Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Toxicogenomics, human risk assessment and possible regulatory uses

Timothy W Gant

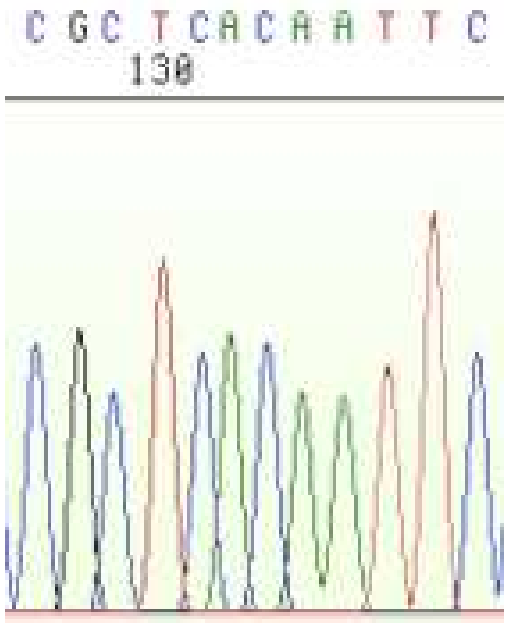
IGHRC OCTOBER 2017

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

20 plus years of 'omics

- Omics has been with us for 20 years plus starting with early metabolomics by NMR and leading into transcriptomics in the late 20th century building on the back of the development of the 96 well capillary sequencer giving rise to clone availability and later the genome sequence (draft 2001; complete 2003).

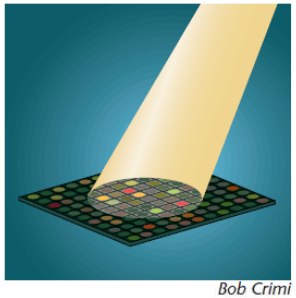


Public Health England
Protecting and improving the nation's health

The Chipping Forecast I

UNIVERSITY OF SURREY

Experimental manipulations will also need to be rigorously controlled. Responses to microenvironment (for example, the position of a culture dish in an incubator or the time of day at which an assay is performed) pose a special risk of misleading global expression studies, in which one is fishing through 100,000 genes to find the small subset that vary. It is well known among *aficionados* that comparison of the 'same' experiment performed a few weeks apart reveals considerably wider variation than seen when a single sample is tested by repeated hybridization.



Bob Crimi

onductor manufacture to produce arrays with oligonucleotides, each in its own 20 μm^2 region[15].

The greatest challenge, however, is analytical. The first expression profiling experiments involved comparing just two samples, with the aim of identifying those genes whose expression levels

extended its dominion: it has domesticated the Megabase and will tame the Gigabase in the not-too-distant future.

The next great challenge is to discern the underlying order. The Periodic Table summarized chemical propensities in its rows and

tories (owing to the actual capital cost of setting up an arraying facility or the amortized capital costs reflected in the purchase price of arrays). Still, these problems are likely to be solved by economies of scale, free-market competition and time—just as

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

The Chipping Forecast II (2002)

forewora

Biology's century: just the beginning for microarrays

doi:10.1038/ng1026

icology. Scientists are conducting profiling studies that may lead to the use of microarrays in personalized medicine, in molecular diagnosis of disease and in predicting drug efficacy and toxicity in different individuals. Just imagine the opportunities to learn even more using this technology!

At times like this, scientists require the utmost flexibility and support from the technologies that they use and from the companies with whom they work. Agilent's vision is to provide scien-

understands
this industry.
and unwaveri
and services t
Agilent Tec
ment of *Nat*
has exemplifi
ward to suppo

in different individuals. Just imagine the opportunities to learn even more using this technology!

At times like this, scientists require the utmost flexibility and support from the technologies that they use and from the companies with whom they work. Agilent's vision is to provide scien-

and services that we provide to help scientists in their research.

Agilent Technologies is proud to sponsor this special supplement of *Nature Genetics*. We salute the spirit of innovation that has exemplified the past 50 years of genetic research and look forward to supporting you for the next 50.

CHRIS VAN INGEN

Agilent Technologies, Life Sciences & Chemical Analysis
395 Page Mill Road

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Genomics in regulatory use

Regulatory Toxicology and Pharmacology xxx (2017) 1–13

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

The challenge of the application of 'omics technologies in chemicals risk assessment: Background and outlook

Ursula G. Sauer [a], Lize Deferme [b], Laura Gribaldo [c], Jörg Hackermüller [d], Tewes Tralau [e], Ben van Ravenzwaay [f], Carole Yauk [g], Alan Poole [h], Weida Tong [i], Timothy W. Gant [j, *]

[a] Scientific Consultancy – Animal Welfare, Germany
[b] ExxonMobil Petroleum and Chemical, Belgium
[c] European Commission, Joint Research Centre, European Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), Italy
[d] Department of Molecular Systems Biology, Helmholtz Centre for Environmental Research - UFZ, Germany
[e] Department of Chemical and Product Safety, German Federal Institute of Risk Assessment (BfR), Germany
[f] BASF SE, Germany
[g] Environmental Health Science and Research Bureau, Health Canada, Canada
[h] European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Belgium
[i] National Center for Toxicological Research (NCTR), U.S. Food and Drug Administration (FDA), USA
[j] Centre for Radiation, Chemical and Environmental Effects (CRCE), Harwell Science and Innovation Campus, Public Health England (PHE), UK

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

How is omics applied in regulation?

Table 1
Summary of responses from the written inquiry: Chemical companies' experiences with the regulatory use of 'omics technologies.

Respondent	Regulatory use of 'omics?	If yes: For which purpose? If no: Why not?	Regulatory acceptance of 'omics data?	Technical comments
A	Yes, transcript-omics.	MoA categorisation for hepatocarcinogenesis; classification based on data similarity by the hierarchical clustering method.	Under consideration.	Procedure conducted in accordance with Affymetrix or Agilent protocols.
B	No.	Results not sufficiently reliable to ensure worker protection; 'omics data do not satisfy REACH provisions.		'Omics technologies must deliver reliable endpoint-relevant results, which can be used for the derivation of Derived No Effect Levels or Predicted No Effect Concentrations and for C&L.
C	Yes, but not in REACH dossiers.	'Omics were applied to support occupational exposure limits.		Data generation is not controlled; the outcome is too experimental condition-specific to be meaningful; it is difficult to correlate 'omics data to actual adverse effects and to determine human relevance.
D	Yes, Next Generation Sequencing, transcript-omics.	For internal decision making and as supporting data for all crop protection products. A few situations where full 'omics data were used for regulatory submission – to elucidate very complex MoAs.	Differently: (1) To help support a MoA and determine relevance, or lack thereof, to humans; (2) To derive NOAEL; (3) One case: 2-year cancer bioassay waived based upon MoA and gene expression data.	
E	Yes, but not in REACH dossiers	Pesticide registrations. For important commodity chemicals, research studies using 'omics approaches have been applied for product stewardship and for establishing MoAs.		

There has never been inclusion of 'omics data in a REACH submission

Public Health England
Protecting and improving the nation's health

EHCA report of 2016 on New Approach Methodologies

UNIVERSITY OF SURREY

ECHA stated in its progress report 2015 (published in 2016), that experience has shown that different advanced techniques such as new approach methodologies [that include 'omics methodologies] are not used in many registration dossiers… This lack of use may be an indication that industry does not consider these NAMs to be sufficiently developed (ECHA, 2016).

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Applications and issues?

- Omics data is sometimes generated with chemical actives; eg: Pesticides and biocides. Data analysis typically follows 'that recommended by the manufacturer'.
- Omics data has never been used to support a submission under REACH
- Some use in establishing MoA particularly for pesticides and for product stewardship
- There are no consistent methods applied to the analysis of omics data
- Used internally for decision making
- If omics output could be standardised then there are opportunities for greater use in chemical grouping and read across as well as the assessment of modes of action.

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Research verses regulation?

Research allows for:

- Individuality in approach
- Peer review of methods
- Justification of choices made
- Repetition and verification

Regulatory use requires:

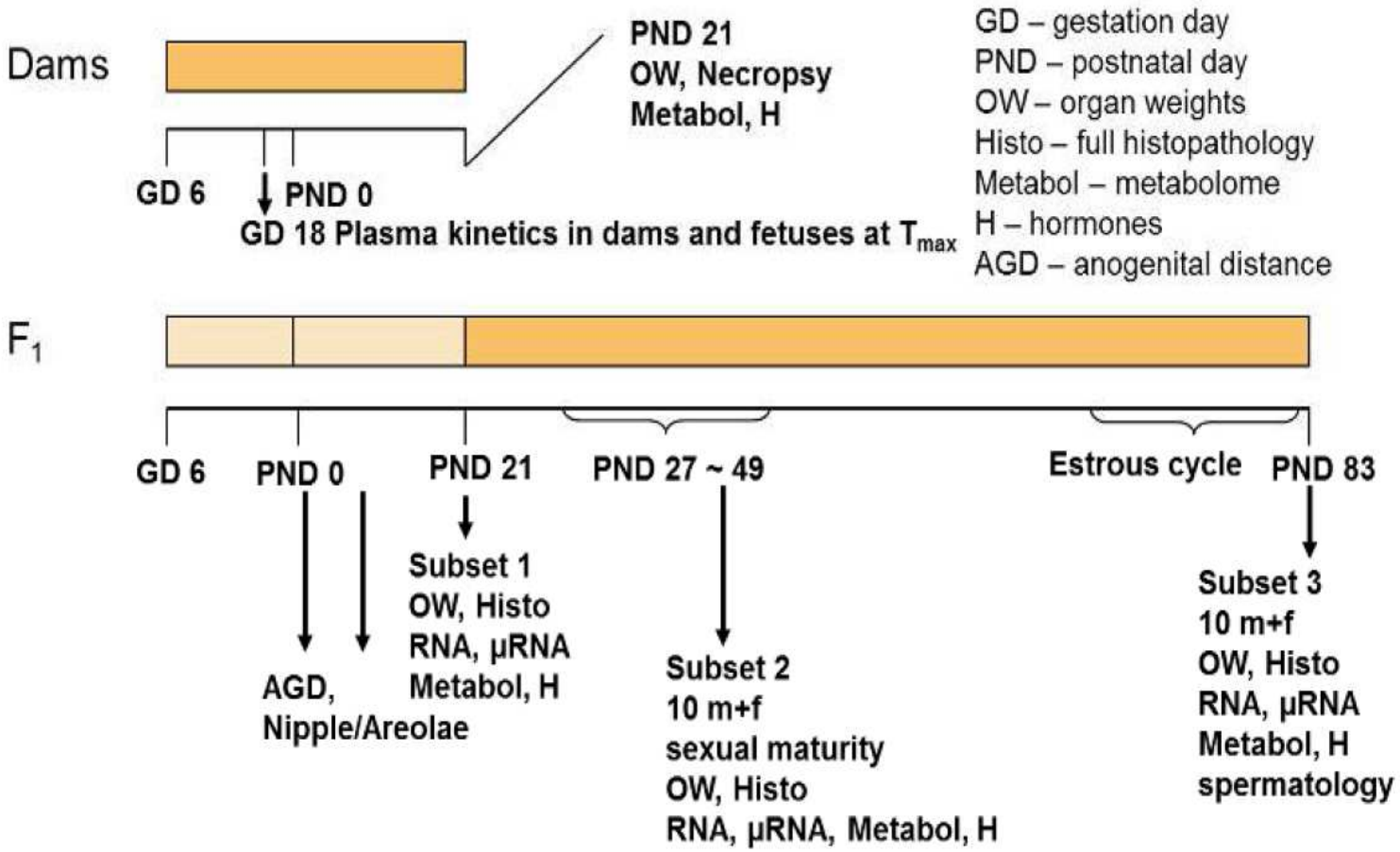
- Common methods and acceptance thereof
- Consistency and reproducibility between laboratories
- Proprietary independence

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Exemplar: The Data Issue: EMSG56

The year is late 2014 - EUROTOX



Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Data mass

Doses
Flutamide – 0.0025, 0.25 and 2.5 mg/kg/day
Prochloraz – 0.01, 5 and 30 mg/kg/day
Vinclozolin – 0.005, 4 and 20 mg/kg/day
Controls – no compound

X

Time points
PND0
PND30-40
PND83

X

Replicates
4

120 data sets of 62976 data points per data set giving 7.56 million data points total

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

EMSG 56

- Searched for a standard, regulatory accepted method for undertaking the bioinformatic analysis of large data sets
- There were no such methods published – OECD does not have a test guideline or a guidance document; MAQC had dealt with data generation but not data processing
- So the data was sent to four houses with specific instructions on how to process the data
- And what did they do……..?

Public Health England
Protecting and improving the nation's health

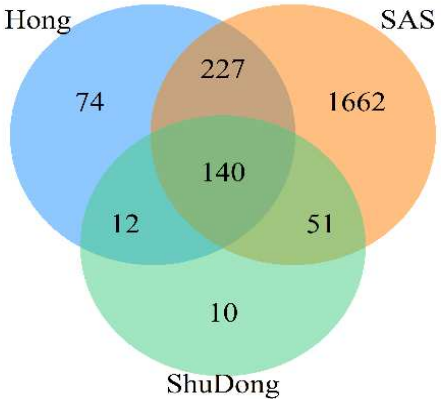
Analysis by three groups – January 2016

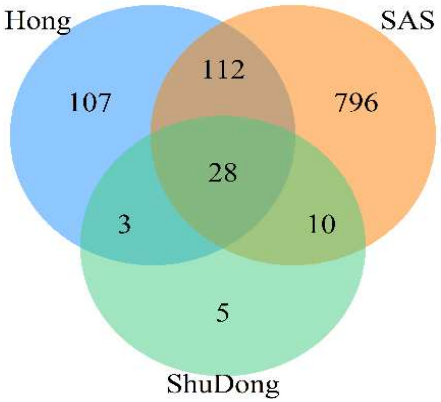
UNIVERSITY OF SURREY

PND30-40

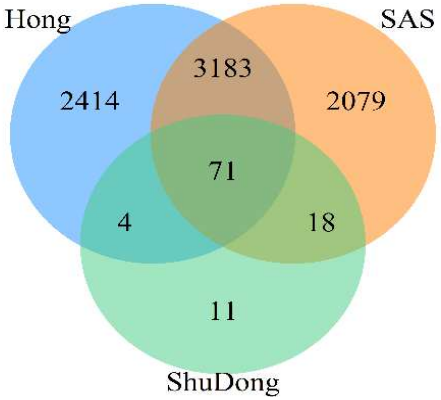
PND83

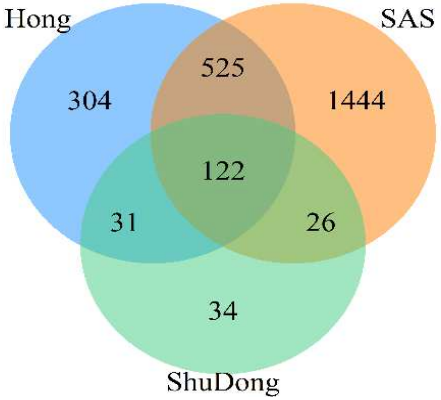
ADI





LOAEL



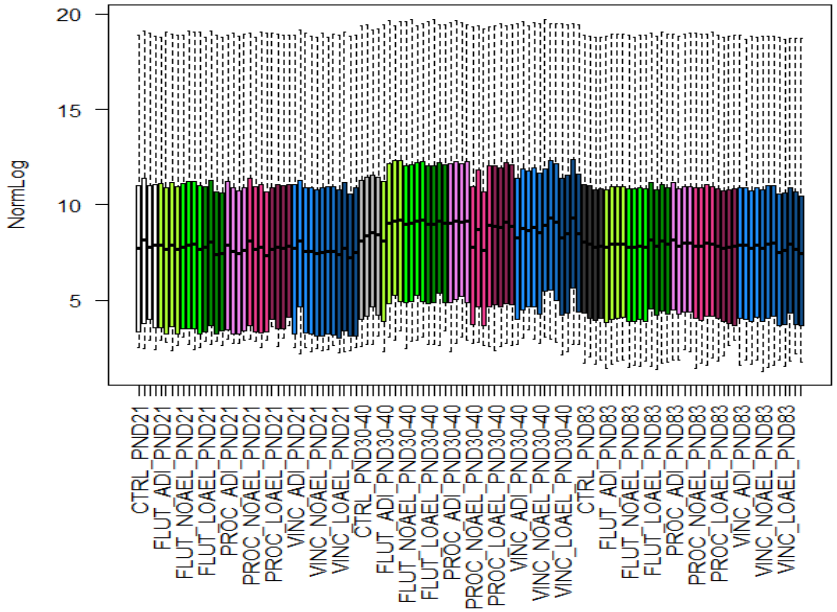


Public Health England
Protecting and improving the nation's health

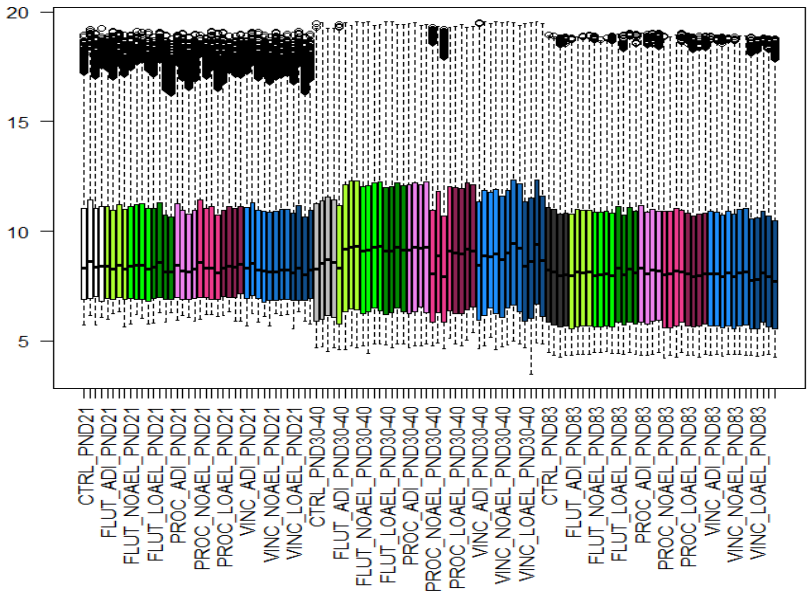
UNIVERSITY OF SURREY

Issue 1 – raw data type

gProcessedData



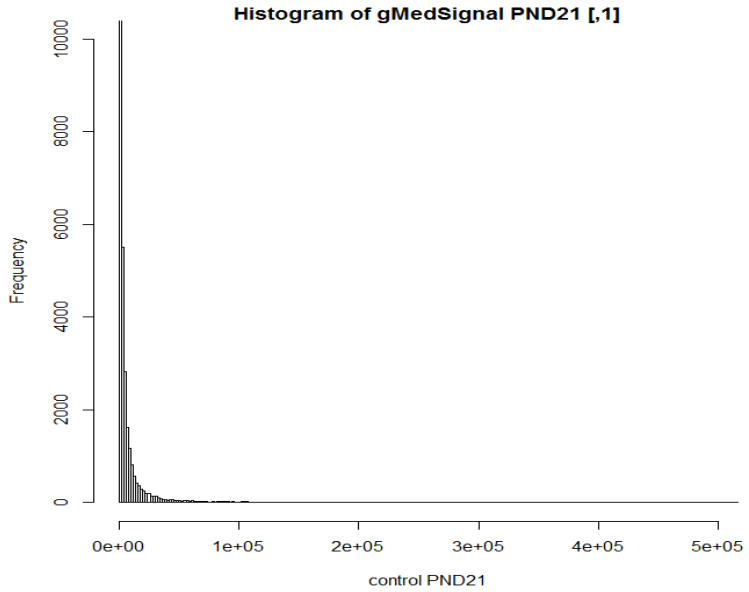
gMedianData

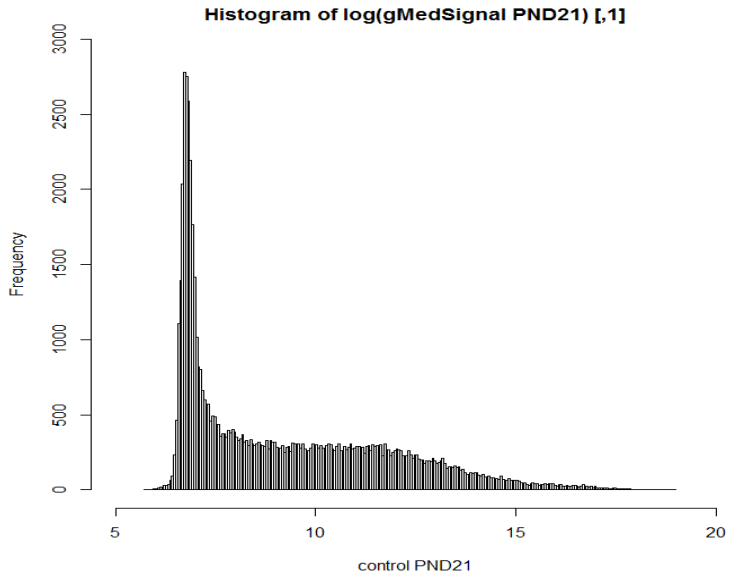


Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Issue 2 – Logs and normality

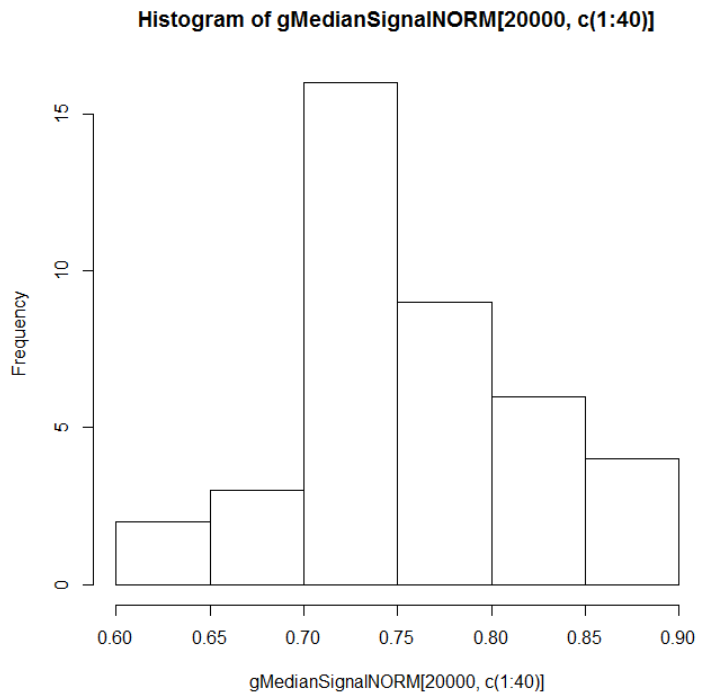


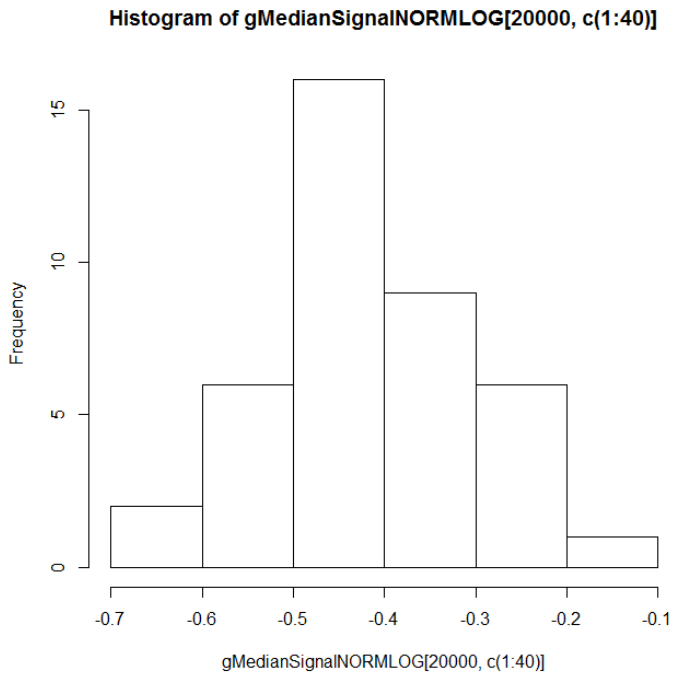


Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Normality: for pairwise comparisons

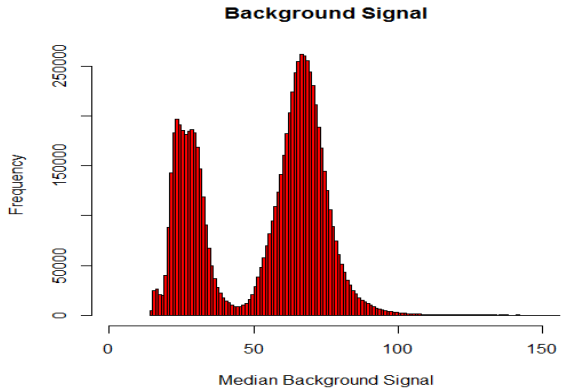


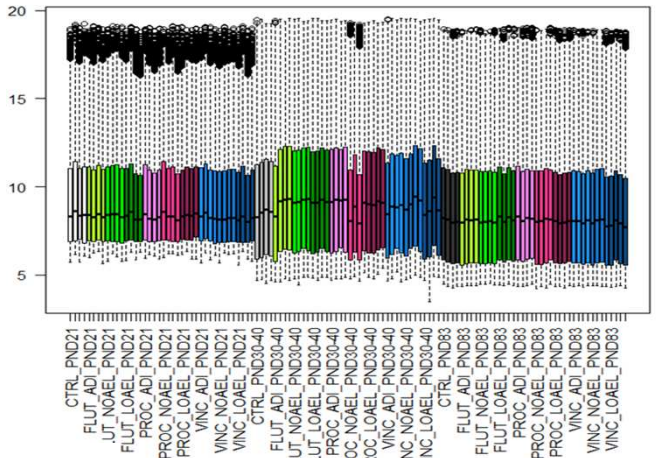


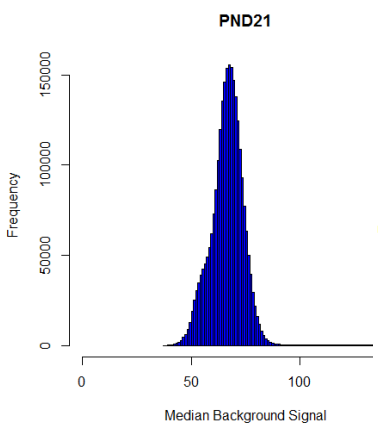
Public Health England
Protecting and improving the nation's health

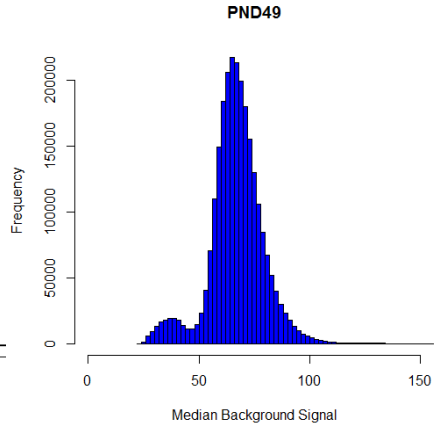
Issue 3 – Background subtraction – yes or no?

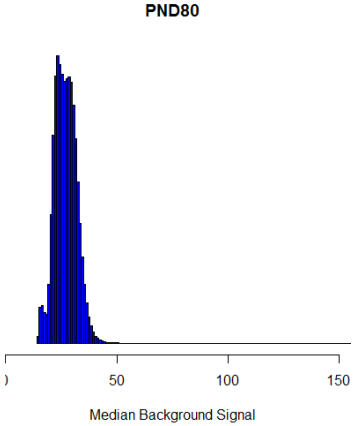
UNIVERSITY OF SURREY







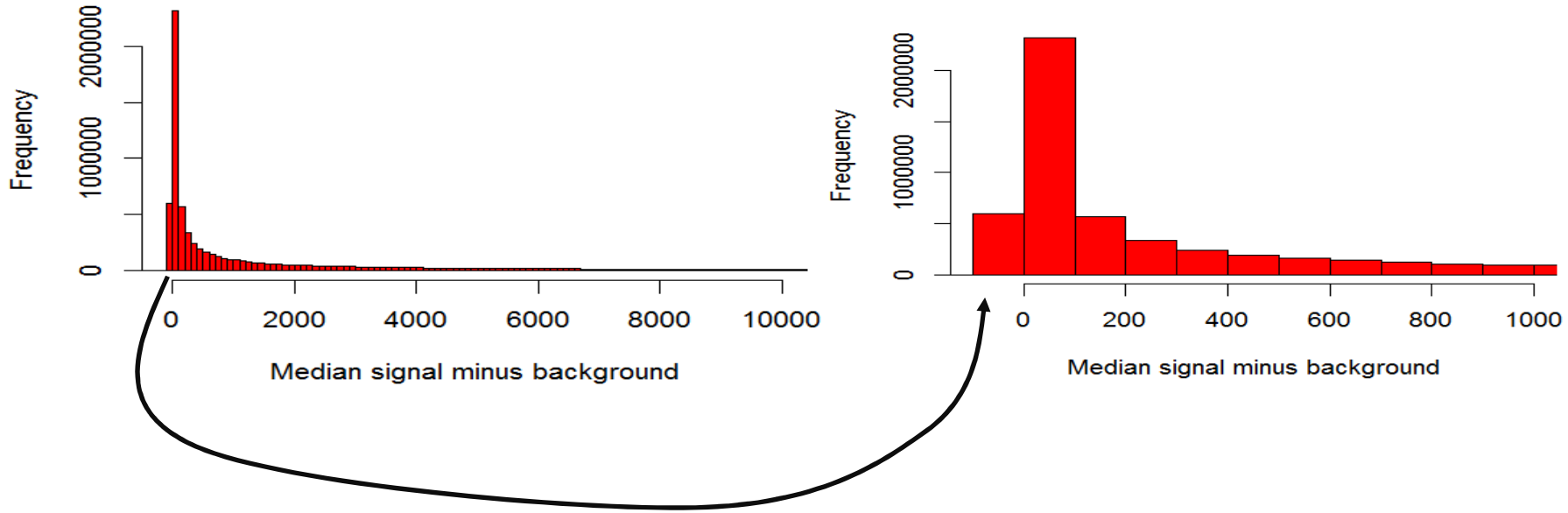




Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Issue 3 – background subtraction



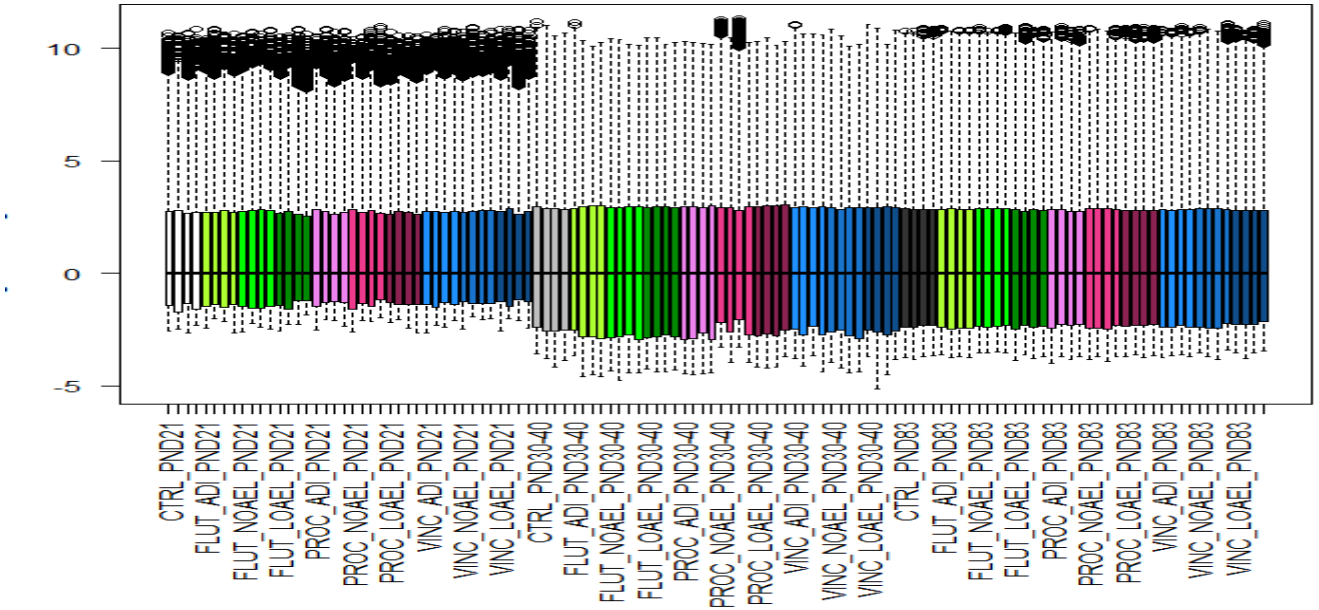
Some signals go below zero because the background is not being measured within the hybridisation area but in the area surrounding and these can be different.

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Issue 4 - Normalization

- Median centering
- LOWESS
- RMA (commonly used in Affymetrix)
- Within sample and across samples



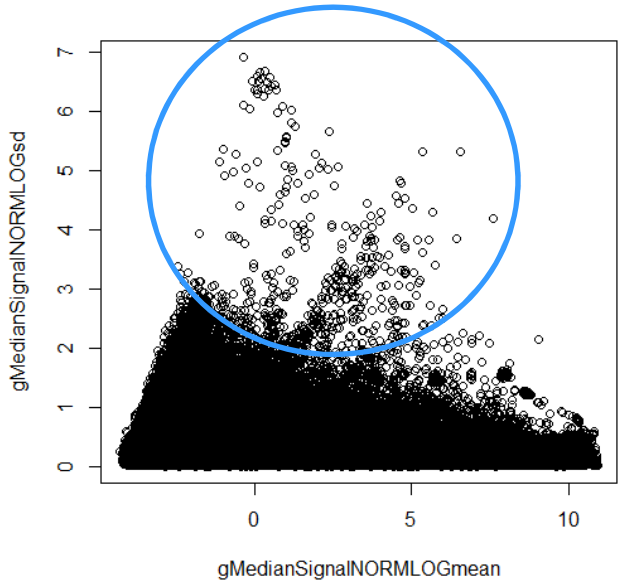
Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Issue 5 – Low signal strength

Greater variation in measurement is associated with a lower signal strength

SD of LOG SIGNAL



LOG SIGNAL

Values are the mean of the four biological replicates for each time and dose.

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Combinations in data analysis

- Image processing
- Background handling
- Transformation
- Normalization
- Gene selection
- Classification
- Biological interpretation
- ……

>10 million combinations

Based on estimation by Dr. Russ Wolfinger (SAS Institute Inc.)
The 4th MAQC Project Meeting, Feb. 3-4, 2006, Boston, MA

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

ECETOC

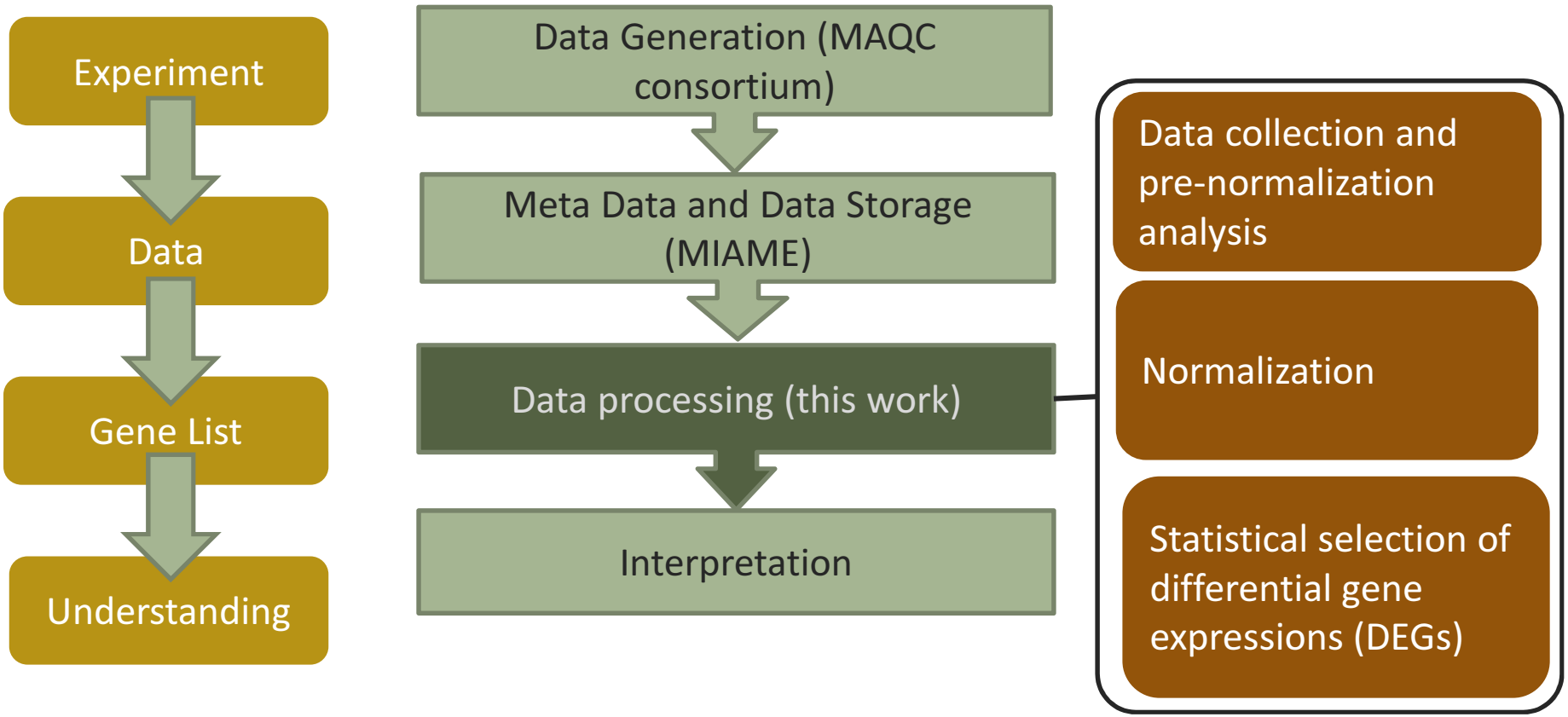
- 6th March 2015 a group convened to look at the data issues from EMSG56 and consider the development of protocols.
- From this a larger group convened July 7-8, 2015 under the auspices of ECETOC to look at the processes of getting from raw data to processed list of differential gene expressions (DEGs).

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Process control in 'omic assessments

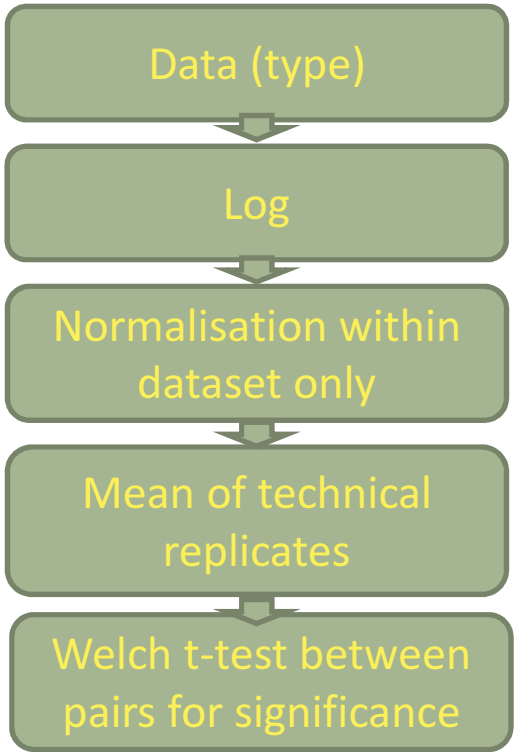
July 2015 in Brussels



Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Outcomes of the July 2015 meeting



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

A reference baseline analysis to act as a denominator for comparison of all other analysis methods

Public Health England
Protecting and improving the nation's health

OECD EAGMST

UNIVERSITY OF SURREY

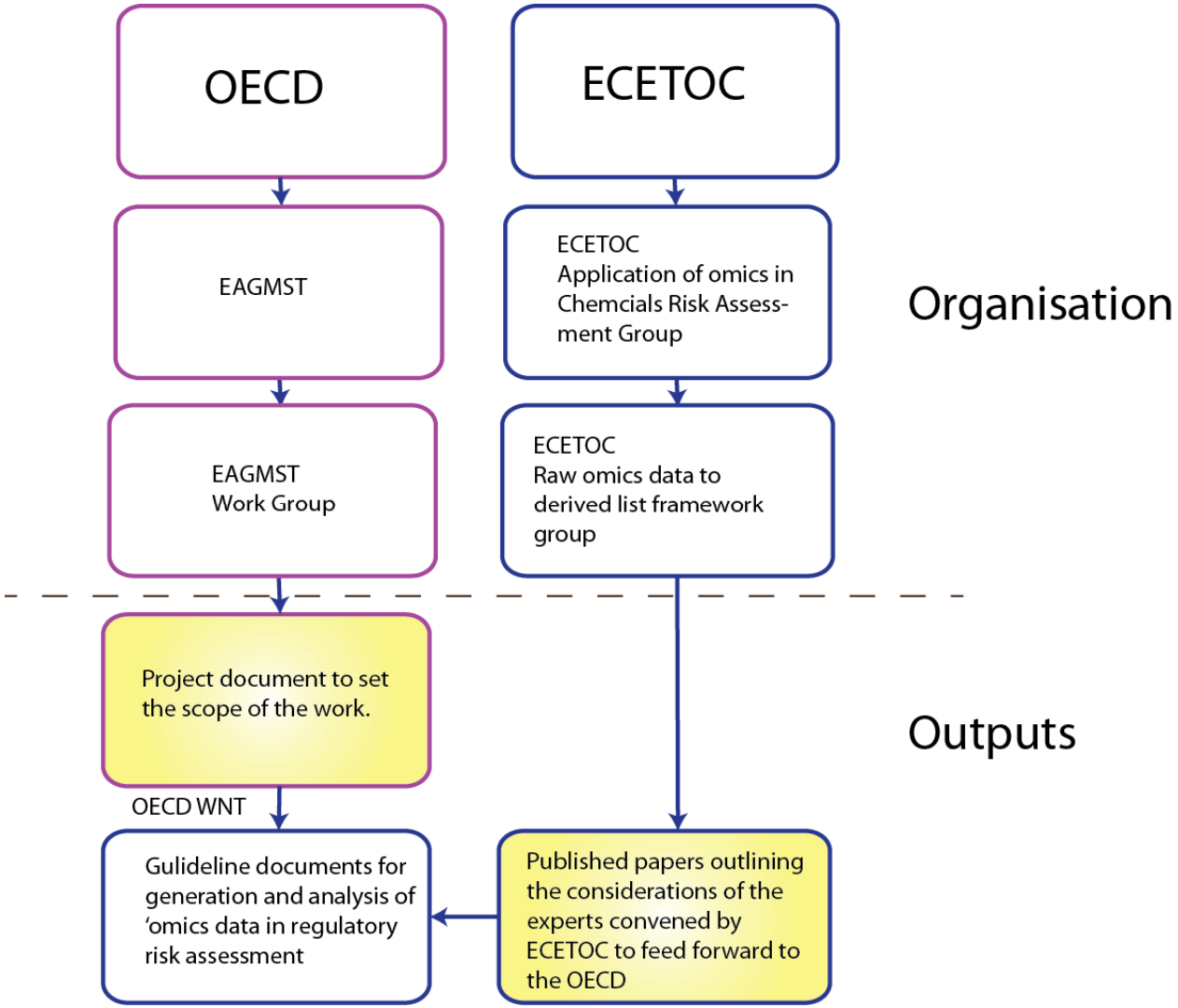
(Extended Advisory Group on Molecular Screening and Toxicogenomics)

- Work from the 2015 workshops presented to the OECD EAGMST meeting in June 2016 (Gant) and agreed for a draft standard project submission form (SPSF) for submission at the December 2016 EAGMST meeting
- Further ECETOC workshop held in Madrid in October 10-12, 2016 led to the development of the concept of 'omics reporting frameworks of which one is the transcriptomics reporting framework. The work to this point is being published in 5 papers currently in press or published with *Regulatory Toxicology and Pharmacology*
- December 2016 – EAGMST acceptance of the SPSF informal agreement to include a project within the EAGMST workplan pending acceptance at the June 2017 meeting
- June 2017 – Acceptance of the project by EAGMST for a series of guidance documents on the three main 'omics to be presented to the OECD Working Group of the National Coordinators for the Test Guidelines Programme (WNT)
- October 2017 – Progress occurring with Transcriptomics and Metabolomics project submission form for the OECD WNT – proteomics is not being pursued at the current time

Public Health England
Protecting and improving the nation's health

OECD/ECETOC workstreams

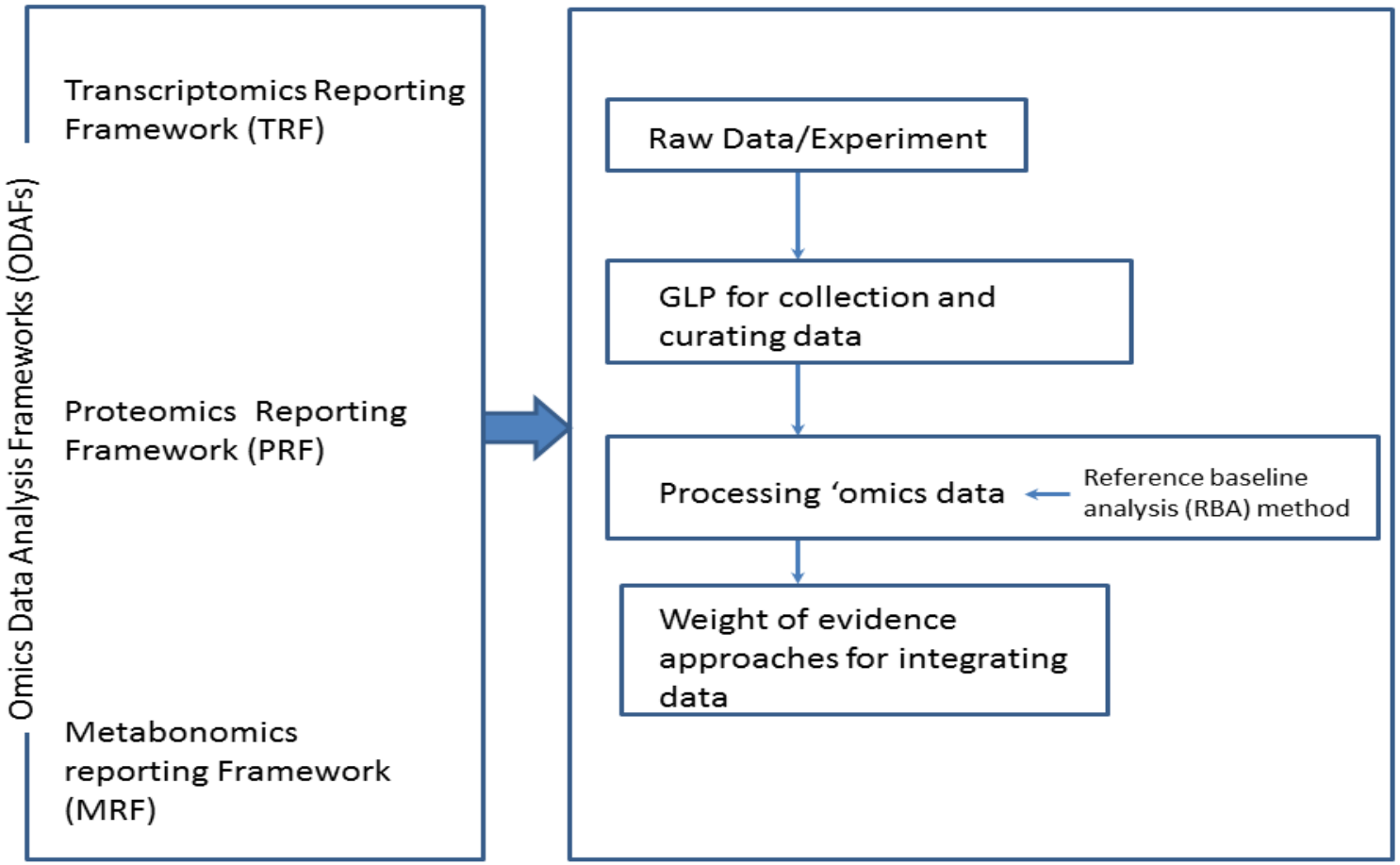
UNIVERSITY OF SURREY



Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

The frameworks – work in progress



Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Conference presentations

- 2015 - workshop outcomes presented at the EUROTOX meeting - Porto
- 2016 – ECHA meeting on NAMS - Helsinki
- 2016 – SETAC meeting - Brussels

(Following this meeting Metabolomics came on board leading to the MERIT project)

- 2017 – SOT - Baltimore

Public Health England
Protecting and improving the nation's health

Where could omics potentially be used in regulation (not exhaustive)

UNIVERSITY OF SURREY

- Classification and labelling (C&L) of substances, for example as part of a tiered testing strategy.
- Weight-of-evidence (WoE) approaches to elucidate the MoA of the substance under investigation.
- Substantiation of chemical similarity for read-across Determination of points-of-departure (PoDs) for hazard assessment.
- Demonstration of species-specific effects and human health relevance (or absence thereof).
- Read across for grouping and hazard identification
- Reduction, Refinement and Replacement.
- Epigenetic assessment for historical exposure

Public Health England
Protecting and improving the nation's health

UNIVERSITY OF SURREY

Thanks

- Wenjun Bao
- Mohamed Bonahmed
- Tim Ebbels
- Karma Fussell
- Madeleine Laffont
- Alan Poole
- David Rouquie
- Steffen Schneider
- Leming Shi
- Tokuo Sukata
- Kayo Sumida
- Weida Tong
- Shu-Dong Zhang

- Hervé Seitz - CNRS France
- Matthew Martin – USEPA

- Miriam Jacobs (OCED WNT for the UK)
- Ursula Sauer
- Rusty Thomas (USFDA)
- Carole Yauk – Health Canada
- Mark Viant