Fast and Integrated Screening Workflow to Assess Gene Expression of Individual Pathways

Olga Lang, Sven Reister, Gerald Schock, Inga Irle
QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Introduction and Methods

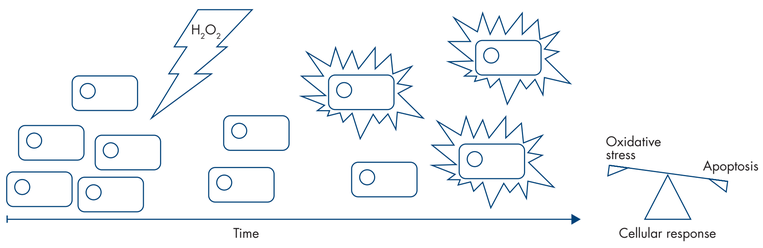
For screening approaches (e.g., drug screening), it is not always clear which gene is the target or effector. Therefore, it is unclear which gene's expression will be assessed, although the general signaling pathway triggered by the compound is known.

Screening

In this study, we describe the use of a quick and simple screening method to assess the consequences of pathway triggering on gene expression. We use as an example, the treatment of Jurkat cells with hydrogen peroxide to induce oxidative stress. Cells were treated with 10 mM H_2O_2 for up to 6 hours. RNA was then extracted using the RNeasy® Plus Mini Kit automated on the QIAcube® Connect. RNA was analyzed using RT² Profiler® PCR Arrays for oxidative stress as a direct response pathway.

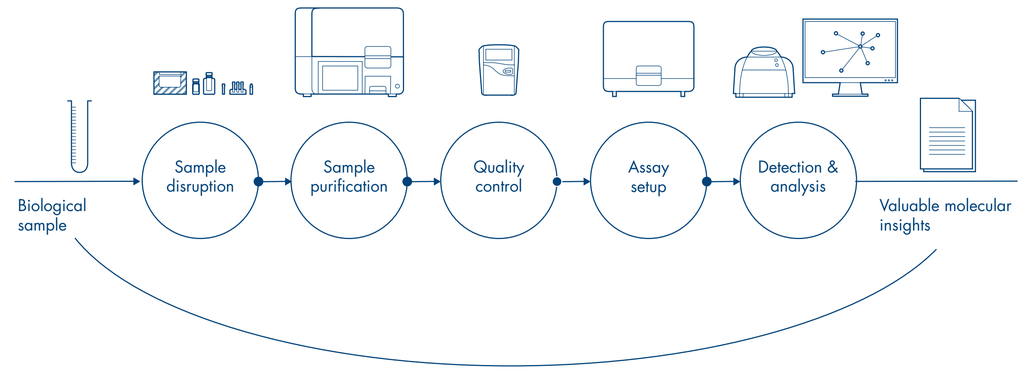
Validation

In a second step, candidate genes identified with these arrays were validated using the QuantiNova® Reverse Transcription Kit and QuantiNova SYBR® Green PCR Kit.



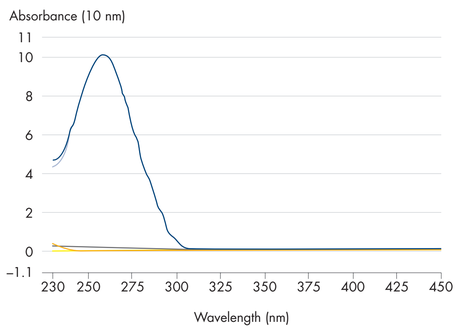
Pathway activation and regulation. Cells can maintain their integrity under oxidative stress, although the likelihood of apoptosis increases over time. This study examines whether the oxidative stress pathway is activated by H2O2 and if so, at what time point the cells induce apoptosis by changes in the gene regulation.

Workflow Overview

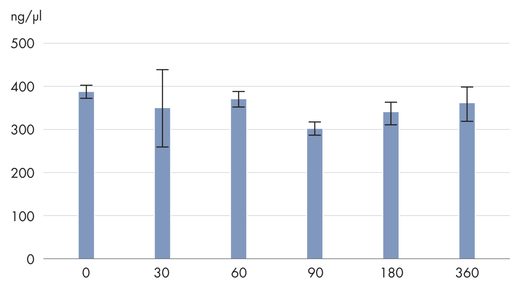


Schematic workflow overview. RNA extraction using the RNeasy Plus Mini Kit was automated on the QIAcube Connect and the purified RNA was quality controlled using the RNA RNeasy App on the QIAxpert. Setup of the RT² Profiler arrays and qPCR for validation using the QuantiNova SYBR® Green PCR Kit was automated using the QIAgility®. Data analysis was performed using GeneGlobe and candidate genes were identified. Analysis of candidate markers was streamlined with Q-Rex and the Gene Expression 2.0 plugin.

Sample Quantification and Quality Control



RNA spectrum from QIAxpert. The QIAxpert was used to quantify the extracted RNA and to evaluate sample purity. The blue curve shows that only RNA is present, while impurities, for example gDNA (orange) and background (grey), are not present.

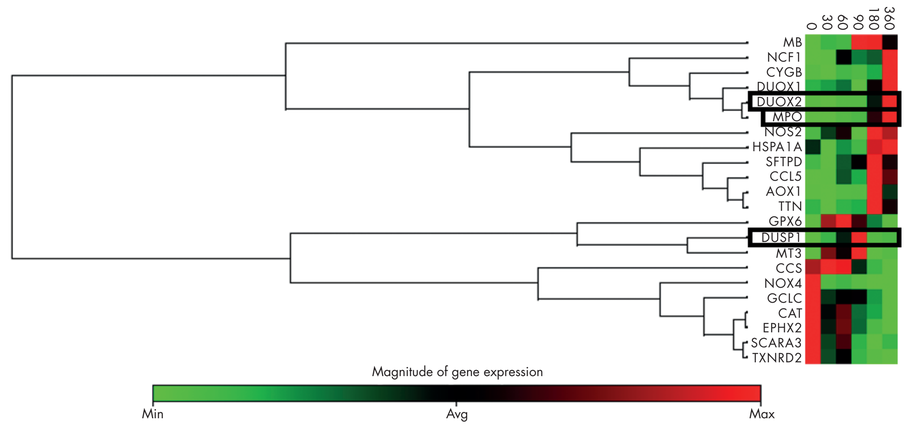


RNA yields from the different samples. For each timepoint, three individual samples were used for nucleic acid extraction. The bar charts indicate an even RNA yield for all samples (n = 3).

Heat Map Analysis of Candidate Genes

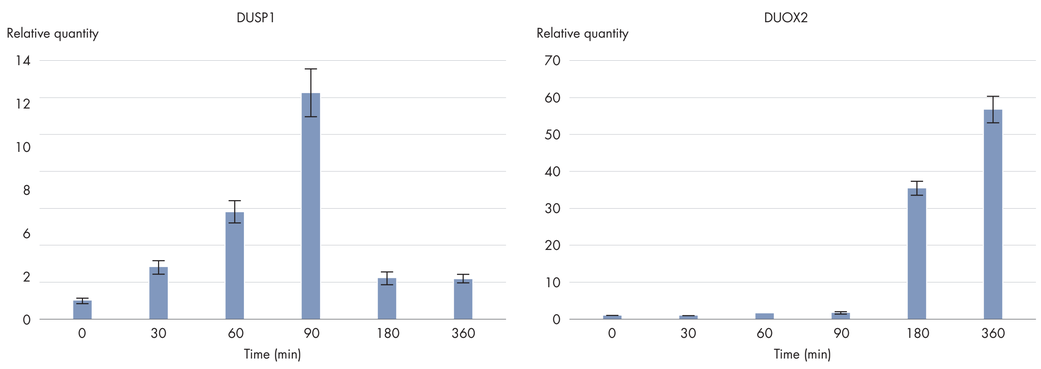
Heat map analysis of the screening candidate genes

Gene symbol	30	60	90	180	360
MPO	>2	9.2	13.61	115.38	188.51
DUOX2	>2	>2	>2	11.96	27.07
DUSP1	2.12	4.73	9.69	>2	>2



Heat map Clustergram shows differential regulated genes. Data from the RT² Profiler arrays was uploaded to the GeneGlobe portal and analyzed for differential gene expression. The three candidate genes (MPO, DUOX2 and DUSP1) were identified for further downstream analysis.

Validation of Target Genes Shows High Concordance



Validation of gene expression using the QuantiNova Reverse Transcription Kit and QuantiNova SYBR® Green PCR Kit. QuantiTect® Primer Assays for DUSP1 and DUOX1 were used to validate the RT² results. The bar charts show concordance with the RT² results indicating that DUSP1 is an immediate early response gene whereas DUOX2 is a late response gene.

Conclusions

- We present an automated workflow to evaluate gene expression of individual pathways. The study showed:
  - Clear upregulation of the oxidative stress-related genes MPO (not shown here) and DUOX2 from 180 min.
  - The DUSP1 gene reaches its highest expression level after 90 minutes and is than downregulated.
  - From 180 min, BIRC5 is downregulated – a clear induction of apoptosis (data not shown).
- The automated RNA extraction using the RNeasy Plus Mini Kit on the QIAcube Connect instrument is highly suited for extraction of high-quality, high-yield RNA. RT² Profiler PCR Arrays are useful tools to evaluate pathway-specific changes in gene expression. In combination with the QIAgility, setup is simplified, offering a comprehensive, walkaway solution.
- Using the Rotor-Gene Q together with the Q-Rex and Gene Globe further simplifies, yet empowers the data analysis, making this an ideal workflow for this type of analysis. A manual setup is also possible.
- Using QuantiNova chemistry combined with QuantiTect Primer assays is a powerful method to validate data — also in larger cohorts.
- The workflow presented enables researchers to identify target genes in treated cells in a straightforward and effective manner.

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Sample to Insight