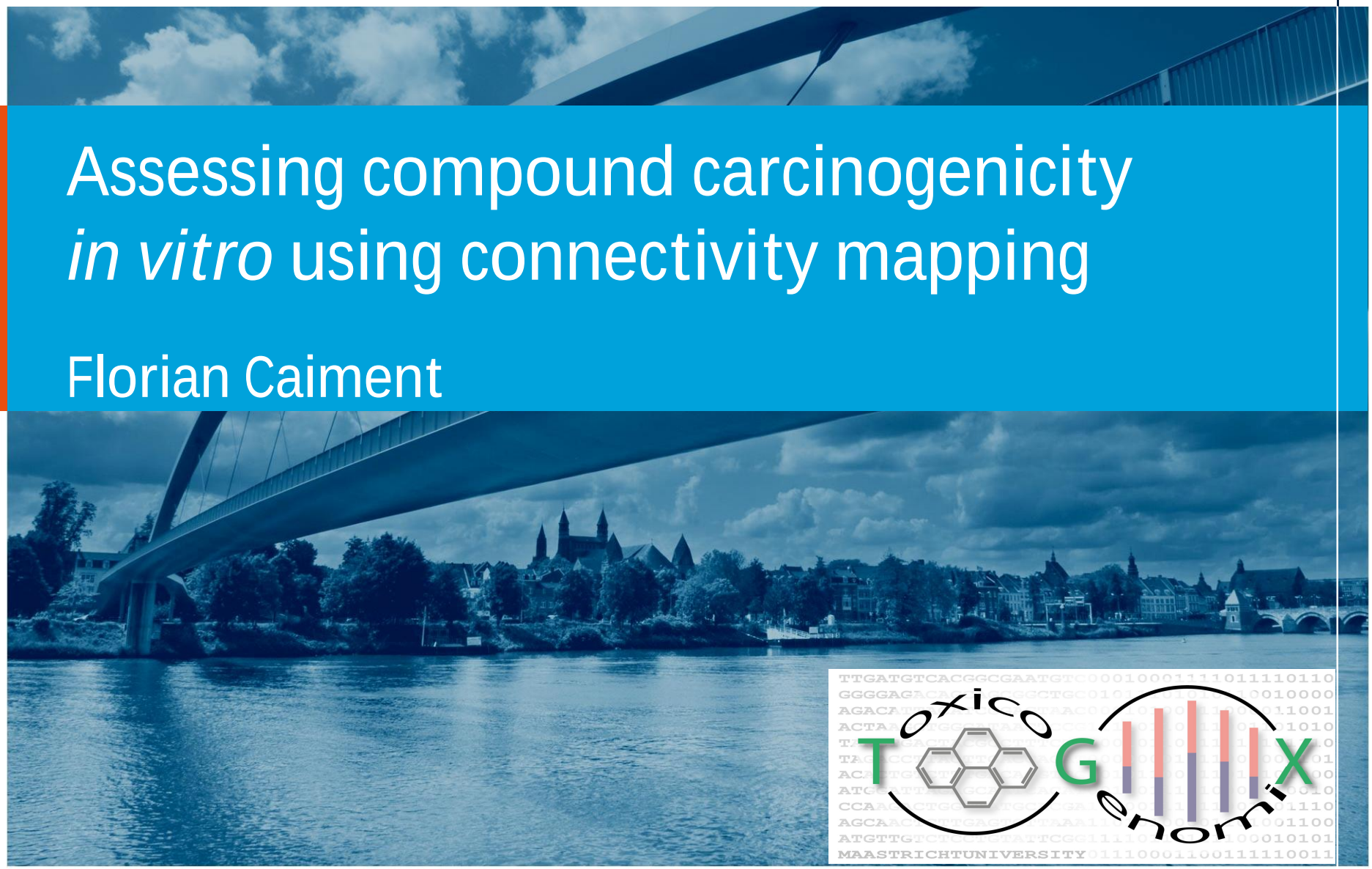
Maastricht University Leading in Learning!

GROW School for Oncology and Developmental Biology

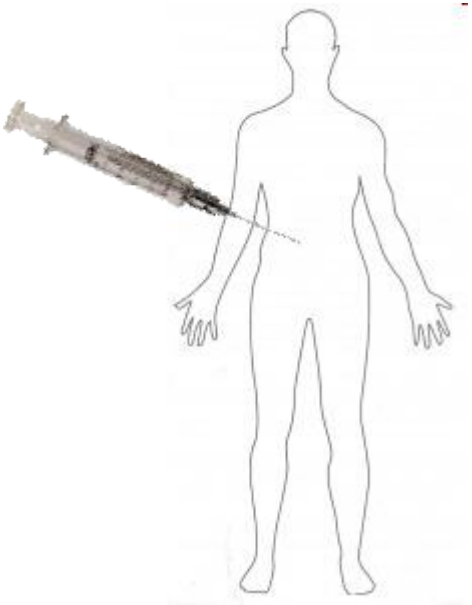
Assessing compound carcinogenicity *in vitro* using connectivity mapping

Florian Caiment

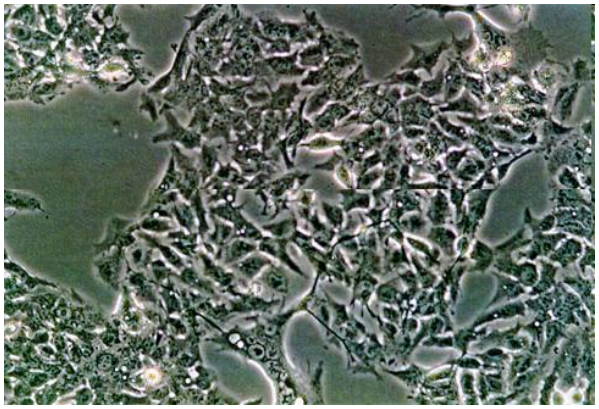


Alternative to animal testing

Compound X



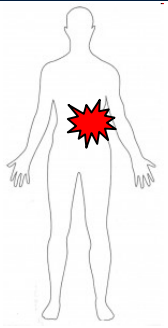
***in vitro* toxicogenomics**

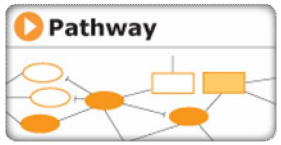


60% accuracy

3R : Reduction, Refinement, Replacement of animal testing.

in vitro toxicogenomics as alternative





Symbol	IGFBP3
AGL	IL13RA1
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COL1A1	MMP2
COL5A2	PLN
CTBP2	POLRMT
DCK	SGCB
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DPP4	VIM
EIF4EBP1	SRPX
FBN1	PROM1
FGFR2	NAT8
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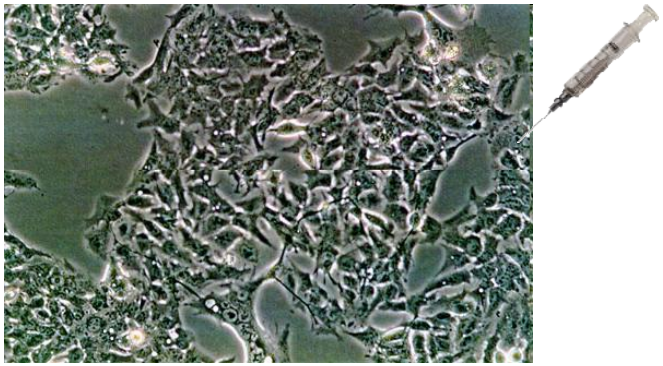
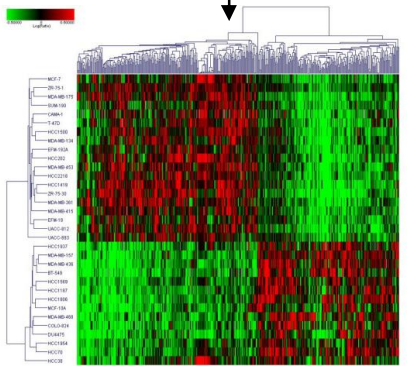
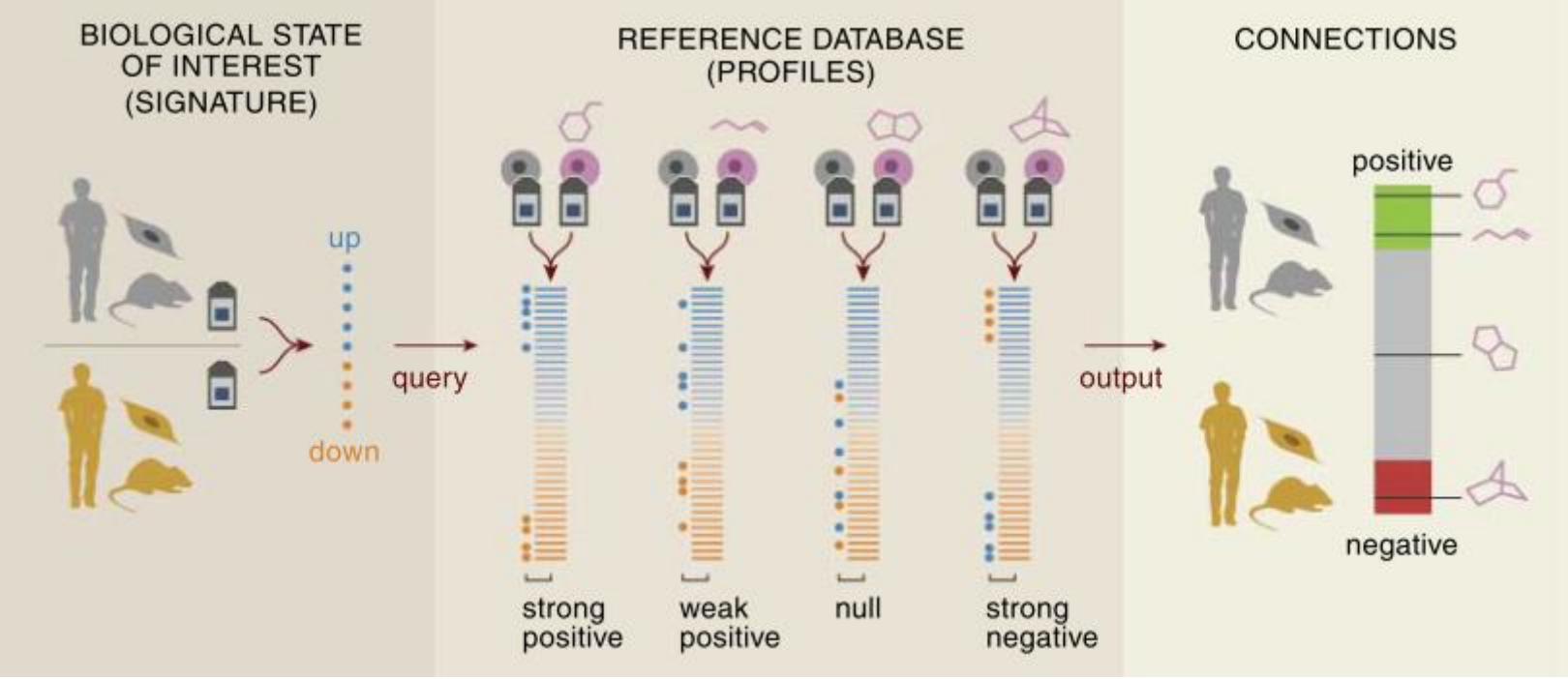
?





The Connectivity Map: Using Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease

Justin Lamb,[1*] Emily D. Crawford,[1†] David Peck,[1] Joshua W. Modell,[1] Irene C. Blat,[1] Matthew J. Wrobel,[1] Jim Lerner,[1] Jean-Philippe Brunet,[1] Aravind Subramanian,[1] Kenneth N. Ross,[1] Michael Reich,[1] Haley Hieronymus,[1,2] Guo Wei,[1,2] Scott A. Armstrong,[2,3] Stephen J. Haggarty,[1,4] Paul A. Clemons,[1] Ru Wei,[1] Steven A. Carr,[1] Eric S. Lander,[1,5,6] Todd R. Golub[1,2,3,5,7*]



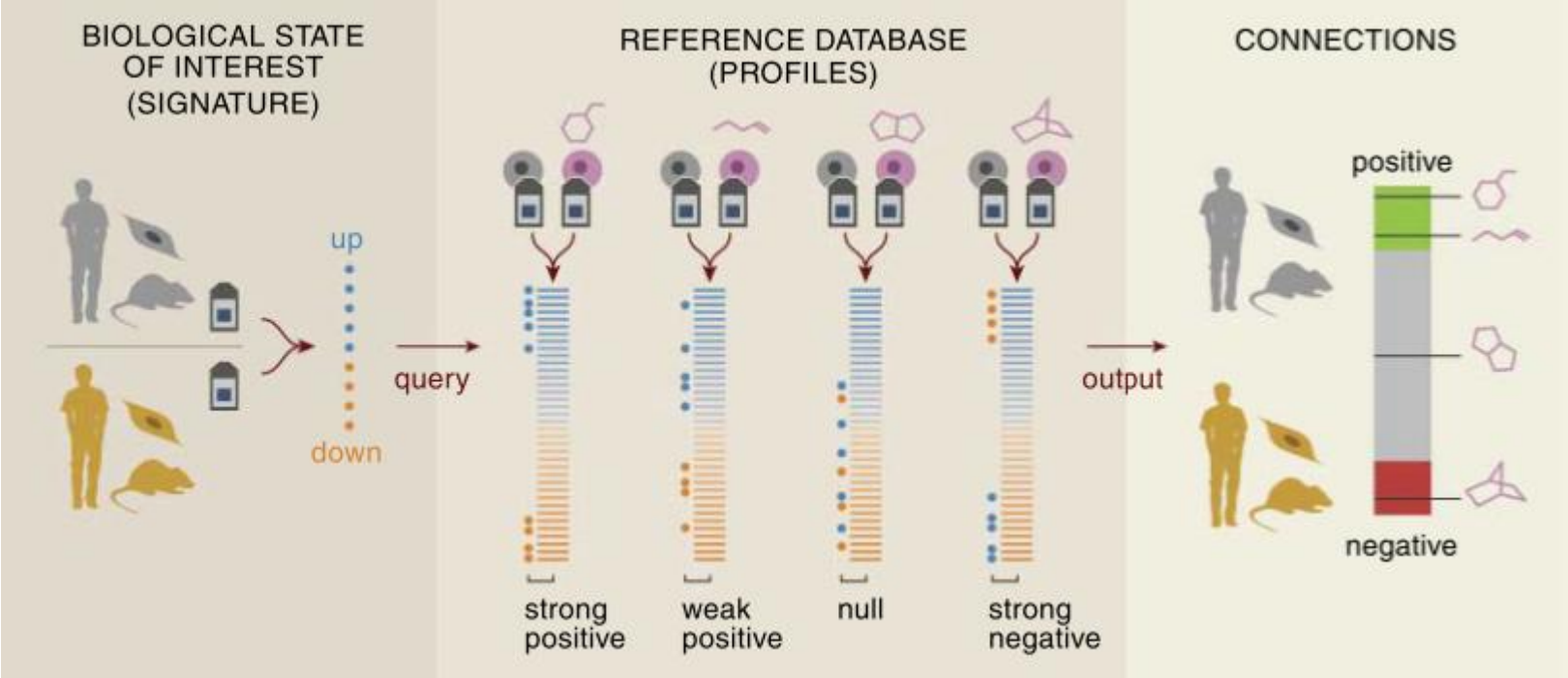
Liver Cancer

HepatoCellular Carcinoma (HCC)

- 5th most common cancer worldwide
- 3rd most common cause of cancer mortality
- Accounts for ~90% of primary liver cancers
- Induced either by hepatic viral infections (HBV/HVC) or hepatic cirrhosis (alcoholism)
  Other known risks: AFB1, cigarette smoking, obesity, diet
- Complex process associated with accumulation of genetic & epigenetic changes that occur during initiation, promotion and progression of the disease

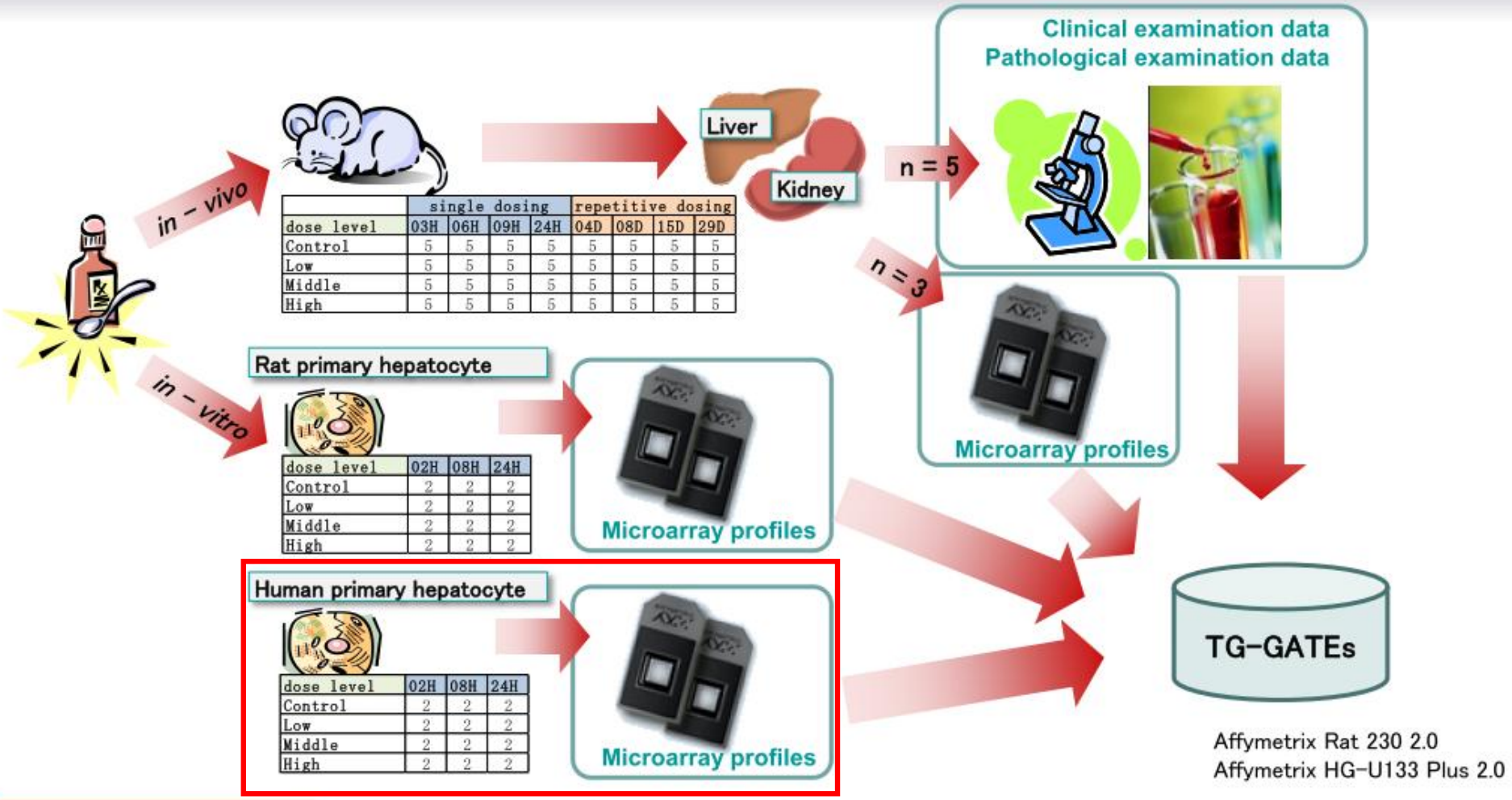
Connectivity Mapping

HCC signature ***in vitro* human liver**




Open TG-GATEs
このDBについて
おしらせ
データ検索
公開データー覧
利用許諾条件
お問合せ
> Top
CompoundかPathologyのどちらか一つの検索条件を選択し[Search]をクリックしてください。
Compound List
select compound
acarbose
acetamidofluorene
acetaminophen
acetazolamide
adapin
ajmaline
allopurinol
allyl alcohol
amiodarone
amitriptyline
上記化合物を一つ選択してください
Search
生物種・実験条件の選択画面に進みます

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**

PHH

158 compounds

Affy_hg u133 plus 2

2 or 3 Times points :
(2h) - 8h, 24h

2 or 3 Doses :
(low)-Medium-High

Control and duplicates
=> 2605 arrays

Compound Classification

- Human Liver Carcinogens (C^{HL}) : 3
  Sufficient data on *human* carcinogenicity on *liver* organ available

- Rodent Liver Carcinogens (C^{RL}) : 23
  Sufficient data on *rodent* carcinogenicity on *liver* organ available

- Non Liver Carcinogens (C^{NL}) : 12
  known human and/or rodent carcinogenic compounds in non-liver target organs

- Non Carcinogens (NC) : 15
  Negative in all available carcinogenicity tests in rodents (often no available information about human studies)

- Unknown Carcinogenicity (UC) : 78
  Unavailable / Unclear *in* all available carcinogenicity tests (mainly in rodents)

International Agency for Research on Cancer

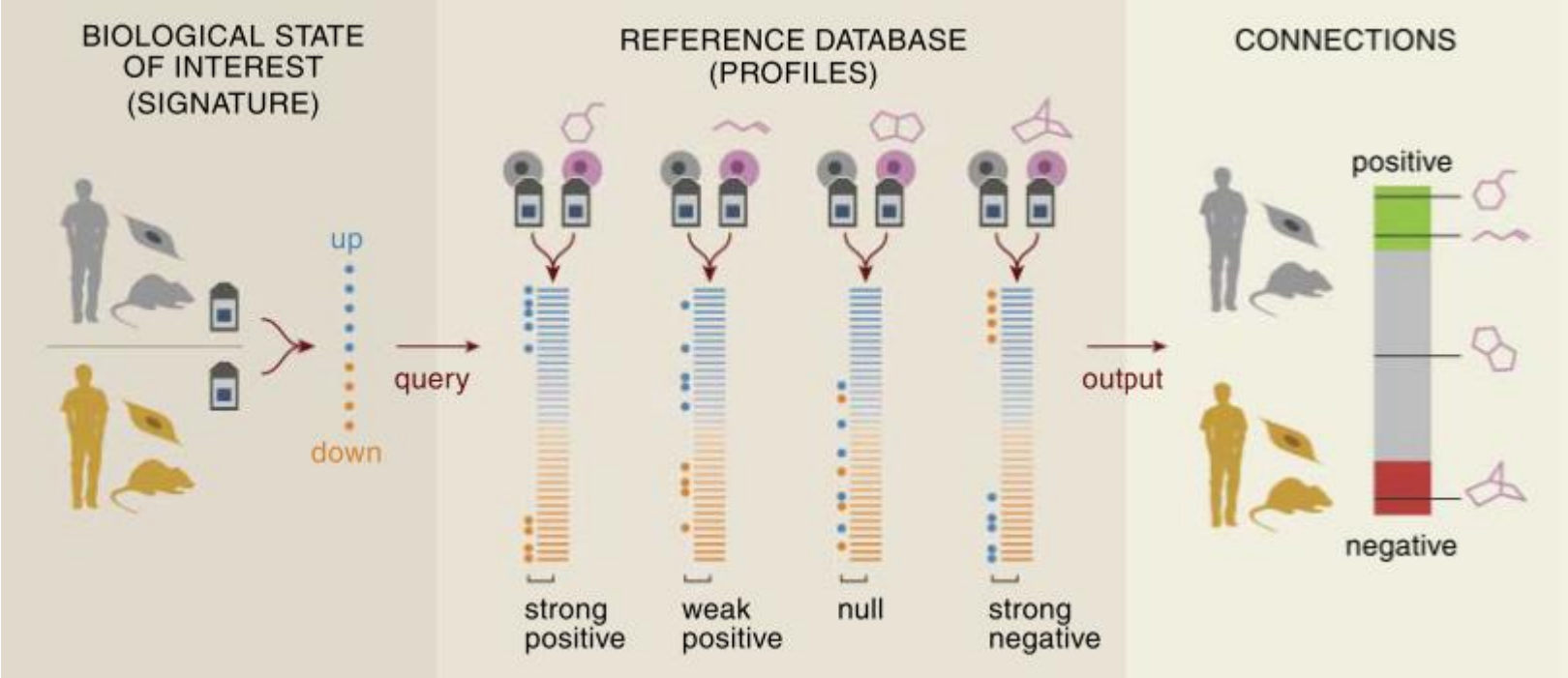
World Health Organization

EPA United States Environmental Protection Agency

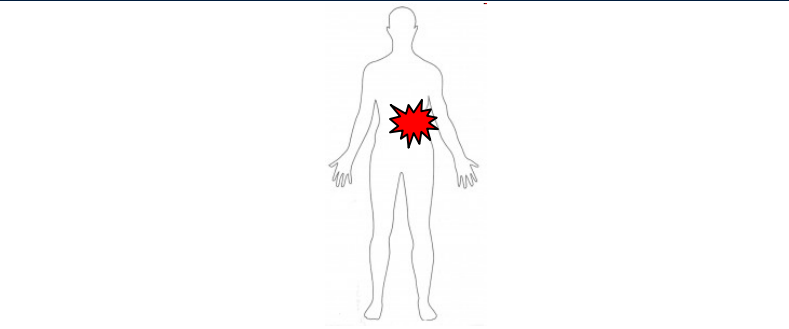
National Toxicology Program
Department of Health and Human Services

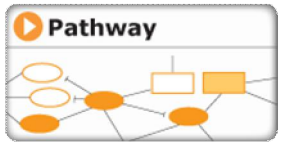
The Carcinogenic Potency Project

Connectivity Mapping



in vitro toxicogenomics as alternative ?





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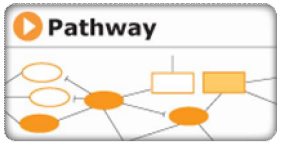
DCK	SGCB
DCN	SP100
DPP4	VIM
EIF4EBP1	SRPX
FBN1	PROM1
FGFR2	NAT8
GJA1	HEPH

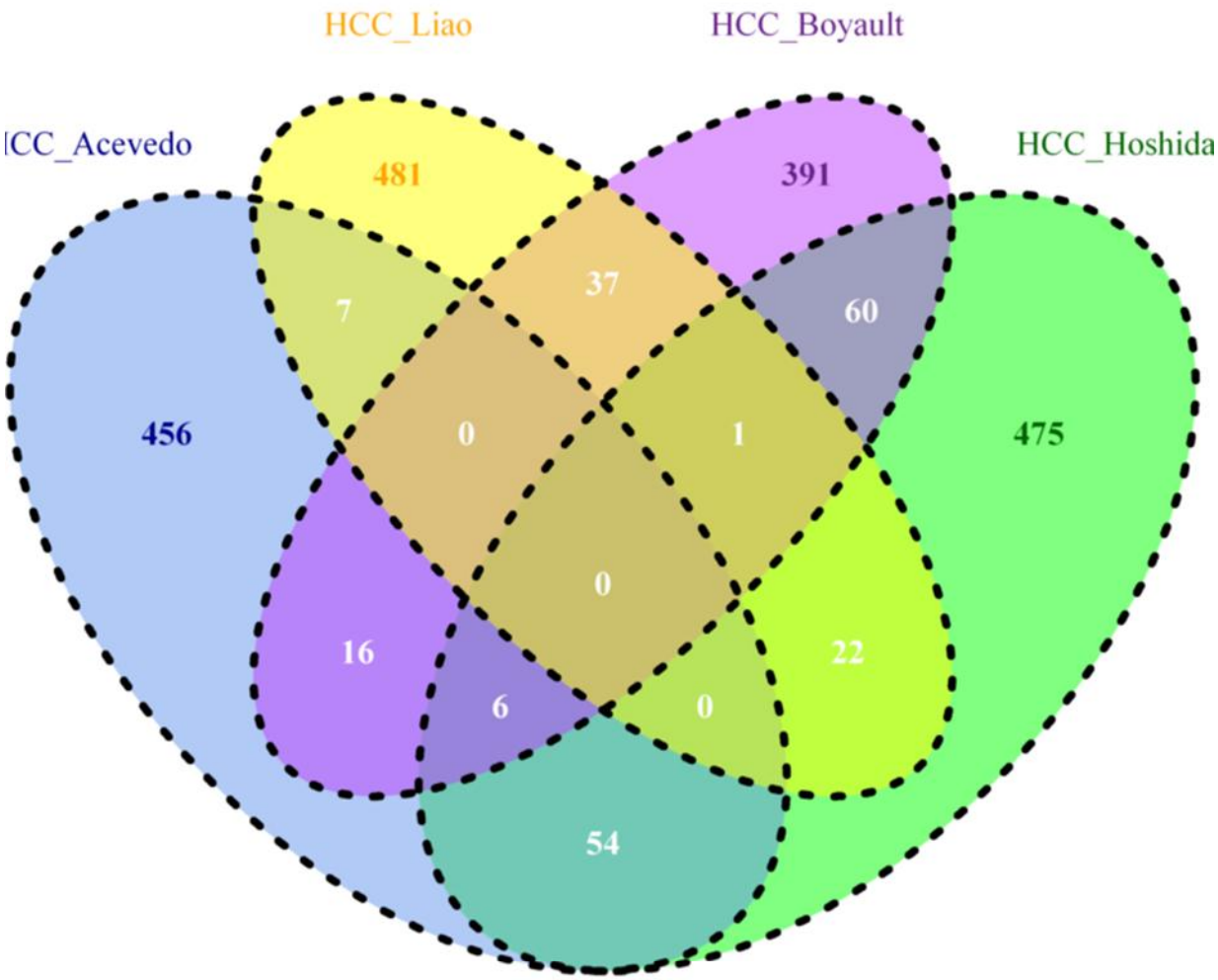
Query signature : genesets



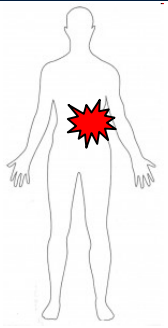
31 genesets selected

5 Databases | **24 Literature** | **2 Pathways**

Genesets Overlap



in vitro toxicogenomics as alternative ?



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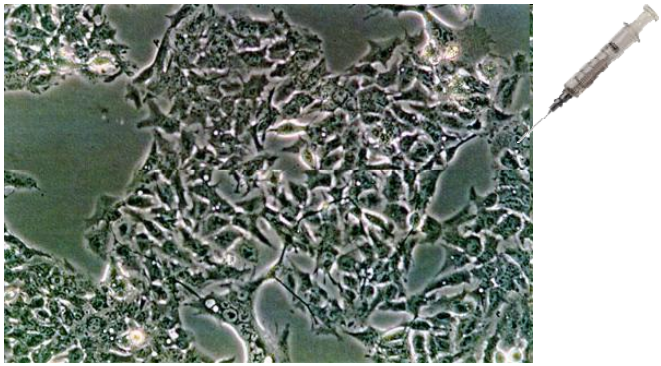
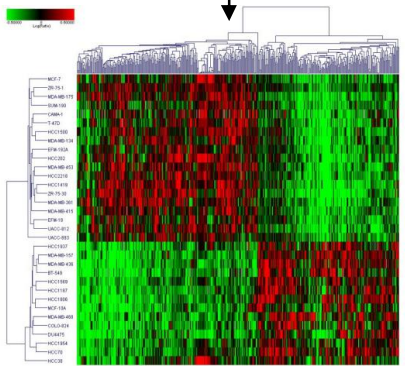
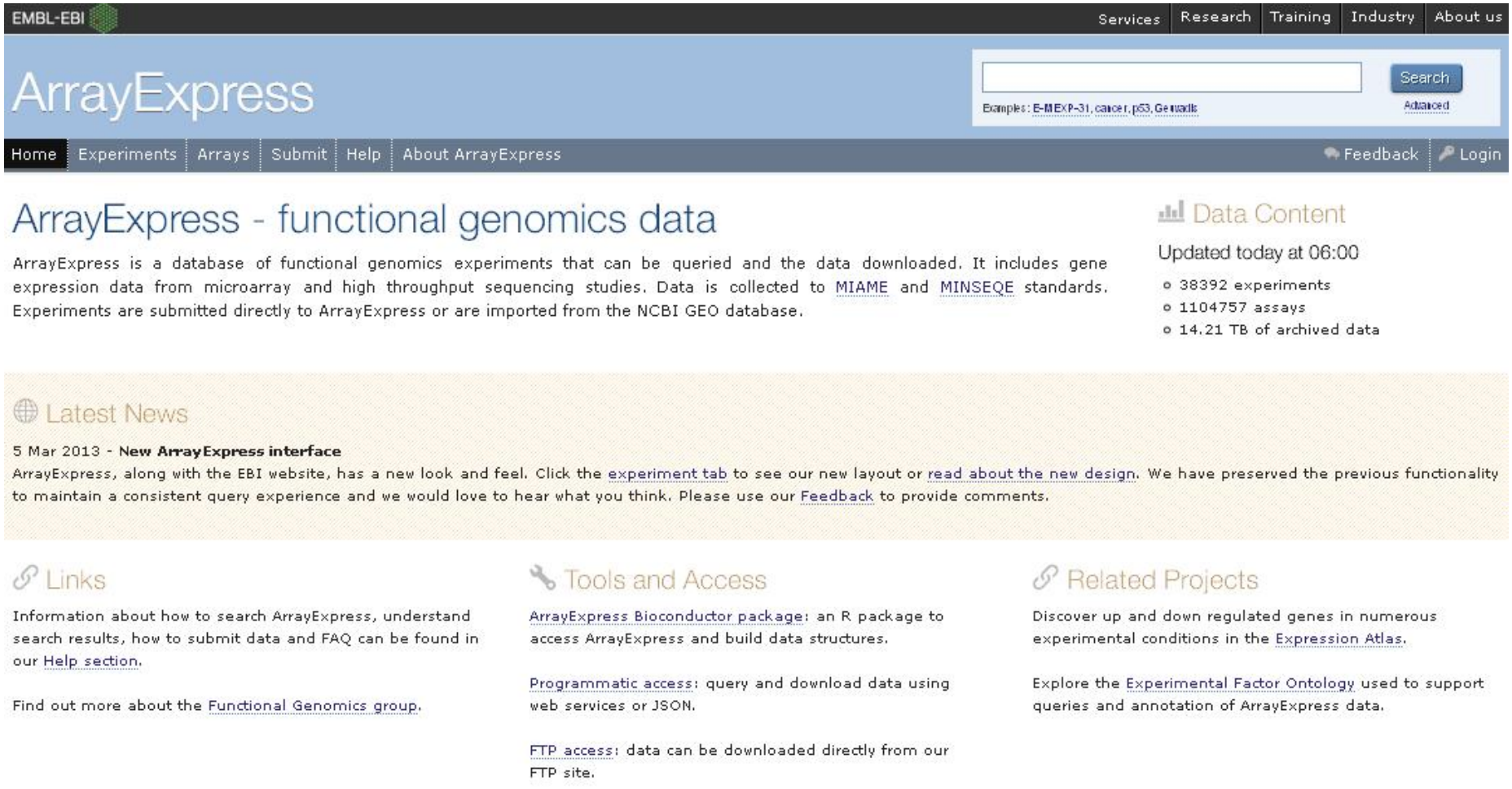
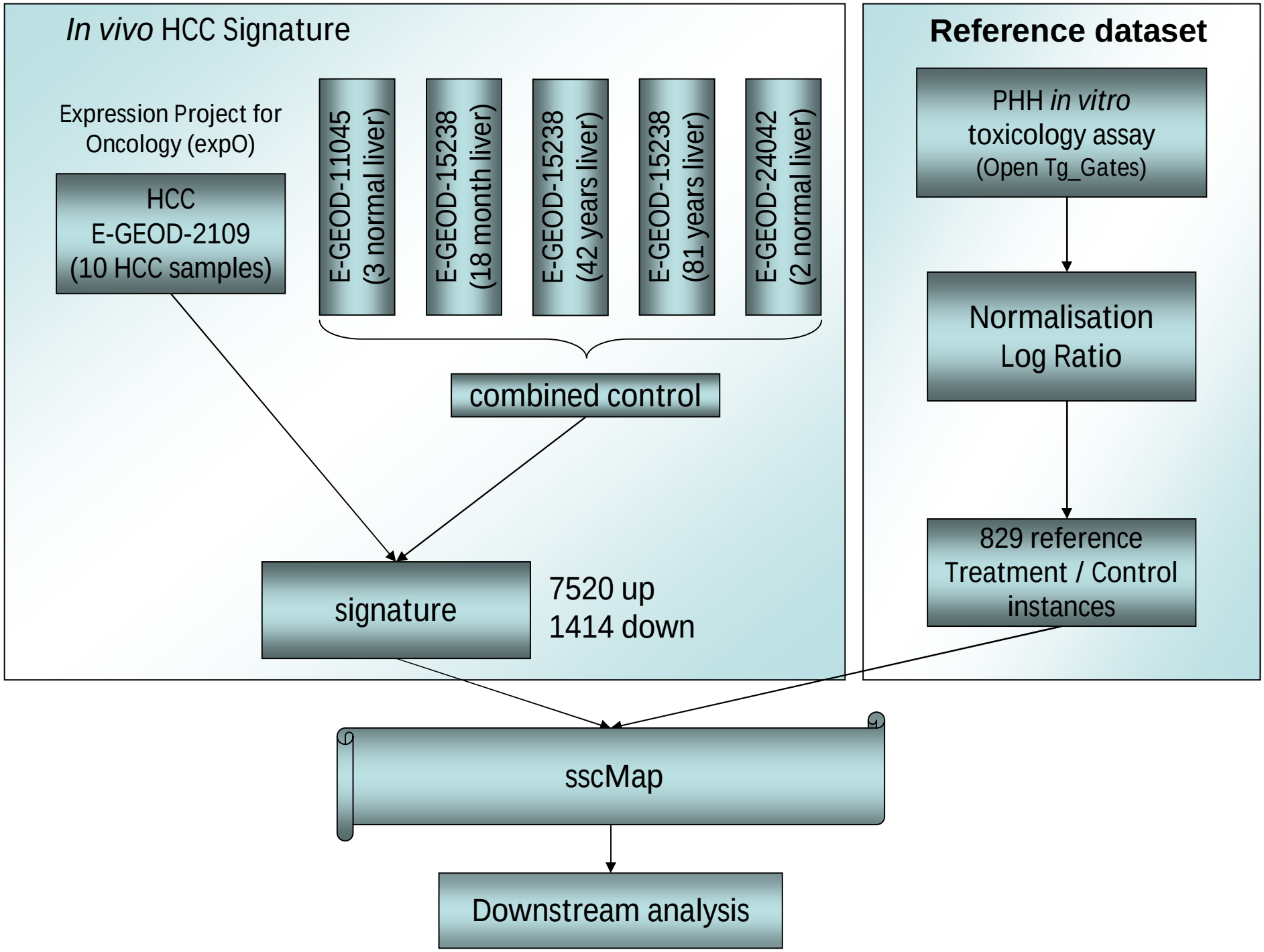
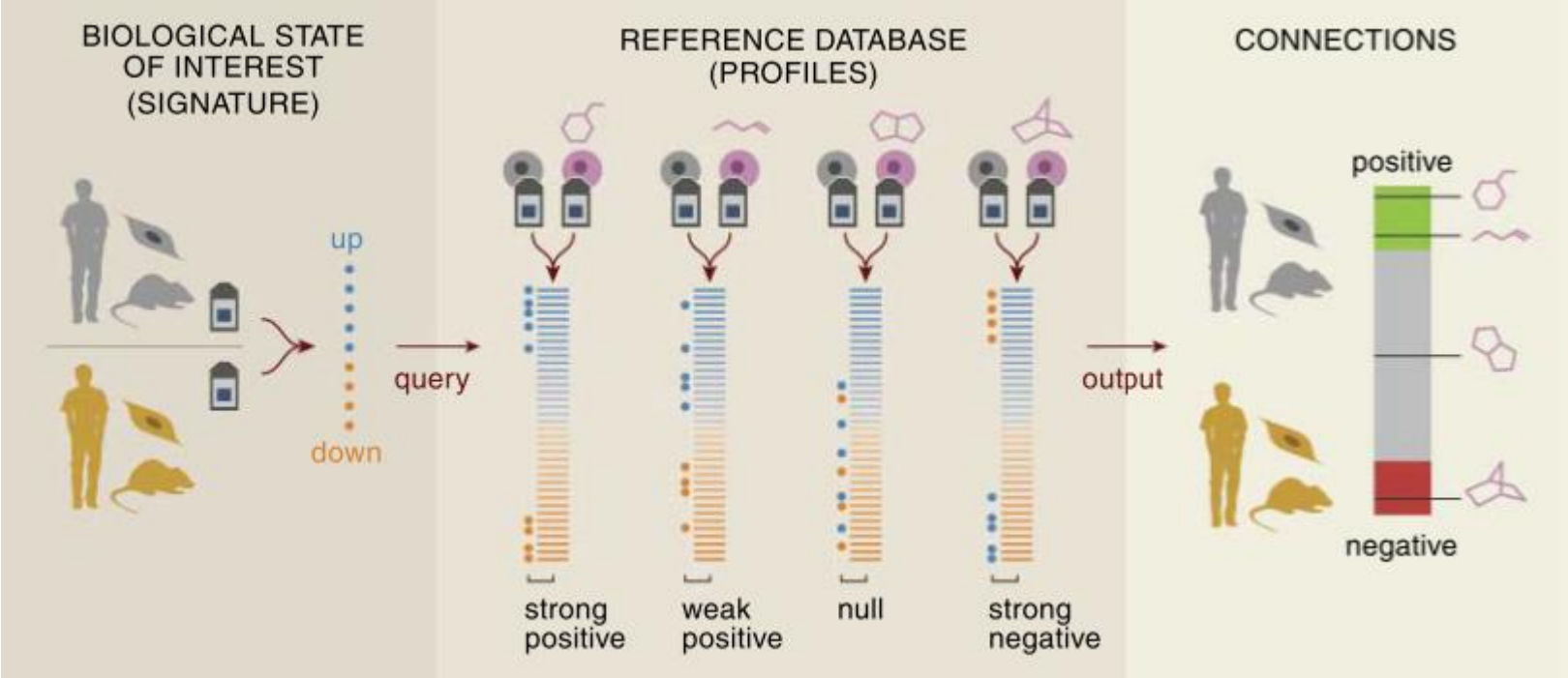




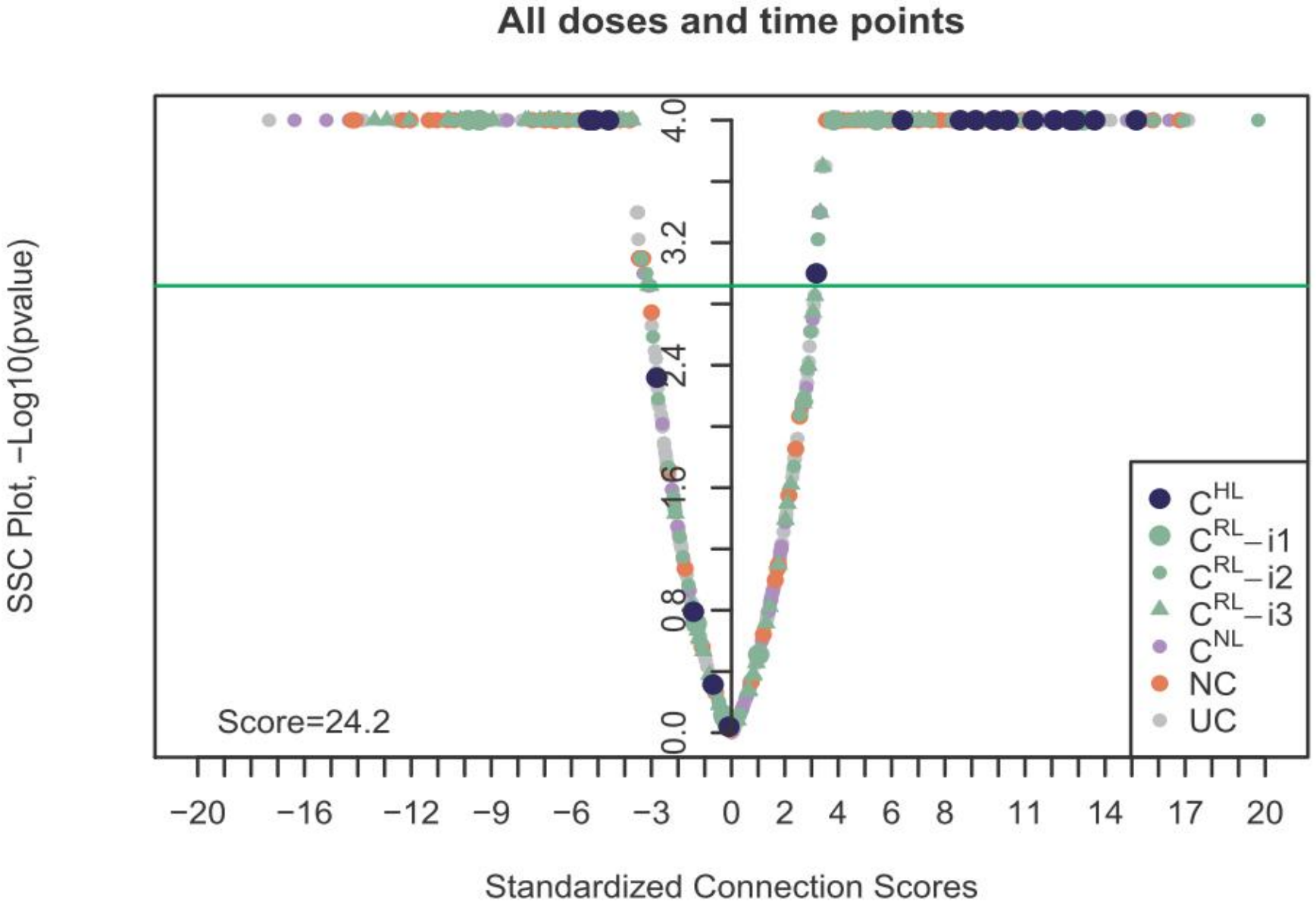
Array Express *in vivo* HCC data



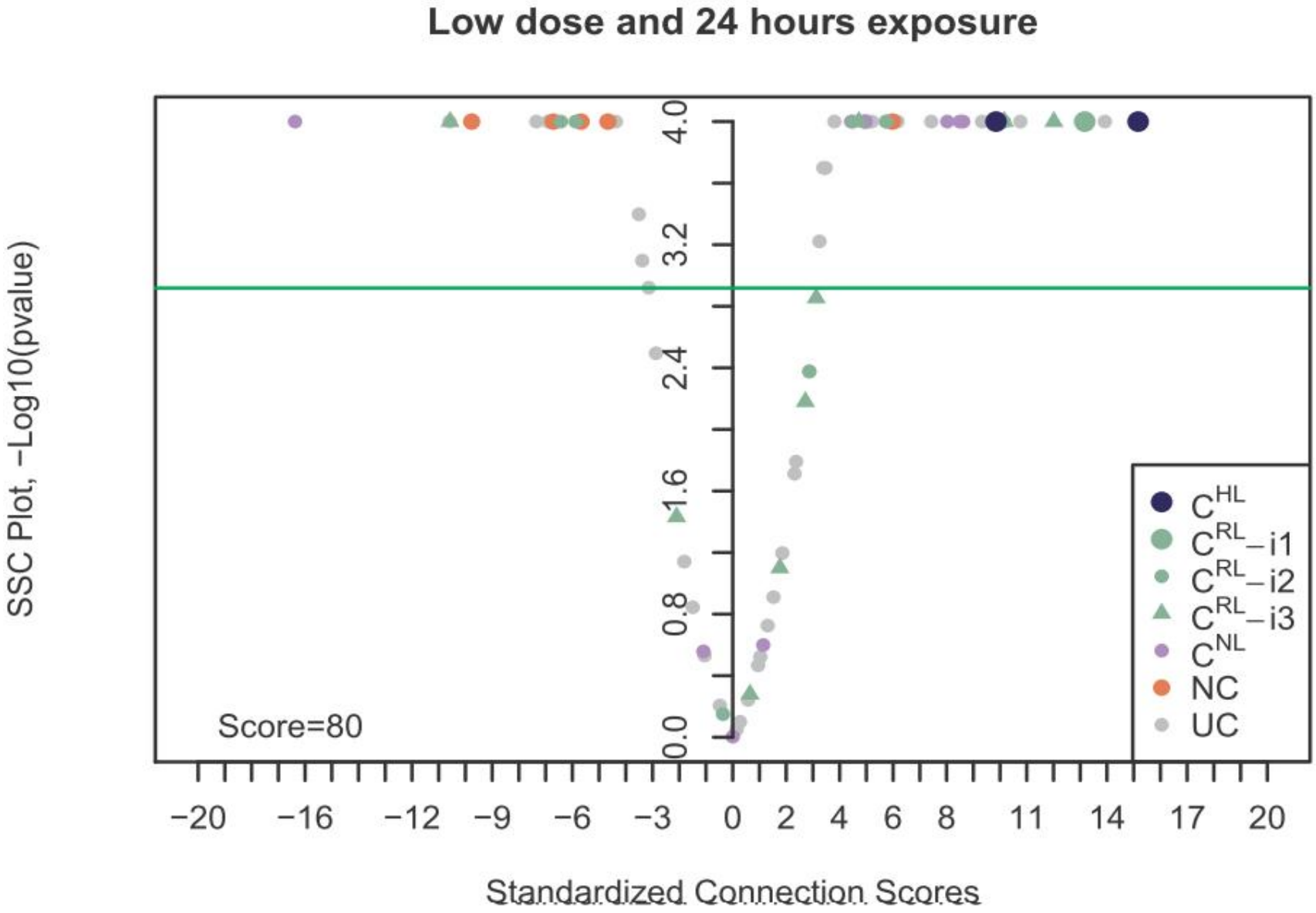

In vivo HCC Signature
Expression Project for Oncology (expO)
HCC E-GEOD-2109 (10 HCC samples)
E-GEOD-11045 (3 normal liver)
E-GEOD-15238 (18 month liver)
E-GEOD-15238 (42 years liver)
E-GEOD-15238 (81 years liver)
E-GEOD-24042 (2 normal liver)
combined control
signature
7520 up
1414 down
Reference dataset
PHH *in vitro* toxicology assay (Open Tg_Gates)
Normalisation Log Ratio
829 reference Treatment / Control instances
sscMap
Downstream analysis


BIOLOGICAL STATE
OF INTEREST
(SIGNATURE)
up
down
query
REFERENCE DATABASE
(PROFILES)
strong
positive
weak
positive
null
strong
negative
output
CONNECTIONS
positive
negative

sscMap results



sscMap results



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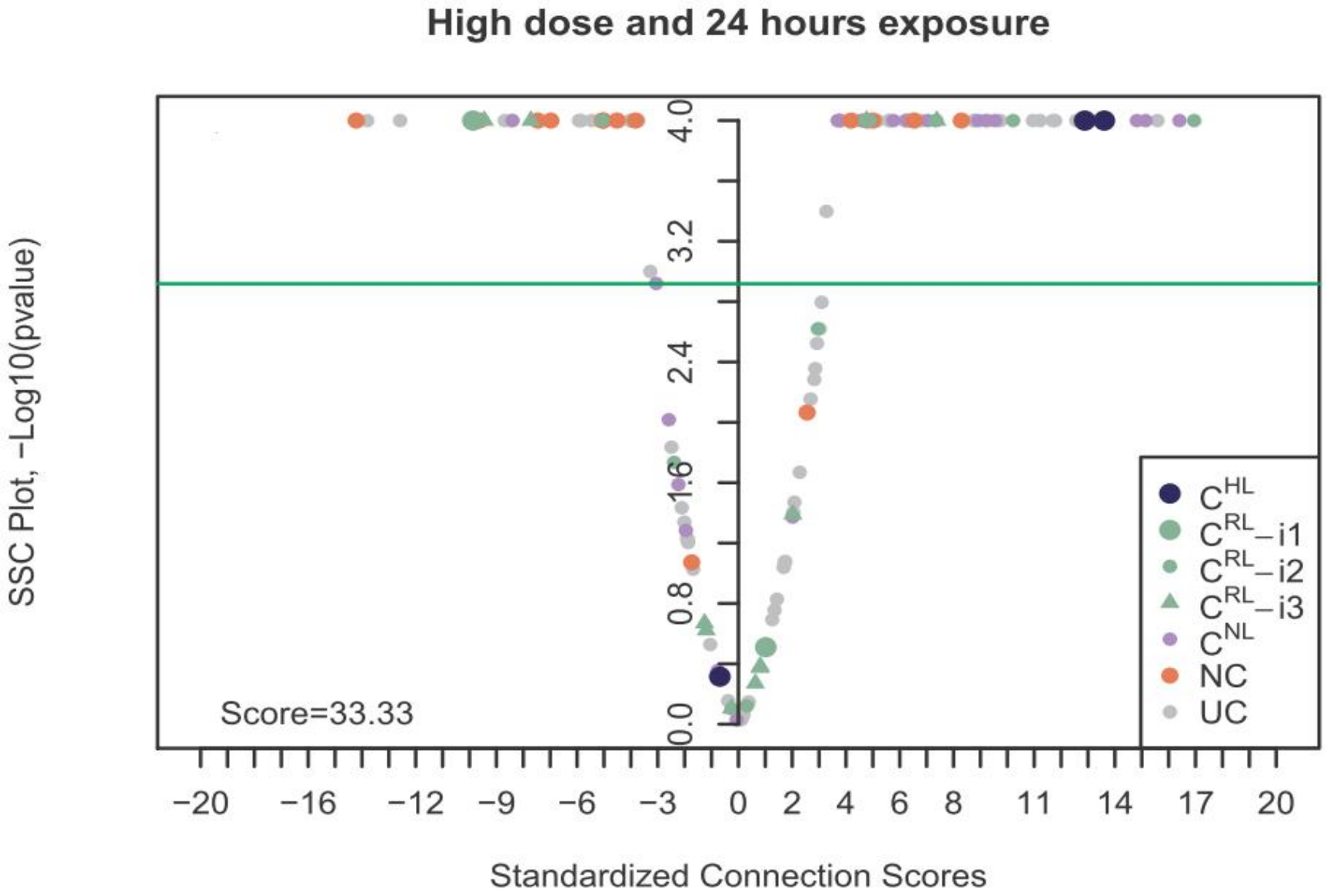
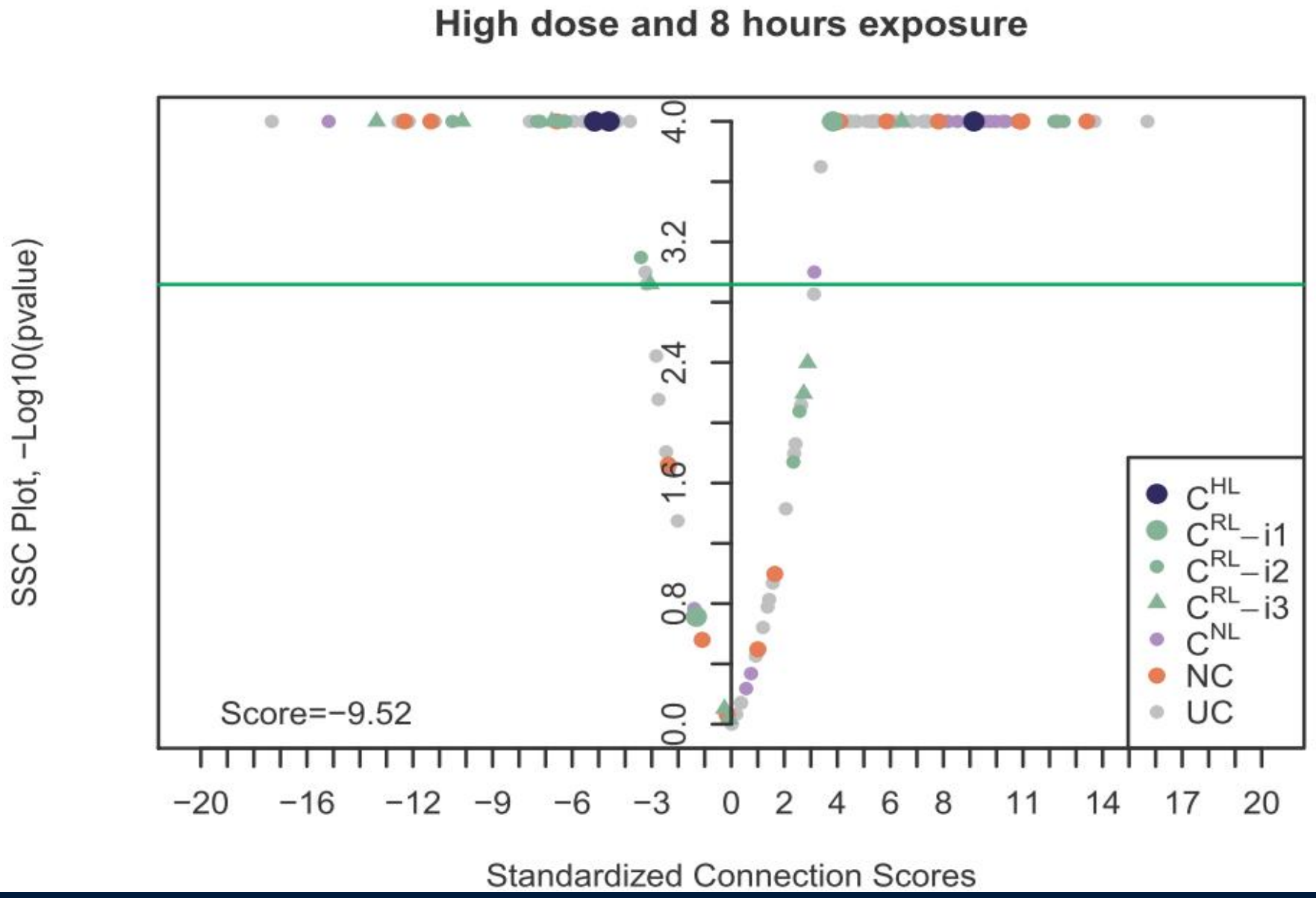
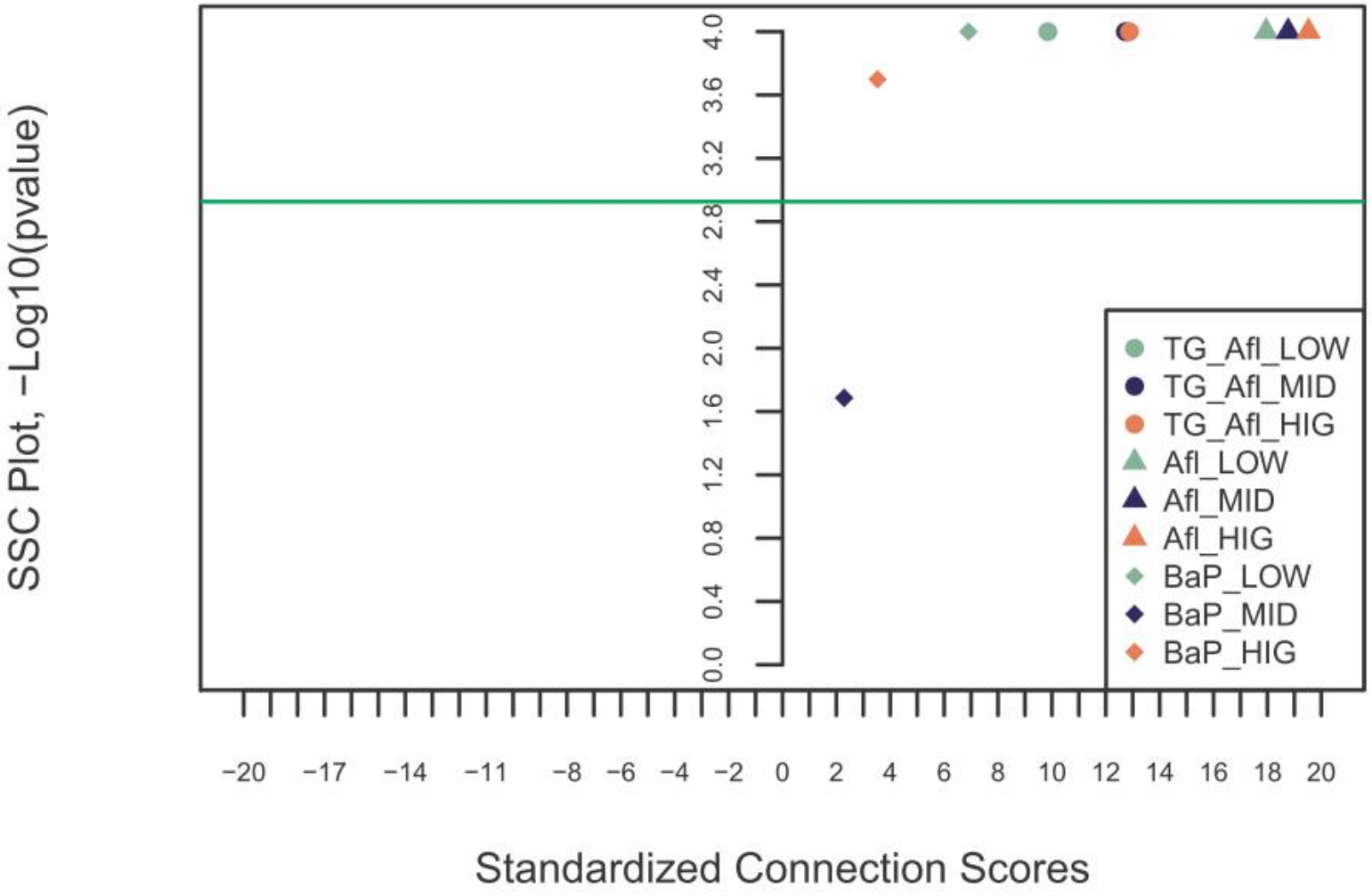


sscMap results



validation set



Conclusion:

- *in vivo* HHC signature with many genes
- best classification at low dose and 24h
- better classification at 24h than 8h
- classification accuracy decrease with concentration

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Assessing compound carcinogenicity *in vitro* using connectivity mapping

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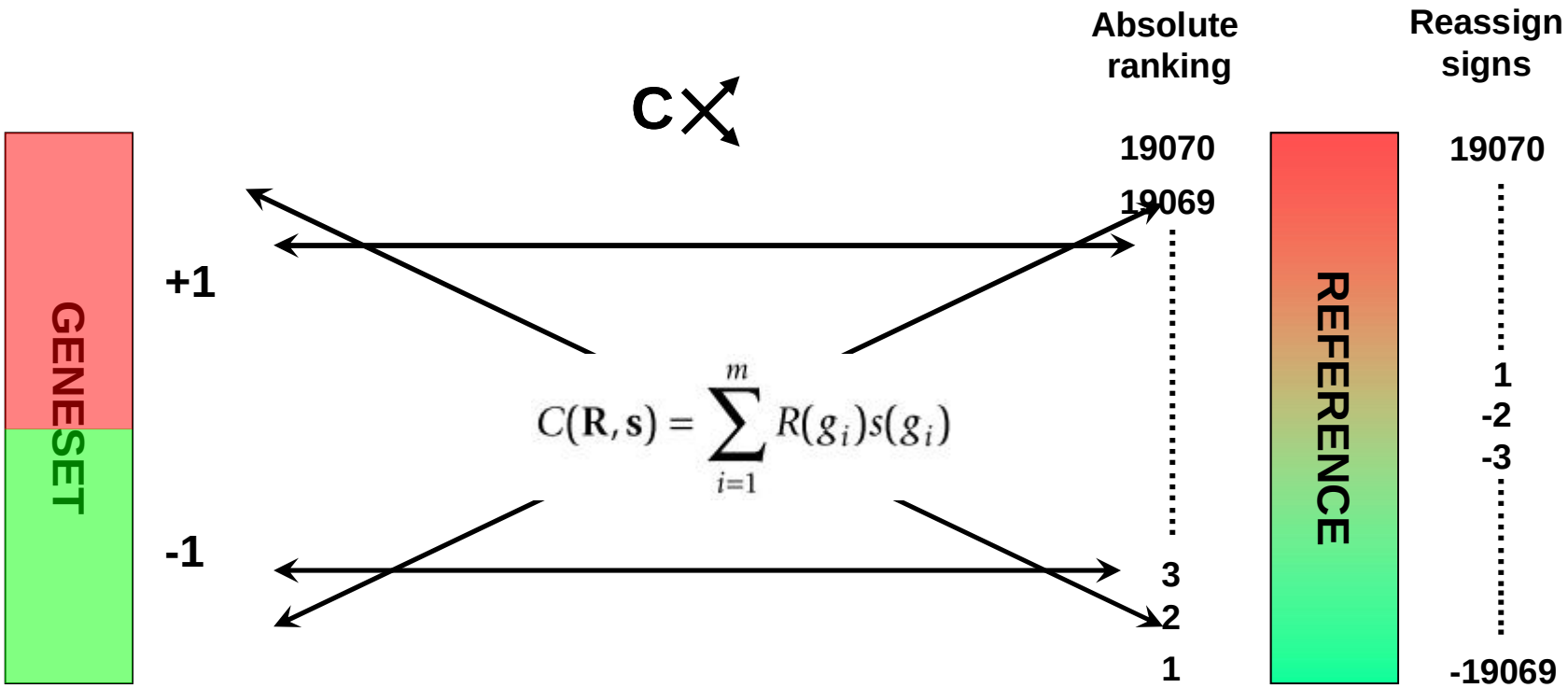
Acknowledgment

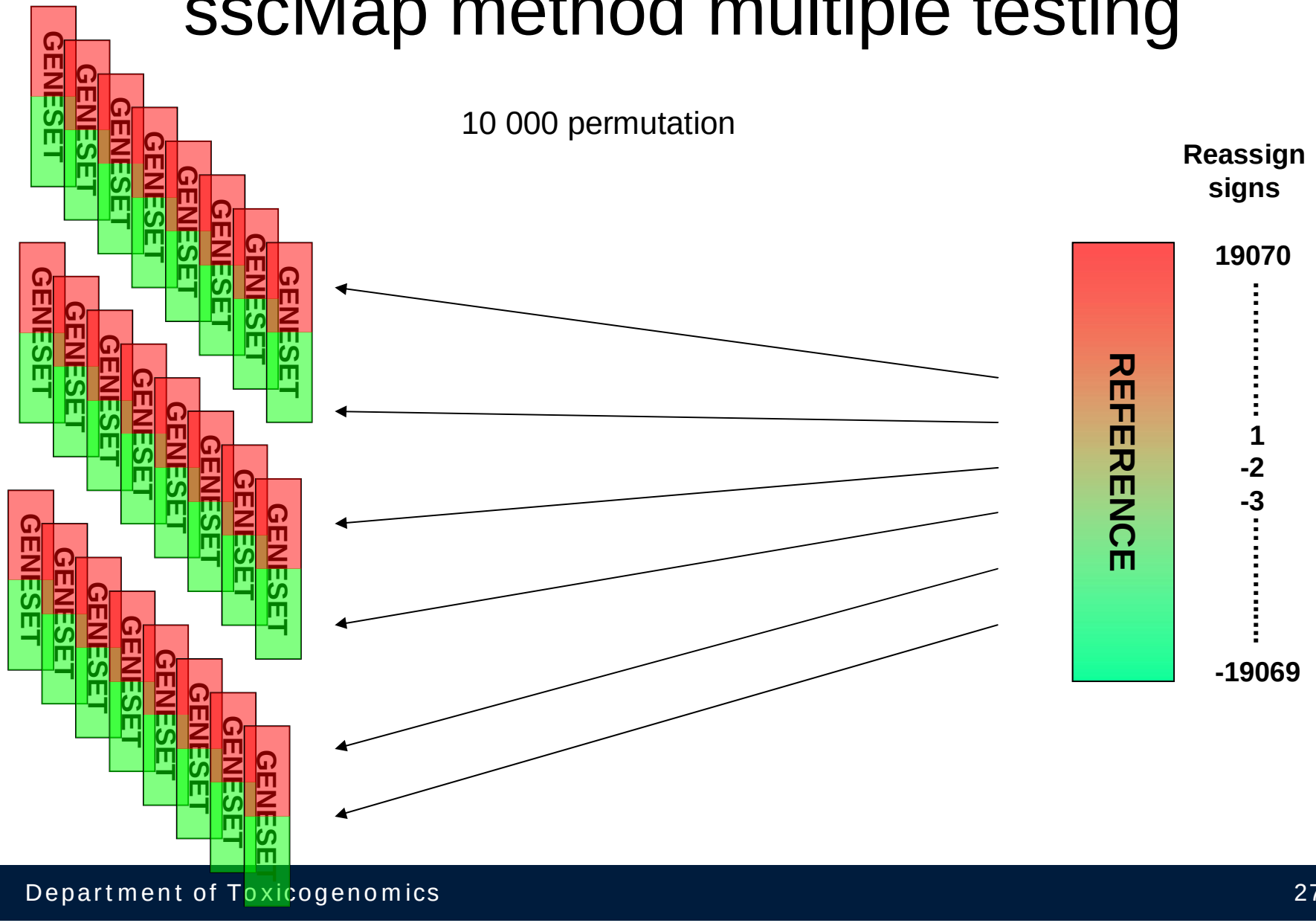
- Maria Tsamou
- Danyel Jennen
- Jos Kleinjans

QUESTIONS?

sscMap method



sscMap method multiple testing



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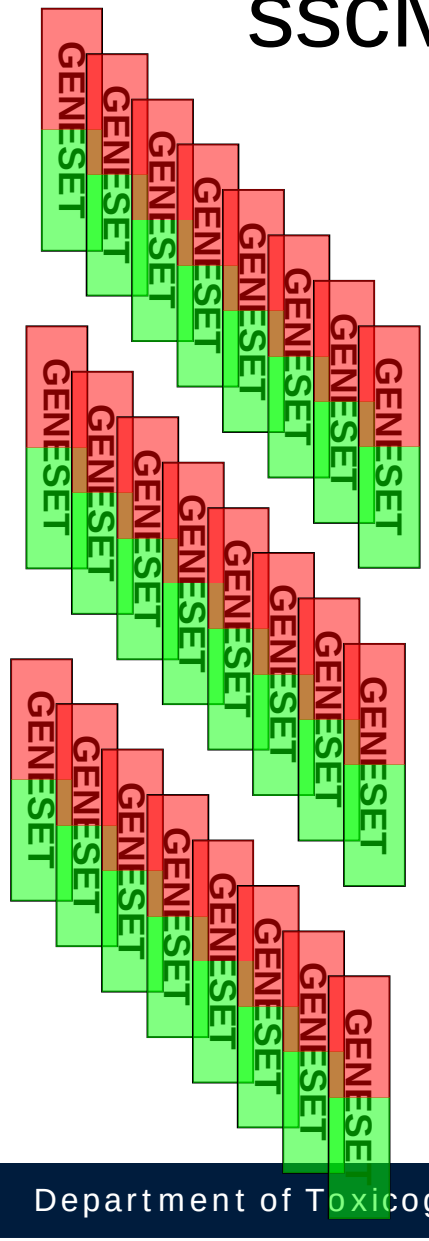
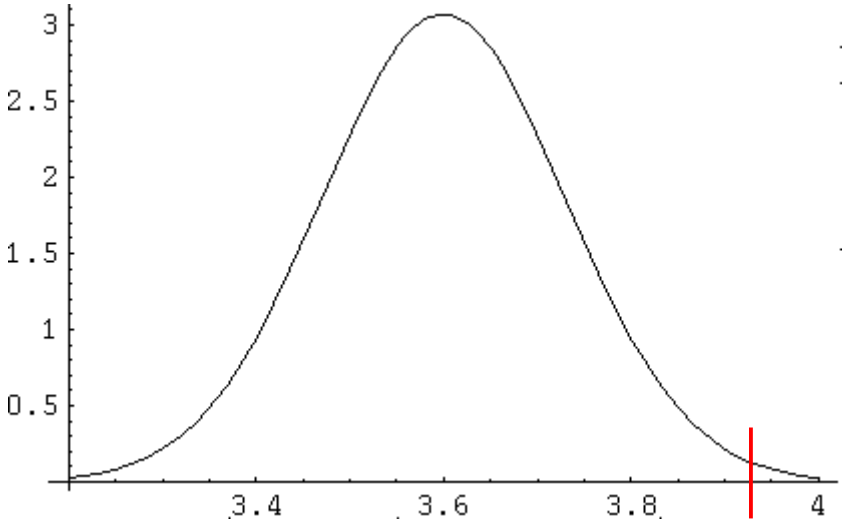


10 000 permutation



Reassign signs

19070

1

-2

-3

-19069