A New Workflow Automating Data Analysis in Gene Expression Screens Across Assay Formats Produces Consistent Results at Scale and Efficiency

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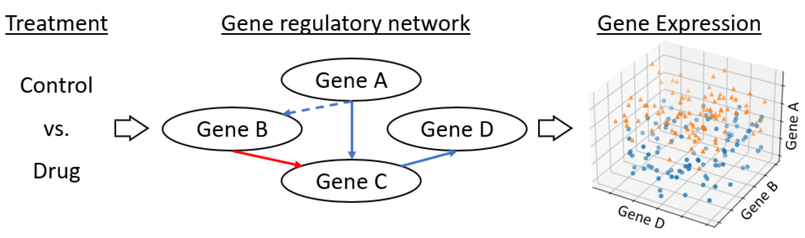
The characterization of gene expression changes has broad applications, including (1) screening of RNA-based or small molecule drugs which function by direct modulation of gene expression, e.g., by altering pre-mRNA splicing, (2) verifying effects of protein-targeting drugs on given cellular pathways, and (3) toxicity profiling or assessing target selectivity. Today, assays such as multiplexed reverse transcription qPCR (RT-qPCR) or bead-based technologies like the QuantiGene™ assay can be performed on automated platforms to enable screening of gene expression at scale. These technologies allow a large increase in throughput, but to date, there exists no common analysis workflow which can consistently and efficiently process high-volume data for all gene expression assays. In this poster, we present a new, highly automated analysis workflow embedded in Genedata Screener which yields major efficiency gains and standardized, high-quality results. This workflow provides built-in functionality for processing and quality control (QC) procedures common to all gene expression assays, such as: normalization to house-keeping genes, which can be assigned per assay; dedicated fits for fold change measurements in dose-response; automated QC including masking of unreliable measurements, flagging of cytotoxic compounds, and dedicated quality plots. Additionally, the workflow features analyses specific to RT-qPCR, such as viewing raw amplification curves for RT-qPCR experiments and robust, automated determination of Ct values. Recently, this workflow was deployed at Evotec to streamline RT-qPCR-based screens, enabling the routine screening of up to 400'000 compounds, resulting in rapid analysis and significantly shortened cycle times for gene expression assays.

Key Applications for Screening of Gene Expression

Elucidation of compound mechanism



Safety screens

Exposure
- Pharmaceutical
- Environmental
- Microbiological

⇒ Gene Expression Screen ⇒

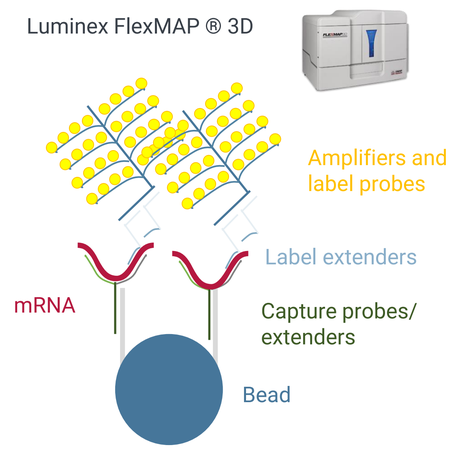
Risk assessment
- Cardiovascular Toxicity
- Infectious Agents
- Hepatic Transplantation
- Anabolic Steroid Use

RNA-based therapeutics

- RNA aptamers for receptor binding, signal transduction inhibition
- Interference RNAs to promote degradation, inhibit translation
- Protein/ antigen expression via mRNA delivery

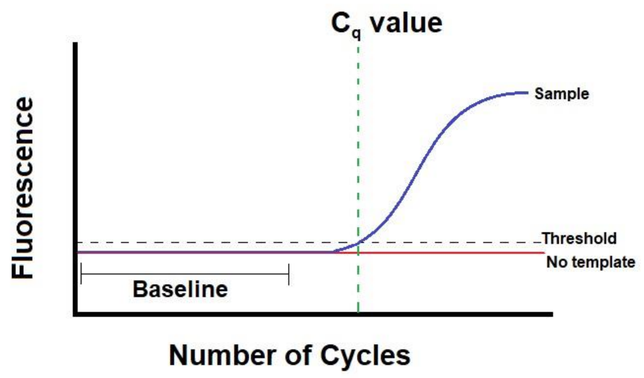
Assay Technologies for Screening of Gene Expression

QuantiGene™



In a QuantiGene assay, mRNAs are captured by hybridization to a set of gene probes each attached to a bead of gene-specific dye color. The Luminex instrument detects dyes and amplified signals. Assay is run in 96- or 384-well plates, measuring expression of 20-80 genes per well.

RT-qPCR



Each cycle in RT-PCR approximately doubles the concentration of transcript for a given gene. Cq, the number of cycles for the signal to cross the quantification threshold, is inversely proportional to the initial gene concentration. Assay is run in 96- or 384-well plates, with 2-3 genes of interest (GOI) and one housekeeping gene (HKG) measured per well.

Data Analysis Automation Yields Efficiency Gains

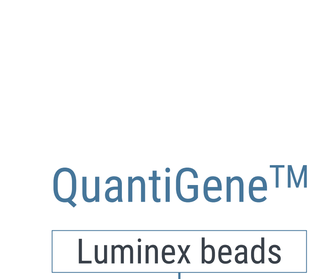
QuantiGene™

Luminex beads → Signal, # beads

Mask wells with low bead count

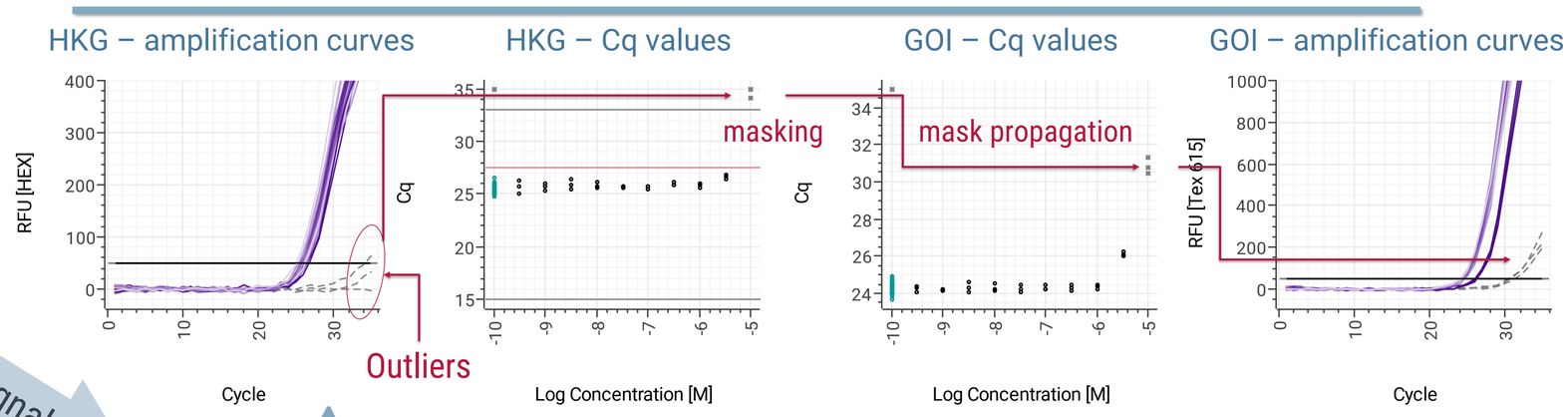
RT-qPCR

Amplification Curves



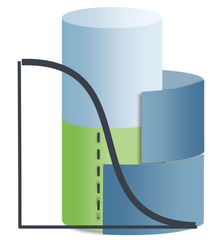
Cq determination

Drift correction, automatic or manual threshold setting for Cq

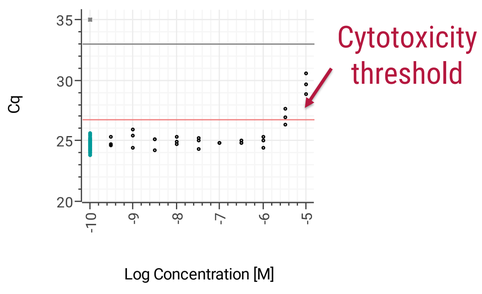


Automatic masking of expression values outside a user-defined range and propagation of the mask to all measured genes if a housekeeping gene shows such aberrant signals.

Signals / Cq values → QC review → Result calculation → Result review → Real-time Reporting

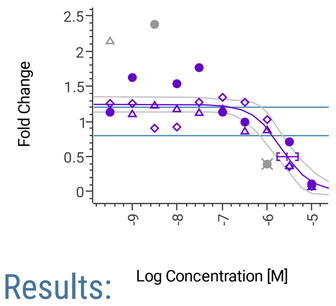


HKG – Cq values

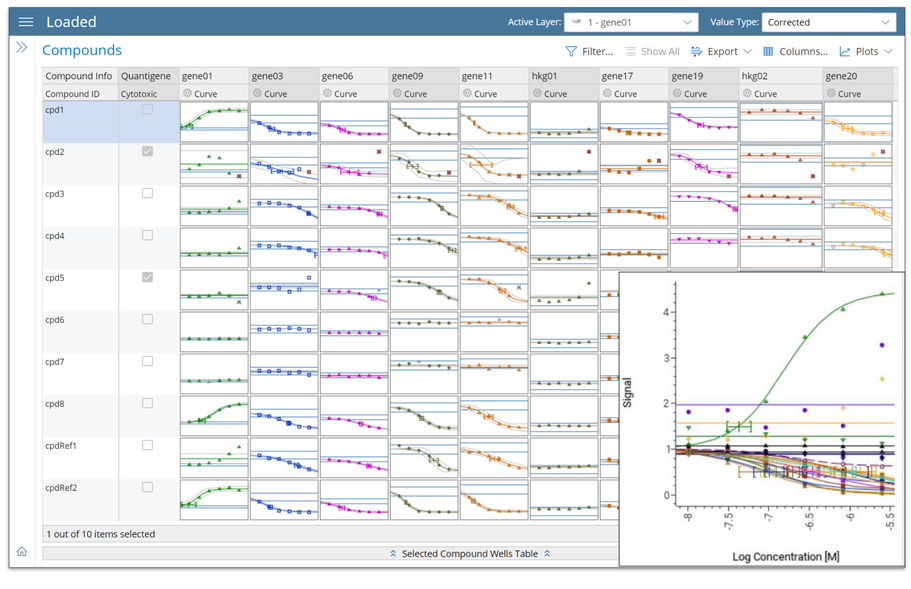


Automatic flagging of compounds that decrease the expression of a housekeeping gene below a defined threshold

GOI – Fold Change Fit



Results:
- dCq, ddCq, Fold Change
- DRC fit parameters
- Fit Validity
- QC Flags



Conclusion

The workflow presented here for automating the analysis of gene expression screens unifies processing, quality control and result review for both RT-qPCR and QuantiGene™ assays while ensuring technology-specific adaptive processing. The entire analysis is embedded in the Genedata Screener software platform, well-integrated with the research IT infrastructure, enabling effortless processing, (re-)analysis and reporting of results from such screens – independently of the number of genes or compounds under investigation. Evotec applies it for routine screening of gene expression at so far unmatched throughput and result consistency, for advancing transcriptome-based drug discovery.