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# Characterization of Bacterial Response to Toxic Chemicals Using DNA Microarrays

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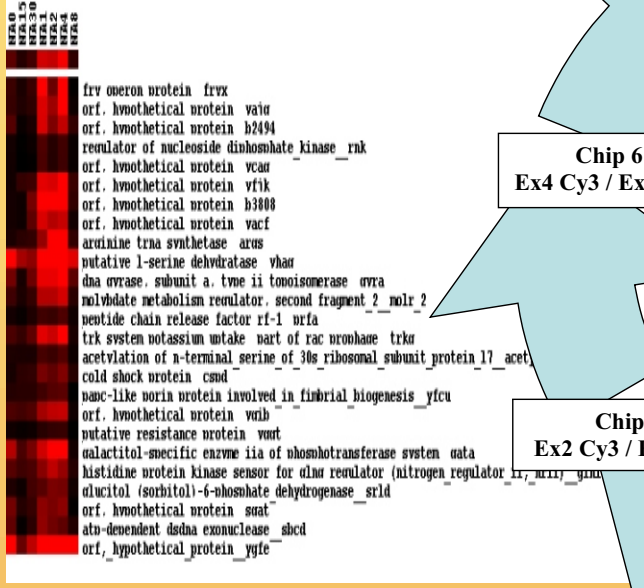
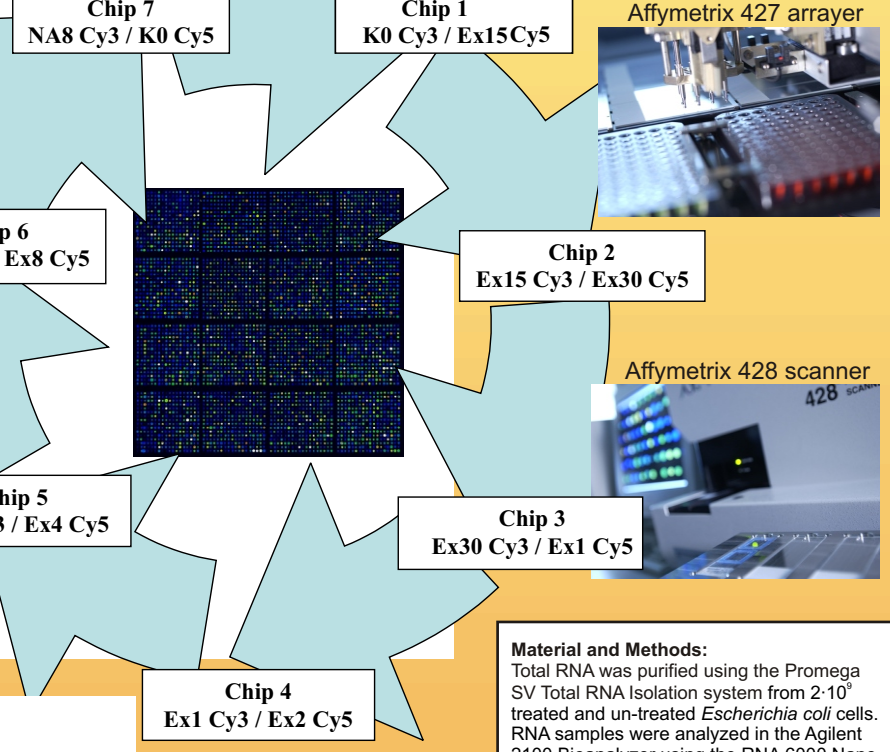
## Introduction

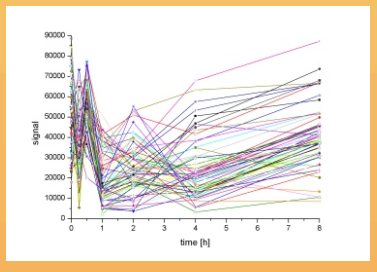
Monitoring for chemical toxins in water and wastewater has become of widespread interest throughout the world. Biosensors sensing systems with biological detection elements have the capacity to detect chemical toxins in a sensitive and selective manner. Recently, the research focused to use recombinant reporter bacteria in optical sensor configurations for the purpose of monitoring toxicity. The bacteria were strains of *Escherichia coli* that were modified to express either green fluorescent protein (GFP) or bioluminescence when genes of the SOS response (DNA repair, *recA*) or heat shock response (*grpE*) were induced (up-regulated or "turned on"). Although these biosensors worked well, their function can be validated and perhaps improved through a functional genomics study in which the induction of several thousand genes is detected simultaneously. Specifically such a study would accomplish the following goals:

- Compare timecourse of sensor signal (GFP fluorescence) with actual SOS response
- Identify genes that respond sooner or more specifically to toxins
- Identify gene induction patterns that can identify one toxin vs. another (which can in turn be incorporated into multichannel sensors)

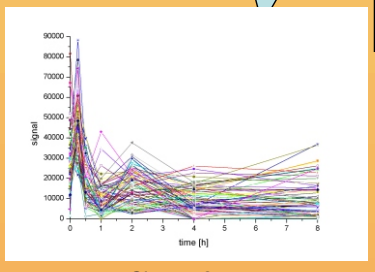
Expression analysis of *E.coli* genes using a loop design




Cluster 5




Cluster 8

**Material and Methods:**
Total RNA was purified using the Promega SV Total RNA Isolation system from $2 \cdot 10^9$ treated and un-treated *Escherichia coli* cells. RNA samples were analyzed in the Agilent 2100 Bioanalyzer using the RNA 6000 Nano LabChip™. During reverse transcription 100 µg of purified total RNA is converted into a Cy3 or Cy5 labeled cDNA and hybridized simultaneously to the same array. Performing a time course experiment we choose two different desings, a common reference design as well as a loop design. Time points are 0, 15min, 30min, 1h, 2h, 4h and 8h.

**Results:** Before the further interpretation of the results, the data has to be cleaned and standardized.
An analysis of correlation was performed and thus the coregulated genes in experiment and reference were deteceted. These genes were also erased from the data set. In order to maintain the amount of regulated genes, the ratio of the signals from different time steps are formed. In addition, the difference between the signal is determined.

Two differend cluster algorithms were applied to the data.
For the hierachical clustering the program "Gene Cluster" (Michael Eisen, Stanford University 1998-9) was used.
k-means clustering was performed with MATLAB, Version 6.5.0196271 a Release 13.01 The MathWorks, Inc., 2003.
The data set, consisting of 3034 genes, was divided into 15 clusters.

The results presented here demonstrate that gene regulation take place between 15 min and 2 h after cell treatment. The gene expression pattern in two cluster consists of initial low-level induction, a sudden large increase in induction which is then followed by a gradual decrease in induction. Most of the genes found to share similarity in cluster 5 are involved in membrane transport and signal transduction. Our results show that it is possible to use microarrays to examine the

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