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## Gene expression and its application in biotechnology

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### Abstract

Advances in biotechnology has been the subject of praise for a decade now, this is due to techniques such as gene expression which has contributed immensely in the success of genetic engineering, medical advancement, vaccine production and agriculture. Gene expression has become a very important tool for the overall improvement of quality of life. This paper tries to look into the development of gene expression in the last forty years and to highlight how technological advancements in the study of gene expression brought about improvements as a focal point. Technological advancements associated with northern blotting, western blotting, and enzyme-linked immunosorbent assays, Polymerase Chain Reaction (PCR) technology with emphasis on quantitative PCR, differential protein display technique and DNA sequencing and hybridization arrays technology with emphasis on macroarrays and microarrays in facilitating gene expression will be discussed.

**Keywords:** Gene expression, Application, Biotechnology, Bioengineering

### 1. Introduction

The emergence of the Watson and Crick model of DNA in 1953 brought an insight into how DNA could function as the molecule of heredity; base pairing explained how genetic information could be copied from the DNA, while the existence of double strands of DNA gave further explanation into how errors could lead to mutations in daughter DNA molecules (1).

From 1960, rapid developments in the field of molecular biology ensued. These developments lead to translation of RNA transcripts from protein-coding sequences using the genetic code, into corresponding amino acid sequences in the protein product (1). This flow of information from nucleic acids to proteins as summarized by Crick, 1958, culminated into what is now termed gene expression. This covers the fact that proteins are not the only products of gene expression since it is proven that some genes code for functional RNA molecules such as rRNA and tRNA as well (1).

The knowledge of the genetic code in 1970 along with sequencing techniques lead to a breakthrough in the field of molecular biology by providing vast information on the organization and expression of genes (1). In 1977, came a technology that enables the analysis of gene expression called the northern blot technique. This technique allows the hybridization of labeled probes of cDNA and RNA to RNA blots so that gene expression patterns of mRNA transcripts can be analyzed. It also provided a means of confirmation of gene expression (2).

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Another novel analytical technique used for detecting proteins from a given sample of tissue extract or homogenate was devised in 1979 called the western blot (3). The technology uses gel electrophoresis for separation of proteins after they have been detected using probes with specific antibodies through transfer to a membrane. The technique is unique in that, not only will the molecular weight of the proteins be measured, their amounts in varying samples can also be accessed. This technology found wide applicability in molecular biology, biochemistry, immunogenetics and other fields of biosciences. (4) Highlighted the use of specific antibodies for binding of a specific protein from a mixture of thousands using the western blot technique. Because of the notable advances in the understanding of gene and protein expression; a more sensitive method for protein detection has emerged using a single western blot available in commercially made detection kits called the Q-dot western detection technology (5).

In the early 1980s also came another breakthrough called the Enzyme-Linked Immune Sorbent Assay (ELISA) to improve on the limitations of radioimmunoassay in the late 1960s to early 1970s. Radioimmunoassay uses radioactively labeled antigens and antibodies for detection of proteins. However, concerns on the hazards caused by radioactive wastes, the expensive nature of counting equipment and the need for special laboratory facilities created the need for a cheaper, more efficient and risk-free assay technique that uses enzymes to label antibodies and proteins for analysis. Today, it has found useful applications in diagnosis of diseases, monitoring therapy in patients and offers insights into pathogenesis of diseases (6).

In 1983, Kary Mullis developed the PCR method for gene amplification. However, developments over the years has seen this technique metamorphosing into a tool for analyzing gene expression in limited number of genes (up to a thousand) called the quantitative PCR (qPCR) technology. The setback of this technique is that only a small number of genes can be analyzed (7).

Differential display technique of gene expression of eukaryotic mRNA came into effect in 1992 as a fast and reliable method of comparison of gene expression of two or more cell populations or tissues in a bi-directional approach, simultaneously comparing and analyzing transcribed genes (2). The serial analysis of gene expression (SAGE) was developed in 1995 shortly afterwards. Although the northern blot, differential display and SAGE technologies all have their own advantages in expression of genes; they are not without their shortcomings which makes them in-effective if large numbers of expression products have to be analyzed. Limited or small amounts of mRNA can be studied at the same time when using the northern blot technique; differential display technique allows only a few numbers of different conditions of gene expression to be compared, although it allows simultaneous detection of many differences in gene expression. This makes the differential display technique unsuitable for quantitative studies. The SAGE technique requires a lot of labor involving complex sample separation procedures, extensive DNA sequencing and lacks sensitivity.

DNA microarray technology has brought in a significant improvement in sensitivity and efficiency of screening gene expression such that, the above problems associated with northern blot, SAGE and differential display techniques can be minimized. In addition, with DNA microarrays, the small size of the array allows for high sensitivity and for parallel screening of large numbers of genes using smaller amounts of starting materials. The comparative analysis of a good number of samples is also achievable with this technique (7). This development was strongly supported by the fact that; microarrays can be used as a powerful tool for comprehensive and quantitative analysis of gene expression profiles of normal, diseased state and developmental processes, thus, paving the way for its application in many conditions that includes; human breast cancer development, human inflammatory diseases and the transcriptional modes associated with human fibroblast (8).

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## **2. Techniques in gene expression**

### **1.1. Northern blotting techniques for gene expression**

Northern blotting method usually involves the fractionation of RNAs based on their sizes by gel electrophoresis accompanied by their transfer onto a membrane using capillary, vacuum or pressure blotting. This results in a permanent binding of the RNA to the membrane through a non-covalent interaction as a consequence of exposure to ultraviolet light or by heating at the temperature of "80°C". in a vacuum or oven (9). Sequences of RNA are then detectable on the blot through hybridization on specific radiolabeled probes. The probes are formed either through enzyme incorporation of <sup>32</sup>P or <sup>33</sup>P nucleotides or with nucleotides joined to biotin so that the probes can be detected through chemiluminescence. X-ray films or phosphor storage plates are used for detecting hybridization signals arising after probe hybridization. However, prior to detection by X-ray or phosphor plates, the probes are incubated with chemiluminescent substrate. Bands identified by the probes highlights the mRNA size while the band intensity shows mRNA abundance (10).

Despite its application in determining the accumulation of transcripts in cells and tissues using radioactive probes, the northern blot is not without its limitations such as, health hazards, the short half-life of the  $^{32}\text{P}$ -labeled probes as well as the high amount of target RNA required for detection of hybrids. Advances have been made in using non-radioactive probes in northern blot analysis as different haptens like digoxigenin-, fluorescein- or biotin-labeled probes were found to be effective for invitro analysis in plants using the northern blot (10). DNA and RNA probes labeled with digoxigenin have been applied in rare mRNA detection in animal system and were found to be a suitable means of analysis (10).

A rapid and optimized northern blot technique that provides for detection and analysis of mature and precursor microRNAs was described by Tran, 2006. This simple method minimizes the use of commercially available components and reduces hybridization. An XcellSoreLock mini-cell apparatus for detection of miRNA genes in mammalian cell lines was used in the procedure. Total RNA was separated using 15% TBE urea gel with the apparatus and after electrophoresis; RNA is transferred onto a nylon membrane. This is accompanied by labeling of a reverse complimentary probe to the miRNA mature sequence with incubation at 37°C for 2hrs. The technique has the advantage of utilizing one electrophoretic unit for both RNA electrophoresis and transfer, reduces the volume of reagents needed for the procedure and provides for detection of precursor miRNAs that are 65-70nt in length. Notable technical advantages associated with the use of the technique includes stripping and reprobing of the membrane for the expression of a different miRNA gene (meaning the membrane can be reused three times), time taken for hybridization is only 2 hours and the use of gels reduces reagent preparation time (10).

## 1.2. Western blotting

Protein transfer from gels through electrophoresis onto a membrane emerged in 1979 (11). Western blotting was described as the electrophoretic transfer of proteins from sodium dodecylsulphate-polyacrylamide gels to nitrocellulose and subsequent detection through radiography with antibody and radioiodinated proteins (11). This was also supported by Tobin et al, 1979, further explaining the transfer of proteins from Sodium Dodecyl Sulphate (SDS) polyacrylamide gels onto the surface of an adsorbent membrane. The employment of antibody probes afterwards, against the nitrocellulose bound proteins has brought about a revolution in the field of immunology (12). Blotting efficiency from gels to solid membrane support is influenced by the nature of the gels, the molecular mass of the protein and the membrane used. It is therefore ideal to use gels that will give the required resolution. Thin inner gels gives complete and faster transfer, however, they may cause handling problems. High molecular mass proteins blot poorly after SDS-PAGE giving a low level of detection on immunoblots. The application of heat, special buffers and partial protein digestion prior to transfer facilitates protein transfer (12).

Consequently, high quality specific antibodies are very crucial for western blotting analysis since they facilitate specific binding to target proteins in a mixture on a standard western blot. Despite the vast commercial availability of antibodies, they remain expensive and unaffordable in some laboratories. Therefore, any method that provides for the use of minute amount of antibodies without affecting result outcome is an added advantage. In lieu of these (13) devised an economical single-sided antibody incubation method for western blot. It was discovered that incubation of membranes in heat sealed bags or plastics or the direct application of reagents to one side of the membrane has been used to reduce cost of expensive reagents (13). Hence, the single-sided antibody incubation saves on the use of antibodies. Comparison of the single-sided membrane incubation to conventional immersion incubation shows that the single-sided membrane incubation provided same result with the conventional method when both techniques were used to detect gene expression of endogenous beta-actin in human embryonic kidney 293 cells (HEK-293). About 80% of primary and secondary antibodies were saved using this method (14).

Owing to the increasing understanding of gene and protein expression pathways, the need for more sensitive, quantitative methods that enables multiple detections of proteins ensued. Two new western blot detection kits from quantum dot corporation (QDC) are now available. These two kits make use of the high brightness, stability and multiple colors of secondary antibody conjugates for quantitative detection of several proteins using a single gel. Pico gram levels of proteins can be detected by using several gel imaging systems. Immunolabeled proteins can be detected by using secondary antibody conjugates which are fluorescent materials consisting of nanocrystal core and shells of CdSe and ZnS respectively. The nanocrystals are coated with an organic molecular layer that allows for water solubility and serves as sites for conjugation of biomolecules. Light absorption over a particular wavelength forms the basis of resolution in using the kits. The blots are labeled with rabbit and mouse primary antibodies which are used for colorimetric or chemiluminescent detection after electroblotting from SDS-PAGE gels. Qdot conjugates are then used for labeling of the blots and imaged with a fluorescence gel imaging system (15).

A recent powerful method of western blot called the mass western experiment for detecting the presence of specific proteins in complex mixtures without employing antibodies in the process emerged. This provides for identification of

proteins as a result of its high sensitivity and selectivity. Relative abundance of proteins between samples can also be compared. The technique involves labeling and digestion of membrane protein extracts with isotope-coded affinity tag reagents and the resulting peptides were analyzed using liquid chromatography-tandem mass spectrometry. Corresponding ions arising from peptides derived from proteins of interest are continuously exposed to collision-induced dissociation in an ion-trap mass spectrometer. This is followed by simultaneous trapping and fragmentation of both heavy and light isotope-coded affinity tag-labeled peptides resulting in identification and quantitation in a single mass spectrum. The procedure maximizes sensitivity allowing for analysis of peptides that would have gone undetected (Arnott *et al*, 2002).

### 1.3. Enzyme Linked Immunosorbent Assay (ELISA)

In the early 1960s to late 1970s radioimmunoassay was the prime antigen-antibody assay system but problems associated with the safety of laboratory personnel, radioactive wastes and special laboratory facilities as well as the purchase of expensive counting facilities became a major concern. Hence, the idea of using enzyme labels was proposed (16). The discovery of antibodies was also crucial for the invention of ELISA. Modifications of radioimmunoassay resulted in the first ELISA technique (17). Three components were found to be crucial in ELISA technique, these are notably; an antibody specific for the desired molecule, a solid-phase for antigen-antibody complex capture and an enzyme mediated colorimetric detection system. Molecules are detected through their binding to a specific primary antibody whereas antigen-antibody complex are captured onto a solid surface which can be a specialized polystyrene material with high protein binding capacity. Unbound reagents are washed leaving only the desired protein bound to antibody attached to the solid support. The antibodies are either conjugated to an enzyme which catalyzes the conversion of a substrate to a product with specific light absorbance or the specific antigen-antibody are recognized using another antibody conjugated to an enzyme capable of catalyzing color conversions. Hence, quantitation is achieved either by intensity of light absorbance or by means of an enzymatic reaction (17). Furthermore, the ELISA technique is sensitive, easy to carry out and has remarkable advantage associated with its use, which includes detection of circulating genes as well as screening of sera for the presence of antibodies in infective diseases (WHO, 1976).

Consequently, to improve on the sensitivity of ELISA, macromolecules that are capable of assembling large number of enzymes and antibodies have been employed in this technique. Notably, two types of macromolecules have been used for this purpose; one allows for the clustering of antibodies and labeling molecules like enzymes, radioactive materials and fluorescence (17). This types of macromolecules were highlighted to include, polymeric horseradish peroxidase (HRP)-Streptavidin conjugate, 3DNA dendrimer and immunoglobulin-G (IgG)-poly-D-glutamic acid-(HRP)<sub>n</sub> conjugate previously used in ELISA (18). The other variant of macromolecules allows the oriented immobilization of antibodies. As an example, nanoparticles were harnessed to display antibodies on their surfaces. Nanoparticles like streptavidin-conjugated nanobeads and biotin-coated liposomes have been found to be effective (18). Bionanocapsules-based enzyme-antibody conjugates for ELISA was generated recently of approximately 30nM displaying immunoglobulin-G Fe-binding ZZ domains derived from *staphylococcus aureus* (designated as ZZ-BNC) and has been shown to be very useful in detection of antigens and antibodies in aqueous phase of conventional ELISA (18).

Owing to low detection limits for protein complexes in samples associated with ELISA, much lower than that achieved for measurement of nucleic acids, there is need for improvement of the technique. As a means of comparison, conventional immunoassay has a typical limit of detection for P24 capsid protein of HIV of only 5pg/ml as compared to PCR which can detect RNA from 50 copies of HIV/ml of blood corresponding to RNA concentration of about 166zM (19). A method based on the counting of single enzyme-labeled immunocomplex of protein by capturing on paramagnetic beads in single microarray (siMoA) called digital ELISA was developed recently which is about 1000 fold more sensitive compared to the conventional ELISA method (19). Addressing specificity issues requires consideration of whether signals generated by target molecules can be recognized above signals generated as a consequence of other molecular interactions arising from non-target molecules. As an instance, if the sensitivity is meant to detect 50 enzyme-labeled target molecules when captured and 1000 enzyme molecules were detected from background signals; then this poses a challenge for detecting specific signals above that generated by the background (19). Against this backdrop, the siMoA was designed as a highly sensitive means for protein detection in blood taking into account the aforementioned theoretical considerations of sensitivity and specificity. Furthermore, high concentrations of reagents are needed for labeling of low amounts of captured proteins resulting in increasing the background and causing limitations in sensitivity (20). It was observed that the interactions between reagents for labeling and the immobilized capture of antibodies can dominate background signals; therefore, an alternative that optimizes sensitivity and specificity of immunoassay in this respect was introduced as digital ELISA (20). This employs the use of beads to capture excess antibodies as the supporting material, making the system more efficient for protein binding by maximizing the number of proteins captured. The proteins are detected using siMoA which is highly sensitive to enzyme and capable of detecting about 220zM (20).

Electrochemical detection of redox active enzyme substrate using another novel technique of sandwich ELISA in disposable plastic microchips consisting of photoablated microchannels with electrodes was also described by (20). The electrodes in the microchannels allows for the detection of immobilized antibodies on photoablated microchannel surfaces in approximately 5 minutes which is faster than microtitreplate technique. The technique is also rapid, portable and does not require a complex sensor.

#### 1.4. Differential Display analysis

In 1992, a fast and reliable method that compares differential expression of genes of two or more cell populations or tissues as mRNA was published. It has now emerged as a powerful tool for the identification of genes that are either over expressed or under expressed in one cell type relative to the other (21). The technique, first described by Liam and Pandee, employs the isolation of total RNA from two cell types that are to be compared and strand copies of the RNA in both cell types are formed by reverse transcription using an oligo-dT primer consisting of a specific dinucleotide at the 3' end (3' amplimers). These are then used (3' and 5' amplimers) in polymerase chain reaction (PCR) for amplification of cDNAs that can hybridize to the 3' and 5' amplimers. The PCR includes the use of a radioactive nucleotide to allow running the product side-by-side on a gel which is simultaneously detected by autoradiography. Bands are used to ultimately distinguish bands from one cell type compared to the other by the display of the mRNA which are differentially expressed (21). Subsequent identification of a differential gene can be confirmed following excision of the bands from the gel and further amplification with the same primers used for the initial display yielding PCR products which can be applied in probing polyA RNA using northern blot.

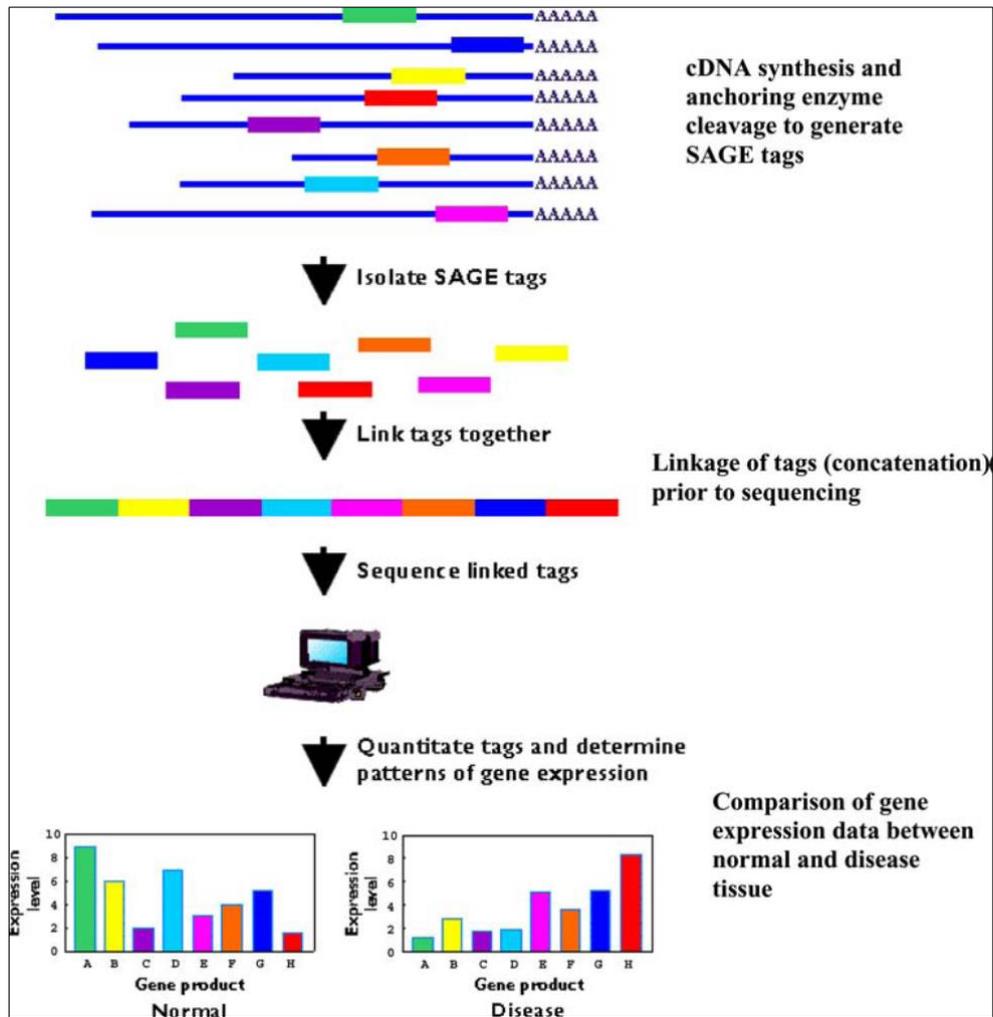
Despite its application in the identification of differential gene expression, a couple of serious problems are noted to limit the usefulness of the technique. First, patterns of differential expression observed on the display is not reproducible using northern blot creating false positive results with a frequency of greater than 70% (22). Secondly, cDNAs obtainable represents only approximately 300bps usually at the 3' end of the mRNA (3'-untranslated region) usually not included in GenBank and varies from one organism to another and as such cannot be matched with already characterized genes. Therefore, obtaining the translated region of the mRNA implies the use of time-consuming protocols for screening cDNA libraries by researchers generating another problem as well, due to similarities of the 3'-untranslated regions of different genes (22).

Consequently, to overcome the problems associated with false positives and labour intensive requirements of conventional differential display; a modified method was described that eliminates such limitations, decreases screening time for a set of primers and minimizes the use of radioactive materials. Such modifications includes, reverse transcription using random hexamers of the total RNA; amplification products are separated using agarose and subsequently visualized with ethidium bromide creating a precision as results of excision of the target band from gels which can be visualized directly and finally; the elimination of a second amplification step associated with conventional techniques, thus, reducing screening time for a set of primers and reduces false results (23)

An improved approach for mRNA differential display was developed to identify genes regulated by muscarinic acetylcholine receptors in HEK293 cells by designing a set of 64 different random primers for comprehensive screening analysis of the mRNA species in cell population. Genes from two cell lines were differentially expressed i.e. cells stimulated with carbacol a cell receptor agonist) and those that are not stimulated with the reagent. This led to identification of 38 different bands in 192 PCR experiments. Northern blotting was used to confirm 81 differential genes in 19 out of 23 bands that were analyzed. The issues arising with the use of equipment, reagents and procedures were also solved by these improvements (24).

#### 1.5. Serial Analysis of Gene Expression (SAGE)

Differential display and subtractive hybridization, although useful in identifying gene differences among transcripts, provide only a small picture and transcripts expressed at low levels can be missed during analysis (25). The SAGE is a highly efficient, high throughput technique that provides for the simultaneous detection and measurement of expression levels of genes in a cell at a given time, rare genes inclusive. This is further supported by (26); indicating that prior knowledge of the genome is not required and that the technique does not require special equipment apart from that found in a molecular biology laboratory, nor is the need for any genomic sequence to influence interpretation of SAGE data required for large number of genes. However, the SAGE is not without its limitations in that, the presence of poly-A tail on the transcript possess a problem to apply the SAGE technique to prokaryotes. The technique is not dependent on information available about the transcripts (25).



**Figure 1** cDNA synthesis and anchoring enzymes cleavage to generate SAGE tags

The SAGE technique is highlighted by two principles. The first is the generation of oligonucleotide sequence tags of approximately 10-11bps with inherent information for identification of a unique transcripts which are subsequently utilized to identify genes and relative abundance of transcript on their mRNA. Secondly, short sequence tags are used to generate a concatamer that allows for efficient transcript analysis in a serial mode since the SAGE involves processing leading to analysis of 25-50 tags on a DNA sequences. Tags are formed by transcription of poly-A tail RNA into double-stranded biotinylated cDNA (ds-cDNA). Anchoring enzyme (Nal III) is used to digest ds-cDNA into fragments with average length of 256bps. Following their isolation with paramagnetic streptavidin genes, the fragments are divided into two equal halves and ligated to two different linkers (25,26). Each linker comprises of a site for tagging enzyme (BsmFI), an anchoring enzyme overhang and a PCR priming site. Digestion of bound linker-cDNA sequences with tagging enzyme releases from the streptavidin beads; fragments that are made up of linker and short cDNA sequence tags. Blunt-ended linker tags are then isolated and the two sets of linker tags are subsequently ligated to linker-ditag-linker constructs and amplified through PCR using specific primers to the linkers. The constructs are finally digested using Nal III to release ditags which are isolated and ligated to generate concatamers for cloning and sequencing. Sequencing of the concatemated tags is utilized for identification of approximately 1000 tags per gel. Quantitation and identification of cellular transcripts can be achieved by this rapid sequencing technique. Sequencing data obtained from the SAGE can be analyzed for identification of every gene expressed in the cell and their level of their expression as well (25). The SAGE methodology was similarly described by Horan, 2009, showing a simplified schematic diagram explaining the process as shown in the figure above.

Furthermore, to improve the efficiency of the SAGE technique, a notable modification that tackles issues relating to mRNA amount needed for library preparation and the use of tag sequencing in identifying corresponding transcripts emerged. Several modifications have resulted in different techniques of SAGE for gene expression analysis such as SAGE-lite have emerged.

Since its introduction, SAGE has found application in the various biological fields, as a result the total tags analysed using the technique are close to five million as shown in the profile below (25). Diverse cells and tissues from a variety physiologic and pathologic situation were identified using this gene expression analysis leading to the collection of various numbers of total tags from different studies involving various cells and tissues (25,26).

### 1.6. Quantitative Real-time/reverse transcriptase-PCR

Many Technological developments have been made using PCR following its discovery in the mid-1980s. Although, quantitative PCR was used before the real-time PCR technique was developed, it has certain limitations such as the requirement of specific training, rigorous testing and lots of pipetting. Real-time PCR brought about rapid development within a short period (27). The real-time PCR which is a recent advancement in PCR technology became a popular technique due to its application in diverse clinical and basic sciences. The technique involves PCR and detection as well as monitoring of accumulated reaction products in every cycle during amplification. One of the outstanding properties of the PCR is its ability of detection of both the presence and quantity of specific target nucleic acids as well as determination of sequence variation (28).

Another sensitive methodology that allows for the rapid quantitation of mRNA levels of selected genes is the real-time reverse transcriptase PCR (RT-PCR) applied in transcriptional profiling and molecular diagnostics. The technique ensures monitoring of fluorescent products accumulation obtained through each PCR cycle (28). In qPCR, the emission of fluorescent signals by the amplified transcripts proportional to the number of genes of amplicons produced in every cycle is estimated. This is achieved by adding a fluorescent detector to the system which is specific to the amplified transcript or one that binds non-specifically to the double-stranded DNA (29). Eventually, the principle of the RT-PCR is based on the use of taq-polymerase, utilizing its 5'-3' exonuclease activity and the use of fluorescent oligonucleotides which are dual-labeled as probes that emit fluorescent signals on cleavage. These two principals were combined in the TaqMan assay which was the first real-time PCR assay developed (29). In addition, the technique has been shown to be advantageous in that it enables gene expression to be compared over a broad range >10<sup>7</sup> -fold, in addition to its high sensitivity, specificity and reproducibility which can be relied upon. Furthermore, its application in a clinical setting is somewhat easier and efficient with regards to the consumption of biological materials (29).

Owing to limitations in conventional PCR techniques to do with labor and expensive nature of the protocol (29) a multiplex format of the PCR was introduced to bring about improvements over the conventional technique in lieu of the above limitations by introducing multiple primers capable of amplifying RNA or DNA from several viruses in a simple reaction; simultaneously (29). A multiplex RT-PCR with enzyme-linked amplicon hybridization assay (ELAHA) was developed, designated the mRT-PCR-ELAHA for detection of amplification products using colorimetric detection methodology (29). This technique uses multiple virus-specific primer pairs in a single reaction coupled with enzyme-linked amplicon hybridization assay (ELAHA) using specific probes for virus to target unique gene sequences. By using this technique, seven respiratory viruses were examined in 598 nasopharyngeal aspirate (NSA) samples from patients. Consequently, other respiratory viruses such as ADV, Flu A and B, PIV types 1,2 and 3 and RSV were studied using this novel technique (30).

Recently, qPCR was applied as a method for detection and quantitation of food-Bourne pathogens. One of the notable advantages of the process is that, it is a much faster technique compared to conventional culture-based methods and was found to be highly sensitive, specific and allows for the simultaneous detection of microorganisms. Due to developments of extraction protocols for various food matrices, either as commercially available kits and simple instruments; RNA integrity can be rapidly checked (30).

### 1.7. DNA Microarrays

Various gene expression techniques for the expression of mRNA levels exists, notably; the northern blotting, differential display (31) serial analysis of gene expression and dotblot analysis. These techniques, although useful, have their inherent limitations that make them unsuitable for the expression of large numbers of gene products. With the northern blot, only a limited number of mRNAs can be studied; differential display can only compare a limited number of different conditions associated with the genes apart from been not quantitative; SAGE involves quite a lot of labor and requires DNA to be sequenced apart from been not very sensitive and the dotblot analysis needs large amount of materials because of the filter size. Against this backdrop, the introduction of DNA microarray that confers high sensitivity and throughput for screening of gene expression is notable development (32). Therefore, the need to analyze large sets of genes in a high throughput manner forms the basis for the development of this technology (32).

DNA microarray has been found to be applied in the survey of thousands of parallel genes as well as expression monitoring (33), in gene polymorphism leading to diseases (33) and for sequencing (33). Two major types of DNA

microarrays have been highlighted as: the oligonucleotide-based arrays and the PCR product-based arrays. The principle involved is the same for both types of arrays; however, differences may arise with regards to its application to prokaryotes or eukaryotes. As an instance, poly-A is used for eukaryotic arrays while prokaryotic arrays require total RNA labeling for a bacterial experiment using the array (34).

The arrays are made through deposition of DNA spots on a solid support usually a coated glass surface which is somewhat different from the conventional nitrocellulose or charged nylon supports. The surface of the glass allows for; arraying molecules in a parallel manner, miniaturizing the procedure and use of fluorescent dyes for detection. No diffusion of materials onto the support occurs, therefore, focusing for laser scanning microscopy can be adopted reduced using either photolithography developed by affymetrix Inc.

Photolithography involves the use of UV light sources passing through a mask that directs the stepwise photochemical reaction (oligonucleotide synthesis) on a siliconised glass surface. Masks are produced with openings that are as small as few micrometers giving a density equivalent to hundred thousand probes per centimeter of the glass. However, multiple probes per gene is allowed when using high-density arrays (32). Mechanical gridding on the other hand, utilizes an inkjet or physical disposal of materials with high precision pins. The system is vulnerable to evaporation and contamination due to surface contact and transport of small amounts of liquids with dust particles. The gridding instrument utilizes an XYZ motion control with high precision based on step engines. It allows spotting of 96 or 384 well plates to glass in a defined pattern (35).

Oligonucleotides can be utilized as probes for both photolithography and gridding while cDNAs are mostly utilized for gridding. Differences exist in terms of hybridization reactions in the two cases, with both requiring special preparations so that the probes can be used optimally. Samples are fragmented to prevent tertiary structures and to attain maximum hybridization when shorter probes of 25-50 nucleotides are employed (35). Fragmentation of samples is not required with larger probes of 300-2000 nucleotides in length (34). Thousands of DNA spots can be found on an array, each containing large number of identical DNA molecules which may vary in length between 25 to hundreds of nucleotides. Labeled gene probes from the biological system to be investigated are then applied on the arrays and washed. Hybridization of the gene products to complimentary sequences in the arrays allows measurement of the amount of samples bound (33). The hybridization conditions like; concentration of samples, ionic strength as well as temperature is shown to be highly dependent on DNA fragments' size present in the array (34).

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### 3. Conclusion

Although various technological approaches are available for the effective gene expression analysis as highlighted in this study, progress are been made to develop new technologies to satisfy our demands to carryout research in the field of molecular biology. It can be noted that developments in technologies have resulted in new throughput techniques that are highly sensitive, specific and reproducible making the old conventional techniques somewhat obsolete due to one or more limitations that are inherent in their applications for gene expression analysis. Health hazards, labor and cost of equipment have been the predominant drawbacks with conventional techniques.

Owing to this, selections of currently available tools have to be made with care. Alternatively, combination of techniques available will be a reasonable approach, if carefully selected. This is because gene expression is done on a global scale and what is obtainable or affordable in one area or region may be a burden for researchers elsewhere. Therefore, one can decide on which technology best suits his demand or needs of the laboratory and project to be undertaken, taking into consideration, the efficiency of the technique of choice as well.

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### Compliance with ethical standards

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The authors declared no conflict of interest throughout the project, however, there are constructive argument at some point in the research but valid agreement was cemented before publishing the result.

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