Application of technology tools in human health assessments of chemical entities.

Timothy W. Gant

MRC Toxicology Unit

LRI Annual meeting Brussels 2010

New tools are available to toxicologists – but first what do we want to achieve?

- Address public/regulator concerns with meaningful and applicable science.
- Use more *in vitro* and less *in vivo* models
- Where *in vivo* models are used ensure they are relevant
- Understand and predict differential sensitivities/resistance in populations and individuals.
- Find better markers that allow for population monitoring with little invasion for sample collection
- Make more reliable predictions of toxicity
- Achieve all of the above at less cost

Outline

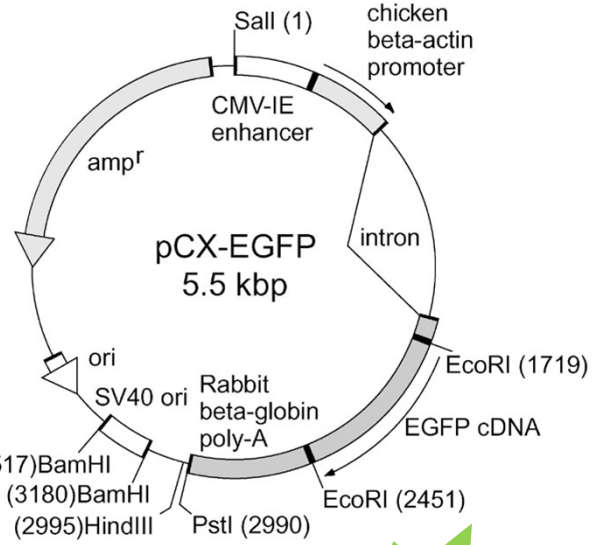
- *In vivo* systems
- *In vitro* systems
- High throughput tools
- Bioinformatics and databases.

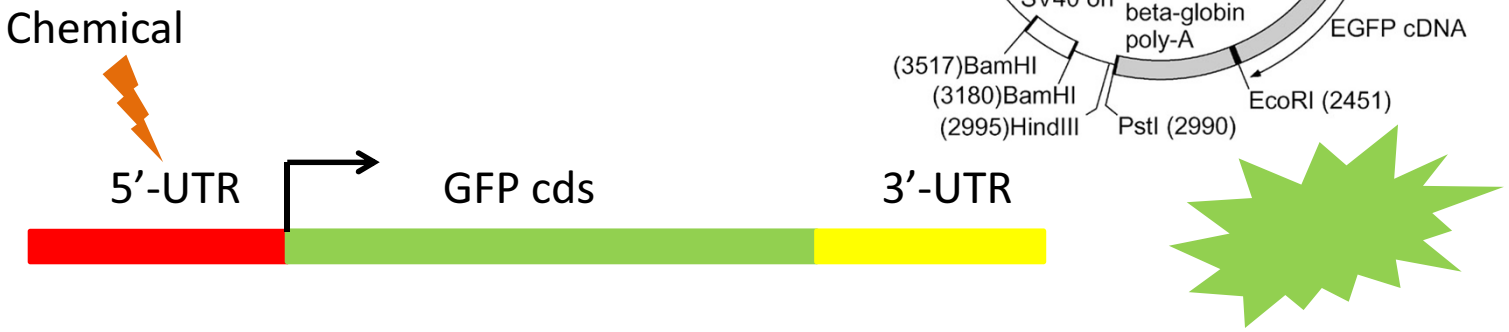
Genetic tools

Modified in vivo and in vitro systems – gene knockin/knockout and mutant

http://kumikae01.gen-info.osaka-u.ac.jp/tg/tg-ad.cfm

Usage as model systems or as reporters for gene events, reporter can be targeted to whole mouse or organs depending on the promoter region used.

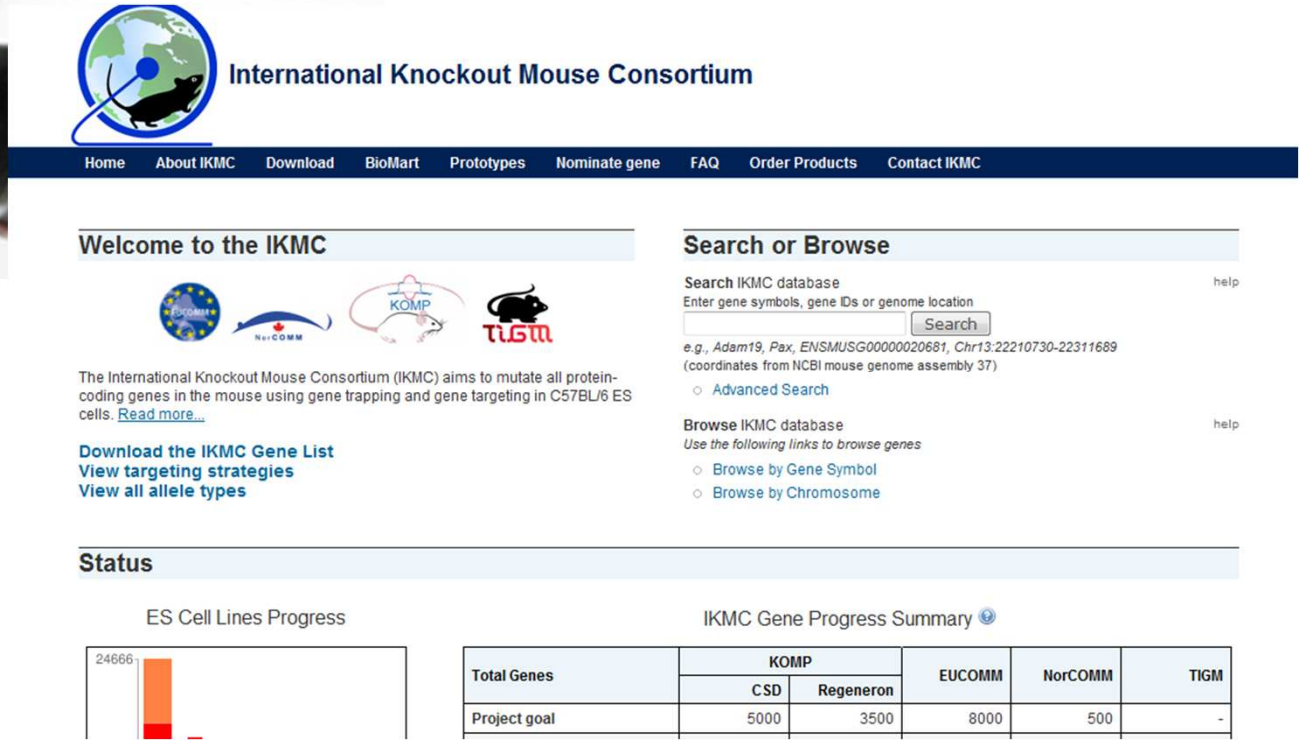




Gene knockout and phenotype

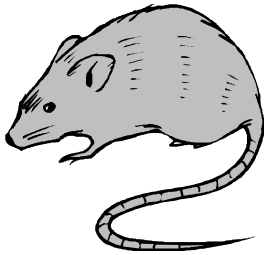
The ob/ob mouse.

Mutants and knockouts are particularly useful for investigating the role of genetic variation in responses to



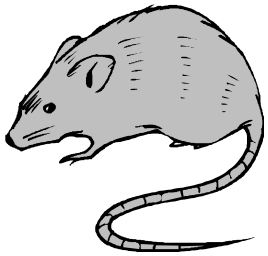
The cre locus

Useful for looking at genes that are embryonic lethal or where there is a requirement to look at the effect in one tissue

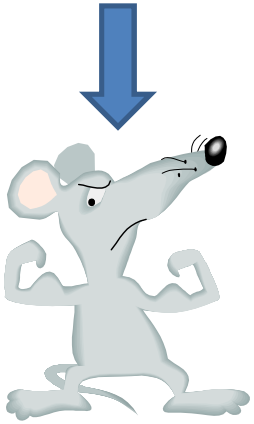


Floxed mouse

X



Cre recombinase mouse



There are many variations on this technology.

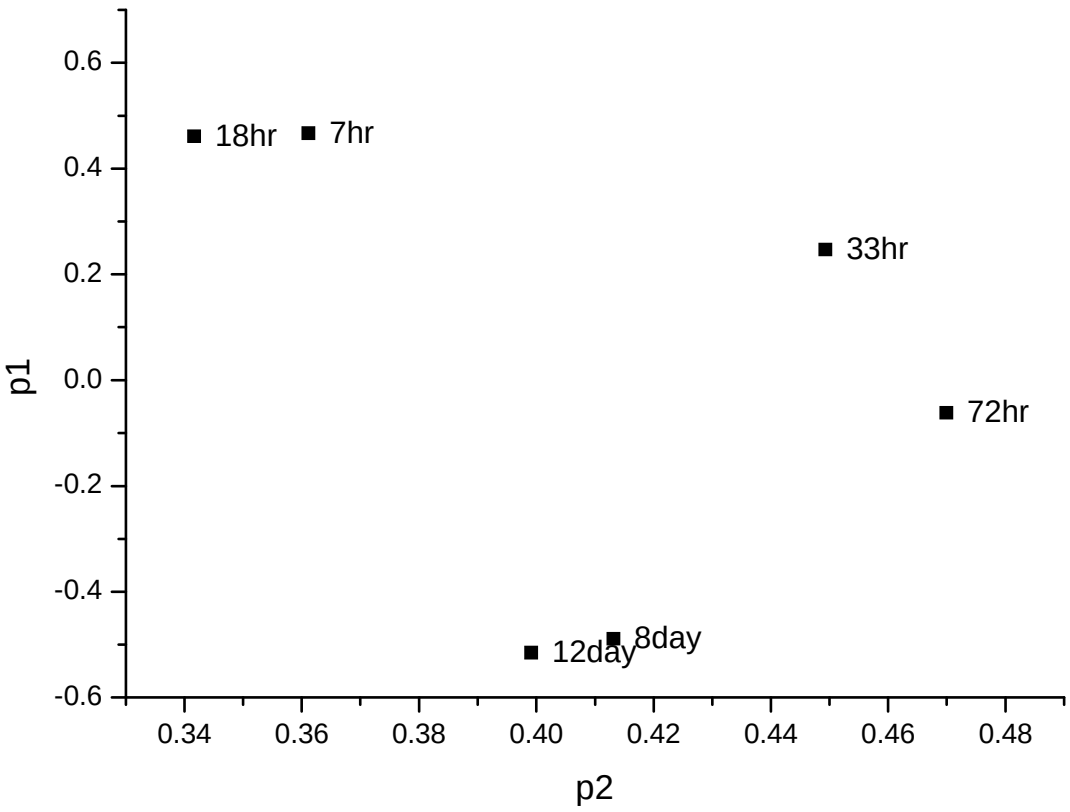
In vitro cell tools

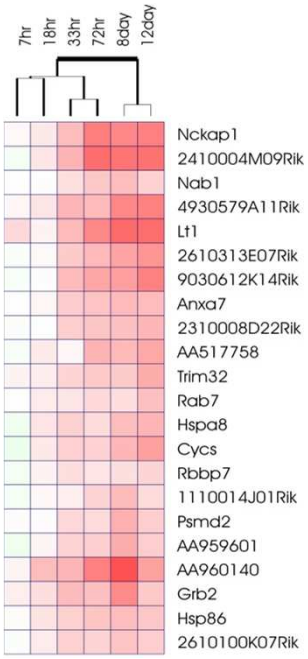
- Primary cells – a useful model but it should always be remembered that they change physiology in culture.

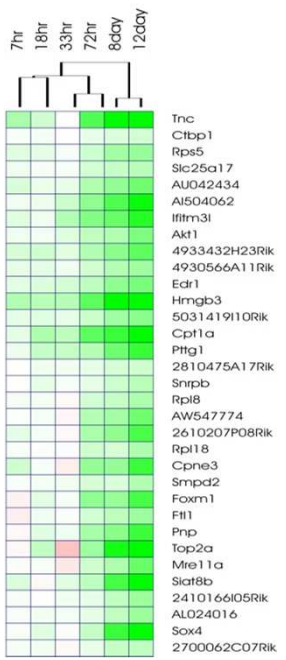
- Cell lines, established and genetically modified

- Stem cells
  - Differentiated from Embryonic cells
  - Differentiated from induced pleuripotent cells

Primary neuronal hippocampal cells

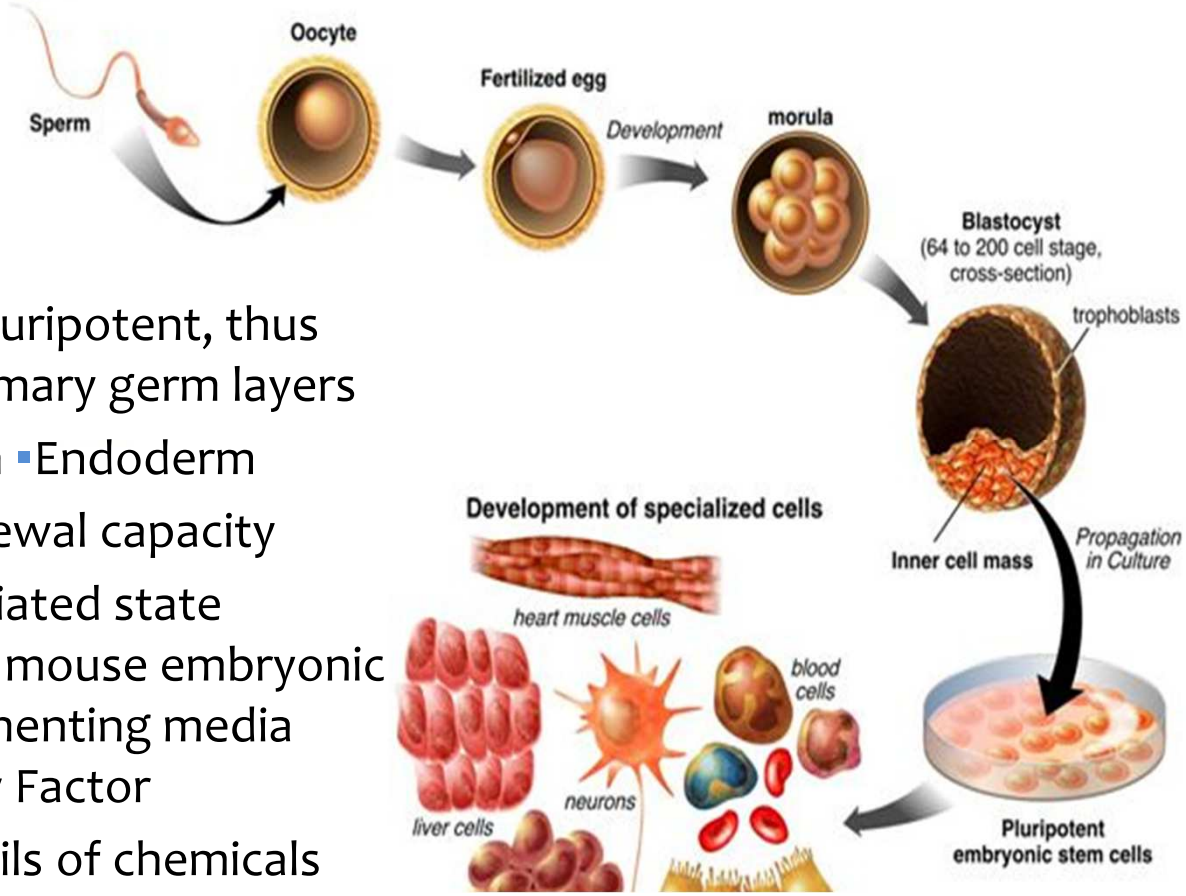






From ArrayExpress – Accession number E-MEXP-11

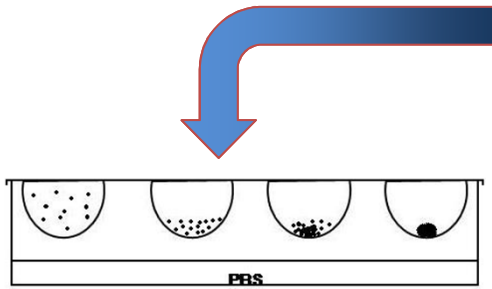
Embryonic Stem cells

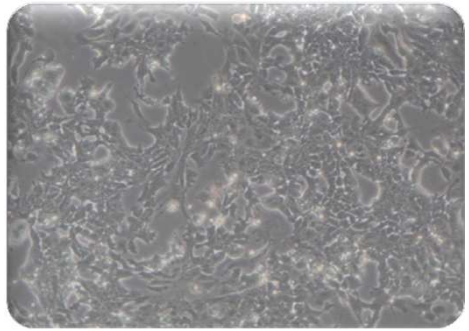


- Inner cell mass cells are pluripotent, thus can develop into the 3 primary germ layers
  - Ectoderm
  - Mesoderm
  - Endoderm
- Unlimited *in vitro* self-renewal capacity
- Maintained in undifferentiated state through co-culturing with mouse embryonic fibroblast and by supplementing media with Leukaemia Inhibitory Factor
- Differentiate using cocktails of chemicals and growth factors

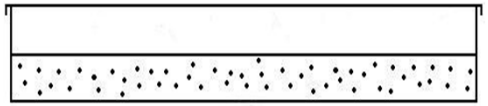
Differential of ES cells.

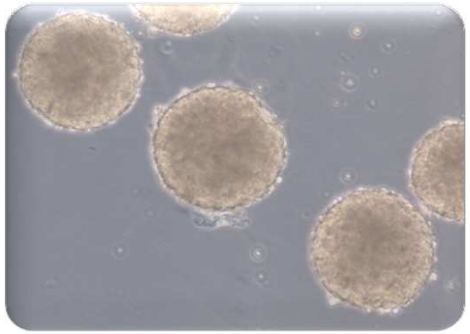
Hanging Drop Culture



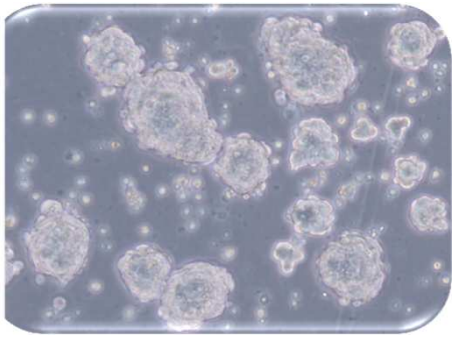


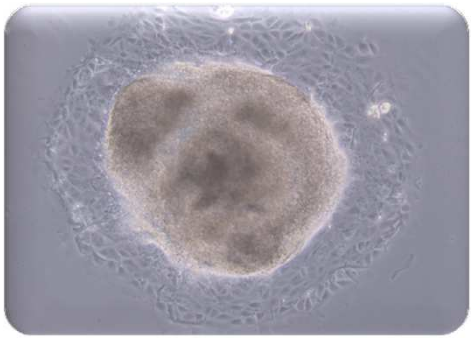
Suspension Culture



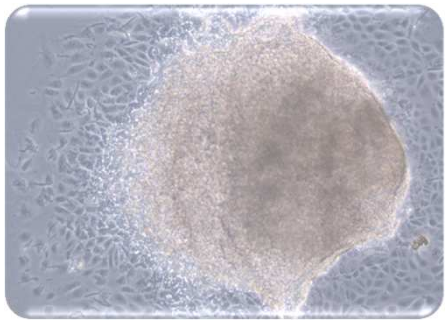


Culture of embryoid bodies

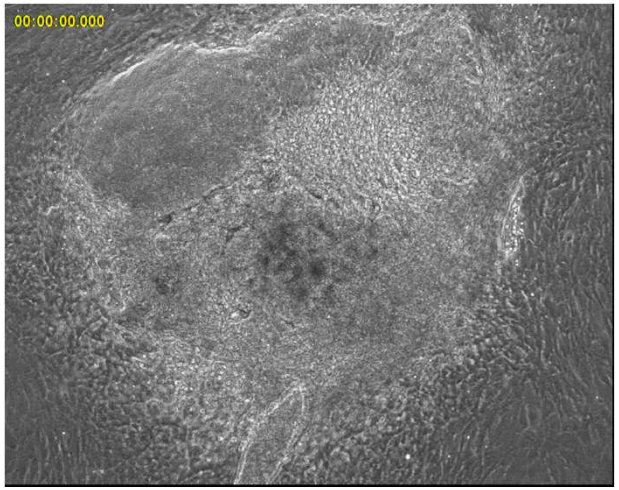


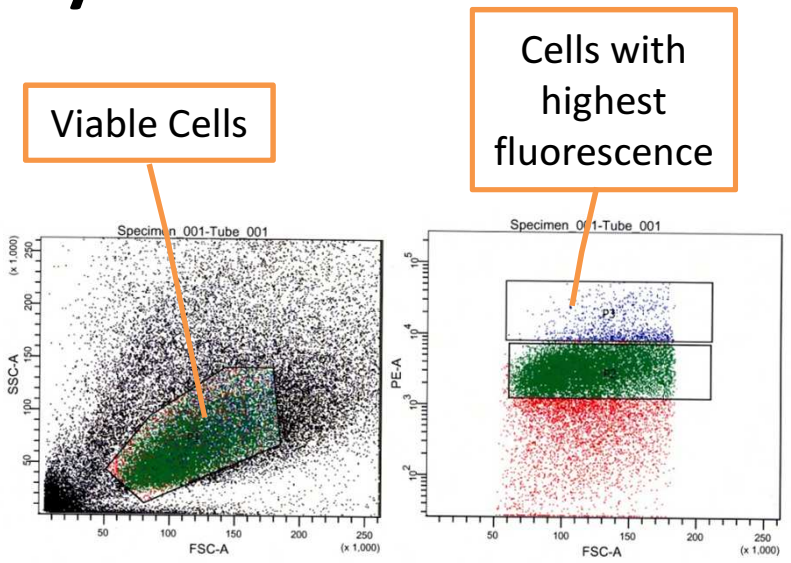


Attachment



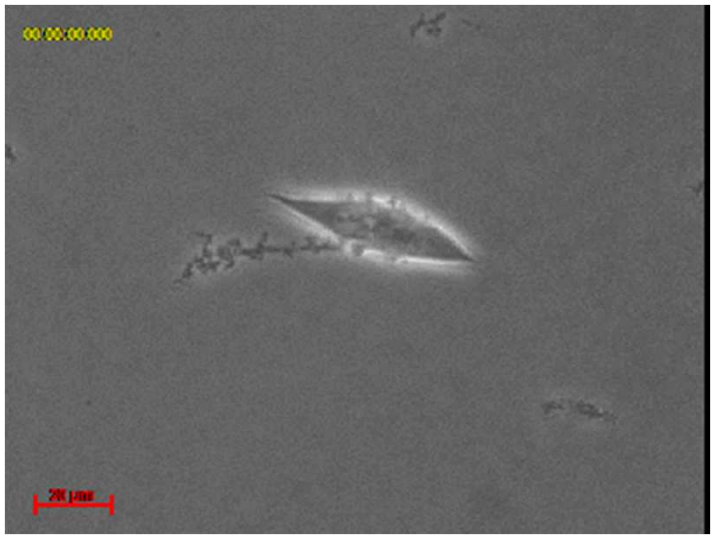
Cardiomyocytes





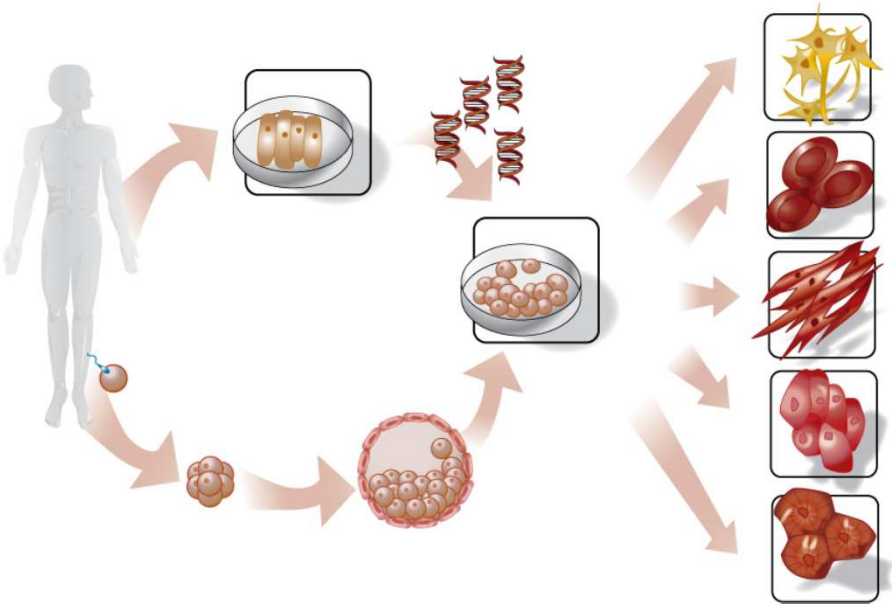
Day 15

Cell suspension treated with a mitochondrial selective dye (TMRE) and sorted according to fluorescence intensity using FACS (adapted from Hattori *et al.*, 2010). Cells with the highest fluorescence intensity re-plated.



Induced pleuripotent cells

- Cells derived from normal somatic cells such as fibroblasts
- Transfection of stem cell like factors cause de-differentiation into stem cells like cells
- From these can then be re-differentiated cells of different types



<u>Advantages</u>

- Do not require foetal material
- Cells can be derived from many individuals reflecting the diversity of human genetic background.

Cell systems – Stem cells

Differentiated from embryonic cells

- Potentially a source of 'primary cells' modeling the normal in vivo human cells

From iPS cells

- Source of human 'primary' cells
- Available with different genetic backgrounds
- Decreased moral issues

Current disadvantages ;

- High maintenance, have yet to prove their worth over well differentiated cell lines, line and particularly genetically modified cell lines
- Different differentiation protocols may give variability in the derived cells.
- Yields of differentiated cells are low
- Yet to achieve protocols that give differentiated cells the are fully matched with those from tissue of the same type.

Technology tools

Systems primary measuring tools – ‘omics

- Genomics
  - microarrays
  - HTS/NGS
- Proteomics
  - Mass spec for total for proteins or protein events such as phosphorylation or ubquitination
- Metabonomics
  - NMR
  - Mass spec
- Lipidomics etc.

Next generation sequencing

Relies on the parallel sequencing of many millions of DNA fragments

What can it be used for generally:

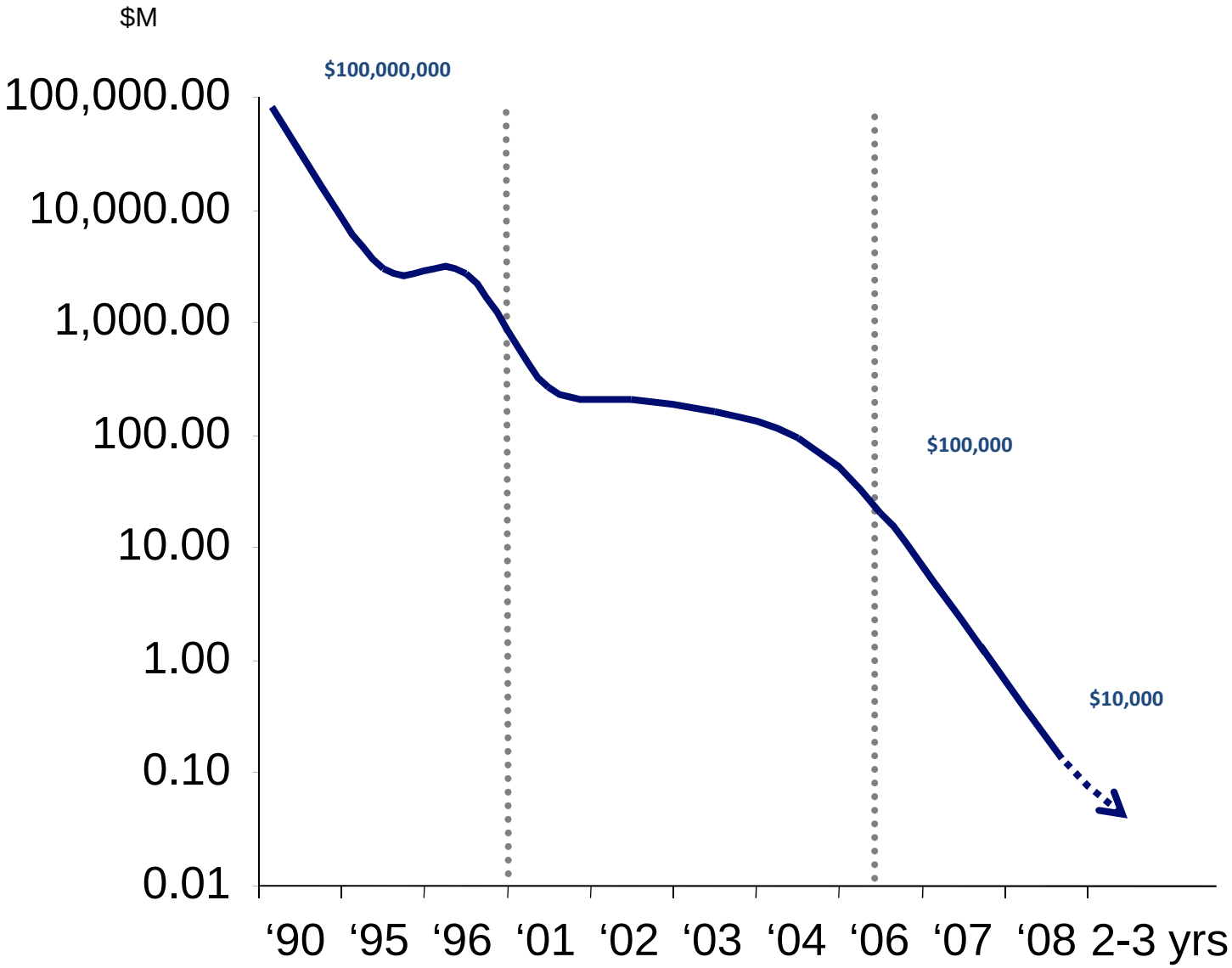
1. Analysis of the genome; SNPs, CNVs, methylations marks, indels and other variants
2. Analysis of the transcriptome; mRNA counting, splice variation

In safety assessment;

Xenobiotic induced changes in mRNA transcripts (much like microarrays)

Contribution of genetic background to individual/strain/species variation in response the chemical exposure.

Where is it going - costs



Remember this guy from ICCA Amsterdam

A genome costs about $30000 and will come down more next year and the year after that .

"Looks like you've got all the data —what's the holdup?"

He has even more data and is still in the office – on the up side data handling is improving

More data = Greater Uncertainty?

- The flow of data will not stop
- It is both generating understanding but also more uncertainty.
- It is neither desirable or possible to sop the data flow. The responsibility here for both academic institutions and the industry is to work together to put it in context and understand it.
- There is a disconnect between our ability to generate data and ability to understand it

ICCA Amsterdam 2008

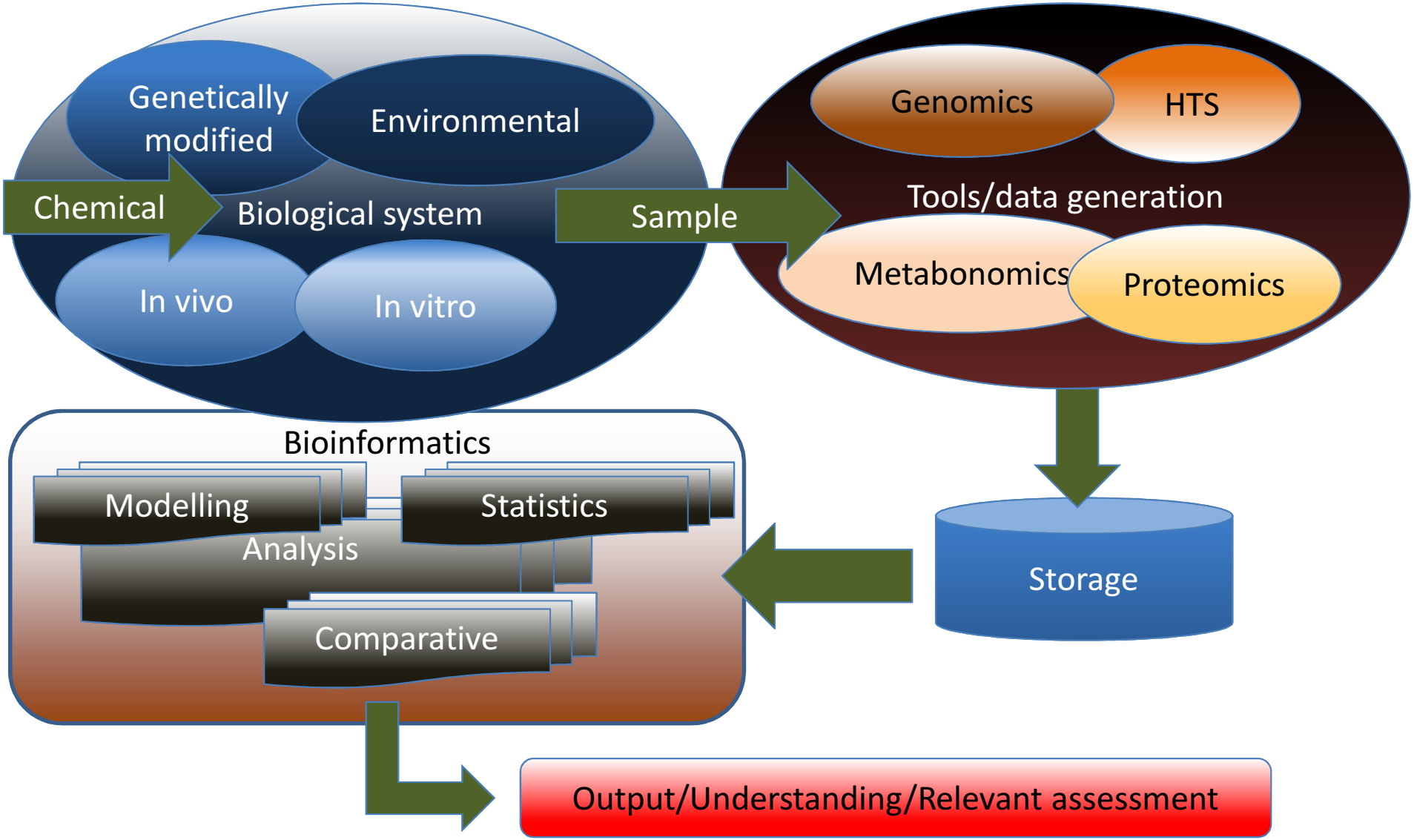
‘There is a need to understand chemical effects in early life and the influence of polymorphisms’

Gary Ginsberg

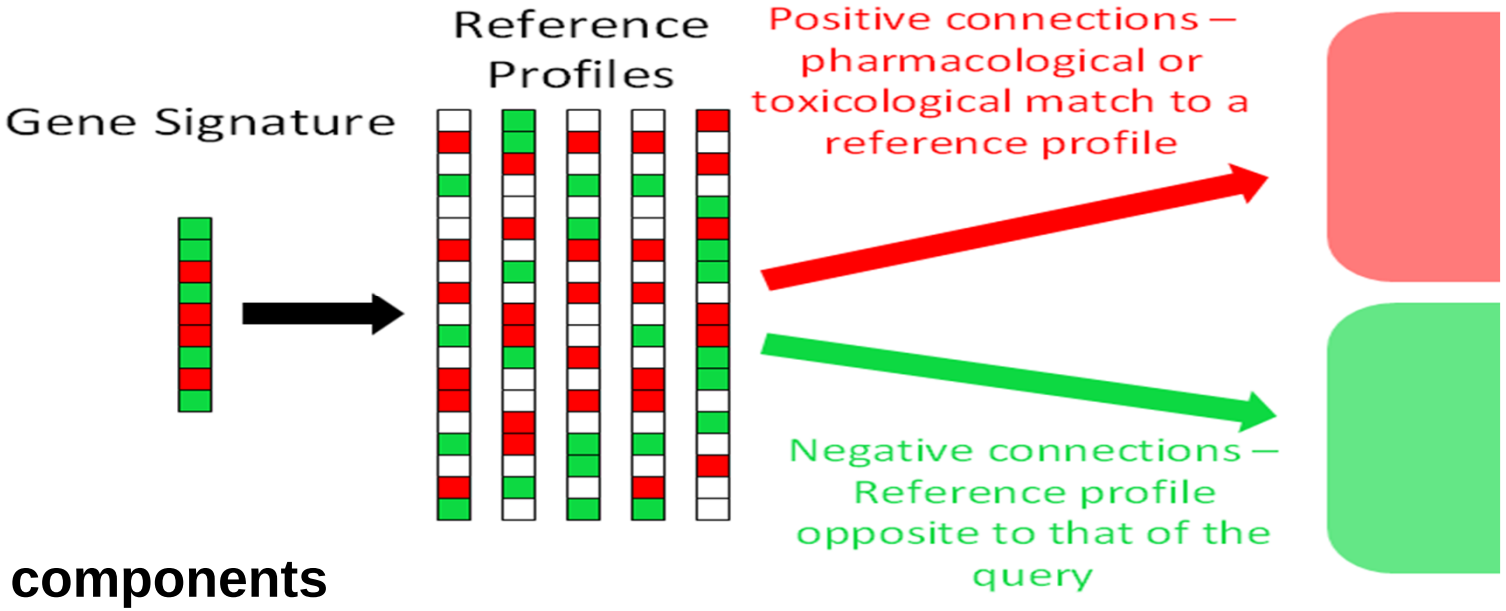
We have fantastic technology to identify gene polymorphisms, but understanding is ~~lacking~~ lagging.

Tim Gant

Bioinformatics – the glue



Connectivity mapping



Key components

1. Reference Profiles: A set of gene expression profiles, obtained from systematic microarray gene expression profiling.

   [MCF7 and other cell lines, 164 bioactive small-molecule compounds, 564 microarrays, 453 individual reference profiles. GEO database GSE5258, J Lamb et al., Science 313, 1929 -1935 (2006)]

2. Gene signature: a short list of important genes, selected by the researchers as a result of some microarray experiments investigating a particular biological condition.

3. Connection score, defined as a function of a Reference Profile and a Gene Signature. It should reflect the underlying biological connection between them.

The algorithm – Zhang/Gant

For an ordered gene list of N genes where g_i represents the gene (g) number (i) in the signature the score for that gene is the product of its signed rank in the query profile (s) and its signed rank in the reference profile (R). Thus the connection score between query signature of m genes and the reference profile is:

$$C(R,s) = \sum_{i=1}^{m} R(g_i)\mathrm{s}(g_i)$$

It is brutally simple.

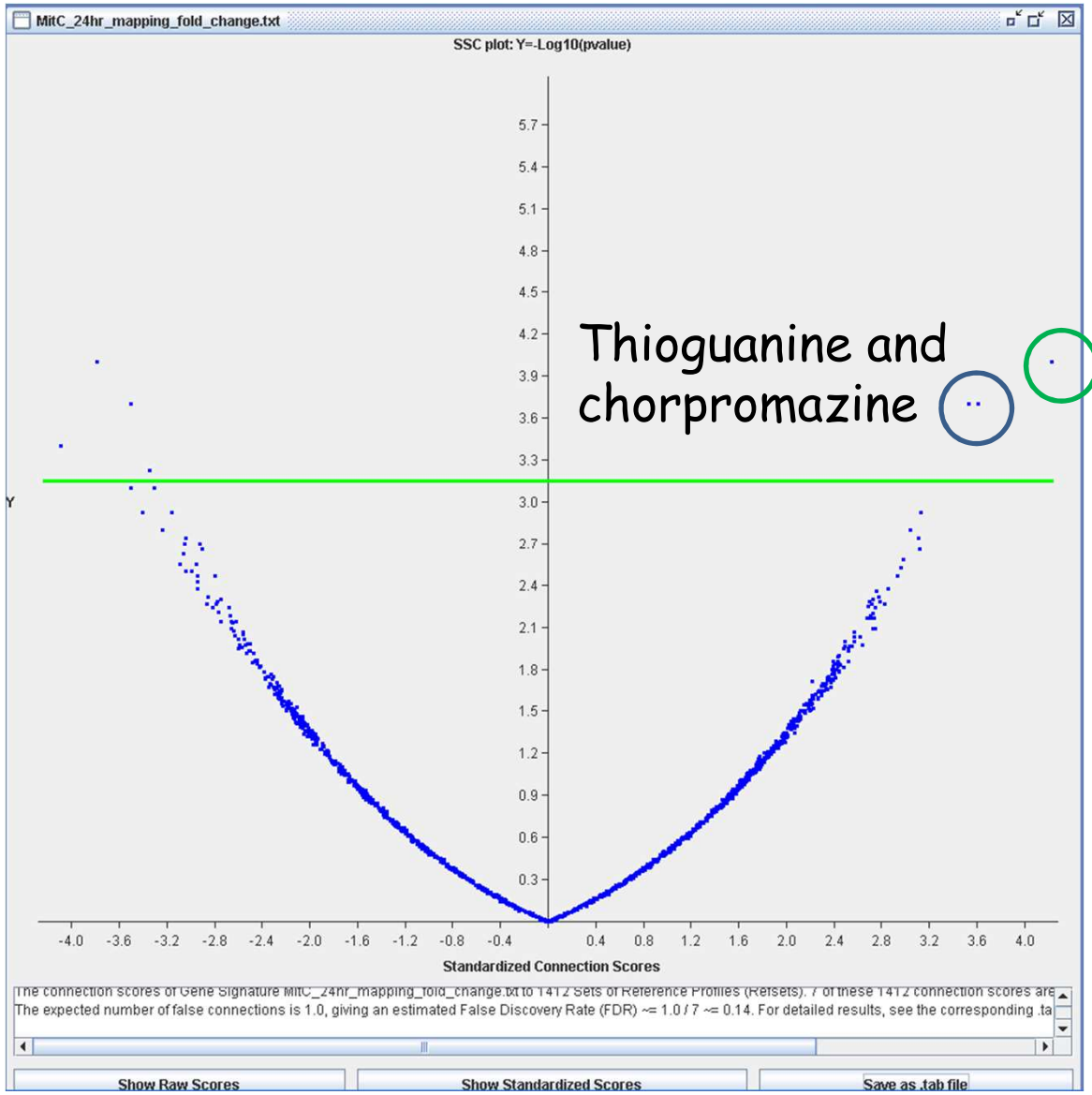
And you don't have to keep reassembling your data as you may have to do for multivariate methods.

Transcriptional patterns of genotoxicity - Mathijs *et al* E-MEXP-2209

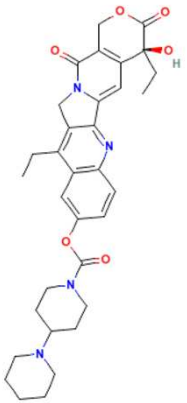
Chemical	DNA effect	Ah receptor effect
Mitomycin C	Genotoxic/Adducts	None
DMN	Methylation	None
Aflatoxin B1	Bulky adducts	Some Ah like effect
Benzo(a)pyrene	Bulky adducts	Ah agonist
TCDD	None	Ah agonist
Cyclosporin A	None	None

Mathijs et al *Tox Sci* 112, 374 (2009).

Mitomycin C 24 hour 50 genes



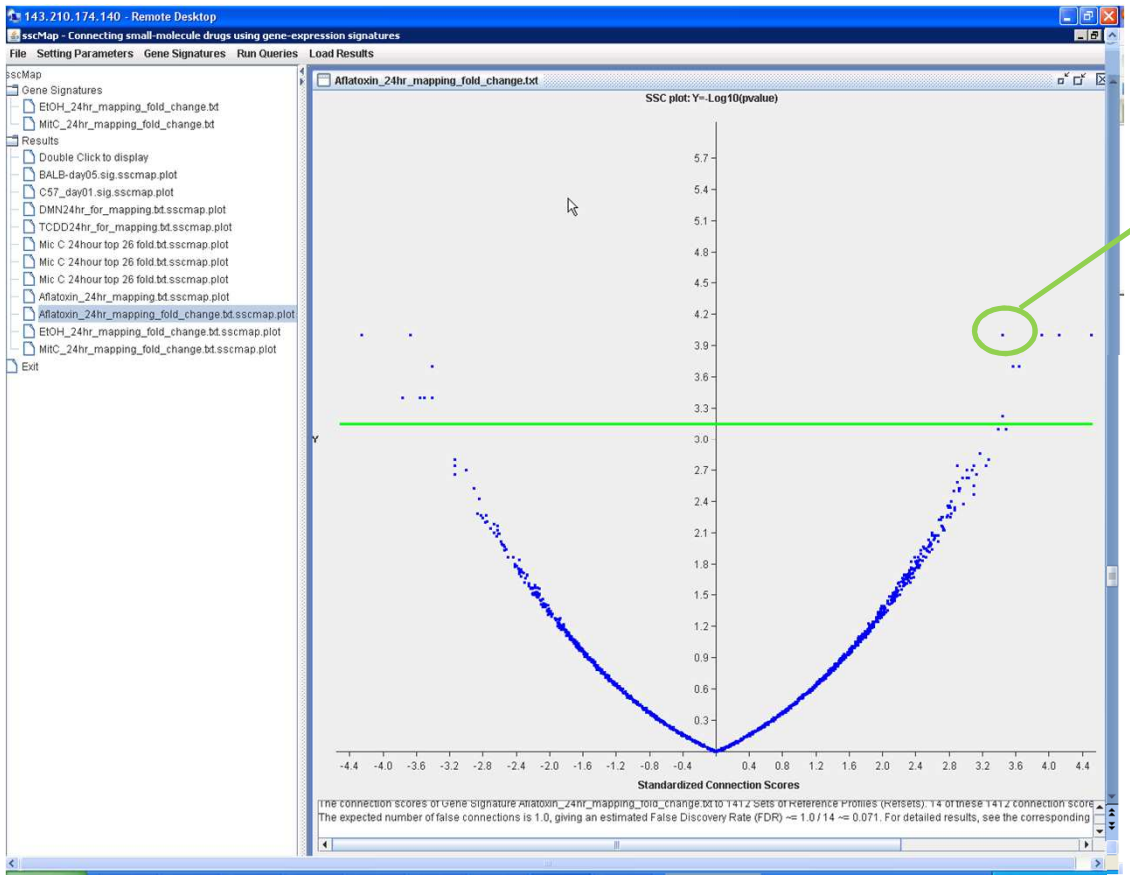
Irinotecan



Topoisomerase I inhibitor – leads to double strand DNA breaks

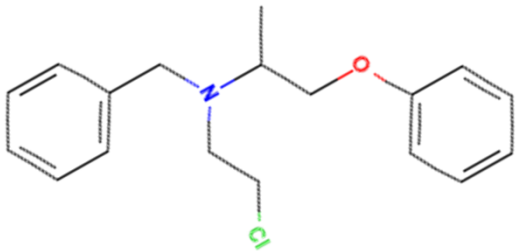
Chorpromazine positive in sister chromatid exchange assay

Aflatoxin B1 - 24 hour 50 genes



Phenoxybenzamine

GENE-TOX Evaluation A (pre-1980):
Species/Cell Type: Nonhuman **Assay Type:**In vivo carcinogenicity studies **Assay Code:**CCG+ **Results:**Positive **Panel Report:**EMICBACK/67174; MUTAT RES 185:1-195,1987



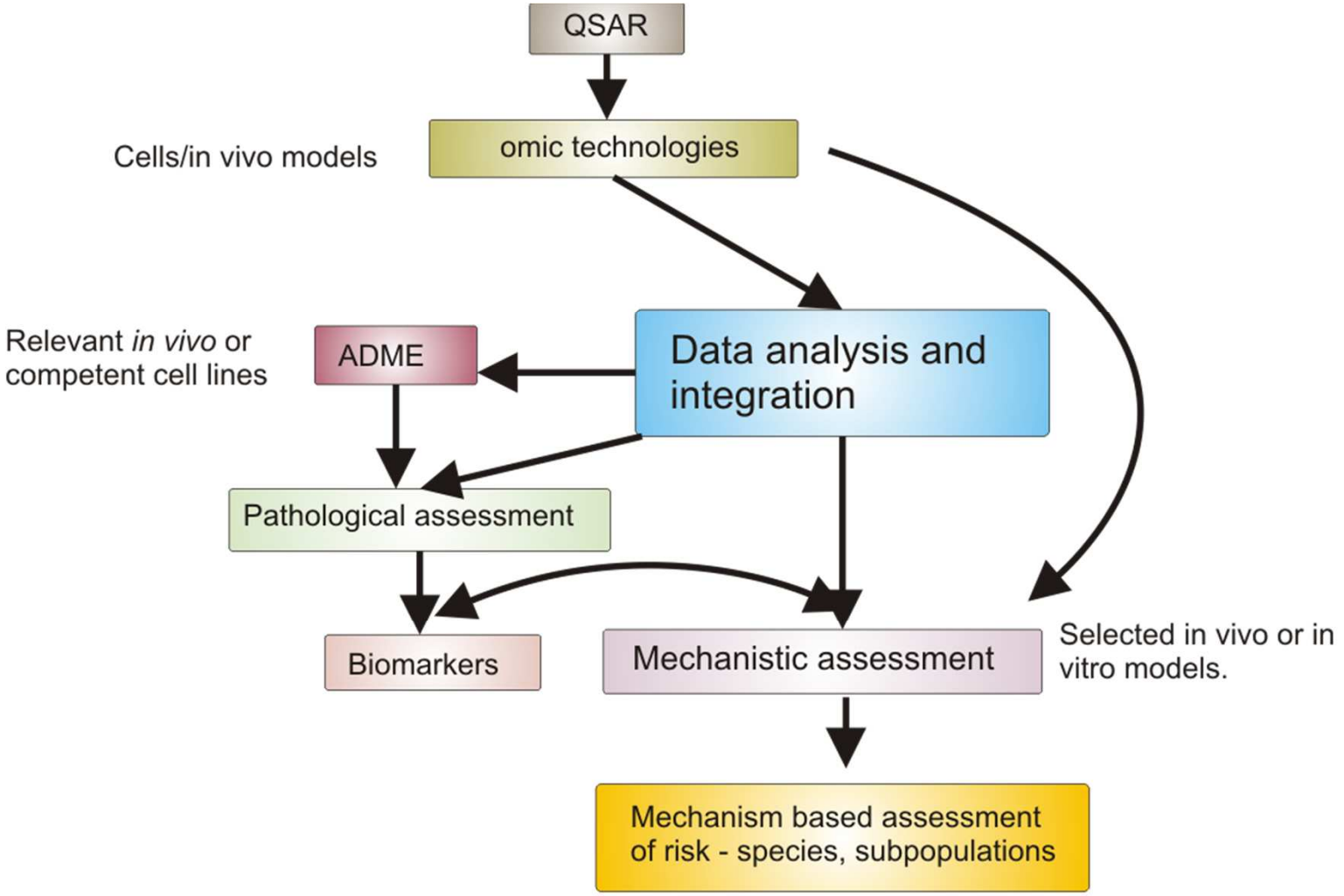
Translation

- Validation of new methods takes so long - by the time it has started the technology has moved on.
- But do we need validated fully tests? Indeed is there any such thing as a validated test. What is right for one chemical class will be wrong for another so what is the point in trying to develop generic tests. What is applicable for on individual will be wrong for another. How are stipulated protocols going to improve risk assessment? Lets get more intelligent.

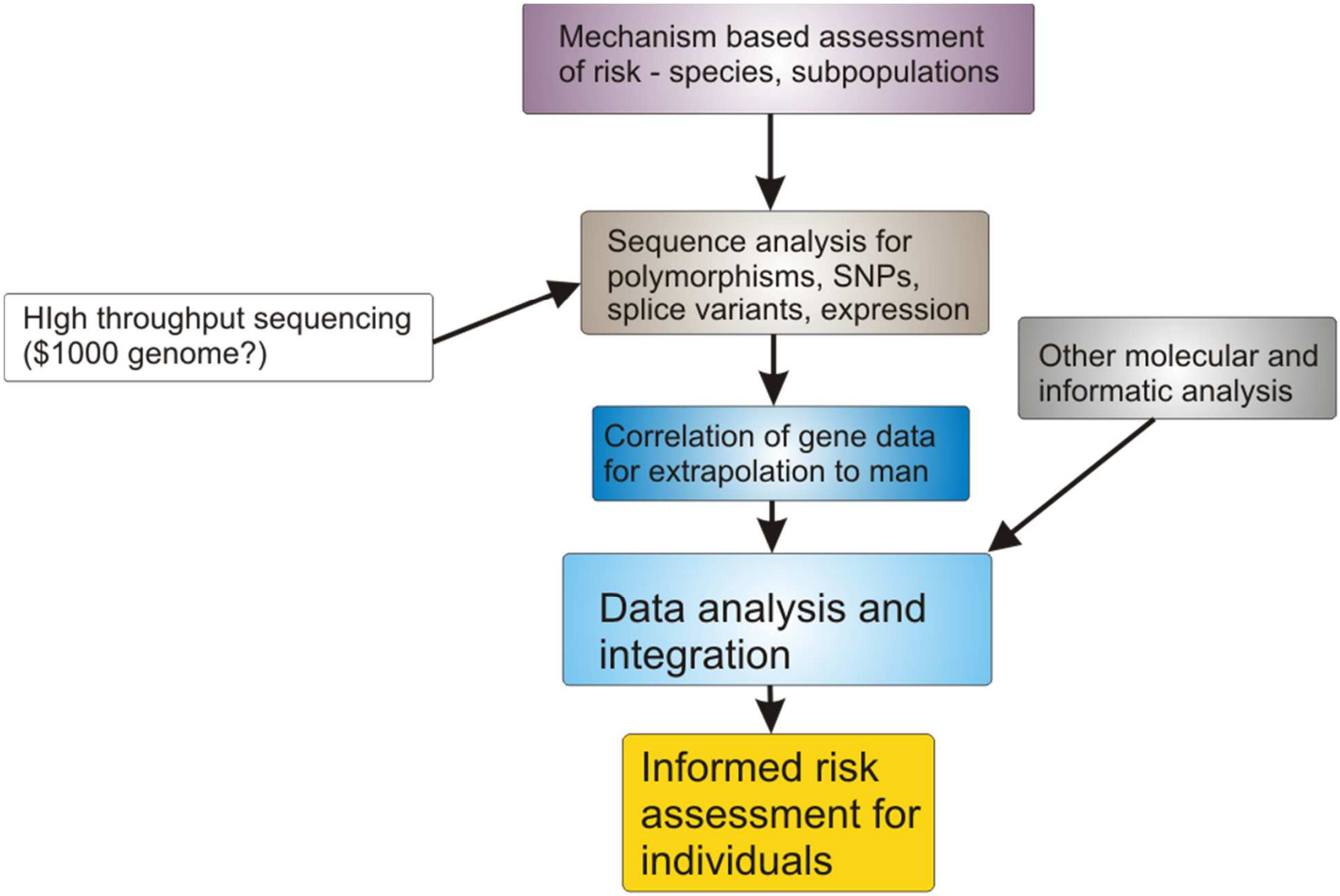
Instead use a toxicity pathway (mode of action) approach* with decision points at each method point. With appropriate positive controls this could work well and allow the immediate incorporation of new methods into chemical risk assessment.

* See US EPA strategic plan for evaluating the toxicity of chemicals 2009.

A flexible and on-going approach to risk assessment



Relevant risk assessment



Acknowledgments

Thanks to:

Shu-Dong Zhang
Harsharon Bahir
Joshua Smalley