



Work Package 1: Transcriptomics

Introduction

The goal of the Transcriptomics work package is to improve food safety by using cultured human cells as biological detectors (“Cytosensors”) for the detection of toxic contaminants in food. This new technique is prompted by findings that living cells respond to toxic chemicals by changing the pattern of genes that are converted into RNA transcripts.

Each individual RNA transcript carries the information for the synthesis of a particular protein product.

The term “transcriptome” refers to a collection of RNA intermediates in a given biological system. Accordingly, “transcriptomics” is defined as a large-scale analytical method that can be used to monitor complex RNA profiles consisting of thousands of transcripts.

When human cells are exposed to food extracts, changes in the overall pattern of RNA transcripts are suggestive of the characteristic reactions triggered by toxic contaminants. WPI have developed a novel assay, based on the detection of RNA “fingerprints” using specially designed microchips. The novel assay allowed the use of cultured human cells as versatile sensors for the detection of hazardous contaminants in food. The principle of this new transcriptomic approach is illustrated in Figure 1 below.

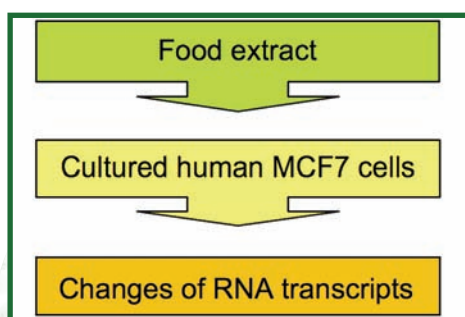


Figure 1. Scheme of contaminant detection by RNA fingerprinting. Cultured human cells are exposed to extracts prepared from food samples. Following an incubation of 3-24 hours, RNA transcripts are isolated, labelled and detected on high-throughput DNA microchips. Toxic contaminants generate characteristic changes in the transcriptional pattern, thus giving rise to diagnostic RNA fingerprints that can be used to recognise and quantify hazardous contaminant detection.

Work Package Progress and Results

By measuring these RNA transcripts in human cells, we have provided screening tests for the following contaminants:

- Dioxins and dioxin-like pollutants
- Phytoestrogens and estrogen-like chemicals
- Mycotoxins belonging to type - A trichothecenes

Dioxins and dioxin-like pollutants

These substances are industrial by-products and a class of highly toxic environmental pollutants that accumulate in meat, fish, eggs and milk. They are known to promote cancer and interfere with the normal function of hormones.

Figure 2 below illustrates how dioxins extracted from contaminated milk, shift the cellular RNA profile, thus generating a molecular fingerprint that is diagnostic for the presence of these toxic compounds.

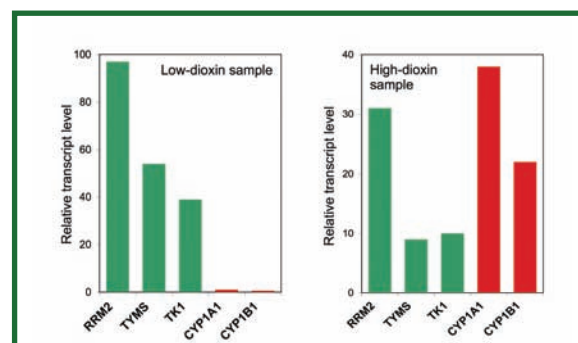


Figure 2. Detection of dioxins in milk using changes of the RNA fingerprint in human MCF7 cells. RRM2, TYMS, TK1, CYP1A1 and CYP1B1 are RNA transcripts that code for different human proteins.

Phytoestrogens and estrogen-like chemicals

These natural compounds are identified as “endocrine disruptors” and known to interfere with the normal functionality of sexual hormones released into the human body. Phytoestrogens are plant-derived components with estrogen-like activity and are found at high concentrations in soy and many other plant products.





The excessive intake of such phytoestrogens particularly during early childhood years is thought to cause long-term health risks such as cancer or reproductive dysfunctions. Figure 3 demonstrates how the RNA fingerprinting of human cells are exploited to detect phytoestrogens in baby food.

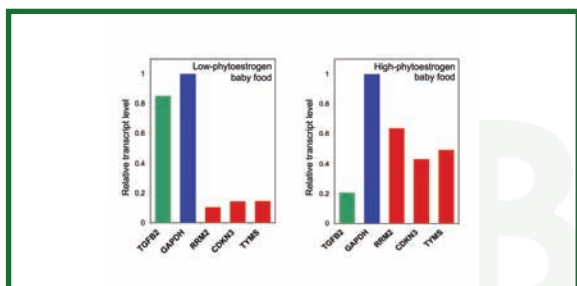


Figure 3. RNA fingerprints induced by food extracts with different phytoestrogen concentrations. TGFB2, RRM2, CDKN3 and TYMS are examples of more than 100 transcripts that code for estrogen-dependent human proteins.

Mycotoxins belonging to Type-A trichothecenes

Type-A trichothecenes are hazardous natural compounds generated by moulds that contaminate field crops (wheat, barley, maize, oats, rye and rice) and manufactured goods (bread, beer, breakfast cereals, and baby food). A major target for these mycotoxins is the immune system, often leading to long-term health effects by reducing the resistance to common viral and bacterial infections. In addition to this, lifelong exposure to trichothecenes has been associated with an increased risk of developing cancer. Figure 4 shows how changes in the transcriptional pattern are used to detect type-A trichothecenes in wheat samples.

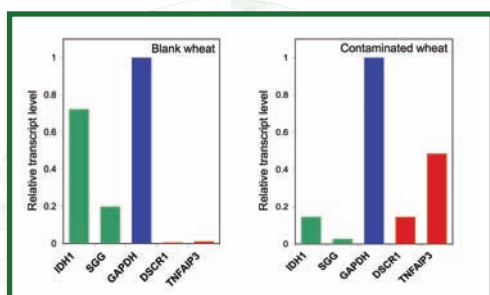


Figure 4. Detection of Type-A trichothecenes in wheat using changes in the RNA fingerprint of MCF7 cells. IDH1, SGG, GAPDH, DSCR1 and TNFAIP3 are individual human transcripts.

Benefits of the BioCop Project

Consumer

The foods we eat may contain a multitude of man-made or natural contaminants that can pose many health hazards. To protect the consumer, WPI has developed a novel RNA fingerprinting method by which a variety of food samples can be screened. This means that the consumer can have more confidence in the food they eat, knowing that the possible presence of multiple groups of contaminants are being monitored.

Food Industry

The newly developed microchip fingerprinting method provides an effect-driven assay that can be integrated into HACCP (Hazard Analysis and Critical Control Points) systems to monitor the true impact of potentially toxic contaminants. Another advantage of this fingerprinting strategy is its ability to detect the additive activity of a mixture of chemicals at any one time.

Scientist

The transcriptomic-based fingerprinting approach will be used by scientists all over the world in the areas of agriculture and food technology. It will also be used to detect new or emerging contaminants that trigger known adverse reactions to human health.

Training/Workshops

With the exception of a portable reader, this user-friendly transcriptomic platform requires no specialised equipment. Therefore the method can be easily disseminated with the aim of being introduced to researchers, food companies, end users and policy makers through a range of workshops, demonstrations/training activities and publications.

Future Activity

The microchip-based fingerprinting assay will be subjected to miniaturisation (to reduce the cost of materials) and automation (to reduce labour time). In addition to this, the fingerprinting strategy will be





developed to detect a wider range of toxic food contaminants.

Frequently Asked Questions

Question:



What are the costs of the RNA fingerprinting assay?

Answer:



The total costs will be comparable to those of other cell-based bioassays.

Question:



What is the total assay time?

Answer:



With further automatisation, the overall assay time will be reduced to approximately 24 hours.

Question:



What is the added value of this assay?

Answer:



A key advantage is that the novel test exploits health-relevant endpoints within a toxicological significant target system. With the widespread use of rapid screening tests, (not related to any toxicological endpoint), effect-driven in-vitro bioassays will become of increasing importance to support risk assessment and to monitor the success of risk management actions.

