DNA Analysis using a Portable Robotic Instrument

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Abstract

Obtaining the sequence of nucleotides that constitute a gene or segment of DNA involves extracting DNA from a cell, amplifying the number of DNA molecules, purifying, tagging the ends of the fragments with fluorescent dyes, and reading off the nucleotide sequence from the ends of the fragments. Currently separate, often large instruments are used at each stage, and manual steps are needed. This paper describes a novel design for which a provisional patent has been submitted, for a portable and automated robotic instrument capable of performing the first four out of the five DNA analysis steps. There are currently no such portable automated instruments available. To prove the concept of the design, the instrument was successfully prototyped and tested. The paper also describes the use of lab-on-a-chip (LOC) technology to improve the design. The electric charge of molecules is used to move solutions through microchannels etched in a plastic or glass substrate. The microfluidic movement can be used to replace the automated robotic equipment normally used to transfer solutions between steps of our new DNA analysis procedure. Areas of application include health–care, agriculture, forensic medicine, the military, remote sensing and environmental monitoring.

1 Introduction

Arguably, the modern revolution in biology can be traced to the discovery of the genetic code, and the role of DNA in transmission of genetic information. The Human Genome Project is seen by many as the culmination of this revolution. By sequencing the 3 billion bases that compose the nuclear genetic material in our cells, it provides us with information to understand what it takes to be human. Unravelling the DNA sequence of a biological sample involves five main steps beginning with extracting it from the cell, followed by amplifying the number of DNA molecules, purifying the amplified product, tagging the ends of the fragments with fluorescent dyes and finally, reading off the nucleotide sequence from the ends of the fragments. Currently separate instruments are used together to carry out this process. The equipment is readily available from a large number of manufacturers. However, some of these instruments are large and require preprocessing stages based on manual laboratory work by staff with technical expertise. Even though automation has played a major role in increasing the throughput and improving the reliability of the process, the instruments are still designed only for use in a laboratory environment. A DNA sample from an animal or plant must be sent to a laboratory for it to be analysed. This is not always feasible and can be inconvenient.

A DNA analysis system that is portable, integrated and automated has several advantages:

- reduced number of components
- reduced labour
- reduced sample reagent consumption
- increased speed of the analysis procedure
- reduced waste
- more robust analysis as a result of automation
- less prone to contamination and evaporation errors as the system is closed

A portable and automated DNA analysis system would find use in a large number of fields such as clinical diagnostics, forensic medicine, research, military applications, remote sensing and food testing. In fact, we were motivated to develop this instrument because some of our collaborators are involved in analysis of genetic material obtained in remote locations and where transport

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of samples back to the laboratory is dangerous or prohibitive.

In this paper, we describe a novel, portable, automated system that is capable of carrying out four of the five steps required for DNA analysis. An improved version is also described, using a microfluidic device to replace the bulky liquid handling equipment. Section 2 explains the steps and equipment involved in DNA analysis. Section 3 discusses the requirements for a portable analyser. Section 4 presents our design for a new protocol and instrument. Section 5 presents experimental results. Section 6 presents a microfluidic system design.

2 Background

The process of analysing DNA starting from a blood or tissue sample involves five main steps. Although the exact procedure varies depending on the equipment used and the starting material, below is given a brief overview of the general steps involved and the required equipment.

Extraction: Before DNA can be used in any process involving its analysis or manipulation, it must be isolated from the cell and purified from the other substances present. The procedures for extraction of DNA involve many disjoint steps using equipment such as centrifuges and incubators. Any automatic system must include not only automatic equipment for each extraction step, but also equipment for automating the transfer of liquid between machines. The liquid handling requirement is the most challenging aspect. The few available automatic extraction machines are large and designed for high throughput rather than for portability.

Amplification: Sequencing DNA involves cleaving the DNA strands randomly at different points. In order to allow this random process to represent at least one strand of every length, we require millions of DNA strands. So DNA is amplified after extraction. Polymerase Chain Reaction (PCR) is the most widely used method for this step. PCR is generally performed in a commonly available instrument known as a thermal cycler or "PCR machine" [Griffin and Griffin, 1994]. The instrument typically comprises a heating and cooling element, a micro-controller to manage the temperature cycling and a feedback mechanism such as a thermocouple to measure and maintain the temperature at the required level. Most thermal cyclers are designed for high throughput and can handle multiple samples. However, they normally weight only between 5-10 kgs and can be used for portable applications.

Quantification (optional step): Extraction and PCR are prone to erros due to contamination so most laboratories include a quantification step after PCR. Either a spectrophotometer or a Reverse Transcriptase PCR (RT-PCR) system is used. A spectrophotometer is a small portable instrument that requires one microlitre of the amplified DNA sample to be placed between the ends of two optical fibres. The quantity of DNA present is measured by the instrument and displayed using a computer. An RT-PCR system is a PCR machine which has an additional fluorescence excitation and detection mechanism. By adding one of several commercially available fluorescent probe molecules to the PCR reaction mix, it is possible to detect the quantity of DNA produced after each amplification cycle. However, these machines are substantially heavier and more expensive than the conventional PCR machine. DNA can also be quantified manually by running a small sample of the amplified DNA product across an electrically charged agarose gel. The time taken to migrate across the gel gives a rough estimate of the quantity of DNA present.

Purification: Once a sufficient quantity has been produced, the next step is to purify the DNA by removing unwanted substances left over from the PCR reaction. This is normally done manually in a lab by filtering and centrifuging the PCR product with purification buffers [Alphey, 1997]. Automating this step requires the use of expensive and bulky liquid handling equipment weighing over 10 kgs [Brush, 1999].

Sequencing: The next step is sequencing, which normally involves adding sequencing solutions to the purified DNA and cycling the mixture through three different temperatures. The result is a set of DNA fragments differing in length by a single nucleotide with the end of each fragment labelled with a fluorescent dye. A thermal cycler is the only instrument required to perform this step.

Separation and Detection: In this final step, the fragments are size-separated by the process of capillary or gel electrophoresis. Of the two, capillary electrophoresis is more commonly used and involves placing the DNA fragments at one end of a capillary filled with polyacrylamide or agarose gel. An electric field is applied between the two ends of the capillary. Since DNA is negatively charged and the fragments are of varying lengths, they take differing amounts of time to migrate through the capillary. As they arrive near the positive electrode, they are irradiated with laser light, which excites the fluorescently labelled base at the end of each fragment. Since each base is tagged with a different colour, it is possible to detect the order of the bases in the sequence. This step of the procedure is normally performed using a 'DNA sequencer' or 'DNA analyser' [Swanson, 2000].

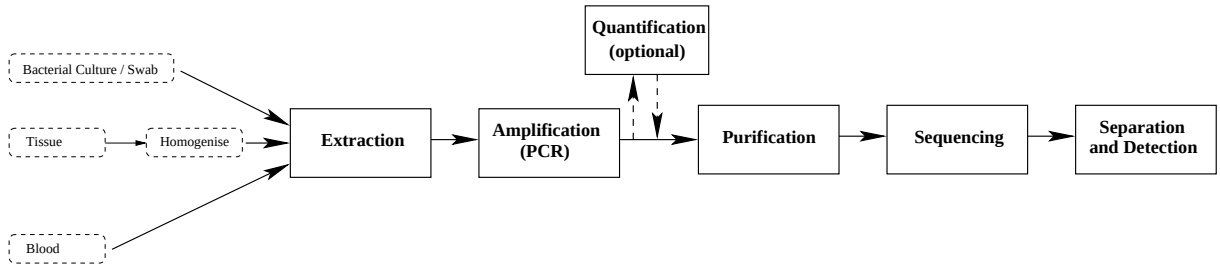


Figure 1: Steps involved in DNA analysis

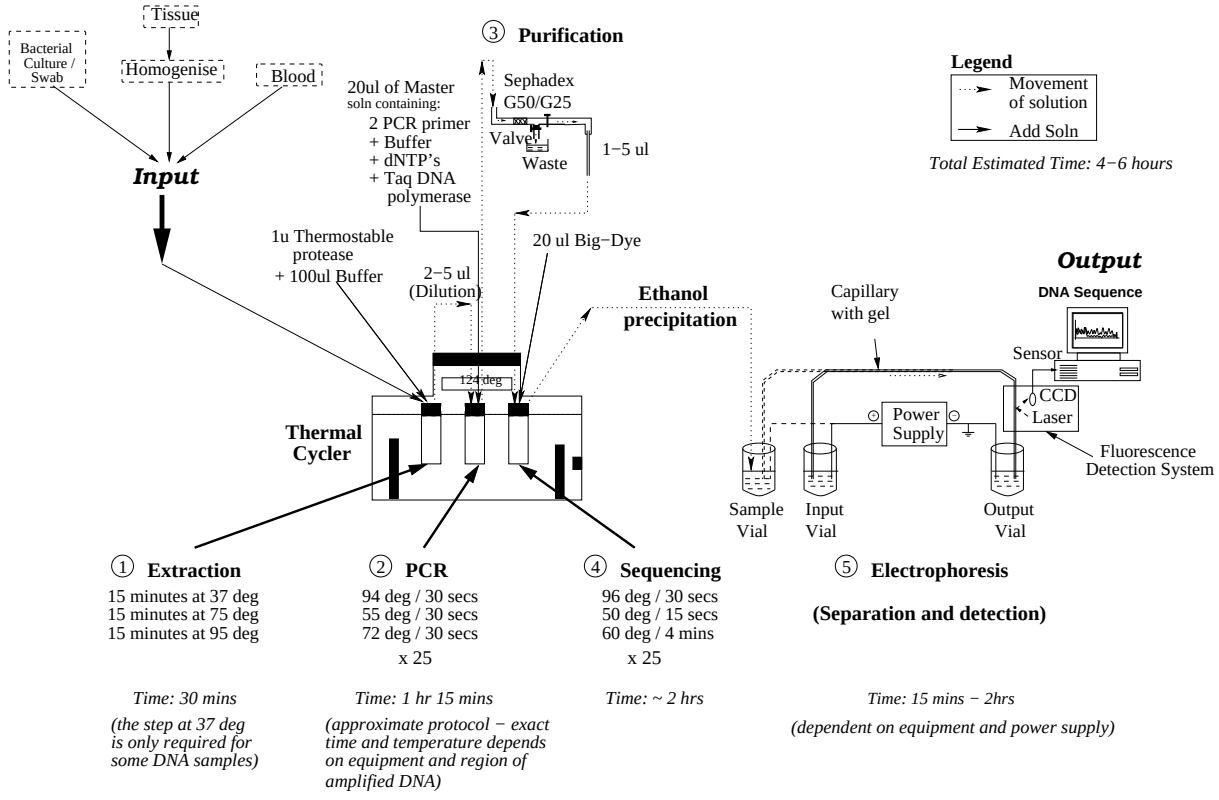


Figure 2: Protocol for DNA analysis. The protocol has been optimised to reduce component parts.

These instruments typically weigh more than 100 kgs and are not portable. It should be noted that before carrying out the electrophoresis procedure, there is a short preparatory step involving precipitation with ethanol or isoproponol [Alphey, 1997].

3 Requirements

The focus of most DNA equipment manufacturers is to produce high-throughput, bulky instruments engineered to be used inside a laboratory. Therefore, a DNA sample that requires analysis must be collected on site and sent to a laboratory to be analysed. In many circumstances this is undesirable and it would be more practical to have a portable instrument that can be taken out of the laboratory. Ideally such an instrument should be controlled by a computer or similar interface and should not require any manual liquid handling during the analysis process. This prevents any contamination from human intervention. In general, the system should be robust enough for outdoor use.

However, integrating the process is not enough to make the equipment portable. Many components such as the thermal cycler, laser, power supply and liquid handling robotics are essential for an automated DNA analysis system, however, each weigh at least 5 kgs. These along with other essential components add on to increase the total weight of the system to over 40 kgs. Therefore, in order to create a truly portable design, either the biological steps of the process must be modified or the technology for some of the essential components must be improved.

4 Design

Using a new method for the extraction step and a non-standard method for the purification step, a new biological protocol for DNA analysis was developed by our research group, one specifically optimised to reduce component parts. Figure 2 describes the steps involved using this new procedure.

4.1 A new protocol

Initially, the DNA is extracted from either blood, bacterial culture or tissue using a new and recently shown method for DNA extraction [Daniel and Saul, 2002]. This extraction method uses a thermostable proteinase that simplifies the bio-chemistry of the process. With this new method, all the steps required to perform the extraction process can be done in a thermal cycler, without the need to handle either the reagents or the DNA sample at any point during the process.

Once the DNA has been extracted, amplification is done in the conventional manner by PCR. A simple yet effective method is used to predict the approximate number of cycles required for the PCR process, without using a quantification step. This is done by controlling the quantity of DNA present before the amplification reaction starts. After extraction, a fixed small quantity of solution (usually between 2-5 μl) is taken, so it is possible to approximately predict the amount of DNA present. The exact quantity of extracted DNA depends on the starting sample. Although this is not the conventional method of quantifying the DNA product, it is effective enough to use in a portable system.

Instead of performing the purification reactions using the established methods which require bulky robotics, the purification is done using Sephadex™ resin, which is a size filtration matrix comprising a gel filtration media incorporating a filtering resin. This matrix allows the larger DNA fragments through while restraining the primers and other unwanted substances.

Sequencing involves cycling the purified PCR product along with sequencing reagents through a pre-defined temperature profile. The product from the sequencing reaction contains fluorescently labelled DNA fragments of varying lengths. The final step of the DNA analysis procedure is to size-separate these fragments in a DNA sequencer.

Thus, with this new protocol, the extraction, amplification and sequencing reactions can all be done in a thermal cycler as all the three procedures require cycling the sample through a pre-defined heat profile. However, the last step of electrophoresis involves separation and detection of migrating DNA fragments and requires a separate DNA sequencer instrument.

4.2 A portable instrument

By improving the purification and quantification procedures and employing a new, more convenient method of extraction, a simplified procedure for DNA analysis has been developed. This procedure is more suitable for automation. The fluorimeter required for real time PCR and the centrifuge are some of the components not required with this new procedure. Also, the functionality of the liquid dispensing mechanism is simplified since the entire extraction procedure can be performed in a single thermal cycler. Using this new DNA protocol, a new portable instrument has been developed that can perform four out of the five steps of the DNA analysis procedure in an automated way. The instrument is capable of performing the processes of DNA extraction, amplification, purification and sequencing but not the electrophoresis step.

Figure 3 shows the prototype portable instrument that can perform all the process from DNA extraction through to sequencing. This instrument consists of a thermal cycler (Techne PHC-3 Dri-Block® Cycler), two

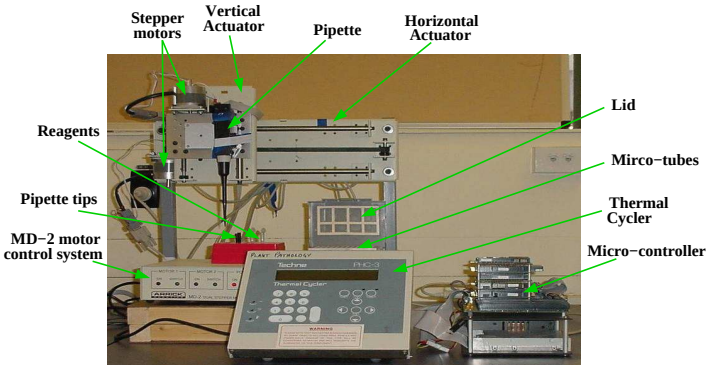


Figure 3: Illustration of the different parts that make up the core of the prototype instrument

linear actuators and an electronic pipette (rLINE BRC 300 manufactured by Biohit Plc, Finland). Any commercially available thermal cycler can be used in the design so long as it includes at least three separate micro-tubes and a heat control lid that can be opened and closed automatically between reactions. Also, it should be possible to pre-program the thermal cycler with the heat profiles for the extraction, amplification and sequencing reactions. The two linear actuators are used to manage the horizontal and vertical movements of the pipette. The linear actuators can be controlled by a computer, they can support the weight of the pipette, and can work together with the top opening lid of the thermal cycler. The actuator used for horizontal movement is attached to a framework built around the thermal cycler to keep the equipment stable. This actuator moves the pipette in the left/right direction. The second actuator acting in the vertical direction, moves the pipette in and out of the micro-tubes.

On the side of the heating chamber are a set of plastic tips that can be used with the pipette. These are within reach of the pipette movement and can be changed between steps to prevent contamination. Big-dye and PCR mix are placed in reservoirs, next to the pipette tips. These too are within reach of the pipette's movement and can be added to the micro-tubes as required.

In the implemented system, the lid of the thermal cycler is not automated, however it is possible to buy a thermal cycler with an automated lid. The thermal cycler is controlled by an integrated microprocessor while the linear actuators and pipette are controlled by external computers. It is possible, if required, to implement a communication protocol to integrate these systems. Thus, the prototype does not incorporate complete 'hands-off' automation. However, there is no manual liquid handling required once the instrument has been started and it is rugged and automated enough for outdoor environments. The instrument weighs ∼19 kgs (excluding the weight of the computers) and costs NZ$12,050 to build (excluding computer and labour costs). The dimensions of the instrument are 50×50×60 cm. However, for transportation the instrument can be separated into three individual components. Also, it will be appreciated that this was only a proof-of-concept implementation. Cheaper, lighter and more effective components could be used for commercial manufacturing.

4.3 Operation of the system

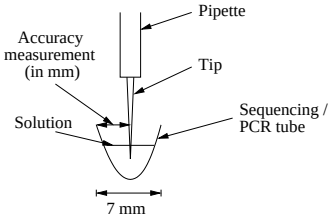
Initially, a pre-prepared mix for the PCR procedure and the sequencing reaction is placed in micro-tubes at the side of the thermal cycler (see Figure 3). Following this, the DNA sample along with a thermostable protease and buffer are added to the extraction micro-tube. Next, the lid is closed and the thermal cycler carries out the pre-programmed temperature profile for the extraction procedure. The extraction procedure takes 45 minutes and once it is completed, the lid is automatically opened and between 2-5 μl of the product (depending on initial sample of DNA) is transferred to the amplification micro-tube. The remaining extracted DNA product can be removed and stored for later use. However, this is optional and is not a requirement to carry out the procedure. Following this, the PCR mix is added to the amplification micro-tube and the lid of the thermal cycler is closed. The cycling protocol for the amplification reaction is now carried out. This takes about 40 minutes. Once this is completed, 1-5 μl of the PCR product is passed through a tube containing Sephadex™ resin and then into the sequencing micro-tube. This is followed by adding the sequencing mix (containing Big-dye® and other sequencing reagents) to the same micro-tube. The lid is now closed and the sequencing reaction is performed in the micro-tube by cycling the temperature between 96℃, 50℃ and 60℃. After close to two hours, the sequencing reaction is complete. Thus, in under four hours and without any manual intervention, fluorescently labelled and sequenced DNA is produced, ready to be analysed in a sequencer.

5 Experimental results

5.1 Performance of the instrument

Tests were performed to show that the instrument is capable of performing the required tasks and reliable enough for field use. The emphasis was on testing the dilution and purification steps. These two steps are associated with the liquid handling component and therefore are dependent on the engineering of the instrument.

To measure the accuracy of the instrument, the protocols for the dilution and purification steps were carried out seven times and each time the distance of the



Test	Accuracy measurement			
	Dilution Step		Purification Step	
	PCR tube	Extraction tube	PCR tube	Sequencing tube
1	4 mm	3 mm	3 mm	4 mm
2	4 mm	2.5 mm	3 mm	3.5 mm
3	3.5 mm	3 mm	3 mm	3 mm
4	4 mm	3 mm	4 mm	4 mm
5	4.5 mm	4 mm	3.5 mm	4 mm
6	4 mm	3.5 mm	4 mm	4 mm
7	3 mm	2.5 mm	4.5 mm	4 mm
** Ideal distance from centre is 3.5 mm*				

Figure 4: The table shows the measurements taken to test the accuracy of the DNA dilution and purification steps. In each test the distance of the pipette from the edge of the extraction, sequencing and PCR micro-tubes was measured. Even though the results show variations of $+/-1$ mm, the accuracy is sufficient since even with the error, the pipette tip is inside the tube when aspirating or dispensing. The accuracy of the measurements is 0.5 mm.

pipette from the edge of the extraction and PCR micro-tubes was measured. The results are listed in Figure 4. The measurements were taken by pausing the movement of the actuators when the pipette was inside the micro-tubes. Since the diameter of the micro-tube is 7 mm, ideally, the centre of the tip should be about 3.5 mm away from the edge of the micro-tube. As can be seen from the table, the measurements show that, in general, the distance of the centre of the pipette tip from the edge is 3-4 mm. Although there is a variation of $+/-1$ mm in the measurements, for our liquid handling requirements, this is manageable since the outer diameter of the pipette tip is only 1-3 mm (depending on how much the tip is immersed inside the tube). It should be noted that the software controlling the actuator was used to improve the accuracy by reseting the positions after each step.

5.2 Proof of the procedure

To show the validity of the new biological protocol developed for a portable instrument, experiments were performed to compare this protocol against the protocol normally used in molecular biology laboratories. The main difference was in the way the extraction and purification steps are performed and the emphasis of the experiments was to show the validity of these two steps and also to qualitatively assess the effectiveness of the procedure.

Since forensic science is one of the possible applications of a portable instrument, a blood stained tissue was used as the source of DNA. The H16539 primer (Invitrogen™ Life Technologies) was used to amplify a 447 base pair long fragment of DNA located in the D-loop region of the human mitochondrial DNA (mtDNA) genome [Greenberg *et al.*, 1983]. This part of the DNA is important for forensic investigations and is routinely employed for identification of individuals, especially at crime scenes [Holland *et al.*, 1993; Stoneking *et al.*, 1991].

The final output from the protocol is an electropherogram, which indicates the amount of fluorescence detected at each base in the amplified DNA molecule. The electropherograms were fed to the ABI® AutoAssembler™ 2.0 software and the output DNA sequence from the software is given in table 1.

The percentage error described in the tables represents the number of peaks in the electropherogram that could not be decoded by the ABI® AutoAssembler™ 2.0 software. The error rate for the Sephadex™ purified reaction was 3% while that for the Roche purified products was <1%. An error rate of up to 5% is normally acceptable from a electropherogram as the error can be corrected using a combination of bioinformatics methods, base calling algorithms and by sequencing the DNA fragment from both sides. Therefore the sequence obtained by the Sephadex™ purified DNA with the H16530

Purification sample: Sephadex™, H16530 primer
Error: 3%

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001 AGATGTCGGATACAGTTCACTTTAGCTACCC--T---TGTTATGGGCC-GGAGCGAGGAGAGTAGCACTCTT
073 GTGCG-CATATTGATTTCACGGAGGATGGTGGTCAAGGGACCCCTATCTGAGGGGGGTCATCCATGGGGACG
145 AGAAGGGATTTGACTGTAATG-GCTATGTACGGTAAATGGCTTTATGTACTATGTACTGTTAAAGATGGGTA
217 GGTTTGTTGGTATCCTAGTGGGTGAGGGGTGGCTTTGGAGTTGCAGTT-ATGTGTGATAGTTGAGGGTTGAT
289 TGCTGTACTTGCTTGTAA-CATGGGGAGGGGGTTTTGATGTGGATTGGGTTTTTATGTACTACAGGTGGTCA
361 AGTATTTATGG-ACCGTGCAATATTCATGGTGGCTGGCAG-AATGTACCAAATA-ATAGCGGT-G-TTGATG
```

Purification sample: Roche, H16530 primer
Error: <1%

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001 AGATGTCGGATACAGTTCACTTTAGCTACCCCCAACTTGTTATGGGCCCGGAGCGAGGAGAGTAGCACTCTT
073 GTGCGG-ATATTGATTTCACGGAGGATGGTGGTCAAGGGACCCCTATCTGAGGGGGGTCATCCATGGGGACG
145 AGAAGGGATTTGACTGTAATGTGCTATGTACGGTAAATGGCTTTATGTACTATGTACTGTTAAAGATGGGTA
217 GGTTTGTTGGTATCCTAGTGGGTGAGGGGTGGCTTTGGAGTTGCAGTTGATGTGTGATAGTTGAGGGTTGAT
289 TGCTGTACTTGCTTGTAAGCATGGGGAGGGGGTTTTGATGTGGATTGGGTTTTTATGTACTACAGGTGGTCA
361 AGTATTTATGGTACCGTGCAATATTCATGGTGGCTGGCAGTAATGTACGAAATACATAGCG-TGG-TTGATG
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Table 1: DNA sequence produced by sequencing the Sephadex™ purified and the Roche purified solutions with the H16530 primer supplied by Invitrogen™ Life Technologies. The '-' represents peaks that could not be decoded by the AutoAssembler software.

primer was well within an acceptable range. Also, since 97% of the DNA fragment could be read by the software, it is clear that the purification of DNA can be successfully done using Sephadex™ as predicted in our design. Although the quality of the electropherogram is not as good as the Roche purified products, it should be noted that this was only a proof-of-concept test of the purification by this method. The Roche purification kit on the other hand is a commercially available DNA purification kit. However, now that the concept of using our optimised protocol for DNA analysis has been validated, experiments can be done to improve the quality of the method.

6 Microfluidics

The previous section described the successful prototyping of a system that is portable and automated, and performs four out of the five steps required for DNA analysis. Even though this instrument is an improvement on current technologies, it uses relatively bulky robotic equipment to add and transfer solutions between steps. Although this is the most natural automated way of moving samples between processes, almost 12 kgs out of the 19 kgs weight of the prototype instrument is for automation. Removing the heavy equipment will reduce the size and weight of the instrument and in addition increase the robustness by eliminating the mechanical movement of the pipette and linear actuators.

One alternative to heavy and bulky automation is to add, transfer and mix solutions on a lab-on-a-chip (LOC) device containing microfluidic channels. Movement of solutions through the microfluidic channels of a LOC device is normally done using electrophoretic and electroosmotic methods which involve varying the electric potential between the ends of the microfluidic channels [Polson and Hayes, 2001; Whitesides and Stroock, 2001; Figeys and Pinto, 2000]. Thus, for such a process to work, the samples involved need to be electrically charged. Since DNA is negatively charged, it is possible to move it through the microfluidic channels.

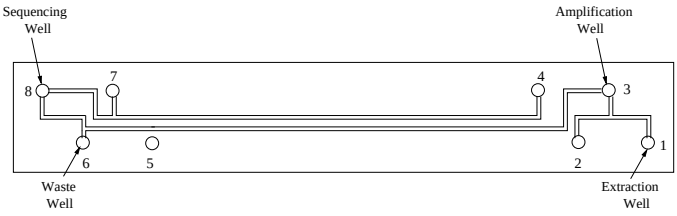

Figure 6: Design of a microfluidic chip capable of being placed in a thermal cycler to perform the DNA extraction, amplification, purification and sequencing reactions

6.1 Design

We have developed a microfluidic device to handle DNA extraction, amplification, purification and sequencing, in conjunction with a thermal cycler and power supply. The extraction, amplification and sequencing reactions are carried out in separate wells of the microfluidic chip. Since thermal cyclers are normally designed to be used with micro-tubes, the casing of the thermal cycler must be altered to enable temperature cycling reactions within the wells of the microfluidic chip. The microfluidic chip uses electrophoretic methods to move DNA between the extraction, amplification and sequencing steps. The power supply and electrodes apply a voltage to migrate DNA across the microfluidic channels. External pipettes integrated with the thermal cycler's lid can be used to dispense reagents into the reaction wells. A computer is used to control the applied voltage, the liquid dispensing and the switching between electrodes.

Thus, by incorporating a microchip into our design for an automated and portable DNA sequencing machine, a number of mechanical processes have been eliminated, such as pipetting and dispensing. It is important to note that the microfluidic device will replace only the automation. Technology for microchip based thermal cycling is still in early development stages. However, with improvements in thermal cycling [Woolley *et al.*, 1996] and power electronics, in the future it may be possible to create a hand-held device to sequence DNA.

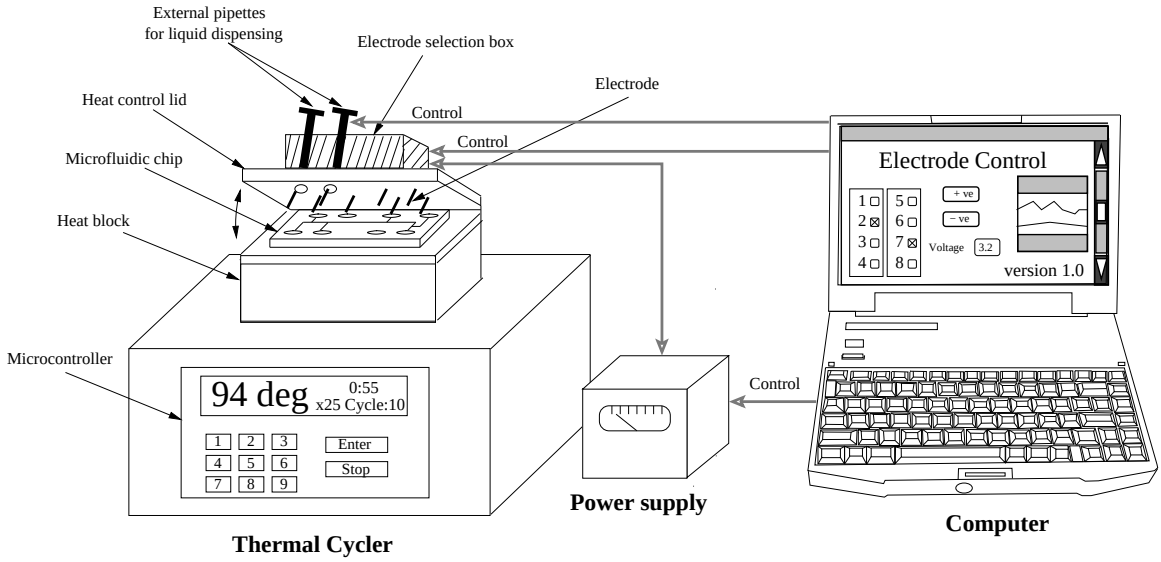

Figure 5: Design for an instrument capable of performing four out of the five steps required for DNA analysis. The design uses microfluidic technology to manage the liquid handling.

6.2 Operation

Initially, the microfluidic chip is placed in a thermal cycler and the extraction procedure is performed in the extraction well (see Figure 6). Next, the PCR procedure used for amplification is reasonably robust and a quantity of 20-50 ng of extracted DNA is sufficient for the reaction to be carried out [Erlich, 1989]. This amount of DNA is present in about 1-2 μl of the extracted DNA solution [Daniel and Saul, 2002]. Therefore, 1-2 μl of extracted DNA must be present in the amplification well for the PCR to be carried out.

At the end of the extraction reaction, a negative voltage is applied to the extraction well and the amplification well is kept grounded. Since DNA is negatively charged, the negative voltage applied to the extraction well repels the DNA out of the well and into the microfluidic channel. Also, since the voltage potential is applied between the extraction and amplification wells, the DNA moves towards the amplification well, but not into the well. This method of moving a sample out of the well and into the microfluidic channel is documented in Footz et al [2001].

Once the DNA has migrated into the channel, the next step is to move it into the amplification well. A positive voltage is applied to the amplification well while the extraction well is kept grounded. Since DNA is negatively charged, it moves towards the amplification well. Also, if the channels are loaded with a sieving medium that hinders diffusion, the DNA will migrate at a relatively slow pace (taking a few minutes) [Crabtree *et al.*, 2001]. Therefore, once the required quantity of DNA has migrated into well 3, the voltage across the wells can be turned off. In this way the required quantity of extracted DNA can be transferred to the amplification well and the amplification reactions can be carried out after adding the required PCR reagents [Waters *et al.*, 1998; Khandurina *et al.*, 2000; Woolley *et al.*, 1996].

The purification step involves applying a positive voltage to the waste well while keeping the amplification well grounded. Since the microchannel contains a sieving matrix and because the amplified DNA molecules are of a different size and have a different electrophoretic mobility in comparison to the primers and other contaminants, they will migrate across the microfluidic channels at a different rate [Chan *et al.*, 1993; Baker, 1995]. The DNA molecules are larger in size by a factor of between four to ten (depending on the amplification primers used) and take at least twice the time (could be much longer) to move across the channel [Baker, 1995]. Thus, after applying the voltage for a short time, the primers and other contaminants will migrate and diffuse into the buffer present in the waste well, while the DNA will still be in the microfluidic channel, about half way between the amplification well and the waste well. At this point in time, the positive voltage applied to the waste well is stopped and instead applied to the sequencing well. Since only DNA is present in the channel, it will migrate towards the sequencing well. The primers and other salts are diluted into the buffer in the waste well and will not enter the channel [Footz *et al.*, 2001]. Thus, eventually the sequencing well will contain purified DNA ready for sequencing. The required reagents can be added to this well and the sequencing

Expt.	Time for which a 2 kV voltage was applied to the amplification well	DNA concentration in the amplification well after the application of the voltage
A	2m	18.33 ng/ul
B	2m30s	22.56* ng/ul
C	3m	41.32* ng/ul
D	3m30s	52.22 ng/ul
E	4m	51.43 ng/ul
**conc. within amount needed for amplification reaction*		

Table 2: Variations in concentration of DNA collected at the amplification well by varying the time for which a positive voltage was applied at the well. For all the experiments, an initial voltage of -2 kV was applied to the extraction well for 30 seconds.

reaction can be carried out. On completion, the product in the well is sequenced DNA ready for separation and detection.

6.3 Evaluation

To show the validity of the design, a microfluidic chip capable of performing the extraction, dilution, amplification, purification and sequencing steps was fabricated. Experiments were performed to show the use of the microchip in carrying out the steps of the DNA analysis process. Since the extraction, amplification and sequencing reactions involve only temperature cycling and the microfluidic chip can be used up to temperatures beyond those required for these reactions, the emphasis was on proving the dilution and purification reactions. Experiments were performed to show the dilution of DNA from the extraction to the amplification step using electrophoretic methods.

The microchip used is a Protolyne™ custom chip made of glass. To manage the power supply requirements, a Spellman CZE1000R 9W auto-reversing power supply was used. This power supply can deliver up to 30 kV output voltage at a maximum load current of 300 μA. Platinum wire (EPSI, .020" 3N5) was used for the electrodes. The glass microchip was placed on a perspex block, which acted as an insulator. The block was placed on a wooden base built to support the electrodes and improve the reproducibility of the experimental setup.

Initially 150 ng of extracted DNA was placed in the extraction well and a voltage of -2 kV was applied for 30 seconds to the well. This was followed by applying a voltage of 2 kV for between 2-4 minutes. Results from the experiments are listed in Table 2. In most of the experiments performed, it was observed that at the end of the electrophoretic movement, the quantity of solution present in the amplification well was in the range of 1-1.5 μl. For the amplification reaction to be successful, about 20-50 ng of DNA is needed in the amplification. Thus, it can be inferred from this set of experiments involving DNA extracted from human blood, that the best protocol to use is to first apply a voltage of -2 kV for 30 seconds followed by a voltage of 2 kV for about 2 minutes 30 seconds to 3 minutes.

Thus, the results from the experiments have been successful in demonstrating the dilution of DNA and its movement from the extraction well to the amplification well.

6.4 Experimental conditions and precautions

The DNA used for the experiments was extracted from human blood. Also, the channels were loaded with POP-6™ matrix and the wells with genetic analyser buffer to allow for electrophoretic movement. In addition, the microchip was also thoroughly cleaned between experiments by flushing deionised water through the channels by means of a 1 ml syringe. This was repeated at least 5 times. Following this, the chip was dried, reloaded and a series of 2 kV, 10 second electrophoretic injections were made between the extraction and amplification wells. These injections were continued until no current was flowing between the channels. This was followed by another series of flushing deionised water through the channels. Finally, the chip was allowed to dry before using for the next experiment. This cleaning procedure was followed between each experiment and was a precautionary measure to prevent any contamination from the previous experiment. Another precaution taken involved cleaning the electrodes with ethanol before each experiment. Common laboratory precautions such as wearing gloves, using filtered pipette tips, etc. were also observed when dealing with DNA.

7 Conclusion

By improving the extraction, purification and quantification steps of the DNA analysis procedure, a novel method of DNA analysis has been devised. This method is more suitable for portability and automation. Using this new protocol for DNA analysis, a portable instrument has been designed that can perform four out of the five steps of the DNA analysis procedure in an automated way. The instrument is capable of performing the processes of DNA extraction, amplification, purification and sequencing but not the electrophoresis step.

The instrument weighs ∼19 kgs (excluding the weight of the computers) and cost NZ$12,050 to build (excluding computer and labour costs). Its dimensions are 50×50×60 cm; for transportation it can be separated into three parts. Also, it will be appreciated that this was only a proof-of-concept implementation. If manufactured, cheaper, lighter and more effective components

would be used.

Reliability tests were performed to measure the accuracy of the movement of the linear actuators. Although there is a variability of $+/-$ 1 mm in the repeatability of the movement, the accuracy is manageable. Experiments were done to show the validity of the novel biological procedure that is employed in the design. Thus, a proof-of-concept portable and automated instrument has been successfully implemented and tested. It is capable of performing four out of the five steps required for DNA analysis.

The quality of the method was not as good as manual procedures used in DNA sequencing laboratories, but it was good enough to give the DNA sequence, which is the required output. Further refinement should improve the quality.

A prototype microfluidic device was designed for DNA extraction, amplification, purification and sequencing.

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