ESR 11:

A transcriptomic and metabolomic approach to biomarkers of exposure and effect

Jinkang Zhang

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Antwerp, Belgium 14-09-2011

ME


•2007-2010, M.S. in Marine Biology (molecular biology),
Institute of Oceanology, Chinese Academy of Sciences (IOCAS), Qingdao, China
Graduate University of Chinese Academy of Sciences (GUCAS), Beijing, China
•Born in Anhui, China
•2003-2007, B.S. in Biotechnology,
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Pyongyang
평양
Tianjin
天津
河北
Seoul
서울
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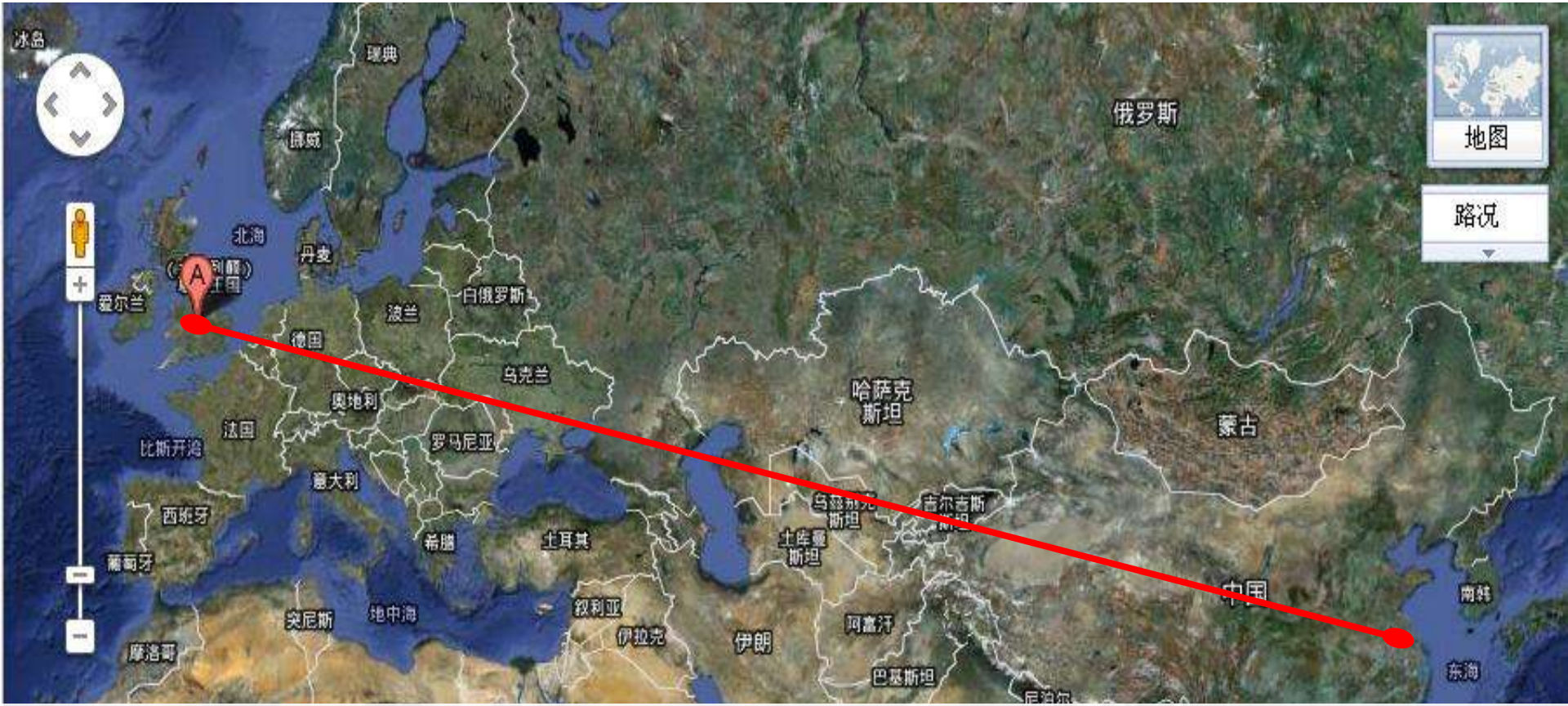
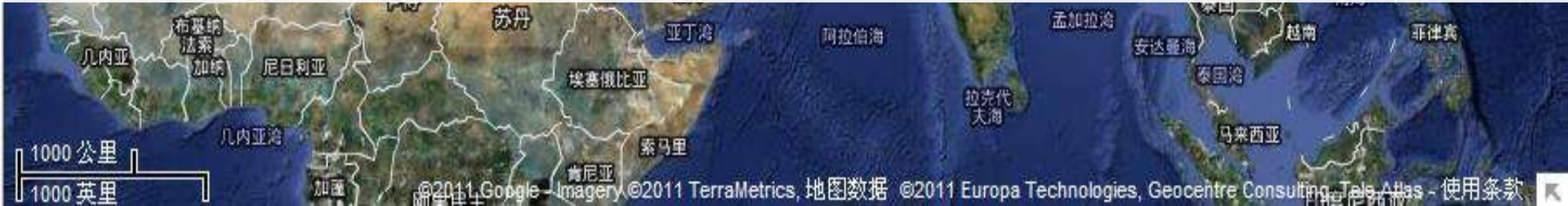
上海
Kagoshima
鹿児島
西藏自治区
Chengdu
成都
四川
湖北
Lhasa
拉萨
Chongqing
重庆
Wuhan
武汉
East China Sea
Changsha
长沙
Arunachal Pradesh
Kathmandu
Sikkim
Bhutan
贵州
湖南
江西
Fuzhou
福州
Naha
那覇
Guiyang
贵阳
福建
Taipei
台北市
广西壮族自治区
广东
Taiwan
Kaohsiung
高雄市
香港
Hong Kong
Myanmar (Burma)
Haikou
海口
澳門
Macau
Mizoram
Laos
海南
200 mi

ME



2011- present, PhD student (INFLAME_ESR11),
University of Birmingham, UK



ESR 11:

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Outline

- Project objectives
- 3 years plan
- Progress to date
  - ✓ Literature review
  - ✓ Cell culture
  - ✓ MTT assay
  - ✓ Training in transcriptomics and metabolomics
- Next 6 months plan (Sept.2011- Feb.2012)

INFLAME-WP3

- **Research Objectives of WP3**: to advance our knowledge of the effects of exposure to FRs.

- ESR10: Mechanistic profiling of flame FRs in general systemic stress and endocrine disruption

- **ESR11: A transcriptomic and metabolomic approach to biomarkers of exposure and effect.**

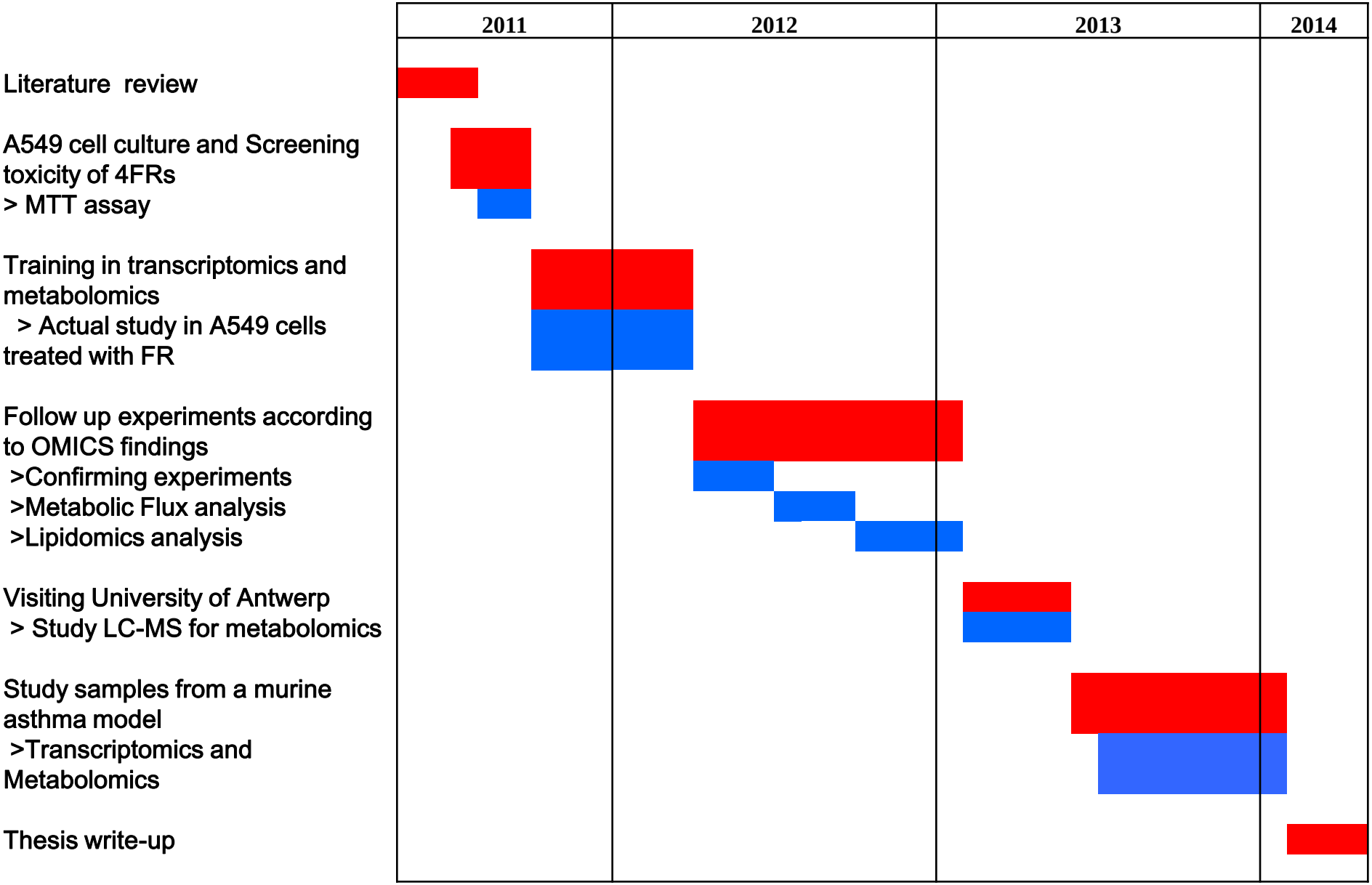
- ESR12: The role of FRs in indoor dust in potentiating or facilitating allergic responses to inhaled allergens

Project objectives of ESR11

- The ESR 11 will focus on the molecular mechanisms via which FRs exert toxicity, using the state-of-the-art metabolomics (FT-ICR mass spectrometry and 1H NMR spectroscopy) and transcriptomics approaches (Agilent microarrays).

- Specifically, the effects of FRs exposures will be studied

3 years plan


2011
2012
2013
2014
Literature review
A549 cell culture and Screening toxicity of 4FRs
> MTT assay
Training in transcriptomics and metabolomics
> Actual study in A549 cells treated with FR
Follow up experiments according to OMICS findings
>Confirming experiments
>Metabolic Flux analysis
>Lipidomics analysis
Visiting University of Antwerp
> Study LC-MS for metabolomics
Study samples from a murine asthma model
>Transcriptomics and Metabolomics
Thesis write-up

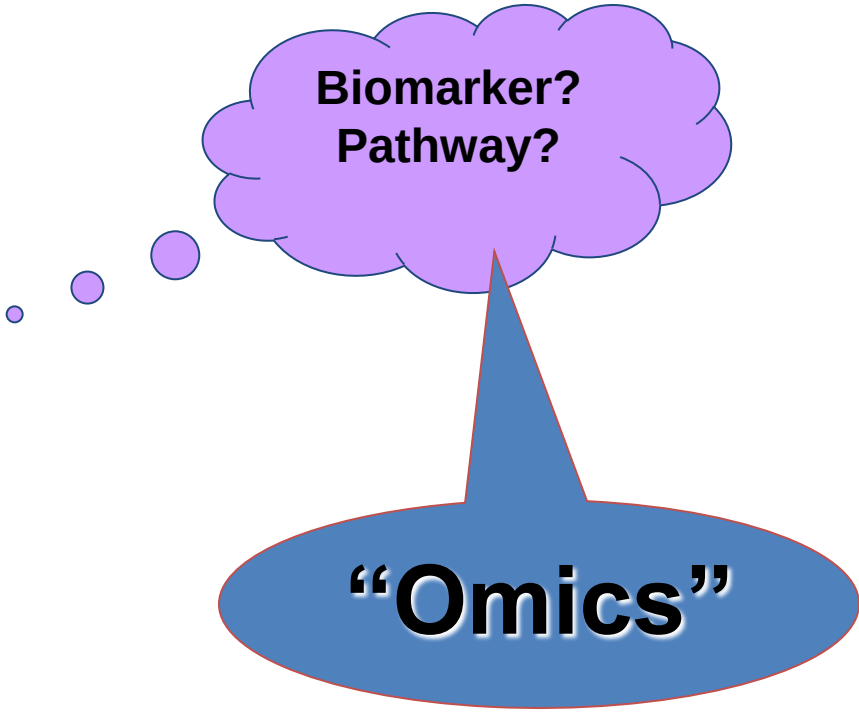
Flame retardants classes

- Inorganic FRs
- Halogenated organic FRs
- Organophosphorus FRs
- Nitrogen based FRs

(van Esch, 1997)

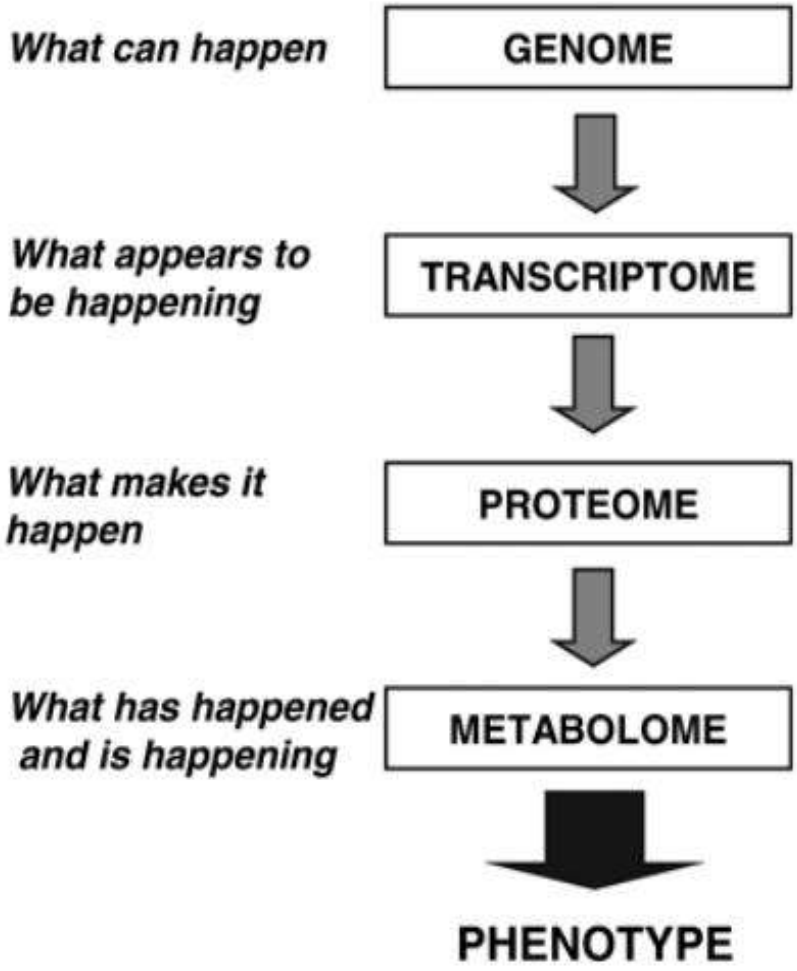
Potential toxicological effects of FRs

- Hepatotoxicity
- Endocrine toxicity
- Developmental toxicity
- Immunotoxicity
- Tumorigenicity



(Sjodin et al., 2003; Per Ola Darnerud, 2003; Dingemans, 2010)

The “Omics” cascade



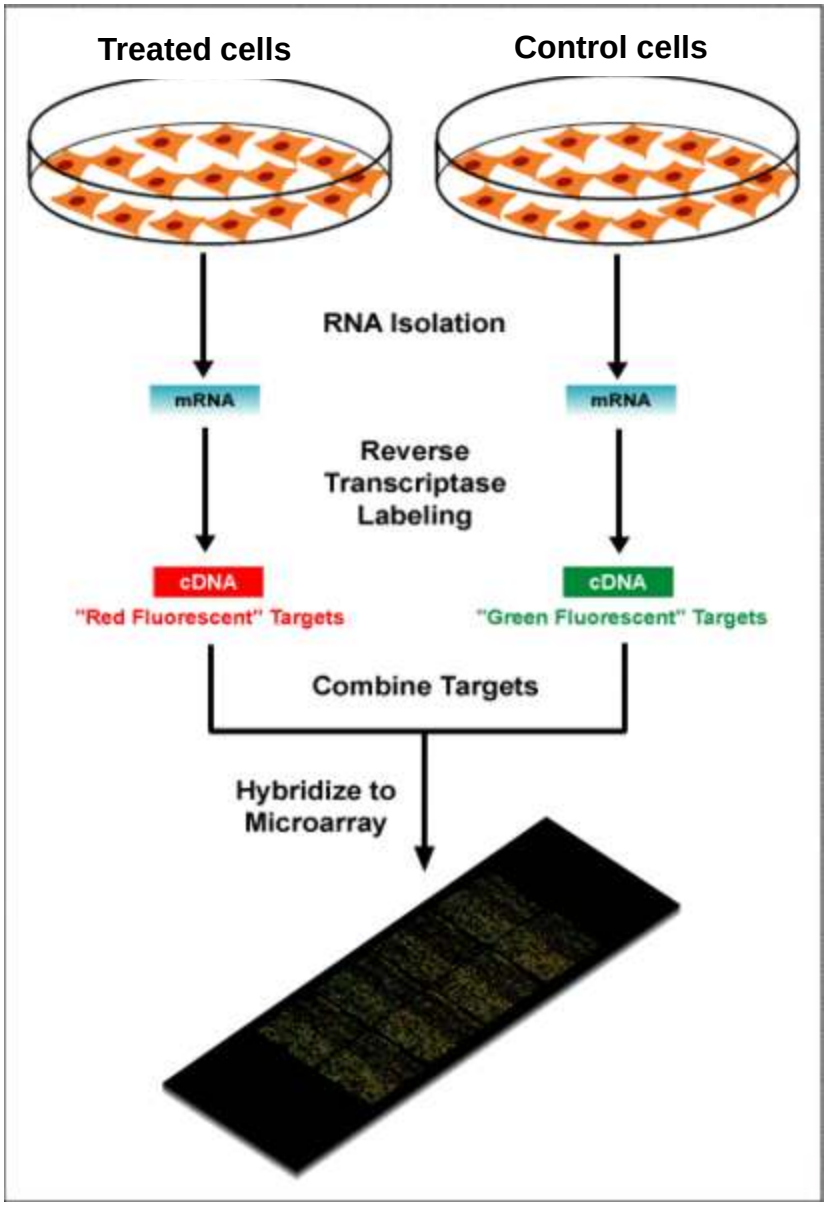
Dettmer et al., Mass Spectrom Rev. 2007; 26(1): 51-78.

The “Omics” cascade comprises complex datasets that as an entity comprehensively describe the response of biological systems to disease, genetic, and environmental perturbations. The most powerful database will integrate data from all omic levels. However, of these databases, the metabolome is the most predictive of phenotype.

Why Omics?

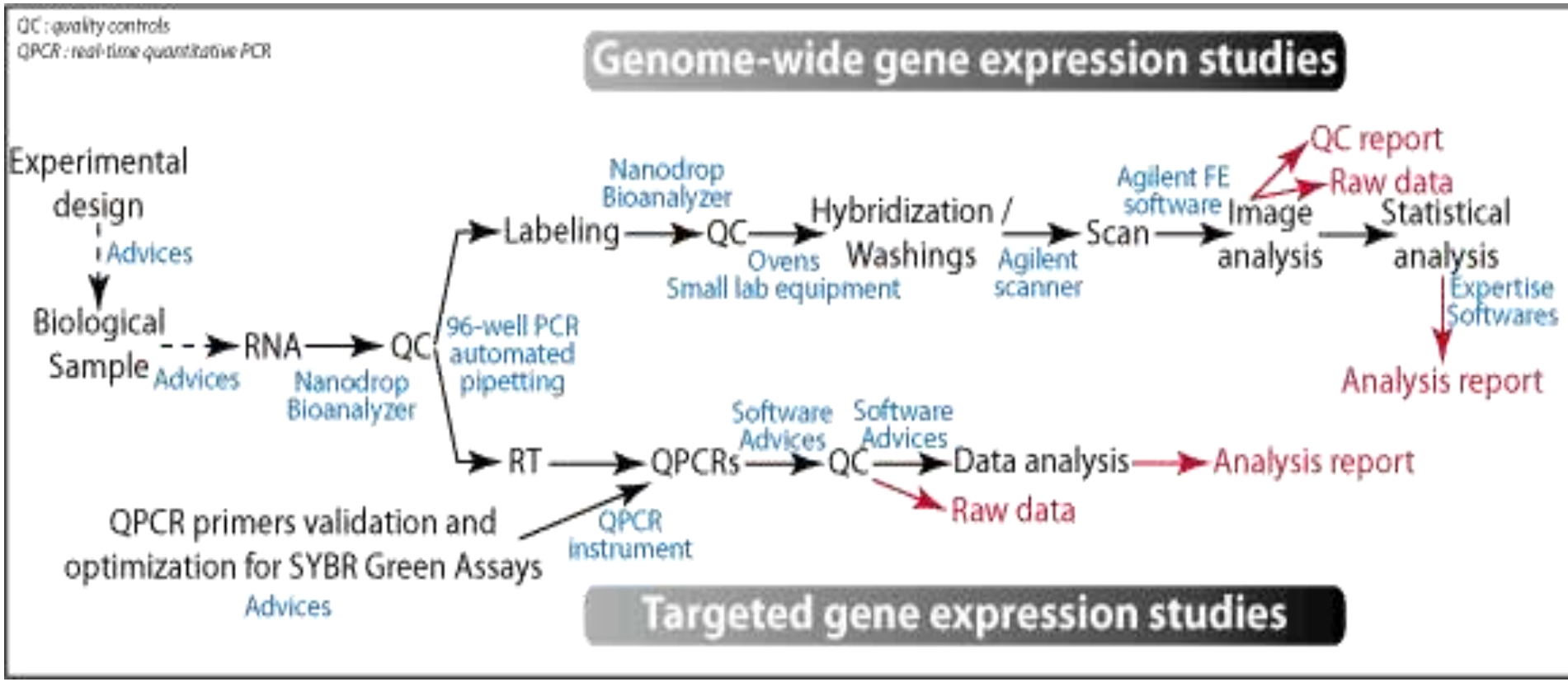
- Can potentially identify presence of toxicity biomarkers
- Enormous potential to examine established and potentially new toxicity pathways
- Widespread use for screening metabolite changes

Transcriptomics workflow



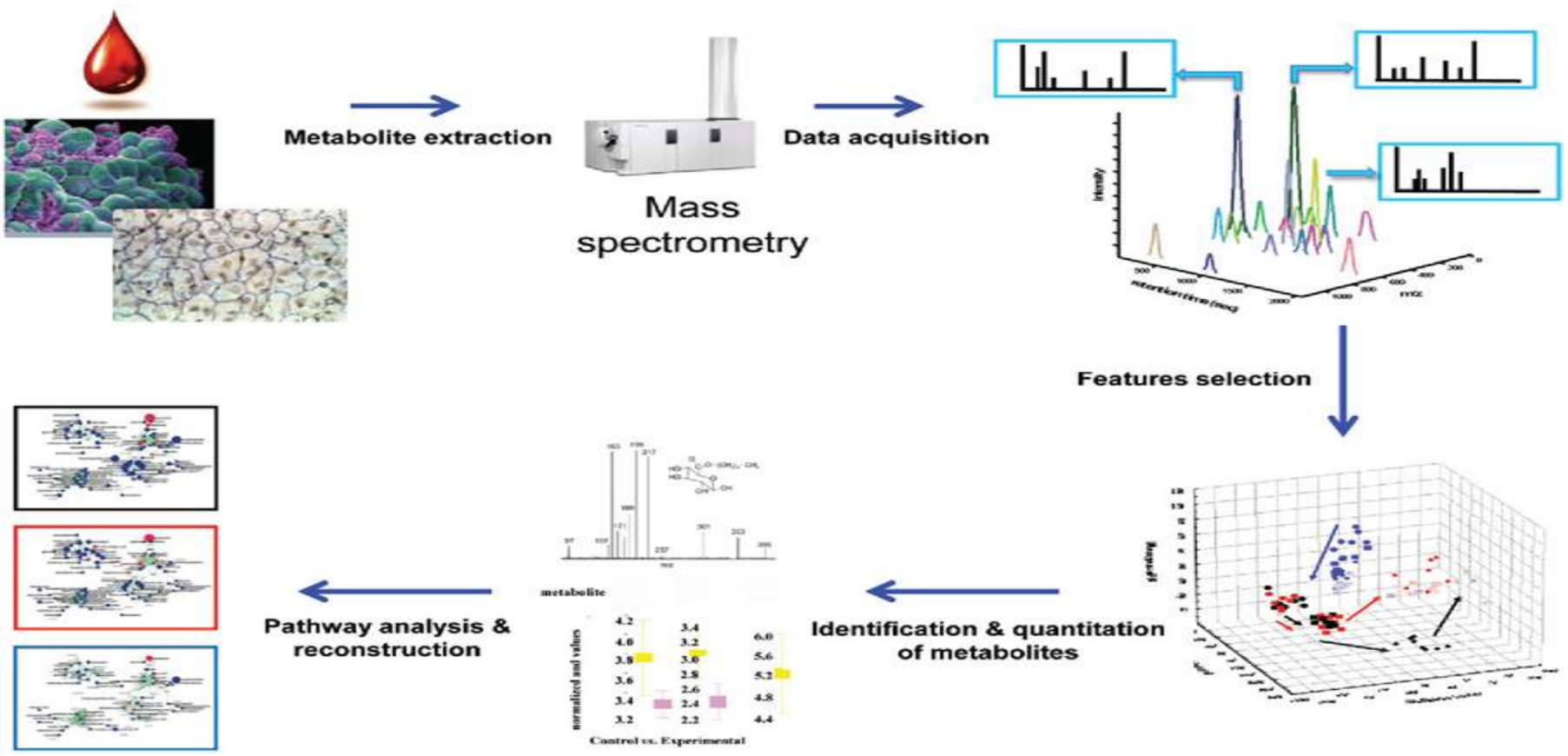
(Picture from website)

Transcriptomics workflow



(Picture from website)

Metabolomics workflow



Lee et al., BioTechniques, 2010, 49(2):557-65

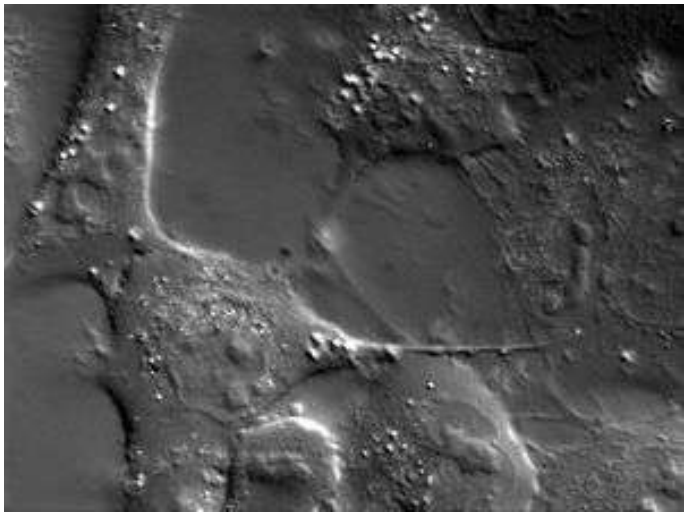
Small-molecule metabolites are extracted from the sample matrix. / Metabolites are separated using chromatographic steps, ionized, and analyzed using MS. / Features of interest are selected from raw data using univariate and multivariate statistical approaches. / Features are then identified using database searches, comparisons to authentic standards, and MS/MS. / Identified features can then be used for reaction monitoring, pathway analysis, and metabolic network reconstruction.

Cell culture

- A549 cells

(adenocarcinomic human alveolar basal epithelial cells)

- Adherent cells
- Doubling time: ~ 22 hrs



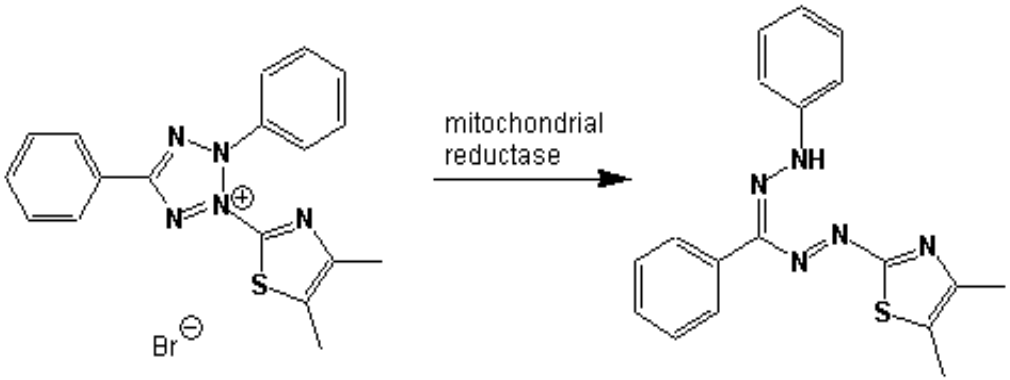
http://en.wikipedia.org/wiki/A549_cell

- Media: DMEM supplemented with 2 mM glutamine, 10% fetal bovine serum (FBS), and 100 U/mL penicillin/streptomycin

MTT assay

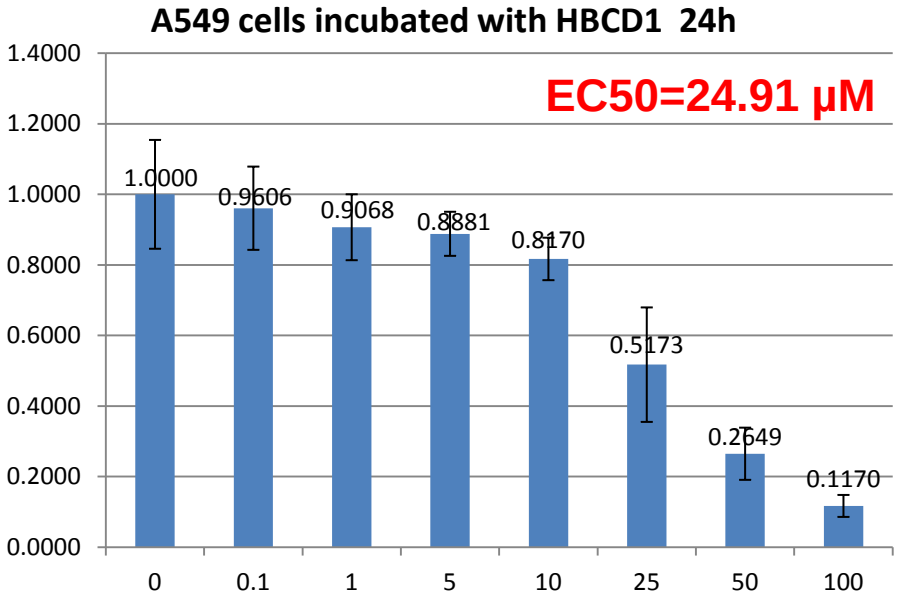
- are colorimetric assays for measuring the activity of enzymes that reduce MTT to formazan dyes, giving a purple colour.
- to assess the viability (cell counting) and the proliferation of cells (cell culture assays).

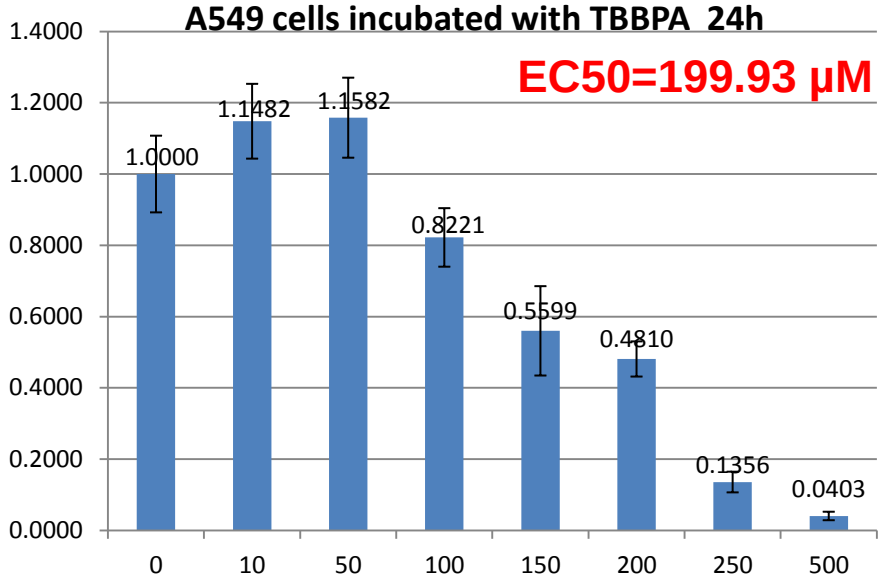
MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

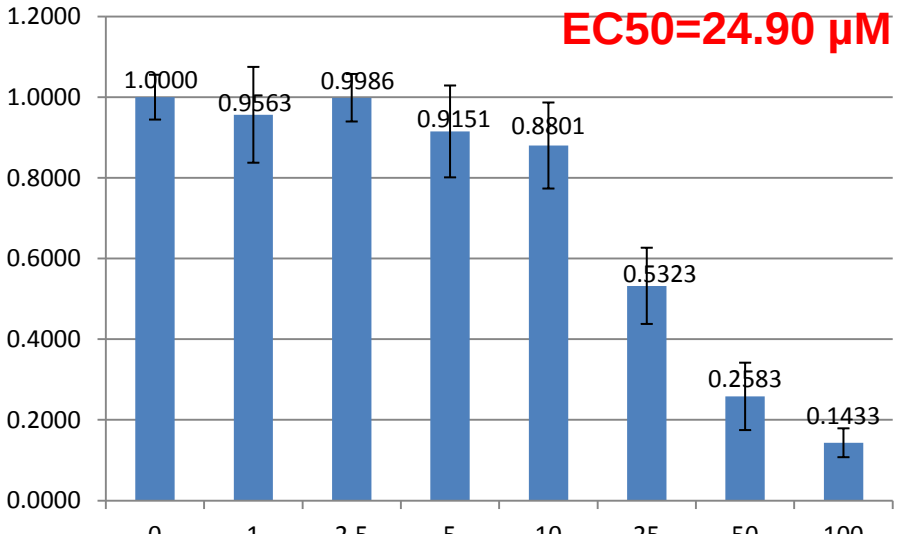


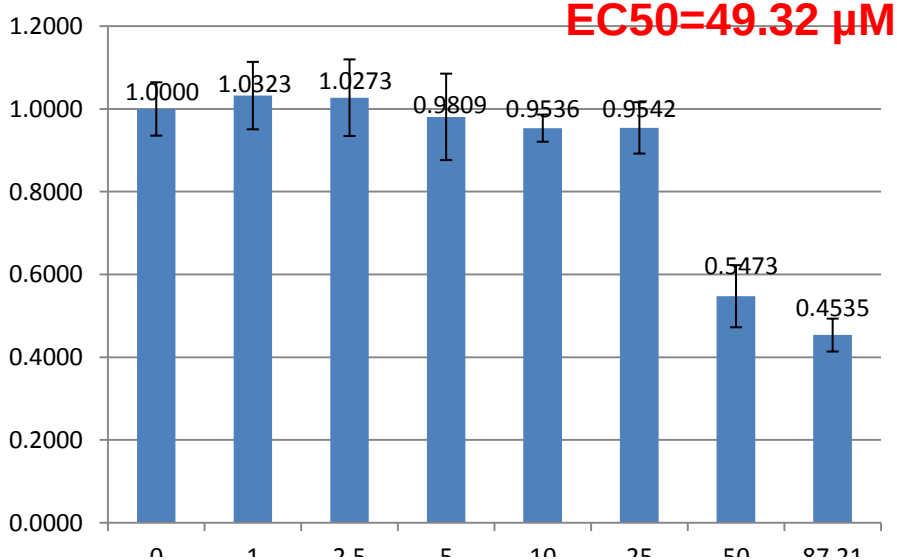
(http://en.wikipedia.org/wiki/MTT_assay)

MTT assay


A549 cells incubated with HBCD1 24h
EC50=24.91 μM
1.4000
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
1.0000
0.9606
0.9068
0.8881
0.8170
0.5173
0.2649
0.1170
0
0.1
1
5
10
25
50
100


A549 cells incubated with TBBPA 24h
EC50=199.93 μM
1.4000
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
1.0000
1.1482
1.1582
0.8221
0.5599
0.4810
0.1356
0.0403
0
10
50
100
150
200
250
500


A549 cells incubated with HBCD2 24h
EC50=24.90 μM
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
1.0000
0.9563
0.9986
0.9151
0.8801
0.5323
0.2583
0.1433


A549 cells incubated with BDE47 24h
EC50=49.32 μM
1.2000
1.0000
0.8000
0.6000
0.4000
0.2000
0.0000
1.0000
1.0323
1.0273
0.9809
0.9536
0.9542
0.5473
0.4535

Preliminary training in transcriptomics and metabolomics

- Preparing protocols for extraction of RNA and metabolites in A549 cells
- ……

Next 6 months plan

(Sept.2011- Feb.2012)

- Finish standard protocols for RNA and metabolites extraction in A549 cells study
- Practical skills training in trancriptomics and metabolomics
- Data collection and analysis

(Multivariate and univariate statistical analysis)

- Primary data of A549 cells exposure to HBCD

(Pathway analysis and mechanism interpretation)

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THANK YOU!