

Module 5

Transcriptome analysis

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1. INTRODUCTION

Organisms have to accommodate changing environmental conditions and their programs of growth and development. Much of the biological regulations assuring these functions occur at the level of transcriptional control of genes involved in these processes. Thus comprehensive knowledge of the genes that change their expression pattern in response to a signal may reveal much on the mechanisms that are turned on.

Genome-wide expression monitoring has recently become feasible with the description of complete genome sequences or with the availability of large EST databases and through the development of cDNA and oligonucleotide array technology. The principle of this technology is very simple. DNA molecules or oligonucleotides corresponding to the genes whose expression has to be analysed (the probes) are attached in an ordered fashion to a solid support that can be a nylon membrane or a glass slide. Miniaturisation and automation of array production with robotic spotters or in situ synthesis of oligonucleotides makes it possible to produce arrays with several thousand genes (i.e. a substantial part of the genome) represented on few square centimetres. To measure the relative abundance of the corresponding transcripts in a RNA preparation, the sample is first labelled with a fluorescent or radioactive marker and then hybridised with the arrays. The intensity of the hybridisation signal is a measure for the relative abundance of the corresponding mRNA in the sample. The expression profile or transcriptome refers to the complete collection of mRNAs present. Thus comparing the hybridisation signals for different mRNA samples allows changes in mRNA levels to be determined under the conditions tested for all the genes represented on the arrays.

The purpose of array experiments and transcriptome characterisation are to address biological issues and this can be achieved at different levels of complexity (Lockhart and Winzeler, 2000). Probably the simplest application of arrays is their use in “gene discovery” experiments. In such an approach, arrays are used to identify novel genes that are associated with a biological process of interest. For example, comparing the transcriptome in control roots of *M. truncatula* with mycorrhizal roots or nodules may lead to the identification of new genes expressed in symbiotic developmental programs, which can then be further functionally characterised. But what may be considered as the main conceptual change brought about by array technology is the possibility to work at the gene network level. Indeed, global analysis of gene expression under a set of physiological conditions allows genes to be clustered into groups sharing a similar expression pattern, and possibly involved in a same cellular function. Combining such global expression analyses with mutant characterisation, whenever possible, may be powerful to conduct a finer study of such gene networks. Arrays can be used in the functional analysis of uncharacterised genes, since they can provide hints for the cellular process in which the genes are involved. They also make possible the comparative analysis of families of related genes and may allow these to be distinguished (for example by use of so-called “dedicated arrays”). Finally, expression profiles can be used as “fingerprints” for a number of applications such as phenotypic characterisation, disease diagnosis, cancer cell classification or drug target identification.

Although the main use of arrays is monitoring mRNA levels, they may also serve in other applications. To name just a few, changes in DNA copy number can be measured, polymorphism can be detected or the DNA-binding sites of transcription factors can be located.

Different array technologies exist and the three major types are “gene or DNA chips”, “microarrays” and “macroarrays” (Lockhart and Winzeler, 2000). Gene chips carry oligonucleotides that are photolithographically synthesized in situ, every gene being represented by a set of specific oligos. For the other two systems, robotic spotters deposit few nanograms of DNA (typically PCR products), generally at 100-400 μm intervals for microarrays on glass slides, or at 1-2 mm intervals for macroarrays on nylon membranes (although the use of microarrays on nylon membranes has also been described, see Bertucci et al., 1999). Chips are hybridised with a single fluorescent-labelled sample. Microarrays are most often used with a two-colour hybridisation strategy: two different samples are labelled each with a different fluorescent-labelled dye, mixed and then co-hybridised to an array. Macroarrays are used with radioactively (^{33}P) labelled samples, each sample being analysed independently. Finally, a confocal laser scanner for the gene chips and microarrays and a PhosphorImaging system for the macroarrays are used for measuring the hybridisation signal.

Gene chips and microarrays are costly and require special equipment. In contrast, macroarray equipment is much more accessible to any molecular biology laboratory. During the course, the manipulation of macroarrays will be taught. Some bioinformatics tools for the treatment of results will be introduced. Finally, a demonstration of the equipment for the fabrication and use of microarrays will illustrate the potential of this other technology. Examples of protocols for handling microarrays can be found in Eisen and Brown (1999). An interesting web site at <http://www.gene-chips.com> contains a large list of academic and industrial links related to the array technology.

2. MATERIALS AND METHODS*

*Methods that will be carried out during the practical course are indicated with *. However, other methods that can be useful in the use of macroarrays are included as well.

2.1. Arrays

The arrays that will be used in the practical course were previously made. The probes that were printed on the arrays were clones from a cDNA library prepared from young nodule mRNA from *M. truncatula* (Györgyey et al., 2000). These clones included genes coding for known nodulins (proteins specifically present in nodules), cell cycle proteins, signal transduction proteins, transcription factors, components and regulators of the cytoskeleton, a group representing diverse proteins and finally a set of ESTs with no homology to previously characterised proteins. The identity of each of the clones will be provided in an Excel file to the participants. In total, 192 unique genes, repeated three times, are represented on the arrays.

The DNA to be spotted was prepared by PCR using for most of the clones universal primers from the library vector. However, for some clones gene specific primers were used (see fig. 1). The spotted DNA fragments had a minimum length of 500bp and maximum of 3000bp. 100 μl PCR reactions were used to obtain sufficient DNA for printing (10 μg for a 100 μl reaction). PCR reactions were done in 96-well microtiter plates, with standard amplification parameters. 1 μl of the PCR products was run on an agarose gel to check the product purity (presence of one band with the expected size) and evaluate its concentration by comparison

with a DNA product of known concentration. For making macroarrays, in contrast to microarrays, these PCR products can be used as such without any further purification.

Before spotting, DNA was denatured by adding 10 μ l of 2 M NaOH giving a final concentration of about 0,2 M. [Alternatively DNA can be denatured in 50% DMSO, which reduces evaporation during the spotting and gives more homogeneous spots but may necessitate concentrating the sample]. The DNA was spotted with a BIOMEK 2000 robot (Beckman, CA) on Hybond N+ membranes purchased from Amersham (Immobilon-Ny+ from Millipore works equally well). The main components of such a robot are a movable print-head that carries pins, a table for attaching the membranes, a stand for microtiter plates containing the DNA solutions, a station for cleaning and drying the pins and finally a computer controlling the movements of the print-head. The membranes were made with an 8-pin configuration. Each of the pins absorbs a droplet from a row of 8 wells from the microtiterplate and deposits this droplet (\pm 150 nl corresponding to \pm 15 ng of DNA) on the membrane at the appropriate location. This movement is repeated until each membrane on the table is printed in triplicate. Such a cycle is repeated for all the rows of, in our case, two 96-well plates with 192 clones. In between cycles, the pins are cleaned in a bath and dried by ventilation. The procedure resulted in arrays that have a configuration as shown in figure 1 with 8 patches of 24 clones that are repeated 3 times. After deposition, DNA is cross-linked to the membranes by UV radiation (125 mjoules) in a standard UV illumination chamber. Membranes are conserved at room temperature until use.

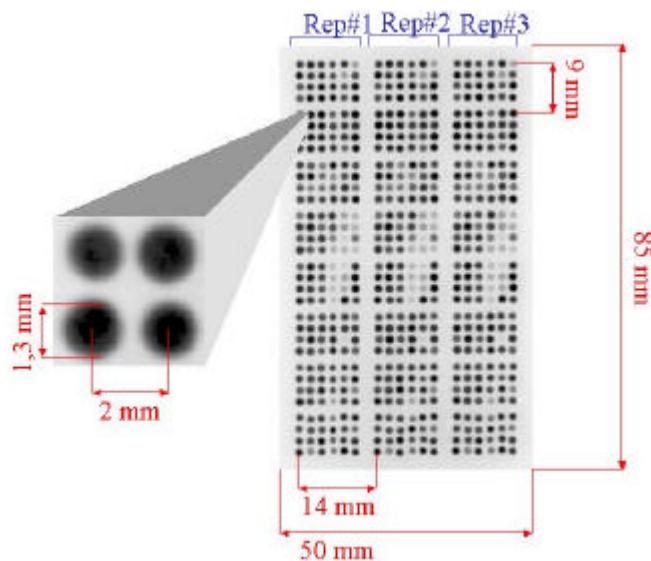


Figure 1. Array configuration. The arrays contain 192 unique DNA sequences, organized in columns of 8 patches with 6 by 4 clones. The columns are repeated 3 times (Rep#1, Rep#2, Rep#3). The dimensions of the array and the spots are indicated. The spots are visualized by hybridisation with a ^{33}P -labelled oligo, common to all clones except for those that show no or little signal (see text for additional information).

2.2. Choice of isotope for labelling

The isotope ^{33}P is preferred to ^{32}P for labelling reactions, even though it is more expensive, because of its lower energy and therefore lower radiation range. This gives sharper signals, and makes it possible to quantify weak signals even when they are close to strong signals. The half-life of ^{33}P is 25,4 days compared to 14,3 days for ^{32}P .

2.3. Membrane quality control by ^{33}P -oligo hybridisation

Unlike microarrays, macroarrays cannot be simultaneously hybridised with two samples. Thus no internal control can be included in the hybridisation experiment. Therefore, it is important to evaluate the quality of the depositions on each membrane before hybridisation with a sample. This can be done by hybridising with an oligo that is common to all probes. In our case most probes that were spotted are PCR products containing part of the vector pADGal4 (Stratagene), which was thus used to design the oligo. The result of such an hybridisation is shown in figure 1. Most clones show an intense hybridisation signal. Those that do not light up are clones that were PCR amplified with gene specific oligos and thus do not contain the pADGal4 vector sequence. The membranes that will be used during the course were verified previously and membranes that did not have the same quality as the example shown in figure 1 were removed. The results of this quality control will be distributed. The protocols for oligo labelling and hybridisation are the following :

Prehybridisation

Incubate membranes in a sufficient volume of Church Buffer containing 0,1 mg/ml salmon sperm DNA, overnight at 65 °C with gentle shaking. From this point, avoid at any moment drying of the membranes.

Oligo labelling

The oligo is labelled by 5' phosphorylation using T4-polynucleotide kinase (PNK). For hybridisation of 10-40 membranes, prepare a 100 µl reaction containing:

- 2 µl 13-mer oligo 100 µM (200 pmol)
- 68 µl H₂O
- 10 µl 10x PNK-buffer (supplied by the manufacturer of the enzyme)
- 10 µl PNK (10 units/µl)
- 10 µl [γ - ^{33}P]-ATP (3000 Ci/mmol; 10 µCi/µl)

Incubate this mix at 37 °C for 1 hour. The labelled oligo can be used as such, without purification, in the hybridisation.

Hybridisation

Hybridisation of oligos is made at low temperature (10 °C). Because SDS precipitates at low temperature, SDS is replaced by sarcosyl in the hybridisation buffer. *[Alternatively, a longer oligo can be used, and hybridisation can be carried out at 2-5 °C below the T_m , with standard Church Buffer].*

- Remove the hybridisation solution and wash the membranes once with SSARC Buffer at room temperature.

- Wash the membranes two more times with cooled (4 °C) SSARC buffer.
- Immerse membranes in SSARC Buffer, add the labelled oligo and hybridise overnight at 10 °C with gentle shaking.
- Remove the hybridisation solution and rinse the membranes shortly in cold SSARC Buffer.
- Wash the membranes for 20 min. at 10 °C in SSARC Buffer with gentle shaking.
- Remove the washing buffer and add fresh SSARC Buffer.

Exposure

- Place the membranes on a piece of 3MM whatman paper that is wetted with SSARC Buffer.
- Wrap the membranes in saran wrap and remove bubbles between the membrane and the foil. At this point, the success of the hybridisation can be monitored with a Geiger-Müller detector.
- Place the membranes in a cassette and expose to a phosphor screen for 30 min. to a few hours.
- Scan the screen with a STORM or PhosphorImager machine (Molecular Dynamics). The ImageQuant software allows visual inspection of the quality of the spots on the membrane.

Membrane regeneration

Immediately after exposure, the oligo has to be removed before storage of the membranes or use in a hybridisation experiment with labelled cDNA.

- Boil 300 ml of Regeneration Buffer.
- Add 100 ml to the membranes, shake gently and remove the solution.
- Add the rest of the Regeneration Buffer and let cool down to room temperature. The success of dehybridisation can be monitored with a Geiger-Müller detector. If detectable radioactivity is remaining, repeat the steps above.
- Expose the membranes to a phosphor screen as described above.
- If the radiolabelled oligo is removed to satisfaction, membranes are dried overnight between two 3MM Whatman papers and stored at room temperature for a short time or at 4 °C in a sealed bag for a long period of time.

2.4. RNA preparation

A relatively high amount of RNA is needed for array hybridisations. For one labelling experiment, 20 µg to 50 µg of total RNA or the corresponding amount of mRNA is used. Every experiment is normally repeated at least once, doubling the amount of RNA to prepare. Therefore, care has to be taken in the preparation of the biological material that has to be produced in sufficient amount and dissected precisely. In addition, RNA must be of high quality to obtain efficient cDNA synthesis with high label incorporation. Therefore, we use routinely a combination of 2 protocols. The first protocol, based on phenol extractions, allows large amounts of starting material to be used and results in high quantities of crude RNA. We tested this protocol for many different tissues of *M. truncatula* with generally satisfying results. In the next step, this crude preparation is further purified with a commercial kit yielding highly pure

RNA. If desired, this total RNA can be further enriched for mRNA by selection of polyadenylated RNA. However, we routinely use total RNA for labelled cDNA synthesis.

- Tissue is dissected and frozen immediately in liquid nitrogen.
- The material is grinded under liquid nitrogen with pestle and mortar to a fine powder. Addition of some quartz sand may help.
- While the material is still frozen in the mortar, add 2 to 4 ml of Solution D.
- Leave the solution to melt and transfer to a 15 ml falcon tube.
- Add $\frac{1}{10}$ volume of a 2 M Na-acetate pH 4 solution. Add 1 volume acidic phenol and $\frac{1}{4}$ volume chloroform/isoamyl alcohol (24/1). Mix well by shaking. Leave 30 min. on ice.
- Centrifuge 4000 rpm for 40 min. at 4 °C.
- Remove the upper phase and transfer to a corex tube.
- Add $\frac{1}{2}$ volume of cold, absolute ethanol, mix well and incubate for 10 min. on ice.
- Centrifuge at 10000 rpm for 30 min. at 4 °C.
- Wash the pellet with 80 % ethanol and dry under vacuum. Take care not to dry longer than necessary: a pellet that is too dry is hard to dissolve.
- Resuspend the pellet in 400 μ l Proteinase K solution (1x Proteinase K Buffer containing 0,16 mg/ml Proteinase K) and transfer as well as possible all the material to a 2 ml eppendorf tube. Depending on the starting tissue, this step can be difficult because the pellet may contain a lot of insoluble material. Incubate 30 min. at 50 °C.
- Add 1 volume acidic phenol and $\frac{1}{4}$ volume chloroform/isoamyl alcohol (24/1). Mix strongly. Leave 10 min. on ice and centrifuge 5 min.
- Transfer the upper phase to a fresh tube. Add 1 volume chloroform/isoamyl alcohol (24/1). Mix strongly. Leave 10 min. on ice and centrifuge 5 min.
- Transfer the upper phase to a fresh tube. Add $\frac{1}{20}$ volume 5 M NaCl and 3 volumes absolute ethanol. Mix and leave overnight at -20 °C.
- Centrifuge 15 min. at 4°C. Wash pellet with 80 % ethanol. Dry under vacuum (not too long!) and dissolve pellet in DEPC treated water.
- Quantify RNA with a spectrophotometer and verify quality of RNA on a non-denaturing 1 % agarose gel.

This crude RNA preparation is very well suited to use in for example Northern analysis. However, if the RNA is to be used in enzymatic reactions, better and more reproducible results can be obtained when the RNA preparation is further cleaned. A fast and easy method relies on commercially available columns (e.g. Qiagen columns; a cheaper kit [E.Z.N.A. Plant RNA kit] working equally well can be obtained from Omega Bio-Tek, Inc.). In these methods, the RNA prep, obtained as described above, is conditioned with a buffer provided by the manufacturer. The solution is applied to a column on which the RNA is specifically retained. The column is then washed with several buffers before the RNA is eluted and obtained in a highly pure form. The RNA quantity is measured with a spectrophotometer and its integrity verified on a non-denaturing 1 % agarose gel. Concentrate the RNA samples under vacuum to obtain solutions of 2,5 μ g/ μ l.

The organizers will provide the RNA preparations that will be used in step 2.5 below. Every participant will use a different RNA preparation, purified from roots, wild type nodules or non-functional nodules formed on plant mutants or induced by mutant bacteria.

2.5. ³³P-labelled cDNA synthesis*

Reverse transcription*

For the reverse transcription, two alternatives are possible : reverse transcription from an oligo-dT primer that selects only the poly-adenylated mRNA or priming using random hexamers. The latter possibility can only be applied when purified mRNA is used in the reverse transcription reaction. During the practical course, total RNA will be used, thus with oligo-dT priming. However, the following protocol can easily be adapted for random priming by using 500 ng mRNA and replacing the oligo-dT primer in the first step with 80 ng random hexamers. The chase reaction with cold dCTP can be omitted, in which case cDNAs molecules will be much shorter.

- Mix 20 µl containing 25 µg of total RNA with 2,5 µl of an 18-mer oligo-dT primer (2,5 µg/µl). Heat for 10 min. at 70 °C to denature RNA secondary structures.
- Place on ice.
- Add :
 - 10 µl 5x first strand RT-buffer (supplied by manufacturer of the enzyme)
 - 5 µl 100 mM DTT
 - 0,5 µl 40units/µl RNasin RNase inhibitor (Promega)
 - 5 µl of a solution containing 10 mM of each dATP, dGTP and dTTP
 - 5 µl [α -³³P]-dCTP (3000 Ci/mmol; 10 µCi/µl)
 - 2 µl PowerScript Reverse Transcriptase (Clontech)
- Incubate at 37 °C for 1 hour.
- Add 2 µl 25 mM cold dCTP.
- Incubate further for 1 hour.
- Add 50 µl STE Buffer.

Purification and determination of label incorporation*

- Take 1 µl of the non-purified labelled cDNA and add to liquid scintillation solution. Put aside.
- Apply the labelled cDNA to a G-50 spin column and centrifuge 2 min. at 750g.
- Recover the flow-through containing the labelled cDNA, take 1 µl and add to liquid scintillation solution.
- Measure the counts in the two scintillation samples with a counter and make the ratio of counts after purification to the counts before purification. Incorporations of 20 % - 40 % should be obtained.

RNA hydrolysis*

- Add 100 µl STE Buffer.
- Add 60 µl 1 M NaOH.
- Incubate 10 min. at room temperature.
- Add 60 µl 1 M HCl.
- Add 60 µl 20x SSPE.
- This ³³P-cDNA is ready to be used immediately in a hybridisation experiment.

2.6. Hybridisation* and membrane regeneration

Prehybridisation*

- Transfer membranes to a 50 ml Falcon tube.
- Incubate membranes in 10 ml Church Buffer containing 0,1 mg/ml denatured salmon sperm DNA, for 2 hours at 65 °C with rotation, in a hybridisation oven. From this point, avoid at any moment drying of the membranes.

Hybridisation*

- Remove the prehybridisation solution and add 10 ml of fresh Church Buffer containing 0,1 mg/ml denatured salmon sperm DNA.
- Add the ³³P-labelled cDNA.
- Incubate overnight at 65 °C with rotation in a hybridisation oven.

Washing*

- Pour off the hybridisation solution.
- Rinse shortly with Washing Buffer.
- Refresh Washing Buffer and incubate with rotation in the hybridisation oven for 20 min. at 65 °C.
- Refresh the Washing Buffer.

Exposure*

- Place the membranes on a piece of 3MM Whatman paper that is wetted with Washing Buffer.
- Wrap the membranes in saran wrap and remove bubbles between the membrane and the foil. At this point, the success of the hybridisation can be monitored with a Geiger-Müller detector.
- Place the membranes in a cassette and expose to a phosphor screen for 30 min. to a few hours.
- Scan the screen with a STORM or PhosphorImager machine (Molecular Dynamics). The ImageQuant software generates an image file (gel).

Membrane regeneration

Membranes can be reused several times but this requires an efficient removal of the hybridised cDNA. The regeneration protocol as described above for oligo removal is insufficient in our hands for dehybridising cDNA molecules. In contrast we find that treatment with NaOH is efficient to remove most of the radioactivity.

- After exposure of membranes, immediately transfer them to a bath at 65 °C containing Array Stripper.
- Incubate 15 min., refresh the Array Stripper solution and incubate another 15 min. at 65 °C. At this point, the success of dehybridisation can be monitored with a Geiger-Müller detector.
- Change solution for Neutralisation Buffer and incubate 15 min. at room temperature. Repeat this step once.

- Change solution for Regeneration Buffer and incubate 15 min. at room temperature. Repeat this step once.
- Place the membranes on a piece of 3MM Whatman paper that is wetted with Regeneration Buffer.
- Wrap the membranes in saran wrap and remove bubbles between the membrane and the foil.
- Expose the membranes to a phosphor screen. If the radioactivity is removed to satisfaction, membranes are dried overnight between two 3MM Whatman papers and stored in a sealed bag at 4 °C.

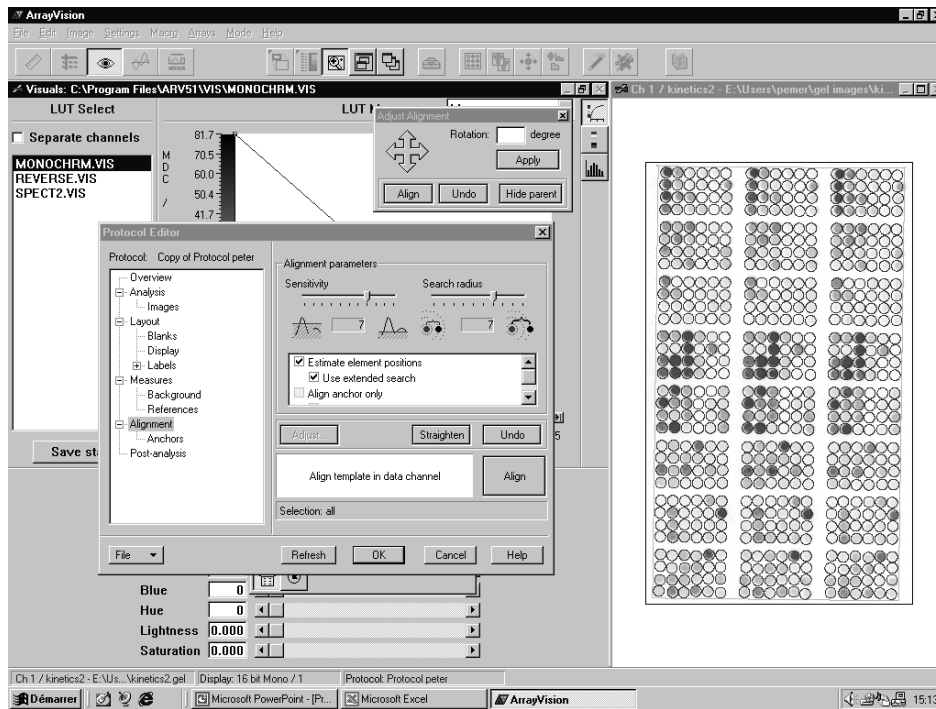
2.7. Result analysis*

After the above described “wet lab” part follows an important “dry lab” work that consists of the computer analysis of the hybridisation results and extraction of useful information from this. To make sense, at least two experiments must be included in the analysis in such a way that two or more different conditions are compared (e.g. wild type and mutant(s)) and differential mRNA levels can be detected for those genes that are affected by the perturbation(s).

The different steps included in such an analysis are quantification of the hybridisation signal for each spot on the array, normalisation of the data for each array allowing comparison of different experiments and finally visualisation of the results in a clear and easily interpretable way. For each of these steps, different options are available. A few of these will be demonstrated and taught during the practical course.

ArrayVision*

It is possible to analyse the results (i.e. the gel file from the STORM or PhosphorImager) directly with the ImageQuant software (Molecular Dynamics). However, there is software on the market that is specifically dedicated to the analysis of array data. During the course, the use of one such program, ArrayVision, will be illustrated (fig. 2). A demo-version of this program can be downloaded at the web site <http://www.imagingresearch.com.eb>.



	A	B	C	D	E	F
1	Spot labels	VOL - MDC	SD - MDC / mm2	Bkgd	sVOL	S/N
2	1 : 1 (a)	3217185,55	616320,071	15018,737	3202146,813	294,905
3	1 : 2 (a)	2360279,452	420803,557	12552,011	2347727,441	229,258
4	1 : 3 (a)	287619,2093	67949,21	11036,003	276583,206	23,885
5	1 : 4 (a)	277971,6185	74104,308	14645,992	263325,627	15,125
6	1 : 5 (a)	162317,2403	35043,509	18328,546	143988,694	7,732
7	1 : 6 (a)	69968,2101	18071,331	19030,594	50937,616	3,513
8	1 : 1 (b)	4028897,998	855878,42	21338,368	4007559,631	235,582
9	1 : 2 (b)	2845910,094	666363,046	15226,911	2830683,183	210,19
10	1 : 3 (b)	363262,1242	85142,079	14950,077	348312,047	28,619
11	1 : 4 (b)	289279,7482	89836,088	15497,365	273782,383	20,876
12	1 : 5 (b)	150593,7836	30945,665	12769,236	137824,548	11,36
13	1 : 6 (b)	81462,8838	17700,695	11463,132	69999,752	5,981
14	1 : 1 (c)	3166942,48	710937,242	17811,053	3149131,427	203,266
15	1 : 2 (c)	2084127,055	429468,286	15853,236	2068273,819	141,506
16	1 : 3 (c)	253984,7545	58350,917	12728,775	241255,979	21,132
17	1 : 4 (c)	321348,4078	80405,616	12094,472	309253,936	25,661
18	1 : 5 (c)	112336,0793	29686,291	12244,689	100091,39	7,369
19	1 : 6 (c)	75949,591	16898,207	16959,808	58989,783	3,284
20	1 : 7 (a)	3306155,677	663007,345	16356,165	3289799,512	262,464
21	1 : 8 (a)	611228,9095	106867,696	13913,768	597315,142	52,677
22	1 : 9 (a)	281658,1894	56739,937	11052,899	270605,291	25,876
23	1 : 10 (a)	276862,9259	74859,105	13275,868	263587,058	15,654
24	1 : 11 (a)	38729,9914	11896,919	17719,91	21010,081	1,183

Figure 2. ArrayVision. A gel-file from a STORM or PhosphorImager analysis of an array is imported in ArrayVision. With the “Protocol Editor”, an overlay is constructed which is first placed manually on top of the scanned image. The “Adjust Alignment” tool then allows calculation of the exact position of the grid. Finally, the program calculates the volumes, means or medians for each spot and generates an Excel output file.

The program works in two steps. First, the user defines a protocol in which a grid is constructed and the type of measurement (total volume of a spot, mean of the pixels in a spot or median value of the pixels), background correction and output format is chosen. The grid is an overlay of the array with the same dimensions and spacing of the spots. In the second step, the user places the grid manually as well as possible on the array and then allows the computer to adjust the exact position of the grid. After this, the investigator verifies the positioning of the grid and if necessary manually corrects individual spots. Since arrays never have perfectly regular shapes and suffer from slight deformations, the capacity of the program to find and calculate exact spot positions is a critical feature. Once the grid is positioned to satisfaction, the program calculates the volumes, means or medians for each spot and generates an output file. The Excel output will be used in the next step.

Excel*

In Excel, two macros will be applied to the output file of ArrayVision. The first macro will sort the data, add the annotation of the spots and calculate the mean of the 3 replicates for each spot. The second macro will normalize the data using a subset of genes present on the arrays that has been shown to have a non-differential expression over a large set of physiological conditions. This step will allow comparing results from different array hybridisations. Finally, results of all the participants will be joined in one Excel table that will be saved as a tab delimited text file.

Cluster and Tree View*

Cluster and Tree View (fig. 3) are programs developed by M. Eisen (Eisen et al., 1998) that can be downloaded from the Internet (<http://rana.stanford.edu/software/>). The first program uses as input a table generated by Excel and allows gene clustering according to similar expression behaviour over the different experiments included in the analysis. And similarly, experiments can be clustered according to the resemblance of the corresponding transcriptomes. Tree View is a program that uses the output file of Cluster and generates a colour-coded visual interpretation of the clustering results (figure 3).

Epclust*

Alternatives to Cluster and Treeview are available on the Internet. One possibility is provided at <http://ep.ebi.ac.uk/EPCLUST/>. Array measurements are sent to this server and clustered results are returned.

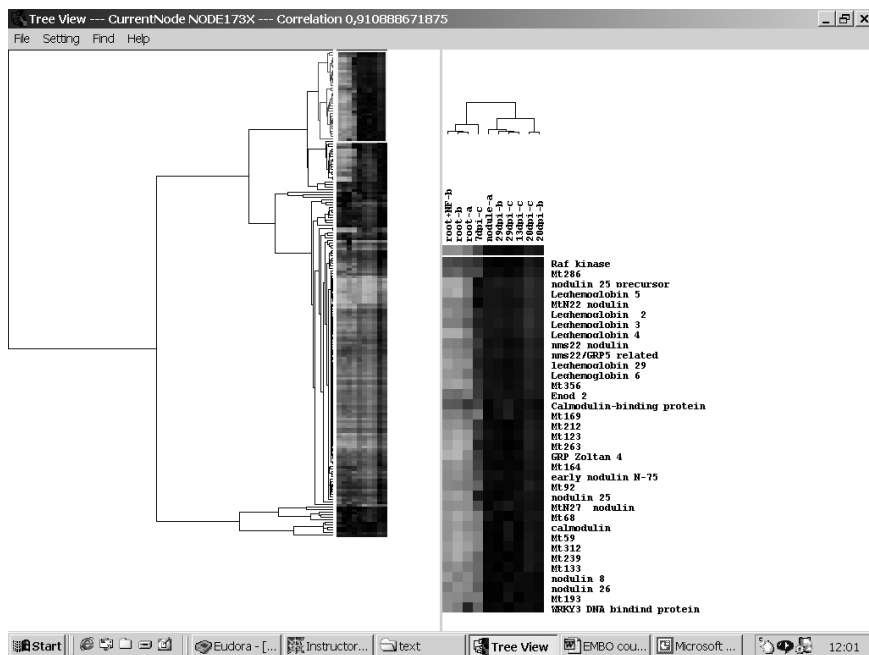
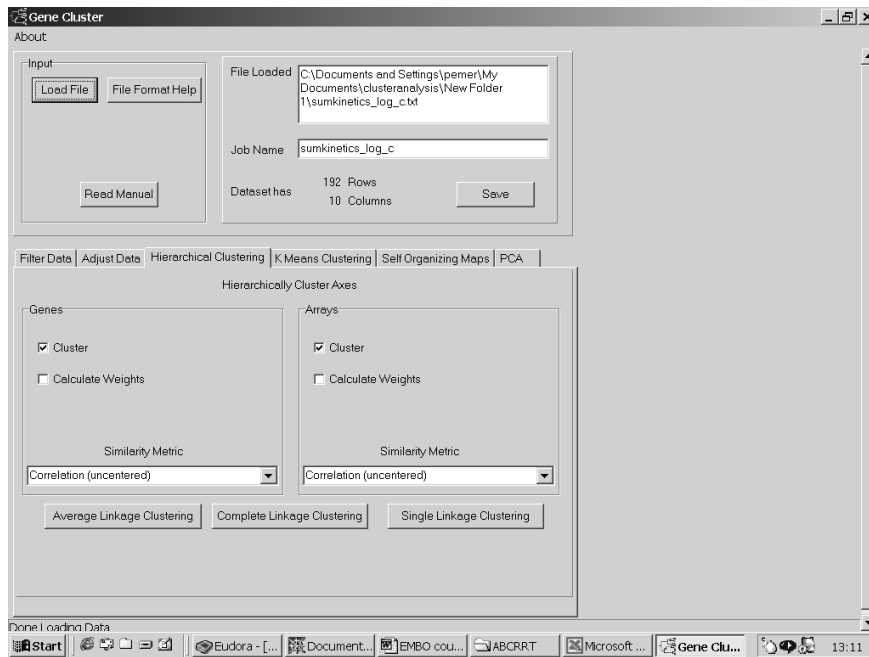


Figure 3. Cluster and Tree View. Data from different array hybridisations are introduced in the form of a tab-delimited table in Cluster. This program can use different algorithms to calculate similarities and cluster the genes and experiments. The Tree View program generates dendrograms and a colour-coded visual interpretation of the clustering results.

2.8. Solutions

Oligos

5' pADGal4 vector oligo : ATT CGA TGA TGA AGA TAC CCC
3' pADGal4 vector oligo : GTA ATA CGA CTC ACT ATA GGG C
13-mer oligo : GAG TCG ACC CGG G

Solution D

4 M guanidium thiocyanide
25 mM Na-citrate
0,5 % Na-sarcosyl
0,1 M β -mercaptoethanol (added freshly)

10x Proteinase K Buffer

100 mM TrisHCl
10 mM EDTA
5 % SDS
pH 7,4

Proteinase K stock solution

20 mg/ml Proteinase K in 1x Proteinase K Buffer

For a new stock, incubate the solution 30 min. at 50 °C to digest any contaminating RNase activity.

DEPC treated water

1 ml DEPC per litre of water
Stir overnight and autoclave.

Church Buffer

0,5 M sodium phosphate
7 % SDS
1 mM EDTA
pH 7,2
Heat to 65 °C and filter before use

Regeneration Buffer

5 mM sodium phosphate
0,1 % SDS
pH 7,2

Washing Buffer

40 mM sodium phosphate
0,1 % SDS
pH 7,2

SSARC Buffer

600 mM NaCl
60 mM sodium citrate
7,2 % Na-sarcosyl

Array Stripper

0,4 M NaOH
0,1 % SDS

20x SSC

3 M NaCl
0,3 M Na-citrate
pH 7,0

Neutralisation Buffer

0,2 M TrisHCl
0,1 % SDS
0,1x SSC
pH 8,0

STE Buffer

0,1 M NaCl
10 mM TrisHCl
1 mM EDTA
pH 8,0

20x SSPE

3 M NaCl
0,2 M Na-Phosphate
20 mM EDTA
pH 7,4

2.9. Demonstration of the microarray facilities at the “Transcriptome Platform”*

The Gif/Orsay DNA microarray platform on the CNRS campus in Gif-sur-Yvette is a voluntary effort to pool expertise, materials and knowledge and to coordinate the activities of two important campuses in the life sciences domain, those of Gif-sur-Yvette (CNRS) and Orsay (the University of Paris XI and the Curie Institute). As a whole, the program federates about 28 research groups composed of 56 scientists, 78 technical assistants, 13 postdocs and 20 doctoral students. The platform is a common service facility (240 square meters), easily accessible on the CNRS campus in Gif-sur-Yvette, in which all the equipment and know-how is found. The platform is implanted in an exceptional technological environment that includes, in particular, a service for imagery and cell sorting on the campus in Gif.

The platform is equipped with 1) a Genomic Solutions Gentac3 Workstation equipped with an autoloader capable of producing microarrays on glass slides as well as doing colony picking, library replication and library management ; 2) an automated hybridisation station capable of processing 12 slides simultaneously ; 3) two fluorescent scanners, one with 4 lasers and the other with 2 lasers ; 4) a liquid handling robot ; 5) several informatics stations for data analysis.

Students will be shown briefly how glass microarrays are typically prepared, hybridised, scanned and analysed on the platform. Time will be allowed for questions.

3. REFERENCES

- Bertucci, F., Beranrd, K., Loriod, B., Chang, Y-C., Granjeaud, S., Birnbaum, D., Ntuyen, C., Peck, K., and Jordan, B. (1999) Sensitivity issues in DNA array-based expression measurements and performance of nylon microarrays for small samples. *Hum. Mol. Genet.*, **8** : 1715-1722.
- Eisen, M.B., and Brown, P.O. (1999) DNA arrays for analysis of gene expression. *Methods Enzymol.* **303** : 179-205.
- Eisen, M.B., Spellman, P.T. Brown, P.O., and Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* **95** : 14863-14868.
- Györgyey, J., Vaubert, D., Jiménez-Zurdo, J.I., Charon, C., Troussard, L., Kondorosi, A., and Kondorosi, E. (2000) Analysis of Medicago truncatula nodule expressed sequence tags. *Mol. Plant-Microbe Interact.* **13** : 62-71.
- Lockhart, D.J., and Winzeler, E.A. (2000) Genomics, gene expression and DNA arrays. *Nature* **405** : 827-836.

4. TIME SCHEDULE

First Day–3 hours (Tuesday 27 November 2001)

Prehybridisation (2.6)

Reverse transcription (2.5)

Purification and determination of label incorporation (2.5)

RNA hydrolysis (2.5)

Hybridisation (2.6)

Second Day–2 hours (Wednesday 28 November 2001)

Washing (2.6)

Exposure (2.6)

Third Day-5 hours (Thursday 29 November 2001)

Data analysis (2.7)

Demonstration of the microarray facilities (2.9)