

Lead compound screening for metabolic disease treatments with a flexible multiplex gene expression assay that monitors genes related to peroxisome proliferator-activated receptors alpha and gamma

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Introduction

Peroxisome proliferator-activated receptors (PPAR's) are the target for many drugs for the treatment of metabolic diseases such as hyperlipidemia, insulin resistance, and coronary artery disease. This is because these receptors have been found to control expression of genes instrumental in physiology related to these diseases. Specifically, PPAR alpha regulates fatty acid metabolism and is the target of fibrates, which lower lipid levels in the blood. PPAR gamma is involved in adipocyte differentiation and when activated by thiazolidinediones induces insulin sensitization. We sought to develop an RT-PCR based assay for monitoring the impact of compounds on the expression of multiple genes regulated by, or related to, these receptors in a single well. By monitoring 20 plus genes in a single well this assay can be adapted to a high throughput format, and used as a compound screen for drugs that target PPAR related disorders. A second feature of this assay is its modularity. The part of the assay representing one pathway can be replaced, or more genes can simply be added that represent another pathway or endpoint, such as toxicity. This feature is also illustrated in the results presented.

Materials & Methods

Animal study and RNA extraction: Rats were treated with clofibrate, rosiglitazone, or pioglitazone and sacrificed after 1 week of treatment. The livers were collected from control and treated animals, and total RNA was isolated and its concentration determined with a ribogreen assay. The RNA was DNAase treated and normalized.

Reverse transcription and the polymerase chain reaction: 25ng of total RNA was used as template for the reverse transcription reactions. Half of these reactions were carried over as templates for the PCR reactions. The PCR products were fluorescently labeled during the PCR reaction and analyzed on a capillary electrophoresis system.

Assay development: RT and PCR primer pairs designed for each gene to be monitored are chimeric, with a gene specific sequence and a universal sequence common to all forward and reverse primers, respectively. A pair of universal primers that recognize the universal sequences in the chimeric primers are included in the reaction in great excess, with one fluorescently labeled. After reverse transcription and a few rounds of PCR, these universal primers drive the reactions, so all the PCR products are essentially amplified by the universal primer set. The chimeric primers are designed to produce PCR products that all have a difference in length of approximately 5 base pairs, resulting in a stratified set of labeled PCR products.

The primer pairs represented genes from the modules found in Table I. They were used to develop two multiplexes (Table II, Plex I; Table III, Plex II). These plexes were used to monitor the expression of the selected genes in total RNA from the livers of treated and untreated animals.

Results

Table I: Genes in Multiplex Modules

PPAR alpha related

Fatty acyl-CoA oxidase
Apolipoprotein AIV
Apolipoprotein CIII
Pyruvate dehydrogenase kinase 4
Phosphoenolpyruvate carboxykinase
PPARalpha

PPAR gamma related

CD36
ABCA1
COX-2
Lipoprotein lipase
UCP2

Controls

Beta actin
GAPDH
Cyclophilin A
External control

Oxidative Stress

DNA topoisomerase IIA
Heme oxygenase-1
Glutathione reductase
p21
Thioredoxin reductase

Glutamyl-cysteinyl ligase (reg. subunit)
HSP70

Drug Metabolism

CYP1A1
CYP3A1
CYP2B1
CYP4A1
CYP2E1

Table II: Plex I

PPAR alpha related

1. Fatty acyl-CoA oxidase
2. Apolipoprotein AIV
3. Apolipoprotein CIII
4. Pyruvate dehydrogenase kinase 4
5. Phosphoenolpyruvate carboxykinase
6. PPARAlpha

PPAR gamma related

7. CD36
8. ABCA1
9. COX-2
10. Lipoprotein lipase
11. UCP2

Controls

12. Beta actin
13. GAPDH
14. Cyclophilin A
15. External control

Oxidative Stress

16. DNA topoisomerase IIA
17. Heme oxygenase-1
18. Glutathione reductase
19. p21
20. Thioredoxin reductase

21. Glutamyl-cysteinyl ligase (reg. subunit)
22. HSP70
23. CYP1A1

Table III: Plex 2

PPAR alpha related

1. Fatty acyl-CoA oxidase
2. Apolipoprotein AIV
3. Apolipoprotein CIII
4. Pyruvate dehydrogenase kinase 4
5. Phosphoenolpyruvate carboxykinase
6. PPARAlpha

PPAR gamma related

7. CD36
8. ABCA1
9. COX-2
10. Lipoprotein lipase
11. UCP2

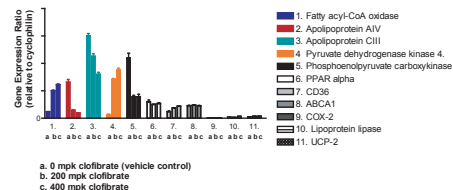
Controls

12. Beta actin
13. GAPDH
14. Cyclophilin A
15. External control

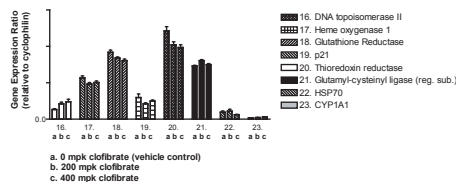
Oxidative Stress

16. HSP70
17. Heme Oxygenase-1
18. CYP1A1
19. CYP3A1
20. CYP2B1
21. CYP4A1
22. CYP2E1

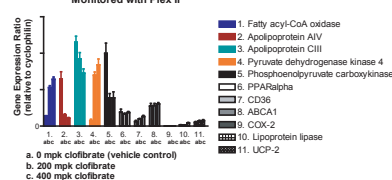
Impact of Clofibrate on Metabolism Related Genes Monitored with Plex I



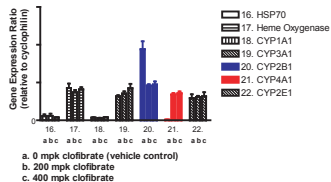
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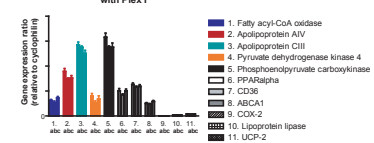
Impact of Clofibrate on Metabolism Related Genes Monitored with Plex II



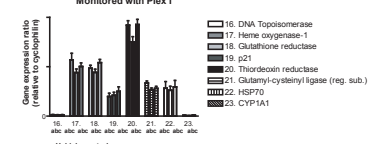
Impact of Clofibrate on Toxicologically Relevant Genes Monitored with Plex II



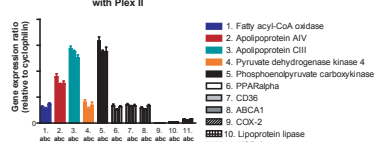
Impact of Glitazones on Metabolism Genes Monitored with Plex I



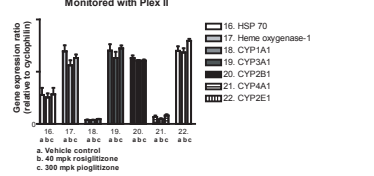
Impact of Glitazones on Oxidative Stress Related Genes Monitored with Plex I



Impact of Glitazones on Metabolism Genes Monitored with Plex II



Impact of Glitazones on Toxicologically Relevant Genes Monitored with Plex II



Conclusion

The results clearly illustrate that a compound's impact on the gene expression of multiple pathways can be monitored by multiplex gene expression analysis (eXpression Profiling), which can monitor 20 plus genes simultaneously in one well. Additionally, the gene content of these single reaction multiplex assays can be easily modified as needed during the drug discovery process. The example we present is the concurrent analysis of genes regulated or related to the PPARs pathways and those that monitor oxidative stress (Plex I) or drug metabolism (Plex II).