The value of Omics to chemical risk assessment

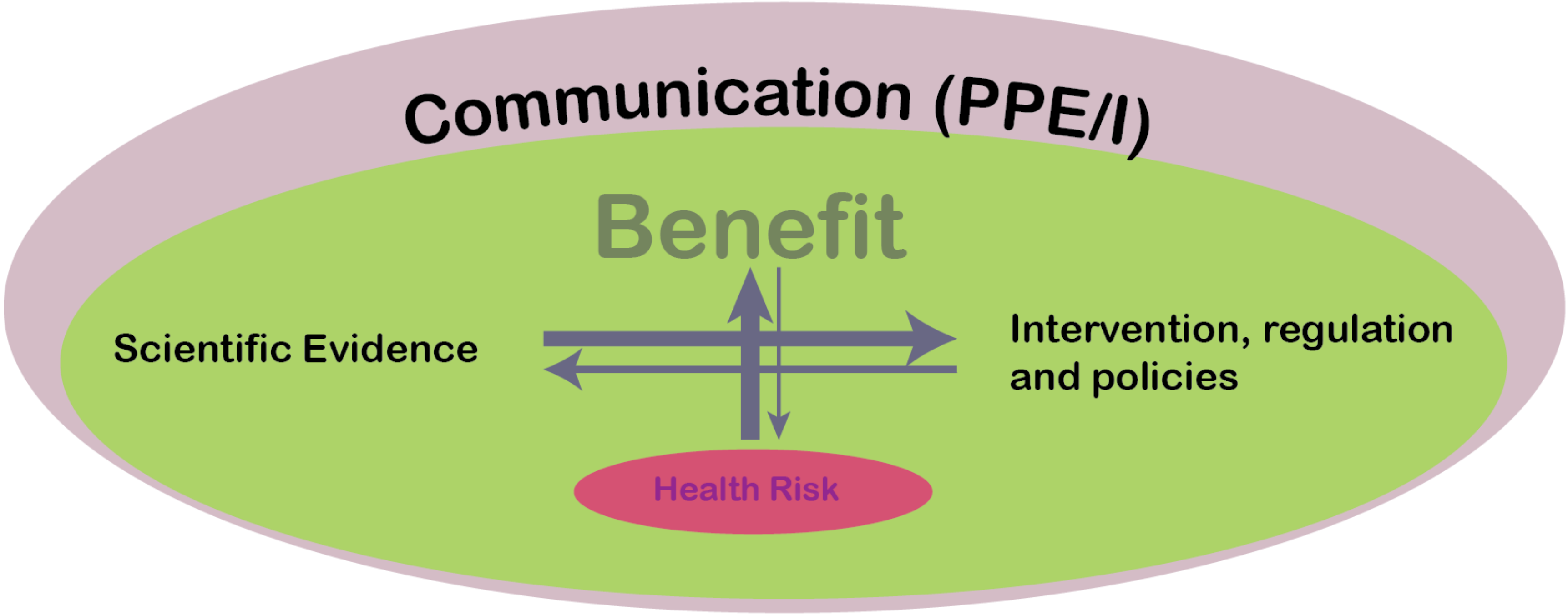
Timothy W Gant

There is a focus on transcriptomics in this talk but for example only.
All omics are useful in risk assessment

Outline

- What are we aiming to achieve?
- What is omics and systems toxicology?
- Hazard and read across
- Modes of Action – and critical points
- Biomarkers
- Exposure
- Outcomes
- A Personal risk assessment
- The challenge of bioinformatics

What do we want to achieve?



What is omics?

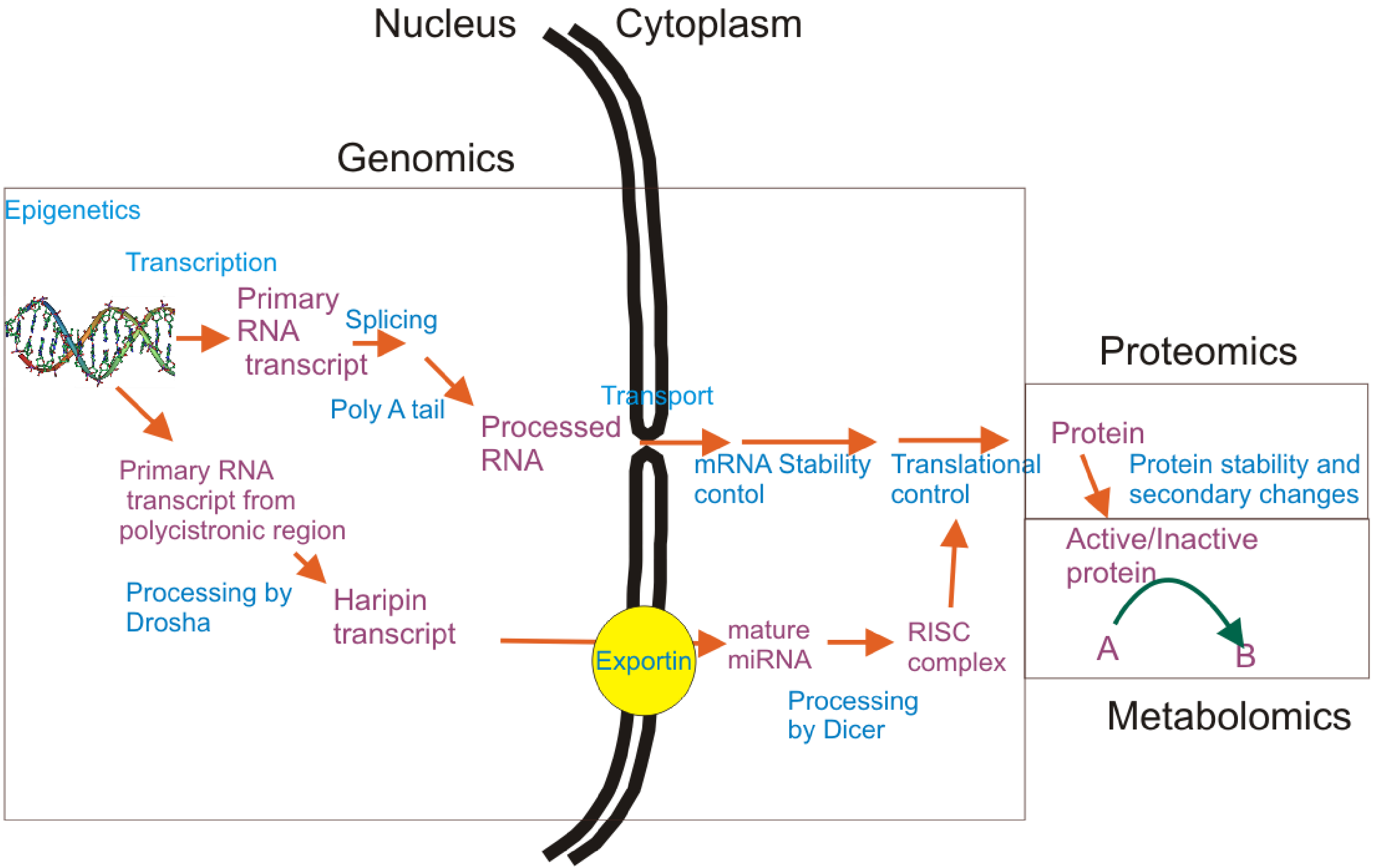
A collection of tools characterised by their ability to measure many constituents of a biological family in concurrently

- **Genomics:** large scale study of the genome including transcription, translation and DNA changes such as mutations, amplifications, deletions, and epigenetic changes.

- **Proteomics:** large scale analysis of protein expression, in particular differential protein expression resulting from biochemical, physiological or pathological change.

- **Metabolomics:** The discernment of chemical footprints in tissues or body fluids resulting from biochemical, physiological or pathological change.

Omics application in the cell



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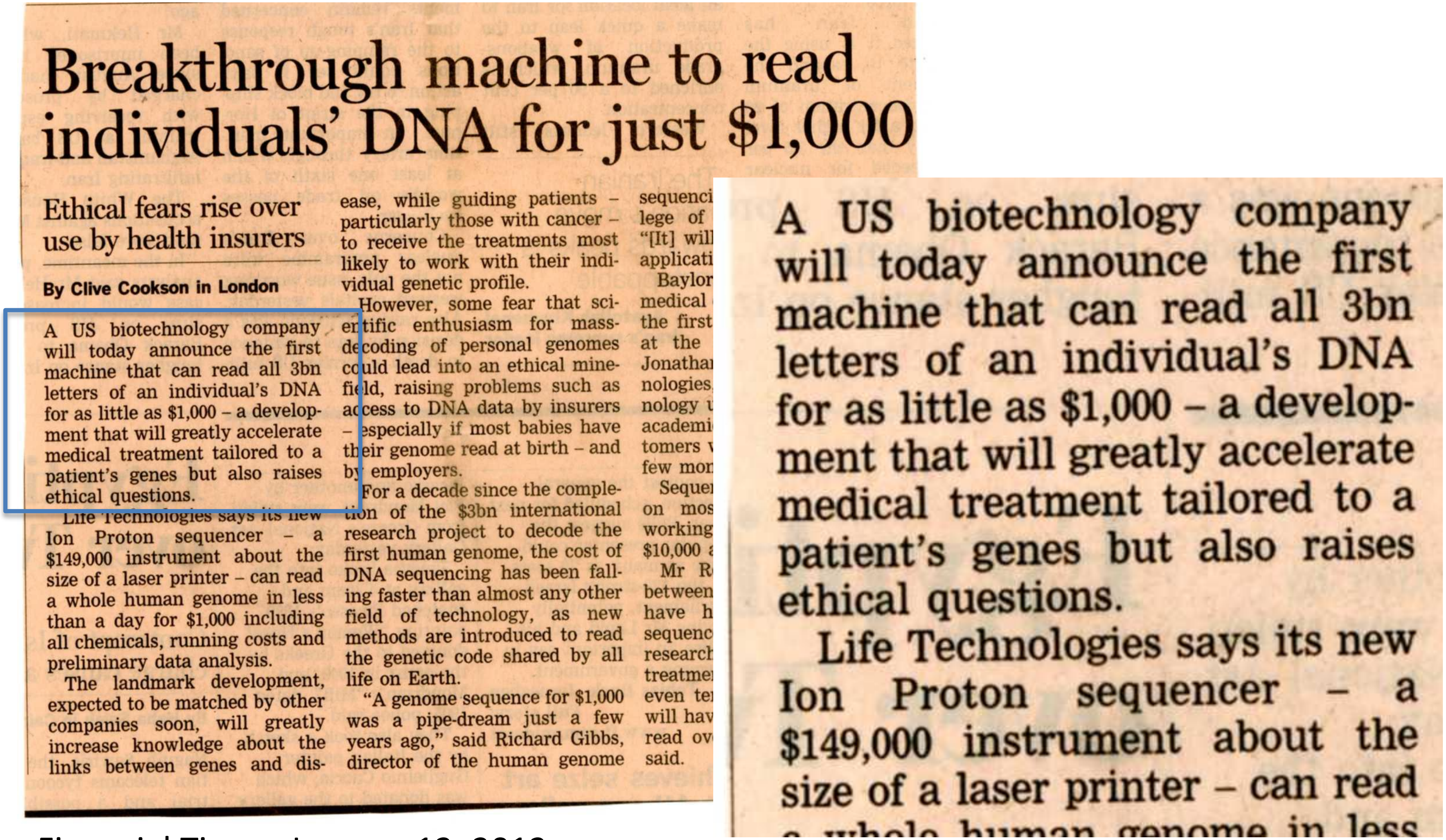

Breakthrough machine to read individuals' DNA for just $1,000

Ethical fears rise over use by health insurers

By Clive Cookson in London

A US biotechnology company will today announce the first machine that can read all 3bn letters of an individual's DNA for as little as $1,000 – a development that will greatly accelerate medical treatment tailored to a patient's genes but also raises ethical questions.

Life Technologies says its new Ion Proton sequencer – a $149,000 instrument about the size of a laser printer – can read a whole human genome in less than a day for $1,000 including all chemicals, running costs and preliminary data analysis.

The landmark development, expected to be matched by other companies soon, will greatly increase knowledge about the links between genes and disease, while guiding patients – particularly those with cancer – to receive the treatments most likely to work with their individual genetic profile.

However, some fear that scientific enthusiasm for mass-decoding of personal genomes could lead into an ethical minefield, raising problems such as access to DNA data by insurers – especially if most babies have their genome read at birth – and by employers.

For a decade since the completion of the $3bn international research project to decode the first human genome, the cost of DNA sequencing has been falling faster than almost any other field of technology, as new methods are introduced to read the genetic code shared by all life on Earth.

"A genome sequence for $1,000 was a pipe-dream just a few years ago," said Richard Gibbs, director of the human genome sequenci
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A US biotechnology company will today announce the first machine that can read all 3bn letters of an individual's DNA for as little as $1,000 – a development that will greatly accelerate medical treatment tailored to a patient's genes but also raises ethical questions.

Life Technologies says its new Ion Proton sequencer – a $149,000 instrument about the size of a laser printer – can read a whole human genome in less

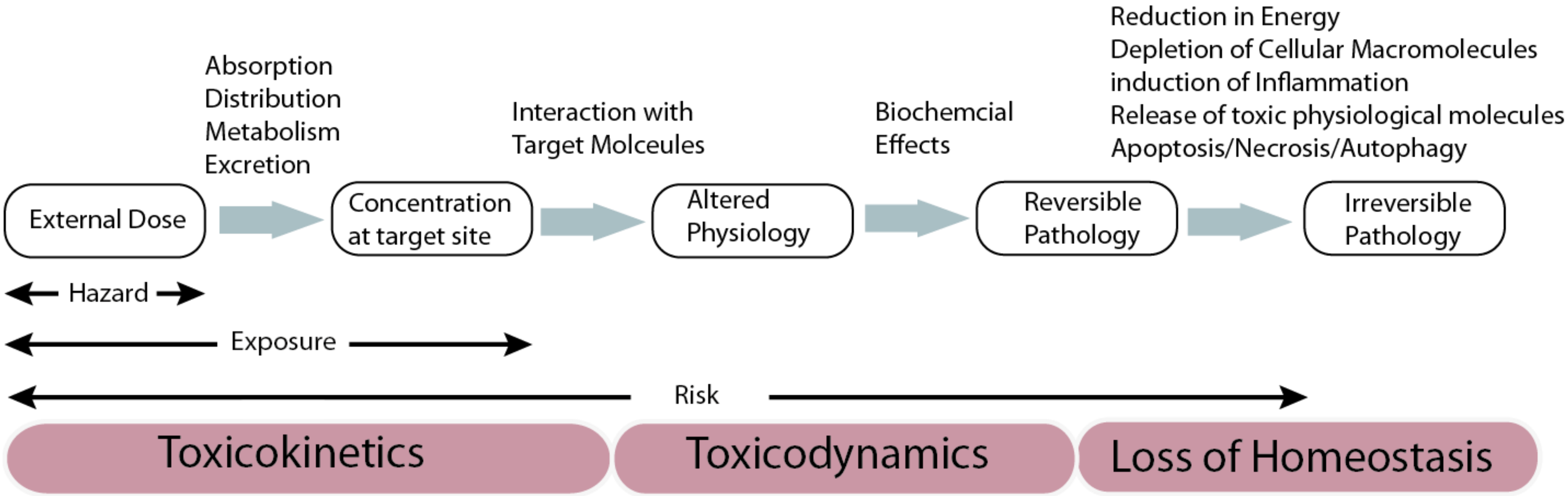
Financial Times; January 12, 2012

Systems toxicology

Systems biology is the integration of genomics, transcriptomics, proteomics, and metabolomics together with computer technology.

Systems toxicology is all of this applied to understanding toxicity particularly pathways from exposure to outcome.

Application to Toxicology



Absorption
Distribution
Metabolism
Excretion

Interaction with
Target Molceules

Biochemcial
Effects

Reduction in Energy
Depletion of Cellular Macromolecules
induction of Inflammation
Release of toxic physiological molecules
Apoptosis/Necrosis/Autophagy

External Dose → Concentration at target site → Altered Physiology → Reversible Pathology → Irreversible Pathology

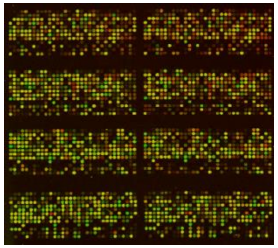
← Hazard →

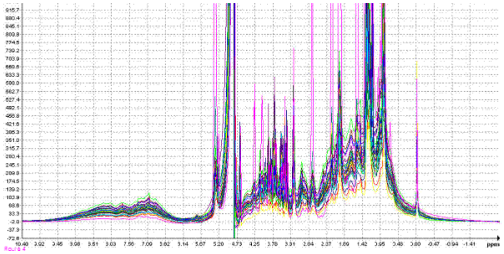
← Exposure →

← Risk →

Toxicokinetics | Toxicodynamics | Loss of Homeostasis

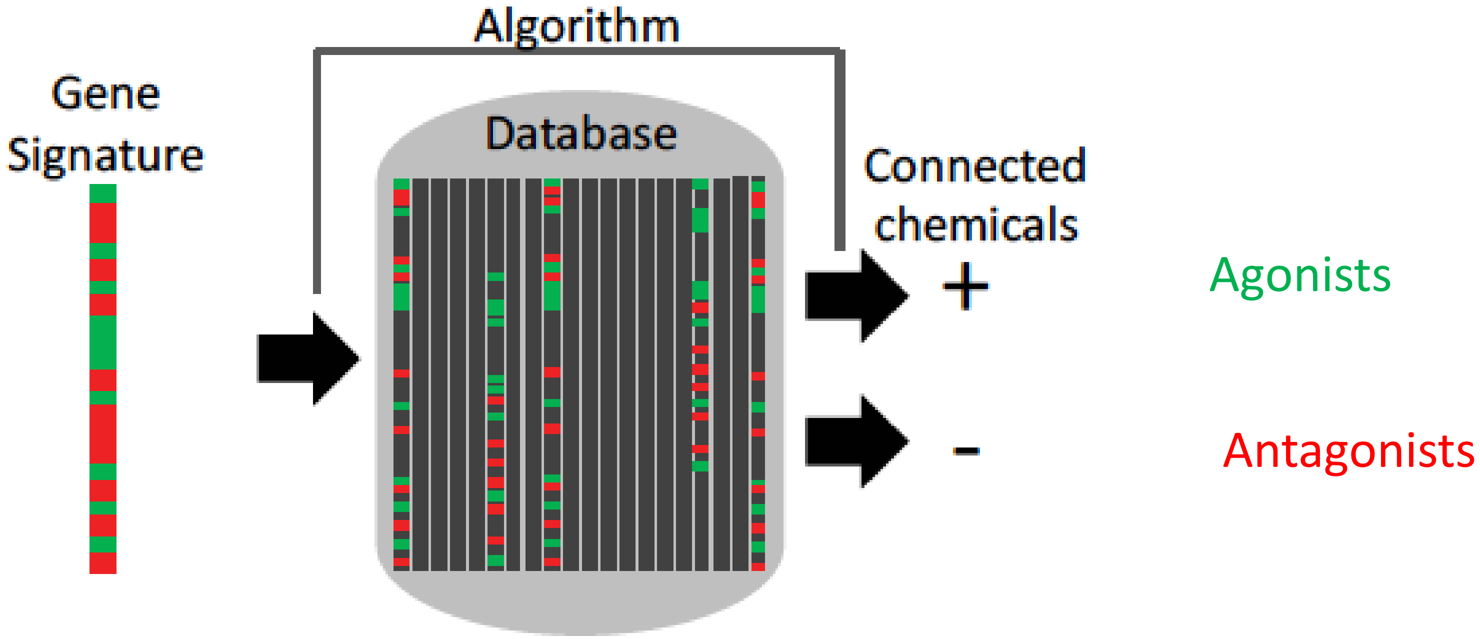
$$\frac{dC}{dt}$$





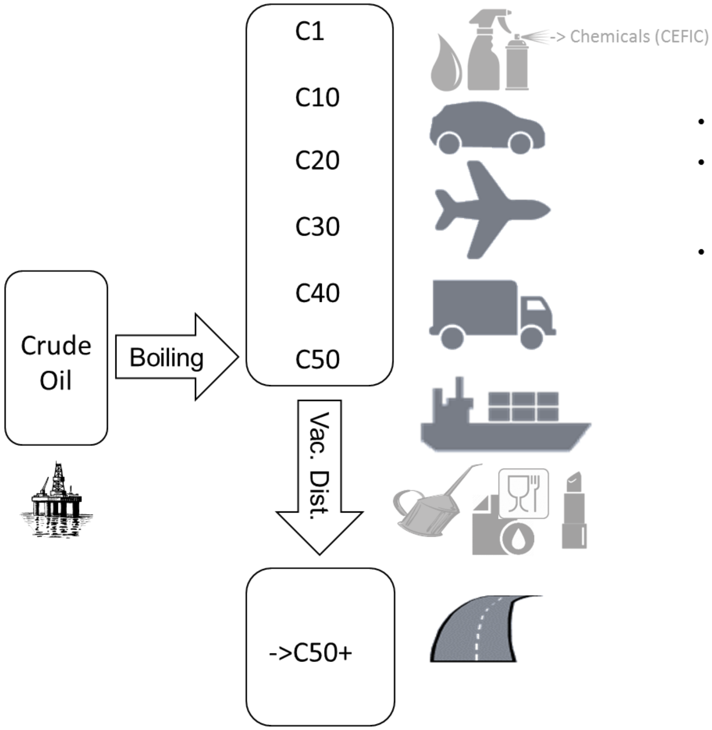
Bioinformatics

Hazards and Read Across - Using pathways for Grouping and Hazard identification



Zhang and Gant BMC Bioinformatics (2008)

UVCBs



Crude Oil

Boiling

C1
C10
C20
C30
C40
C50

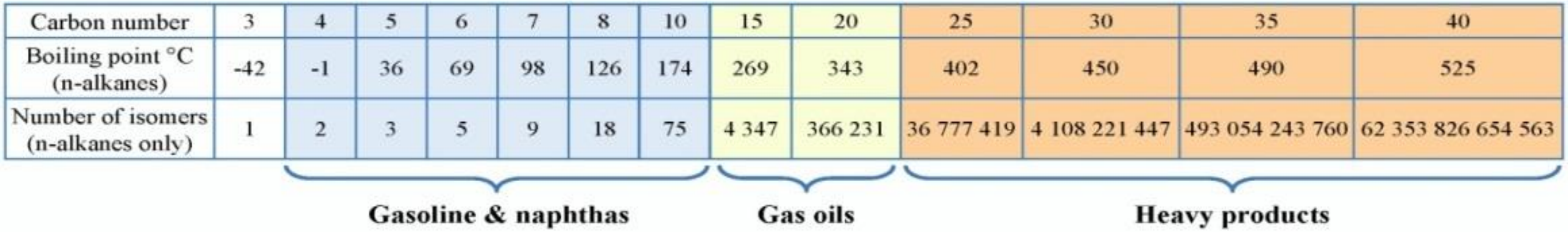
Vac. Dist.

->C50+

-> Chemicals (CEFIC)

- 207 petroleum products
- Thousands to billions of molecules (isomers) per product
- **U**nknown or **V**ariable composition, **C**omplex reaction products, **B**iological materials (UVCB)

Oil products are identified by their CAS number manufacturing stream, and physico-chemical parameters such as energy density, boiling and freezing points.



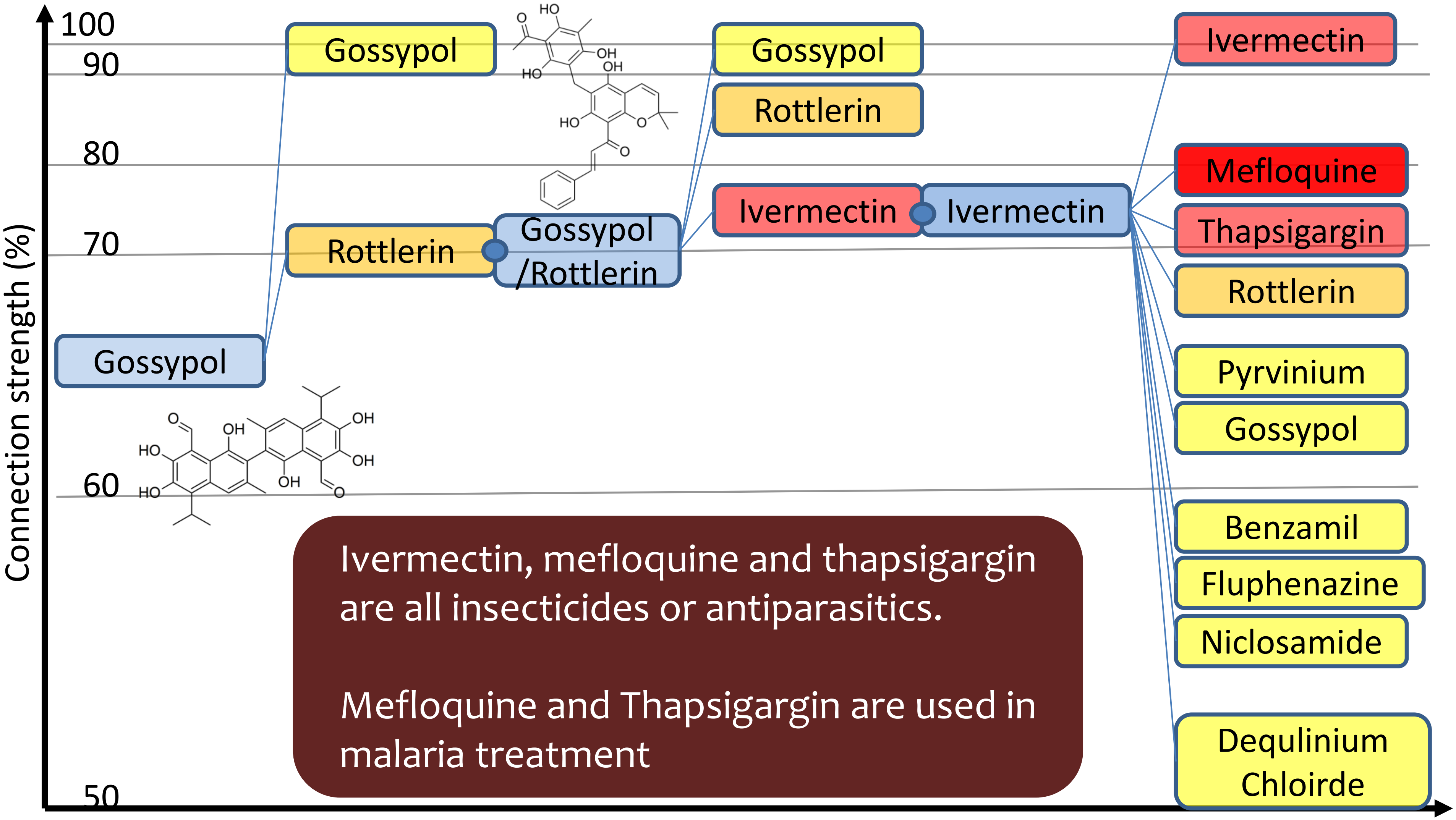
Carbon number	3	4	5	6	7	8	10	15	20	25	30	35	40
Boiling point °C (n-alkanes)	-42	-1	36	69	98	126	174	269	343	402	450	490	525
Number of isomers (n-alkanes only)	1	2	3	5	9	18	75	4 347	366 231	36 777 419	4 108 221 447	493 054 243 760	62 353 826 654 563

Gasoline & naphthas

Gas oils

Heavy products

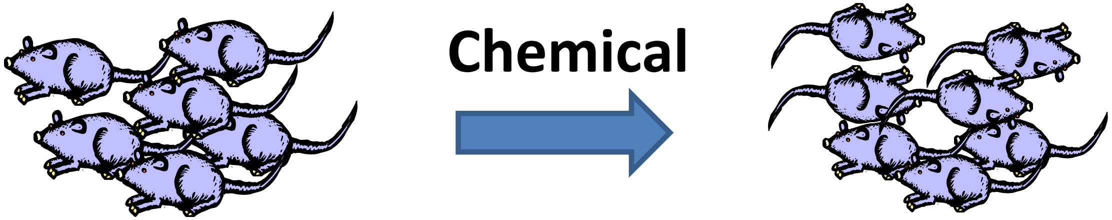
Read Across

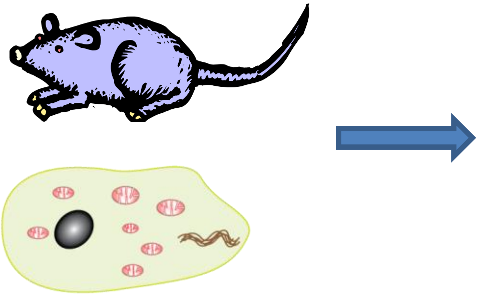


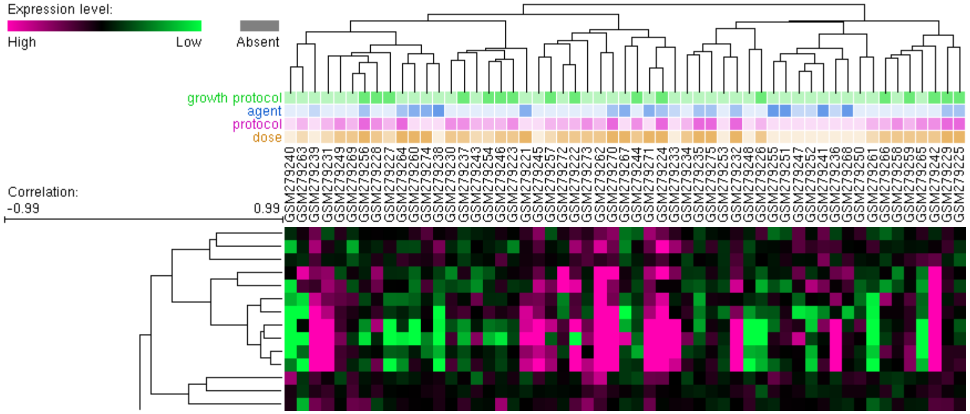
Ivermectin, mefloquine and thapsigargin are all insecticides or antiparasitics.

Mefloquine and Thapsigargin are used in malaria treatment

Hazard – new end points

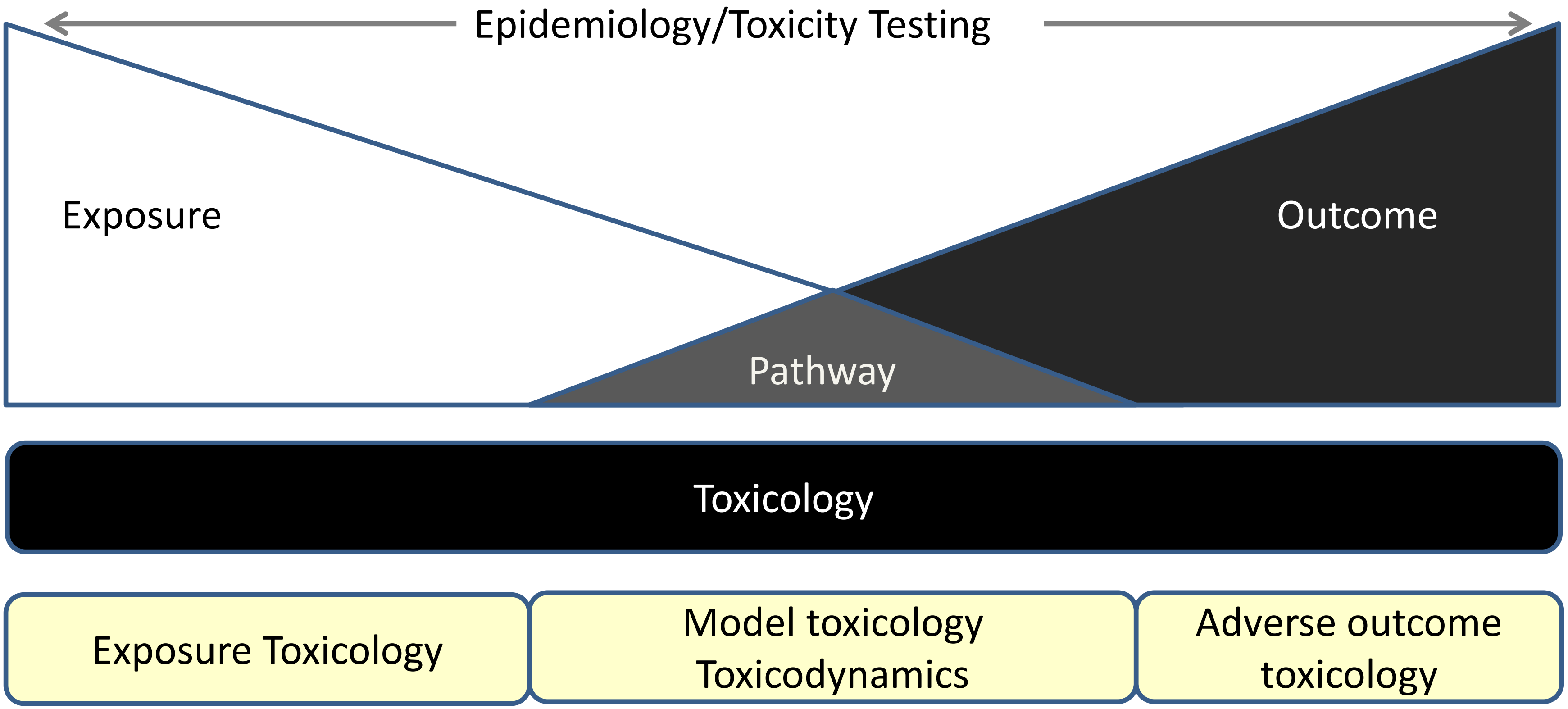






New high throughput technologies give the opportunity to measure more end points; the challenge is that it is not always clear what constitutes adversity and what is homeostasic response

Understanding pathways to discern risk



Epidemiology/Toxicity Testing

Exposure

Outcome

Pathway

Toxicology

Exposure Toxicology

Model toxicology
Toxicodynamics

Adverse outcome
toxicology

Adapted from thoughts of Dan Costa US EPA/ORD

Public Health England

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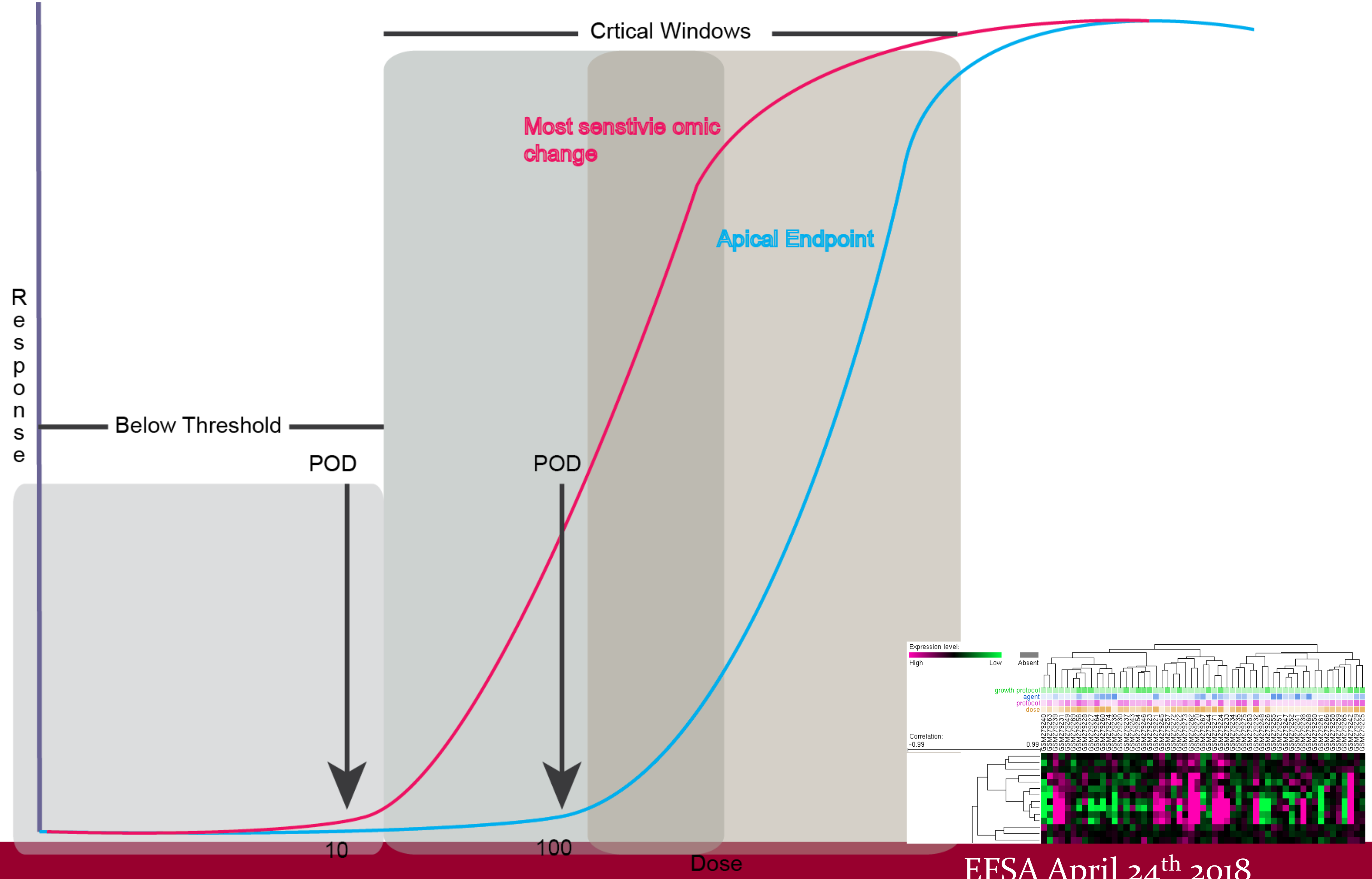
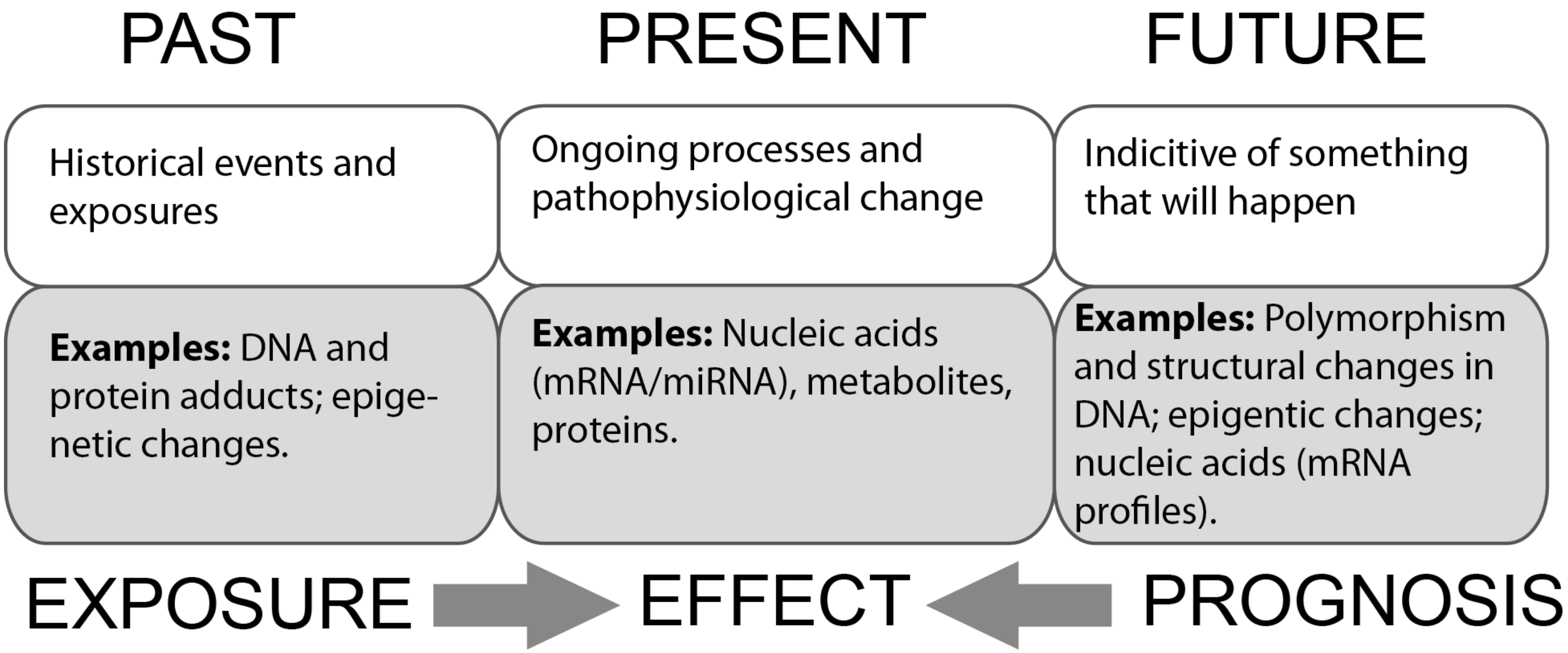
Hazard – Critical points?



MOAs and AOPs

- MOAs and AOPs are conceptually similar
- AOP and MOAs are very similar but have one molecular initiating event (MIE) in the pathway linked to one exposure type and outcome – the same MIE can be reused for other outcomes.
- Both are a linear sequence of key events connecting one exposure to one outcome
- MOAs can take into account modifying factors; AOP don't
- AOPs are generic; MOAs are for one chemical.

Biomarkers



PAST	PRESENT	FUTURE
Historical events and exposures	Ongoing processes and pathophysiological change	Indicitive of something that will happen
Examples: DNA and protein adducts; epigenetic changes.	**Examples:** Nucleic acids (mRNA/miRNA), metabolites, proteins.	**Examples:** Polymorphism and structural changes in DNA; epigentic changes; nucleic acids (mRNA profiles).

EXPOSURE → EFFECT ← PROGNOSIS

T Gant. E Marczylo, M Leonard : Discovery and Application of Novel Biomarkers, in Predictive Toxicology Vision to reality Eds. Pfannkuch F and Suter-Dick L. Wiley (2014)

Release of miRNAs from tissues

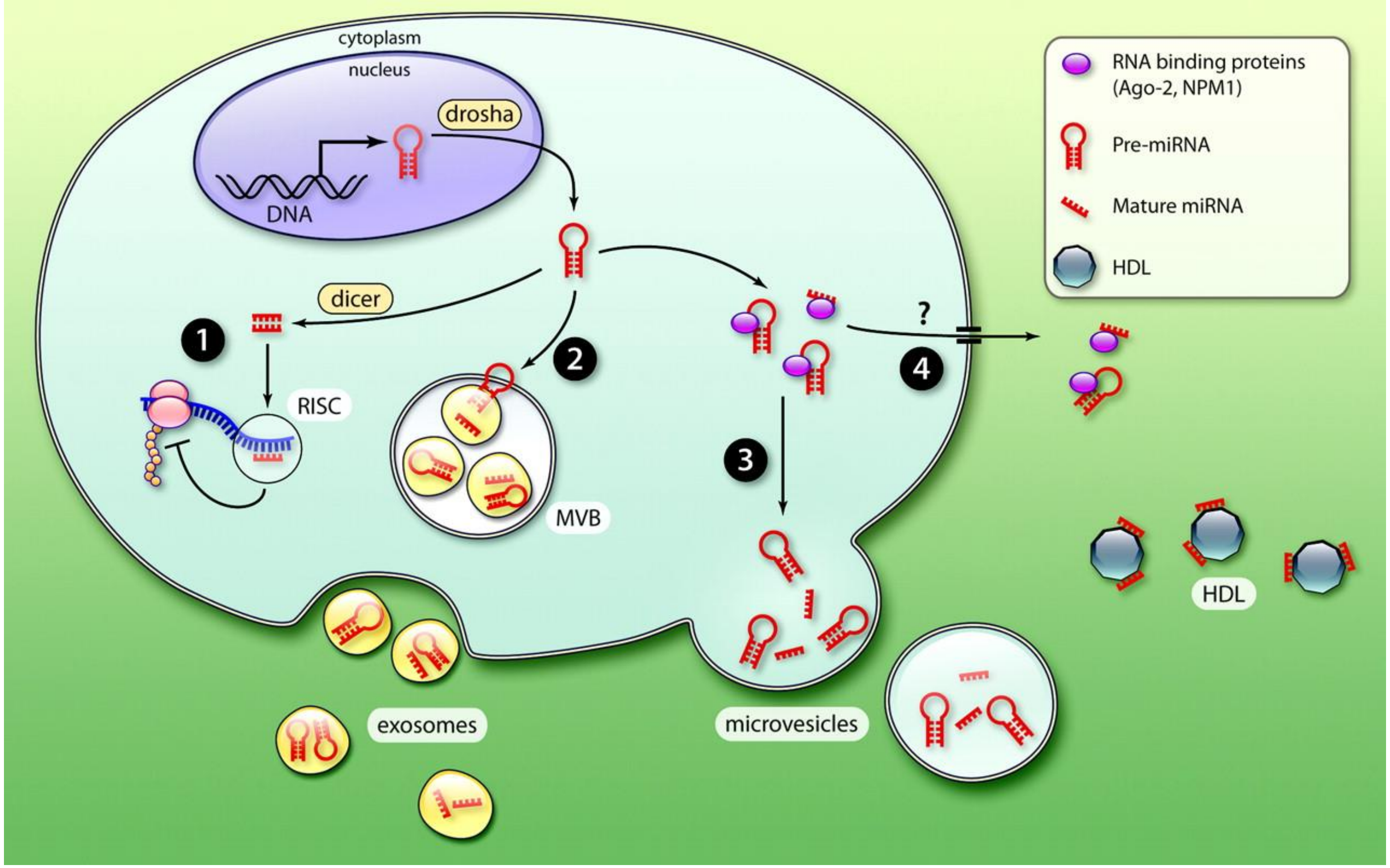
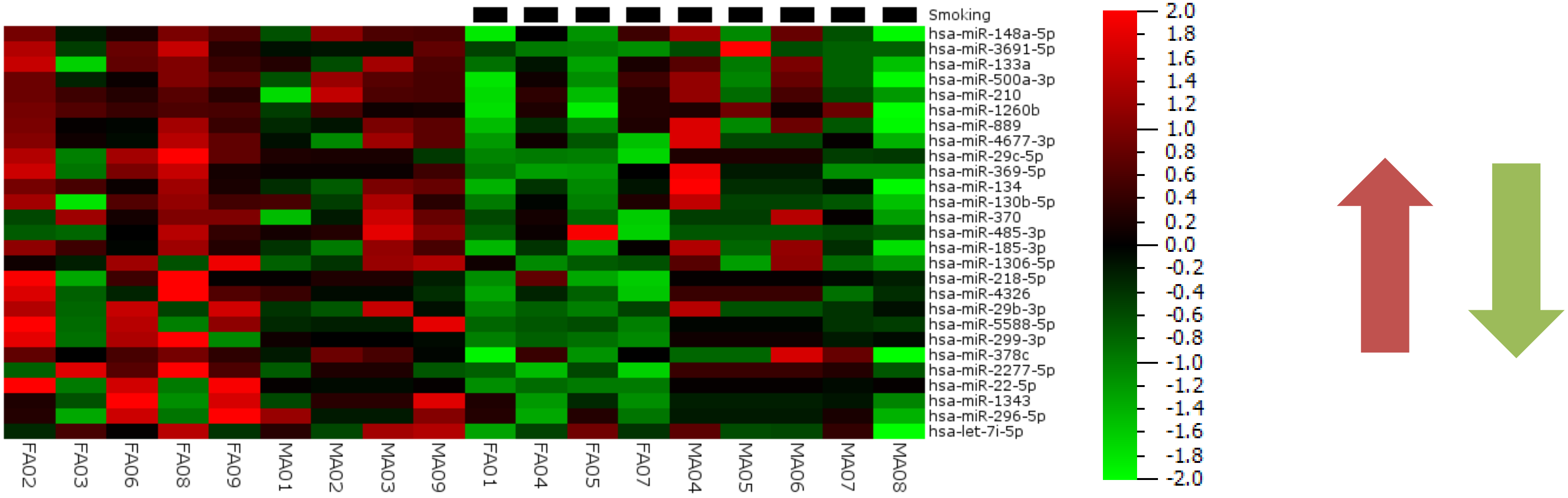


Illustration credit: Cosmocyte/Ben Smith

miRNAs in plasma and smoking status

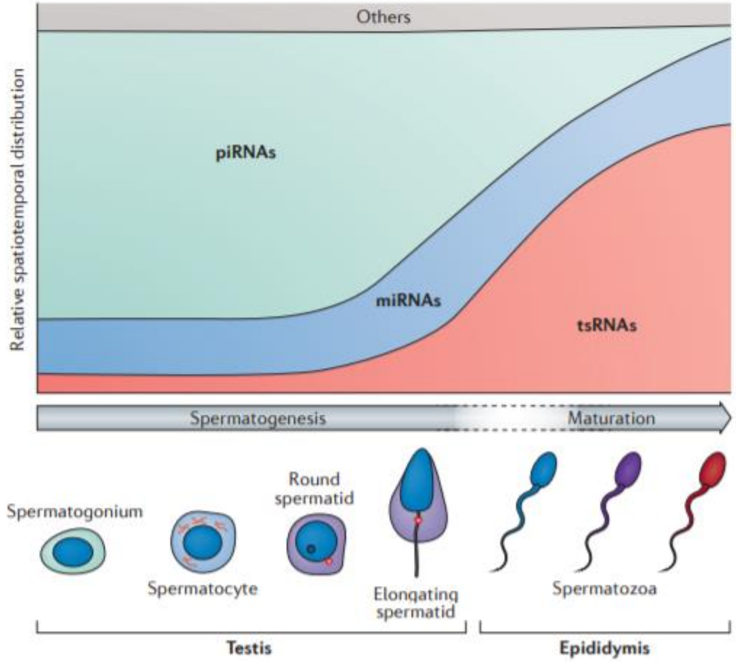


Small RNA species in sperm

EPIGENETICS

Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications

Qi Chen[1], *Wei Yan*[1] *and Enkui Duan*[2]



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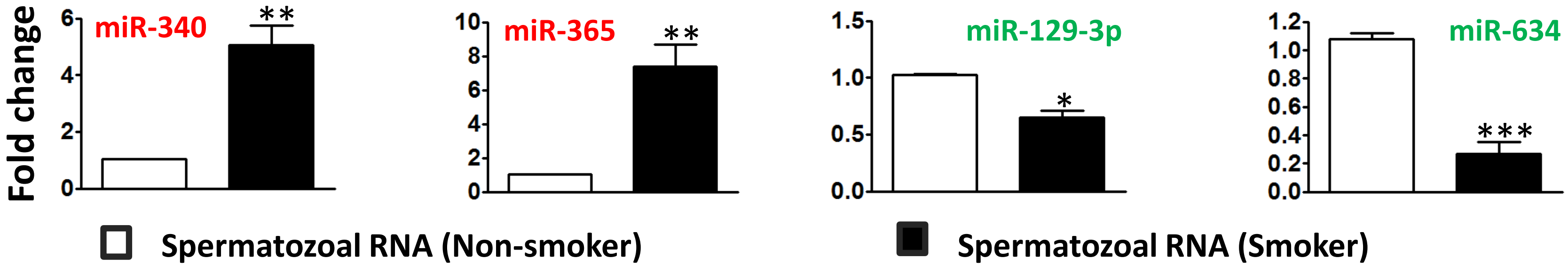
miRNA biomarkers in sperm

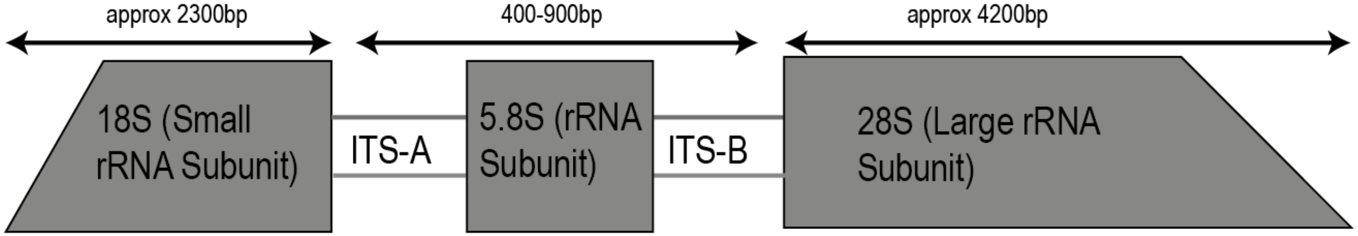
- Smoking induced changes in the miRNA content of human sperm (28, top 5 ↑↓):

miRNA	Fold Change	p value
miR-365	1.6	0.001
miR-944	1.6	0.027
miR-1267	1.5	0.016
miR-340	1.5	0.006
miR-4513	1.5	0.021

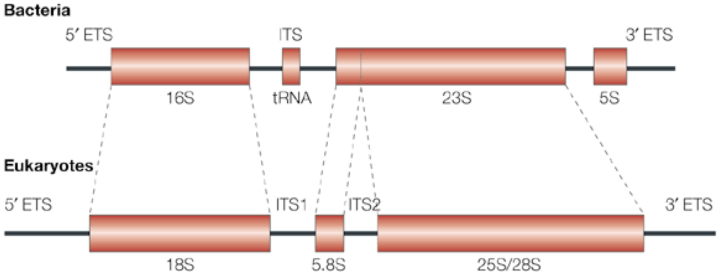
miRNA	Fold Change	p value
miR-146b-5p	0.7	0.023
miR-634	0.7	0.011
miR-129-3p	0.7	0.010
miR-652	0.7	0.002
miR-4723-5p	0.5	0.015



Exposure – environmental microbiome



Microbiome



Nature Reviews | Molecular Cell Biology

16 S region is the ribosomal subunit region of bacteria

Purify DNA from bacterial sample

PCR amplify across the 16S region using a conserved primer set and tags

Sequence PCR products

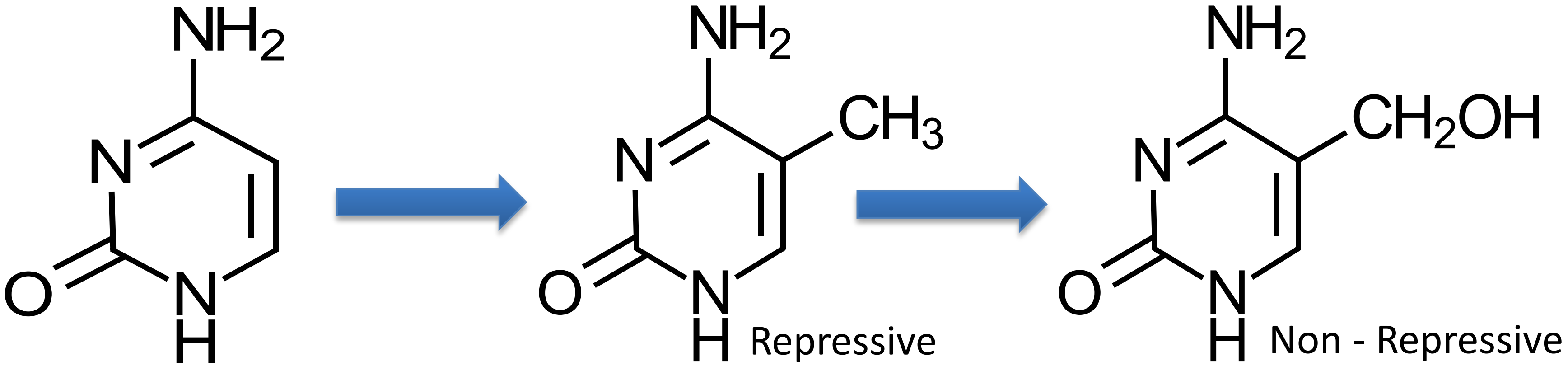
Compare to database

Identify bacterial species

Exposure - Fingerprints in DNA

I was here

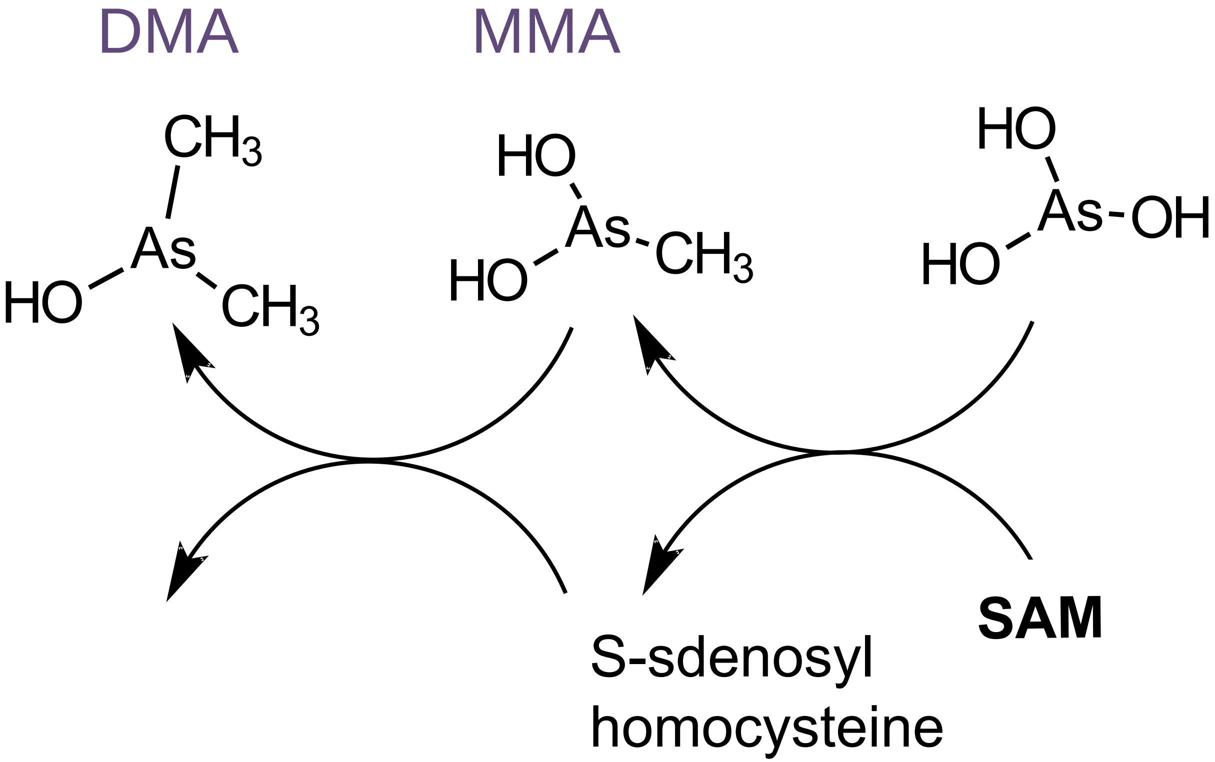
Methylation



Repressive

Non - Repressive

Arsenic metabolism



The metabolism of As can deplete the cells of S-adenosyl-Methioinine leading to methylation changes

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Arsenic effects on cell methylation

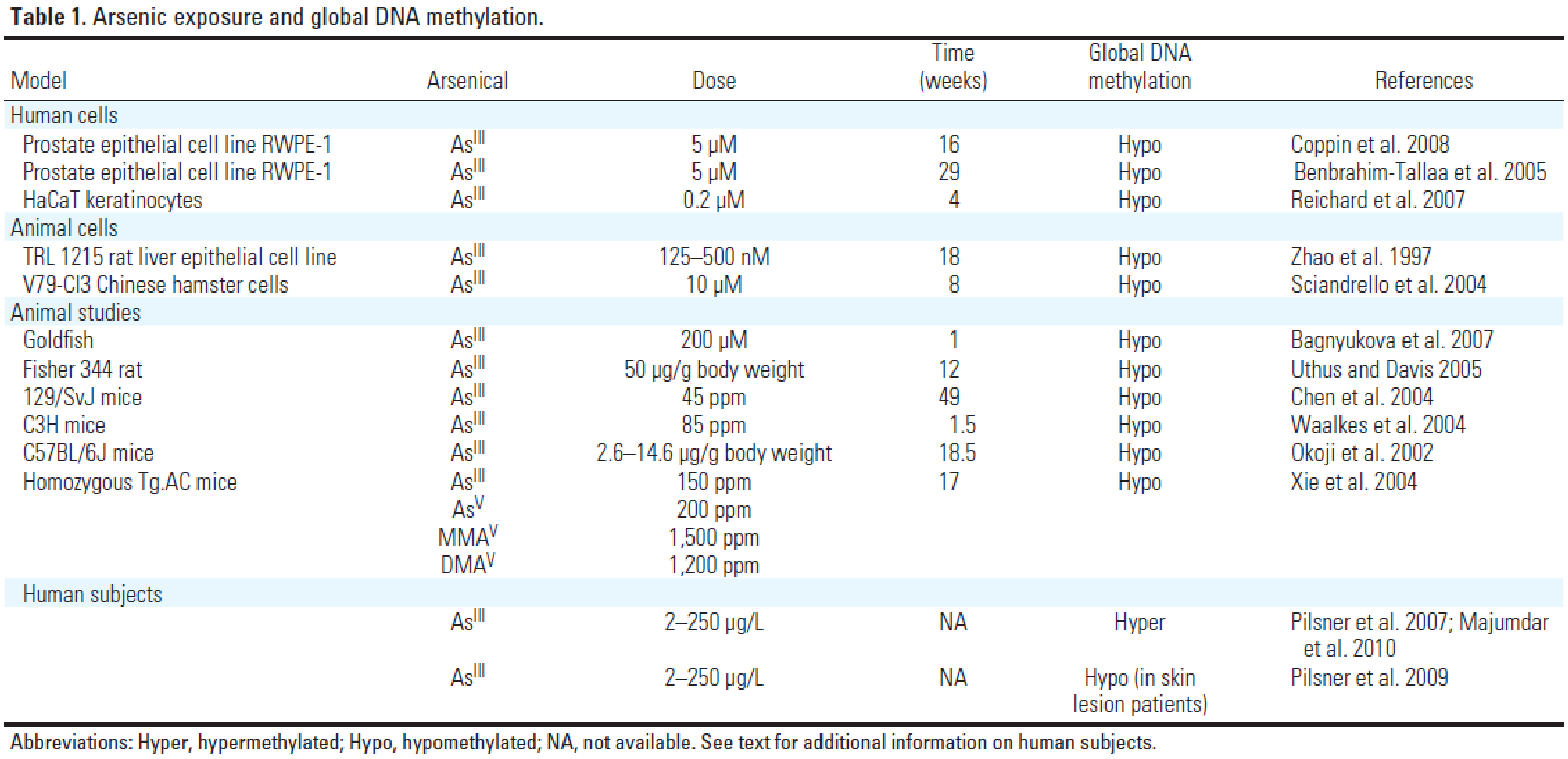

Table 1. Arsenic exposure and global DNA methylation.

Model	Arsenical	Dose	Time (weeks)	Global DNA methylation	References
Human cells					
Prostate epithelial cell line RWPE-1	As^{III}	5 µM	16	Hypo	Coppin et al. 2008
Prostate epithelial cell line RWPE-1	As^{III}	5 µM	29	Hypo	Benbrahim-Tallaa et al. 2005
HaCaT keratinocytes	As^{III}	0.2 µM	4	Hypo	Reichard et al. 2007
Animal cells					
TRL 1215 rat liver epithelial cell line	As^{III}	125–500 nM	18	Hypo	Zhao et al. 1997
V79-Cl3 Chinese hamster cells	As^{III}	10 µM	8	Hypo	Sciandrello et al. 2004
Animal studies					
Goldfish	As^{III}	200 µM	1	Hypo	Bagnyukova et al. 2007
Fisher 344 rat	As^{III}	50 µg/g body weight	12	Hypo	Uthus and Davis 2005
129/SvJ mice	As^{III}	45 ppm	49	Hypo	Chen et al. 2004
C3H mice	As^{III}	85 ppm	1.5	Hypo	Waalkes et al. 2004
C57BL/6J mice	As^{III}	2.6–14.6 µg/g body weight	18.5	Hypo	Okoji et al. 2002
Homozygous Tg.AC mice	As^{III}	150 ppm	17	Hypo	Xie et al. 2004
	As^{V}	200 ppm			
	MMA^{V}	1,500 ppm			
	DMA^{V}	1,200 ppm			
Human subjects					
	As^{III}	2–250 µg/L	NA	Hyper	Pilsner et al. 2007; Majumdar et al. 2010
	As^{III}	2–250 µg/L	NA	Hypo (in skin lesion patients)	Pilsner et al. 2009

Abbreviations: Hyper, hypermethylated; Hypo, hypomethylated; NA, not available. See text for additional information on human subjects.

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CRITICAL REVIEWS IN TOXICOLOGY, 2016
http://dx.doi.org/10.1080/10408444.2016.1175417

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REVIEW ARTICLE

OPEN ACCESS

Environmentally induced epigenetic toxicity: potential public health concerns

Emma L. Marczylo[a], Miriam N. Jacobs[a] and Timothy W. Gant[a]

[a]Toxicology Department, CRCE, PHE, Chilton, Oxfordshire, UK

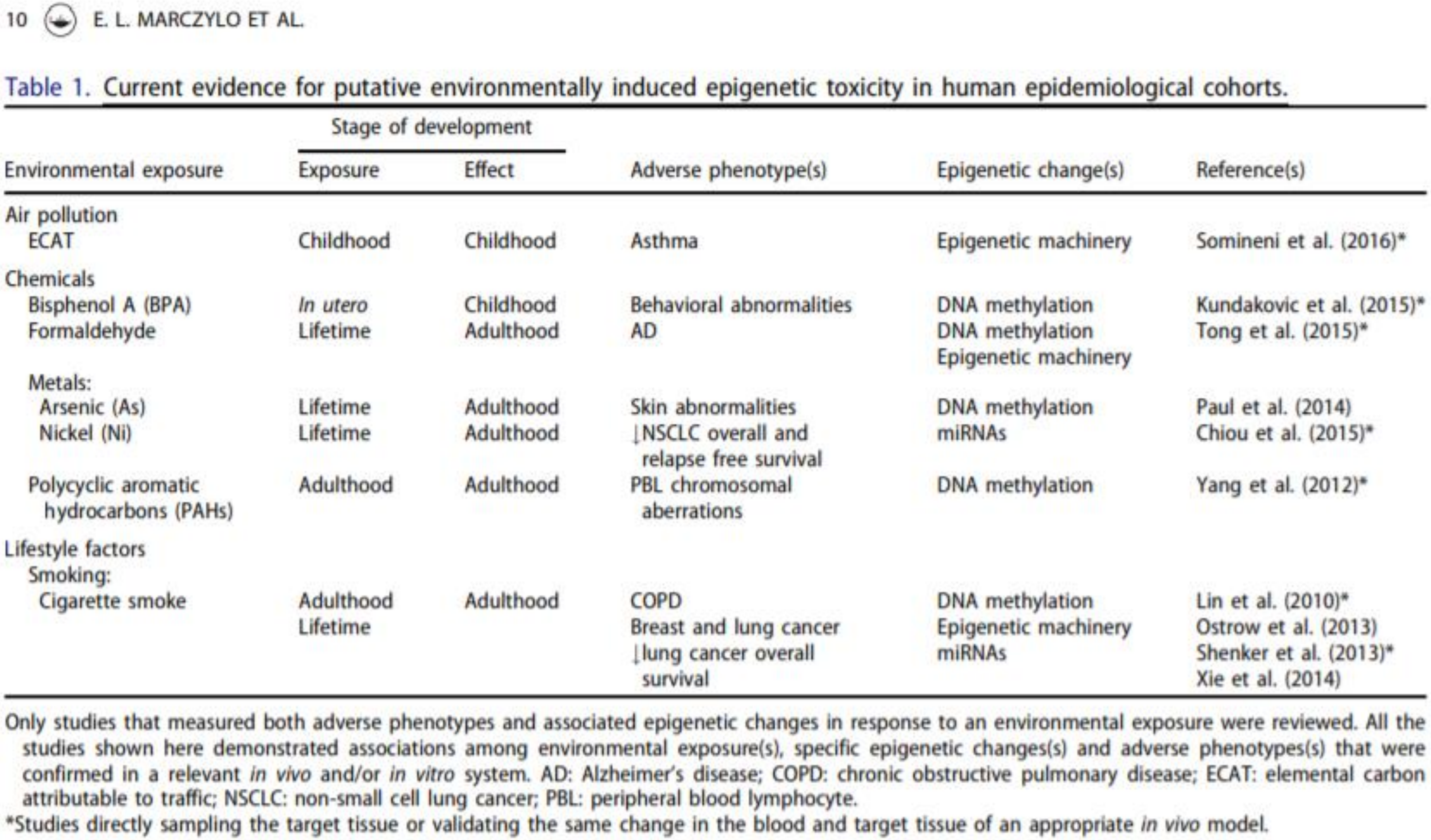


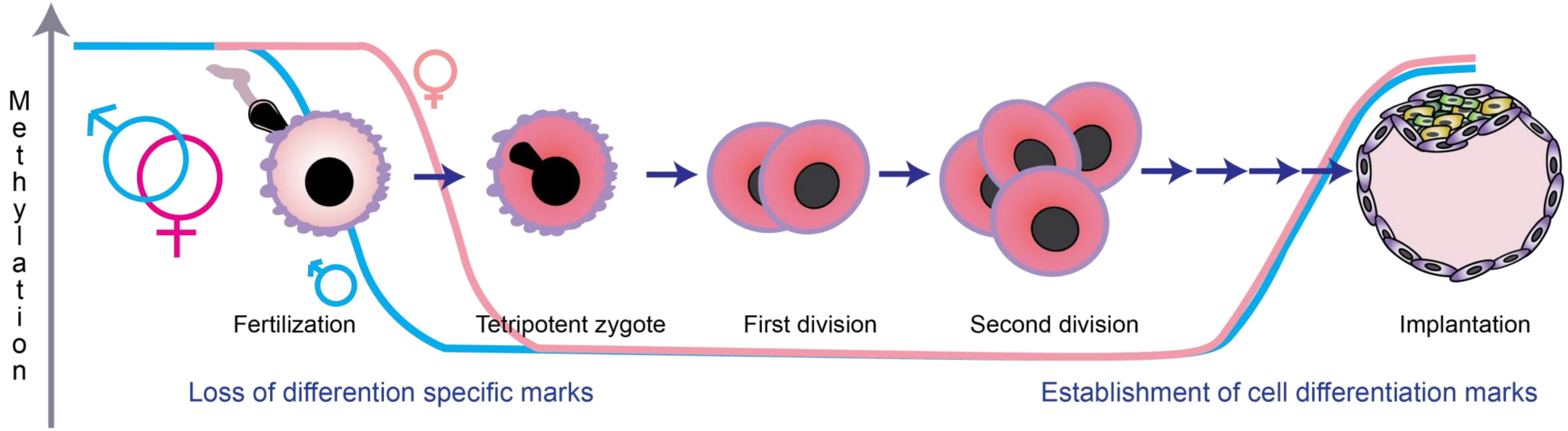
Table 1. Current evidence for putative environmentally induced epigenetic toxicity in human epidemiological cohorts.

	Stage of development				
Environmental exposure	Exposure	Effect	Adverse phenotype(s)	Epigenetic change(s)	Reference(s)
Air pollution					
ECAT	Childhood	Childhood	Asthma	Epigenetic machinery	Somineni et al. (2016)*
Chemicals					
Bisphenol A (BPA)	*In utero*	Childhood	Behavioral abnormalities	DNA methylation	Kundakovic et al. (2015)*
Formaldehyde	Lifetime	Adulthood	AD	DNA methylation Epigenetic machinery	Tong et al. (2015)*
Metals:					
Arsenic (As)	Lifetime	Adulthood	Skin abnormalities	DNA methylation	Paul et al. (2014)
Nickel (Ni)	Lifetime	Adulthood	↓NSCLC overall and relapse free survival	miRNAs	Chiou et al. (2015)*
Polycyclic aromatic hydrocarbons (PAHs)	Adulthood	Adulthood	PBL chromosomal aberrations	DNA methylation	Yang et al. (2012)*
Lifestyle factors					
Smoking:					
Cigarette smoke	Adulthood Lifetime	Adulthood	COPD Breast and lung cancer ↓lung cancer overall survival	DNA methylation Epigenetic machinery miRNAs	Lin et al. (2010)* Ostrow et al. (2013) Shenker et al. (2013)* Xie et al. (2014)

Only studies that measured both adverse phenotypes and associated epigenetic changes in response to an environmental exposure were reviewed. All the studies shown here demonstrated associations among environmental exposure(s), specific epigenetic changes(s) and adverse phenotypes(s) that were confirmed in a relevant *in vivo* and/or *in vitro* system. AD: Alzheimer's disease; COPD: chronic obstructive pulmonary disease; ECAT: elemental carbon attributable to traffic; NSCLC: non-small cell lung cancer; PBL: peripheral blood lymphocyte.

*Studies directly sampling the target tissue or validating the same change in the blood and target tissue of an appropriate *in vivo* model.

Outcomes - Fertilisation, methylation and early life



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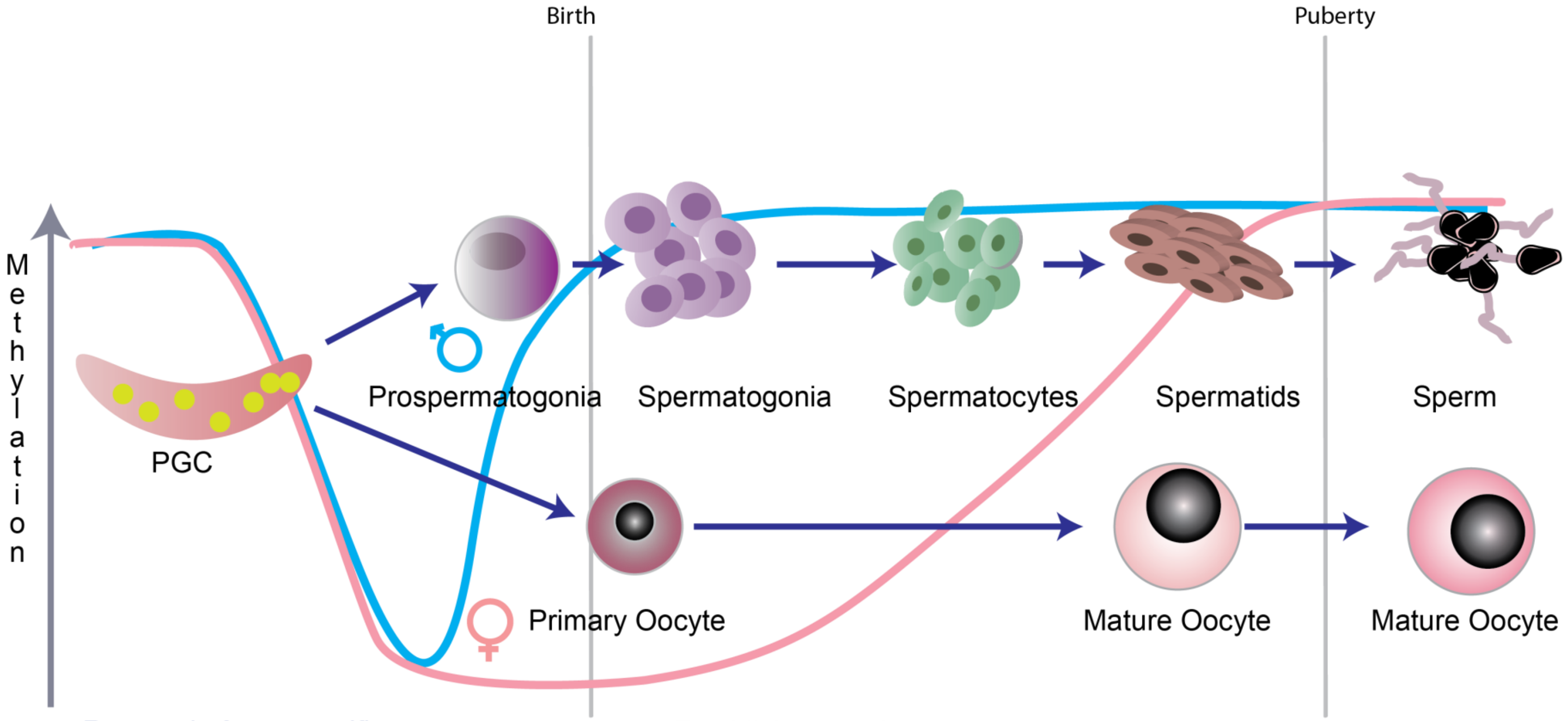
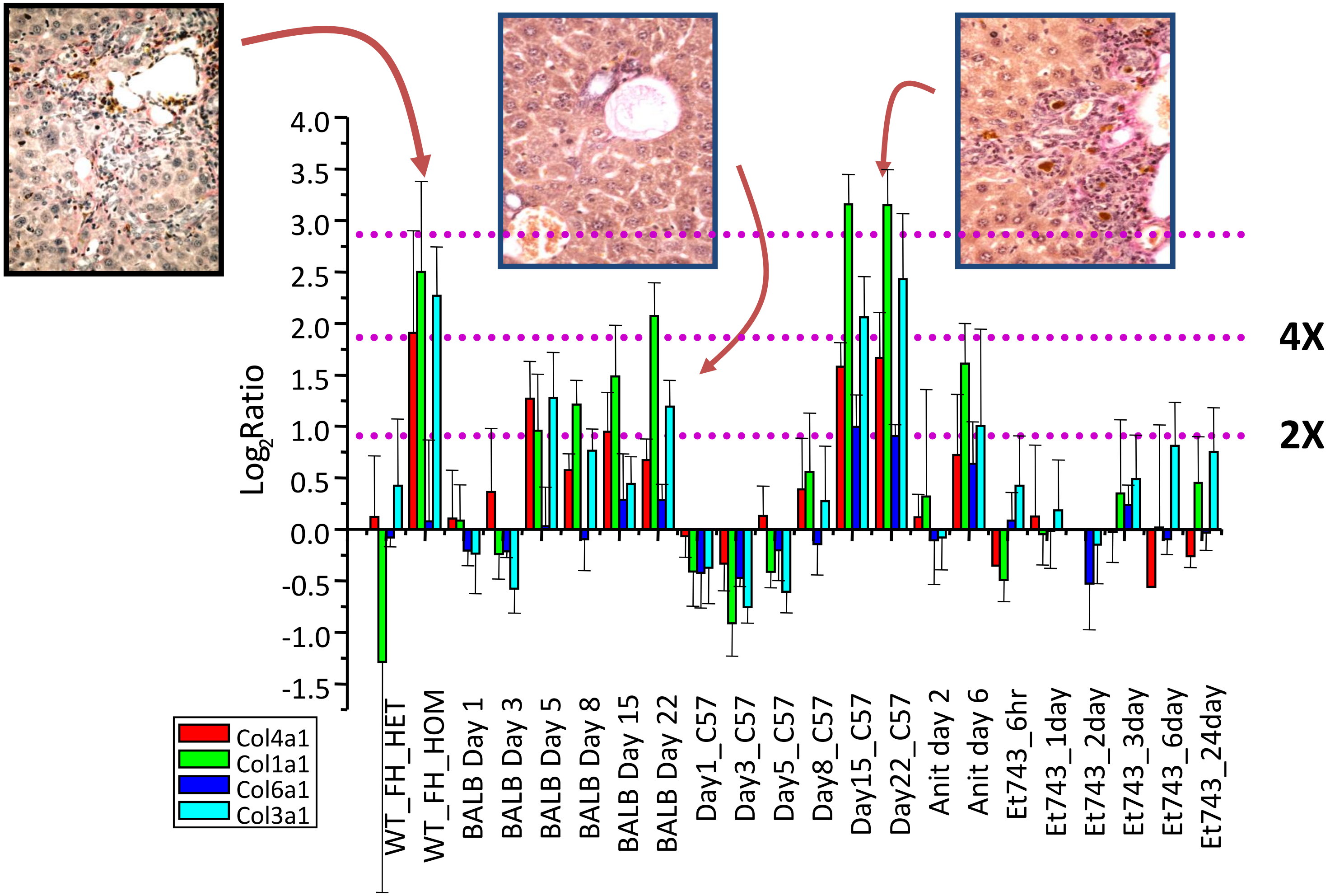
Gamete formation



Removal of sex specific marks in primordial germ cells in embryogenesis

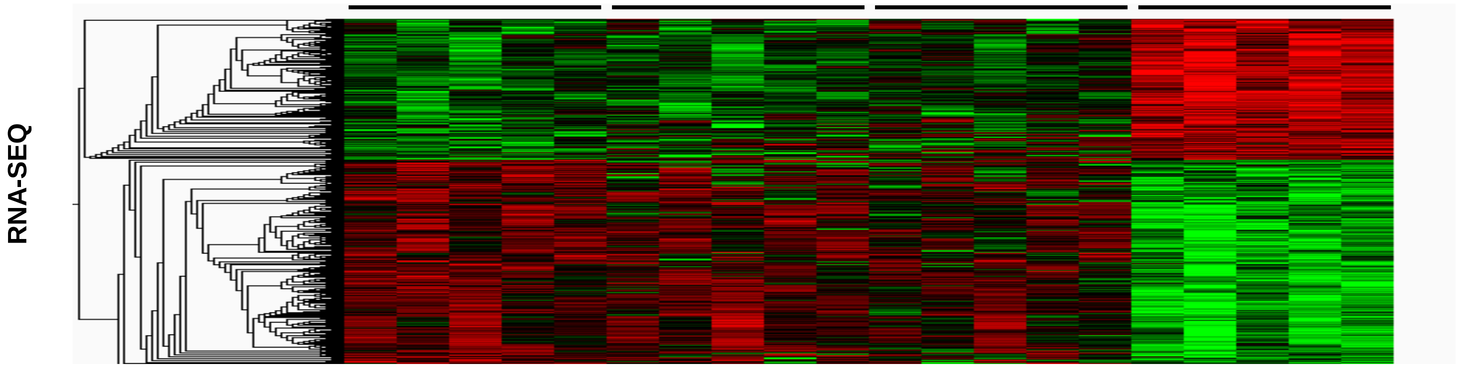
Establishment of gender specific marks

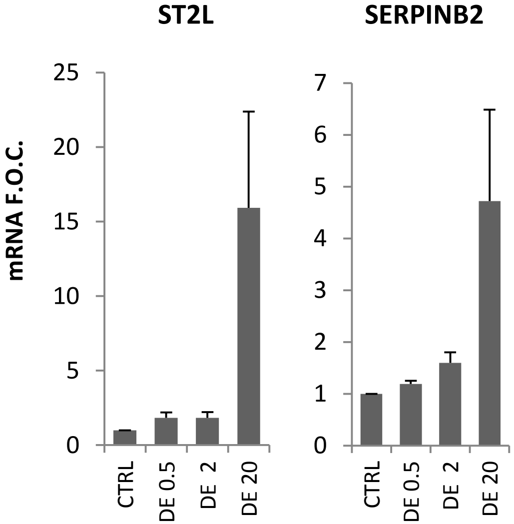
Outcomes - Phenotypic anchoring

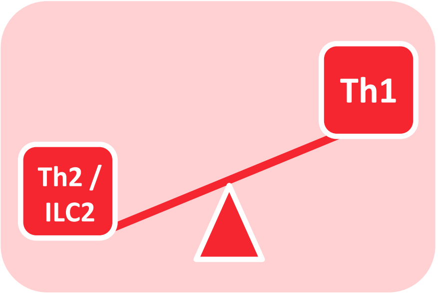


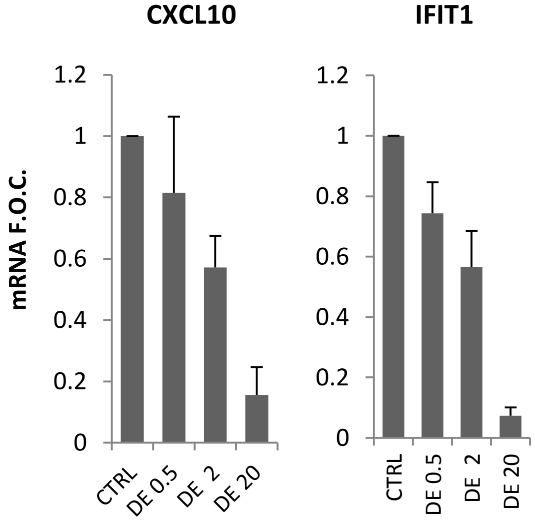
Phenotypic anchoring – increase expression of collagen genes can be associated with fibrosis in the liver.

Outcomes - Phenotype changes in diesel particulate exposure

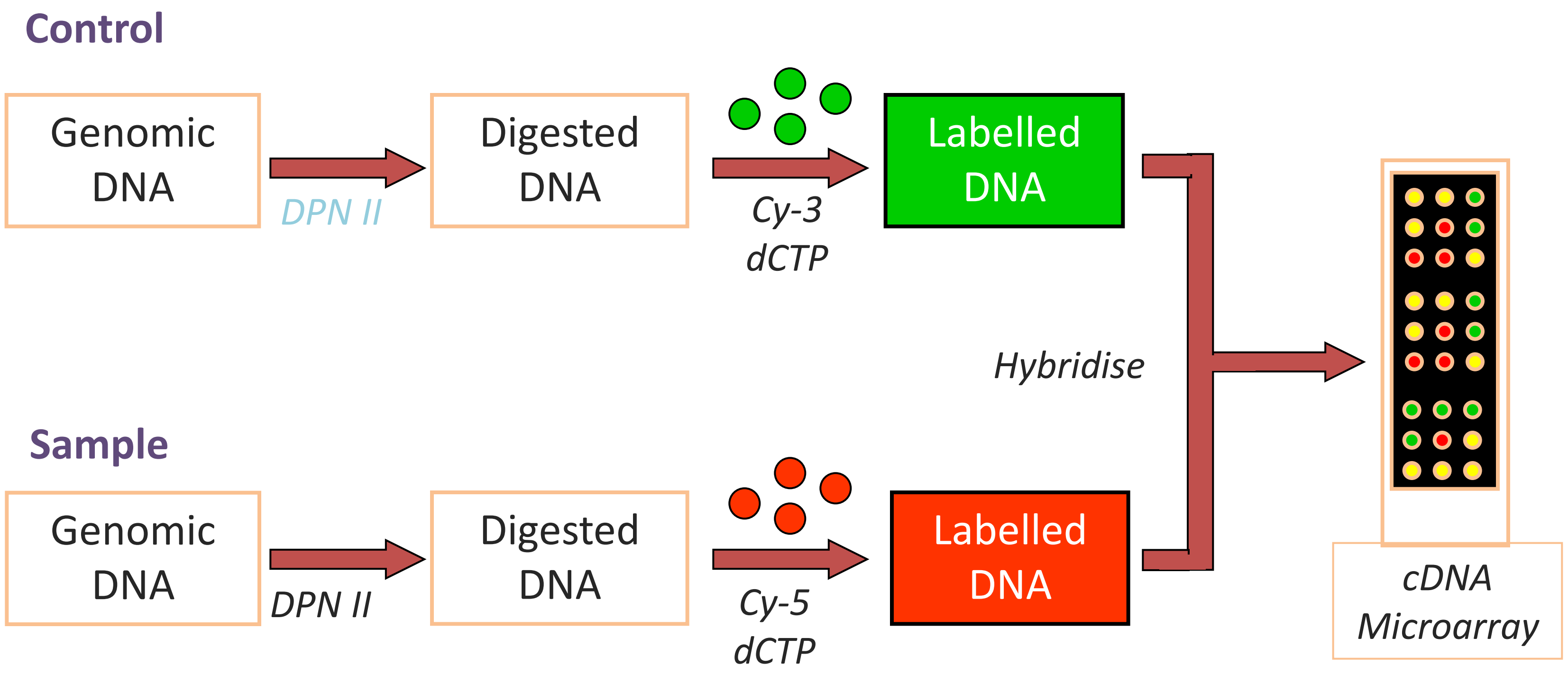




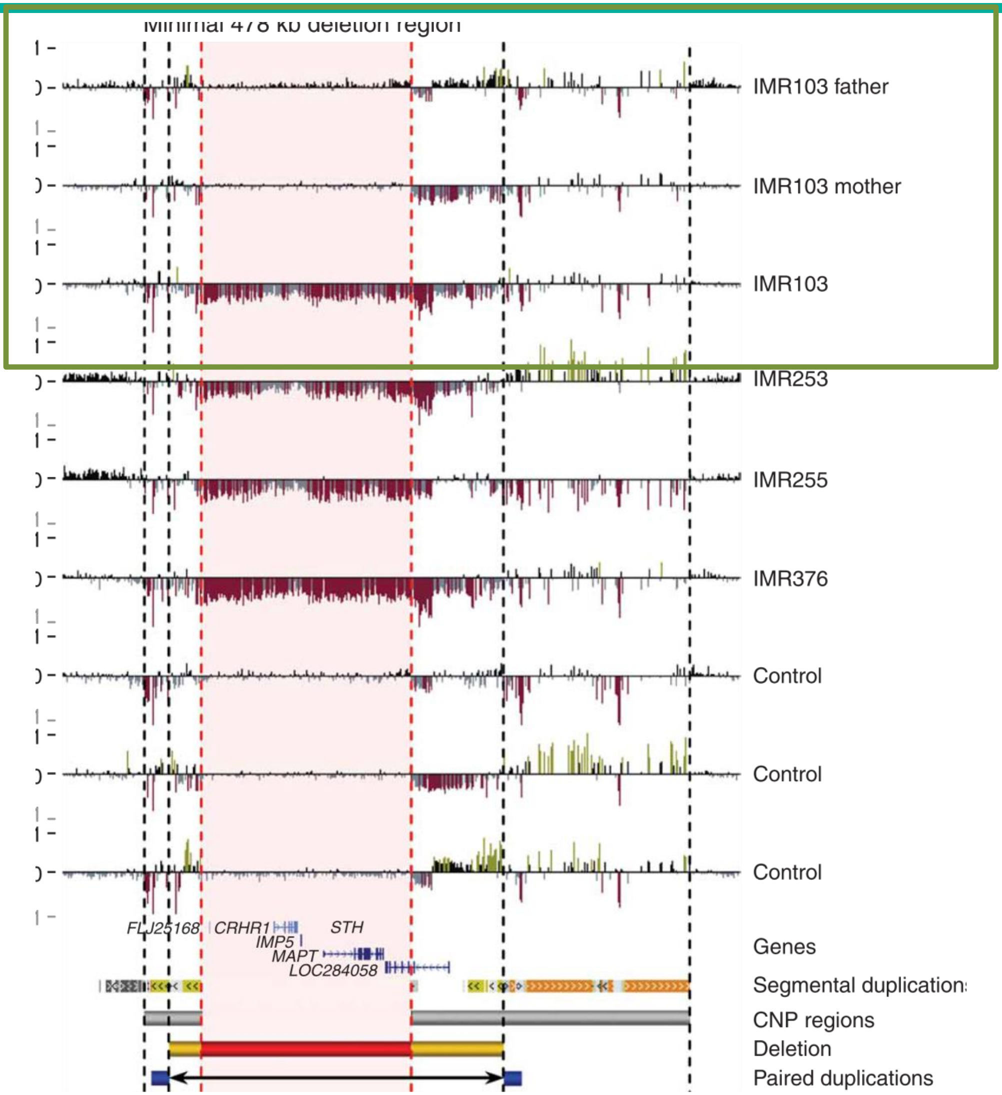




Personal risk - ArrayCGH



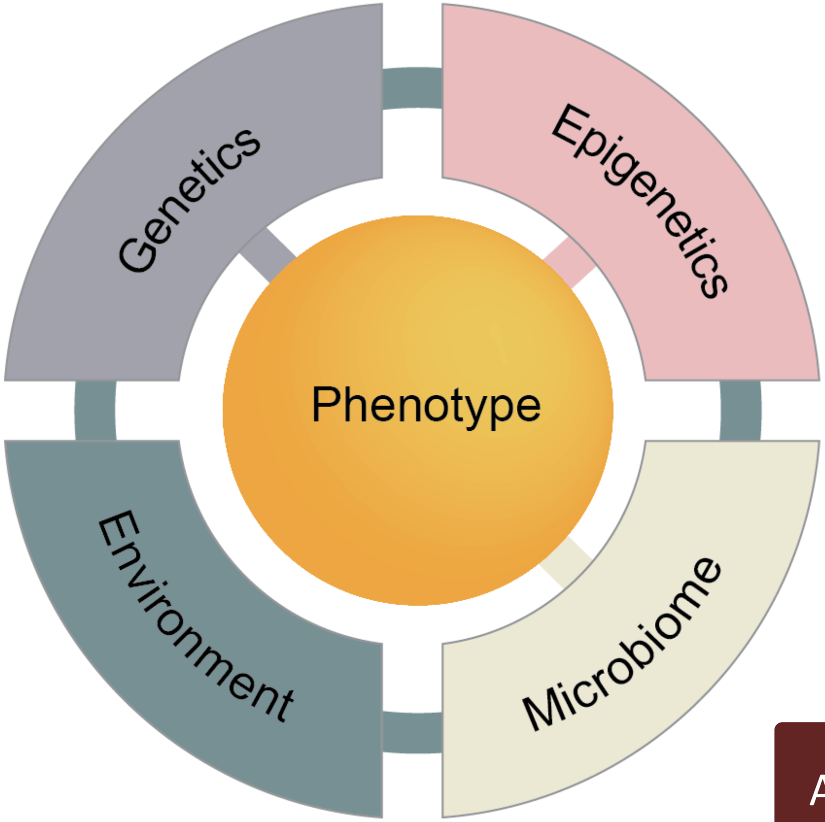
Can also show where the mutation arose



No indication of the same deletion in parents of affected individual 103 – suggests the deletion is a *de novo*.

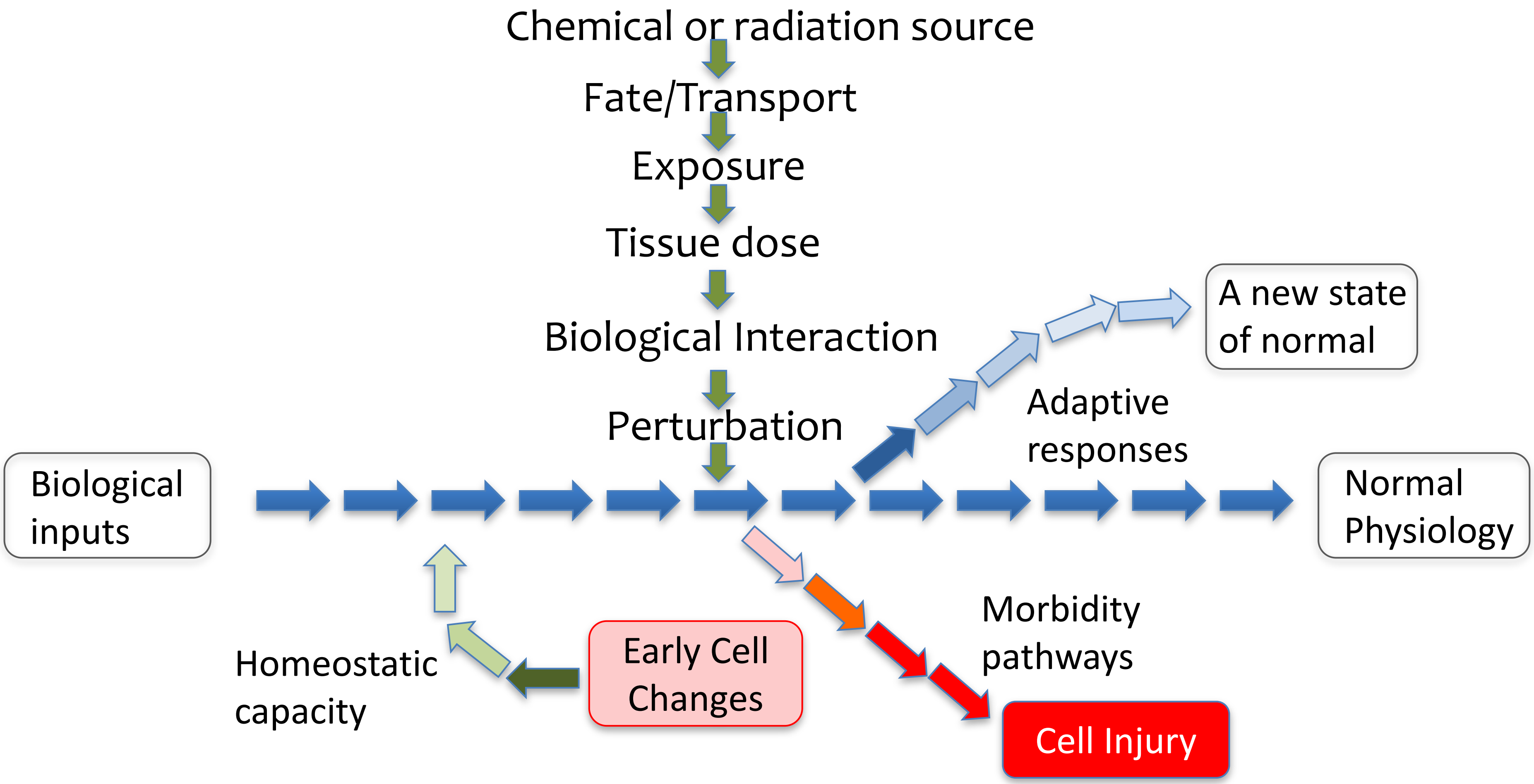
Sharp AJ et al
Nature Genetics 38(9), 1038 (2006)

Factors affecting personal risk



All addressable with Omics

Omics is useful at all cellular response levels



Chemical or radiation source

Fate/Transport

Exposure

Tissue dose

Biological Interaction

Perturbation

Biological inputs

A new state of normal

Adaptive responses

Normal Physiology

Homeostatic capacity

Early Cell Changes

Morbidity pathways

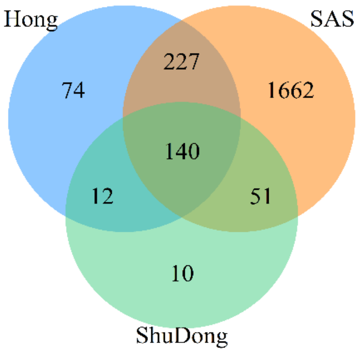
Cell Injury

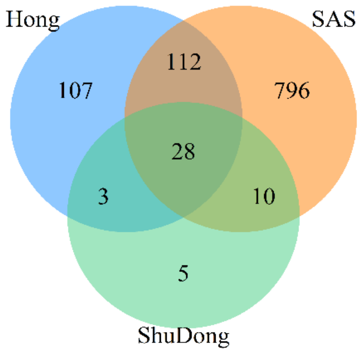
The challenge of bioinformatics

PND30-40

PND83

ADI





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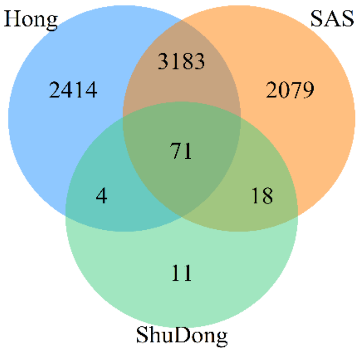
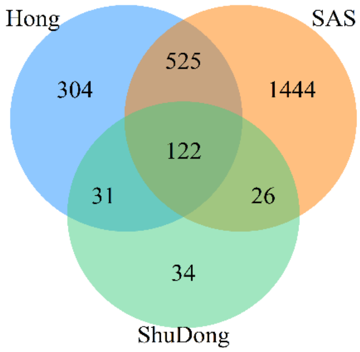
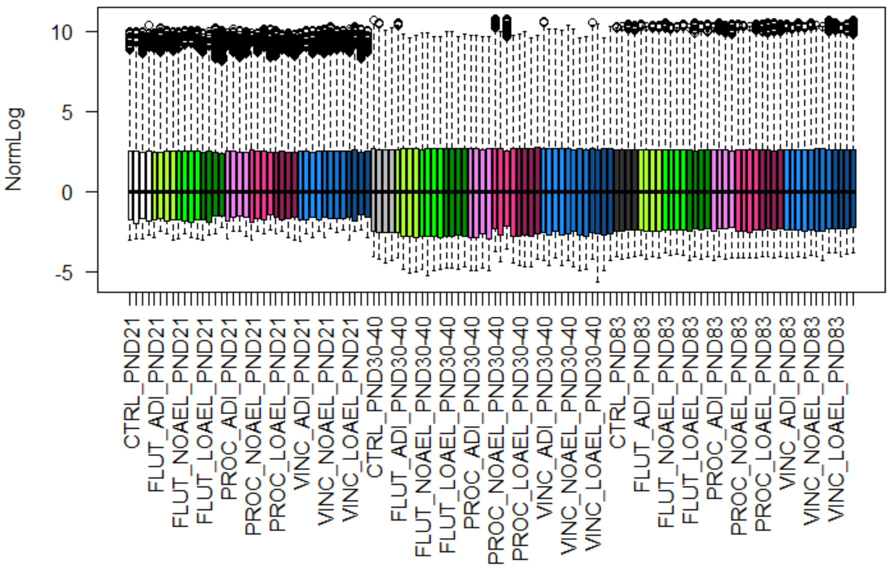
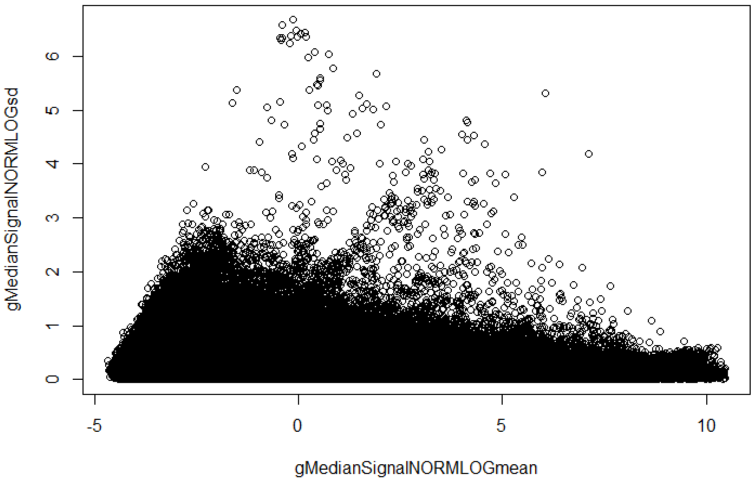




The data issue





Plot first used in –
Lu et al; Nature (2005); 435, 834
(supplementary).

The reference method

- Data (type)
- Log
- Normalisation within dataset only
- Mean of technical replicates
- Welch t-test between pairs for significance

How to recognise outlier data sets?

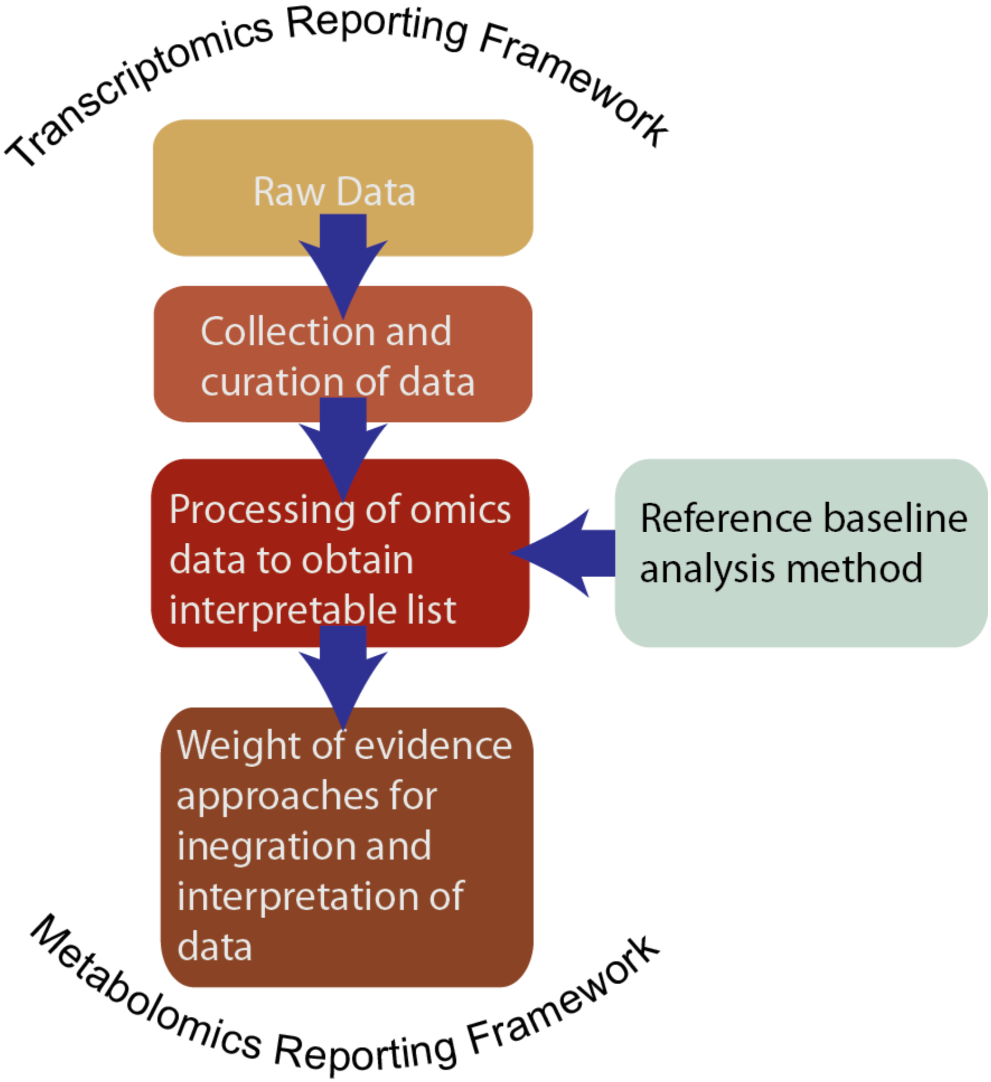
How to deal with low signal strength?

Background subtraction?

Type of normalisation and why not between data sets?

Welch (deals with unequal variance better than Students t-test)

A fold change of 1.5 and p value of $p<0.05$ should be used as a cut-off



Transcriptomics Reporting Framework

Raw Data

Collection and curation of data

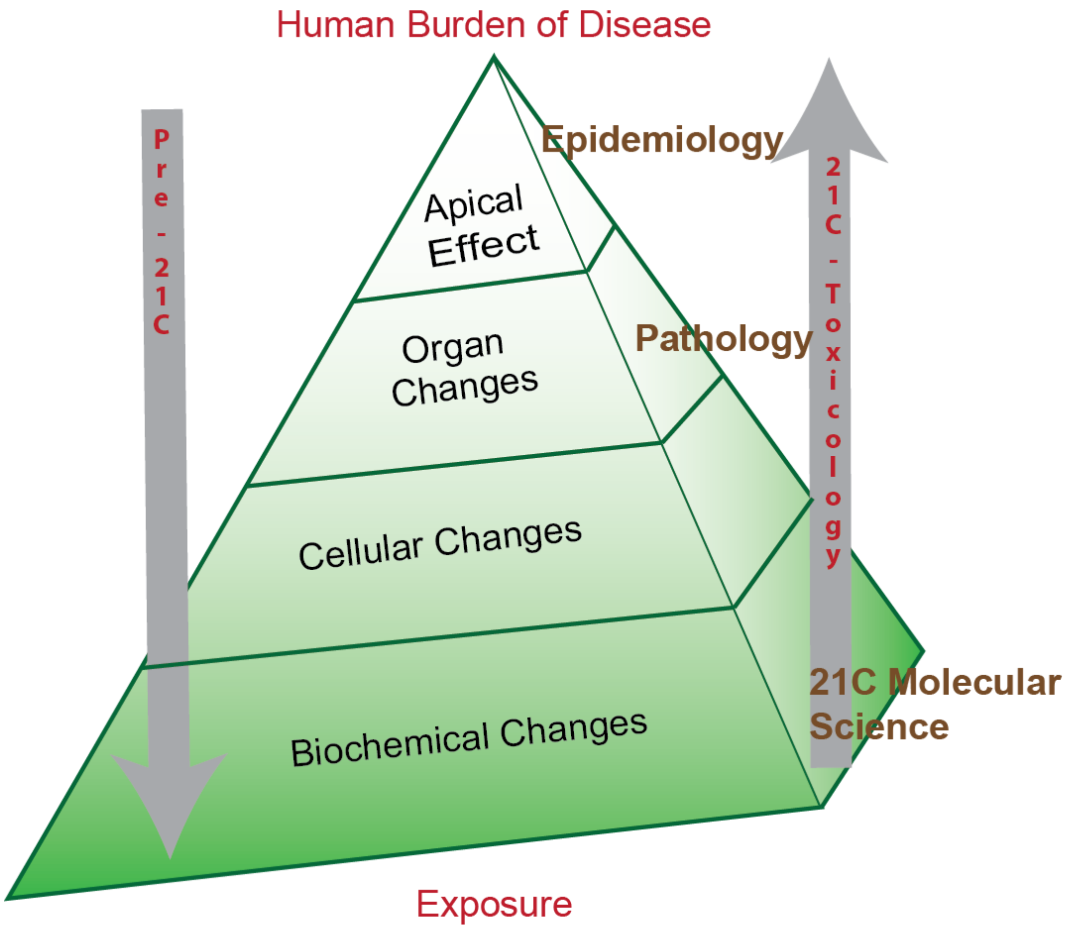
Processing of omics data to obtain interpretable list

Reference baseline analysis method

Weight of evidence approaches for inegration and interpretation of data

Metabolomics Reporting Framework

21 Century Toxicology



Does omics have value to chemical risk assessment?

Yes; with care