HIGH CONTENT GENE EXPRESSION ANALYSIS USING TRAC: A MAGNETIC PROPOSITION

For a full understanding of gene expression patterns related to transcriptional changes, a high content system capable of analysing multiple genes in many samples at once could provide greater efficiency, both in terms of direct costs and time. The new TRAC (TRanscriptional Analysis with the aid of Affinity Capture) technology is a novel assay procedure enabling simultaneous multiplex gene expression analysis from large sample numbers.

Understanding the changes in the expression of genes as a result of disease or environmental pressures is key, not only to the advancement of science, but also to the discovery of targets for pharmaceutical intervention. It is, though, influenced by many factors and understanding the full gamut of control and regulation requires in-depth analysis of each of the up- or down-regulated genes under various conditions. Years of intensive global gene expression studies have yielded an abundance of genome-wide expression data enabling the identification of gene expression signatures for diverse biological situations, such as disease states and toxicological responses. The subsequent need is to analyse focused gene sets from large data samples in a time- and cost-efficient manner for research, target discovery and drug screening. TRAC (Transcriptional analysis with aid of affinity capture, Figure 1) is a novel hybridization and bead-based assay that enables multiplex mRNA target detection from large sample numbers. Originally developed by Hans Söderlund and others at VTT Technical Research Centre of Finland, TRAC is now available from PlexPress Oy (www.plexpress.fi). When used with a Thermo Scientific KingFisher Flex sample processor (Figure 2), the TRAC assay offers an efficient and automated solution for target capture, washing and elution. The functionality of TRAC using KingFisher Flex sample processing has been shown in a number of applications, including molecular toxicology, gene expression-based monitoring of biotechnical processes, cell-based cancer marker gene screening, siRNA research and pathway studies.

Getting on TRAC

The TRAC workflow combines hybridization in a solution, efficient magnetic separation and capillary electrophoresis quantification for the highly reproducible analysis of multiplex gene expression. The TRAC format is highly flexible and can accommodate any set of genes, be it a particular target pathway, disease progression or other biological system. The entire process occurs in the mobile phase and sample transcripts can be introduced either as extracted RNA or directly from cell lysates — as biotin-Oligo(dT)

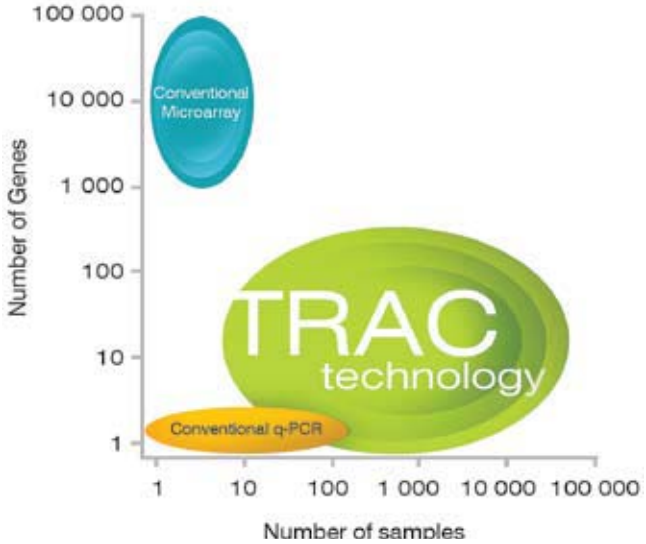


Figure 1: TRAC provides a solution to specific requirement – high throughout gene expression analysis.

capture probes are used to single out mRNA transcripts via their poly-A tails. Assay specificity is introduced through the use of fluorescently labelled gene-specific probes, which hybridize to their complementary target sequence on the transcripts. Once hybridization of these various components has been completed, the biotin-oligo(dT) probes (and the attached transcripts) are affinity captured by streptavidin coated onto magnetic beads. As a result, the transcript–probe complexes can be extracted from the rest of the lysate and unbound material using a high speed magnetic particle processor, such as the Thermo Scientific KingFisher Flex, which greatly simplifies the wash and elution steps. To analyse the assay, the labelled probes are separated using capillary electrophoresis. Each labelled probe defines the presence of a specific gene, and the recorded fluorescent signal for each probe is directly proportional to the number of probes bound to a particular transcript during the assay. As a result, as the number of transcripts for a gene increases, the number of probes present at the analysis stage also increases. Therefore, the relative expression of each gene is recorded and, with suitable controls, the absolute expression can be calculated.

Figure 2: Thermo Scientific KingFisher Flex

KingFisher Flex Process

Samples within the hybridization mix in each of the 96 wells of the microtitre plate are magnetically attracted to the probes of the KingFisher Flex. This enables the samples to be lifted out of the plate and then placed into wash buffer in another plate; this can be repeated a number of times to ensure that there is no unwanted material at the analysis stage. The samples are then transferred from the wash buffer into the elution buffer, where the labelled probes dissociate from the transcript-probe complex. Importantly, the transcripts remain attached to the beads because of the biotin–streptavidin affinity, ensuring that they can be removed from the elution buffer, leaving just the labelled probes. This entire process can be automated on the KingFisher Flex.

Application Examples

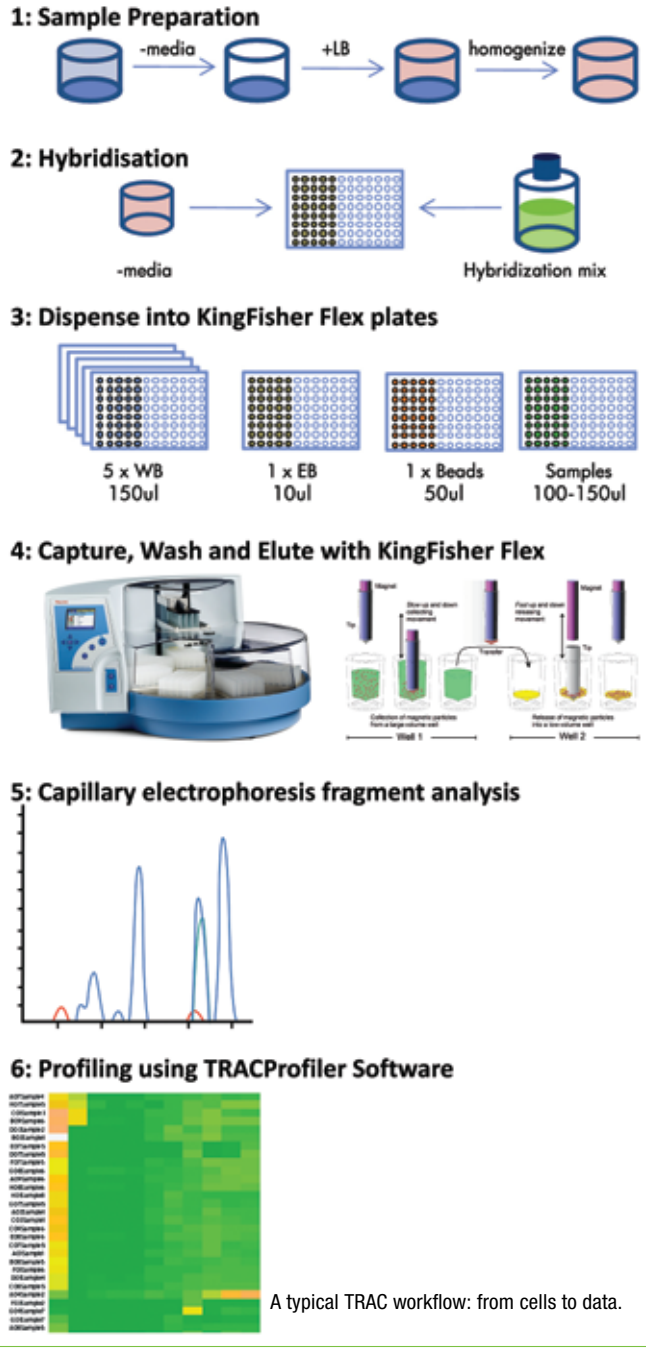
siRNA knockdown validation

Objective: The TRAC assay was used to optimize siRNA delivery conditions (transfection, exposure times and cell amounts) and to evaluate androgen receptor (AR) siRNA knockdown efficiency. The TRAC results were compared with a luciferase reporter gene assay.

Results and conclusions: TRAC and luciferase assays showed consistency in the efficiency variations of target silencing by different siRNA products. Also, some non-targeting control siRNAs led to decreased levels of AR transcript when added at high concentrations. In addition to AR transcription, TRAC enabled the simultaneous detection of 14 other genes related to AR response, interferon response and cell viability. With this type of direct analysis of transcript levels, the construction of cell lines with reporter genes could be avoided altogether (Figure 3).

Cancer marker screening in cell cultures

Objective: The TRAC assay with the KingFisher Flex instrument was used to screen the expression signatures of 20 cancer-related gene markers in colon cancer cell lines cultured on 96-well plates. The gene markers were related to cell adhesion,



A typical TRAC workflow: from cells to data.

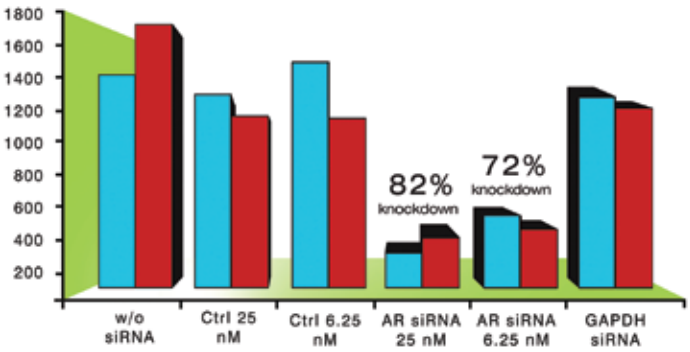


Figure 3: Androgen receptor (AR) gene silencing efficiency by targeting and non-targeting control siRNAs measured by TRAC analysis. HEK cells (12,000 cells/well) were transfected with different siRNAs for 72 hours after which AR mRNA levels were measured by TRAC analysis.

	1h	2h	3h	6h	8h	24h
CCND1	0,0	0,0	0,0	0,0	0,0	0,0
CDH1	0,0	0,0	0,0	0,0	0,0	0,0
CDK4	0,0	0,0	0,0	0,0	0,0	0,0
COX1	-0,2	0,6	0,2	0,0	-0,2	0,0
CTSB	0,0	0,2	0,0	0,0	0,0	0,0
ITGA3	-0,7	0,0	0,2	0,0	0,0	0,0
MLH1	-1,0	0,2	-0,1	0,1	-0,1	0,0
MMP14	0,0	0,0	-0,7	-0,7	0,0	0,0
MMP2	-0,6	0,2	-0,1	0,0	0,2	0,0
MMP7	-0,4	0,0	0,0	0,1	0,2	0,0
MMP9	0,0	0,0	0,0	0,0	0,0	0,0
MSH2	-0,2	0,3	0,0	0,1	0,2	0,0
p53	-0,2	0,4	0,2	0,3	0,3	1,9
PLAUR1	-1,3	0,0	0,1	0,0	0,0	0,0
PRSS123	-0,1	0,2	0,0	0,0	0,1	0,0
SPINK1	0,0	0,0	0,0	0,0	0,0	0,0
TEK	0,0	0,0	0,0	0,0	0,0	0,0
uPA	0,0	0,0	0,0	0,0	0,0	0,0
VEGFA	0,0	0,0	0,0	0,0	0,0	0,0

Figure 4: Expression signature of cancer-related genes in COLO cell line after drug candidate treatment.

angiogenesis and plasminogen activation. The assay was set up for use with chemical-based gene expression screening of cell cultures. Expression profiles of four different cell lines (COLO, HT-29, CaCo2 and DLD) were compared with the gene markers after treatment with a drug candidate. Samples were collected six times during 24 hours. **Results and conclusions:** The expression signatures could be detected directly from 10–100 x10^3 cells grown on 96-well plates. The dynamics of gene expression for the analysed set could not have been observed at a single point in time. Reproducibility was good with CVs below 12% for the system (Figure 4).

The Power of TRAC

By implementing a purely liquid phase assay, there are no surface binding steps, which ensures a faster throughput. Furthermore, the careful selection of probes enables at least 30 different genes to be studied simultaneously in each of the 96-wells in a standard microtitre plate, producing more than 2880 data points per plate in 2–3 hours. The magnetic bead-based affinity capture also plays an important role in the overall speed of the assay, as well as the ability to provide a highly purified pool of labelled probes for analysis. This is because the samples themselves are moved between different predispensed solutions, removing the need for sample concentration and supernatant removal at each recovery and wash stage.

Complementing TRAC's ability to explore the role of system parameters is its flexibility to accommodate workflow. As TRAC stabilizes the sample at the very first step, researchers can more simply analyse time-based differential expression, extract a sample for subsequent analysis or run a TRAC experiment concurrent with other tests in the same sample well of a 96-well plate. This greatly simplifies experiments such as cell signalling, cell cycle analysis, stage of disease assessments, stem cell differentiation studies and many more. With such great flexibility, TRAC can be applied to a wide range of diverse applications, from toxicology studies through to assessing gene stability in bioprocessing environments.

Conclusion

Characterizing the expression of genes in complex biological systems is essential to understanding the broader implications of gene function. Without an indication of the conditions from which gene expression patterns emerge, it is very difficult to fully map the downstream system effects. Such multidimensional sets of conditions are key to gaining insights into gene expression behaviour and essential for improving both our fundamental biological understanding, but also our capability to develop better pharmaceutical intervention strategies. **Pharma**

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