

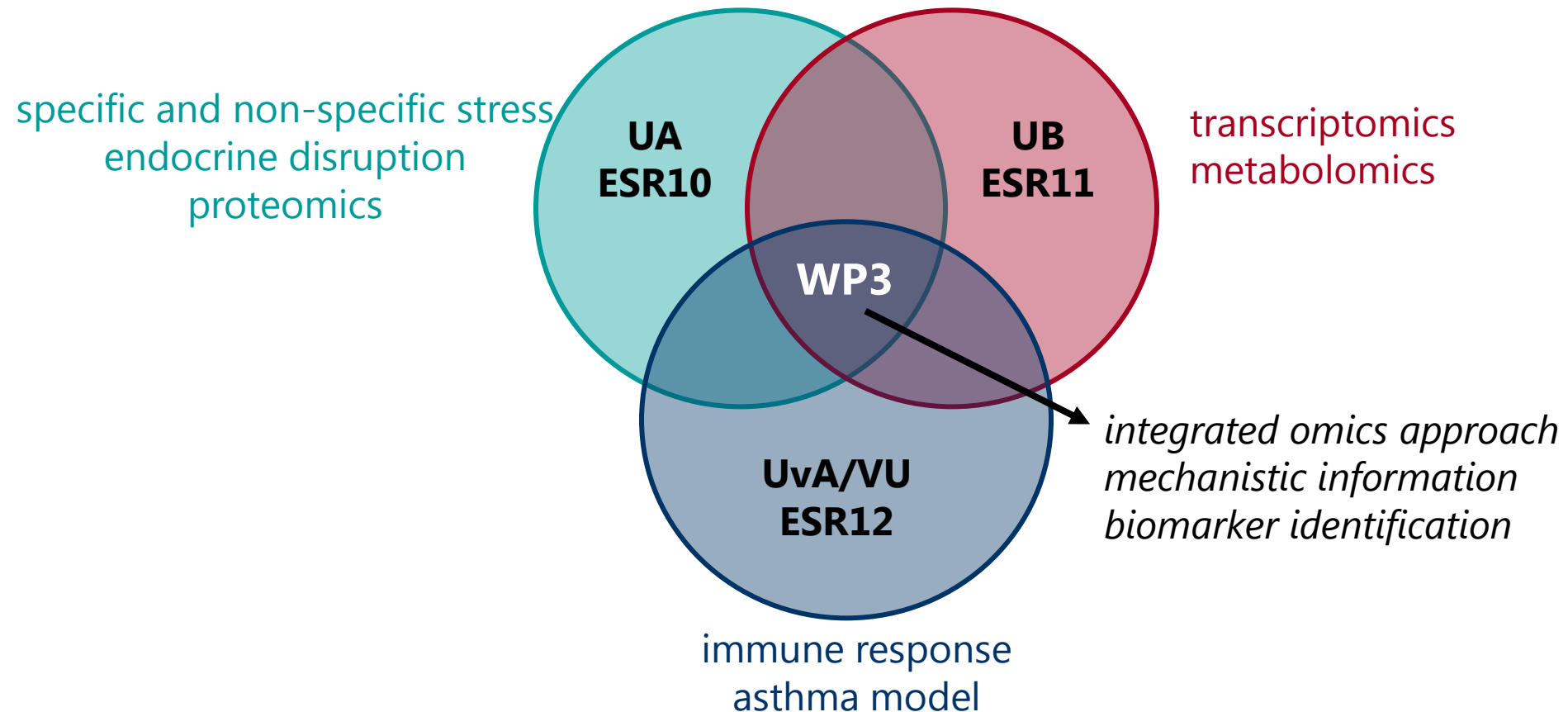
INFLAME

WP3: Understanding Effects of Human Exposure (UA ESR10)

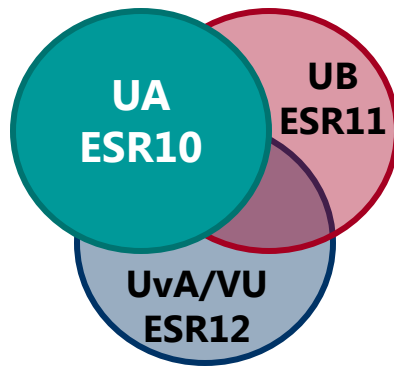
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WP3: Understanding effects of human FR exposure



WP3: Understanding effects of human exposure



***UA-ESR10: mechanistic profiling of FRs
in general stress and endocrine disruption***

targeted approach

specific cellular endpoints
different stress responses
endocrine disruption

flow cytometry, reporter assays
real-time PCR

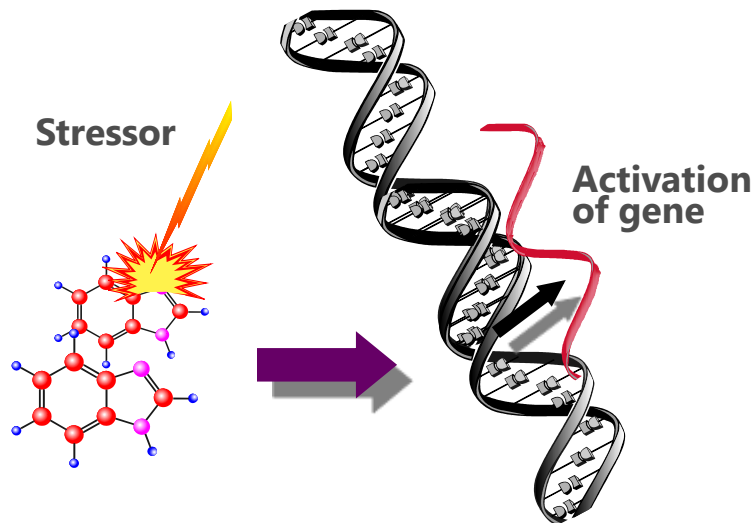
omics approach

proteomics

***in vitro* screening assays**

- Bacterial stress gene profiling → different stress responses
- Human stress gene profiling → different stress responses
- HepG2 cytotoxicity assay → cytotoxicity
- HepG2 MitoStress assay → mitochondrial oxidative stress
- MCF7 cell proliferation assay → estrogenicity
- H295R steroidogenesis assay → disruption of steroid synthesis

Bacterial/Human stress gene profiling



promotor:reporter constructs

Bacterial
E. coli

Human
HepG2

Promoter

CAT Reporter Gene

β -galactosidase activity determined spectrophotometrically (ONPG)
CAT activity determined with ELISA

Stress genes

Oxidative stress
Protein perturbation
DNA damage
Membrane effects
Cellular stress
Biotransformation

E. coli

KatG, Zwf, Soi28, Nfo, MerR
ClpB
Nfo, RecA, UmuDC, Ada, PQ37, DinD
MicF, OsmY
UspA

Human

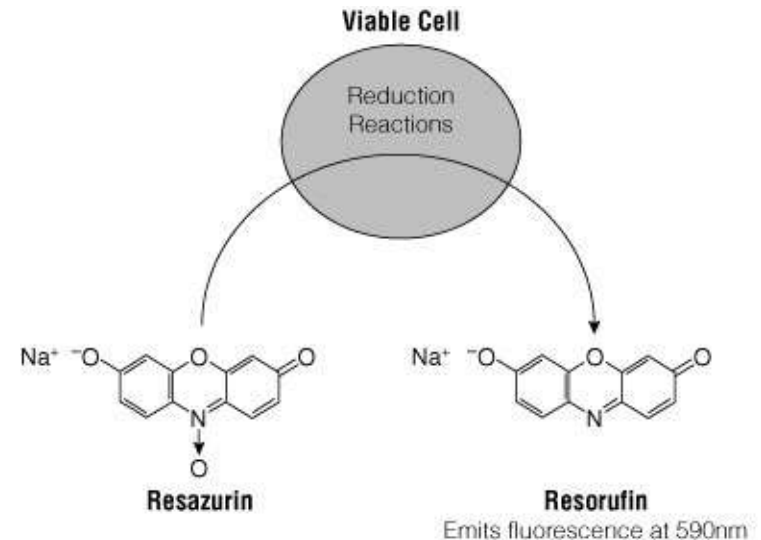
NFkBRE, FOS
HSP70, GRP78
GADD45, GADD153

RARE, HMTIIA, CRE
CYP1A1, GSTY α , XRE ess

HepG2 cytotoxicity assay

AlamarBlue assay or Resazurin assay

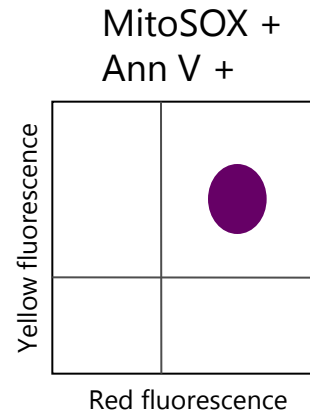
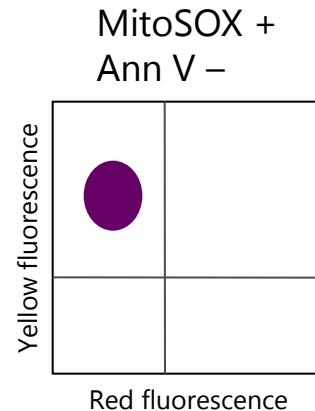
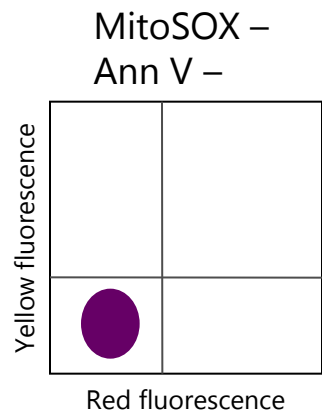
- standard cytotoxicity assay (O'Brien et al. 2000)
- enzymatic conversion of resazurin → fluorescent resorufin
- 96-well plate spectrophotometric assay
- also used as range finding test in other in vitro assays



HepG2 MitoStress assay

MitoStress kit (Millipore)

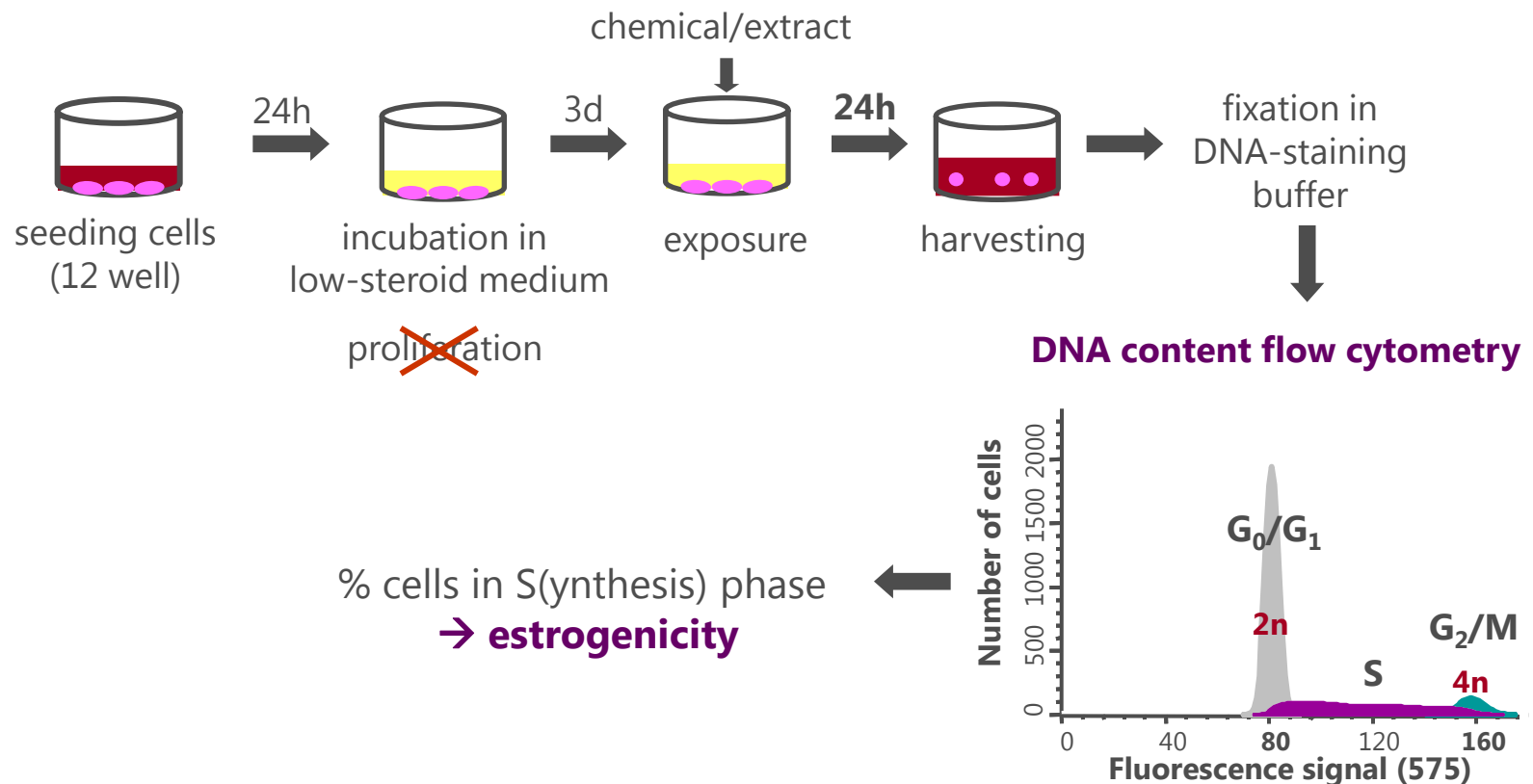
- Flow cytometric 2-dye assay
 - MitoSOX Red reacts with mitochondrial superoxide radicals, live cell-permeable dye
 - Annexin V: binds with phosphatidylserine on surface of apoptotic cells



MCF-7 cell proliferation assay

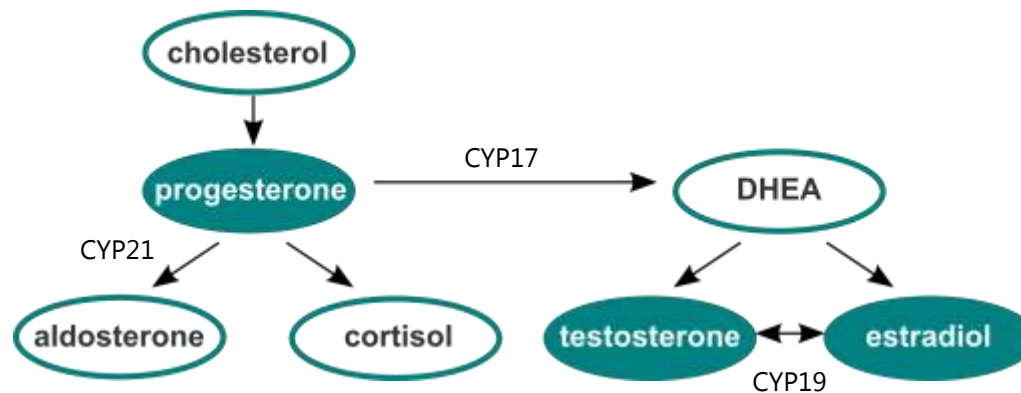
= E-screen assay (Soto et al. 1995)

estrogen dependent proliferation of MCF-7 breast cancer cells



H295R steroidogenesis assay

H295R adrenocortical carcinoma cell line
expresses the complete steroid synthesis pathway



Gene expression analysis – real time PCR

CYP21 – CYP17 – CYP19(aromatase)

Hormone profiling – ELISA

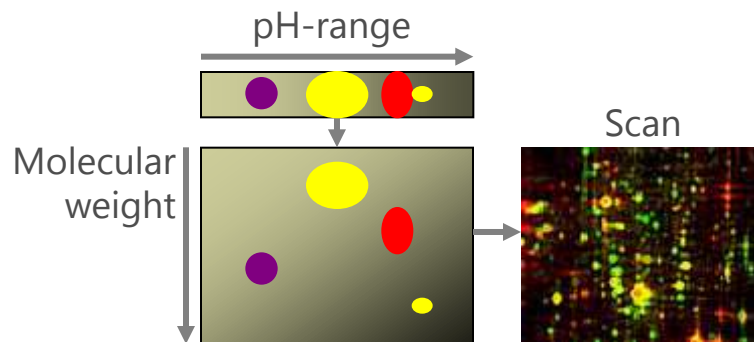
progesterone – testosterone - estradiol

→ **OECD DTG
H295R Steroidogenesis Assay**

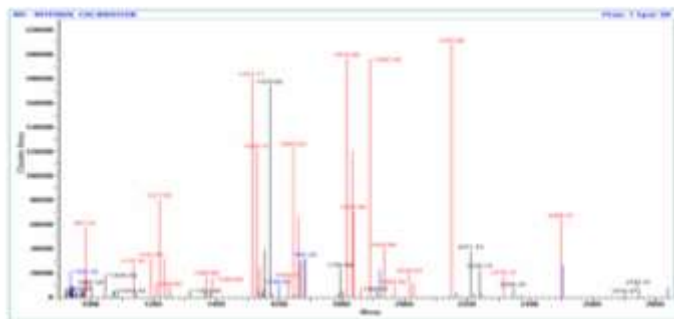
Protein expression analysis

DiGE - Difference in Gel Electrophoresis

1. 2D-electrophoresis



2. Peptide identification



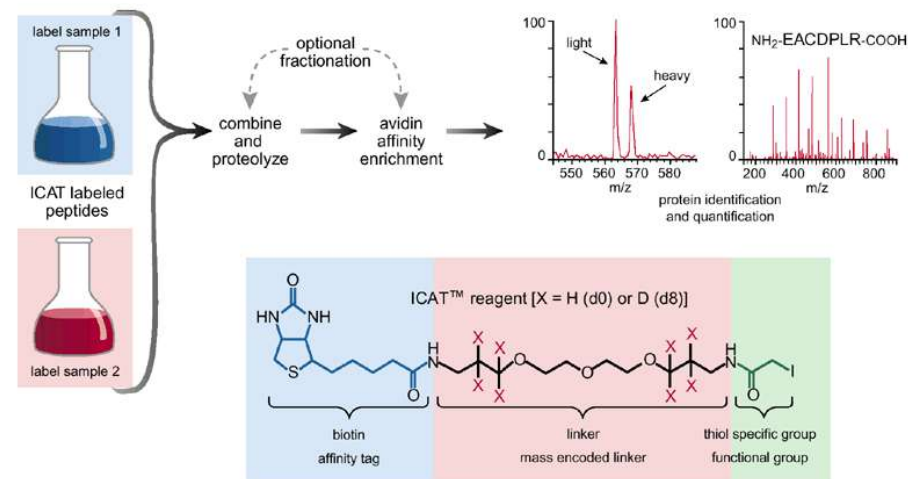
Mass Spectrometry

ICAT – Isotope Coded Affinity Tags

gel-free method

uses isotope coded affinity tags to label samples

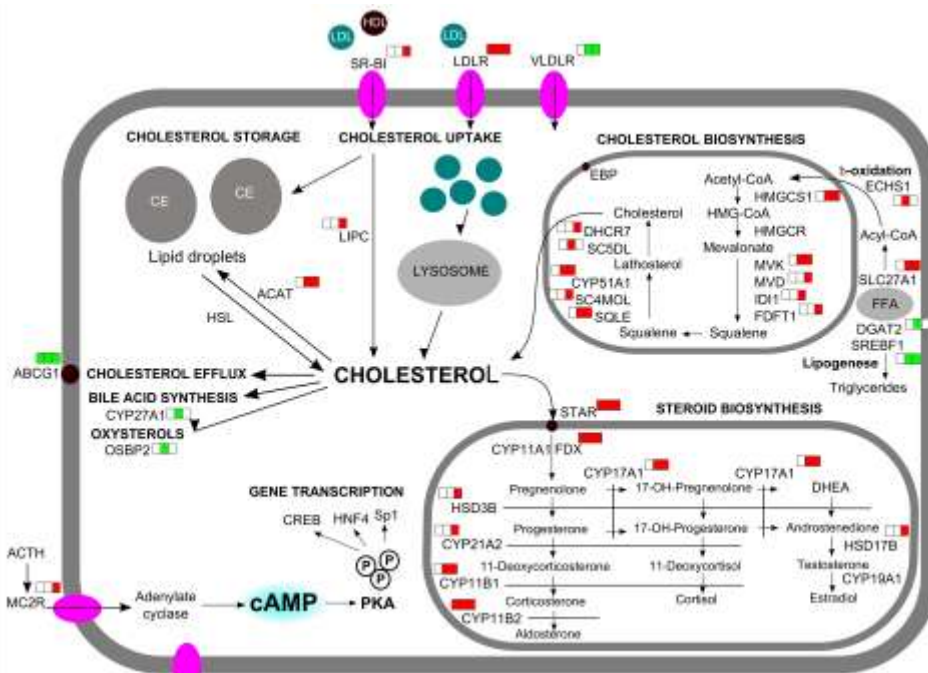
isotopically light (d0) probe
isotopically heavy (d8) probe



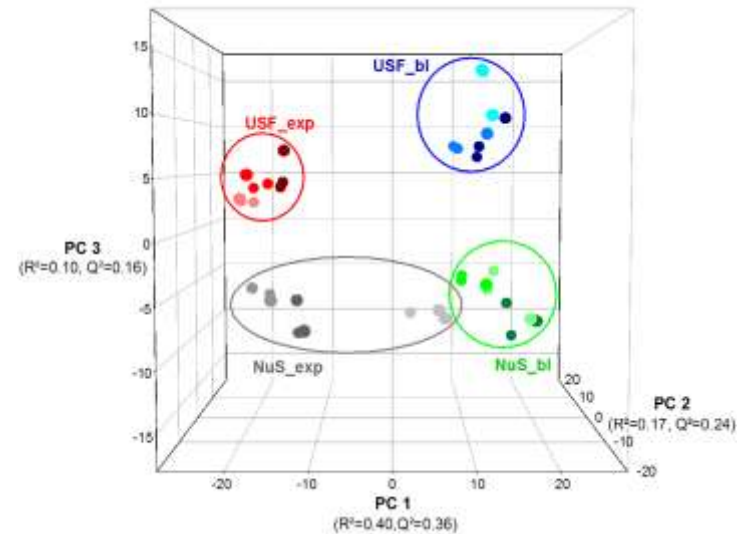
Integrated omics analysis

→ in collaboration with UB – ESR11

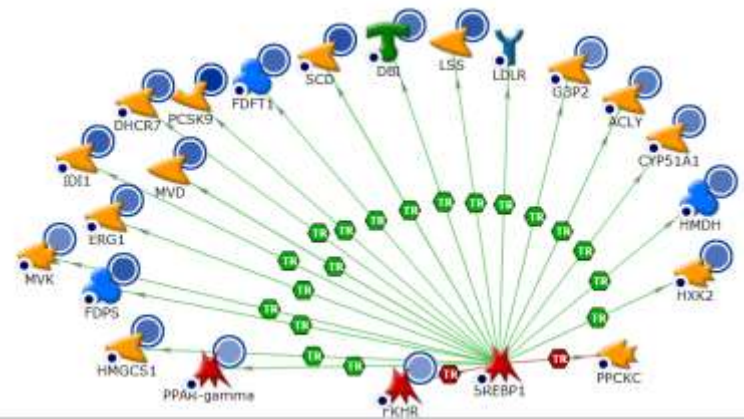
Pathway analysis



Multivariate statistics



Gene/protein networks



introduction

general stress

endocrine disruption

final remarks

EB&T

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EB&T - Ecophysiology, Biochemistry and Toxicology

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