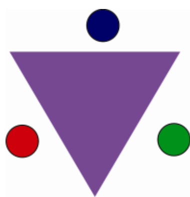

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MICROARRAY DETECTION OF MICROBIAL PATHOGENS AND THEIR EXPRESSION PROFILES

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ABSTRACT

Early diagnosis is crucial for disease treatment and control as it reduces the inappropriate use for antimicrobial therapy and surveillance activity. This requires the ability to detect and accurately diagnose infection at or close to the source/outbreak with minimum delay the need for specific, approachable point-of-care diagnosis able of selective between Causative Pathogens and their subtypes. Nothing of the available Pathogen diagnostic assays combines a point-of-care format with the complex capability to identify a large repertoire of human and. The ability of the microarray to identify divergent Pathogens is based on a balance between the stringency of the hybridization conditions and the signal of the virus relative to background noise on the array. Although Microbial microarrays can test for all Pathogens simultaneously, the requirement for nonspecific amplification by random priming reduces their analytical sensitivity, as compared with assays that detect a single or few agents at one time. This review discusses the applications of microarray technology has turn out to be a shows potential new tool for the detection and identification of Microbial pathogens in human Serum, plasma and cell cultures.

Key words: Microarray, Microbial Pathogen, infectious diseases, diagnosis.

INTRODUCTION

The rising accessibility of rapid and responsive nucleic acid testing assays for Microbial diseases will modernize the practice of medicine by gradually reducing the need for standard culture-based Microbiological methods that take time [1]. Molecular theranostics in Microbial diseases is a promising conception in which molecular biology tools are used to provide rapid and accurate diagnostic assays to enable improved initial management of patients and more efficient use of antimicrobial [2]. It is a multiplex technique used in combination of bioinformatics and statistical data analysis [3]. With the huge number of tests accessible for the finding of Microbial agents affecting humans, it is commonly not predictable that the majority of these tests are indirect. For infections caused by agents, commonly used tests determine the presence of specific antibodies produced by the patient's immune system in response to infectious agent [4]. These tests are useful for blood screening and in a limited extent as diagnostic tools, but, because they offer only an indirect measure of infection, they do not tell the clinicians whether the infection is past or current, or if there is a response to the therapy [5]. An antibody based test can also miss a recent infection, because generally it may take several

days to weeks for the immune system to mount an antibody response to the infectious agent[6].

Microarrays can be used for the identification of a defined set of known Pathogens using specific or random PCR primers, the resequencing of known Pathogens to identify sequence variants, or for the discovery of novel Pathogens using Microbial microarrays [7]. Microbial microarrays attempt to represent all known Pathogens using tens of thousands of oligonucleotide probes [8]. Known Pathogens can be detected on the microarray, as well as novel Pathogens with at least some degree of relatedness to known Pathogens [9]. Although Microbial microarrays can test for all Pathogens simultaneously, the requirement for nonspecific amplification by random priming reduces their analytical sensitivity, as compared with assays that detect a single or few agents at one time [10].

Microarray:

Biomedical research evolves and advances not only through the compilation of knowledge but also through the development of new technologies [11]. Using traditional methods to assay gene expression, researchers were able to survey a relatively small number of genes at a time [12]. Microarray technology aims to monitor the whole genome on a single chip so that researchers can have a better picture of the interactions among thousands of genes simultaneously[14]. They represent a major methodological advance and illustrate how the advent of new technologies provides powerful tools for researchers 15. Scientists are using microarray technology to try to understand fundamental aspects of growth and development as well as to explore the underlying genetic causes of many human diseases [16].

Microarrrinciples

Microarrays are typically composed of DNA “probes” that are bound to a solid substrate such as glass (Figure 1). Each spot (50 to 150 μm) in the array lattice is composed of many identical probes that are complementary to the gene of interest [17]. During hybridization DNA “targets” diffuse passively across the glass surface, when sequences complementary to a probe will anneal and form a DNA duplex [18]. Hybridized targets can then be detected using one of many reporter molecule systems [19]. In essence, a microarray is a reverse dotblot that employs the same principles of hybridization and detection used for many years with membrane-bound nucleic acids (e.g. Southern and Northern blots) [20].

Microbial source and nucleic acid isolation

Microbial samples are purified from whole blood, plasma, and serum throat swabs, cerebral spinal fluid, amniotic fluid, Urine, Stool, Genital tract, Throat, Microbial culture supernatants, Virus-infected supernatants and other cell-free body fluids [21-23].

Primer design, Probes and array for confirming the identity of Microbial strains

Most critical parameter for successful PCR is the design of Primers. All things being equal, a poorly designed primer can result in a PCR reaction that will not work .The primer sequence determines several things such as the length of the product, its melting temperature and ultimately the yield . A poorly designed primer can result in little or no product due to non-specific amplification and/or primer-dimmer formation, which can become competitive enough to suppress product formation[24-25]. This application note is provided to give rules that should be taken into account when designing primers for PCR. More comprehensive coverage of this subject can be found elsewhere [26-28]. Several variables must be taken into account when designing PCR Primers. Among the most critical are: Primer length, Melting Temperature (Tm), Specificity, Complementary Primer

Sequences, G/C content and Polypyrimidine (T, C) or polypurine (A, G) stretches ,3'-end Sequence [29].

Sample amplification and labeling

The C-DNA from the cells in two different conditions is extracted and labeled with two different fluorescent labels: for example a green dye (Cyanine 3) for cells at condition 1 and a red dye (Cyanine 5) for cells at condition 2 (to be more accurate, the labeling is typically done by synthesising single stranded DNAs that are complementary to the extracted mRNA by a enzyme called reverse transcriptase). Both extracts are washed over the microarray. Labeled gene products from the extracts hybridize to their complementary sequences in the spots due to the preferential binding - complementary single stranded nucleic acid sequences tend to attract to each other and the longer the complementary parts, the stronger the attraction [30].

Microarray hybridization

The core principle behind microarrays is hybridization between two DNA strands, the property of complementary nucleic acid sequences to specifically pair with each other by forming hydrogen bonds between complementary nucleotide base pairs. A high number of complementary base pairs in a nucleotide sequence mean tighter non-covalent bonding between the two strands. After washing off of non-specific bonding sequences, only strongly paired strands will remain hybridized [31].So fluorescently labeled target sequences that bind to a probe sequence generate a signal that depends on the strength of the hybridization determined by the number of paired bases, the hybridization conditions (such as temperature), and washing after hybridization . Total strength of the signal, from a spot (feature), depends upon the amount of target sample binding to the probes present on that spot. Microarrays use relative quantization in which the intensity of a feature is compared to the intensity of the same feature under a different condition, and the identity of the feature is known by its position. An alternative to microarrays is serial analysis of gene expression, where the transcriptome is sequenced allowing an absolute measurement[32].

Image acquisition

Microarray images are acquiring through with laser scanner. The scanner executes a region scan of the slide and creates for each dye a digital map or an image, of the fluorescent intensities for each pixel[33]. For a distinctive microarray examination, the scanner generate two 16-bit tagged image file layout (TIFF files), one for each fluorescent dye. Dissimilar dyes attract and emit luminosity at diverse wavelengths[34]. In classify to calculate the large quantity of the two luminous dyes for every spot, the scanners are intend create excitation luminosity at diverse wavelength and perceive dissimilar discharge wavelength. The dyes used usually are Cy3 and Cy5 having discharge in 510-550nm and 630-660nm ranges respectively [35].

Data Analysis

Signal potency is commonly sequence dependent Intended for this cause, averaging indicate intensities is not suitable and probe variant should be investigate separately in expectancy of the concluding data evaluation steps. Probe *reproduces* have the similar progression, by the way of various instances on the arrangement. In theory, these should have the same expression, and their justification is to amplify. The self-assurance in the dependability of the gene investigates authentication development [36]. Probe replicates are developed in two most important approaches [37]The subsequent alternative is to comprise all replica values in the occupied dataset (e.g. to apply

ANOVA-type investigation methods afterward [38]. Investigational inaccuracy is then pooled with genetic variation. It is essential not to puzzle this supplement in the numeral of data values for each gene in the company of the true statistical sample size, which is purely the number of biologically autonomous objective samples [39].

Data normalization

There are two types of variation in the data, the biological variation causal the study plan which one would like to notice and compute and the deviation due to the technology, the handling and processing, which one would like to minimize in order to not obscure the biology [40]. For this reason we compared the variation reduction efficiency of our *Z*-normalization approach with three other methods by calculating three measures of data technical variation: We can be use the following notation to represent the normalization methods we compared:

$NLRi= \log_2 (Ri/Gi)^{-} \text{median} (\log_2)^{(R/G)}$

The median of all logs (ratios) is subtracted from all individual logs (ratios), resultant in a new taken as a whole median log (ratio) of zero.

Log-ratios can be non-linear with respect to signal magnitude. Once applying a locally robust LOWESS function to this graph, the following serves as normalization procedure:

$$LRi=\log_2 (Ri/Gi)- \log_2(2y(xi))$$

Log-ratios can be non-linear with respect to signal magnitude [41]. Use of the R-I plot, namely, $\log_{10}$ (R/G) vs log2(R/G) will show if this effect is present. After applying a locally robust LOWESS function to this method, the following serves as normalization procedure:

$NLRi=\log_2 (Ri/Gi) -\log_2 (2^{y(xi)})$

Provided a sufficient number of probes or control probes are contained within each subarray, the options above can be expanded to normalize these within an array, adjusting for possible print-tip or hybridization gradient problems [42].

Analysis

It is significant to note down that for microarrays projects intended to study distinct gene pathways and communications, a greatest of explanation and statistical consistency is required. For minimum result set for each gene should comprises mean expression prominence per condition, *P* values, and fold-changes from consequence testing[43]. Data points are usually the mean values of biological chip replicates of each gene in a given condition [44]. Limited subsets of interesting genes can evidently be plotted by means of simple vertical bar charts [45]. ANOVA-type methods are somewhat more involved, and appropriate where there is more than one experimental factor under investigation[46,47]. It is significant to note that the expression of personality genes of attention is typically backed up by substantiation using other techniques such as PCR, *in situ* hybridization and Northern blotting[48,49].

CONCLUSIONS

Accessible techniques to screen a broad range of Pathogens are intrinsically biased and there by constrained to detecting a restricted number of candidate Pathogens. To prevent this difficulty, we required to expand a Microbial recognition line of attack based on a combination of Microbial genomics and long oligonucleotide microarray technology. To accomplish this objective, the extremely conserved nucleotide sequences within a Microbial family can be selects for illustration on the microarray. By using the most conserved sequences, we anticipate maximizing the prospect that all members of each Microbial family, as well as unsequenced, unknown, or newly evolved family member can be detected. Secondary, but corresponding, ambition, we required to take advantage of the elevated resolution of

microarray hybridization to distinguish among Microbial subtypes, which is a complex and difficult job with conventional methods.

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