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# Transcription Profiling

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## Introduction

Research hypotheses can now be addressed in the context of entire developmental or biochemical pathways under a large array of experimental or field conditions. Access to complete genomic sequences, coupled with rapidly accumulating data related to RNA and protein expression patterns, have made it possible to determine comprehensively how genes contribute to complex phenotypes. The genetic inheritance of transcript profiles can be used to map the chromosomal location of relevant, regulatory loci. Transcription profiling involves the quantification of gene expression of many genes in cells or tissue samples at transcription (RNA) level.

## DNA microarray

- DNA chip or biochip
- Collection of microscopic DNA spots attached to a solid surfaces.
- Measures the expression levels of large numbers of genes simultaneously.
- Each DNA spot contains picomoles (10-12 moles) as a specific DNA sequences, known as probes.
- These can be short section of a gene or other DNA element that are used to hybridize a cDNA or cRNA (also called anti-sense) sample (called target) under high-stringency conditions.

## RNA Seq Protocol

- Get your sample
- Lyse the cell and extract RNA
- Convert the RNA TO cDNA
- The cDNA pool get sequenced

| description | small\miRNA | Degradome-seq | Digital gene expression | Poly[A]-RNA Seq | Total RNA seq |
|---|---|---|---|---|---|
| Library preparation-RNA selection | Size selection-small RNA | Poly[A] tail selection-miRNA | Poly[A] tail selection-mRNA | Poly[A] tail selection-mRNA | rRNA depletion - mRNA+lncRNA |
| Sequencing cycles | 50 | 50 | 50 | 2×100 | 2×100 |
| Single vs. paired end | Single end | Single end | Single end | Paired end | Paired end |
| **Data analysis** | **discovery expression annotation** | **miRNA target ID degradation plots** | **Expression annotation** | **discovery expression annotation** | **discovery expression annotation** |

## Uses of transcription profiling

- Transcript profiling has been utilized effectively to drive biological discovery in host-pathogen interactions.
- With the increasing availability of a large number of microarray data sets in public repositories, it is also beneficial to use comparative meta-profiling strategies to draw conclusions that bridge multiple experiments for profiling.
- High-density DNA arrays are now available for barley, wheat, rice, maize, sugarcane, grape, citrus, poplar, tomato, *Arabidopsis*, *Brassica*, and cotton, among others.
- In addition to using these arrays to monitor the expression of thousands of transcripts in parallel, many projects are accessing this technology for both host and pathogen through community-driven development of multispecies arrays, such as soybean/*Phytophthora sojae* (root rot)/ *Heterodera glycine* (soybean cyst nematode), *Medicago truncatula/ Medicago sativa/ Sinorhizobium meliloti*, *Fusarium graminearum* (scab), and rice/ *Magnapothe grisea* (rice blast).

## Profiling plant *R* gene-mediated responses to pathogenic bacteria

- A number of studies have yielded global views of the transcriptional response of plants undergoing *R* gene–mediated defense against pathogenic bacteria.
- Mysore et al. utilized Gene Calling technology in tomato to define the gene expression changes in resistance to bacterial speck, and the specific contributions of the *R* gene *Pto* and the gene *Prf*, which is required for *Pto* function. They defined an early role for *Prf* in the response pathway, and also identified changes dependent on *Prf* but not *Pto*, suggesting a distinct, independent role for *Prf* in pathogen recognition.
- In a subsequent study that used a variety of transcript profiling techniques and resources (including subtractive-suppressive hybridization and cDNA microarrays), it was shown that over expression of *Pto* induced gene expression changes similar to those observed during immune responses in animals.
- To gain insight into the molecular basis of tomato resistance to *Xanthomonas axonopodis* pv. v*esicatoria* strains expressing the effector Avr Rxv, which is governed by three, nondominant resistance genes, Bonshtein *et al*. used a similar profiling approach.

## Profiling fungal gene expression within the host

- In addition to differential gene expression occurring within host cells, it is necessary to consider the gene expression of pathogens within the infection site as well.
- A study to monitor expression of genes in *Colletotrichum graminicola*, causal agent of maize anthracnose stalk rot, used laser capture microscopy (LCM) in combination with fluorescent AmCyan protein-tagging, to produce samples for microarray analysis.
- This approach identified over 8000 genes as significantly expressed in *C. graminicola* as early as 2 days after inoculation, which is an early stage of infection with relatively little fungal biomass accumulation.
- Studies with another comparable host-pathogen interaction at 2 days after inoculation, but not employing LCM, led to the identification of only about 900 expressed fungal genes.
- Thus, LCM clearly provided for greater enrichment of fungal mRNA and an order of magnitude increase in power to detect fungal mRNA transcripts.
- Comparison of gene expression between in vitro grown cultures and in planta grown cells showed significant up-regulation of secreted proteins, perhaps signifying the production of effectors and other proteins required for pathogenicity.
- It would be interesting to determine how the maize cells collected in these samples were also responding to *C. graminicola* in these samples. Both and colleagues found many pattern of coordinate expression among *B. graminis hordei* genes in defined metabolic

pathways from cDNA microarray profiling experiments monitoring the infection cycle in barley.

- This allowed an assessment of the metabolic status of the fungus during asexual development as it infected the host plant. Genes encoding several glycolytic enzymes are significantly up-regulated as mature appressoria form, and in the infected epidermis, which contain fungal haustoria.
- Concomitantly, host plants show up-regulation of sugar transport and utilization-related genes after powdery mildew infection, providing a source for nutrient acquisition. Obligate biotrophic rust fungi infect host tissue via intercellular mycelia that form haustoria within the living plant cells.
- Jakupovic and colleagues identified genes expressed during biotrophic growth of the bean rust *Uromyces fabae* by EST sequencing of a haustorium-specifc cDNA library.
- Several of the *Uromyces* ESTs were identical to the *in planta* induced genes (*PIG*s) identified in earlier studies. Virus-encoded sequences were identified, providing evidence for two RNA mycoviruses in *U. fabae*.
- To monitor the ascomycete *B. cinerea*, a broad-spectrum plant pathogen, in real-time infection conditions, Gioti and associates infected *Arabidopsis* leaves with *B. cinerea* and assayed transcript accumulation using a custom macroarray.
- Seven percent of *B. cinerea* genes were differentially expressed during infection, and 27 genes were significantly up-regulated *in planta*.
- Two of the genes, trichodiene oxygenase and pentalenene synthase, had already been associated with fungal pathogenicity, whereas eight have unidentified functions.
- The 27 genes were clustered into three groups; the first group showed maximal expression at the early stage following fungal penetration, the second showed maximal expression at the outset of the colonization of plant leaves, and the third showed maximal expression when the colonization of plant leaves was completed.
- Guldener and colleagues took advantage of the genome sequence of *F. graminearum*, the causal organism of *Fusarium* head blight of wheat and barley, to design a whole genome (18 K) Affymetrix Gene Chip.
- To establish a baseline set of gene expression data, *F. graminearum* Gene Chips were interrogated with RNA isolated from fungus grown in culture under three nutritional regimes, in addition to *in planta* growth in infected barley.
- During the barley infection time course 7132 *Fusarium* probe sets were called present, even though the fraction of fungal transcripts in the total RNA from infected plants is quite low, notably during the early stages of infection.

## Applications of Transcriptional Profiling

- Dissection of changes in gene expression levels
- Categorization of tissues based on expression patterns
- Application of technologies to diverse genotypes
- Measurement of allele-specific differences