Maastricht University Leading in Learning!

GROW School for Oncology and Developmental Biology

TG Gates: a large-scale transcriptomics and pathology database

Florian Caiment

National Projects of Toxicogenomics in Japan (TGP)

- Toxicogenomics Project / TGP (2002-2007)
  - National Institute of Health Science (NIHS)
  - National Institute of Biomedical Innovation
  - 15 Japanese pharmaceutical companies
- Generating data for toxicology:
  - Compounds
  - Rat and human
  - *in vivo* and *in vitro*
  - liver and kidney
  - different doses
  - different exposure duration
  - single dose and repetitive dose

National Projects of Toxicogenomics in Japan (TGP)

- **T**oxico**g**enomics **G**enomics-**A**ssisted **T**oxicity **E**valuation **s**ystem : TG_Gates
  - Approximately 170 compounds (both of in-vivo and in-vitro)
  - clinical, pathological and expression data
  - >20,000 microarray profiles (Affymetrix GeneChip)

National Projects of Toxicogenomics in Japan 2 (TGP2)

- TGP2 (2007-2012): Developing biomarker for :
  - desease diagnosis
  - predicting compound toxicity
- Publications available on :
  - hepatotoxicity
  - phospholipidosis
  - coagulopathy
  - acetaminophen toxicity
  - chemical-induced glutathione depletion
  - ...

National Institute of Biomedical Innovation

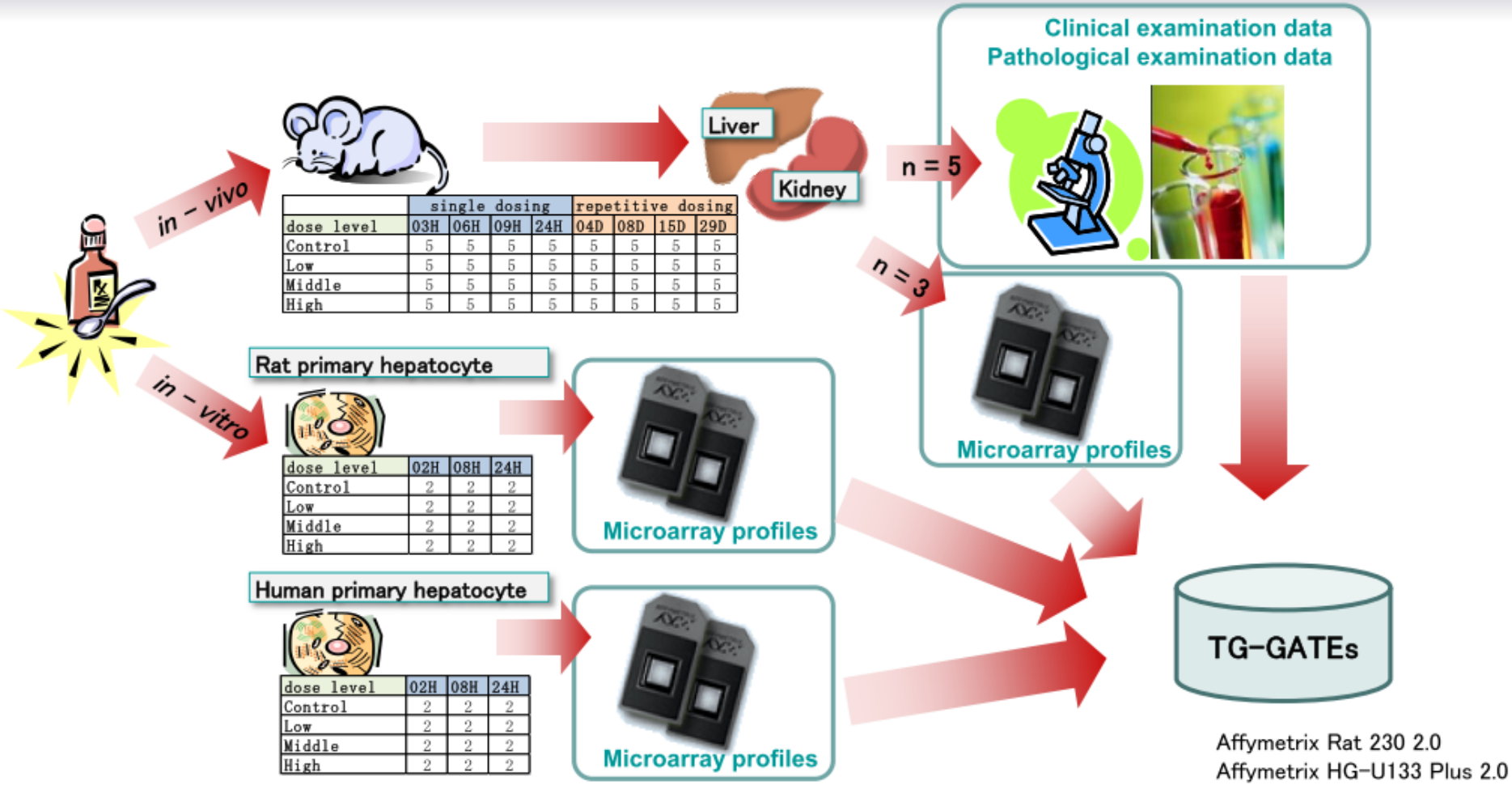
> Japanese

Open TG-GATEs

About this database | News | Data search | List of TGP documents | List of public data | Database License

http://toxico.nibio.go.jp/

Typical Experimental Design for Each Compound



	single dosing				repetitive dosing			
dose level	03H	06H	09H	24H	04D	08D	15D	29D
Control	5	5	5	5	5	5	5	5
Low	5	5	5	5	5	5	5	5
Middle	5	5	5	5	5	5	5	5
High	5	5	5	5	5	5	5	5

dose level	02H	08H	24H
Control	2	2	2
Low	2	2	2
Middle	2	2	2
High	2	2	2

dose level	02H	08H	24H
Control	2	2	2
Low	2	2	2
Middle	2	2	2
High	2	2	2

Up to 240 microarray profiles for each compound

3 (n) x 8 (time-point) x 4 (dose-level) = **96 microarray profiles from vivo liver**
3 (n) x 8 (time-point) x 4 (dose-level) = **96 microarray profiles from vivo kidney**
2 (n) x 3 (time-point) x 4 (dose-level) = **24 microarray profiles from vitro rat hepatocyte**
2 (n) x 3 (time-point) x 4 (dose-level) = **24 microarray profiles from vitro human hepatocyte**

Compound list

1% cholesterol	carboplatin	ethinylestradiol	mefenamic acid	promethazine
2,4-dinitrophenol	cephalothin	ethionamide	meloxicam	propranolol
2-nitrofluorene	chloramphenicol	ethionine	metformin	propylthiouracil
3-methylcholanthrene	chlormadinone	etoposide	methapyrilene	puromycin aminonucleoside
acarbose	chlormezanone	famotidine	methimazole	quinidine
acetamide	chlorpheniramine	fenofibrate	methyldopa	ranitidine
acetamidofluorene	chlorpromazine	fluoxetine hydrochloride	methylene dianiline	rifampicin
acetaminophen	chlorpropamide	fluphenazine	methyltestosterone	rosiglitazone maleate
acetazolamide	cimetidine	flutamide	mexiletine	rotenone
adapin	ciprofloxacin	furosemide	monocrotaline	simvastatin
aflatoxin B1	cisplatin	galactosamine	moxisylyte	sulfasalazine
ajmaline	clofibrate	gefitinib	naphthyl isothiocyanate	sulindac
allopurinol	clomipramine	gemfibrozil	naproxen	sulpiride
allyl alcohol	clozapine	gentamicin	nefazodone	tacrine
alpidem	colchicine	glibenclamide	nicotinic acid	tamoxifen
amiodarone	coumarin	griseofulvin	nifedipine	tannic acid
amitriptyline	cycloheximide	haloperidol	nimesulide	terbinafine
amphotericin B	cyclophosphamide	hepatocyte growth factor	nitrofurantoin	tetracycline
aspirin	cyclosporine A	hexachlorobenzene	nitrofurazone	theophylline
azathioprine	danazol	hydroxyzine	nitrosodiethylamine	thioacetamide
bendazac	dantrolene	ibuprofen	N-methyl-N-nitrosourea	thioridazine
benzbromarone	desmopressin acetate	imatinib, methanesulfonate salt	N-nitrosomorpholine	ticlopidine
benziodarone	dexamethasone	imipramine	omeprazole	tiopronin
bortezomib	diazepam	indomethacin	papaverine	TNFα
bromobenzene	diclofenac	interferon alpha, human	pemoline	tolbutamide
bromoethylamine	diethyl maleate	interleukin 1 beta, human	penicillamine	TGF beta 1
bucetin	diltiazem	interleukin 6, human	perhexiline	triamterene
buspirone	disopyramide	iproniazid	phalloidin	triazolam
buthionine sulfoximine	disulfiram	isoniazid	phenacetin	trimethadione
butylated hydroxyanisole	doxorubicin	ketoconazole	phenobarbital	tunicamycin
caffeine	enalapril	labetalol	phenylanthranilic acid	valproic acid
captopril	erythromycin ethylsuccinate	lomustine	phenylbutazone	venlafaxine
carbamazepine	ethambutol	lornoxicam	phenytoin	vitamin A
carbon tetrachloride	ethanol	LPS	phorone	WY-14643

Open TG-GATEs

About this database | News | Data search | List of TGP documents | List of public data | Database License

> Top

Select a single search condition from either Compound or Pathology and click [Search]

Compound List

select compound

benzbromarone
benziodarone
bortezomib
bromobenzene
bromoethylamine
bucetin
buspirone
buthionine sulfoximine
butylated hydroxyanisole
caffeine

Select one of the above compounds

Clicking [Search] will take you to the species and experimental conditions selection screen

Compound: bromobenzene

- Human
  - in vitro
    - CEL files(24)
- Rat
  - in vitro
    - CEL files(24)
  - in vivo
    - Liver
      - CEL files(96)
      - Pathological items(45)
    - Kidney
      - CEL files(96)
      - Pathological items(5)

CEL files:

To Archive Site
CEL files:zip

	2hr	8hr	24hr
Control	003016034012 (CEL) 003016034013 (CEL)	003016034020 (CEL) 003016034021 (CEL)	003016034028 (CEL) 003016034029 (CEL)
Low	003016034014 (CEL) 003016034015 (CEL)	003016034022 (CEL) 003016034023 (CEL)	003016034030 (CEL) 003016035001 (CEL)
Middle	003016034016 (CEL) 003016034017 (CEL)	003016034024 (CEL) 003016034025 (CEL)	003016035002 (CEL) 003016035003 (CEL)
High	003016034018 (CEL) 003016034019 (CEL)	003016034026 (CEL) 003016034027 (CEL)	003016035004 (CEL) 003016035005 (CEL)

Detail Data

Compound	Species	Test Type
bromobenzene	Human	in vitro
Organ	**Time(Sacrifice)**	**Dose Level**
Liver	8 hr	High
Dose	**Chip Type**	**Sample ID**
200 μM	HG-U133_Plus_2	5097112LRA

Barcode	CEL File
003016034027	Download

Cell Viability

Cell Viability (%) (LDH)	100.7
Cell Viability (%) (DNA)	98.8

Compound: bromobenzene

- Human
  - in vitro
    - CEL files(24)
- Rat
  - in vitro
    - CEL files(24)
  - in vivo
    - Liver
      - CEL files(96)
      - Pathological items(45)
    - Kidney
      - CEL files(96)
      - Pathological items(5)

CEL files: (Single)

To Archive Site
CEL files.zip

	3hr	6hr	9hr	24hr
Control	003017697026 (CEL) 003017697027 (CEL) 003017697028 (CEL)	003017698008 (CEL) 003017698009 (CEL) 003017698010 (CEL)	003017697002 (CEL) 003017697003 (CEL) 003017697004 (CEL)	003017697014 (CEL) 003017697015 (CEL) 003017697016 (CEL)
Low	003017697029 (CEL) 003017697030 (CEL) 003017698001 (CEL)	003017698011 (CEL) 003017698012 (CEL) 003017698013 (CEL)	003017697005 (CEL) 003017697006 (CEL) 003017697007 (CEL)	003017697017 (CEL) 003017697018 (CEL) 003017697019 (CEL)
Middle	003017698002 (CEL) 003017698003 (CEL) 003017698004 (CEL)	003017698014 (CEL) 003017698015 (CEL) 003017698016 (CEL)	003017697008 (CEL) 003017697009 (CEL) 003017697010 (CEL)	003017697020 (CEL) 003017697021 (CEL) 003017697022 (CEL)
High	003017698005 (CEL) 003017698006 (CEL) 003017698007 (CEL)	003017698017 (CEL) 003017698018 (CEL) 003017698019 (CEL)	003017697011 (CEL) 003017697012 (CEL) 003017697013 (CEL)	003017697023 (CEL) 003017697024 (CEL) 003017697025 (CEL)

CEL files: (Repeat)

To Archive Site
CEL files.zip

	4day	8day	15day	29day
Control	003017751015 (CEL) 003017751016 (CEL) 003017751017 (CEL)	003017751027 (CEL) 003017751028 (CEL) 003017751029 (CEL)	003017749003 (CEL) 003017749004 (CEL) 003017749005 (CEL)	003017749015 (CEL) 003017749016 (CEL) 003017749017 (CEL)
Low	003017751018 (CEL) 003017751019 (CEL) 003017751020 (CEL)	003017751030 (CEL) 003017752001 (CEL) 003017752002 (CEL)	003017749006 (CEL) 003017749007 (CEL) 003017749008 (CEL)	003017749018 (CEL) 003017749019 (CEL) 003017749020 (CEL)
Middle	003017751021 (CEL) 003017751022 (CEL) 003017751023 (CEL)	003017752003 (CEL) 003017752004 (CEL) 003017752005 (CEL)	003017749009 (CEL) 003017749010 (CEL) 003017749011 (CEL)	003017749021 (CEL) 003017749022 (CEL) 003017749023 (CEL)
High	003017751024 (CEL) 003017751025 (CEL) 003017751026 (CEL)	003017752006 (CEL) 003017752007 (CEL) 003017752008 (CEL)	003017749012 (CEL) 003017749013 (CEL) 003017749014 (CEL)	003017749024 (CEL) 003017749025 (CEL) 003017749026 (CEL)

High	003017751024 (CEL) 003017751025 (CEL) 003017751026 (CEL)	003017752006 (CEL) 003017752007 (CEL) 003017752008 (CEL)	003017749012 (CEL) 003017749013 (CEL) 003017749014 (CEL)	003017749024 (CEL) 003017749025 (CEL) 003017749026 (CEL)

Compound	Species	Test Type
bromobenzene	Rat	in vivo
Organ	Time(Sacrifice)	Dose Level
Liver	8 day	Low
Dose	Chip Type	Sample ID
30 mg/kg	Rat230_2	0186061LRB

Barcode	CEL File
003017751030	Download

Hematology

RBC ($x10^4$/uL)	580	Neutrophil (%)	17
Hb (g/dL)	12.7	Eosinophil (%)	1
Ht (%)	36.8	Basophil (%)	0
MCV (fL)	63.5	Monocyte (%)	2
MCH (pg)	21.9	Lymphocyte (%)	79
MCHC (%)	34.5	PT (s)	13.1
Ret (%)	6.3	APTT (s)	16.2
Plat ($x10^4$/uL)	115.5	Fbg (mg/dL)	247
WBC ($x10^2$/uL)	133.1		

Biochemistry

ALP (IU/L)	2456	Cl (meq/L)	102
TC (mg/dL)	73	Ca (mg/dL)	10.8
TG (mg/dL)	79	IP (mg/dL)	6.8
PL (mg/dL)	145	TP (g/dL)	5.6
TBIL (mg/dL)	0.05	RALB (g/dL)	2.6
DBIL (mg/dL)	0.01	A / G	0.9
GLC (mg/dL)	200	AST (GOT) (IU/L)	71
BUN (mg/dL)	14	ALT (GPT) (IU/L)	40
CRE (mg/dL)	0.2	LDH (IU/L)	91
Na (meq/L)	141	gamma-GTP (IU/L)	0
K (meq/L)	3.7		

Organ Weight

Terminal Body Weight (g)	255		
Liver (g)	13.597	Relative Liver Weight (%)	5.33
Kidney Total (g)	2.314	Relative Kidney Total Weight (%)	0.91
Kidney Right (g)	1.159	Relative Kidney Right Weight (%)	0.45
Kidney Left (g)	1.155	Relative Kidney Left Weight (%)	0.45

Organ Weight

Terminal Body Weight (g)	255		
Liver (g)	13.597	Relative Liver Weight (%)	5.33
Kidney Total (g)	2.314	Relative Kidney Total Weight (%)	0.91
Kidney Right (g)	1.159	Relative Kidney Right Weight (%)	0.45
Kidney Left (g)	1.155	Relative Kidney Left Weight (%)	0.45

Body Weight

Day	1	3	7						
g	196.2	211.9	241.6						
Amount of Change	---	15.7	29.7						

Image Data

Zoom
Fit
1X
2X
4X
5X
10X
20X
0.4X

Pathological items: 45 items

Refine by Finding x Dose x Time:

x x GO

No	CEL	Finding	Topography	Grade	Dose	Time	Image	Whole slide glass Image
1	003017697024 (CEL)	Cellular infiltration	Centrilobular	minimal	High	24 hr	-	30276
2	003017751025 (CEL)	Cellular infiltration	Centrilobular	minimal	High	4 day	-	30510
3	003017751026 (CEL)	Cellular infiltration	Centrilobular	minimal	High	4 day	-	30516
4	No ChipData (---)	Cellular infiltration	Centrilobular	minimal	High	4 day	-	30512
5	003017697023 (CEL)	Cellular infiltration	Centrilobular	slight	High	24 hr	-	30272
6	003017697025 (CEL)	Cellular infiltration	Centrilobular	slight	High	24 hr	-	30278
7	No ChipData (---)	Cellular infiltration	Centrilobular	slight	High	24 hr	-	30270
8	No ChipData (---)	Cellular infiltration	Centrilobular	moderate	High	24 hr	o	30274
9	003017697023 (CEL)	Degeneration, hydropic	Centrilobular	minimal	High	24 hr	-	30272
10	No ChipData (---)	Degeneration, hydropic	Centrilobular	minimal	High	24 hr	-	30270
11	003017697025 (CEL)	Degeneration, hydropic	Centrilobular	slight	High	24 hr	-	30278
12	No ChipData (---)	Degeneration, hydropic	Centrilobular	moderate	High	24 hr	o	30274
13	003017697012 (CEL)	Hypertrophy	Centrilobular	minimal	High	9 hr	-	30264
14	003017751024 (CEL)	Hypertrophy	Centrilobular	minimal	High	4 day	-	30509
15	003017751026 (CEL)	Hypertrophy	Centrilobular	minimal	High	4 day	-	30516
16	003017752006 (CEL)	Hypertrophy	Centrilobular	minimal	High	8 day	-	30518
17	003017752008 (CEL)	Hypertrophy	Centrilobular	minimal	High	8 day	-	30525

Data quality

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.37, No.4, 791-801, 2012

Original Article

Evaluation of DNA microarray results in the Toxicogenomics Project (TGP) consortium in Japan

Nakatsu Noriyuki[1], Yoshinobu Igarashi[1], Atsushi Ono[2], Hiroshi Yamada[1], Yasuo Ohno[3], and Tetsuro Urushidani[1,4]

- inter and intra-laboratory reproducibility of the replicates by:
  - Clustering
  - Spearman correlation
  - Overlap in lists of DE genes

Data quality

ARRAYANALYSIS.ORG welcome page

- Quick & easy on-line Affymetrix arrays Quality Control -

Get started | Download sources | Pipeline description | Documentation | Bug tracker

[Pipeline Description]

Sample quality
- Sample prep controls
- 3'/5' for control genes
- RNA degradation plot

Hybridization quality
- Spike-in controls
- Background intensity
- Percent present
- PMA calls table
- Pos/Neg controls
- Affx control profiles

Signal comparability
- Scale factor
- Boxplot
- Density histogram
- MA plots
- Reference Layout
- Pos/Neg COI plot
- 2D spatial images
- NUSE plot
- RLE plot

Array correlation
- Correlation plot
- PCA analysis
- Clustering

BiGCaT bioinformatics

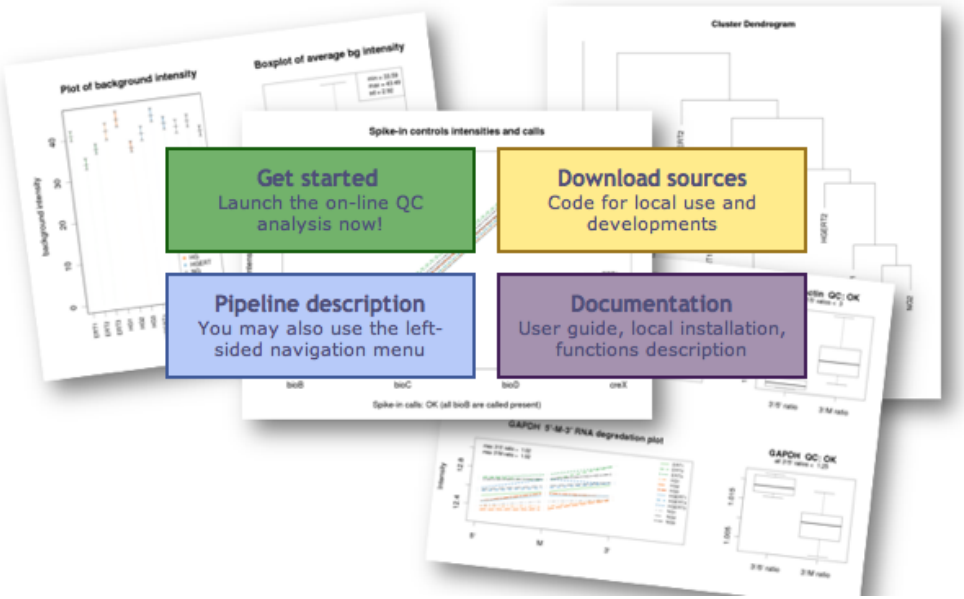
Welcome to ArrayAnalysis.org !

Discover the integrated Affymetrix array Quality Control pipeline

Wednesday, June 1st, arrayanalysis.org may encounter some difficulties between 15:00 and 18:00. Thank you for your comprehension.

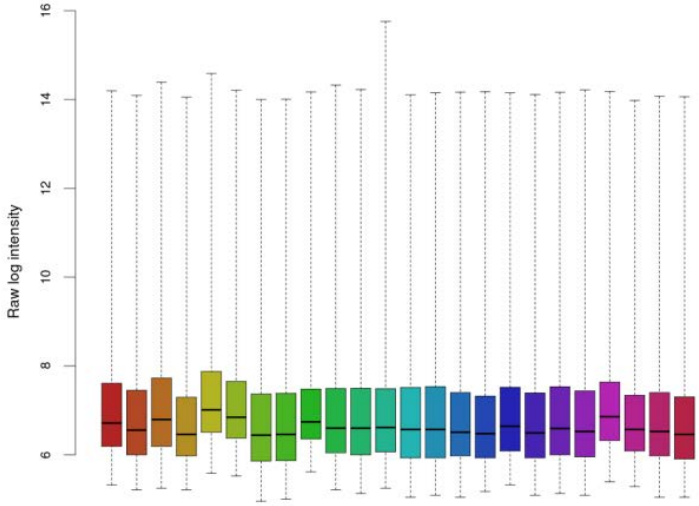
Affymetrix microarrays of different technology versions are very often used in transcriptomics analysis.
Quality control and normalization approaches do exist, especially as packages in Bioconductor/R. However:
- Procedures are often different between teams.
- They are not always easy to access as they run through command lines.
- It is often not clear what the meaning of the specific settings and results are
- They are not always usable for the newer technology types of arrays.

To tackle this, we proposed an automated well-documented and user-friendly pipeline for Affymetrix microarray quality control and normalization.

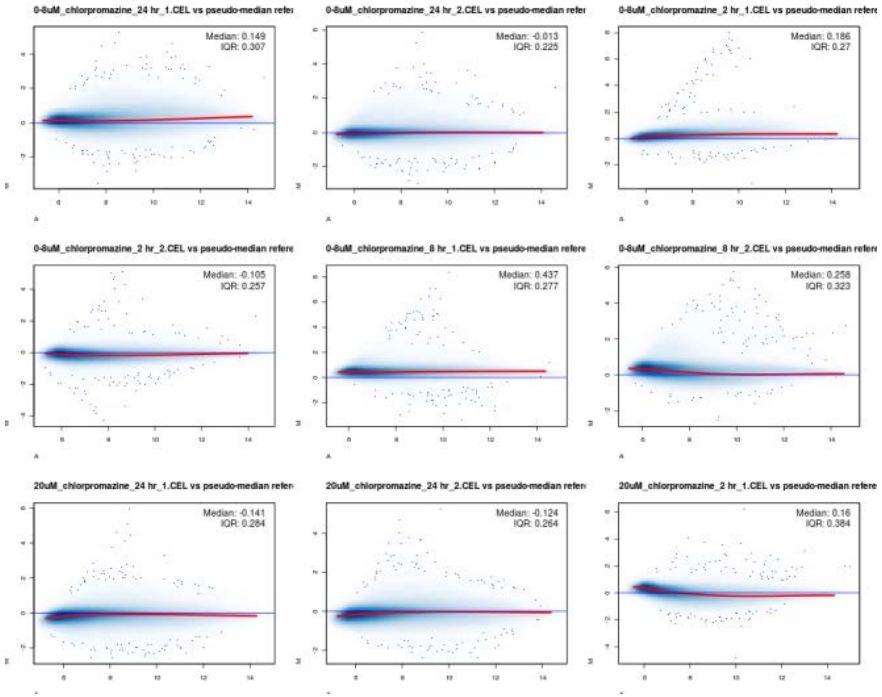


Boxplot of raw intensities

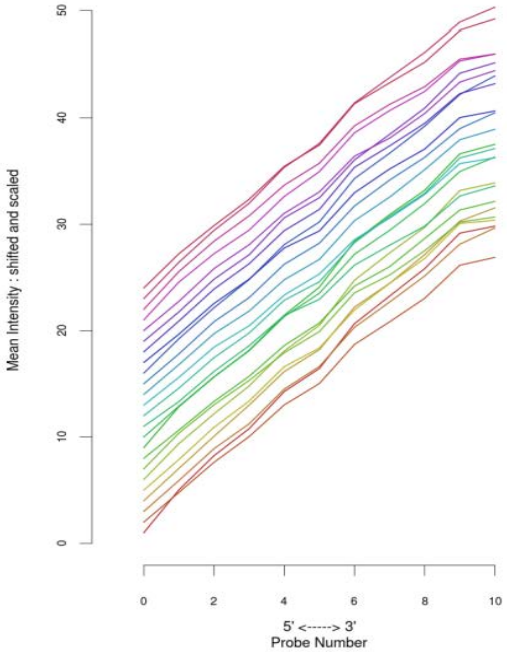
Distributions should be comparable between arrays

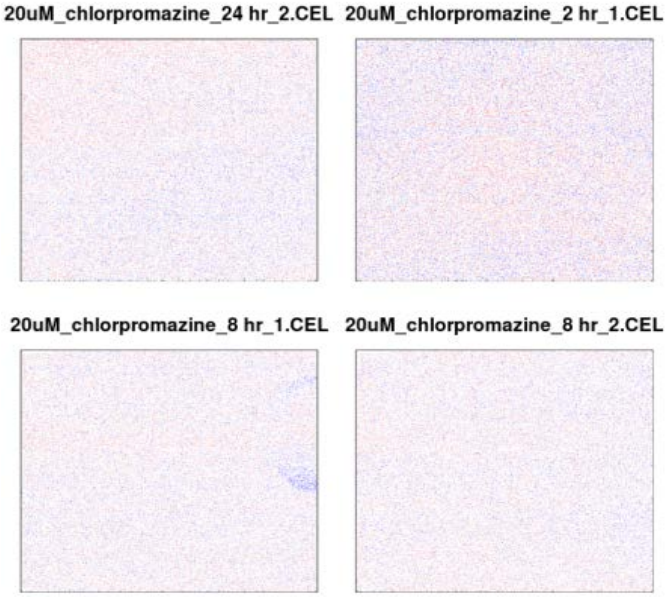


MA plots of raw data 1 / 2



RNA degradation plot





Toxygates

http://targetmine.nibio.go.jp:8080/otgviewer/

Toxygates

Human, Vitro, Single

Compounds

Sel	
☐	2,4-dinitrophenol
☐	2-nitrofluorene
☐	LPS
☐	N-methyl-N-nitrosourea
☐	N-nitrosomorpholine
☐	TNFalpha
☐	WY-14643
☐	acarbose
☐	acetamide
☐	acetamidofluorene
☐	acetaminophen
☐	acetazolamide
☐	adapin
☐	aflatoxin B1
☐	ajmaline
☐	allopurinol
☐	allyl alcohol

Sample groups | Compound ranking (optional)

Sample group definition

Please select at least one compound

Toxygates

Sample groups | Compound ranking (optional)

Sample group definition - editing Liver Tox

	Low	Medium	High
aflatoxin B1	☐ 2 hr ☑ 8 hr ☑ 24 hr ☐ All	☐ 2 hr ☑ 8 hr ☑ 24 hr ☐ All	☐ 2 hr ☑ 8 hr ☑ 24 hr ☐ All
cyclosporine A	☐ 2 hr ☐ 8 hr ☐ 24 hr ☐ All	☐ 2 hr ☑ 8 hr ☑ 24 hr ☐ All	☐ 2 hr ☐ 8 hr ☐ 24 hr ☐ All
ethanol	☐ 2 hr ☐ 8 hr ☐ 24 hr ☐ All	☐ 2 hr ☑ 8 hr ☐ 24 hr ☐ All	☐ 2 hr ☐ 8 hr ☑ 24 hr ☐ All

Save group as Liver Tox Save

Active	Group	Sample count		
☑	Liver Tox	20	Edit	Delete

Next: Select probes

Toxygates

KEGG pathway search

GO term search

CHEMBL targets

DrugBank targets

Free selection

Enter a list of probes, genes or proteins, one per line, to display only those.

Add manual list

Begin typing a gene name to get suggestions.

Add gene

Selected probes

Clear selected probes

Proceed with 0 selected probes >>

Proceed with all probes

Toxygates

KEGG pathway search

GO term search

This lets you view probes that correspond to a given GO term. Enter a partial term name and press enter to search.

liver

determination of liver left/right asymmetry
epithelial cell proliferation involved in liver morphogene
human liver aminopeptidase
intracellular copper delivery
liver aminopeptidase activity
liver cell differentiation
liver development
liver lipase
liver morphogenesis
liver regeneration
liver starch binding
liver trabecula biogenesis
liver trabecula formation

Add selected probes >>

CHEMBL targets

DrugBank targets

Free selection

Selected probes

CADM1 (244345_at)
ALDOB (243901_at)
NF1 (243476_at)
PROX1 (242119_at)
CCNE1 (242105_at)
ARID5B (241969_at)
ALDOB (241280_at)
ASNS (241082_at)
RPGRIP1L (241028_at)
JARID2 (240674_at)
ALDH1A2 (240435_at)
HMGCS2 (240110_at)
ONECUT2 (239911_at)
QDPR (238616_at)
SP3 (238035_at)
NODAL (237896_at)
LOC100506403 (237697_at)
ARID5B (235404_at)
MAN2A1 (235103_at)
ERBB2 (234354_x_at)
VWF (233980_s_at)
ONECUT2 (233446_at)
ONECUT2 (233441_at)
CADM1 (232767_at)
UGT1A1 (232655_at)

Clear selected probes

Proceed with 240 selected probes >>

Proceed with all probes

TGFBR3

transforming growth factor, beta receptor III

Toxygates

Human, Vitro, Single | Liver Tox: aflatoxin B1/Low/8 hr, aflatoxin B1/Middle/8 hr... | Non Liver Tox: aspirin/High/2 hr, aspirin/Middle/2 hr...

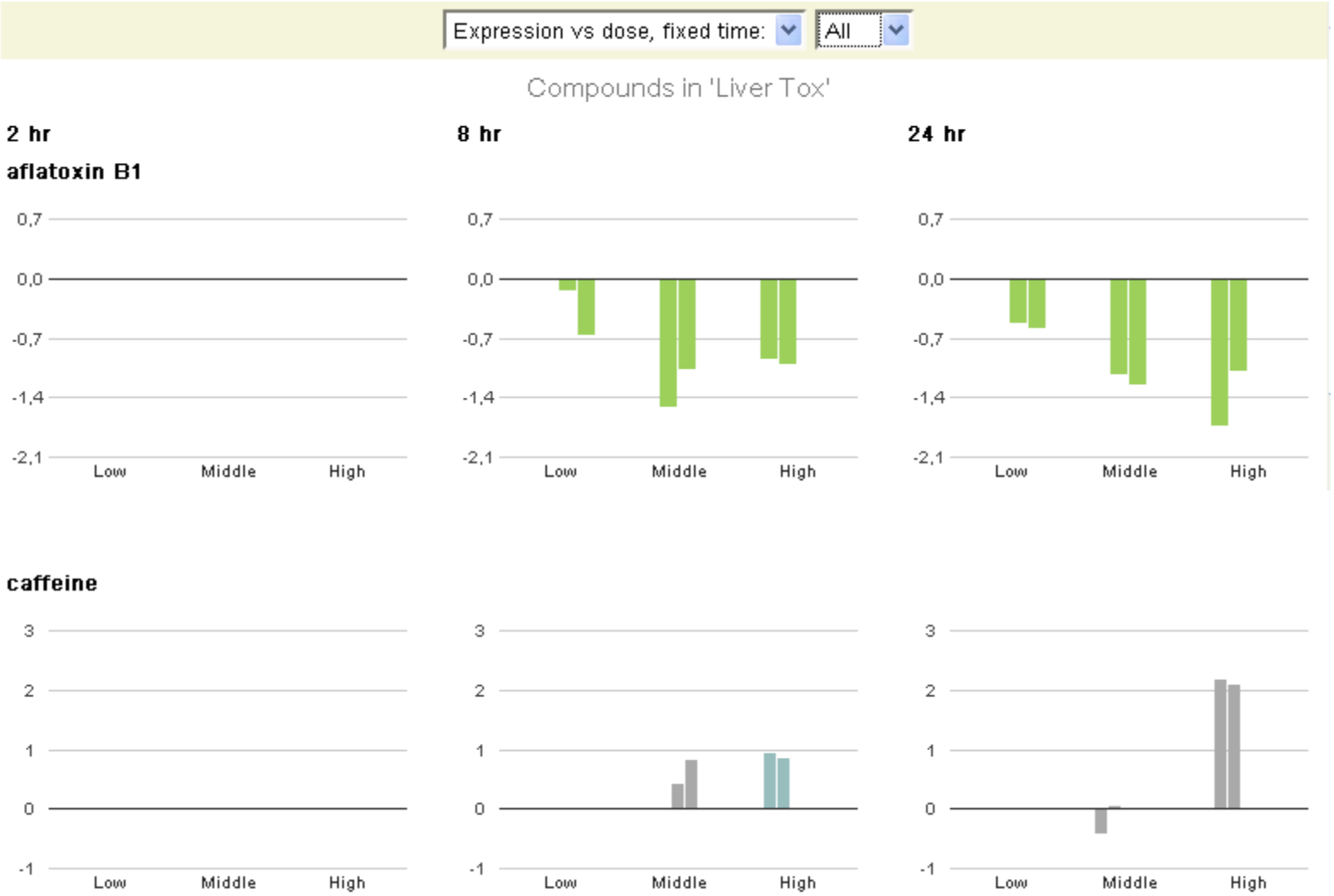
Log2 (fold change) | 1-25 of 240 | Show More | Magnitude >= 0.00 | Apply | No filter | Analysis

Gene Sym	Probe title	Probe	Liver Tox	Non Liver Tox
TGFBR3	transforming growth factor, beta receptor III	226625_at	-0.672	-0.033
TGFBR3	transforming growth factor, beta receptor III	204731_at	-0.636	0.18
MET	met proto-oncogene (hepatocyte growth factor receptor)	213807_x_at	-0.592	0.024
FOXM1	forkhead box M1	202580_x_at	-0.565	0.041
CCNE1	cyclin E1	213523_at	-0.56	-0.081
ANXA1	annexin A1	201012_at	-0.55	-0.116
NF1	neurofibromin 1	210631_at	-0.549	0.853
PROX1	prospero homeobox 1	229376_at	-0.527	-0.115
BAAT	bile acid CoA: amino acid N-acyltransferase (glycine N-choloyltransferase)	206913_at	-0.505	-0.259
PROX1	prospero homeobox 1	228656_at	-0.497	-5E-4
HNF1B	HNF1 homeobox B	205313_at	-0.483	0.07
JUN	jun proto-oncogene	201466_s_at	-0.45	-0.144
MET	met proto-oncogene (hepatocyte growth factor receptor)	213816_s_at	-0.421	-0.035
ARF6	ADP-ribosylation factor 6	203311_s_at	-0.417	0.05

TGFBR3

transforming growth factor, beta receptor III

Toxygates



Conclusion

- Huge open access toxicogenomics dataset
- Liver and Kidney
- *In vivo* (rat) and *in vitro* (human & rat)
- dose effect, time effect
- Single and repetitive dose (in vivo)
- Toxygates