Speeding up Eco'n'OMICs – High throughput hazard assessment by multiplexed bead-based analysis of molecular biomarkers



F. Essfeld<sup>1</sup>, H. Reinwald<sup>1</sup>, F. Marghany<sup>1</sup>, S. Ayobahan<sup>1</sup>, S. Eilebrecht<sup>1</sup>

<sup>1</sup> Fraunhofer Attract Eco'n'OMICs, Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany

## Background

As part of the Eco'n'OMICs project, a number of different reference compounds with known mechanisms of action were tested using an adapted fish embryo toxicity assay, in which zebrafish embryos were exposed to low sublethal concentrations of the compounds. RNA was extracted and sequenced to identify differentially expressed genes specific for the mode of action (MoA) of the test compound<sup>1,2,3,4</sup>. Based on these potential biomarkers, a high-throughput gene quantification assay (QuantiGene<sup>™</sup>) will be developed to analyze up to 80 different

hi	gh-throughput approa	ach	QuantiGene	CC	onventional approach	
rangefinding tests	main tests	extraction	Assay	biomarker database	main test & extraction	rangefinding tests
		<image/>				
<ul> <li>low effect concentrations are determined for a set of up to 11 substances</li> </ul>	<ul> <li>maintest with all substances and 2 exposure concentrations</li> <li>transfer of larvae into 96 deep well plate</li> </ul>	<ul> <li>automated bead-based extraction of RNA from 96 samples</li> </ul>	<ul> <li>simultanous bead-based analysis of up to 80 biomarkers in 96 samples</li> <li>signal amplification by branched DNA technique</li> </ul>	<ul> <li>MoA-specific biomarkers and reference genes using transcriptome analysis</li> <li>over 50 Substances covering wide range of different MoA</li> </ul>	<ul> <li>manual extraction of RNA from low and high exposure concentration (LE &amp; HE)</li> </ul>	<ul> <li>low effect concentrations were determined for well known reference substances</li> </ul>

**Figure 1:** Graphical abstract. Relevant biomarkers are defined by performing RNA sequencing on reference compound treated embryos (right side). Automated extraction of embryos treated with up to 11 substances of interest is performed by using a 96-well format extraction platform (left side). Screening for previously defined biomarkers is then performed using a bead-based gene expression assay enabling to analyze up to 80 targets in 96 samples simultaneously. Created with Biorender.com

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Substance 1								
Substance 2								
Substance 3								
Substance 4								
Substance 5								
Substance 6								
Substance 7								
Substance 8								
Substance 9								
Substance 10								
Substance 11								

Week 2

## Table 1: Comparison RT-qPCR vs. QuantiGene Assay

RT-qPCR	QuantiGene
1 target per reaction	Up to 80 targets per reaction
Reverse transcription is biased and requires no-RT controls necessary	Direct quantification of RNA
Signal amplification by biased target amplification	Signal amplification by branched DNA technique
	LOD: < 1000 transcripts per reaction

Substance 4	
Substance 5	
Substance 6	
Substance 7	
Substance 8	
Substance 9	
Substance 10	
Substance 11	

Substance 7

Substance

Substance 3

**Figure 2:** A rough conservative approximation of the time required for the conventional screening approach using RT-qPCR compared to the multiplexed QuantiGene assay. Created with Biorender.com

## Compared to our conventual testing approach this workflow offers:

- Significant decrease in time consumption for exposure experiment and gene expression analysis
- Increased throughput
- Reduced errors and contaminations by automation

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[1] Essfeld et al. "Transcriptomic profiling of clobetasol propionate-induced immunosuppression in challenged zebrafish embryos." *Ecotoxicology and environmental safety* vol. 233 (2022): 113346. doi:10.1016/j.ecoenv.2022.113346
 [2] Reinwald et al. "Toxicogenomic fin(ger)prints for thyroid disruption AOP refinement and biomarker identification in zebrafish embryos." *The Science of the total environment* vol. 760 (2021): 143914. doi:10.1016/j.scitotenv.2020.143914
 [3] Reinwald et al. "Toxicogenomic profiling after sublethal exposure to nerve- and muscle-targeting insecticides reveals cardiac and neuronal developmental effects in zebrafish embryos." Chemosphere vol. 291,Pt 1 (2022): 132746. doi:10.1016/j.chemosphere.2021.132746
 [4] Ayobahan et al. "Comprehensive identification of gene expression fingerprints and biomarkers of sexual endocrine disruption in zebrafish embryo." Ecotoxicology and environmental safety vol. 250 (2023): 114514. doi:10.1016/j.ecoenv.2023.114514