

DNA Microarrays: Application to Personal Health Care and Cosmetic Industries

Authors: Robert Holtz, William Vitz, BioInnovation Laboratories Inc, Texas, USA

Abstract

While DNA microarrays have been widely used in many areas of research, their application to screening materials for use in the Personal Health Care and Cosmetic industries is relatively new. This article is intended to provide an overview of the DNA microarray procedure to those newly acquainted with the technique and also to provide items of consideration to aid in designing an array-based experiment. The advantages of using DNA microarrays to screen active ingredients for biological effects and the limitations of arrays will also be discussed.

Introduction

Skin care research is continually taking advantage of advanced technologies as they become available. As Personal Health Care and Cosmetic companies strive to screen materials for beneficial biological effects which can be marketable, the use of new technological advances in human skin tissue equivalents and *in vitro* testing methodologies has been a great asset in the screening process. In recent years, technological advances have produced another highly valuable tool for skin care research, the DNA microarray.

DNA microarrays are extremely powerful tools that allow users to analyze changes in gene expression by monitoring changes in the mRNA of hundreds to thousands of genes in a single experiment. All cells function by using their genes to make protein products. This process starts by making an mRNA copy of the gene through a process called transcription. The mRNA copy is then translated into a protein that plays a functional role within the cell or the cell's environment. Since the process of gene expression is highly regulated, the amount of mRNA can be a good indicator of the level of activity for a specific gene. For many years gene expression research was limited to studying a handful of genes in a single experiment through a process called Northern Blotting. However, with the introduction of DNA

microarrays skin care researchers can now rapidly obtain a much more global view of what is happening inside of a cell since the results of one or two array experiments can potentially generate data on changes in gene expression across the entire known human genome.

Microarrays come in many forms, however the most popular form is comprised of glass slides which have been spotted with a large number of DNA fragments called *features*. Each feature contains a nucleotide sequence that corresponds to a single specific gene. With the DNA chips that we use, each feature on the chip is approximately 120 μm in diameter, or roughly the size of a single human cell. This small feature size is what allows for thousands of different gene sequences to be printed in an array pattern on the DNA microarray chip. Within our laboratory DNA microarrays are most commonly used to compare cells or human skin tissue equivalents that have been treated with an active ingredient to untreated cells or tissues.

Overview of DNA Microarray Procedure

The first step in the microarray process is to decide which cell or tissue type to use as the model. For example, if one wishes to look at the effect of a material on keratinocyte gene expression then one can use a keratinocyte cell culture model (keratinocytes grown in a monolayer), a simple keratinocyte tissue equivalent (keratinocytes grown in a three dimensional tissue model), a full thickness tissue equivalent (keratinocytes and fibroblasts grown in a three dimensional model), or a pigmented tissue equivalent (keratinocytes and melanocytes grown in a three dimensional model). In the first two examples, the microarray will show only the changes in keratinocyte genes. In the latter two examples, the microarray will show the combined changes that occur in keratinocytes and fibroblasts or keratinocytes and melanocytes, respectively. It should be noted in the latter two examples that if the tissues are processed as a whole it is not possible to detect changes in only one of the two cell types.



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The microarray will only show the combined changes in gene expression for both cell types in the tissue. This would not pose a problem, for example, if one wanted to look at a gene that was more or less specifically expressed in one cell type (i.e. keratin for keratinocytes or tyrosinase for melanocytes). However, if one wanted to look at changes in genes common to both cell types in the tissue (superoxide dismutase, or glutathione peroxidase for example) then it would not be possible to partition the result from the array analysis to one cell type or the other. In addition, when using tissue equivalents composed of more than one cell type, the mRNA from the predominant cell type can dilute the mRNA in the secondary cell type. For example, in full thickness tissue equivalents the keratinocytes in the epidermal layer greatly outnumber the fibroblasts in the dermal layer. As a result, it can sometimes be difficult to detect changes in certain fibroblasts genes since the fibroblast mRNA is greatly diluted by the keratinocyte mRNA. Thus, there are important considerations to keep in mind when deciding upon the model to use for the screening procedure.

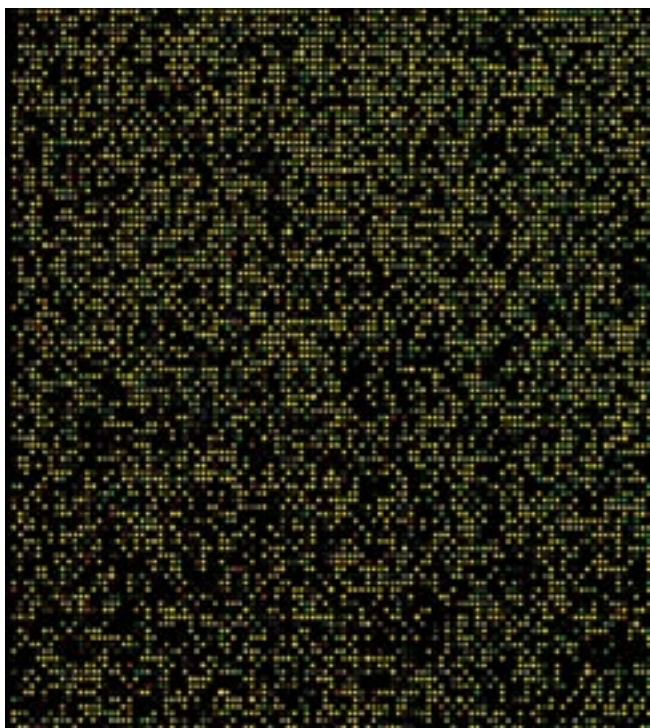


Figure 1. DNA microarray. This is a scanned image of a DNA microarray which contains over 22,000 features. Since each feature denotes a different human gene then this single array chip can generate data on a significant portion of the known human genome. Each feature is approximately 120 μm in diameter, while the approximate dimensions of the array itself are roughly 1.5 cm by 4.5 cm.

After deciding upon a model, the next phase is the treatment phase. Typically, when a material stimulates a change in gene expression the changes in the mRNA can be observed within 6-24 hours. Some materials may require longer treatment times when used on tissue equivalents, especially if the material is intended to exert an effect on the fibroblasts in full thickness tissue models. However, normally a 24 hour treatment of the tissues is sufficient to get an idea of what the material is doing. After the treatment, the cells need to be broken open and the RNA must be recovered. RNA is extremely susceptible to degradation by RNases, and so the cell/tissue lysis step is commonly performed in the presence of guanidinium salts and other potent RNase inhibitors. After the cells have been broken open, the cell lysate is mixed with ethanol. This will allow small nucleic acids, such as RNA, to bind to glass fibres enabling the RNA to be separated from the rest of the cellular components through the use of special glass fibre filter cartridges.

After purifying the RNA, mRNA must be isolated from the total RNA, and then the mRNA must be converted into antisense RNA (aRNA) so that it can bind to the nucleotide sequences spotted on the DNA microarray. The RNA purification process will isolate all of the different types of RNA from the cell, which includes ribosomal RNA (rRNA), messenger RNA (mRNA) and transfer RNA (tRNA). rRNA is the most abundant source of RNA, making up 80-90% of the RNA in the cell and this type of RNA is the dominate feature observed on RNA gels using total RNA. The processes of isolating the mRNA from this mix and converting it to aRNA are intertwined. The first step takes advantage of the poly A tail on mRNA. A short oligonucleotide containing a small region of poly T residues is added, which binds to the poly A tail on the mRNA only, and serves as a primer for a reverse transcriptase enzyme. The reverse transcriptase enzyme will then generate a complementary cDNA chain to the mRNA, forming a cDNA/mRNA heteroduplex strand. The next step is to add a strong RNase, which will degrade all of the RNA (rRNA, mRNA and tRNA), but leave the cDNA copy of the mRNA intact. At the same time a DNA Polymerase is added. As the RNase is removing the mRNA in the cDNA/mRNA heteroduplex, the remaining mRNA serves as a template for the DNA Polymerase allowing the mRNA to be replaced by DNA. The result is a double stranded DNA copy of the mRNA, with the entire original RNA destroyed by the RNase. It should be noted that the short oligonucleotide with the poly T region used as the initial primer for cDNA synthesis also contains a bacterial T7 promoter



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sequence. After the cDNA double strand is generated, adding a bacterial T7 RNA Polymerase and various transcription enzymes can generate aRNA. The *in vitro* aRNA transcription will generate multiple aRNA copies of the original mRNA which amplifies the amount of mRNA.

After the aRNA is generated, it is labelled with a fluorescent probe. Typically, our laboratory uses Cy3 (green fluorescent) and Cy5 (red fluorescent) as the labels. For most array experiments, aRNA derived from cells or tissues treated with the active ingredient of interest is fluorescently labelled (i.e. red) and combined with aRNA from untreated cells or tissues that have also been fluorescently labelled (i.e. green). The combined aRNA are then mixed with a hybridization solution and applied to the microarray. The aRNA and the microarray are allowed to hybridize overnight at a high temperature. The combination of the hybridization solution and the high temperature promotes conditions of very high stringency. This allows only those aRNA and nucleotide sequences on the DNA microarray to interact and bind via Watson-Crick base pairs that share exact complementary base sequences. After hybridization, the microarray is washed to remove any unbound aRNA probe and then scanned with a microarray scanner.

The scanning procedure is a two-part process. The first step is to scan the array and measure the amount of labelled aRNA from the treated cells or tissues (i.e. red labeled) that is bound to each feature. To do this, the scanner uses a laser to excite the fluorescent label and then measures the emitted fluorescent signal for each feature. Multiple, non-overlapping measurements are made in 10 μm blocks of each feature and then the median fluorescent intensity for each feature is calculated. The second step is to then repeat the measurement procedure for the labelled aRNA from the untreated cells or tissues (i.e. green labelled). We typically report results as a ratio of median fluorescent intensity for the treated group (red) to the untreated group (green). If a treatment does not exert an effect on a gene then the median fluorescent intensity of the feature corresponding to that specific gene will be the same for both the scan from the red laser and the scan from the green laser. This would result in a ratio of 1. If a treatment increases the expression of a gene, then the median intensity of the feature would be greater in the scan from the red laser than the green, resulting in a ratio that is greater than 1. A ratio that is less than one would, therefore, indicate that the treatment reduced the expression of a gene. Our laboratory

typically uses a ratio of greater than or equal to 1.3 as the cut-off for upregulated genes and a ratio of less than or equal to 0.7 as the cut-off for downregulated genes. These broader ranges are a more conservative judging criterion and account for possible sources of variation in fluorescence intensity that are not associated with the treatment.

Benefits of DNA Microarrays for Cosmetic and Personal Health Care

Microarrays are unique in terms of the scope of data they provide. For example, when cultured fibroblasts are used as the model, the arrays can provide data on changes in most of the targets that are of interest in the Personal Health Care and Cosmetics industries. Changes in extracellular matrix proteins such as the collagens (all known isoforms), elastin, fibronectin, the fibulins the fibrillins (again, all known isoforms) can all be easily determined within the same experiment. In addition, the same experiment can provide data on the changes in the enzymes

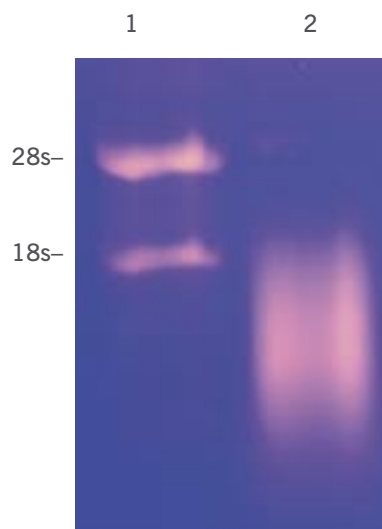


Figure 2. RNA samples. Lane one displays a sample of total RNA while lane two display a sample of aRNA generated by the *in vitro* transcription reaction. The two samples were resolved on a 1.5% agarose gel and the RNA was stained with ethidium bromide and visualized under UV light. The total RNA sample in lane one is dominated by two distinct bands of ribosomal RNA, the 28s and 18s bands. In contrast, the aRNA sample exhibits a broad staining pattern representing strands of aRNA that vary greatly in length. As part of the process that generates the aRNA, the ribosomal RNA has been removed from the sample as evidenced by the absence of the 28s and 18s bands.



responsible for remodelling the ECM as well, such as the matrix metalloproteinases (all known isoforms) and other skin proteases. Due to these global types of measurements on genomic changes, DNA microarray based experiments allow for the observation of more complex interactions between skin components. The data can not only show if an active ingredient increases Type 1 α 1 collagen expression but also if there is an accompanying increase in Type 1 α 2 collagen as well. In addition, the data can show if there are corresponding alterations in the mechanisms involved in collagen breakdown as reflected, for example, by changes in the expression of MMP-1 and TIMP-1.

With respect to the fibroblast example given above, the data would not be limited to just collagen metabolism since the DNA microarray would also present data on the pathways of synthesis and degradation for other ECM components as well. This is very beneficial since observations from our laboratory have shown that active ingredients can be very selective for the ECM components they influence. While some active ingredients can promote changes in all of the major ECM proteins, others may affect change in only a few components. And while materials that affect only a few ECM components may be better suited for use in products since their specificity may better target marketable areas of skin care and reduce potential side effects, these specific effects are more likely to be missed in non-DNA microarray-based screening processes. This is especially true of novel compounds whose beneficial effects have not yet been fully characterized. If the novel compound is screened solely on the basis of its ability to promote elastin synthesis using an ELISA-based method, then any other effects the material has will not be measured and a potentially marketable effect could be missed.

In addition to providing information on how active ingredients affect systems of immediate interest, the data from microarray experiments also provides a valuable library that catalogs in detail the effects of a specific material which can be reviewed at a future time. For example, while a company may immediately review the results of a DNA microarray experiment performed on epidermal keratinocytes for changes in markers of proliferation and differentiation, the array data will also contain information on other aspects of keratinocyte function that may be important in future products. The array will also have data on enzymes involved in lipid metabolism which can be a factor in barrier function, DNA repair enzymes which can be factor in ageing, and antioxidant enzymes which can be a factor in combating UV and ozone induced damage.

Aside from proteins of known function, DNA microarrays also contain gene sequences for proteins that currently do not have a characterized function. While their function is unknown, many of these uncharacterized proteins are expressed in the various cells that make up human skin. As the functions of these unknown proteins are elucidated, or even as the functions of known proteins are expanded to show new and important roles

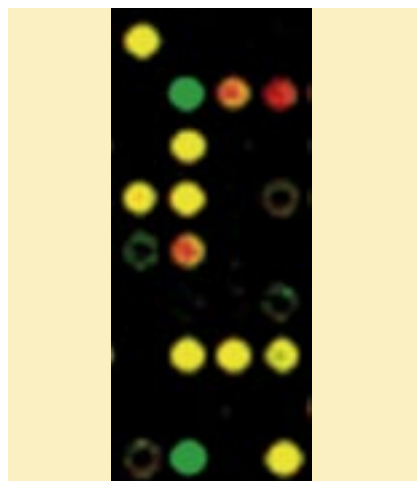


Figure 3. Detailed view of the features on a DNA microarray. The computer will use the fluorescence intensity measurements obtained during the scanning process to generate a digital image of the DNA microarray. A separate image is created for each of the two lasers used to excite the fluorescently labeled aRNA, and then the two images are overlaid to create the images shown above and in Figure 1. If the intensity measurements of a feature are the same for both the scan from the red laser and the scan from the green laser, then when the two scans are overlaid the feature will have a yellow colour. The yellow colour would correspond to a median fluorescent intensity ratio of 1.0 and would indicate that the treatment did not alter the expression of that particular gene. If the intensity measurement of a feature is greater in the scan from the red laser than the scan from the green laser then the feature will take on more of a red colour. This would correspond to a median fluorescent intensity ratio that is greater than 1.0 and would indicate that the treatment is increasing the expression of the gene that the feature represents. If a treatment reduces the expression of a gene then the opposite is true. The median fluorescent intensity measurement from the green laser would be greater giving the feature more of a green colour on the overlay image. This would correspond to a median fluorescent intensity measurement that is less than 1.0. This figure also shows that not all of the genes represented on the DNA microarray are expressed in a given cell type as evidenced by the dark areas that do not contain fluorescently labelled features.

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in skin biology, a company that has used DNA microarrays to characterize the effect of an active ingredient would already have data on how their active ingredient affects these newly characterized genes. Again, this reflects the beneficial effect of the library of data the DNA microarray provides.

Finally, in addition to the information on active ingredients and raw materials that the DNA microarray can provide, the arrays can be used to better characterize the models used in skin care testing as well. Data from the gene expression profiles of full thickness tissues or pigmented tissues allows skin care researchers to see how well these *in vitro* models relate to real human skin. The array data also allows the verification that a gene of interest is expressed in a certain model before running an experiment. In addition, array data from cells or tissues exposed to harmful stimuli, such as UV irradiation, ozone or hypoxia allows researchers to better understand the genetic changes these stimuli can provoke. Alternatively, the effects of ageing on the skin can be further explained by comparing skin cells from young and old individuals to see how ageing related changes are expressed on a genetic level. Once these genetic changes are further characterized, then it is easier to screen active ingredients to combat these changes.

Limitations of DNA Microarrays

The results of a DNA microarray reflect changes that occur at the mRNA level. Whilst for the most part the changes in mRNA are reflected at the protein level there are some exceptions. Since the regulation of gene expression occurs at both the level of transcription and the level of translation, it may sometimes be necessary to confirm DNA microarray observations by measuring changes in the protein of interest with Western Blotting ELISA-based techniques.

Additionally, whilst the results of an array can reflect changes in the amount of the protein products of a gene, the array can not directly show changes in a protein's activity. This is especially relevant to enzymes. For example, materials that inhibit elastase activity may not produce changes that are detectable by a DNA microarray. On the other extreme, materials that activate enzyme activity may not produce changes that are detectable by an array. For example, materials that increase mitochondrial respiration will induce changes in multiple enzymes; however there may be little change in the global pattern of gene expression.

Another important limitation of DNA microarrays is a factor that influences all experiments: timing of the measurement. The data

from the array represents a snapshot of what is occurring within the cell at the moment the RNA is collected. Materials tested may produce changes that are very rapid in onset and yet very transient in duration. If a gene response occurs within 6 hours of a treatment and is over by 10 hours, an array conducted at 24 hours post treatment will miss these effects.

Conclusion

DNA microarrays are powerful tools and are well suited for screening new and existing active ingredients for Personal Health Care and Cosmetic applications. The results generated by a well designed DNA microarray-based screening process can provide detailed information regarding the capabilities of an active ingredient, including information on biological effects which could be overlooked by other screening procedures. It is this depth and scope of information that makes the DNA microarray a valuable method for researching the marketing potential of an active ingredient.

Authors' Biographies

Robert Holtz earned his B.S. from Colorado State University and his M.S. from Texas Christian University. After completion of his M.S. he joined a laboratory at the University of Houston researching the process by which cardiac myocytes became less responsive to stress with age and how this loss of response impacted the normal gene expression profile of the heart and lead to heart failure. Robert is currently the Director of Laboratory Studies at BioInnovation Laboratories, a testing laboratory in McKinney, Texas, where his background in cell culture, ageing and gene expression research has facilitated his company in offering the powerful technology of DNA microarrays and other innovative methods to skin research in the Cosmetic and Personal Health Care Industries.

William Hamilton Vitz: BioInnovation Laboratories was founded in McKinney, Texas by attorney William Hamilton Vitz. Bill earned his bachelor's degree from Texas Tech University and his Juris Doctorate degree from South Texas College of Law. His law career has included the position of Criminal Prosecutor as well as having acted as General Counsel for Collin County Memorial Hospital, National Banks and various Insurance Companies. In addition to handling the business aspects of BioInnovation Laboratories, he has maintained a private law practice for over 35 years. He is also very active in his local community and is a member of the McKinney City Council.

