Application Note:

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High-content gene expression analysis with TRAC using Thermo Scientific KingFisher Flex sample processing

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Introduction

Years of intensive global gene expression studies have yielded an abundance of genome-wide expression data enabling identification of gene expression signatures for diverse biological states such as disease states, patient responses or toxicological responses. The subsequent need is to analyze focused gene sets from large data samples efficiently and cost efficiently for research, drug screening and diagnostic purposes. TRAC (Transcript analysis with aid of affinity capture) is a novel hybridization and bead-based assay enabling multiplex mRNA target detection simultaneously from large sample numbers.

When used with a Thermo Scientific KingFisher sample processor, the TRAC assay, offers an efficient and automated solution for target capture, washing and elution. The functionality of TRAC using KingFisher 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.

This Application Note describes the use of the KingFisher instrument as a component of the TRAC protocol in magnetic bead-based processing of large sample volumes.

TRAC technology

The TRAC method enables rapid quantification of focused gene sets from a large number of samples. Cellular material is collected and lysed. Transcript levels are measured directly from the resulting cell lysate without the need for RNA purification or qRT-PCR amplification. Each gene of interest is recognized by a specific complementary fluorophore-labeled probe. A pool of probes with different lengths or types of labels is added to each sample with a hybridization buffer. Biotin-oligo-dT is used to capture targets from their polyA tails. Hybridization of probes and transcripts takes place in the solution, a faster and more reproducible process than hybridization in which one partner is bound to a surface.

The following steps are automatically performed by the KingFisher instrument in a 96-well microtiter plate format. The probe-transcript complexes are captured by streptavidin-coated magnetic beads. Unbound material is washed off and probes are eluted from the beads. Hybridization, capture, washing and elution are completed in 2-3 hours with little hands-on time.

The probe pools in each sample are separated by capillary electrophoresis, which sorts the probes according to size and label. Separated probes are quantified by their fluorescence signal. A single assay with 96 samples with probes and 30 chosen transcripts yields 2,880 data points. [1]

Materials and methods

Instruments

- Thermo Scientific KingFisher Flex with 96-well magnet head
- Thermo Scientific Multidrop 384 liquid dispenser

Microplates / beads

- KingFisher 96 KF plates (200 µl)
- TRACPACK™ magnetic streptavidin-coated beads

Assay Procedure

1) Cell culture is lysed with buffer and homogenized directly on the plates
2) Lysates are transferred to a 96-well plate together with hybridization buffer that includes target-specific probes
3-4) After hybridization, samples are transferred to the KingFisher instrument together with plates that incorporate magnetic beads and buffers for washing and elution
5) Eluents are analyzed with a DNA sequencer capillary electrophoresis unit
6) Data is analyzed by TRACParser software

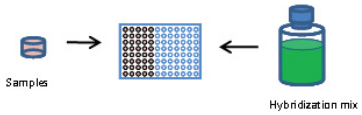
WORKFLOW - Cells to data

1) Sample Preparation

- Remove media
- Add lysis buffer

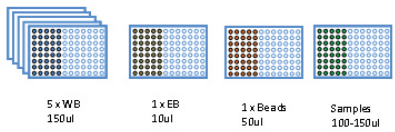
2) Hybridization

- Add samples and hybridization buffer on 96-well plate
- Hybridize at +60 °C, 30-90 min.



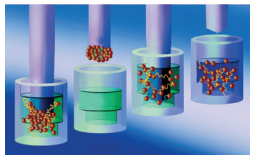
3) Dispense on KingFisher Plates

- 5x Wash buffer (WB) plates
- 1x Elution buffer (EB)
- 1x Bead plate
- Samples after hybridization



4) Capture, Wash and Elute with KingFisher Instrument

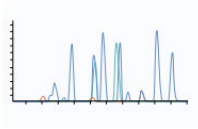
- Insert plates and proceed according to TRAC-specific protocol



STEP	Time (min)	TEMP (°C)	Volume (µl)
Capture	30	RT	50-100
Wash	1 x 5	RT	100-150
Elution	20	37	10

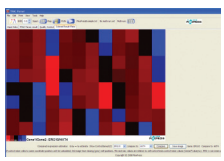
5) Fragment Analysis

- Insert plates to a DNA sequencer and proceed according to TRAC-specific fragment protocol



6) Parsing

- Analyze the raw data from the DNA analyzer using TRACParser Software



Results

TRAC application examples
siRNA knockdown validation

Objective

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

Results and conclusions

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

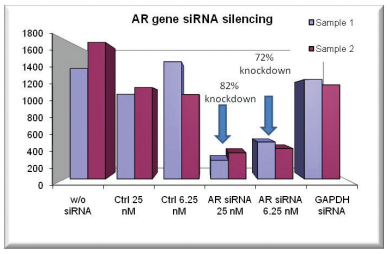


Figure 2. Androgen receptor gene silencing efficiency by targeting and non-targeting siRNA measured by TRAC maps to reporter assay performance.

Cancer marker screening in cell cultures

Objective

TRAC assay with the KingFisher instrument was used to screen 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, 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 over 24 hours.

Results and conclusions

The expression signatures could be detected directly from 10 – 100 x 10^3 cells grown on 96-well plates. The dynamics of gene expression for the analyzed set could not have been observed at a single point in time. Reproducibility was good with CVs below 12% for the system.

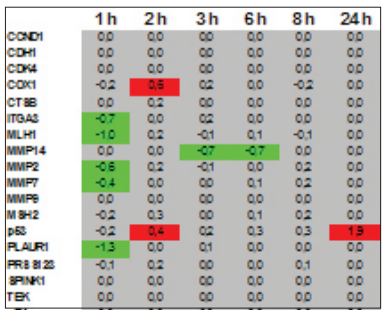


Figure 3. Expression signature of cancer-related genes in COLO cell line after drug candidate treatment.

User benefits

TRAC assay with KingFisher enables high-content gene expression analysis with these user benefits.

High information value:

- Multiplexing allows for a thorough study of gene function
- Observing the dynamics of gene expression gives greater insight into gene functions
- Powerful research data improves decision making to reduce project time and lower project costs

Simplicity:

- Direct use of cell lysates; no RNA extraction
- No cDNA conversion required
- Simple assembly of new, custom-made gene sets
- Easy implementation; experiments are simple to set up

Speed:

- 96-Well plate format enables automated, high throughput sample processing
- Rapid assay protocol with little hands-on time

Accuracy:

- Liquid carryover between processing steps is minimized to reduce the risk of contamination
- Degradation of RNA is eliminated
- Control genes are incorporated into the multiplexed mixture, results are normalized

Reproducibility:

- Intra- and inter-assay CVs <8% (typically <5%)
- Sample-to-sample and day-to-day processing reproducibility is greater with KingFisher instruments compared to manual options

Literature

[1] Rautio JJ et al (2008) TRAC in high-content gene expression analysis: applications in microbial population studies, process biotechnology and biomedical research. Expert Rev. Mol. Diagn. 8, 379-385.

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