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Pathway-Based Concentration Response Profiles from Toxicogenomics Data

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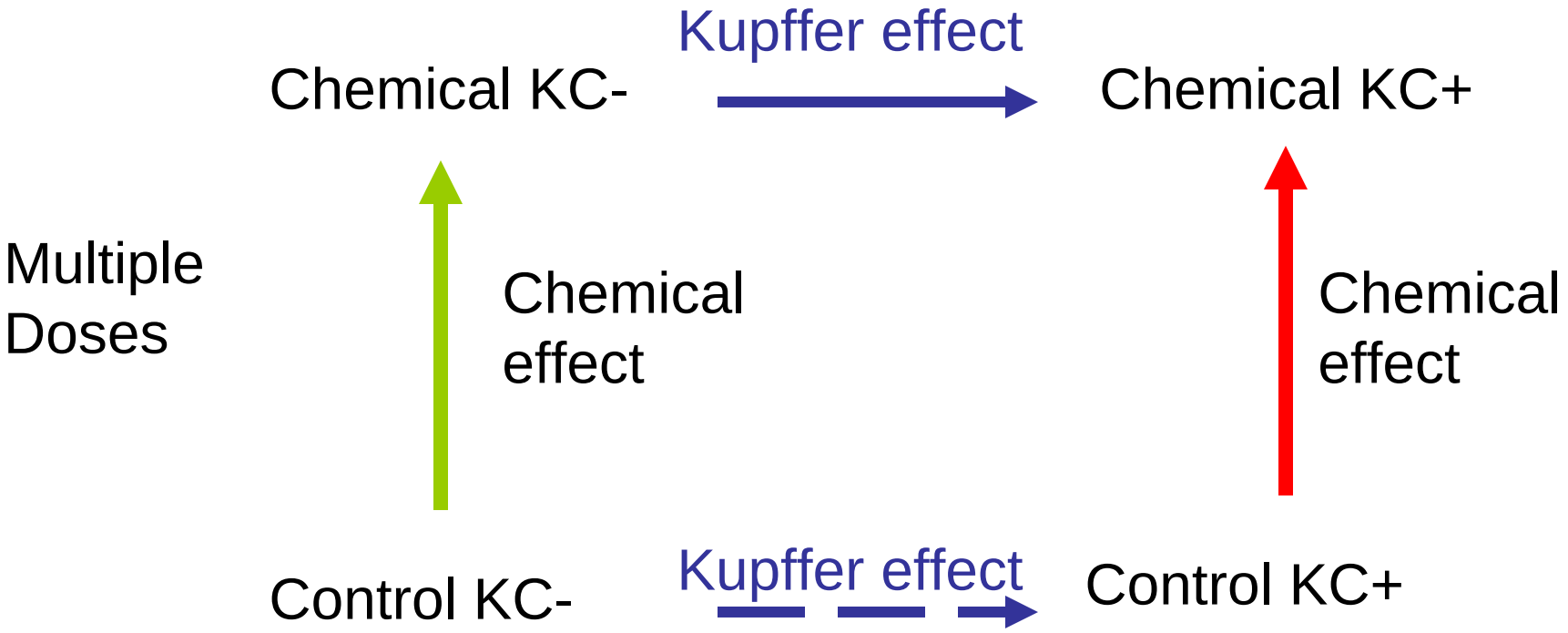
Motivation

- Want to find *in vitro* models that predict *in vivo* toxicity
  - ToxCast program
  - NRC Toxicity Testing in the 21st Century
- Build models around Toxicity Pathways
- Dose-response behavior is critical
  - Internal dose must be > pathway activation dose (Clewell, et al.)
- Evaluate potential use of microarray genomics for discovering pathway LOAEL-like values

Lowest Observed Pathway Response Level

- Combine dose-response microarray data and pathway information
  - Find robust method to derive LOPRL
- Test LOPRL approach in cell system / model of liver toxicity
  - Primary rat hepatocytes
  - With and without Kupffer cells
- Need method that can be run on 100's to 1000's of chemicals
  - Minimize # of replicates needed

Study Design Overview



Kupffer cells have a genome-wide effect on expression data
Difficult to distinguish signaling vs. cellularity

Cell System

- Primary rat hepatocytes
  - 5 replicates / pools
  - 3 animals per replicate / pool
  - With or without Kupffer cells
    - 180,000 hepatocytes/well
    - ±60,000 Kupffer cells/well

- Cells were overlaid with Matrigel and cultured overnight

- Cell preparation performed by IVAL

Chemical and Targets

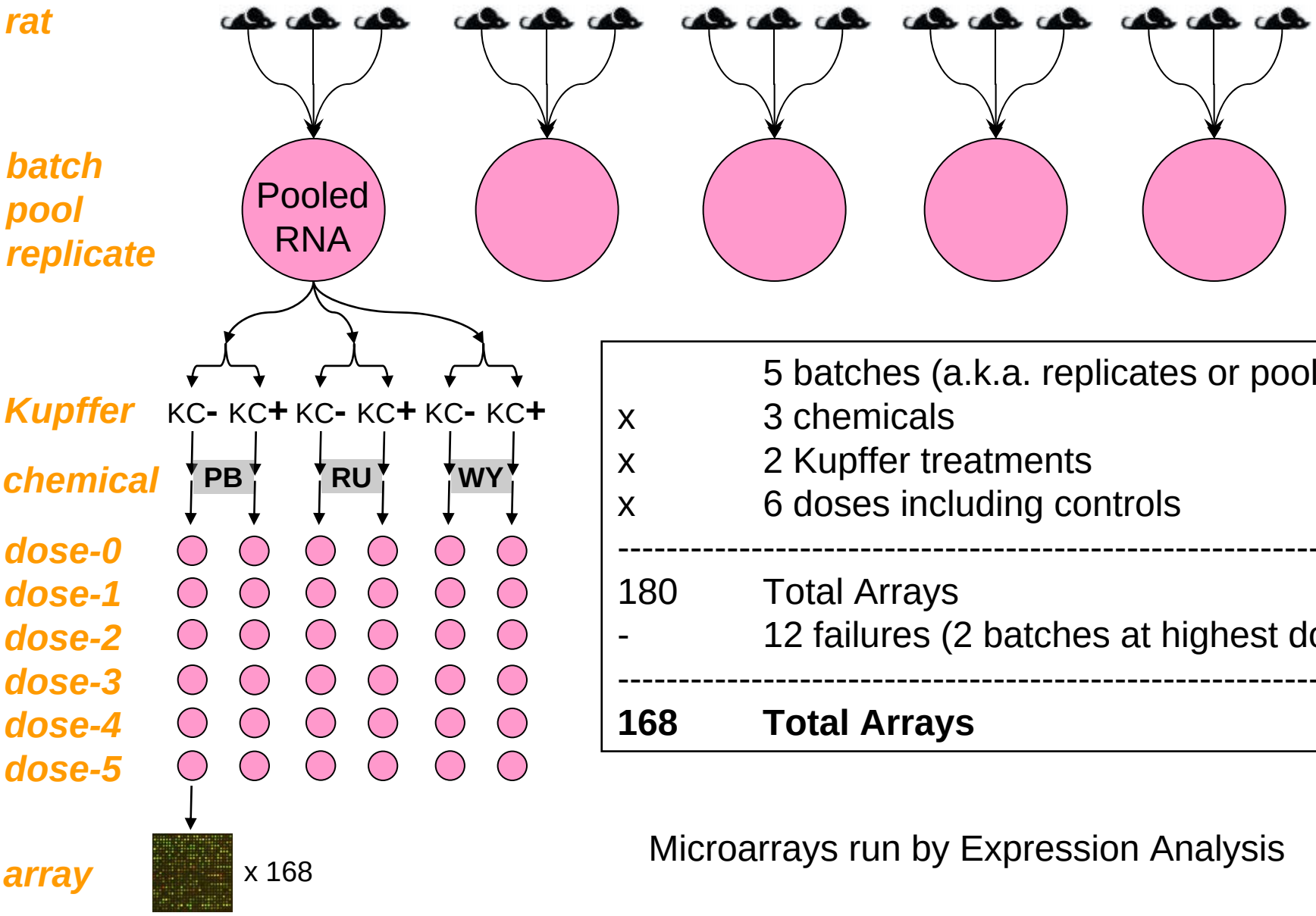
Chemical	Nuclear Receptor Targets	Rat Genes targets
Phenobarbital	CAR	Cyp2b2
WY-14643	PPAR alpha	Cyp4a22
RU-486	PXR	Cyp3a1

Chemical Treatment

- Chemical Treatment
  - RU-486
    - 3, 10, 30, 100, 300 μM
  - Phenobarbital
    - 20, 60, 200, 600, 1800 μM
  - WY-14643
    - 0.3, 1, 3, 10, 30 μM
- Incubate for 24 hours

- Upregulate XMEs through Liver Nuclear Receptors
- Good for testing pathway responses

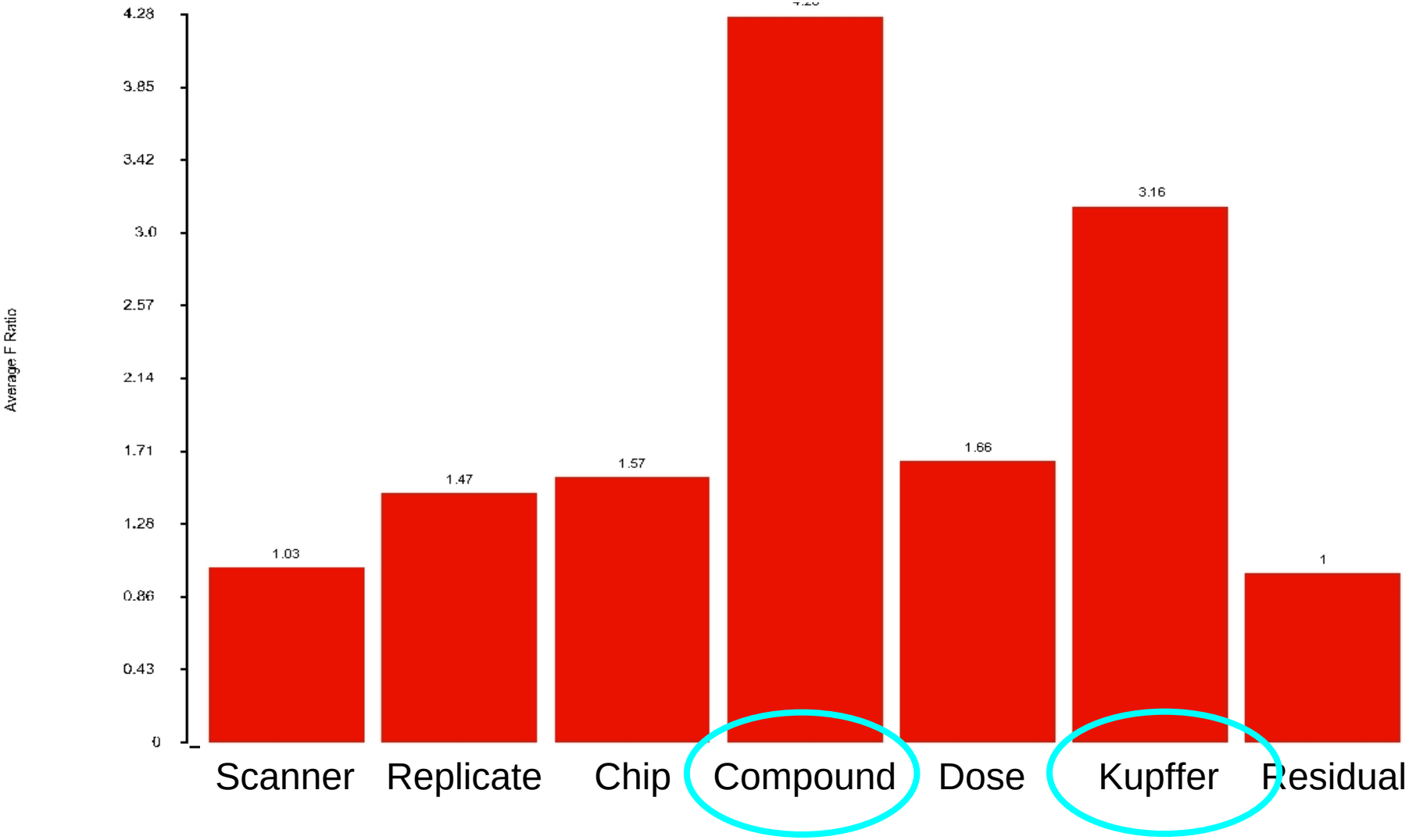
Experimental Design



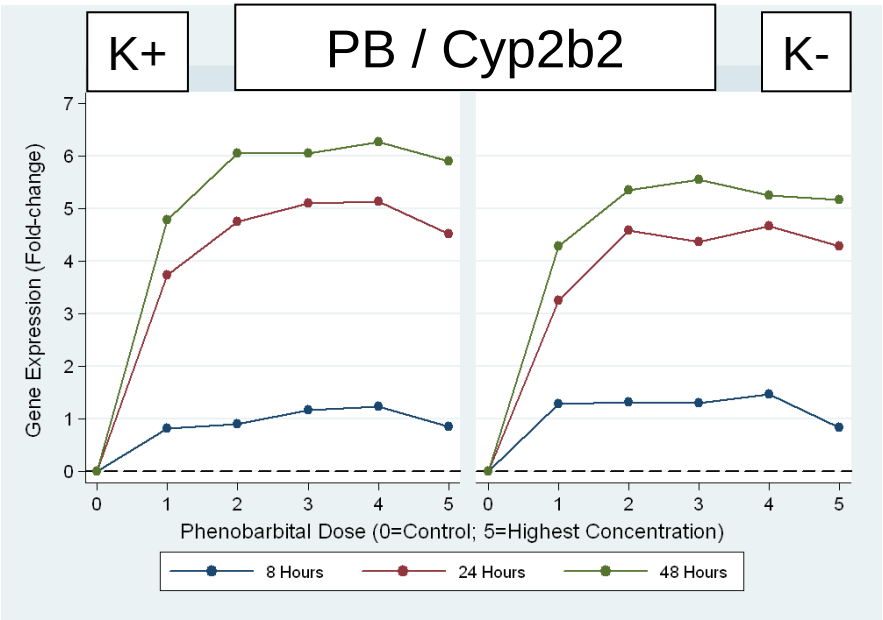
	5 batches (a.k.a. replicates or pools)
x	3 chemicals
x	2 Kupffer treatments
x	6 doses including controls
180	Total Arrays
-	12 failures (2 batches at highest dose)
168	**Total Arrays**

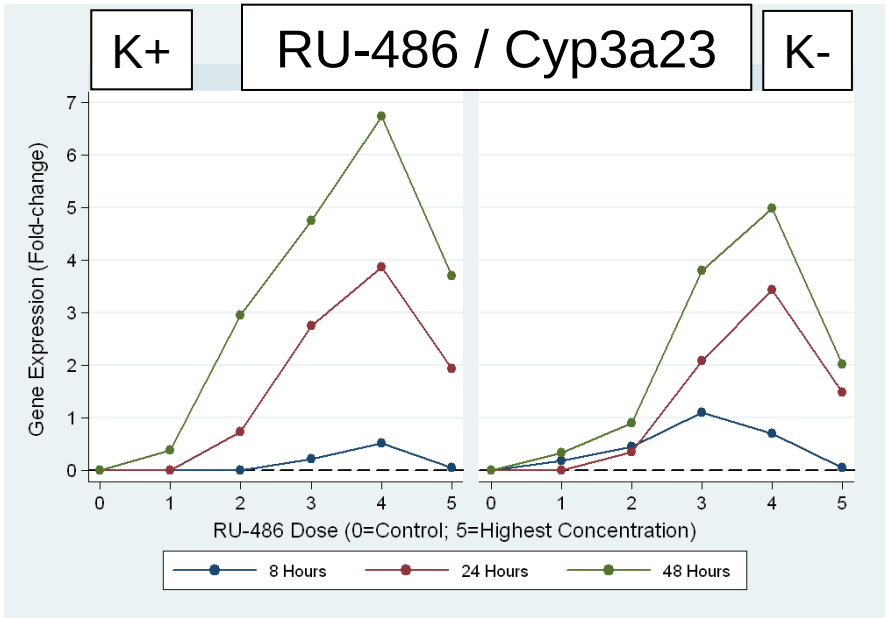
Microarrays run by Expression Analysis

Microarray Variance Component Analysis
Kupffer Cells Do Matter



Single Target Gene PCR

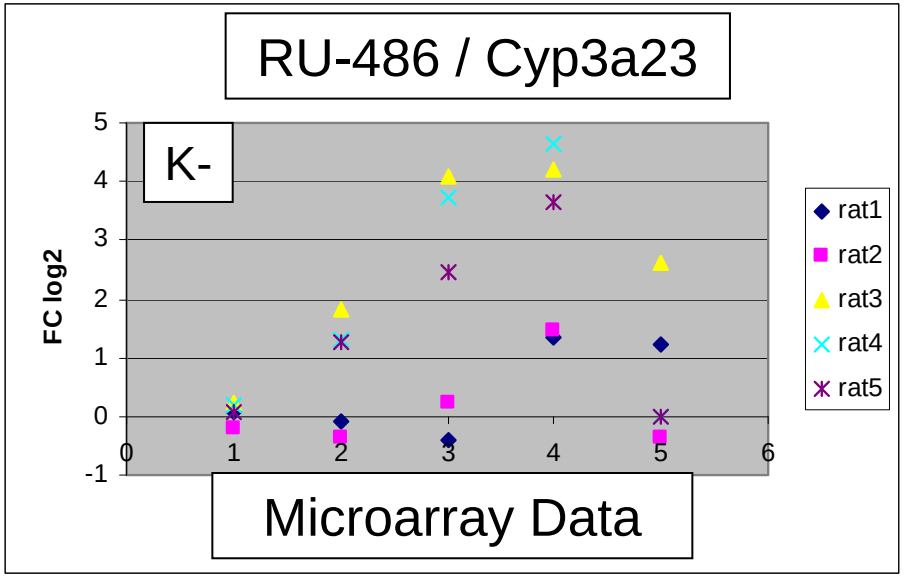




8 hours
24 hours
48 hours

Mild dose-response behavior

Mild Kupffer (K+/-) effect that retains dose-response shape



Pathway Analysis (one of several tried)

1. For each chemical / dose / replicate (pool)
   - Select significantly differentially expressed genes
   - Assign each gene to a pattern [next slide]
   - Calculate enrichment of pathways for patterns
   - Focus initially on KEGG pathways

2. Record how many replicates had pathway signature
   - Indicates reproducibility of method

Generating Signatures or Patterns for Genes

- For each Gene / Dose/ replicate
- Is gene significantly changed from baseline? (0 or + or -)
- Assign each gene to one of $2^{N\text{-dose}}$ patterns

Pattern ID	dose1	dose2	dose3	dose4
0	0	0	0	0
1	0	0	0	+
2	0	0	0	-
3	0	0	+	0
4	0	0	+	+
…	…	…	…	…
80	-	-	-	-

No change any dose

Up-regulated at only the highest dose

Down-regulated at all doses

Derive LOPRL: Combine Genes into Pathways

Pattern	Path 1	Path 2	Path 3	LOPRL
++++	0	1	4	Dose 1
0+++	0	4	1	Dose 2
00++	5	0	0	Dose 3

Number of replicates (out of 5)

Results

Xenobiotic metabolism

LOPRL=Dose 1
Phenobarbital, Kupffer + or –
4/5 replicates

LOPRL=Dose 1
RU-486, Kupffer +

LOPRL=Dose 3
RU-486, Kupffer –
3/5 replicates

LOPRL=Dose 1
Wyeth, Kupffer + or –
5/ 5 replicates

Immune System

LOPRL=Dose 2
RU-486, Kupffer + or -
4/5 replicates

clust ID	signature	chemical	Metabolism: CarboHydrate Metabolism	Energy Metabolism	Lipid Metabolism	Amino Acid Metabolism	Metabolism of other am	Metabolism of cofactors	Biosynthesis of secondary	Xenobiotics Biodegradation	EP: Membrane transport	CP: Endocrine system	Immune system	
40	+ + + +	PB KC-			4					4				PB
13	0 + + +	PB KC-			3					4				
1	0 0 0 +	PB KC-			4	3				3				
40	+ + + +	PB KC+			4					4				
13	0 + + +	PB KC+											3	
4	0 0 + +	PB KC+			3									
1	0 0 0 +	PB KC+	4		4			3		4				
40	+ + + +	RU KC-			3									PU
26	0 - - -	RU KC-	3			4	3							
13	0 + + +	RU KC-											4	
12	0 + + 0	RU KC-											3	
8	0 0 - -	RU KC-				3			3					
4	0 0 + +	RU KC-								3				
1	0 0 0 +	RU KC-											3	
40	+ + + +	RU KC+								3				
26	0 - - -	RU KC+			4	4	4							
13	0 + + +	RU KC+											4	
12	0 + + 0	RU KC+											3	
10	0 + 0 +	RU KC+											3	
8	0 0 - -	RU KC+	3			3								
4	0 0 + +	RU KC+									3			
3	0 0 + 0	RU KC+											3	
2	0 0 0 -	RU KC+		3										
80	- - - -	WY KC-				4								WY
40	+ + + +	WY KC-	4		5	4	4		4	5		4		
39	+ + + 0	WY KC-			4									
13	0 + + +	WY KC-											4	
4	0 0 + +	WY KC-			3									
80	- - - -	WY KC+				3								
40	+ + + +	WY KC+	4		5	4	4		4	5		5		
13	0 + + +	WY KC+											3	
4	0 0 + +	WY KC+	3											

Summary

- Method to use genomics microarray data to find a pathway-based dose-response metric: LOPRL

- Cell system: primary rat hepatocytes
  - Model for chemical-induced liver toxicity
  - Addition of Kupffer cells shows some effect on XME induction

- Approach is not yet reproducible enough to eliminate need for replicates