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

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## 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[1,2,3,4]. Based on these potential biomarkers, a high-throughput gene quantification assay (QuantiGene™) will be developed to analyze up to 80 different markers in 96 samples simultaneously.

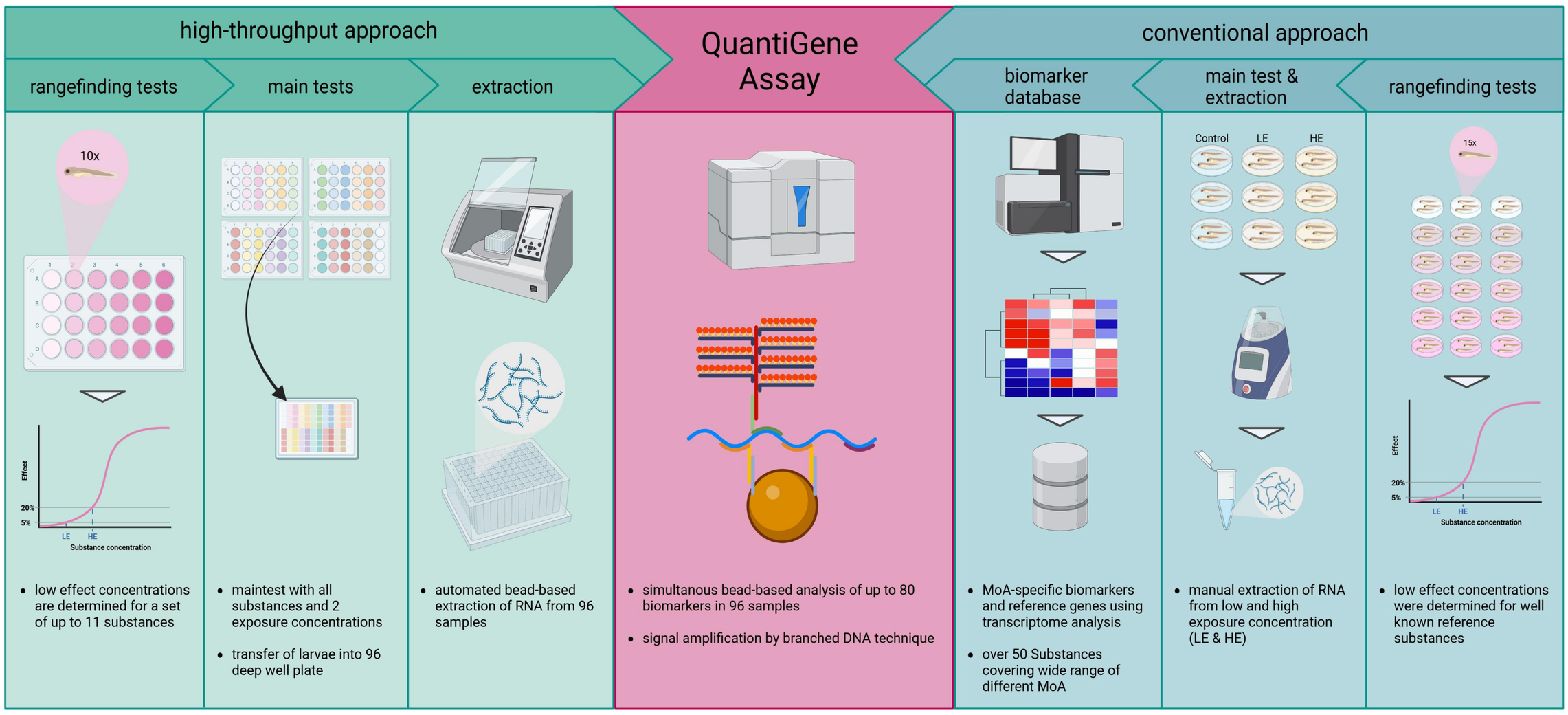


*Figure 1: Graphical abstract. Relevant biomarkers are defined by performing RNA sequencing on reference compound treated embryos (right side). Automated extraction of embryos treated with up to 11 substances of interest is performed by using a 96-well format extraction platform (left side). Screening 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*

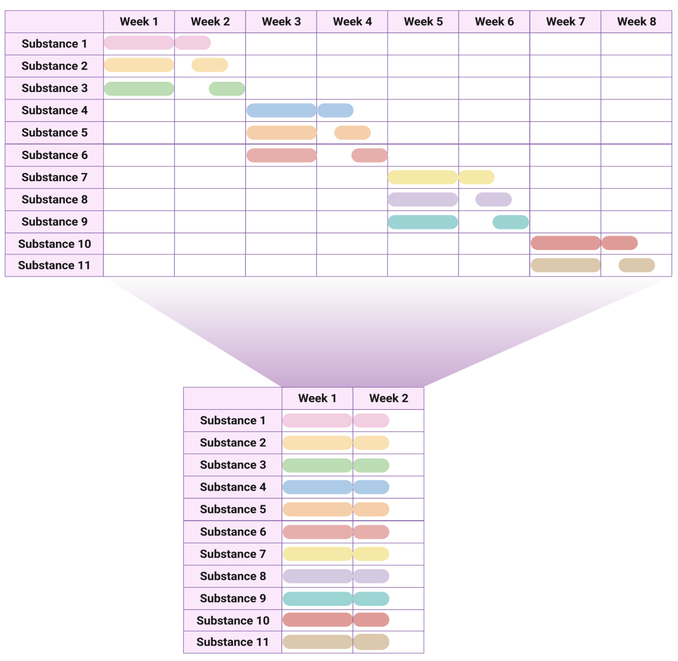


*Figure 2: A rough conservative approximation of the time required for the conventional screening approach using RT-qPCR compared to the multiplexed QuantiGene assay. Created with Biorender.com*

**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 |

**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

**Scan me for more information on our work:**

[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
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