

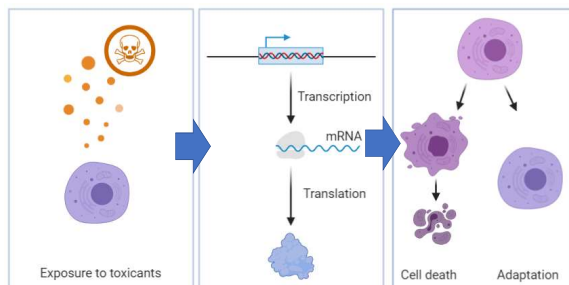
High throughput transcriptomics technology for hazard characterization (WP5)

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Background Information

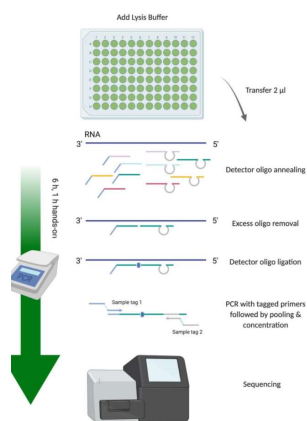
- Endpoints of adverse outcomes are difficult to qualify & quantify in test systems.
- Mechanism-based toxicity testing is required for a shift towards animal-free chemical safety testing.
- Transcriptomics enables quantitative mode-of-action evaluation of chemicals.
- Cost-effective high-throughput transcriptomics (HTTr) based on TempO-Seq technology allows for BMC calculation of biological perturbations.
- ~40,000 transcriptomics were analysed in EU-ToxRisk.
- How many test systems are required to cover MoA by HTTr?
- Can HTTr contribute to read-across?



Infographic of cellular response to toxicants

The technology

TempO-Seq sequencing

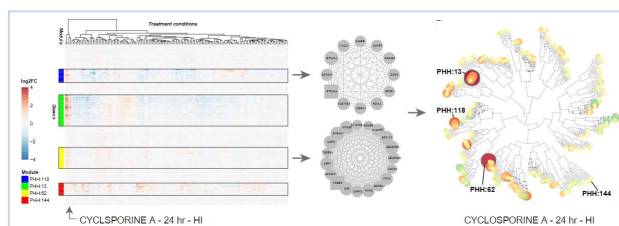


EU-ToxRisk TempOseq panel: ~3000 genes or whole transcriptome

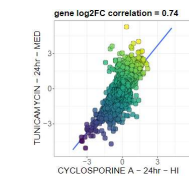
- Normalization
- Log2 count tables
- DEG calculation
- Fold changes and p-values

Pipeline for targeted transcriptomics

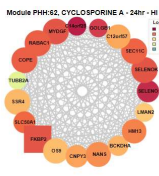
PHH TXG-MAPr tool



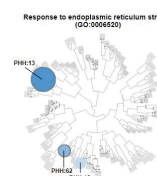
Compound or module correlation



Grouping genes into modules



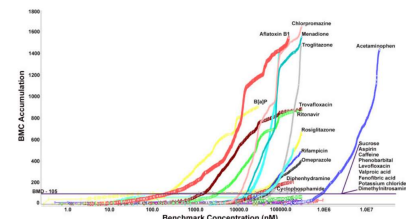
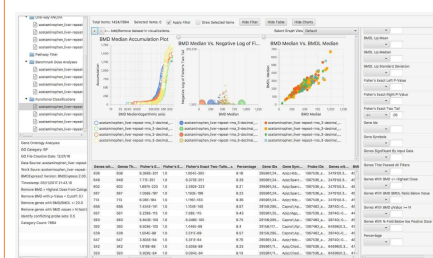
Annotating the modules



Quantitative interpretation of toxicogenomics data of PHH HTTr data. Callegaro *et al.*, (2021)

Gene modules were established by grouping of genes based on weighted gene co-expression in primary human hepatocytes (PHH) exposed with compounds from the TG-GATES database.

BMDExpress



Analysis of dose response data.

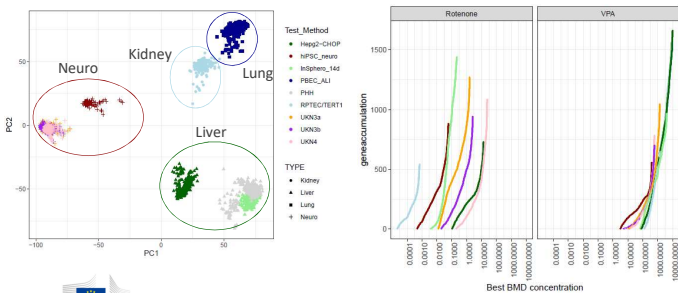
<https://www.sciome.com/bmdexpress>; Ramaiahgari *et al.*, (2019)

Benchmark concentration (BMC) calculation in combination with functional classification analysis.

Application examples

HTTr for cross systems testing with reference chemicals

| Name | Target organ | Cell type | Donor variance in replicates | Culture status upon exposure | Exposure time | Partner |
|--------------|--------------|----------------------------|------------------------------|------------------------------|---------------|----------|
| UKN3a | Neuro | LURMES cells | no | static culture | 72h | UKN |
| UKN3b | Neuro | LURMES cells | no | static culture | 24h | UKN |
| UKN4 | Neuro | LURMES cells | no | differentiated | 24h | UKN |
| hNPC-neuro | Neuro | hNPC-derived neurons | no | differentiated | 72h | BICIT |
| PBEC-ALI | Lung | bronchial epithelial cells | yes | differentiated | 72h | LUMC |
| Insphero 14d | Liver | liver microtissues | yes | 3D culture | 72h | Insphero |
| PHH | Liver | primary human hepatocytes | yes | static culture | 48h | IFADO |
| HepG2-CHOP | Liver | HepG2 (GFP-reporter) cells | no | proliferating | 24h | IE |
| RPTEC/TERT1 | Kidney | RPTEC/TERT1 cells | no | differentiated | 24h | VUA |



HTTr for read-across of VPA analogues in PHH

