246	0.776	0.999	3574
11	KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	Details ...	29	-0.31	-1.12	0.278	0.767	1.000	1448
12	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	Details ...	150	-0.23	-1.11	0.155	0.754	1.000	2317
13	KEGG_FRUCTOSE_AND_MANNOSE_METABOLISM	Details ...	31	-0.30	-1.10	0.290	0.717	1.000	30
14	KEGG_PRIMARY_IMMUNODEFICIENCY	Details ...	25	-0.30	-1.02	0.417	0.981	1.000	2135
15	KEGG_GLYCOLYSIS_GLUCONEOGENESIS	Details ...	51	-0.25	-1.01	0.441	0.990	1.000	275
16	KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	Details ...	56	-0.24	-1.00	0.449	0.961	1.000	2591
17	KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	Details ...	20	-0.30	-0.96	0.495	1.000	1.000	1181
18	KEGG_BUTANOATE_METABOLISM	Details ...	28	-0.27	-0.94	0.561	1.000	1.000	2049
19	KEGG_PROPANOATE_METABOLISM	Details ...	31	-0.25	-0.93	0.550	1.000	1.000	1453
20	KEGG_PENTOSE_PHOSPHATE_PATHWAY	Details ...	24	-0.27	-0.90	0.628	1.000	1.000	1764

Steroid biosynthesis;
lysosome

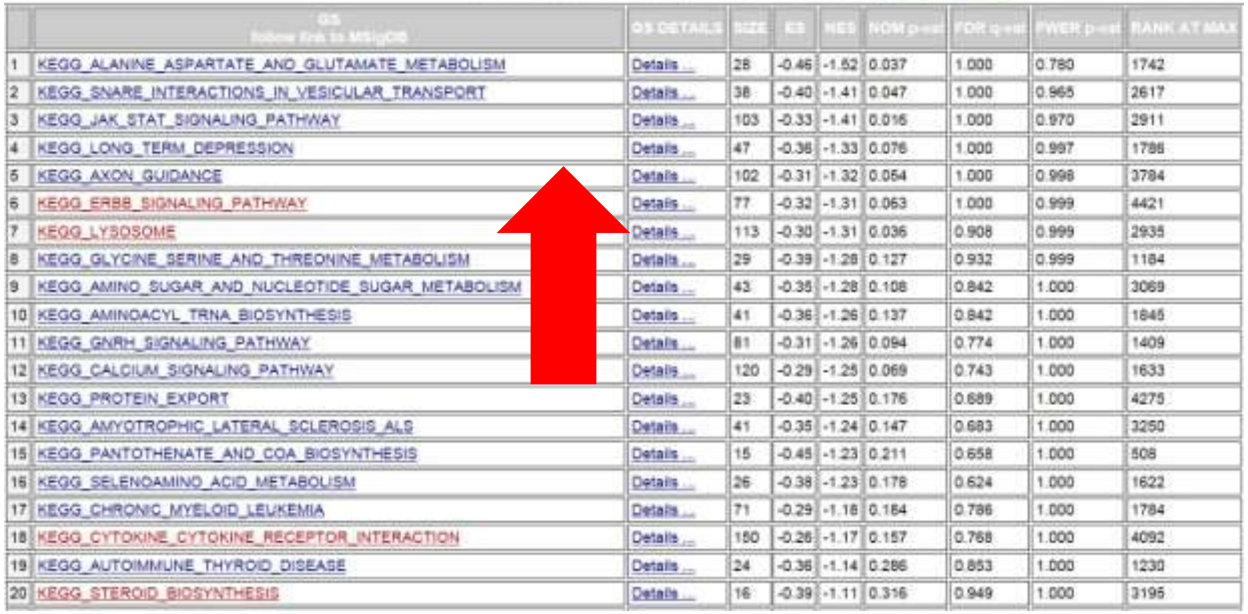


	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	Details ...	100	0.67	2.55	0.000	0.000	0.000	2637
2	KEGG_SPLICEOSOME	Details ...	126	0.55	2.15	0.000	0.000	0.000	4974
3	KEGG_PYRIMIDINE_METABOLISM	Details ...	90	0.54	2.03	0.000	0.000	0.001	5381
4	KEGG_DNA_REPLICATION	Details ...	36	0.63	2.02	0.000	0.000	0.002	3976
5	KEGG_RNA_POLYMERASE	Details ...	28	0.63	1.93	0.001	0.002	0.010	3210
6	KEGG_CELL_CYCLE	Details ...	120	0.50	1.92	0.000	0.002	0.012	5661
7	KEGG_PROTEASOME	Details ...	41	0.56	1.83	0.000	0.008	0.051	5919
8	KEGG_HUNTINGTONS_DISEASE	Details ...	159	0.45	1.82	0.000	0.007	0.056	6472
9	KEGG_RIBOSOME	Details ...	87	0.49	1.81	0.000	0.007	0.062	7705
10	KEGG_PARKINSONS_DISEASE	Details ...	115	0.45	1.73	0.000	0.023	0.203	6634
11	KEGG_NUCLEOTIDE_EXCISION_REPAIR	Details ...	43	0.51	1.67	0.003	0.044	0.375	5440
12	KEGG_MISMATCH_REPAIR	Details ...	23	0.58	1.67	0.009	0.042	0.382	5844
13	KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	Details ...	51	0.49	1.66	0.004	0.045	0.431	4985
14	KEGG_OOCYTE_MEIOSIS	Details ...	100	0.43	1.62	0.002	0.062	0.586	2730
15	KEGG_OLFACTORY_TRANSDUCTION	Details ...	71	0.45	1.61	0.002	0.062	0.615	2178
16	KEGG_PURINE_METABOLISM	Details ...	135	0.40	1.57	0.002	0.089	0.757	5913
17	KEGG_BASAL_TRANSCRIPTION_FACTORS	Details ...	31	0.51	1.57	0.018	0.085	0.761	5141
18	KEGG_OXIDATIVE_PHOSPHORYLATION	Details ...	118	0.41	1.57	0.002	0.080	0.761	6634
19	KEGG_BASE_EXCISION_REPAIR	Details ...	33	0.49	1.55	0.023	0.094	0.830	5271
20	KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	Details ...	41	0.47	1.54	0.015	0.100	0.863	5830

Transcription;
oxidative phosphorylation

A549 cells_High vs. Ctrl

Table: Gene sets enriched in phenotype 100 (4 samples) [plain text format]



	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	Details ...	28	-0.46	-1.52	0.037	1.000	0.780	1742
2	KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	Details ...	38	-0.40	-1.41	0.047	1.000	0.965	2617
3	KEGG_JAK_STAT_SIGNALING_PATHWAY	Details ...	103	-0.33	-1.41	0.016	1.000	0.970	2911
4	KEGG_LONG_TERM_DEPRESSION	Details ...	47	-0.36	-1.33	0.076	1.000	0.997	1786
5	KEGG_AXON_GUIDANCE	Details ...	102	-0.31	-1.32	0.054	1.000	0.998	3784
6	KEGG_ERBB_SIGNALING_PATHWAY	Details ...	77	-0.32	-1.31	0.063	1.000	0.999	4421
7	KEGG_LYSOSOME	Details ...	113	-0.30	-1.31	0.036	0.908	0.999	2935
8	KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	Details ...	29	-0.39	-1.28	0.127	0.932	0.999	1184
9	KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	Details ...	43	-0.35	-1.28	0.108	0.842	1.000	3069
10	KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	Details ...	41	-0.36	-1.26	0.137	0.842	1.000	1845
11	KEGG_GNRH_SIGNALING_PATHWAY	Details ...	81	-0.31	-1.26	0.094	0.774	1.000	1409
12	KEGG_CALCIUM_SIGNALING_PATHWAY	Details ...	120	-0.29	-1.25	0.069	0.743	1.000	1633
13	KEGG_PROTEIN_EXPORT	Details ...	23	-0.40	-1.25	0.176	0.689	1.000	4275
14	KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS	Details ...	41	-0.35	-1.24	0.147	0.683	1.000	3250
15	KEGG_PANTOTHENATE_AND_COA_BIOSYNTHESIS	Details ...	15	-0.45	-1.23	0.211	0.658	1.000	508
16	KEGG_SELENOAMINO_ACID_METABOLISM	Details ...	26	-0.38	-1.23	0.178	0.624	1.000	1622
17	KEGG_CHRONIC_MYELOID_LEUKEMIA	Details ...	71	-0.29	-1.18	0.184	0.786	1.000	1784
18	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	Details ...	150	-0.26	-1.17	0.157	0.768	1.000	4092
19	KEGG_AUTOIMMUNE_THYROID_DISEASE	Details ...	24	-0.36	-1.14	0.286	0.853	1.000	1230
20	KEGG_STEROID_BIOSYNTHESIS	Details ...	16	-0.39	-1.11	0.316	0.949	1.000	3195

N/A

Table: Gene sets enriched in phenotype 0 (4 samples) [plain text format]

	GS follow link to MSigDB	GS DETAILS	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX
1	KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	Details ...	100	0.79	3.22	0.000	0.000	0.000	1997
2	KEGG_DNA_REPLICATION	Details ...	36	0.86	2.87	0.000	0.000	0.000	938
3	KEGG_SPLICEOSOME	Details ...	126	0.62	2.62	0.000	0.000	0.000	3882
4	KEGG_CELL_CYCLE	Details ...	120	0.60	2.47	0.000	0.000	0.000	2314
5	KEGG_MISMATCH_REPAIR	Details ...	23	0.74	2.24	0.000	0.000	0.000	1265
6	KEGG_HOMOLOGOUS_RECOMBINATION	Details ...	28	0.68	2.11	0.000	0.000	0.001	1910
7	KEGG_PYRIMIDINE_METABOLISM	Details ...	90	0.51	2.05	0.000	0.000	0.001	3065
8	KEGG_BASE_EXCISION_REPAIR	Details ...	33	0.62	2.03	0.000	0.001	0.004	2458
9	KEGG_PARKINSONS_DISEASE	Details ...	115	0.48	1.99	0.000	0.001	0.009	5817
10	KEGG_OOCYTE_MEIOSIS	Details ...	100	0.48	1.96	0.000	0.002	0.018	1981
11	KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION	Details ...	75	0.49	1.89	0.001	0.004	0.040	1981
12	KEGG_OXIDATIVE_PHOSPHORYLATION	Details ...	118	0.43	1.81	0.000	0.009	0.105	4800
13	KEGG_NUCLEOTIDE_EXCISION_REPAIR	Details ...	43	0.52	1.80	0.002	0.010	0.118	2681
14	KEGG_PURINE_METABOLISM	Details ...	135	0.41	1.72	0.000	0.022	0.262	3065
15	KEGG_GLYCOLYSIS_GLUCONEOGENESIS	Details ...	51	0.47	1.72	0.002	0.021	0.265	2278
16	KEGG_DRUG_METABOLISM_OTHER_ENZYMES	Details ...	33	0.48	1.60	0.018	0.066	0.648	2581
17	KEGG_CARDIAC_MUSCLE_CONTRACTION	Details ...	58	0.43	1.59	0.008	0.067	0.674	4724
18	KEGG_PROTEASOME	Details ...	41	0.46	1.59	0.009	0.063	0.675	6892
19	KEGG_LYSINE_DEGRADATION	Details ...	43	0.45	1.56	0.018	0.075	0.757	4107
20	KEGG_HUNTINGTONS_DISEASE	Details ...	159	0.36	1.55	0.002	0.075	0.769	4800

Oxidative phosphorylation;
Cell cycle

Preliminary conclusion:

- **Gene sets (pathways) related into energy metabolism (e.g. oxidative phosphorylation pathway), transcription pathway were disturbed in A549 and HepG2 cells after exposure to TDCPP by bioinformatics analysis.**

RT- real time PCR to be employed to validate some genes expressions;

……

……

then,
a story might be expected...

Next 5 months Plan

1) Finish supplemented experiments in TDCPP study

2) Omics study of effect of dust extracts?? to cells

3) Thesis writing up

4) Job hunting:
Postdoc in toxicology (flame retardant's toxicity), omics (toxicogenomics), molecular biology…

Please email to: j.zhang.6@bham.ac.uk (Jinkang Zhang)

THANK YOU!