preparation of Microarrays for the Global Analysis of Differential Gene Expression

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Introduction

At the MRC Toxicology Unit, University of Leicester, we are interested in the altered gene expression between biological systems that are responsible for conferring differential sensitivity (or conversely resistance) to xenobiotics. Such differential gene expression can occur, for example, in cells that are resistant to chemotherapeutic agents, or between species and strains of organisms that show differential sensitivity to environmental contaminants. An understanding of the fundamental genetic basis for differential xenobiotic sensitivity between strains would allow much greater predictive ability to be gained from *in vivo* and *in vitro* toxicology testing as well as being fundamentally interesting to the mechanistic toxicologist. An understanding of the basis of chemotherapeutic resistance in cancer cells would allow for a more targeted approach to chemotherapeutic treatment to be adopted.

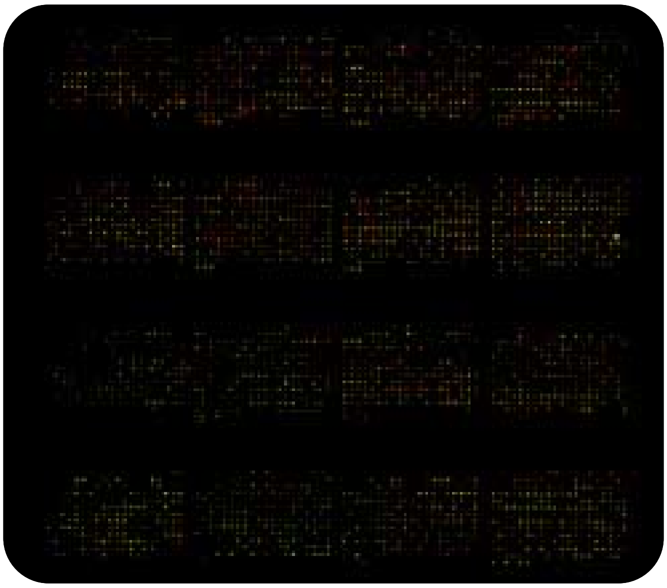
In order to explore these questions we have set up a microarray system which is based around a home built Stanford type arrayer with scanning resource provided by an Axon GenePix 4000A scanner running GenePix 3.0 as the image acquisition and interrogation software. To data output from this image capture and interrogation program we apply various downstream analyses.

Full details of our program and the equipment in use can be found at:
www.le.ac.uk /cmht/twg1/array-fp.html.

Materials

EST clones are obtained from the I.M.A.G.E. collections held at HGMP (Cambridge), and all are sequenced to verify identity. ESTs from the I.M.A.G.E. collection are in a number of different vectors but there are five that predominate; pBluescript® SK-, one of the pSPORT family, lafmid BA, pME18S-FL3, and pT7T3-pac. Of these the pT7T3-pac is the most common.

Figure 1:
Image results of a microarray produced using our home built Stanford type arrayer.



We have chosen to produce our own microarrays over buying them from commercial array manufacturers for a number of reasons listed below:

1. *Cost.* Commercial microarrays are expensive, and though at the initial stages of a project it is easy to think that the amount of data from one microarray is so large that you are never likely to want to run another, in fact the adage of 'one is never enough' applies. Currently our lab uses some 20–30 a week of 4–6,000 gene microarrays.

2. *Control over clones*. By having our own system we can choose the clones that we want to put on it, and the format in which they are placed. This allows us to mix EST's for named and unnamed genes, put down characterised collections or un-sequenced libraries depending on our need.

3. *Integrity, reproducibility and format*. The high throughput nature of the facility allows us to produce replicate sample arrays and control the quality and format of the arrays. These factors allow us to have a very high confidence in our data, and the consistent format is allowing the formation of a database of microarray data.

Insights 12 (2001)

Some of the clones we use have been obtained through Research Genetics (ResGen™). These clones are also drawn from the I.M.A.G.E. collections but are selected and sequence-verified prior to distribution.

Methods

Preparation and PCR of the EST clones

PCR of the EST insert is carried out using ABgene® PCR Master Mix* either directly from the bacteria or from the purified plasmid. In general, it is preferable to PCR directly from the bacteria, but in some cases where the I.M.A.G.E. clones are older this does not work well and a plasmid mini-prep has to be made. For PCR carried out directly from bacteria, an overnight culture is grown in a 96-well 2ml volume plate. From each culture, 2µl of medium is then removed and placed in an ABgene® Thermo-Fast® 96 PCR Plate. To this is added 80µl of master mix that contains either universal or plasmid-specific primers. As a general rule it is preferable to use plasmid-specific primers. PCR is then carried out for 30 cycles using a 94°C denaturing step and 72°C extension temperature. The temperature of the annealing step is varied according to the primers in use. The same conditions are used for PCR reactions from either bacteria or plasmids. These reaction conditions include a five-minute initial 94°C step, which is sufficient to lyse the bacteria.

Following PCR, an aliquot is withdrawn from the reaction, loaded directly onto a 96-well format 1% agarose gel. The ReddyMix™ format of the master mix means that there is no need to add gel loading buffer to the reaction. Ethidium bromide for DNA visualisation is contained within the gel itself and 1x TBE is used as the running buffer. Following electrophoresis, the gel images are electronically stored and analysed for PCR failures and double bands indicating mixed clones or mis-priming.

After PCR, the products are precipitated using isopropanol and stored dry until required for the arrayer, at which point they are re-suspended in 30µl of 3x SSC and aliquoted into 384-well plates. Once re-suspended, the clone solutions are stored at -20°C between print runs.

Results

Images of four gels are shown constituting different clone sets and PCR products from both bacterial culture and plasmid mini-preps. As shown in Figure 2, there are fewer successes in the library clones set, which is typical of clones from an uncontrolled source. As different clones are being PCR amplified in a 96-well format there will inevitably be some compromise in the conditions being utilised. This is particularly true where universal primers have to be employed. Amongst the I.M.A.G.E. clones we also observe about a 2% failure due to mixed clones. Mixed clones appear in the PCR gel as 2 bands of equal intensity that have clearly come from a mix of clones in the collection. A lot of these apparent failures are in fact short EST products.

Discussion and conclusions

As a general rule we find that PCR from the I.M.A.G.E. mouse clones will work well from the bacteria directly but that we have to make mini-preps from the human clones. This probably has much to do with the age of bacteria in the collection, and additionally with the number of times that the bacteria have been accessed. The success rate of the PCR for the EST clones is very dependent on the clone source. A lower success rate is observed from clones obtained directly from libraries where there has been no selection, whereas a high success rate (95–98%) is seen in sequence-verified and selected I.M.A.G.E. clone sets. PCR deficiency can be due to a number of factors, but is most commonly due to either long EST inserts or high GC content. Use of the master mix greatly decreases the possibility of unsuccessful PCR due to reagent failure or mistakes in the reaction set-up.

ABgene® PCR Master Mix* has the advantage of being ready to go. A set of four 96-well PCR plates can be set up and running in 10–15 minutes, so high throughput amplification is possible with minimal labour. The master mix format also provides excellent reproducibility and consistency of results.

Acknowledgements

I would like to thank Joan Riley and Reginald Davies for their help in carrying out the gel electrophoresis, and David Judah for the microarray printing.

Figure 2: Products from a mouse embryo library in pSPORT. These are unverified clones and there is a lower success rate than is obtained with selected clones.

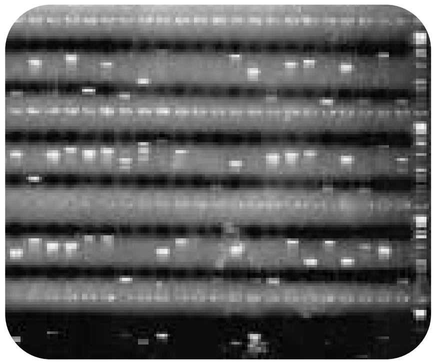
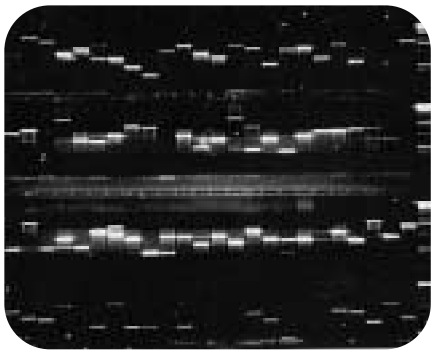
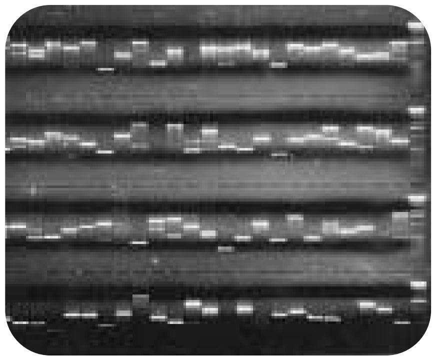
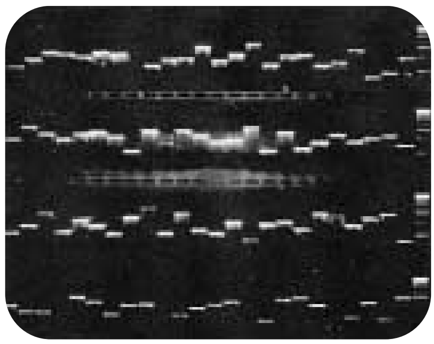


Figure 3: Human PCR products from I.M.A.G.E. clones, selected and sequence verified. These clones are in mixed vectors and a universal primer set has been utilised.



Figures 4 and 5: Mouse EST I.M.A.G.E. PCR products. These are selected and sequence verified I.M.A.G.E. clones that have been amplified using plasmid-specific primers.





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Please see page 6 for product details.