Microarray Technology: Transcending Towards New Era of Gene Expression and Its Potential Applications

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Abstract: Examination of the transcriptome utilizing DNA microarrays has turned into a standard methodology for examination of the atomic premise of human malady in both clinical and trial settings. Microarray innovation has gained solid ground over the previous decade, and there have additionally been critical changes in application territories, from nucleic acids to proteomics and from research to clinical applications. This audit gives a far reaching review of microarrays in diagnostics and biomarker, covering DNA, peptide, protein exhibits. DNA microarrays, or quality chips, permit reviews of quality articulation, (i.e., mRNA articulation) in a very parallel and complete way. The example of quality articulation created, known as the articulation profile, portrays the subset of quality transcripts communicated in a cell or tissue. At its most crucial dimension, the articulation profile can address subjectively which qualities are communicated in ailment states. Microarrays can be utilized to describe the elements of novel qualities, distinguish qualities in a biologic pathway, investigate hereditary variety, and recognize remedial medication targets. Late advances in microarray-based innovations take into account vast scale quality articulation examination in a solitary trial, which have been connected to far reaching tests, mutational investigation, medicate revelation, formative science, and sub-atomic investigation of different infections.

Keywords: DNA microarray; gene expression; biomarker; Gene regulation.

1. Introduction

Every single living life form contain DNA, a particle that encodes all the data required for the advancement and working of a living being. Finding and interpreting the data encoded in DNA, and seeing how such a basic atom can offer ascent to the stunning natural assorted variety of life, is an objective partaken here and there by all life researchers. Microarrays give an astounding perspective into the investigation of DNA, and in this way a rich strategy to take a gander at living systems. DNA is a physical molecule that can encode information in an immediate structure. Cells express information from different parts of this structure in a setting subordinate shape. DNA encodes for characteristics, and authoritative parts control whether characteristics are on or off. For instance, all of the cells of the human body contain a comparative DNA, yet there are a few particular sorts of cells, each imparting a fascinating game plan of characteristics from the DNA. In such way, DNA could be portrayed as existing in some number of states. Microarrays are an instrument used to examine the states of DNA [1].

After finishing of the human genome that contains 3 billion nucleotides encoding the 30,000 qualities. It was discovered that a quality and a protein ponder and research an alternate strategy does not deal with the issue. Since the strategy is mind boggling to the point that once in a while included numerous few quality drew in with the headway of an activities. Essentially, proteomic and genomic progressions could inspect the activities and Changes of characteristics and proteins. DNA microarray progressions is an open utility in sub-nuclear science .The rule advantage is that not typical for customary procedures, it isn't compelled to assemble one quality at some random minute [2]. Amid the last 50% of the twentieth century, the examination of the control and capacity of qualities has to a great extent been driven by well-ordered investigations of individual qualities and proteins. In the previous decade, a change in outlook has developed in which we are currently ready to create a lot of information about numerous qualities in a very parallel and quickly serialized way. A vital device in this procedure has been the improvement of DNA microarrays [3].

Tissue microarray is an ongoing development in the field of pathology that is relied upon to beat these critical issues. The strategy was planned as a high-throughput atomic science procedure for scientists that considers appraisal of articulation of intriguing competitor diseaselrelated qualities or quality items all the while on many tissue tests [4]. It additionally permits parallel atomic profiling of clinical examples at the DNA, RNA, and protein level. This system empowers pathologists to perform expansive scale investigations utilizing immunohistochemistry, fluorescence in situ hybridization (FISH), or RNA in situ hybridization (ISH) at significantly quicker and at extraordinarily bring down expenses contrasted and customary methodologies. This innovation ought not be mistaken for DNA microarrays where each little spot speaks to a one of a kind cloned cDNA or oligonucleotide. In tissue microarrays, the spots are bigger and contain little histologic areas from interesting tissues or tumors.

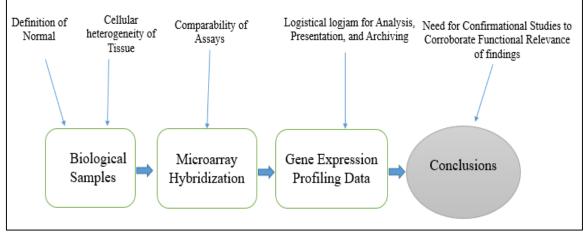


Figure 1. Possible pitfalls in Microarray gene expression profiling Analysis

2. The importance of Microarrays:

Regardless of whether acquired or coming about because of the body's reaction to natural anxieties like infections or poisons, all maladies have a hereditary part. Numerous human maladies are not caused by one hereditary variety inside a solitary quality, however are dictated by complex associations among different qualities, natural and way of life factors. A man's obstruction or weakness to an ailment and the seriousness and movement of the infection are altogether engaged with the many-sided pathways of sickness are not yet known, it has been troublesome for analysts to create screening tests for most maladies and scatters, for example, diabetes, cardiovascular illnesses, Alzheimer's ailment and joint pain. By utilizing microarray innovation, specialists may start to uncover applicable qualities related with a sickness.

Microarrays have made a noteworthy commitment to science both in light of the fact that they can overview countless rapidly or think about a little example measure. Microarrays have been utilized to examine quality articulation inside a solitary example or to think about quality articulation in two distinctive cell types or tissue tests, for example, in sound and ailing tissue. In any case, this innovation is spreading out into a wide range of utilizations, including genotyping, arrangement investigation and a wide range of kinds of microarrays, including sugars, peptides, RNAi, microRNA, proteins and antibodies.

3. Microarray Technology

The improvement of DNA microarray innovation in mid 1990s took into account the first run through to all the while profile and concentrate the transcriptome, as it were to contemplate cells' continuous "prattle" in more detail. The innovation abused the specific same rule that makes nucleic corrosive so basic to data stockpiling: hybridization to correlative successions. Hybridization between the cDNA invert translated from a natural example to a pre-planned reciprocal DNA test masterminded on a slide, or cluster, is the premise of DNA microarrays. A microarray hence comprises of a pre-structured library of engineered nucleic corrosive tests that are immobilized and spatially showed on a strong network. Microarrays advanced from a procedure known as Southern smudging, where DNA pieces are joined to a substrate and after that tested with a realized quality arrangement.

The main DNA clusters were developed by immobilizing cDNAs onto channel paper. In any case, it was not until 1995 that the primary DNA microarrays equipped for breaking down a huge number of arrangements were built by "spotting", or appending short engineered tests to assigned areas on the strong surface, normally glass or silicon chip (see Fig. 2). There are a few different ways that such spotted clusters can be delivered. A few techniques fundamentally utilize a robot to "print" pre-planned tests that have been joined to fine needles onto a concoction grid surface utilizing surface building (models incorporate fine-pointed sticks, needles and ink-fly printing). Different techniques utilize photograph enacted science and concealing to blend tests one nucleotide at any given moment on a strong surface in rehashed ventures to develop tests of explicit grouping in assigned areas.

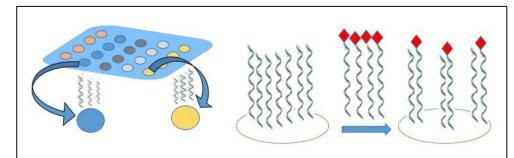


Figure 2. DNA is attached to the specific location or a spt. The hybridization of a probe made grid and complement with the targeted sequence to determine the expression of particular mRNA in sample.

3.1 Spotted Arrays

Poly-lysine covered glass magnifying instrument slides gave great authoritative of DNA and a mechanical spotter was intended to recognize various glass slide clusters from DNA put away in microtiter dishes. By utilizing opened pins (like wellspring pens in plan) a solitary plunge of a stick in DNA arrangement could detect different slides. Spotting onto glass enabled one to fluorescently mark the example. Fluorescent location gave a few points of interest with respect to the radioactive or chemilluminescent marks regular to channel based clusters. To start with, fluorescent identification is very delicate and has a genuinely substantial powerful range. Second, fluorescent marking enabled one to name two (or possibly more) examples in various hues and cohybridize the examples to a similar cluster. As it was exceptionally hard to reproducibly deliver spotted exhibits, examinations of exclusively hybridized tests to apparently indistinguishable clusters would result in false contrasts because of cluster to-exhibit variety. Be that as it may, a two-shading approach in which the proportion of signs on a similar cluster are estimated is significantly more reproducible.

3.2 DNA Microarray measurement of gene expression

Segregate and sanitize mRNA from tests of intrigue. Since we are occupied with contrasting quality articulation, one example typically fills in as control, and another example would be the investigation (sound versus ailment, and so on) Reverse translate and mark the mRNA. With the end goal to recognize the transcripts by hybridization, they should be named, and on the grounds that beginning material perhaps restricted, an enhancement step is additionally utilized. Naming more often than not includes playing out a turn around interpretation (RT) response to create a reciprocal DNA strand (cDNA) and consolidating a brilliant color that has been connected to a DNA nucleotide, delivering a fluorescent cDNA strand. Sickness and sound examples can be marked with various colors and cohybridized onto the equivalent microarray in the accompanying advance. A few conventions don't mark the cDNA yet utilize a second step of enhancement, where the cDNA from RT step fills in as a format to create a named cRNA strand. Hybridize the named focus to the microarray. This progression includes putting marked cDNAs onto a DNA microarray where it will hybridize to their manufactured reciprocal DNA tests appended on the microarray. A progression of washes are utilized to evacuate non-bound arrangements.

Sweep the microarray and quantitate the flag. The fluorescent labels on bound cDNA are energized by a laser and the fluorescently named target groupings that dilemma to a test produce a flag. The aggregate quality of the flag relies on the measure of target test authoritative to the tests present on that spot. In this manner, the measure of target succession bound to each test relates to the articulation dimension of different qualities communicated in the example. The signs are distinguished, evaluated, and used to make an advanced picture of the cluster.

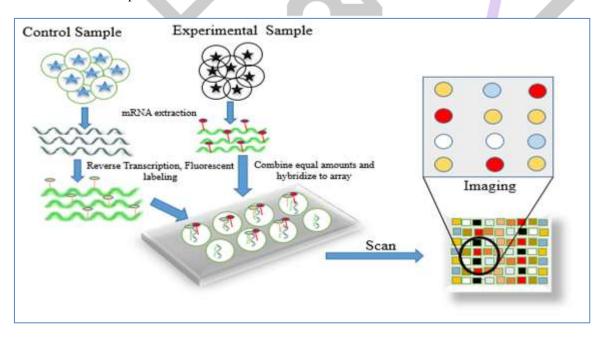


Figure3. A typical DNA microarray co-hybridization (2 dye)

4. ARRAY PRODUCTION

A chip or microchip, in PC innovation, is a little bit of semiconducting material containing an electronic circuit. Such chips are commonly little, typically under 5 cm for each side. Their little size makes current PCs quick, conservative and generally economical. A tantamount marvel is seen in sub-atomic science. The scaling down of specific devices is appropriate for the development of a savvy and convenient gadget — the spotted cluster framework, which offers the pharmaceutical, biotechnology Chips are worked of semiconducting materials. A semiconductor is amaterial that is neither a decent conveyor of power nor a decent

encasing. The most widely recognized semiconductor materials utilized in making chips are the components: silicon and germanium. Be that as it may, about all chips are produced using silicon. Generation of microarrays starts with the choice of tests to be imprinted on the cluster. By and large, these are picked straightforwardly from databases including GeneBank [5] and UniGene [6]. There are three distinct techniques for making the microarray: spotting long DNA parts, spotting pre-assembled oligonucleotides, and in situ (on-chip) combination of oligonucleotides (Table 1).

Table-1 Methods for creating a microarray

Method	Features and/or applications
I) spotting long DNA fragments: Fragment based DNA printing	Array of spotted PCR productsGene expression analysis
II) array of prefabricated oligonucleotides: Gel pads Microelectrodes	 Oligonucleotides are attached to patches of activated polyacrylamide Controlled electric fields for immobilisation
III) <i>in situ</i> synthesis of oligonucleotides: Photolithography Inkjet technology	 Light-directed oligonucleotide-synthesis on chip Adapted from semiconductor industry Oligonucleotides are synthesised drop-by-drop Adapted from the technique used in ink-jet printers

For prokaryotes, tests are normally produced by enhancing genomic DNA with quality explicit groundworks. Spotting PCR items (of roughly 0.6–2.4 kb) speaking to explicit qualities onto a lattice produces DNA exhibits. These PCR items are created utilizing chromosomal DNA as a layout, and hence cleansed by precipitation or gel-filtration, or both. For both, glass and film each cluster spot is created by keeping anfew nanoliters of filtered PCR item, for the most part at 100–500 mg/ml [7]. The printing is done by a robot (arrayer) that detects an example of every quality item onto various lattices in a sequential activity. The films generally utilized are industrially accessible nitrocellulose and charged nylon that are utilized in standard blotching examines (Southern smudge, province and plaque smear, speck and space smudge). The disservices of this technique are that the hereditary material is non-covalently appended which may result in its misfortune from the help, and that just a little measure of the DNA is accessible for hybridisation [8]. Glass-based clusters are regularly made on magnifying instrument slides. They are covered with poly-lysine, amino silanes or amino-receptive silanes [9] which improve both the hydrophobicity of the slide and the adherence of the saved DNA. As a rule, DNA is cross-connected to the framework by bright illumination.

After obsession, leftover amines on the slide surface are responded with succinic anhydride to diminish the positive charge at the surface As the last advance, the stored DNA is part single-stranded by warmth or soluble base. Oligonucleotide chips are created by adjusting semiconductor photolithography to blend oligonucleotide tests in situ on glass or layer substrate. These chips are structured and created based on arrangement data alone, without the requirement for any clones, PCR items, DNA, etc. Test exhibits are produced by light-coordinated compound union, which joins strong stage concoction union with photolithographic manufacture strategies utilized in the semiconductor business [10, 11]. Using a movement of photolithographic spreads to portray chip presentation districts, trailed by specific substance mix steps, the methodology manufactures high-thickness assortments of oligonucleotides, with each test in a predefined position in the bunch. Various test bunches are joined in the meantime on a broad glass wafer. This parallel technique redesigns reproducibility and achieves economies of scale [12]. Oligonucleotides can't be coupled specifically to the surface by silanol gatherings of silicate glass or to generally plastics. It is important to make the surface with a gathering from which the [12, 13]. Oligonucleotides on long spacers expand far from their neighbors and from the surface, and in this manner they permit more productive connection with the objective. The spacer's length has impact on hybridisation yield. It has been demonstrated that the ideal spacer length offers up to 150-overlap increment in a denoted the yield of hybridisation [13]. The effects of different charged social affairs in the spacer were in like manner examined, and it was exhibited that both unequivocally and conflictingly charged get-togethers in the spacer diminish the yield of hybridisation [13]. Besides, the base game plan and progression of the oligonucleotides have in like manner a generous effect on duplex advancement. The effect is clearly a direct result of the lower soundness of A: T than G: C sets. According to this, the oligonucleotides of a comparable length have correspondingly phenomenal Tm regards. Counting an A: T base consolidate extends Tm by for the most part 2°C, differentiated and 4°C for a G: C coordinate [12].

The development for spotting groups (DNA chips) is undoubtedly more clear than that for in situ creation. Synchronous making of advancement of the oligonucleotide chain can be begun. Such spacers in like manner help to crush steric impediment, containing in the manner in which that the terminations of the tests closest to the surface are less accessible than the completions encourage

away numerous bunches with a comparative course of action of tests makes the declaration more saving than in situ association. Furthermore, declaration is moreover a procedure for choice for long progressions, which are open as PCR things.

5. Microarray-based Comparative Genomic Hybridisation (a-CGH)

The first efficient approach to scanning the entire genome for variations in DNA copy number was Comparative Genomic Hybridisation (CGH). Array-based CGH is a powerful technology capable of identifying chromosomal imbalance at a high resolution. While it began as an effective tool for genome scanning, array-CGH is becoming one of the most powerful diagnostic and prognostic tests in clinical pathology. Congenital abnormalities, haematopoietic and solid tumours and infertility are just a few of the areas where CGH microarrays are being applied. The application of comparative genomic hybridisation microarrays has been focused in the field of oncology. Application of array CGH to tumour specimens makes genetic diagnosis of cancers possible and may help to differentiate tumour subtypes allowing for more accurate diagnosis and treatment decisions.

5.1 Gene Expression Is Detected By Hybridization

The motivation behind a microarray is to look at articulation of different qualities at the same time in light of some natural annoyance. All the more by and large, a microarray serves to cross examine the groupings of particles in a mind boggling blend and in this way can fill in as an incredible systematic instrument for some sorts of tests. To see how this happens, it might be valuable to survey the structure of DNA and analyze how the remarkable structure of this particle assumes a job in recognizing itself. Despite the fact that DNA is strikingly instructively intricate, the general structure of the atom is extremely very basic. DNA is comprised of four concoction building squares called bases: adenine, cytosine, guanosine, and thymidine (truncated individually as A, C, G, or T). As individual subunits these building squares are additionally alluded to as nucleotides. A strand of DNA comprises of a sugar phosphate spine to which these bases are covalently connected to such an extent that they frame an arrangement. Since these four bases can shape arrangements, it is conceivable to utilize them to encode data dependent on their examples of event. Without a doubt, from a data perspective, DNA has a potential information thickness of 145 million bits for every inch and has been considered as a substrate for calculation whereby the groupings are alluded to as programming [14, 15].

Like strings of content in a book, the arrangements that make up a strand of DNA have directionality with the end goal that data can be encoded in a provided guidance. The measure of DNA, and in this manner the measure of arrangement, shifts from life form to creature. For example, the microorganism Escherichia coli have 4.5 million bases of succession, though human cells have around 3 billion bases. Correctly how much natural information is encoded in these progressions is dark, addressing a standout amongst the most significant riddles of science, yet microarrays give a way to deal with get snippets of data. Cell DNA frequently contains one strand and in addition of two strands antiparallel to each other. The two strands are hydrogen strengthened together by associations between the bases, molding a structure in the cell. The structure is helical, similar to a twisting staircase in which the bases are associated with each side and convey in a plane to shape the methods for the staircase. Other than the hydrogen bonds between the bases of converse strands, the covering and closeness of the bases to each other provoke a second kind of noncovalent force considered a stacking affiliation that adds to the constancy of the twofold stranded structure. The bases of one strand communicate with the bases of the other strand as per an arrangement of blending rules, to such an extent that A sets with T and C sets with G. Along these lines, on the off chance that one knows the arrangement of one strand, by definition, one, knows the grouping of the contrary strand. This property has significant outcomes in the investigation of science. It is likewise what the cell uses to imitate itself. As the cooperation between the bases is noncovalent, comprising just of hydrogen securities, the strands can basically be softened separated and isolated, in this way opening the route for a duplicating system to peruse each single strand and re-make the second corresponding strand for every 50% of the match, bringing about another twofold stranded particle for every phone. This is additionally the instrument by which cells express qualities. The strands are opened by the quality articulation hardware with the goal that some number of RNA duplicates of a quality can be orchestrated. The RNA transcript has indistinguishable succession from the quality with the special case that uracil (U) replaces T, however the hybridization blending rules continue as before (U and T can both match with A).

This property of complementarity is additionally what is utilized for estimating quality articulation on microarrays. Similarly as vitality can soften strands separated and separate them into single particles, the procedure is reversible with the end goal that solitary strands that are correlative to one another can meet up and reanneal to shape a twofold stranded complex. This procedure is called hybridization and is the reason for some examines or examinations in atomic science. In the cell, hybridization is at the focal point of a few natural procedures, though in the lab complementarity is character and hence hybridization is at the focal point of numerous in vitro responses and investigative strategies. The particles can originate from totally extraordinary sources, yet on the off chance that they coordinate, they will hybridize.

6. Microarrays: Exceptional Substitute to Traditional Technique

Microarrays give an approach to quantify numerous qualities on the double by turning around and parallelizing this procedure. Rather than marking what is known and examining an unpredictable blend of questions to feature a solitary target, microarray strategies name the mind boggling blend that is in an answer and use a two-dimensional surface of known atoms or tests in discrete areas, as readout. In this specific circumstance, the cell RNA blend is named, making a marked complex blend of questions. Complementarity between target particles in the mind boggling blend and tests exhibited on the strong surface will bring

about strengthening and hybridization, consequently catching the named atoms at first glance. Unhybridized particles are washed away preceding measurement. Despite the fact that this procedure has been utilized in the past with films to make macroarrays, normally to screen libraries of clones [16], including distinguishing proof of differentially communicated qualities [17], the accomplishment of microarrays has to do with scaling down. The communication between a test and its objective is a coupling test. The same number of qualities are communicated at low dimensions, affectability is regularly an issue. In a discovering most would think about outlandish, a progression of concentrates on restricting examines in the mid-1980s found that diminishing the span of a coupling target does not lessen the affectability or exactness of a test and really prompts an expansion in affectability [18,19]. Consequently, scaling down of the examine is conceivable. As the extent of a spot diminishes, the partial inhabitance of the immobilized test is needy just on the convergence of the relating target particle in arrangement. This is the rule by which microarrays work. Different advancements have added to productive parallelization through scaling down. The utilization of glass substrates rather than permeable channels as a mode for catching nucleic corrosive in a cluster arrange implies that little hybridization volumes can be utilized. The rate of hybridization is fixation subordinate. In this manner, littler volumes mean higher rates of hybridization and expanded affectability. What's more, strong glass surfaces have low characteristic fluorescence, permitting the utilization of fluorescent colours for naming examples, and alongside strategies adjusted from confocal microscopy, they take into consideration productive measurement of a profoundly parallel scaled down examine. A few gatherings created and connected these ideas, joining scaling down, robots, and genomes to grow new examines for the estimation of quality articulation in parallel [20,21].

7. Applications of Microarrays

The utilization of microarray innovation has been stretched out to the exploration of useful protein pathways. Lackner *et al.* built up a turnaround stage protein exhibit to break down the phosphorylation status of 100 proteins with various bosom malignant growth cell lines [22]. Cell lysates from different cell lines were spotted onto the slide in sequential weakening and examined with various antibodies that perceive phosphorylated proteins. The investigation enabled the analysts to perform flagging pathway organize examination and characterize bosom malignant growth cell lines into various subtypes. Besides, microarray examination can likewise yield profitable data on the deregulated flagging pathway in individual malignant growths.

7.1 Gene Expression Analysis

The prevail use of DNA microarrays has been to gauge quality articulation levels. In this application, RNA is removed from the cells of intrigue and either, marked straightforwardly, changed over to a named cDNA or changed over to a T7 RNA advertiser followed cDNA which is additionally changed over to cRNA through the Eberwine enhancement process [23]. A wide assortment of strategies have been produced for marking of the cDNA or cRNA including: fuse of fluorescently named nucleotides amid the combination, joining of biotin named nucleotide which is in this way recolored fluorescently named streptavidin, fuse of an altered receptive nucleotide to which a fluorescent tag is included later, and an assortment of flag intensification techniques (an early survey of various naming strategies is given in [24]. The two most every now and again utilized strategies are the consolidation of fluorescently marked nucleotides in the cRNA or cDNA combination step or the joining of a biotin named nucleotide in the cRNA blend venture (as is finished by Affymetrix). The named cRNA or cDNA are then hybridized to the microarray, the exhibit is washed and the flag is identified by estimating fluorescence at each spot. On account of biotin named tests, the cluster is recolored post-hybridization with fluorescently marked streptavidin. Laser instigated fluorescence is commonly estimated with an examining confocal magnifying instrument.

7.2 In cancer

Tumor development includes concurrent changes in many cells and varieties in qualities. Microarray can be an aid to scientists as it gives a stage to concurrent testing of a huge arrangement of hereditary examples. It helps particularly in the distinguishing proof of single-nucleotide polymorphisms (SNPs) and transformations, arrangement of tumors, ID of target qualities of tumor silencers, recognizable proof of malignant growth biomarkers, ID of qualities related with chemoresistance, and medication revelation. For instance, we can think about the diverse examples of quality articulation levels between a gathering of malignant growth patients and recognize the quality related with that specific disease.

Quality microarrays have been utilized for similar genomic hybridization. In this strategy, genomic DNA is fluorescently named and used to decide the nearness of quality misfortune or intensification [25-27] Array-based similar genomic hybridization (aCGH) has been utilized to delineate variations from the norm in an extensive variety of tumors, including bosom carcinoma, [27] bladder carcinoma, [28] fallopian tube carcinoma, [29] gastric carcinoma, [30] melanoma, [31] and lymphoma. [32] Gene articulation information can recognize gatherings of cases with fundamentally extraordinary results where routine histopathologic examination does not allow subclassification.

7.3 Antibiotic treatment

Increment in the quantity of safe microscopic organisms and superadded contaminations has prompted disappointment of anti-infection agents. Destructiveness of the bacterial strains too influences the result of the illness procedure. In oral pit where anaerobic microscopic organisms may be the infective specialist, they regularly are not effectively culturable, particularly living beings, for example, actinomyces. DNA microarray examination helps as the bacterial genomic DNA frequently outlives the

suitability of the microscopic organisms and a finding can be made utilizing a little measure of DNA, instead of the expansive quantities of microbes required for culture. In future, a canker example may be sent not for culture and affectability testing, but instead for DNA microarray examination.

For the most part, the counter acting agent microarrays are perfect for discovery of protein plenitude the natural example having a moderately huge unique range [33]. For instance, Haab made utilization of counter acting agent microarrays for serum-protein profiling with the end goal to distinguish potential biomarkers in prostate malignant growth [33]. Utilizing this methodology, the creators could recognize five proteins (immunoglobulins G and M, α 1-against chymotrypsin, villin and the Von Willebrand factor). They had essentially unique dimensions of articulation between the prostate malignancy tests and control tests from sound people.

7.4 Drug Discovery

Microarray innovation has broad application in Pharmacogenomics. Pharmacogenomics is the investigation of connections between's restorative reactions to drugs and the hereditary profiles of the patients. Relative investigation of the qualities from an unhealthy and a typical cell will help the recognizable proof of the biochemical constitution of the proteins orchestrated by the sick qualities. The scientists can utilize this data to blend drugs which battle with these proteins and decrease their impact.

7.5 Toxicological Research

Microarray innovation gives a strong stage to the exploration of the effect of poisons on the cells and their passing on to the descendants. Toxicogenomics builds up connection between's reactions to toxicants and the adjustments in the hereditary profiles of the cells presented to such toxicants.

7.6 Delineating molecular pathways

Experimentation utilizing DNA microarrays requires no theory about which qualities may be of enthusiasm since articulation from vast arrangements of qualities can be inspected in a fair-minded way. This implies the innovation can be utilized to recognize unforeseen transcriptional targets downstream of any phase in a sub-atomic pathway, if that stage can either be explicitly initiated or stifled tentatively. Useful trial techniques incorporate overexpressing or 'thumping down' specific proteins of intrigue, or utilizing inhibitors or agonists of their capacity. Changes in interpretation profiles would then be able to be resolved. An incessant imperative on this methodology is the powerlessness to segregate between changes that are specifically downstream of that arrange in the pathway, for instance coordinate focuses of a translation factor of enthusiasm, from changes related with a flagging course including numerous stages. A precedent is the examination of the downstream impacts of human hormones; a huge and differing scope of qualities were observed to be thyroid hormone responsive and suddenly a vast extent were contrarily controlled [34]. Along these lines, thyroid hormone was appeared to have an effect on numerous novel cell capacities [35]. A further model is the investigation of focuses of the interpretation factor p53. p53 was known to have a basic job in the DNA harm reaction, yet recognizable proof of the qualities included has permitted expanded comprehension of why the quality is so habitually changed in disease [36,37].

8. Limitations of DNA Microarrays

At their center, microarrays are basically gadgets to at the same time measure the overall centralizations of various DNA or RNA groupings. While they have been unfathomably valuable in a wide assortment of uses, they have various constraints. To start with, clusters give a roundabout proportion of relative fixation. That is the flag estimated at a given position on a microarray is commonly thought to be corresponding to the grouping of an assumed single animal types in arrangement that can hybridize to that area. Be that as it may, because of the energy of hybridization, the flag level at a given area on the cluster isn't straightly corresponding to grouping of the species hybridizing to the exhibit. At high focuses the exhibit will end up immersed and at low fixations, balance supports no official. Consequently, the flag is direct just over a constrained scope of fixations in arrangement. Second, particularly for complex mammalian genomes, usually troublesome (if certainly feasible) to configuration exhibits in which numerous related DNA/RNA successions won't tie to a similar test on the cluster. An arrangement on a cluster that was intended to distinguish "quality A", may likewise identify "qualities B, C and D" if those qualities have noteworthy grouping homology to quality A. This can especially hazardous for quality families and for qualities with numerous join variations. It ought to be noticed that it is conceivable to configuration clusters explicitly to identify join variations either by making exhibit tests to every exon in the genome [38] or to exon intersections [39]. Nonetheless, it is hard to configuration exhibits that will particularly distinguish each exon or quality in genomes with numerous related qualities. At long last, a DNA cluster can just identify successions that the exhibit was intended to distinguish. That is, if the arrangement being hybridized to the exhibit contains RNA or DNA species for which there is no complimentary grouping on the cluster, those species won't be distinguished. For quality articulation examination, this ordinarily implies qualities that have not yet been clarified in a genome won't be spoken to on the exhibit. What's more, non-coding RNA's that are not yet perceived as communicated are normally not spoken to on a cluster. Additionally, for very factor genomes, for example, those from microbes, exhibits are normally planned utilizing data from the genome of a reference strain. Such clusters might miss an extensive part of the qualities present in a given disconnect of similar species. For instance, in the bacterial species Aggregatibacter actinomycetemcomitans, the quality substance varies by as much as 20% between any two disengages [40].

Subsequently an exhibit planned utilizing quality explanation from a "reference separate" won't contain a significant number of the qualities found in different disengages.

9. Future Utilizations of Microarray Innovation

The greatest challenge in using this technology is its expense. Making microarray cost efficient by developing the related software and science technology further, can make it more reliable in any field. Enhancing reproducibility and reliability of the data accessed through this method can also boost the applications of the microarray. Microarrays have the potential of multiplexed detection, but they are not portable (due to heavy and expensive scanners) and additionally require long incubation (with the sample) time. However protein microarrays, and particularly antibody microarrays (or immunoarrays), have the potential of becoming portable devices capable of detecting multiple biological targets, thus serving as ideal diagnostics and monitoring tools. A number of characteristics of the present methods are holding back the realization of this potential such as large dimensions of the active sites (spots) that result in the requirement of scanners, thus hindering the portability of the technology; lack of integration of the microarray with detection, sample (liquid) handling, read-out, power and remote reporting systems preventing autonomous operation (requiring human handling).

The quantities of microarray-based examinations distinguishing new qualities or sub-atomic pathways associated with tumor characterization, malignant growth movement, or patient result are developing exponentially. We are currently moving toward what is being alluded to as the "postgenomic time", amid which the symptomatic, prognostic, and treatment reaction biomarker qualities recognized by microarray screening will be examined to give customized the executives of patients. Clinicians will have the capacity to utilize microarrays amid early clinical preliminaries to affirm the components of activity of medications and to survey sedate affectability and danger. Combined with more traditional biochemical examination, for example, IHC and ELISA, microarrays will be utilized for analytic and prognostic purposes.

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REFERENCES

- 1. Seidel, C. Analysis of Microarray Data: A Network-Based Approach. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. 2008, 1-26. ISBN: 978-3-527-31822-3.
- 2. Dadkhah, K.; Anijdan, S.H.M.; Karimi, M.; Abolfazli, M.K.; Rezaei, F.; Adel, F.; Ataei, G. DNA microarray, types and its application in medicine. *Scholars Acad J Biosci.* **2015**, 3(7), 598-602.
- 3. Harrington, C.A.; Rosenow, C.; Retief, J. Monitoring gene expression using DNA microarrays. *Curr. Opin. Microbiol.* 2000, 3(3), 285–291.
- 4. Kumar, B.; De Silva, M.; Venter, D.J.; Armes, J.E. Tissue microarrays: a practical guide. Pathol. 2004, 36(4), 295-300.
- 5. Benson, D.A.; Boguski, M.S.; Lipman, D.J.; Ostell, J., GenBank. Nucleic Acids Res. 1997, 25(1), 1-6. 10.1093/nar/gks1195.
- 6. Schuler, G.D. et al. A gene map of the human genome. Sci. 1996, 274(5287), 540-546
- 7. Cheung, V.G.; Morley, M.; Aguilar, F.; Massimi, A.; Kucherlapati, R.; Childs, G. Making and reading microarrays. *Nat. Genet.* **1999**, 21(1), 15-19.
- 8. Gingeras, T.R.; Kwoh, D.Y.; Davis, G.R. Hybridization properties of immobilized nucleic acids. *Nucleic Acids Res.* **1987**, 15(13), 5373-5390.
- 9. Schena, M.; Shalon, D.; Heller, R.; Chai, A.; Brown, P.O.; Davis, R.O. Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*(20), 10614-10619.
- 10. Fodor, S.P.A.; Read, J.L.; Pirrung, M.C.; Stryer, L.; Lu, A.T.; Solas, D. Light-directed, spatially addressable parallel chemical synthesis. *Sci.* **1991**, 251(4995), 767–773.
- 11. McGall, G.; Labadie, J.; Brook, P.; Wallraff, G.; Nguyen, T.; Hinsberg, W. Ligh-directed synthesis of high-density oligonucleotide arrays using semiconductor photoresists. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, 93(24), 13555-13560.
- 12. Southern, E.; Mir, K.; Shchepinov, M. Molecular interactions on microarrays. Nat. Genet. 1999, 21(1), 5-9.
- 13. Shchepinov, M.S.; Case-Green, S.C.; Southern, E.M. Steric factors influencing hybridisation of nucleic acids to nucleotide arrays. *Nucleic Acids Res.* **1997**, 25(6), 1155-1161.
- 14. Adleman, L.M. Molecular computation of solutions to combinatorial problems. Sci. 1994, 266 (5187), 1021-1024.

- 15. Amos, M. Theoretical and Experimental DNA Computation. Springer. 2005
- 16. Gergen, J.P.; Stern, R.H.; Wensink, P.C. Filter replicas and permanent collections of recombinant DNA plasmids. *Nucleic acids res.* **1979**, 7(8), 2115–2136.
- 17. Riggleman, R.C.; Fristensky, B.; Hadwiger, L.A. The disease resistance response in pea is associated with increased levels of specific mRNAs. *J. Plant Mol. Biol.* **1985**, 4(2–3), 81–86.
- 18. Ekins, R.P.; Chu, F.W. Multianalyte microspot immunoassay microanalytical "compact disk" of the future. *Clin. Chem.* **1991,** 37(11), 1955–1967.
- 19. Ekins, R.P. Ligand assays: from electrophoresis to miniaturized microarrays. Clin. Chem. 1998, 44 (9), 2015–2030.
- 20. Schena, M.; Shalon, D.; Davis, R.W.; Brown, P.O. Quantitative monitoring of gene Expression patterns with a complementary DNA microarray. *Sci.* **1995**, *270*(*5235*), 467-470.
- Lockhart, D.J.; Dong, H.; Byrne, M.C.; Follettie, M.T.; Gallo, M.V.; Chee, M.S.; Mittmann, M.; Wang, C.; Kobayashi, M.; Horton, H.; Brown, E.L. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat. Biotechnol.* 1996, 14(13), 1675–1680.
- Boyd, Z.S.; Wu, Q.J.; O'Brien, C.; Spoerke, J.; Savage, H. et al. Proteomic analysis of breast cancer molecular subtypes and biomarkers of response to targeted kinase inhibitors using reverse-phase protein microarrays. *Mol. Cancer Ther.* 2008, 7(12), 3695-3706.
- 23. Van Gelder, R.N.; von Zastrow, M.E.; Yool, A.; Dement, W.C.; Barchas, J.D.; Eberwine, J.H. Amplified RNA synthesized from limited quantities of heterogeneous cDNA. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, 87(5), 1663–1667.
- Richter, A.; Schwager, C.; Hentze, S.; Ansorge, W.; Hentze, M.W.; Muckenthaler, M. Comparison of fluorescent tag DNA labeling methods used for expression analysis by DNA microarrays. *BioTechniques*. 2002, 33(3), 620–628. 630.
- 25. Pollack, J.R.; Perou, C.M.; Alizadeh, A.A.; Eisen, M.B.; Pergamenschikov, A.; Williams, C.F. et al. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat Genet.* **1999**, 23(1), 41–46.
- Pollack, J.R.; Sorlie, T.; Perou, C.M.; Rees, C.A.; Jeffrey, S.S.; Lonning, P.E.; et al. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. *Proc. Natl. Acad. Sci. U.S.A.* 2002, 99(20), 12936–12968.
- 27. SolinasToldoS, S.; Lampel, S.; Stilgenbauer, S.; Nickolenko, J.; Benner, A.; Döhner, H. et al. Matrix-basedcomparative genomic hybridization: Biochips to screen for genomic imbalances. Genes *Chromosomes Cancer.* **1997**; 20(4), 399–407.
- Veltman, J.A.; Fridlyand, J.; Pejavar, S.; Olshen, A.B.; Korkola, J.E.; DeVries, S. et al. Array-based comparative genomic hybridization for genome-wide screening of DNA copy number in bladder tumors. *Cancer Res.* 2003; 63(11), 2872–2880.
- 29. Snijders, A.M.; Nowee, M.E.; Fridlyand, J.; Piek, J.M.; Dorsman, J.C.; Jain, A.N. et al. Genome-wide arraybased comparative genomic hybridization reveals genetic homogeneity and frequent copy number increases encompassing CCNE1 in fallopian tube carcinoma. *Oncogene.* **2003**, 22(27), 4281–4286.
- 30. Weiss, M.M.; Snijders, A.M.; Kuipers, E.J.; Ylstra, B.; Pinkel, D.; Meuwissen, S.G. et al. Determination of amplicon boundaries at 20q13.2 in tissue samples of human gastric adenocarcinomas by high-resolution microarray comparative genomic hybridization. *J. Pathol.* **2003**, 200(3), 320–326.
- 31. Buckley, P.G.; Mantripragada ,K.K.; Benetkiewicz, M.; Tapia-Páez, I.; Diaz De Ståhl, T.; Rosenquist, M. et al. A fullcoverage, high-resolution human chromosome 22 genomic microarray for clinical and research applications. *Hum. Mol. Genet.* **2002**, 11(25), 221–229.
- 32. Martinez-Climent, J.A.; Alizadeh, A.A.; Segraves, R.; Blesa, D.; Rubio-Moscardo, F.; Albertson, D.G. et al. Transformation of follicular lymphoma to diffuse large cell lymphoma is associated with a heterogeneous set of DNA copy number and gene expression alterations. *Blood.* **2003**, 101(8), 3109–3117.
- 33. Miller, J.C.; Zhou, H.; Kwekel, J.; Cavallo, R.; Burke, J. et al. Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. *Proteomics.* **2003**, 3(1), 56-63.
- 34. Feng, X.; Jiang, Y.; Meltzer, P.; Yen, P.M. Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. *Mol. Endocrinol.* **2000**, 14(7), 947-955.
- 35. Yen, P.M. Studying hormonal regulation by microarrays: distinguishing the trees from the forest. *J. Clin. Endocrinol. Metab.* **2005**, 90(2), 1241-1242.
- 36. Kannan, K.; Kaminski, N.; Rechavi, G.; Jakob-Hirsch, J.; Amariglio, N.; Givol, D. DNA microarray analysis of genes involved in p53 mediated apoptosis: activation of Apaf-1. *Oncogene*. **2001**, 20(26), 3449-3455.
- 37. Sax, J.K.; El-Deiry, W.S. p53-induced gene expression analysis. Methods Mol. Biol. 2003, 234, 65-71.
- Gardina, P.J.; Clark, T.A.; Shimada, B.; Staples, M.K.; Yang, Q.; Veitch, J.; Schweitzer, A.; Awad, T.; Sugnet, C.; Dee, S.; Davies, C.; Williams, A.; Turpaz, Y. Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array. *BMC Genomics*. 2006, 7,325. <u>10.1186/1471-2164-7-325</u>.
- Castle, J.; Garrett-Engele, P.; Armour, C.D.; Duenwald, S.J.; Loerch, P.M.; Meyer, M.R.; Schadt, E.E.; Stoughton, R.; Parrish, M.L.; Shoemaker, D.D.; Johnson, J.M. Optimization of oligonucleotide arrays and RNA amplification protocols for analysis of transcript structure and alternative splicing. *Genome Biol.* 2003, 4, R66. <u>https://doi.org/10.1186/gb-2003-4-10-r66</u>.
- 40. Kittichotirat, W.; Bumgarner, R.E.; Asikainen, S.; Chen, C. Identification of the pangenome and its components in 14 distinct Aggregatibacter actinomycetemcomitans strains by comparative genomic analysis. *PLoS ONE*. **2014**, 6(7), e22420.