NANOGENESEQ CHIP DESIGN TECHNOLOGY

Executive Summary

NANOGENESEQ founders are Drs. Ajith Kumar (Ajith) and Arun Kumar (Arun), Medical Graduates from Medical College, Trivandrum actively engaged in several advanced mathematics, biotechnology and nanotechnology projects with national and international research organizations.

Drs Ajith & Arun conceptualized the launch of NANOGENESEQ

Since the discovery of the DNA, sequencing has become the most significant routine activity in the field of genetics and drug discovery and their related applications. However, there are still many caveats and science is away still from translating genome sequence data in useful preventive or therapeutic remedies for diseases. The new race is in assigning to genes and to variations in genes, a role in disease initiation, progression and drug response. The goal is to identify genes expressed in disease and not in healthy tissues and once such task is accomplished, to design molecules that interact or block the harmful proteins made by these genes. Both require sequencing and proteomics technology.

Current DNA and protein separation and detection technologies are far from being perfect. They are still complex, time consuming, expensive, relatively inefficient and, henceforth susceptible to substantial improvements and open to innovative approaches. In the high-throughput

sequencing sectors, such as modern drug discovery, the improvements needed are in efficiency and cost of running automated systems.

NANOGENESEQ Inc. technologies address these two needs with a highly innovative and efficient system, the NANOGENESEQ System, which optimize DNA and protein sequencing at both the efficiency and cost level. NANOGENESEQ as a system comprises both hardware and software elements.

The NANOSEQ HARDWARE system consists of two components: the NANOCHIP (NC) and the HELICAL LASER SCANNER (HLS).

NC is a cube like structure composed of juxtaposed silicon plates (wafers). It has a multiple sieve architecture made possible by nanoscale channels etched in the wafers. The nanochannels of each wafer are connected to the channels of the wafer lying immediately below , thus forming a sponge-like device. The size of the channels in each wafer decreases from top to bottom, i.e. they are largest in the top wafer and smallest in the bottom wafer. It is this multiple sieve sponge-like architecture that allows NC to separate large molecules according to mass and shape.

HLS is a moving semiconductor laser incorporated within the NC, which is capable of detecting the movement of the molecules and DNA segments flowing through the NC. Its capability to move in a spiral fashion allows it to measure the size and shape of molecules as they arrive at the end of each of the nanochannels and empty into the detection chamber.

NC and HLS, being made of semiconductor materials that can be wired to microprocessors down to the nanoscale level, can be remotely

controlled as to modify key parameters, such as flow rate of molecules within the NC and the temperature in discrete portions of the device. This in turn allows to remotely control, through the use of advanced self-learning algorithms, the order in which molecules are separated and detected.

The key advantages of NC are:

- *NC allows zero resistance flow and avoids overlapping of molecules NC triples the separation and detection efficiency of the most advanced of existing systems*
- *NC allows heterogeneous separation.*
- *NC favors sphere head formation and flow*
- *NC increases the parameters that can be measured.*
- *The entropy of flow of molecules within the device can be measured and controlled*
- *The flexibility of the NC device itself can be controlled*
- *With NC the choice of fluorescent dyes that can be used is greatly increased*

The key advantages of HLS are:

- *HLS is a semiconductor micro-laser with nanoscale level sensitivity*
- *HLS measures the changes in shape of molecules as they pass through the NC device*

- *HLS enormously increases the amount of information that can be gathered on molecules behavior.*
- *HLS will allow multicolor detection and use of a much wider variety of fluorescent dyes.*
- *HLS will make heterogeneous detection a reality*
- *HLS allows to isolate single molecules within a mixture and selectively separate it from the mixture*
- *HLS can be remotely controlled by feed-back loops*
- *With HLS, molecular self-assembly becomes possible*

NANOGENESEQ HARDWARE is complemented by proprietary NANOSEQ algorithms used techniques to design novel, monitor and control the HARDWARE.

NANOGENESEQ has designed novel algorithms based on mathematical models that are used in industry and university laboratories to simulate chaos, atomic explosions, particle physics experiments, fluids dynamics, turbulent flow and control of micro electronic devices and smaller scale structures, etc.

Software based on fractal geometry principles, an ideal model since it is readily convertible into binary code, was used to design NANOCHIP.

Self-learning algorithm , which are iterative in nature, originate from simple basic rules called initiators and generators, and are capable of performing very complex analytical studies, have been developed to control both NC and SLS.

NANOGENESEQ SOFWTARE is strategic to the company and hence constitutes a key component of its intellectual property.

The market for genetic data and technology is projected to be worth several of billions of dollars within the next decade. Products provided by genomics and proteomics firms include not only databases and specialized software to search the databases, but also automated sequencers, and DNA microarray systems. The sequencing and microarray systems are the reference markets for NANOGENESEQ products.

. The key competitors (potential partners!) in the next 5-10 years that NANOGENESEQ will face are the manufacturers of conventional and next generation electrophoretic based systems being developed by Lab-on-a-Chip companies such Affymetrix, Amersham Pharmacia, Agilent Technologies, Rosetta Inpharmatics and the PE Corporation , which includes Celera and Applied Biosystems.

The key advantages described above, when compared with the most advanced systems available today translate into highly competitive advantages:

Inherent advantages

- *NANOGENESEQ (NC and HLS) allows to selectively separate and detect individual molecules within an heterogeneous mixture*
- *NANOGENESEQ increase the data readout on single molecules allowing to take 3D measurements, the changes in shape, the speed of changes in shape the entropy of molecule flow through the device and the flexibility of the device itself; all these measurements allow to predict the*

molecule type and are the data feed for the self-learning algorithms , thus improving their predictive capacity over time. This is especially useful for proteomics studies and for molecular self-assembly applications.

- *NANOGENESEQ allows to measure the entropy of flow and device flexibility, thus allowing to control the separation and detection process. The entropy of flow be modified via software so that the chip can be commanded to perform different tasks, according to the detection target.*
- *NANOGENESEQ is modular, thus allowing to build arrays of NCs & HLS to perform multiple functions and tasks.*
- *NANOGENESEQ has longer chip life cycle and reduces energy costs during operations . The flexibility of the chip reduces the frictional and heating effects, so the chip doesn't need any cooling devices, like cooling fans. This drastically reduces the cost during operations and results in longer chip life.*

Sequencing advantages:

- *In the case of DNA, NANOGENESEQ triples the amount of base pairs that can be sequenced on a given segment to 2100 from the current 700;*
- *NANOGENESEQ widens the choice of fluorescent dyes that can be used , thus making multicolor detection a reality; multicolor detection , i.e. the use of multiple sets of 4 dyes each, will allow multiple simoultaneous sequencing of more than one fragment within the same sequencing step.*

Proteomics advantages

- *NANOGENESEQ allows 3D scanning of molecules as they flow within the*

device, thus allowing to selectively detect individual protein forms as they move through the device. This translates into a much improved efficiency in screening studies

- *Integrating several NCs into a larger composite NANOGENESEQ (as in a chessboard arrangement) provides an extraordinary platform to do more integrative protein detection tasks with respect to current methods and this a key advantage for proteomics applications.*

Molecular self-assembly

- *HLS provides the 3D measurements needed to identify and select possible self-assembly candidates within a mixture and NC can selectively separate them as to bring them within the same environment where they can explore self-assembly arrangements. This can be exploited to develop novel chemistries for use in nearly all fields, from advanced materials to pharmaceuticals.*

The considerations so far described have encouraged the founders to launch a start-up company whose mission will be to further development, final engineering and prototyping of its DNA sequencing systems and the acquisition of one or more strategic partners for their manufacturing and commercial development.

During the startup phase NANOGENESEQ will achieve the following milestones:

- *Experimental device development and testing*
- *Integration of the device to a microchip & filing of patents*
- *Testing sample DNA and comparing it with conventional methods*
- *Development of a device incorporating laser scanners and development of a full scale working prototype*

Acquisition one or more strategic partners for commercial development

The Founders

NANOGENESEQ founders Drs. Ajith Kumar (Ajith) and Arun Kumar (Arun), Medical Graduates from Medical College, Trivandrum actively engaged in several advanced mathematics, biotechnology and nanotechnology projects with national and international research organizations.

Market Need Rationale

Since the discovery of the DNA, sequencing DNA has become the most significant routine activity in the field of genetics and drug discovery and their related applications. Year 2000 was the annus mirabilis of biology, the year when scientists announced that their mission to compile the human genome was essentially complete. The biologists' equivalent of

landing man on the moon. However, despite the fanfare, there are still many caveats and science is far away still from translating genome sequence data in useful preventive or therapeutic remedies for diseases.

The human genome is by no means complete and sequencing alone will not lead to new drugs. It is just one step in a long, very long road to routine and effective use of genetic sequencing data to support drug discovery and development efforts. With the genome sequencing race finished, a new one is on. The new race is in assigning to genes and to variations in genes, a role in disease initiation, progression and drug response. This is a huge task currently being tackled with modern drug discovery tools (functional genomics, proteomics, bioinformatics, etc.) . The goal is to identify genes expressed in disease and not in healthy tissues and once such task is accomplished, to design molecules that interact or block the harmful proteins made by these genes. The bottom line is that DNA sequencing, far from being over, now that a rough map of the human genome has been produced, has become a mainstay technique in biomedical research at all levels, in both academic and commercial environments.

Current DNA and protein separation and detection technologies are far from being perfect. Notwithstanding the enormous progress made in recent years with automated sequencing robots, they are still complex, time consuming , expensive, relatively inefficient and , henceforth susceptible to substantial improvements and open to innovative approaches. In the high-throughput sequencing sectors, such as modern drug discovery, the improvements needed are in the sequencing efficiency and cost of running

automated sequencing systems, often operated 24 hours a day every day of the year. At the bench level in academic labs, the market need is for simple –to-use devices that are easy to adapt in "home brew" systems specific for the individual scientist's needs.

NANOGENESEQ technologies address these needs with a highly innovative and efficient system which optimize DNA and protein sequencing at both the efficiency and cost level.

Scientific Rationale

There are various methods to separate DNA fragments or proteins and to detect them. The most relevant ones are based on electrophoresis, capillary action, capillary gel electrophoresis, ion separation, phase transition etc. In the case of DNA, the most widely used are based on capillary electrophoresis separation of DNA strands followed by detection, again by various methods.

All current sequencing techniques involve similar steps and the process is exemplified below:

- *first the DNA stretch to be sequenced is cut down in segments of a size small enough,100.000-200.000 base pairs , so that they can be individually stored in E.coli clones; the collection of clones is called a clone library;*
- *for sequencing purposes the size of the clone is further reduced by cutting each single stretch down to 2000 base pair fragments which , again, are stored individually in E.Coli sub-clones;*

- *the sequencing of each of the stored fragments starts by producing billions of copies of the DNA fragment by colturing the E.coli subclone that contains it overnight, and recovering the fragments by means of chemical digestion & separation from bacterial debris*
- *the sequencing reaction requires four components:*
  - *the template DNA , i.e. the billions of copies of DNA fragments)*
  - *free bases, the building blocks of DNA (A, T, C, G) ; about 5% of the free bases are tagged with a fluorescent dyes of a color specific for each of the bases (yellow, red, green, blue); apart from identifying the base, the dye-labeled bases have the purpose of halting the copying reaction after they are matched with the complementary base by the polymerase enzyme;*
  - *the primer, a short sequence of DNA used to initiate the DNA copying reaction*
  - *DNA polymerase, an enzyme which guides the copying of the fragment sequence by ensuring that each base is matched with its complementary case (C with G – T with A)*
- *The template copying process starts when the primer attaches to template DNA; this is the signal for polymerase to cause the free bases to attach to its complimentary base on the template by reading one by one the template sequence and choosing the proper match;*
- *Approximately 5% of the time , instead of a free base, polymerase will incorporate a dye-labeled base in the sequence; at this point copying stops; this is a key step of the sequencing reaction; in fact, given that the fragment to be sequenced is 2000 bases long, and given the fact that*

billions of copies abound in the reaction vessel, it is 100% sure that a series of different length template-complementary fragments will be produced spanning the entire template sequence, starting from the first base to approximately 700 bases . This process is exemplified below:

primar-template sequence: *primer -----**ATCG**……*

*primer -----**T****

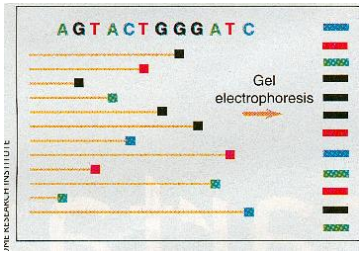
*primer -----T**A****

*primer-----TA**G****

*primer-----TAG**C****

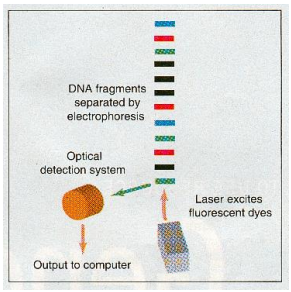
*where * stands for the dye-labeled bases.*

The different length complementary fragments will have a different molecular weight and hence it will be possible to separate them by electrophoresis , where the electrical field causes the flow of the fragments through the gel and the rate of flow of each fragment is directly related to its molecular weight:

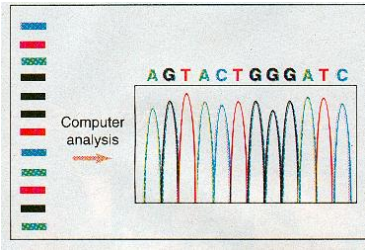


The fluorescent tag at the end of the fragment will identify the specific base and by reading the fluorescence tags, the template complementary

sequence is obtained; in automated sequencers the fluorescent dyes are excited by lasers and are read by optical detection systems ;



the data output is then fed to a computer which provides an analysis of the sequence



The majority of DNA sequencers available on the market today incorporate the basics of the method described above.

Although capillary electrophoresis sequencers have undergone several evolutionary improvements, they all share the following common disadvantages:

- *They are plagued by overlapping problems: overlapping of different DNA fragments, as they move across the gel or membrane, makes*

sequencing and detection inefficient and expensive, since it slows down the sequencing process and imposes the use of expensive reagents which brings the commercial cost of sequencing at $10-12 dollars per base pair.

- *Only few DNA fragments can be flown in the separation columns at any given time, to avoid the overlapping problem*
- *Can only sequence up to a maximum of 600 to 700 Base pairs at any given time, again , due to severe overlapping among the base pairs that lead to high levels of inaccuracy.*
- *Use traditional laser detection systems that severely restrict the use of dyes to only few low molecular weight dyes with fluorescent properties. This means that high molecular weight dyes, soluble dyes, heat-stable dyes, etc. cannot be used in conventional systems.*

NANOGENESEQ has designed, by using highly innovative fractal algorithms, separation and detection systems which eliminates most of the disadvantages, dramatically improve sequencing precision and throughput and, as result, significantly reduce sequencing cost per base pair. The novel design, based on nanotechnology approaches, can be rightly considered the next generation of high throughput sequencing technology.

1. Mission and Goals

Mission

NANOGENESEQ's mission is to further development, final engineering

and prototyping of its DNA sequencing systems and the acquisition of one or more strategic partners for their manufacturing and commercial development.

Goals

During the startup phase NANOGENESEQ will achieve the following milestones:

- *Experimental NC device development and testing*
- *Integration of the device to a microchip & filing of patents*
- *Testing sample DNA and comparing it with conventional methods*
- *Development of a device incorporating the SLS and development of a full scale working prototype*
- *Acquire one or more strategic partners for commercial development*

The Core Technology

NANOGENESEQ as a system comprises both hardware and software elements.

NANOGENESEQ HARDWARE SYSTEM

*The NANOSEQ HARDWARE system consists of two components: the NANOCHIP (**NC**) and the HELICAL LASER SCANNER (H**LS**).*

NC is a cube like structure composed of juxtaposed silicon plates (wafers). It has a multiple sieve architecture made possible by nanoscale channels etched in the wafers. The nanochannels of each wafer are connected to the channels of the wafer lying immediately below , thus forming a sponge-like device. The size of the channels in each wafer decreases from top to bottom, i.e. they are largest in the top wafer and smallest in the bottom wafer. It is this multiple sieve sponge-like architecture that allows NC to separate large molecules according to mass and shape.

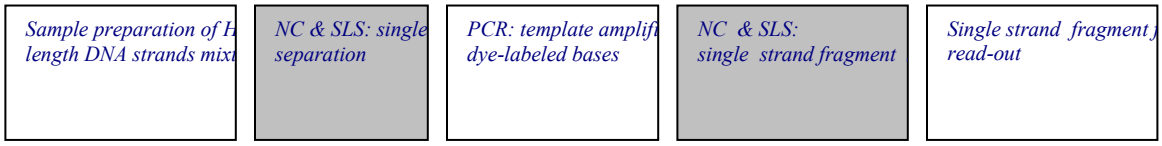
HLS is a moving semiconductor laser incorporated within the NC, which is capable of detecting the movement of the molecules and DNA segments flowing through the NC. Its capability to move in a spiral fashion allows it to measure the size and shape of molecules as they arrive at the end of each of the nanochannels and empty into the detection chamber.

NC and HLS, being made of semiconductor materials that can be wired to microprocessors down to the nanoscale level, can be remotely

controlled as to modify key parameters, such as flow rate of molecules within the NC and the temperature in discrete portions of the device[1,2,3,4]*. This in turn allows to remotely control, through the use of advanced self-learning algorithms, the order in which molecules are separated and detected*[5,6,7]*.*

As such, taken together, NC and HLS allow to separate and detect single molecules within an heterogeneous mixture, or allow multiple detections. They will allow moreover to characterize the 3D shape of complex molecules, such as proteins or cells and, ultimately, allow single fragments of molecules, possessing complementary surfaces, to self assemble and form self assembly linkages within the device, by selectively separating and detecting such fragments according to the characteristics of such surfaces and shapes they possess so as to align them in the proper sequence.

In their initial configuration NC and SLS will form the critical components of high-speed sequencing systems. Their functional location within such systems is exemplified in ***Figure 1****, below , by taking DNA fragments as an example:*



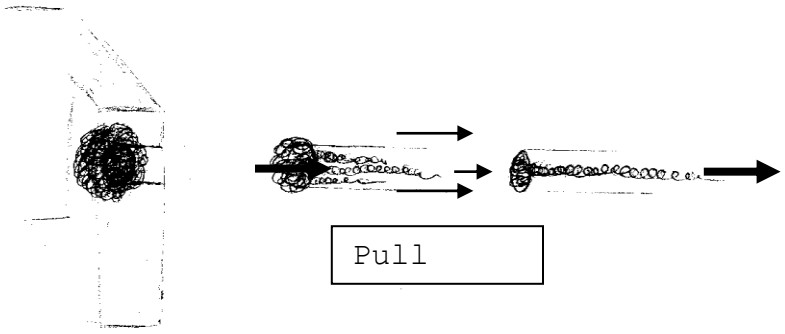
Theoretical & experimental basis and key advantages

The development of NC and HLS embody the most advanced theories in mathematics and semiconductor science[8,9,10].

NANOCHIP

Development of the NC device hinges on studies done by several labs in observing the behavior of large molecules, such as DNA, flow in microscopic passage ways and the specific shapes and patterns it produces, namely, the roughly spherical shape that it assumes in deep sections, the stretching shape and behavior in the shallow sections.

Recent work has shown that when DNA samples are placed in a water solution and are flown through channels, consisting of deep and shallow alternating areas, by applying an electric field to draw the DNA fragments towards the other end of the channel, what happens is that in the deep section the chain-like DNA fragments contract into a roughly spherical shape, too large to fit through the shallow sections. Hence, at the border between a deep and a shallow section, the fragment is trapped until it can stretch out thin enough to fit through the shallow section of the channel. When this happens the large fragment zips through the shallow channel section faster than short segments, the opposite of what happens in organic gels. The reason is that longer strand form larger spheres which in turn exercise pressure against a larger area of the barrier, meaning that there are more parts of the sphere that extend into the shallow area to form a sort of beachhead which pulls the rest of the fragment through, as shown in ***Figure 2****, below:*



This work, conducted by advanced nano technology laboratories (Cornell Nano Biotechnology Center) is focused on the development of microchips, incorporating the above nanochannel design with deep and shallow sections, but on a planar , 2D, design based on the use of plane geometrical principles[11].

NANOGENESEQ scientists have instead arrived at the conclusion that a 3D approach would represent a true innovation for the field. The effort required the use of fractal geometry and chaos theories,[12,13,14,15] the only ones powerful enough to design complex 3D structures comprising up of 2400 nanoscale channels, each divided in alternating shallow and deep segments, to wire each channel for readout purposes and to simulate the fluidics behavior of large molecules in a nanochannel environment. To optimize fluidics, holes (less than 1mm diameter) were designed in an alternating pattern in areas with no channels.

In the end, their efforts yielded a design which is superior to planar geometric designs since it is a 3D design, where asymmetric channels provide easy stretching and contracting of molecule fragments forming larger and smaller spheres and various other shapes and allow for much greater separation efficiency.

This theoretical work " in silico" was based on advanced mathematics algorithms that are by themselves an invaluable proprietary asset.

To test the in silico model in real world situation, the scientists resorted to inspiration from the work done by Sir Geoffrey Taylor with the "Heleshaw cell". Taylor, a British physicist first used this cell to study and recognize fluidics problems for commercial applications in the oil industry. The Heleshaw cell allows to simplify the flow of fluids by confining it to a thin layer, to trap fluids so that they can then be detected and photographed.

The Heleshaw cell was adapted to monitor the flow of fluids in 3D porous structures and to produce flow patterns in microfluidics[16]. Moreover, the Heleshaw cell , which has a fractal architecture, allowed to study the flow properties of heat producing materials, such as organic molecules. The assumption was that the fractal architecture would minimize the production of heat, which is produced by the flow of molecules through a matrix. In the case of DNA, heat makes the DNA fragments unstable and causes uncoiling. The instability and the uncoiling, in turn, slow down the flow of the fragment, due to increased friction, and produce even more heat.

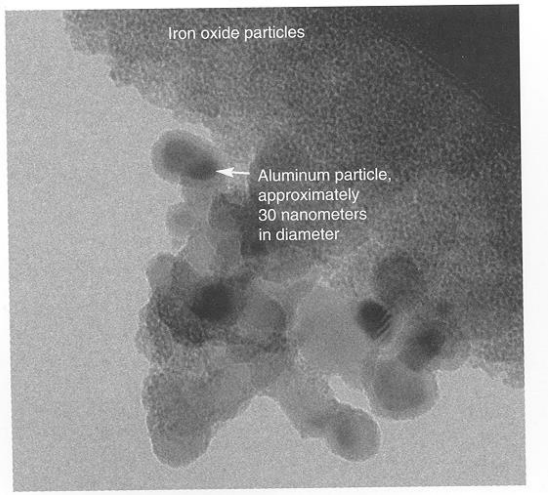
This is one reason why in conventional gel methods different segments are not easily separated. NANOGENESEQ scientists reasoned that

if they were able to lower the overall heat output in the separation matrix, this would facilitate separation of segments of different lengths. They further reasoned that the flow of heat producing materials could be controlled by designing a matrix with a fractal design, and that this feature could be used to avoid instability and uncoiling of the DNA fragments so that they could be very easily moved and controlled through the micro and nano structures of the matrix. Finally they concluded that this design would increase the possibility to obtain spherical structures, which in turn would facilitate the flow. They also developed novel ways to improve upon certain advanced etching methods on a nanotechnology scale[17,18] .

*To simulate the flow of heat producing materials, also called thermitic material, they used a mixture of aluminum oxide and iron oxide. This is a standard mixture used to study the impact of heat producing mixtures on different materials . One of the major property of such a mixture is that it is highly reactive, produces intense heat and is uncontrollable when mixed in conventional ways. But using fractal geometries these materials can be made to mix very effectively without such intense heat emitting property and it can be controlled very well. It also allowed the flow of thermitic material effectively by lowering the overall heat output of the system and by allowing for the formation of spherical heads , as shown in **Figure 3**, below.*

Figure 3 shows how the aluminum oxide & iron oxide mixture flows through a fractal geometry and produces spheres that regulate flow. It also

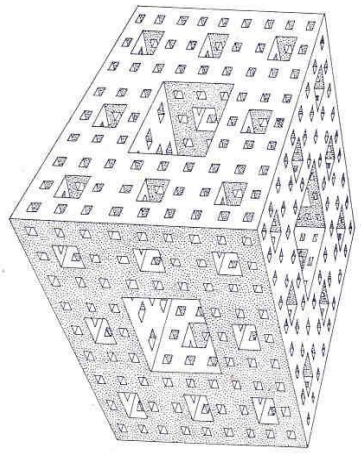
gives evidence of formation of larger number of spheres when the scales are reduced to micro & nano levels.



The first experimental device they constructed consisted of 12 square acrylic plates measuring 2 inches on a side and with a thickness of 1 mm, stacked on one another other in the same manner as a cover slip is placed over a microscopy slide containing a specimen. Each plate contained shallow grooves and rough surfaces. To facilitate injection of fluids containing colloid materials and suspended solutions, they drilled holes (less than 1mm diameter) in an alternating pattern in areas where there were no grooves, to facilitate the charging of the cell. The 12 plates were spaced by the use of spacers, adjustable to within millimeter width.

The finished device architecture, consisting of stacked plates gave the 3D architecture needed for their purposes, and it closely simulated a "Menger" sponge-like structure which allowed to carry out fluidics studies on a macroscopic scale. For Micro and Nanoscale they are currently using a new innovative method called discrete branched etching.

A graphical representation of a "Menger" sponge - a cube-like (12 µm x 12 µm x 12 µm) fractal sponge architecture with the smallest square measuring 100 nm, the larger square measuring 3 µm and central holes measuring 5 µm - is provided in ***Figure 4****, over:*



They then passed colloids, dyes and solutions containing suspended particles to check and understand their flow pattern and behavior and photographed it. This device has allowed them to record the peculiar behavior of large size and high molecular weight molecules when drifted through the device to which an electric field had been applied. As it happens in gel electrophoresis, the molecules assumed various shapes.

Armed with this knowledge they then proceeded to simulate fluidics with large size and high molecular weight molecules at both microscopic and nano scale. Their experimental assumptions were strongly backed by studies performed at Cornell with plane geometrical architectures, to study the behavior of, and to DNA fragments in microscopic passage ways.

Key advantages

- *NC allows zero resistance flow and avoids overlapping of molecules*

In conventional systems, as molecules slowly make their way through the tiny pores of the gel, one of the main problems is their overlapping caused by the intense density of the media (gel or membrane) which acts as a "viscous" substrate.

NC has been designed specifically to avoid molecule overlapping. This is achieved thanks to its porous fractal architecture, which acts as a separation sieve, and to the fact that molecules can be carried by non-viscous, low/zero resistance media, such as water, thus acting as a highly transparent medium. This allows molecules to travel freely, without overlapping and also makes molecules visible thanks to this high transparency.

- *NC triples the separation and detection efficiency of the most advanced of existing systems*

The most advanced capillary electrophoresis systems existing today can separate DNA fragments up to a maximum of 700 base pairs. It cannot handle larger sized DNA fragments due to the inherent smaller dimensions of the gel pores. In one hour, the most advanced system available today is generally capable of separating 500 base pairs

NC, thanks to its inherent porous characteristics and to the zero resistance media, can handle larger size molecules, up to 2100 base pairs,

and can separate 1500 base pairs or more in one hour. This is at least 3 times as much as the most advanced system today.

- *NC allows heterogeneous separation.*

NC acts like a multiple sieve where each sieve (corresponding to each wafer in the stack forming the cube-like structure) has a particular dimension and is connected in parallel to the sieve (wafer) immediately below. Each sieve constitutes a separation element, with the first for the largest molecules and the last for the smallest. The sieving elements are the nanochannels which have a discrete geometry etched in the wafer that allows to separate the smaller molecules from the larger.

Once the test solution is injected into the system, all the molecules are channeled in one chamber on each wafer from which, thanks to the sphere head formation phenomenon previously described, the longest ones to flow first, followed by medium size and small size molecules, respectively.

- *NC favors sphere head formation and flow*

The nanochannel system in NC, having alternating deep and shallow sections, allows the formation of sphere heads which will differ in size according to the mass and length of the molecules. Larger and longer molecules form larger sphere heads. The larger the sphere head the faster the flow through the channels. This means that larger DNA fragments or molecules flow out of the system first.

The relative speed by which molecules flow out of the system is also greatly increased. This phenomenon is totally the opposite of what happens in conventional and capillary electrophoresis, where shorter fragments travel faster through the gel due to a lower mass.

- *NC increases the parameters that can be measured.*

In conventional and capillary electrophoresis systems the amount of information that can be obtained on the characteristics of molecules is limited to 2 parameters, length and speed, since flow of the molecules in such systems happens according to a planar , 2D, mode.

With NC, in which flow happens in a 3D environment, in addition to length and speed, also depth, entropy of flow and device flexibility can be measured. This allows to measure the speed at which sphere heads form and at which they zip through the channels, their 3D dimensions, the entropy of flow and the flexibility of the device. The additional parameters amplify exponentially the data output of the system and allow to build parameters databases on which advanced algorithms can feed to predict, detect and control the separation and detection process.

The entropy of flow of molecules within the device can be measured and controlled[6] *. The fractal architecture of NC minimizes the production of heat (entropy of flow), which is produced when flowing molecules through a matrix. In the case of DNA, heat makes the DNA fragments unstable and causes uncoiling. The instability and the uncoiling,*

in turn, slow down the flow of the fragment, due to increased friction, and produce even more heat.

NC , fabricated with semiconductor materials at a nanoscale level which allows to wire each single channel to a microprocessor, allows to measure the entropy of flow in each single channel and to control it via software and microprocessors.

This in turn allows to control the entropy of flow , to avoid instability and uncoiling of the molecules, to modulate the speed at which sphere head form and zip through the device, and ultimately, to selectively control the speed and order with which molecules flow through the device.

The possibility to control entropy makes NC function intelligently. Entropy in NC can be controlled, as in a switching circuit, through software. Control of entropy will allow to select and detect a specific DNA fragment or protein from a heterogeneous mixture. This has never been done before and it is a truly novel feature which only NC has.

- *The flexibility of the NC device itself can be controlled*

NC has inherent flexibility properties thanks to its fractal geometry (variable architectures with expanded and contracted – i.e., deep & shallow - regions which minimize friction and flow entropy) and to its microprocessor-controlled nanochannel architecture.

These two features allow to optimize the flow path of molecules directing it (remotely by the use of advanced algorithms) through the least resistance (i.e. lowest entropy) route and to modify the entropy of the

flow[6,7]. In this respect, NC is a flexible device in the sense that its functions can be modified according with the molecules to be separated and detected. This is another novel feature that only NC has.

- *With NC the choice of fluorescent dyes that can be used is greatly increased*

The choice of dyes that can be used with existing systems is limited to few low molecular weight dyes with fluorescent properties. This is due to the thermal instability of low molecular weight dyes and to the fact that the laser equipment used with existing systems distort fluorescent dyes, thus limiting their number to very few low molecular weight dyes with fluorescence wavelengths restricted to a very narrow band. (see also the section on HLS, over)

With NC , thanks to its inherent fractal design and to the possibility to control the entropy of flow, the choice of dyes is greatly enhanced to include large molecular weight, heat resistant and soluble dyes. This novel feature of NC enables multicolor detection to enhance pattern recognition[16]. Pattern recognition, which is made possible by the detection of large number of frequencies and wavelengths, is especially useful in the identification and detection of a far larger number of molecules in heterogeneous detection and of proteins of different classes in proteomics applications.

HELICAL LASER SCANNER

HLS is a moving semiconductor laser incorporated within the NC, which is capable of detecting the movement of the molecules and DNA segments flowing through the NC. Its capability to move in a spiral fashion allows it to measure the size and shape of molecules as they arrive at the end of each of the nanochannels and empty into the detection chamber.

- *.*

Current scanning systems used in micro electronic devices can only scan in a 2D scheme and have, hence, inherent limitations in terms of the number of molecules that they can detect and in terms of the amount of data that they can generate. Their use with NC would be highly inefficient, given the large number of molecules that it can handle and the great amount of data that it can generate.

Hence, to take full advantage of the NC features NANOGENESEQ scientists resorted to the most advanced laser technology available at the nanoscale level, namely semiconductor lasers which can be incorporated directly on a silicon chip. This revolutionary design, drawing form the most recent advances in material science, would allow to build a microlaser, possessing the capability to take 3D measurements, directly into the NC. . The HLS architecture is based on the "microcavity" laser strategy where the compounds to be tested are placed between gallium arsenide and one of the mirrors that reflects light in a circular fashion[23,24,25,26]*.*

The HLS micro-laser is made up of tiny pieces of gallium arsenide sandwiched between two mirrors. In this type of laser gallium arsenide, the

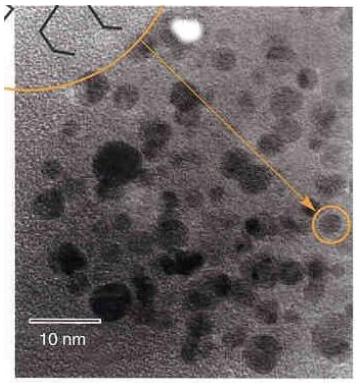
light excitation source, emits infrared light that repeatedly bounces between the mirrors, intensifying it, until it finally bursts out in a concentrated laser beam.

The capability to take 3D measurements was achieved by designing a set of mirrors where one of the mirrors moves in a circular fashion and hence allows a spiral scanning read-out pattern by the gallium arsenide beam.

The rotating mirror function in HLS is provided by quantum balls. Nanometer scale quantum balls can be made by bonding quantum wires to the surface of a quantum dot. The quantum dot in HLS acts as a rotating mirror and it emits light of very high frequency. It is this property which makes higher level detection possible as compared to conventional lasers. The quantum dot is able to detect molecules at nano level as the laser threshold is in the nano ampere range.

The quantum dot laser takes 3D measurements, a key feature to detect the dimensions of sphere heads and to take 3D measurements of proteins in proteomics applications.

*A photo of a quantum dot is provided below in **Figure 5**, below, where the arrow head points to a quantum dot.*



To improve detection efficiency, voltage controlled spatial light modulators, based on polymer films containing oriented light adaptive biomaterials (e.g. DNA base pairs with fluorescent tags, bacteriorhodopsin etc.) will be incorporated in the future[9]. This improvement can further greatly increase detection and effectively enhance and speed up the sequencing procedure.

HLS is a true nanoscale 3D imaging system. Spatial light sensing in sequencing and proteomics systems has not been used by anyone yet. It represents a true innovation all by itself.

Key advantages

- *HLS is a semiconductor micro-laser with nanoscale level sensitivity*

The quantum dot, the scanning element of SLS, is extremely sensitive, with a threshold in the nano ampere range, and thus is able to scan molecules at the nanoscale level. This features allows it to scan and

measure multiple points of the molecules' surfaces, thus allowing to plot accurate descriptions of the molecules 3D structure.

- *HLS measures the changes in shape of molecules as they pass through the NC device*

HLS is in-built into the NC device. It takes 3D measurements of molecules as they pass through NC and hence records over time the changes in shape of the molecules, thus providing a wealth of information on speed of sphere head formation, length and width of molecules and their behavior in different flow entropy environments.

- *HLS enormously increases the amount of information that can be gathered on molecules behavior.*

As molecules pass through the NC, they change shape and form sphere heads, according to their size and length. The information gathered by HLS, over time, on the behavior of different molecules will allow to build, over time databases on the different molecule parameters which can feed information to self-learning algorithms[7] capable of predicting key parameters of molecules to be separated and detected.

- *HLS will allow multicolor detection and use of a much wider variety of fluorescent dyes.*

Conventional laser systems distort low molecular weight dyes. This limits the number of fluorescent dyes that can be used to very few low molecular weight dyes and limits detection to fragments with fluorescence wavelength restricted to a very narrow band. The end result is that the number of multicolor bands that can be detected at the same time is severely limited. In fact, the argon ion laser (which is the excitation source in many flow cytometres, confocal laser scanning microscopes, laser scanners, sequencers etc.) the wavelengths used to excite green, yellow, orange, red fluorescent dyes are limited primarily to the 488 and 514 nm spectral lines. It can also go higher to ranges like 430-470 etc, but in these ranges the intensity (frequency) is reduced and the detection capabilities are distorted. In fact in these ranges it cannot detect any specific frequency but only average weighted ones.

HLS is able to scan and detect wavelenghts, in the 380-430 nm spectral lines, specifically, and average weighted ones in the 330-360 nm spectral lines. This will allow to use a much wider variety of dyes (novel multicolor, high molecular weight, soluble and heat resistant dyes) and enabling multicolor detection to levels never experienced before, also thanks to the lack of overlapping problem afforded by NC and to the inherent spiral scanning mode of HLS.

- *HLS will make heterogeneous detection a reality*

In conventional systems, which rely totally on 2D flow, the amount of information that can be obtained on any given molecule is limited to 3

parameters: the length , flow speed and fluorescence peak. No more, due to lack of additional parameters for detection.

In NC, which allows flow in a 3D environment, SLS provides the measurements of many more parameters which relate to behavior of molecules when they flow in a 3D environment (formation of sphere heads, their 3D dimensions, the speed by which they zip through the nanochannels, the entropy of flow). This in turn allows to selectively control the path of molecules flow and to selectively separate molecules out of an heterogeneous mixture.

- *HLS allows to isolate single molecules within a mixture and selectively separate it from the mixture*

SLS is very effective in measuring and detecting formation of sphere head properties and this feature can be used to predict and in turn isolate single molecules within a mixture. In fact, plotting formation of sphere heads against time, larger molecules form sphere heads faster than smaller molecules.

SLS is able to measure and isolate the time sequence of sphere head formation, thus allowing to predict the dimensions of the molecules and to selectively control their separation from the heterogeneous mixture. This feature is particularly suitable for the isolation and detection of specific proteins in proteomics applications.

- *HLS can be remotely controlled by feed-back loops .*

The components of the laser field, such as pulse duration, phase and amplitude, not only can be remotely controlled but they can be automatically adjusted through the use of self-learning algorithms[6,7] which, feeding on the information that becomes available during the separation and detection process , as in a continuos feed-back loop, make the necessary adjustments.

- *With HLS, molecular self-assembly becomes possible*

The molecular basis of self-assembly hinges on complementary of surfaces and fast spontaneous motion. To self-assembly molecular parts must have surfaces that match in shape and electrostatic parameters and must move quickly without degrading in the process.

Brownian motion provides the speed of movement: in aqueous solution it makes protein sized parts shift by their own diameter roughly 10^6 times per second, turn by a radian roughly 10^7 times per second and shift by an atomic diameter 10^{10} times per second. Thus, molecular parts are able to thoroughly and rapidly explore their environments and possible self-assembly arrangements.

The results can be rapid, reliable self-assembly of complex structures. However , for this to happen, molecules with complementary surfaces must come in contact in the shortest time possible. This requires upstream

selection, based on information about their 3D structure, and realignment as to favor self-assembly by Brownian motion linking.

HLS can provide the 3D measurements needed to identify and select possible self-assembly candidates within a mixture and NC can selectively separate them as to bring them within the same environment where they can explore self-assembly arrangements.

Molecular self-assembly is a new extraordinary tool in nano-biotechnology whose use , although well researched at the research level, has not found practical applications yet, due to the lack of useful technology. HLS and NC have the potential to reduce it to practice to develop novel chemistries for use in nearly all fields, from advanced materials to pharmaceuticals.

NANOGENESEQ SOFTWARE

NANOGENESEQ HARDWARE is complemented by proprietary NANOSEQ algorithms used techniques to design novel, monitor and control the HARDWARE.

NANOGENESEQ has designed novel algorithms based on mathematical models that are used in industry and university laboratories to simulate chaos, atomic explosions, particle physics experiments, fluids dynamics, turbulent flow and control of micro electronic devices and smaller scale structures, etc.

Software based on fractal geometry principles, an ideal model since it is readily convertible into binary code, was used to design NANOCHIP.

Self-learning algorithm , which are iterative in nature, originate from simple basic rules called initiators and generators, and are capable of performing very complex analytical studies, have been developed to control both NC and SLS.

NANOGENESEQ SOFWTARE is strategic to the company and hence constitutes a key component of its intellectual property.

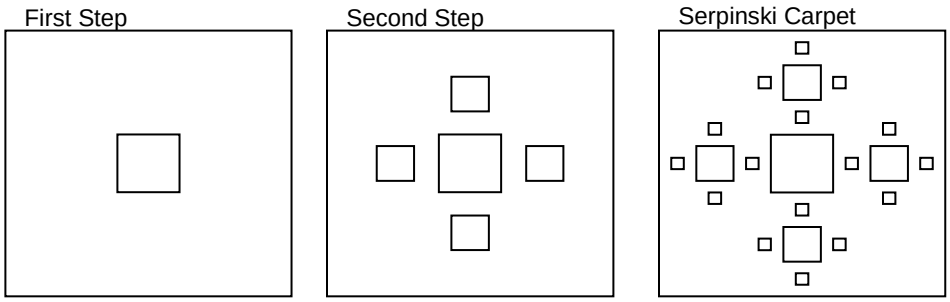
NC fractal design algorithms

Software based on fractal geometry principles, an ideal model since it is readily convertible into binary code, was used to design NANOGENESEQ. The rules for the development of the fractals are very simple.

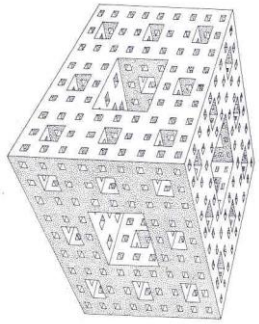
For instance, to generate a fractal square the steps, represented by ***Figure 6*** *below, are :*

- *Start with a square of side length 1, divide it in to 9 equal parts and remove the central part: this is the basic transformation.*
- *Apply the same transformation to the remaining squares and iterate or repeat the process indefinitely. The resulting object after all iterations is called a Serpinski carpet.*

Figure 6: How to generate Serpinski carpets



A 3D analogue of the above is called menger sponge.



A menger sponge, the design at the heart of NANOCHIP , can be fabricated using two methods:

- *by stacking the above mentioned Serpenski carpet one above the other ;*
- *by taking a cube of side 1, dividing into 27 small cubes, removing the central cube and each cube on the middle of the 6 phases of the cube, applying the same transformation to the remaining 20 cubes and iterating until we a finite structure is obtained.*

The side of the cube with each iteration is reduced to one third, while the number of cubes increases by a factor of 20. The dimensions of the menger sponge is therefore log 20/log 3 = 1.3010 / 0.4771 = 2.73 approximately (a fractional dimension, hence called a fractal.)

With each iteration the volume reduced by a factor of 20/27=0.741, so that ultimately the volume is reduced significantly, while generating additional surfaces i.e., the overall surface area increases significantly.

This provides the following benefits:

- *by increasing the surface area more data can be stored and/or flown through.*
- *by reducing the volume of the device, retrieval of data from the storage site is accelerated.*

For these reasons we used the fractal concept and therefore the menger sponge as tools to design NANOCHIP.

Data Storage Software

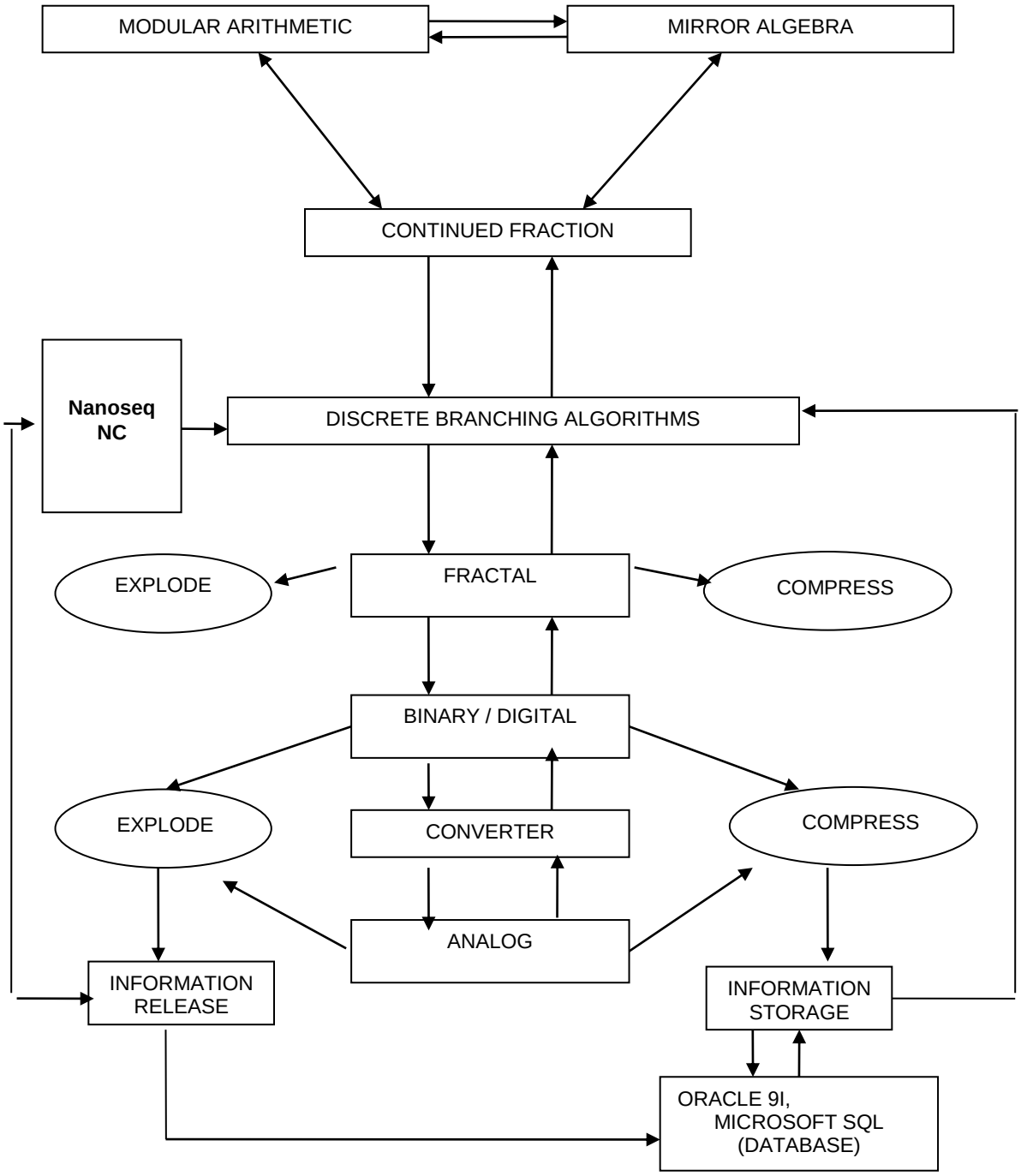
For Data Storage standard software platforms like Oracle 9I, Microsoft SQL Server, My SQL, etc. are used. Data storage is done allocating specific parameters like length, breadth, depth, sphere head properties, temperature, entropy etc., to each DNA fragments separated, by allocating the specific locations in the data storage software for individual channels. It is then stored and retrieved accordingly.

Self Learning Algorithms

The basic concepts of the learning algorithm has been illustrated in ***Figure 7****. These algorithms are iterative in nature, originate from simple basic rules called initiators and generators, and are capable of performing very complex analytical studies. They are also defined as self-learning algorithms.*

Figure 7: Self learning Algorithms and learning loops connected to Nanoseq NC

2.



Product Description

The configuration of NANOGENESEQ sequencing system will comprise the following elements

a) Novel and proprietary components developed by NANOGENESEQ:

- *NANOCHIP : will consist of a micro-fluidics silicon device, capable of comprising up to 2400 nanochannels, each consisting of alternating deep & shallow sections, each wired to a microprocessor and all channels flowing into the detection chamber.*
- *HLS: the microcavity semiconductor spiral laser scanner, fabricated with gallium arsenide, will be in built into the detection chamber and will provide 3D measurements of molecules flow through NC.*
- *Physical appearance will resemble currently available DNA micro-array chips.*

b) Off-the-shelf components to be adapted to create automated systems:

- *Electrophoresis components*
- *PCR amplification systems*
- *Traditional opto-electronic laser scanners.*
- *Software linking the different hardware components*

These components are currently used in various amplifications as well as analytical devices including spectroscopy and can be readily adapted to NANOGENESEQ specification.

Research & Development

R&D Goals during the plan period

- *Final design, simulation and testing protocol for the construction and testing of device with nano-channels in the 100 nanometer diameter range. This goal will be achieved with in-house resources and by the recourse to advanced chip-making facilities .Manufacturing of nano-channel device and coupling of the nano-channel device to a microchip processor.*
- *Completion and functional testing of prototype device in comparative studies with conventional systems.*
- *Incorporation of laser based detection system and final hardware architecture development. This goal will be achieved with in-house resources.*
- *Integration of the hardware with the OP software to automated the system.*

R&D Strategy and Plans during the plan period

- *Nano-channel device coupled with a microchip processor.*

Design and engineering of the NANOSEQ channel device will be done with in-house resources. Manufacturing of the nano-channel device prototypes will be outsourced. Current chip-making technology already allows to manufacture nano-scale circuitry in the 100 nano-meters range by the use of deep ultraviolet light (molecular beam epitaxy) and light sensitive stencils.

Quality control of etched channel circuitry will be achieved with scanning tunneling microscopy . Manufacturing facilities to manufacture the nano-channel device are available both commercially as well as academically in the USA, Europe & Japan.

NANOGENESEQ will outsource the manufacture of the nano-channel device prototypes to one of the labs involved in nanotechnology .

Further improvements in material flow through the device will be achieved by decreasing material electrical resistance (increasing electron mobility in solid state or semi solid state devices by chemically modifying the lattice structure) and by further improvement of the innovative structural geometry. This is expected to enhances the movement of the material significantly and to increase the spherical shape of the DNA Segments.

- *Incorporation of laser sensor technology*

Incorporation of laser sensor technology to scan the flow of DNA fluid in the micro-device and detecting it based on optic emissional properties

will be done in house. The laser device will be arranged at a degree to the axial plane to enhance 3D viewing and also to allow full-scale scanning, in a way analogous to that used in MRI.

Further improvements of the detecting efficiency will be achieved by the means of voltage-controlled spatial light sensing devices that can greatly increase the detection and effectively enhance and speed up the sequencing procedure.

- *Off-the-shelf Hardware components*

The other components of NANOSEQ architecture include flow channels, microelectronic circuit boards, sample storing segments etc. All these are already available and will be adapted on a off-the-shelf basis.

- *Software*

Fractal geometry algorithms for NANOGENESEQ-channel design and laser sensor technology have been developed and will be improved upon with in-house resources.

Commercially available software will be adapted to create the Operating System for the automated full scale system architecture.

R&D Milestones during the plan period

The R&D Milestones that NANOGENESEQ has posed for itself as a

*goal are summarized in **Table 1**.*

Table 1	***Months 0 - 1***	***Months 1- 2***	***Months 2- 3***	***Months 3 - 4***
NANOGENESEQ channel prototyping	**Design , Simulation *Protocol for development and testing of device *Prototype Manufacturing Specifications*	**Prototype manufacturing & testing*		
NANO Chip integration		**Integration with microchip processor *Filing of patents*	**Comparative testing of sample DNA vs. conventional methods*	
Laser sensor detection integration			**Incorporation of laser scanners *Full scale working prototype*	**Final stage testing, final product development for demonstration and testing samples*
Software Integration & Automation			**Commercial software adaptation*	**Completion of Operating System software for final totally automated system*

R&D Skills & Resources

NANOGENESEQ, during the startup period, will draw heavily from resources

available in the South India's academic community . The heart of the development plan will involve the following resources:

Hardware Development

Design and in silico simulation will be done with in-house resources with contribution from the academic facilities mentioned earlier.

Fabrication of the final NC prototypes will be achieved by outsourcing the task, under a contract agreement, to one the following nanotechnology groups, known and accessible to NANOGENESEQ scientific founders:

- *POLAND – Nano Tech Institutes & Government / Defense / Space Agencies*
- *Hewlett Packard, USA (Stan Williams, Head of Nanotechnology fabrication group)*
- *Foresight institute, California , USA (Dr. Eric Drexler , Head of the Institute, a pioneer in the world of Nanotechnology)*
- *Xerox Corporation, California , USA (Ralf Mercle is Head of the Group)*
- *NASA (National Aeronautics and Space Administration), USA . NANOSEQ founders have collaborated in the past with Dr. Arnold Nicagaussian, Chief Health Officer at NASA Headquarters.*
- *Cornell Nanobiotechnology (Harold Craighead, Head of the unit).*
- *The few micro electronic device manufactures in Silicon Valley having medium scale nanotechnology facilities.*

- *Cannon Inc, Hitachi Inc., Sony Inc, Hamastu photonics, Osaka University, in Japan.*
- *University of Hamburg and Siemens Corp , in Germany.*
- *University of Oxford , University of Cambridge , U.K.*

Fabrication of final HLS prototype will be will be achieved by outsourcing the task, under a contract agreement, to one the following Gas Chromatography Mass Spectroscopy developers: IBM, Hewlett Packard, Sony Inc., Hamastu Photonics, Xerox Corp., Toshiba, Siemens, Samsung, Hitachi, LG Electronics etc.

Quality control of the device will be done in house by the use of nano-manipulator microscopes.

Software Development

The algorithms embedding fractal geometrical methods and self-learning algorithms have been developed and will continue to be improved upon with in-house resources and academic expertise.

Biotechnology reagents, materials, expertise

All these materials are available commercially and hence will not be developed. Facilities , expertise and clinical sample s will be made available by the academic groups mentioned earlier.

Medium-Range R&D Plans

During the startup period NANOGENESEQ will focus efforts on the

development of the core constituents of the NANOSEQ system, i.e. NC and HLS.

After the start-up period NANOGENESEQ intends to use its core technology to develop further applications for use in:

- *the separation & detection of proteins in proteomics research*
- *the separation & detection of inorganic polymers*
- *the separation & detection of CD 4 & CD8 cells, MHC class molecules and chemokines .*

In fact both the NANOCHIP and the LASER SCANNING DEVICE are ideal for separation and detection of large molecules in high throughput systems. The NANOCHIP, thanks to its sponge-like structure and its 3D detection capabilities, is ideal for the detection of large molecules .

Preliminary small scale work done with light scattering micrograph techniques allow us to believe that the core technology can be combined with this technique to separate and detect CD 4 & CD8 cells, MHC class molecules and chemokines. This application is based on the observation that different patterns responds to light stimuli with different light emitting and absorbing properties. This causes multiple reflections and refraction. Light scattering micrograph can be used to separate and detect reflections of interest. According to the intensity of light the micrograph produces graphs which can be identified for plotting wave length on the X- axis and the intensity on the Y - axis. This graphical pattern, plotted on a computer screen can be evaluated and recognized against standards.

Markets & market size

Markets

Genomics is quietly transforming the pharmaceutical industry. Companies are moving from drug discovery and development based on medicinal chemistry to the design of drugs based on information provided by genomics and proteomics. Virtually all of the major pharmaceutical houses either have formed partnerships with genomics and/or proteomics firms that began to emerge in the early 1990s or have created in-house divisions. As little as 2 years ago, there were only a dozen or so firms in the genomics industry.

In 1999, there were estimated to be more than 200 companies worldwide that list genomics as one of their businesses. The market for genetic data and technology is projected to be worth several of billions of dollars within the next decade. The reason for this rapid interest in genomics is simple: genomics allows greater efficiency in identifying therapeutic targets by determining which genes are responsible for the creation or enabling of disease processes, how these genes control these processes, and what might be done to stop them.

Products provided by genomics and proteomics firms include not only databases and specialized software to search the databases, but also automated sequencers, and DNA microarray systems.

The sequencing and microarray systems are the reference markets for NANOGENESEQ products.

The global DNA & gene chip market size was valued at USD 4.50 billion in 2017 and is projected to exhibit a CAGR of 11.4% during the forecast period. The ability of DNA microarrays to allow comprehensive simultaneous analysis of thousands of genes has resulted in the success of this technology in the past decade. Technological advancements in computational capabilities, robotics, and fabrication methods for microarrays have led to rapid uptake of gene chips in clinical laboratories across the globe. Although the popularity of Next Generation Sequencing (NGS) has skyrocketed in the recent past, NGS is not yet able to completely replace microarrays in diagnostics and cytogenetics due to its relatively high price and steady rate of clinical implementation.

Market Size

The microarray market is estimated to be the fastest growing of all bio-instrumentation markets, with a compound growth rate of more than 45% from 1999 to 2001, according to Strategic Directions International Inc., Los Angeles.

Microarrays are quickly making their way into different areas of pharmaceutical R&D, the most notable being expression analysis, genotyping, and sequencing.

Microarrays allow researchers the opportunity to monitor the activity of thousands of different genes in parallel. By quantitatively measuring expression changes in genes of interest, researchers can define the phenotypic condition of the cell in terms of its expression profile.

Typical experiments range from comparing diseased tissue with normal tissue, to understanding the effects of a drug on gene expression in a given tissue, to simply defining the "normal" condition.

In an effort to correlate genetic variation with disease or drug response, researchers will be screening large populations for single nucleotide polymorphisms. Each association study may require thousands of patients, each being evaluated on thousands of polymorphic markers.

Taking a further look, the number of biochips used in the pharmaceutical industry is expected to triple in the next 5 years, from 200,000 used in 2000 to 600,000 in 2005, according to a report from London-based Informa Pharmaceuticals entitled "Biochips and Micro-arrays: Technology and Commercial Potential." But the largest expected microarray market is not in drug discovery and development. Molecular diagnostics will achieve wider applications due to its spread into health care, and it is expected to exceed $1 billion by 2005. It would not be surprising to see that figure go as high as $1.5 billion, but that will depend upon the speed with which the various technological developments see market launch.

Microarrays are also expected to fill a very wide range of applications: from pharmaceutical, point of care diagnostic and medical laboratory uses, to agriculture. As the genetic markets become identified and some new medicines come bundled with a genotyping test, microarrays will make way into their largest potential market.

With the chip prices falling by that time, microarrays will provide a perfect tool for clinical diagnostics due to their ease of use, integration of sample preparation into the chip, and easily detectable readouts. We may soon see doctors performing disease diagnosis, personalizing drug prescriptions, monitoring disease state and progression, evaluating disease susceptibility, and classifying pathogenic

The global DNA sequencing market was valued at $6,243 million in 2017, and is expected to reach $25,470 million in 2025, registering a CAGR of 19.0% from 2018 to 2025. DNA sequencing is a technology in which several DNA strands can be sequenced through massive parallelization.

The global DNA & gene chip market size is expected to reach USD 10.7 billion by 2025, at an 11.4% CAGR during the forecast period. DNA and gene chips have gained much success in providing high throughput capabilities for comprehensive genome studies to enhance disease knowledge and target them. This technology has emerged as a valuable and promising solution across various aspects of disease management. These factors have been driving the market.Conventional means of gene analysis allow investigation of relatively small amounts of genes at a time, which further emphasizes on the uptake of DNA microarray technology. DNA microarray technology allows concurrent analysis of very large numbers of nucleic acid fragments in a single experiment.

Continuous development of new tools to support and enhance reliability of DNA microarray technology is likely to drive this market at a significant pace in the foreseeable future. Furthermore, healthcare entities are engaged in exploiting novel solutions for enhancing and combining data generated from gene chip with data generated from high throughput technologies. Mutually beneficial partnerships between microarray developers and pharmaceutical/biopharmaceutical companies for acceleration of genomic and biomedical research activities is anticipated to spur market growth in the coming years.

Competition and Competitive Advantages

The Industry

DNA sequence analysis is a multistage process that includes the preparation of DNA, its fragmentation and base analysis, and the interpretation of the resulting sequence. Gel electrophoresis is used to determine the order of bases on a strand. DNA molecules that differ in size by one base are labeled with nucleotide-specific fluorescent dye and loaded onto a slab of acrylarnide gel. An applied voltage causes the DNA fragments to separate by size as they migrate through the gel. At the bottom of the apparatus, a detector, such as a charge-coupled device (CCD) or photomultiplier tube (PMT), reads the color of the fluorescent dye at the end of each DNA fragment.

The DNA microarray industry is comprised of companies which supply

- *micro-array slides : Corning and Surmodics*
- *micro-arrayers (eg. robotic spotters and photolithographic equipment) : Affymetrix, Amersham Pharmacia Biotech, Biorobotics , Cartesian Technologies, Virtek, GeneMachines , Genomic Solutions , Nimblegen , Packard Instruments*
- *scanners : Affymetrix, Agilent Technologies, Applied Precision, Axon Instruments, GSI-Lumonics, Virtek*
- *software for designing and analyzing microarrays : Affymetrix , Agilent Technologies, Axon Instruments , BioDiscovery , Clontech Laboratories , GeneLogic , GeneMachines , Rosetta Inpharmatics , Silicon Genetics , Spotfire*
- *pre-spotted slides : Affymetrix Agilent Technologies Clontech Laboratories GeneLogic Incyte Genomics Rosetta Inpharmatics*

Competition

A. Conventional Systems : Capillary Sequencers

The main competitors are Applied Biosystems and Molecular Dynamics. The Applied Biosystems PRISM 3700 and the Molecular Dynamics MegaBACE 1000 capillary sequencers enable rapid DNA analysis used for the determination of the human sequence.

These instruments, which incorporate capillary tubes to hold the sequence gel, automate sample loading, separation matrix loading and removal, data collection and analysis, and provide significant improvement over the manual gel preparation and lane tracking required in slab-gel

sequencers. Sequencing time per sample is about 10 hours in slab gels but only 3 hours in capillaries.

These sequencers maintain efficiency with high-throughput, upstream automated sample preparation and sample tracking throughout the process to avoid mix-up, and real-time quality control.

B: Novel "Conventional" Approaches

Mass spectrometry

Matrix-assisted laser desorption ionization (MALDI) time-of-flight (TOF) mass spectrometry has been adapted primarily for detecting sequence variations between individuals. It uses the chemistry established for conventional sequencing but replaces the size separation of DNA in gels with mass-dependent strand separation of gas phase ions in a vacuum.

The chemistry specifies the base at the end of a fragment, and the mass of the fragment specifies the location of the corresponding base in the sequence. This approach, much like electrophoresis in a vacuum, permits simultaneous analysis of many DNA strands in seconds. Although the technique is fast, the sequence length that can be achieved currently is only about 100 bases.

Pyrosequencing

Pyrosequencing is a nonelectrophoretic DNA sequencing method used primarily for mutation analysis and genotyping. It enables sequencing of

DNA strands up to 200 nucleotides long and is currently one of the fastest methods for analyzing a primed DNA strand. The technique takes advantage of four enzymes [DNA polymerase, sulfurylase (from yeast), luciferase (from the firefly), and apyrase (from potato)] cooperating in a single tube to signal the incorporation of a nucleotide to a growing DNA strand. Detection is based on the visible light produced by coupling the pyrophosphate released during nucleotide incorporation with the enzymes sulfurylase and luciferase. By sequentially adding nucleotides and observing the flash of light each addition causes, the sequence of the template can be determined as the DNA strand is copied.

Capillary-Based Submicroliter Fluid Handling Systems

An important consideration in increasing sequencing efficiency is reducing fluid volumes of the sequencing and separation steps to reduce costs and processing speeds. The Genomation Laboratory at the University of Washington is developing an instrument that prepares 5000 microliter samples in 8 hours for high-tbroughput large-scale DNA sequencing.

The reaction preparation module of this automated, multipurpose, fluid-handling system aspirates submicroliter DNA samples, delivers precise picoliter volumes of reagents using piezoelectric dispensers, and mixes fluids.

Next generation of electrophoresis-based sequencers

The next generation of electrophoresis-based sequencers will most likely comprise microcharmel plates or "microchips." The advantages of

microchip sequencers over capillary electrophoresis systems include more efficient sample injection; higher speed, resolution, throughput, and detection sensitivity; lower costs and sample and reagent consumption; and the potential integration on a single chip with up stream sample processing steps.

Robert Mathies's (UCA-Berkeley) rotary design runs 96 samples in parallel on individual channels in a radial configuration, like the spokes of a wheel. Samples are detected in the center of the microplate by a laser-excited rotary confocal scanner and reduces sequencing times from hours to minutes.

An integrated' microfabricated system or "lab-on-a-chip" under development by researchers at the University of Michigan prepares DNA samples and sequences them. It includes a system to control fluid motion, an air-driven fluid pump, a temperature-controlled reaction chamber, and an electrophoresis and fluorescence detection system. All of the components are microfabricated on the same wafer and require no external lenses, heaters, or mechanical pumps.

Plastic electrophoresis chips have also been constructed on polycarbonate substrates and have successfully separated doublestranded DNA. This technology has the potential for low-cost fabrication of disposable, single-use electrophoresis devices.

Caliper Technologies recently released their AMS 90 or "LabChip" that takes 96 DNA samples directly from microplates and performs

electrophoretic size-based separation on a microchip. Small (10 nl) samples can be processed in 5 to 30s.

Agilent Technologies Bioanalyzer 2100 detects the separated DNA bands using laser-induced fluorescence.

C. Future developments

DNA sequencing by size fractionation is generally a slow and indirect method of obtaining sequence data. In the future, detection and analysis of single molecules may provide a very rapid' low-cost DNA sequencing option.

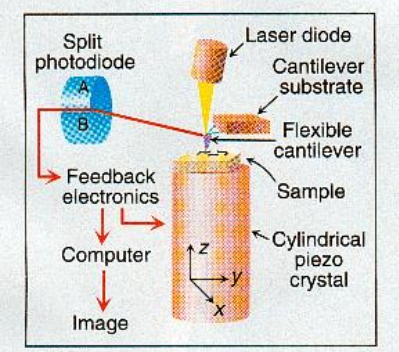
Nanopore detection

One technique being developed at the National Institute of Standards & Technology may allow direct analysis of individual DNA molecules at rates of up to 1000 bases per second. Bases on a single-stranded DNA molecule are forced, under an electric potential difference, single-file through a nanopore less than 2 nm in diameter (see figure, right). An integral detector in the pore translates the characteristic physical and chemical properties of a base or sequence of bases into an electrical signature.

When no DNA is present, the pore exhibits an ionic current of 120 pA at 120 mV potential. When the pore is occupied by polyoucleotides, the ionic conductance decreases according to the nucleotide composition of the DNA. To achieve single-nucleotide sequence resolution, several improvements are needed, not least of which includes the coupling of more sensitive detectors to the pores to discern single nucleotides inthe signature.

Atomic Force Microscopy

Another single-molecule analysis technique, atomic force microscopy (AFM) involves scanning a nanometerscale tip across a surface to read the surface of DNA, much as a blind person reads Braille. Intermolecular forces between the tip and surface move a flexible cantilever up and down. The corresponding deflections are measured with a laser, and a topographic map of the surface is generated. The ultimate goal of AFM is to scan single-stranded DNA with single-nucleotide resolution.



Schematic of AFM.

Nanopore and AFM sequencing have the potential to sequence very long DNA molecules, obviating the need for cloning, fragmenting, and reassembling used with current approaches. Thus, they would also dramatically reduce the need for preparation of large numbers of samples.

However they are believed to be far ahead in time.

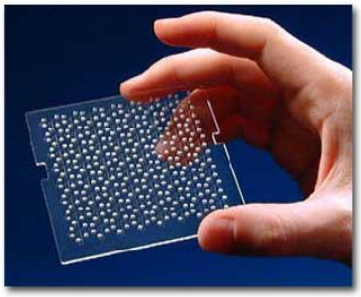
In conclusion the key competitors (potential partners) in the next 5-10 years that NANOSEQ will face are the manufacturers of conventional and next generation electrophoretic based systems being developed by Lab-on-a-Chip companies/labs. Among those, the most prominent are described below and over.

Competitors

ACLARA BioSciences, Inc

ACLARA BioSciences develops microfluidics array technologies, often referred to as lab-on-a-chip system. Its proprietary technology targets genomic applications (RNA and DNA analysis) and high-throughput pharmaceutical drug screening. Movement of tiny (pico/nanoliter) volumes of fluids through interconnected networks of microscopic channels enables a range of common laboratory procedures: mixing, incubation, metering, dilution, purification, capture, concentration, injection, separation, and detection.

Its plastic LabCard microfluidic array platform is mass produced and disposable, allowing researchers to rapidly perform large numbers of bio/chemical measurements in a format that is at once inexpensive, miniaturized, automated,and free from cross-contamination worries.



Affymetrix Technology

Develops state-of-the-art GeneChip Probe Array Technology for acquiring, analyzing and managing complex genetic information for use in

biomedical research. GeneChip® technology uses miniaturized, high-density arrays of oligonucleotide probes. The set of oligonucleotide probes to be synthesized is defined, based on its ability to hybridize to the target loci or genes of interest. With this information, computer algorithms are used to design photolithographic masks for use in manufacturing the probe arrays.

Probe arrays are manufactured by Affymetrix's proprietary, light-directed chemical synthesis process, which combines solid-phase chemical synthesis with photolithographic fabrication techniques employed in the semiconductor industry. Using a series of photolithographic masks to define chip exposure sites, followed by specific chemical synthesis steps, the process constructs high-density arrays of oligonucleotides, with each probe in a predefined position in the array. Multiple probe arrays are synthesized simultaneously on a large glass wafer. This parallel process enhances reproducibility and helps achieve economies of scale. The wafers are then diced, and individual probe arrays are packaged in injection-molded plastic cartridges, which protect them from the environment and serve as chambers for hybridization. Once fabricated, the GeneChip® probe arrays are ready for hybridization. The nucleic acid to be analyzed (the target) is isolated, amplified and labeled with a fluorescent reporter group. The labeled target is then incubated with the array and stained with florescent dye using the fluidics station and hybridization oven. After the hybridization reaction is complete, the array is inserted into the scanner, where patterns of hybridization are detected.

The hybridization data are collected as light emitted from the fluorescent reporter groups already incorporated into the target, which is

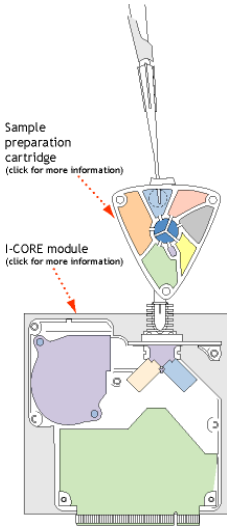
now bound to the probe array. Probes that most clearly match the target generally produce stronger signals than those that have mismatches. Since the sequence and position of each probe on the array are known, by complementarity, the identity of the target nucleic acid applied to the probe array can be determined.

Caliper Technologies

Develops, manufactures, and sell proprietary microfluidic LabChip systems to pharmaceutical and other companies. To create LabChip® products, Caliper combines manufacturing methods from the microchip industry with expertise in fluid dynamics, biochemistry and software and hardware engineering to develop miniature, integrated biochemical processing systems.

Cepheid

New company dedicated to the application of microfluidics and microelectronics technologies to test systems for DNA analysis technology. Cepheid has a portfolio of technology which enable automation of complex DNA detection procedures, from sample preparation and processing through real-time analysis and detection. The company is developing fully integrated portable instruments and laboratory systems that can be used to detect infectious disease agents, human genes, and industrial and environmental contaminants . Cepheid' technologies are : ***I-CORE module****: enables rapid amplification and DNA detection to be carried out in a single reaction tube.* ***Fluidic systems****: designed to carry out preparation and processing of raw specimens and reagents, in a rapid, automatic, hands-off manner.Cepheid's I-CORE and fluidic systems are designed as modules, to be integrated into a wide range of system configurations for rapid, automatic DNA based analyses.*



Motorola BioChip Systems

Motorola BioChip System integrate biochips with instrumentation, bioinformatics and communications. They include microarray-based single nucleotide polymorphism and gene expression analysis systems, as well as microfluidics and other advanced technology platforms. Its proprietary array technology is centered around a three-dimensional gel pad format consisting of flexible content architectures. These integrated systems can be used to achieve high throughput or portability for sample preparation and a variety of diagnostic functions. The company's microarray-based integrated systems will support a wide variety of applications including DNA and RNA analysis, as well as proteomics.

Nanogen Inc

Microelectronics meets molecular biology."Microarrays are considered by many to have a very promising future in genetic research and

diagnostics. Nanogen extends the power of microarrays through the use of electronics. Most biological molecules are charged and can be moved and concentrated electronically. The NanoChip™ takes advantage of this by connecting each test site on the NanoChip array to an electrode. By manipulating the charge at each test site, DNA, RNA and other molecules can be rapidly moved and concentrated enhancing the flexibility and accuracy as compared to other array systems.

As discussed in the preceding sections there are many companies which manufacture and market both microarray equipment and microarrays themselves. This is unlikely to continue. Consider the analogy in the microelectronics industry: Intel sells the ready-to-use chips and Applied Materials sells the equipment to make the chips, but no one makes both. In the microarray market, advances in technology and fiscal reality will force companies to focus.

This is the strategy that NANOSEQ will pursue early on, and that is to become the definitive supplier of NANOCHIP and SPIRAL LASER SCANNER to the genomics , proteomics and diagnostics industries worldwide.

Competitive Advantages

The key advantages of NC are:

- *NC allows zero resistance flow and avoids overlapping of molecules*
- *NC triples the separation and detection efficiency of the most advanced of existing systems*
- *NC allows heterogeneous separation.*
- *NC favors sphere head formation and flow*
- *NC increases the parameters that can be measured.*
- *The entropy of flow of molecules within the device can be measured and controlled*
- *The flexibility of the NC device itself can be controlled*
- *With NC the choice of fluorescent dyes that can be used is greatly increased*

The key advantages of HLS are:

- *HLS is a semiconductor micro-laser with nanoscale level sensitivity*
- *HLS measures the changes in shape of molecules as they pass through the NC device*

- *HLS enormously increases the amount of information that can be gathered on molecules behavior.*
- *HLS will allow multicolor detection and use of a much wider variety of fluorescent dyes.*
- *HLS will make heterogeneous detection a reality*
- *HLS allows to isolate single molecules within a mixture and selectively separate it from the mixture*
- *HLS can be remotely controlled by feed-back loops*
- *With HLS, molecular self-assembly becomes possible*

The key advantages described above, when compared with the most advanced systems available today translate into highly competitive advantages:

Inherent advantages

- *NANOGENESEQ (NC and SLS) allows to selectively separate and detect individual molecules within an heterogeneous mixture*
- *NANOGENESEQ increase the data readout on single molecules allowing to take 3D measurements, the changes in shape, the speed of changes in shape the entropy of molecule flow through the device and the flexibility of the device itself; all these measurements allow to predict the molecule type and are the data feed for the self-learning algorithms , thus improving their predictive capacity over time. This is especially useful for proteomics studies and for molecular self-assembly applications.*

- *NANOGENESEQ allows to measure the entropy of flow and device flexibility, thus allowing to control the separation and detection process. The entropy of flow be modified via software so that the chip can be commanded to perform different tasks, according to the detection target.*
- *NANOGENESEQ is modular, thus allowing to build arrays of NCs & SLS to perform multiple functions and tasks.*
- *NANOGENESEQ has longer chip life cycle and reduces energy costs during operations . The flexibility of the chip reduces the frictional and heating effects, so the chip doesn't need any cooling devices, like cooling fans. This drastically reduces the cost during operations and results in longer chip life.*

Sequencing advantages:

- *In the case of DNA, NANOGENESEQ triples the amount of base pairs that can be sequenced on a given segment to 2100 from the current 700;*
- *NANOGENESEQ widens the choice of fluorescent dyes that can be used , thus making multicolor detection a reality; multicolor detection , i.e. the use of multiple sets of 4 dyes each, will allow multiple simoultaneous sequencing of more than one fragment within the same sequencing step.*

Proteomics advantages

- *NANOGENESEQ allows 3D scanning of molecules as they flow within the device, thus allowing to selectively detect individual protein forms as they move through the device. This translates into a much improved*

efficiency in screening studies

- *Integrating several NCs into a larger composite NANOGENESEQ (as in a chessboard arrangement) provides an extraordinary platform to do more integrative protein detection tasks with respect to current methods and this a key advantage for proteomics applications.*

Molecular self-assembly

- *HLS provides the 3D measurements needed to identify and select possible self-assembly candidates within a mixture and NC can selectively separate them as to bring them within the same environment where they can explore self-assembly arrangements. This can be exploited to develop novel chemistries for use in nearly all fields, from advanced materials to pharmaceuticals.*

Business Development Strategy and Plans

In the foreseeable future, NANOGENESEQ will derive its revenues from strategic alliances for its core technology. These alliances will provide the opportunity to receive royalties and profit sharing, if the Company and its partners are successful in developing and commercializing products. They will provide substantial funding for R&D and access to important technology, will broaden the product development pipeline and reduce

product development risks. These alliances, finally, enhance the Company's ability to bring products to market because of the partners' substantial resources and expertise in regulatory issues, manufacturing and marketing.

The strategy of the company is to seek strategic alliances as soon as working prototypes of NANOCHIP and HELICAL LASER SCANNING devices are developed. The founders are confident to be able to close very early on alliances at very favorable terms to the Company.

Intellectual Property Issues

Key aspects of the technology described by this plan are based on the use of novel fractal and self-learning algorithms. Public disclosure of the algorithms before the company will have had the opportunity to consolidate its technology lead over the competition would undermine the value of the Intellectual Property portfolio that NANOSEQ is developing. In light of this, and also of the strategic nature that high-throughput sequencing technology will play in modern drug discovery, the Founders have taken the decision to postpone filing for patent protection until all key claims of future patents are embedded in working hardware prototype devices, to obtain the highest protection as possible for NANOSEQ core technologies.

Location & Facility

NANOGENESEQ will have its hardware and software development facility in Trivandrum, India, in laboratory space leased at favorable

terms, where most of the development work on NANOCHIP and HELICAL LASER SCANNING will be carried out. The facility will consist of two building omprising the following labs:

- *First Building: clean room , microscope room, computer room, cold and storage room, inventory room*
- *Second Building: administration, office rooms, connference and library room.*

The facility will not include, at least initially, nano-fabrication which requires an STM (Scanning Tunneling Microscope) capabilities . The STM work will be done in out-sourcing.

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Financial Strategy

NANOGENESEQ intends to raise start-up funding from Venture Capital sources to carry it to prototyping completion and validation .

Immediately following prototyping completion and validation, anticipated at the end of year 2, NANOGENESEQ intends to enter into one or more strategic partnerships to develop Client-specific applications for NANOCHIP and HELICAL LASER SCANNER. The companies to be approached are those described in the Competition and Market sections. To

those companies NANOGENESEQ will offer limited license right to uses of its technology in exchange for equity, cash & royalties.

As soon as a major strategic partnership is established, the company will start selection of investment banks for an IPO to be achieved at the earliest possible date, provided market and pricing conditions are favorable.

Corporate and Capital structure

Corporate structure

NANOGENESEQ will be incorporated as a limited responsibility company, in U.K (depending on how discussions with venture capital companies proceed)..

NANOGENESEQ Ltd. will be the legal entity responsible for the contract leases and contract work to be done in Trivandrum, India , and elsewhere.

A wholly owned Ltd. company will be incorporated in India, immediately following prototyping completion and validation. Until then , all activities of the company will be carried out on a contracted-out basis.

Capital Structure

NANOGENESEQ will have a total capital of Euro 6 million, divided into Privileged Shares (PS) and Common Shares (CS). The investors are asked for 2 million Pound Sterling in exchange for 20% of the post-funding capital of NANOSEQ.

NANOGENESEQ post-funding capital is shown in Table 1.

Table 1: Capital structure of NANOSEQ		
Shareholders	**Share type**	**Shares holding**
VCI	Privileged	50%
Founders & Management	Common	50%

There will be no difference of an economic nature between PS and CS shares. Extraordinary Shareholders Assembly deliberations will require the majorities dictated by governing law.

Privileged Shares (PS)

PS will have the following rights:

- *PS will represent x 20% of the post-funding capital and CS shares 80%;*
- *Should the company be put into liquidation, liquidation surplus proceeds (after payment of creditors) will be distributed first to PS shareholders until concurrence of an amount equivalent to the initial investment plus interest accrued over the years; the interest rate applied will be 80% of commercial lending rates for the years prior to liquidation.*
- *VCI will convert all its PS into CS, should NANOSEQ be quoted on the market, sold or issue new shares;*
- *PS shares will express 2 Board members, including the Chairman and will nominate the President of the Internal Auditing Committee*

Common Shares

- *CS will be attributed to the Founders*
- *CS will express 3 board members, including the CEO, and will nominate one member of the Internal Auditing Committee*
- *Ordinary Management Powers will be attributed to the CS*

Stock Options

The Company will have a stock option plan for the Management Team and the SAB, that on a fully diluted basis will represent a total of 10% of the capital. The options, when exercised, will dilute all the shareholders in proportion.

Exit

Once NANOGENESEQ has built an established portfolio of patents, completed prototyping and entered into a major strategic partnership with a commercial company, it is possible to consider an IPO on a technology stock market place in Europe or on Nasdaq in the US. Provided the shares perform in the after market this would provide a way for investors to exit their investment.

Pro-forma Financial Statements

Pro-forma statements have been based on the following assumptions:

- *a startup venture round of 2 million Pound Sterling will be closed before inception*
- *at least 2 million Pound Sterling contribution (any combination of up-front, equity, contract research and milestones) will be obtained through a strategic alliance during the period.*

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