DESIGN OF A ROBOTIC DEVICE FOR AUTOMATED NUCLEIC ACID EXTRACTION FROM BIOLOGICAL SAMPLES

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ABSTRACT

DESIGN OF A ROBOTIC DEVICE FOR AUTOMATED NUCLEIC ACID EXTRACTION FROM BILOGICAL SAMPLES

Nucleic Acids (DNA or RNA) present the genetic structure of the cell or the organism and so are the essential components to make genetic testing. Molecular genetic testing allows one to analyze the genetic structure of an organism to have an idea about the present temporary or hereditary characteristics of the tissue or the whole organism, or specifically define its species. In order to analyze the genetic structure, one must extract and isolate the nucleic acids (NA), which are most of the time inside the cell. The aim of this thesis study is to design and manufacture an automated device with low throughput DNA extraction. Currently, the automated devices used for extraction of genetic material are being manufactured only by the foreign companies. Automated commercial devices used for this purpose were investigated in detail as well as the manual NA extraction hand tools for use in NA extraction. Commercially available components (pipette tip, reagent cartridges, tubes, etc.) to isolate NA were reviewed. Mechanism design process for a low cost and high precision system that requires minimal human operator intervention is carried out. The conceptual designs were developed and the final design of the device was made to comply with the selected components. Electronic equipments (motors, drivers, interface card, etc.) and a suitable graphical user interface compatible with the electronic components was selected and adapted to the system. Finally, a device which is competitive with the commercial ones has been designed and its prototype has been manufactured as a result of this thesis study.

ÖZET

BİYOLOJİK NUMUNELERDEN NÜKLEİK ASİDİN OTOMATİKLEŞTİRİLMİŞ İZOLASYONU İÇİN ROBOT TASARIMI

Nükleik asitler (DNA veya RNA) hücrenin veya organizmanın genetik yapısını temsil ederler ve bu nedenle genetik testler için gerekli bileşenlerdir. Moleküler genetik testler organizmanın anlık, geçici ya da kalıtımsal karakteristikleri hakkında bilgi sahibi olunabilmesi için genetik yapısının analiz edilmesini sağlar. Genetik yapının analiz edilebilmesi için çoğu zaman hücre içinde bulunan nükleik asitlerin (DNA veya RNA) izole edilmesi gerekmektedir. Bu çalışmanın amacı düşük çıktılı, otomatize edilmiş bir DNA izolasyon cihazının tasarlaması ve üretilmesidir. Günümüzde, genetik bileşenlerin elde eidlmesi için kullanılan otomatize cihazlar yalnızca yabancı şirketler tarafından üretilmektedir. Otomatik DNA izolasyon cihazlarının yanı sıra manuel DNA izolasyon ekipmanları da araştırılmıştır. Ticari olarak piyasada bulunan, DNA izolasyonunda kullanılan parçalar (pipet uçları, reaktif kartuşlar, tüpler, vb.) gözden geçirilmiştir. Düşük maliyetli, yüksek hassasiyetli ve minimum operatör müdahalesini gerektirecek mekanizma tasarımı ortaya çıkarılmıştır. Kavramsal tasarımlar geliştirilmiş ve seçilmiş olan parçalarla uyumlu olacak şekilde son tasarım elde edilmiştir. Elektronik ekipmanlar (motor, sürücü, arayüz kartı, vb.) ve uyumlu bir kullanıcı arayüzü seçilmiş ve sisteme adapte edilmiştir. Bu çalışmanın sonucu olarak, piyasada bulunan ticari cihazlar ile rekabet edebilir bir tasarım ortaya konmuş ve bir prototip üretilmiştir.

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CHAPTER 1

INTRODUCTION

Molecular tests start to have an upward tendency to have importance. Everyday more genetic tests are being introduced to the routine use for medicine after the completion of human genome sequencing which marked the end of the first phase of the genomics revolution. Microbiology, biochemistry, pathology, pharmacology and many branches of biology recruit genetic based tests for diagnosis as well as in vitro research purposes. Molecular genetics is not only used in medicine area but veterinary, zoology, agriculture and all areas related with organisms involve in the genetic tests. Molecular genetic testing allows one to analyze the genetic structure of an organism to have an idea about the present temporary or hereditary characteristics of the tissue or the whole organism, or specifically define its species. Nucleic Acids (DNA or RNA) present the genetic structure of the cell or the organism and so are the essential components to make genetic testing. Laboratories have been forced down the route of expensive systems which is restricting their use to laboratories with the best research financing (NGRL 2009). On the other hand, manual preparation kits continue to be extremely time consuming and stressful. Thus the importance of the low throughput automated DNA purification devices are increasing rapidly.

1.1. Objective of the Thesis

The aim of this thesis study is to design and manufacture an automated device with low throughput DNA extraction. Automated commercial devices used for this purpose were investigated in detail as well as the manual NA extraction hand tools for use in NA extraction. The NA extraction methods are studied and the most appropriate one for automation is selected. A robotic workstation which can extract NA's has been designed and its prototype has been manufactured as a result of this thesis study.

1.2. Contents of the Thesis

In the folowing chapters NA's, types of NA's are explained detailing with the chemical structure. Then the need for nucleic acid extraction, NA sources and exraction methods are told and the most appropriate one for automation are decided. Currently available NA extraction devices are examined in detail.

Next mechanical design of the workstation is mentioned with the design specifications, design criterias and conceptual designs. After the final design is decided with the suitable components, manufacturing of the NA extraction device are explained.

Electronics and computer software are talked. A suitable graphical user interface selection is explained and the adaptation of this graphical user interface to the system is presented.

And finally test results of the NA exractor are submitted, critical coding steps are talked. Problems during testings and the solutions for these problems are explained. The accomplished sytem is discussed and the future works are determined.

CHAPTER 2

NUCLEIC ACIDS

Living organisms are complex systems. Hundreds of thousands of proteins exist inside each one of us to help carry out our daily functions. These proteins are produced locally, assembled piece-by-piece to exact specifications. An enormous amount of information is required to manage this complex system correctly (Roberts et al. 2002) (Seanger 1984). This information, detailing the specific structure of the proteins inside of our bodies, is stored in a set of molecules called Nucleic Acids (NA) (Figure 2.1). There are several types of NA's which serves at different levels of this act, given in detail in the following sections of this chapter.
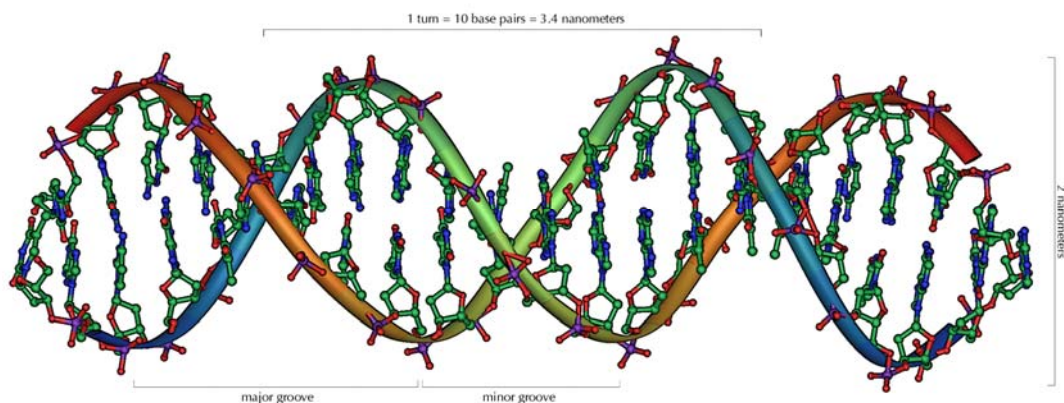
Figure 2.1. Schematic representation structure of a nucleic acid (DNA molecule)
(Source: Wikipedia 2009)

The discovery of the genetic material was done by a Swiss physician and biologist, Johannes Friedrich Miescher. He isolated various phosphate-rich chemicals, which he called nuclein (now nucleic acids), from the nuclei of white blood cells in 1869 at Felix Hoppe-Seyler's laboratory at the University of Tübingen, Germany.

Miescher devised different salt solutions eventually producing one with sodium sulfate. The cells were filtered. Since centrifuges were not present at this time the cells

were allowed to settle at the bottom of a beaker. He then tried to isolate the nuclei free of cytoplasm. He subjected the purified nuclei to an alkaline extraction followed by acidification resulting in a precipitate being formed which Miescher called nuclein (now known as DNA). He found that this contained phosphorus and nitrogen, but not sulfur (Watson 1969) (Dahm 2008).

2.1. Types of Nucleic Acids's

There are mainly two kinds of nucleic acids; deoxyribo-nucleic acid (DNA) and ribonucleic acid (RNA). Detailed information about these nucleic acids is given in the following.

2.1.1. Deoxyribo-Nucleic Acid (DNA)

In most living organisms (except for some viruses), initial and hereditary genetic information is stored in the molecule deoxyribonucleic acid (or DNA). DNA is made and resides in the nucleus of living cells. DNA gets its name from the sugar molecule contained in its backbone-deoxyribose; however, it gets its significance from its unique structure.

2.1.2. RiboNucleic Acid (RNA)

RiboNA, or RNA, gets its name from the sugar group in the molecule's backbone–ribose. RNA is important in the production of proteins in other living organisms that it can move around the cells and thus serves as a sort of genetic messenger, relaying the information stored in the cell's DNA out from the nucleus to other parts of the cell where it is used to help make proteins. RNA is also the main genetic material in some viruses. RNAs are named like messanger (mRNA), transfer (tRNA), ribosomal (rRNA), (small interfering) siRNA, (micro) miRNA and so according to their functions in the cell.

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2.2. Chemical Structure of the NA's
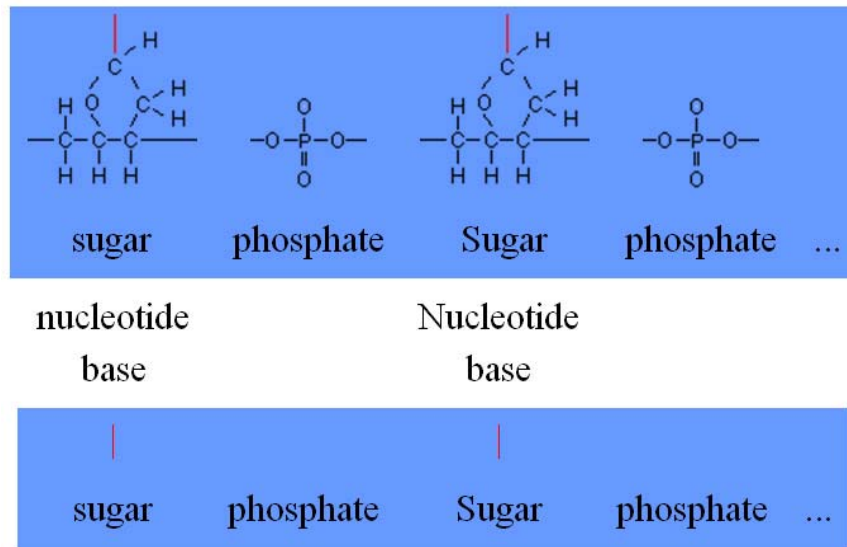
Figure 2.2. Molecular structures of sugar and phosphate molecules
(Source: Vision Learning 2009)

The NAs are very large molecules that have two main parts. The backbone of a NA is made of alternating sugar and phosphate molecules bonded together in a long chain, represented in Figure 2.2.

Each of the sugar groups in the backbone is attached (via the bond shown in red) to a third type of molecule called a nucleotide base. Though only four different nucleotide bases can mainly occur in a NA, each NA contains millions of bases bonded to it. The order in which these nucleotide bases appear in the NA is the coding for the information carried in the molecule. In other words, the nucleotide bases serve as a sort of genetic alphabet on which the structure of each protein in our bodies is encoded.

2.2.1. Chemical Structure of the DNA

Four main nucleotide bases are present in DNA: adenine (A), cytosine (C), guanine (G), and thymine (T). These nucleotides bind to the sugar-phosphate backbone of the molecule, shown schematically in Figure 2.3 as follows.
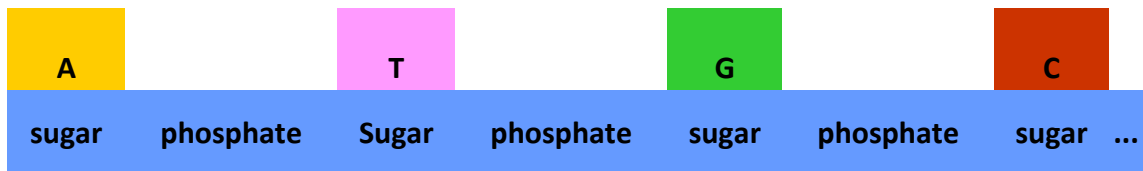
Figure 2.3. Schematic representation of the molecular structure of nucleotides bind to the sugar-phosphate backbone (Source: Vision Learning 2009)

2.2.2. The DNA Double Helix

In the early 1950s, four scientists, James Watson and Francis Crick at Cambridge University and Maurice Wilkins and Rosalind Franklin at King's College, determined the true structure of DNA from data and X-ray pictures of the molecule that Franklin had taken (Watson 1969). In 1953, Watson and Crick published a paper in the scientific journal *Nature* describing this research. Watson, Crick, Wilkins and Franklin had shown that not only is the DNA molecule double-stranded, but the two strands wrapped around each other forming a coil, or helix. The true structure of the DNA molecule is a double helix shape as shown in Figure 2.4 below.
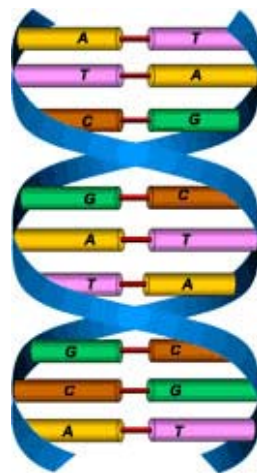
Figure 2.4. Schematic representation of the molecules and structural shape of the DNA molecule (Source: Vision Learning 2009)

The versatility of DNA comes from the fact that the molecule is actually double-stranded. The nucleotide bases of the DNA molecule form complementary pairs: The nucleotides hydrogen bond to another nucleotide base in a strand of DNA opposite to the original. This bonding is specific, and adenine always bonds to thymine (and vice

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versa) and guanine always bonds to cytosine (and vice versa). This bonding occurs across the molecule, leading to a double-stranded system as represented in the following (Figure 2.5).

Sugar	phosphate	sugar	phosphate	sugar	phosphate	sugar ...
T		A		C		G
A		T		G		C
Sugar	phosphate	sugar	phosphate	Sugar	phosphate	sugar ...
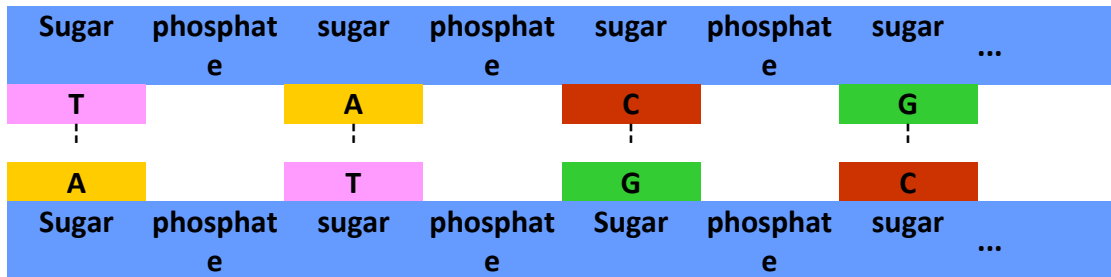
Figure 2.5 Double-stranded system of bonding of molecules
(Source: Vision Learning 2009)

The double-stranded DNA molecule has the unique ability that it can make exact copies of itself, or self-replicate. When more DNA is required by an organism (such as during reproduction or cell growth) the hydrogen bonds between the nucleotide bases break and the two single strands of DNA separate. New complementary bases are brought in by the cell and paired up with each of the two separate strands, thus forming two new, identical, double-stranded DNA molecules.

2.2.3. Chemical Structure of the RNA

Several important similarities and differences exist between RNA and DNA. Like DNA, RNA has a sugar-phosphate backbone with nucleotide bases attached to it. Like DNA, RNA contains the bases adenine (A), cytosine (C), and guanine (G); however, RNA does not contain thymine, instead, RNA's fourth nucleotide is the base uracil (U). Unlike the double-stranded DNA molecule, RNA is a single-stranded molecule (Figure 2.6).
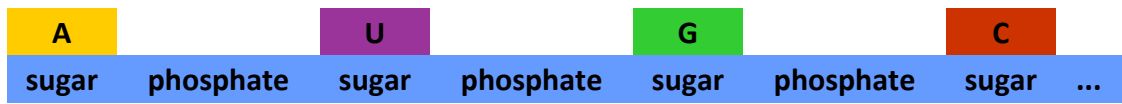
Figure 2.6. Molecular structure of RNA
(Source: Vision Learning 2009)

CHAPTER 3

NUCLEIC ACID EXTRACTION

Molecular tests start to have an upward tendency to have importance. Everyday more genetic tests are introduced to the routine use for medicine after the completion of human genome sequencing which marked the end of the first phase of the genomics revolution. Microbiology, biochemistry, pathology, pharmacology and many branches of biology recruit genetic based tests for diagnosis as well as in vitro research purposes. Molecular genetics is not only used in medicine area but veterinary, zoology, agriculture and all areas related with organisms involve in the genetic tests. Molecular genetic testing allows one to analyze the genetic structure of an organism to have an idea about the present temporary or hereditary characteristics of the tissue or the whole organism, or specifically define its species.

3.1. The Need for NA Extraction

NAs (DNA or RNA) represent the genetic structure of the cell or the organism and so are the essential components to make genetic testing. In order to analyze the genetic structure, one must extract and isolate the NAs (NA) which are most of the time inside the cell. Isolation of NAs are performed in the laboratory for a variety of reasons such as cloning of desired genes, comparisons of different genomes, study of expression patterns, or forensic evaluation. No matter the reason for the isolation of the NA, the general procedures are similar.

3.2. Sources of NA's

The source for the NA are various: Basically it can be isolated from any living or dead organism, Body fluids containing cells or free genetic material like blood, sputum, urine, feces or any other tissue specimens like buccal swaps, biopsy material or hair follicles can serve as the source in human and other animals; while in vitro cultures

can be used for viral or bacterial genome or plasmid DNA extraction as well as eukaryotic NA isolation. NA isolation is also possible from various plant tissues.

3.3. NA Extraction Process

NA isolation process can be summarized as cellular lysis, decreasing the solubility of the NAs, specific compartmentalization of lysate soup according to characteristic intermolecular forces or buoyancy, washing steps of the unwanted debris and finally isolation and elution of the NA's.

There are several different ways for this process changing in types of the chemicals used, labor time and standardization and resulting in different yield and purity. Traditionally, NA extraction from mammalian cells has been a tedious and time-consuming process (Cler, et al. 2006).

3.4. Commonly Used NA Extraction Methods

The rapidly growing field of molecular diagnostics and molecular phylogeny requires a need for quick, simple, robust, and high-throughput procedures for extraction of NA from diverse organisms and tissues. It is quite clear that the extraction methods have to be adapted in such a way that they can efficiently purify DNA from various sources. Another important factor is the sample size. If the sample is small (for example sperm, or a single hair) the method has to be different to the method used in isolating DNA from a couple of milligrams of tissue or mililiters of blood. Another important factor is whether the sample is fresh or has been stored. Stored samples can come from archived tissue samples, frozen blood or tissue, exhumed bones or tissues, and ancient human, animal, or plant samples (E-notes 2009). As an alternative to traditional extraction protocols (e.g., phenol/chloroform procedure), newer approaches like single-step proteinase K digestion (without the use of organic solvents), simplified approaches for sequentially precipitating proteins and then DNA or adsorption separation techniques have been applied for DNA purification. The most commonly used supports to adsorb DNA include silica-based particles, glass fibers, anion-exchange carrier and modified magnetic beads. Summary of the DNA extraction methods are summarized in Table 3.1 below.

Table 3.1 Summary of five extraction methods
(Source: Cler, et al. 2006)

Summary of the five DNA extraction methods

Method	Name	Vendor	Catalog no.	Salient features
1	QIAamp blood Mini-kit	Qiagen	51104	Silica-gel membrane binds DNA, contaminants pass through spin column
2	Puregene	Gentra	D-5500A	Protein precipitation followed by DNA precipitation
3	Dynabeads DNA DIRECT universal	Dynal	630.06	DNA adsorbed onto magnetic beads
4	Phenol/ chloroform	N/A	N/A	Proteinase K, followed by organic extraction, followed by DNA precipitation
5	Proteinase K	N/A	N/A	Proteinase K without DNA precipitation

3.4.1. Organic Extraction

The classical approach to DNA extraction employs organic solvents to dissolve DNA after which it is precipitated in absolute alcohol (Valgren, et al. 2008). Though suitable for highly cellular samples, this approach requires multiple centrifugation steps and often results in poor yields of amplifiable DNA when the starting material is limited. Organic extraction (phenol/chloroform) not only utilizes hazardous chemicals but also requires multiple centrifugation steps.

3.4.2. Chelex Extraction

Chelex extraction is rapid and relatively cheap method but it can leave inhibitors for consequent reactions, like PCR (Polymerase Chain Reaction) in the final extract. Chelex ion exchange resin binds multi-valent metal ions and is particularly useful in removing inhibitors from DNA (Valgren, et al. 2008). It can be used with any type of sample, including whole blood, bloodstains, seminal stains, buccal swabs, or hair. The only difference from the previous method is the presence of resin, which binds the

impurities from the solution, while DNA is being left in the solution. By centrifuging the samples, the resin is brought to a pellet and separated.

3.4.3. Silica Matrices Extraction

Purification on silica matrices seems to be the best candidate for automation as the extraction process does not require centrifugation or application of pressure or vacuum to move solutions through a chromatography bed, gives high yield and possible PCR inhibitors are efficiently removed. The silica based fiber or bead technology to isolate NAs exploits the reversible boundary properties of NAs to silica derivatives.

3.4.4. Paramagnetic Particles Extraction

One of the most recent and popular technology recruits the microbeads with silica shell and paramagnetic cores collect the NAs in a solution and to collect the beads in a magnetic field. Magnetic microparticles (Fe_3O_4 or magnetite) are applicable as a medium for NA purification with the aim of producing a universal approach for extraction of NA from biological samples like blood leukocytes, cultured cells, tissues, body fluids, bacteria and plants. The magnetic DNA extraction is very efficient in removing inhibitors of the PCR reaction. During recent years, magnetic separation techniques using magnetizable solid-phase supports have become increasingly applied to a number of biological applications.

3.5. Automation in Laboratories

The process of NA isolation is performed manually or at different grades of automation in the laboratories which offers genetic testing. Many laboratories worldwide still continue to isolate the DNA manually due to cost, when high throughput ones prefer fully automatized systems more due to lots of benefits like less hands on time, standardization, less exposure to toxic chemicals and good yield and purity results. In order to automate DNA extraction it is necessary to use a suitable purification method when cost aspects, automation friendliness, and chemistry characteristics such

as DNA binding capacity and recovery rates are considered. The extraction method recruiting paramagnetic microbeads appear to be a practical choice for automation (Valgren, et al. 2008).

3.6. Paramagnetic Microbeads

Separation technology is one of the most complex and important areas of biotechnology and biomagnetic separation techniques are becoming increasingly important with a wide range of possible applications in the biosciences. One of the most recent and popular technology recruit the microbeads. Microbeads are small particles (around 30 nm diameter spheres) with silica shell and paramagnetic cores to collect the NAs in a solution. Then the beads are collected in a magnetic field (Figure 3.1). Magnetic microparticles (Fe_3O_4 or magnetite) are applicable as a medium for NA purification with the aim of producing a universal approach for extraction of NA from biological samples like blood leukocytes, cultured cells, tissues, body fluids, bacteria and plants (Haak, et al. 2008). The magnetic DNA extraction is very efficient in removing inhibitors of the downstream reactions.
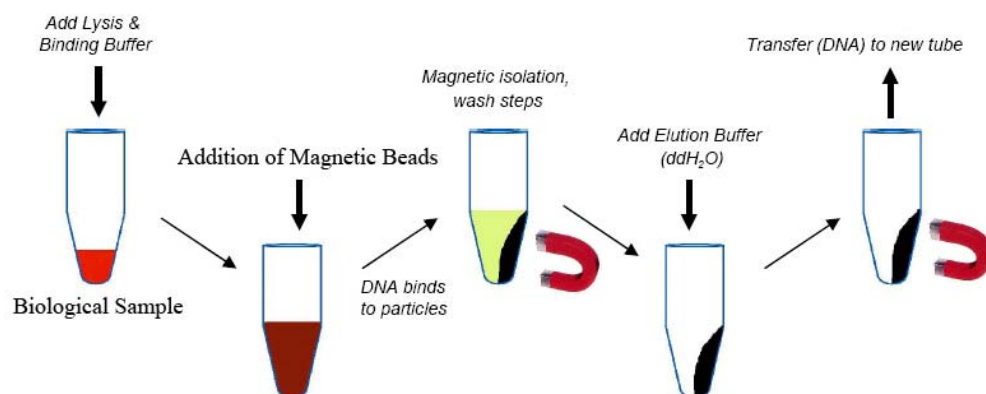
Figure 3.1. Schematic representation of extracting genetic material from organic sample using magnetic beads (Modified from Source: Chemicell 2009).

The main advantages of magnetic separation techniques are; fast and simple handling of a sample vial and the opportunity to deal with large sample volumes

without the need for time-consuming centrifugation steps. This also makes biomagnetic separation ideal for automated assay/analysis systems (Saiyed, et al. 2006).

3.6.1. Structure of the Microbeads

Magnetic particles for bioseparation consist of one or more magnetic cores with a coating matrix of polymers, silica or hydroxylapatite with terminal functionalized groups. The magnetic core generally consists either of magnetite (Fe_3O_4) or maghemite (gamma Fe_2O_3) with superparamagnetic or ferromagnetic properties (Figure 3.2).
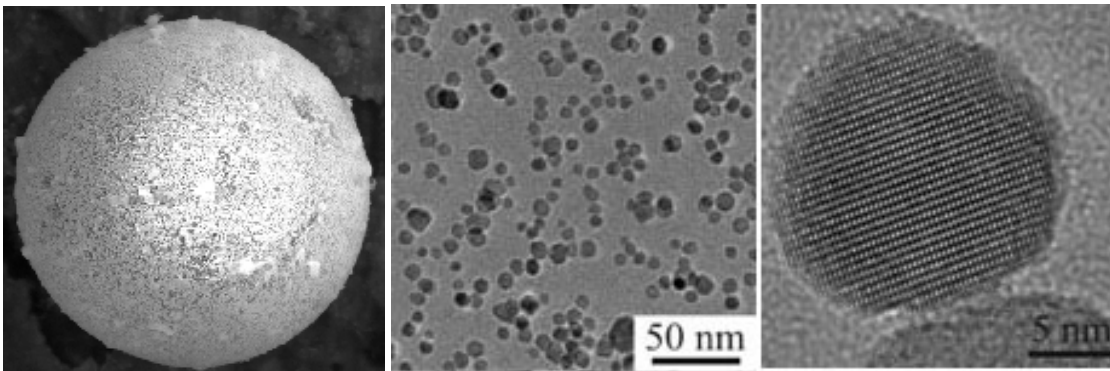
Figure 3.2. Microscopic views of magnetic beads
(Source: Wikipedia 2009)

Particles can be produced with magnetic cores made with magnetic ferrites, such as cobalt ferrite or manganese ferrite. Superparamagnetism is when the dipole moment of a single-domain particle fluctuates rapidly in the core due to the thermal excitation so that there is no magnetic moment for macroscopic time scales. Therefore, these particles are non-magnetic when an external magnetic field is applied but do develop a mean magnetic moment in an external magnetic field. In contrast, ferromagnetism means that the particles have a permanent mean magnetic moment. Here, the larger effective magnetic anisotropy suppresses the thermally activated motion of the core-moments.
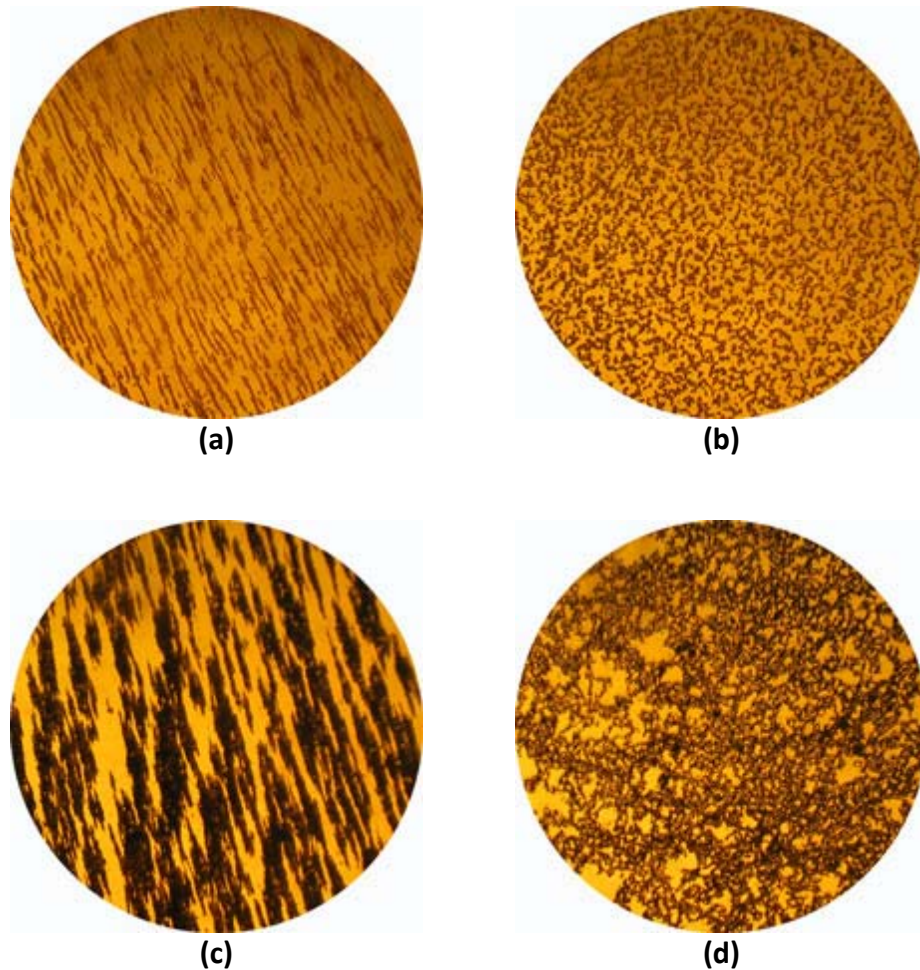
Figure 3.3. (a) Superparamagnetic particles under the influence of an external magnetic field (b) superparamagnetic particles in absence of an external magnetic field, monodisperse particle distribution (c) Ferromagnetic particles under the influence of an external magnetic field (d) Ferromagnetic particles in absence of an external magnetic field, lattice form (Modified from Source: Chemicell 2009).

Advantages of the superparamagnetic particles are easy resuspension, large surface area, slow sedimentation and uniform distribution of the particles in the suspension media. Once magnetized, the particles behave like small permanent magnets, so that they form aggregates or lattice due to magnetic interaction (Figure 3.3 (a) and (b)). Advantages of ferromagnetic particles are very strong magnetic properties and therefore the fast separation with an external magnetic field even in viscous media (Figure 3.3 (c) and (d)). Most commonly ferromagnetic particles are used for the separation of DNA/RNA (SiMAG/MP-DNA), whereas superparamagnetic particles are more suitable for all other applications.
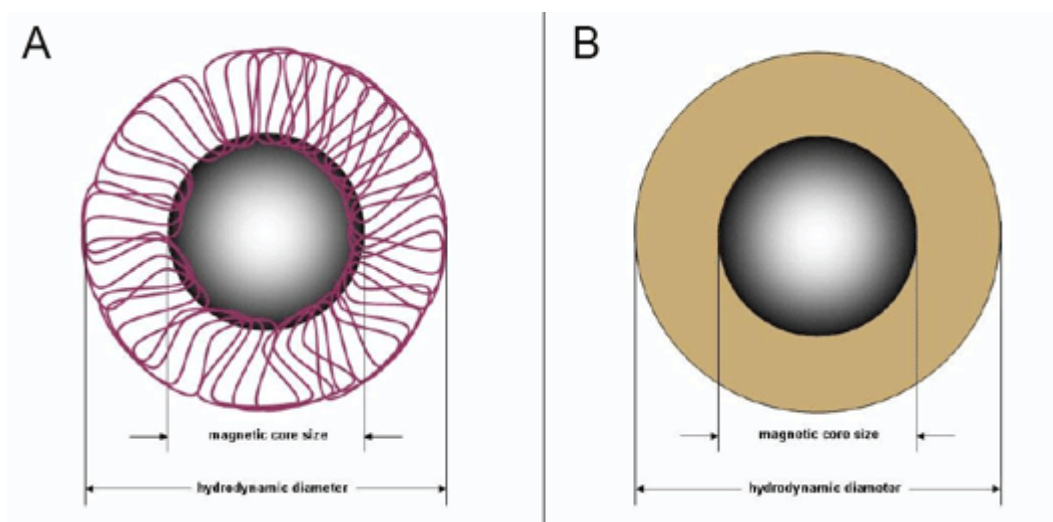
Figure 3.4. Schematic representation of the assembly of (a) fluidMAG and (b) SiMAG particles (Modified from Source: Chemicell 2009).

The particle size (particle diameter) is given as hydrodynamic diameter, which includes the core diameter and two times the diameter of the cover matrix. The hydrodynamic diameter is determined by Dynamic Light Scattering (DLS) also known as Photon Correlation Spectroscopy (PCS). As fluidMAG particles offer a very hydrophilic with water molecules filled polymer matrix a smooth transition appears between the matrix and the surrounding liquid of the suspension (Figure 3.4 (a)). Hence no exact defined surface edge is given. However SiMAG particles possess a solid cover matrix (Figure 3.4(b)). Therefore a distinct transition between the solid matrix and liquid suspension medium exist and the hydrodynamic diameter is accurately determinable.

The amount of magnetic particles in gram per volume unit and is gravimetrically determined from a well measured volume of the suspension medium, and is defined as the weight of volume. The weight of volume is not identical with the particle concentration as the numbers of particles per volume unit in a suspension will change due to the force of gravity.

3.6.2. Availability of the Microbeads

The commercial availability of the chemicals is of cardinal importance when designing the system. As every other part, consumables have to be easily supplied and if alternative ways are possible, the most cost-effective one has to be selected. Magnetic particles for bioseparation is readily available in commercial forms consisting of one or more magnetic cores (*magnetite* (Fe_3O_4) or *maghemite* (gamma Fe_2O_3) with superparamagnetic or ferromagnetic properties) with a coating matrix of polymers, silica or hydroxylapatite with terminal functionalized groups. With the increasing demand of these particles in especially biotechnology areas, tens of companies can offer particles on their web sites, as off the shelf. Companies also provide particles in different sizes and concentrations and different shell coats for the particles or offer any custom structure modifications.

3.6.3. NA Isolation Procedure Using Paramagnetic Particles

NAs are mostly present inside the cells even they can be in free form in biological fluids. To isolate the NA inside the cell, one must first burst the cell to release the NA inside by using several chemical solutions (Figure 3.1). This robotic workstation is a liquid handling platform to process the mixing steps of chemical solutions in NA isolation.

The first step of the NA isolation from the biological fluids aims to burst the cell membrane and nuclear membrane if present. These membranes are composed of lipid and proteins and so are susceptible to detergents and protein lyzing enzymes. So, adding a solution of detergents and protein lyzing enzymes in a buffer over the cell containing fluids; mixing and waiting for several minutes will be the first step in the procedure. After several minutes, the solution will turn into a soup of cell debris, protein, lipid and polysaccharide with free floating NA.

The next step aims to decrease the solubility of the NA in the solution and involves addition of chaotrophic salts in a high concentration.

This is followed by collecting the NA on the silaxate coated beads with paramagnetic core (FeO_3). Silaxate has an affinity for NA at right pH and in high salt and polyalkylene concentrations and reversibly binds to free NA in the solution. This

allows one to easily isolate the NA bound beads from the rest of the soup, by applying a magnetic field. But the isolated beads do not only contain NA in pure form, since some debris remnants are trapped between the filamentous structures of the NA.

Removing the magnetic field, washing the beads with fresh buffer, while keeping the pH and high salt solution constant, causes beads to resolve and reapplying the field will again collect the NA bound beads together but leaving some of the debris dissolved in the wash buffer. Repeating this last step for several times will cause the NA on the beads to have a better purity every time. These washing steps must be optimized for wash buffer concentrations, washing periods and number of washes for the best effective purity and yield combination, since every wash also causes loosing some of the NA also in the supernatant.

The procedure ends with elution of the NA from the magnetic beads, by lowering the salt concentration and changing the pH. Applying the magnetic field, this time causes the empty beads to aggregate on the magnet, leaving the free NA dissolved in the elution buffer. The supernatant containing the isolated NA can be collected easily without removing the magnetic field.

3.6.4. Commonly Used Laboratory Tools for Magnetic Bead NA Exraction

There are several types of hand pipetters for use in magnetic bead NA extraction (Figure 3.5). Basicly they are all composed of accurate sringe pumps including a pipette tip exracting mechanism and a ml scale. Hand pipetters are capable of sucking a pre adjusted volumes by the adjustable scale. Volumetric capacities start from 5-50 µl and rises up to 2 ml. These hand pipetting equipments also have multi head models for multiple testing in a shorter period of time.

Figure 3.5. Some examples of types of hand pipetters.

In the following picture (Figure 3.6), an example of 100-1000 µl pipetter, volumetric scale and pipette tip exracting mechanism can be seen. The picture of 5-50 µl pipetter is given in Figure 3.7.

5 cm

Figure 3.6. Picture of 100-1000 µl pipette and mechanisms of application.

Figure 3.7. Picture of 5-50 µl pipetter with 50 µl pipette and volumetric scale.

Some of these hand tools for extraction have multi-pipette holding capacity, like the one shown in Figure 3.8 below. The multi-pipette holder has a mechanism for extracting the pipettes and activated by the movement of the arm and the pipettes are extracted at the same time (Figure 3.9).

Figure 3.8 Picture of multi-head hand pipette.

Figure 3.9. 20-200 µl Multy head hand pipetter and pipette tip extracting mechanism.

In the picture below, several kinds of pipette tips for different volumes handling, are represented (Figure 3.10). Detailed picture from some of the pipettes are also given in the following picture (Figure 3.11).

5 cm

Figure 3.10. Views of various types of pipettes used
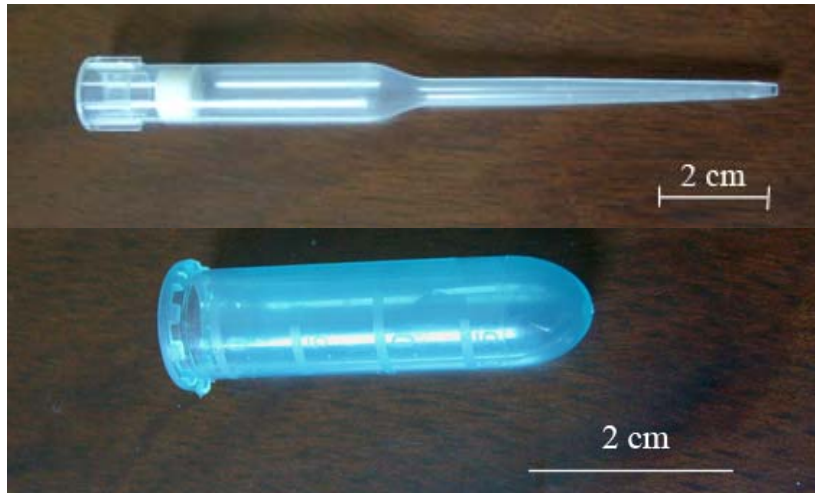
Figure 3.11. Detailed views of different types of pipettes used

3.6.5. A Manually Performed Magnetic Bead NA Extraction

In this section manually NA extraction from blood sample using magnetic beads will be presented in detail. To be able to do the analysis kit of chemicals is neccessary; hence it is decided to use BILATEST® Genomic DNA Kit 250 for extraction of DNA manually due to its availibility advantages. The BILATEST® Genomic DNA Kit 250 is designed for the simple and fast isolation of genomic DNA from 250 µl whole blood. The complete protocol takes approximately 30 minutes; the expected yield from 250 µl normal healthy whole blood is 5-10 µg DNA. This chemical kit consists of 8 chemicals and is optimized for use with BILATEC Magnetic Separators (Figure 3.12). These included reagents are as follow:

- Lysis Buffer   17.5 ml
- Magnetic Beads   2.5 ml
- Binding Buffer   47.5 ml
- Washing Buffer A   40 ml
- Washing Buffer B   40 ml
- Washing Buffer C   40 ml
- Washing Buffer D   75 ml
- Elution Buffer   10 ml

Figure 3.12. View of BILATEST® Genomic DNA Kit 250 for extraction of DNA
with 8 chemicals and magnetic seperator

Purification Protocol for 250 µl of Blood starts with placing 250 µl blood sample in a tube and adding 350 µl Lysis Buffer (1). After mixing properly (8 to 10 pipetting strokes) it is incubated 5 minutes at room temperature (Figure 3.13). Then 50 µl resuspended Magnetic Beads (2) is added and then directly 950 µl DNA Binding Buffer (3) is mixed with 10 pipetting strokes and incubated 5 minutes at room temperature.
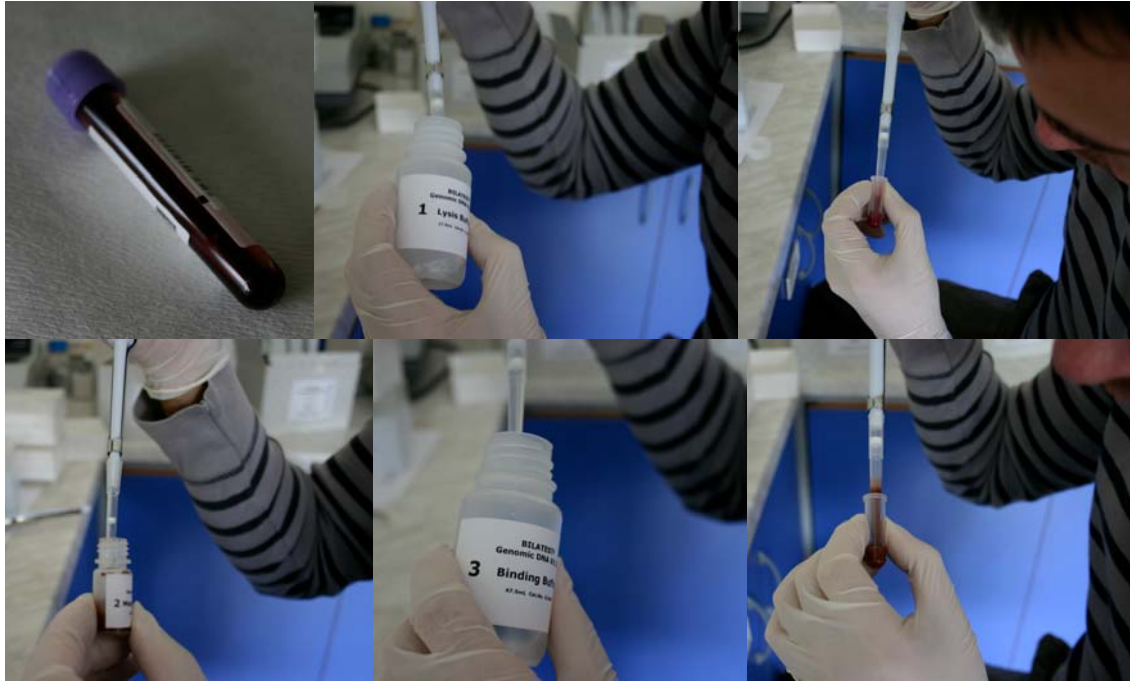
Figure 3.13. Pipetting of the reagents
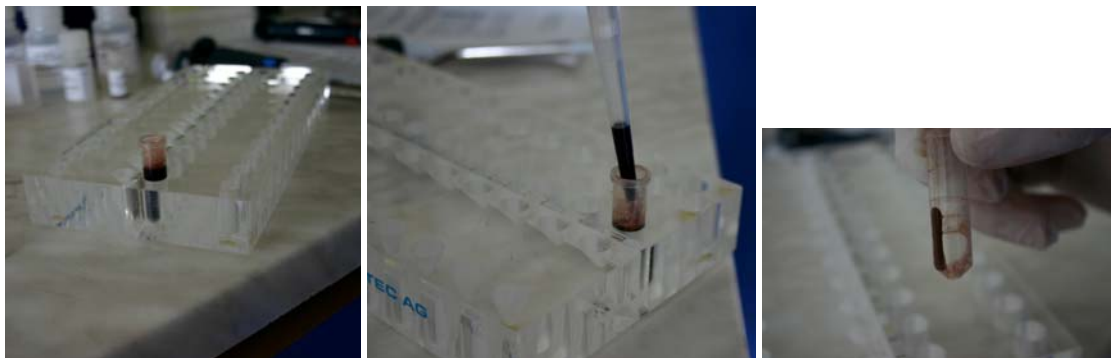
Figure 3.14. Magnetic separation

Tube is then placed in a magnetic separator to draw the Magnetic bead/DNA complex to the side of the tube (Figure 3.14). Left 2 minutes, then discarded supernatant and removed from the magnet position (The magnetic beads will not be visible in this step. Therefore, it is important to remove the supernatant from the opposite side of the magnets, in order not to aspirate magnetic beads). After, 800 µl Washing Buffer A (4) is added to the tube and thoroughly resuspend the beads in the washing buffer by aspirating the bead pellet for 15 times. Then separate the Magnetic bead/DNA complex

in the magnetic separator for 1 minute, discard supernatant and remove tube from the magnet position (Figure 3.15).

Figure 3.15. Wash buffer 4 and separated magnetic beads.

Washing procedure is repeated using Washing Buffer B (5) and then Washing Buffer C (6). After completely removing the last traces of Washing buffer C (6), the tube is left in the magnetic separator (Figure 3.16).

Figure 3.16. Wash buffer C.

With the tube in the magnetic separator (the beads attracted to the side of the tube (Figure 3.17)), 1.5 ml of Washing Buffer D (7) is gently added not to disrupt the pellet. Left for 90 seconds without resuspending the pellet and then carefully removed and discard the supernatant. (Note: a longer incubation time or resuspension of the bead pellet in Washing Buffer D (7) may reduce the final DNA yield.)
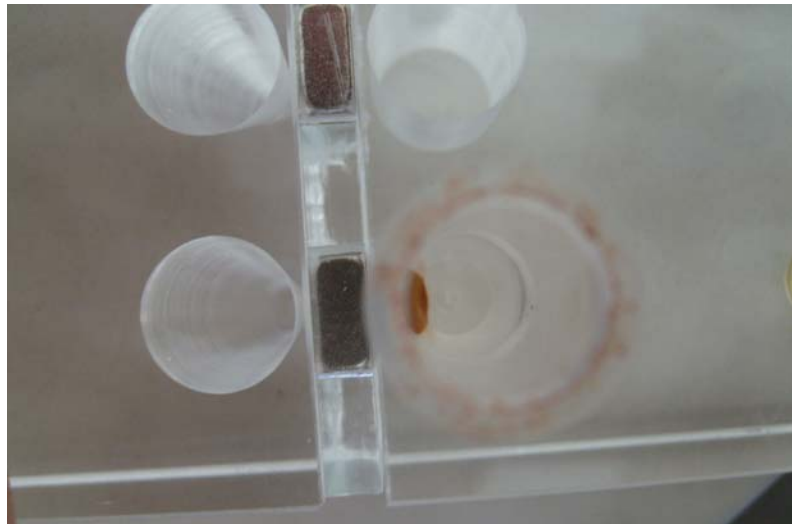
Figure 3.17. Beads under magnetic field.

After 200 μl (or another suitable volume) of Elution Buffer (8) is added to the tube and thoroughly resuspended the Magnetic bead/DNA complex by mixing the pellet with 10 to 15 pipetting strokes. Suspension is then incubated for 5 to 10 minutes at 55 °C (occasional agitation may facilitate complete DNA elution). Following DNA elution place the tube in the magnetic separator for 2 minutes or until all the beads have separated from the eluate. The eluate containing the purified DNA is transfered to a clean tube (Figure 3.18). At the end of the process we have succeeded handling purified DNA in 35 minutes (Figure 3.19). During the isolation 17 pipette tips and 3 of 2 ml tubes were used.

Figure 3.18. Incubation step.

Figure 3.19. Extracted DNA.

3.6.6. Robotic versus Manual Extraction

While many analysis methods have been automated, most research laboratories still extract DNA and RNA manually. Equipment for automated NA extraction has in the past been too expensive or too advanced for laboratories with few or moderate numbers of samples. Several bench-top extraction instruments for low sample throughput have recently been introduced in the market. These systems replace the manual mini-kits. Even if only processing a few samples, automation offers a number of advantages.

Repetitive strain injuries account for the highest percentage of illness caused in the laboratory. These injuries arise from repetitive motions such as the extensive pipetting required for manual NA extraction. Extraction instruments substitute hand pipetting, thereby contribute to a healthier working environment. Closed automated systems also reduce exposure to harmful extraction chemicals and infectious agents in the samples.

Automated NA extraction is not necessarily faster than manual methods when we only have a few samples, but it allows us to spend our time on other tasks. The downstream analysis can be preapared while the extraction instrument works. Automation means less routine work for highly qualified personnel and more resources available for scientific exploration.

The quality of the isolated NAs may affect the outcome of the genetic analysis. Automated extraction of NAs offers an extra security for precious samples. More consistent results are generally obtained with an instrument. Errors associated with human handling such as mixing up of sample tubes and contamination, are highly reduced.

3.6.7. Automation of the Process

This robotic workstation is planned to give ability for easy purification of NAs from a wide range of sample types relevant for clinical research. All processing steps are performed on the worktable; from opening of the reagent cartridge to elution of highly pure NAs. In the design of the workstation following tasks are considered: liquid handling with the pipet tips which function as reaction chambers, increasing the efficiency of magnetic separation and eliminating the need for centrifugation steps.

Some of the NA extraction devices that can be found commercially in the market, which are based on the magnetic separation technology are mentioned in detail in the following subsections below.

3.6.8. Qiagen BioRobot M48

The Qiagen BioRobot M48 is a desktop robotic workstation designed for magnetic particle based sample preparation (Figure 3.20). The BioRobot M48 uses a patented technology to perform magnetic separation, but can also perform simple liquid handling tasks like mixing, aspiration, dispensing, etc. The Qiagen BioRobot M48 uses a linear array of syringes (the Nozzle Head or Syringe Head) to treat 6 samples simultaneously. The instrument has the capacity to treat 6-48 samples per run. Depending on the protocol, operation takes 1.6 to 4.0 hours for 48 samples, which are placed in 4 sample racks, each of which can hold 12 samples (GMI 2009).

The Nozzle Head moves along the X, Y, and Z-axes to perform reagent dispensing as well as the NA extraction. The BioRobot M48 holds up to 6 Reagent containers (small) and 4 Reagent containers (Large) for up to 48 tests per run.

A dispensing head with 6 syringes treats all preparation processes including sample and reagent dispensing as well as magnetic particle separation. Syringe pumps allow small-volume liquid handling. Syringe pumps are operated simultaneously. Specially designed tips handle 25 µl to 1000 µl volumes. Volumes 25 µl Accuracy <5% Volumes > 50 µl Accuracy <2%.

For temperature control, the BioRobot M48 has 2 Peltier heating/cooling units laid out on a nozzle movement area (the Work Platform) Temperature range from 4 ^{o}C to 90 ^{o}C, monitored by PC. The 6×8 tube racks are used in the heating and cooling units for incubation, elution, and storage. For ambient temperature step, a Sample Preparation Plate, which has 6×7 wells, is used. Up to 8 plates can be used. 1×7 wells are used for one sample preparation.

(a)
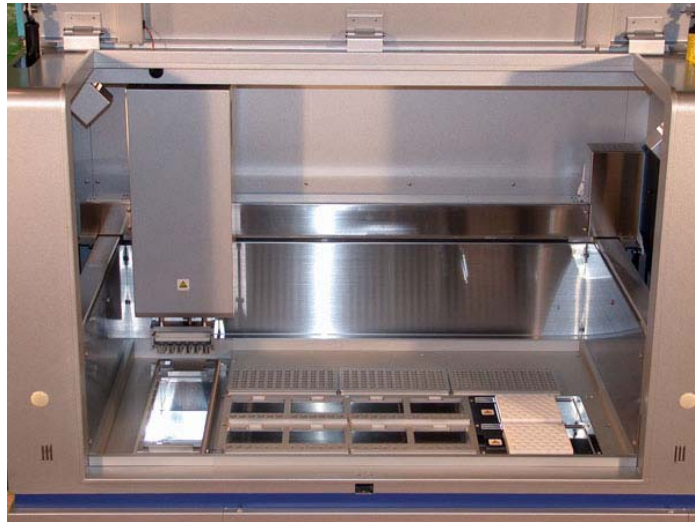
(b)

Figure 3.20. Qiagen BioRobot M48, (a) outside and (b) inside view
(Source: GMI 2009)

3.6.9. Mole Genetics Genemole

GeneMole is a closed and fully automated system comprising a liquid-handling robot capable of running 1 to 16 samples at a time (Figure 4.21). Operation is simple via the intuitive touch-screen menu. Set-up time is about 3 minutes (GeneMole 2009). Samples are loaded in a rack and then transferred by the robot to sealed and disposable cartiges which are pre-filled with reagents. Sample volumes are from 50-200 µl with launch protocols available for DNA DNA blood, DNA tissue, RNA cells and RNA

tissue. Using purification based on magnetic beads, NAs are ready for downstream applications. Use of the disposable cartiges eliminates crossover or contamination and makes GeneMole particularly suitable for multi-user environments.

Figure 3.21 View of GeneMole
(Source: GeneMole 2009)

3.6.10. QiaGen Biorobot EZ1

The BioRobot EZ1 System (Figure 3.22) offers fully automated purification of NAs from a wide variety of samples using magnetic particle technology (Qia Gen 2009). The system provides rapid and reliable isolation of NAs, optimal for downstream applications. The main features of the BioRobot EZ1 include:

- Closed system which purifies high-quality NAs from 1–6 samples per run.
- Small footprint with no external computer — saves laboratory space.
- Rapid purification of NAs — 6 samples processed in 15–20 minutes.
- Pre-programmed EZ1 Cards — contain protocols for NA purification.
- Pre-filled, sealed reagent cartridges — for easy, safe, and quick .
- Complete automation of NA purification, from opening of the reagent cartridges to elution of NAs — no centrifugation steps required.

Figure 3.22 QiaGen Biorobot EZ1 (a) general, (b) detailed view.
(Source: Qia Gen 2009)

The magnetic particle technology used by the BioRobot EZ1 System combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. NAs are isolated from lysates in one step through their binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnetic source and then efficiently washed. The NAs are eluted in water or low-salt buffer.

Protocols for NA purification are stored on pre-programmed EZ1 Cards (integrated circuit cards). The user simply inserts an EZ1 Card into the BioRobot EZ1 and the instrument is then ready to run a protocol. The availability of various protocols increases the flexibility of the BioRobot EZ1 System.

3.6.11. Comparison of the Commercial Bio-robots

The general technical specifications of the commercial deviced, explained in detail in the previous sections, are summarized and tabulated in the following table (Table 4.1). As the technical properties of all the devices vary in a wide range, there is not an exact best combination of these parameters. It is mostly dependant to the application details for the purpose thought to be used for.

Table 3.2. Technical specifications of the most common commercial devices for comparison.

	Qiagen		GeneMole
	BioRobot M48	BioRobot EZ1	
Dimensions (mm)	1070×600×875	600×420×550	600×500×350
Weight (kg)	130	~50 kg	40
Interface	Software operated by computer	Footprint with no external computer	Intuitive software operated by a touch screen
Protocols	Programmable Computer Software	Pre-programmed EZ1 cards	Pre-installed protocols. New/upgrated protocols easily transferred via a USB; stick
Disposables	Standard pipette tips Standad sample and elution tubes	Standard pipette tips Standad sample and elution tubes	Standard pipette tips Standad sample and elution tubes
Reagents	6 small reagent containers and 4 large reagent containers	Pre-filled, sealed reagent cartridges	Pre-filled disposable reagent strips
Throughput	6-48 samples per run 6 samples simultaneously	1-6 samples per run	1-16 samples per run
Dispensable Volume	25 µl to 1000 µl	up to 1000 µl	up to 2000 µl
Operation Time	1.6-4.0 hours depending on protocol	15 - 20 minutes	40 minutes
Workspace coordinates	X-Y-Z	X-Y	X-Y

CHAPTER 4

MECHANICAL DESIGN OF A ROBOTIC DEVICE FOR NA EXTRACTION FROM BIOLOGICAL SAMPLES

This robotic workstation is planned to give ability for easy purification of NAs from a wide range of sample types relevant for clinical research. All processing steps are performed on the worktable; from opening of the reagent cartridge to elution of highly pure NA's. The operation of the workstation is mainly composed of a series of liquid handling with the pipette tips which act as reaction chambers, increasing the efficiency of magnetic separation and eliminating the need for centrifugation steps.

4.1. Design Specifications

The device is planned to be a liquid handling robot. It mixes different chemicals in exact amounts and at the exact time in-between the reaction tubes. In other words, it ensures that each chemical is transferred to the right target in exact amounts and as soon as the mixture is homogenized. It moves on to the next step untill all reactions are complete and the desired sample is extracted.

For each sample, 9 separate disposable reaction tubes (+1 extra) (QIA-GEN brand), 1 incubation tube, one pipette, 2 final sample tube will be used. The robot should be able to move between these 13 stations and do precise metering. During processing the chemical agents, it has to position itself at different heights, grip and release the associated pipette. For incubation it has to keep the temperature of a specific tube constant at 56 °C.

During magnetic separation, it must expose the pipette to magnetic field and hold it within the magnetic field between stations. It should be able mantain the field during pipetting as well. During all of these steps, the most accurate one is the amount of required liquid volume. The system basically consists of 2 separate sections. Disposable tubes, pipette tips and a tray carrying chemicals and fine print pumps and suction system that includes a magnet for magnetic separation unit.

4.1.1. Design of the Workspace

The workspace of the device is designed according to commercially available chemical reagent kit, pipette type and 2 ml sandart tubes by QIA-GEN. The workspace should be easy to use. Sample tubes and disposable pipette tip inserted into the device is difficult to place due to limited working space. Also one substance can be polluted by another. To prevent all these possible problems, the worktable will be a 2-piece design. First section holds the sample tube, the result tubes and pipette tip. Second section will carry the reagents. Both two parts should be removable, filled outside and reloaded. By this way the installation of the device can be done outside for easy use.

4.1.2. Pipette tip and tube carrier

An appropriate place for an example tube, a pipette tip, a reaction tube and an empty tube shall be considered. In Figure 4.1, the pipette tip and the sample tube can be seen. Sample tubes have a volume of 2 ml, and they are 40 mm long and 11 mm in diameter cylindrical shaped tubes. Close to the top region there is a 13 mm diameter flange. The bottom parts have a conical structure, and can be inserted into their 11.1 mm slot can. Similarly, the pipette is 100 mm long, 9 mm wide at the neck part, has a 10 mm flange at the top. By making 9.1 mm diameter holes there will be enough space to place them. As a result, for each sample analysis to be done, one hole for the sample tube, one hole for the result tube and one hole for the pipette tip, we need 3 holes in total. But to have one more tube place can be useful for the future applicatons. Therefore, that will require one more hole for the extra tube. Consequently there should be four holes total per sample.
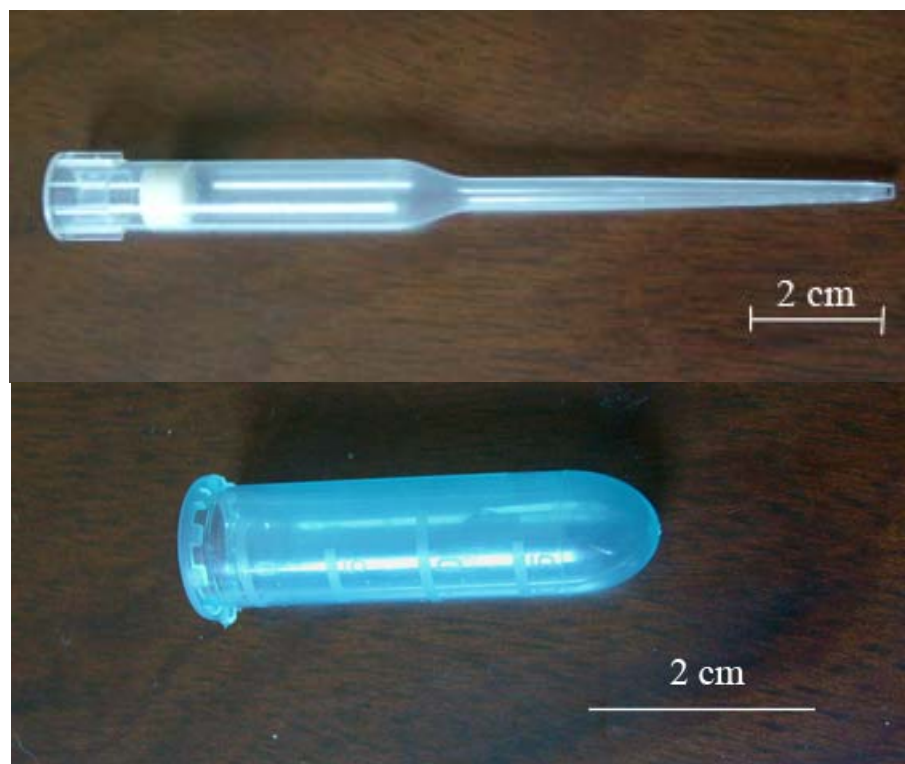
Figure 4.1. Type of pipette and tube used (1200 µl and 2 ml)

4.1.3. Chemical kit (reagent) carrier

When designing the chemical kit carrier, it is necessary to design according to the kits being used (Figure 5.2). As seen from the figure below, these chemical kits are composed of 11 tubes in a line. The reason for one of them being separated from the others is that the mixture has to stay in the heating section during the incubation process at constant temperature of 56 °C. These kits can be positioned in the slots in a row. This slot system provides ease of loading, besides these kits are already designed to be used in such positioning of slots. Both sides of the upper part of these kits have 1 mm extensions, which helps them to fit these slots. It is compulsory to arrange 10 of these chemical reagents in parallel position and easily handled in and out.

Figure 4.2. View of chemical reagent

The kit holder should be able to stand alone on a smooth surface for need of one operator to load and unload. The design of the holder according to the defined parameters is shown in the figure below (Figure 4.3).
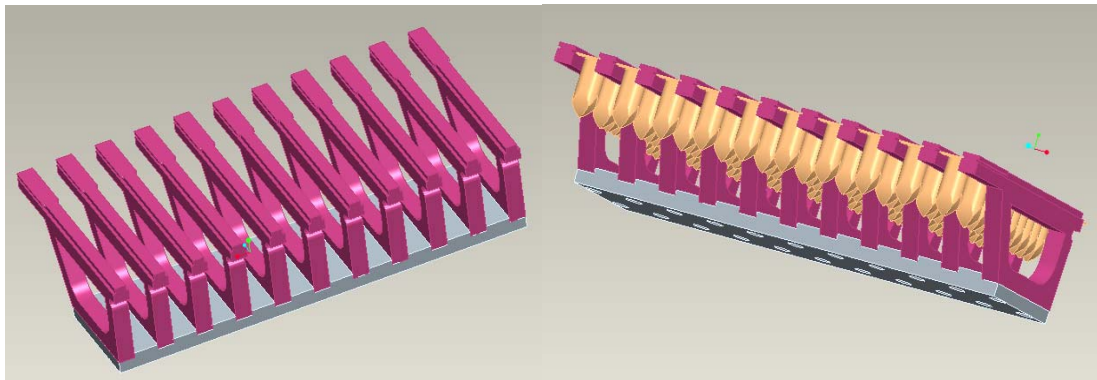
Figure 4.3. Design views of the chemical reagent holder of the workstation

4.1.4. Design of Accurate Syringes

The system can work similar to the syringe. The amount of liquid to be transferred can be achieved by positioning the syringe piston properly. Let the cross sectional area of the piston be A, and the piston is moved for a distance of h, the volume change will be V which we can not use as a reaction chamber. Contact of these chemicals to the cylinder surface results in crosscontamination for the other samples;

and that effects the test results. Therefore, a single-use reaction chamber and a specially designed pipette volume are needed. A micro particle filtering system attached to the pipette opening will help for the sterilization of the cylinder system. Similar to the hand pipetting tools, at the end of the piston-cylinder system a nozzle can be attached to fit the pipett in the opening (Figure 4.4).
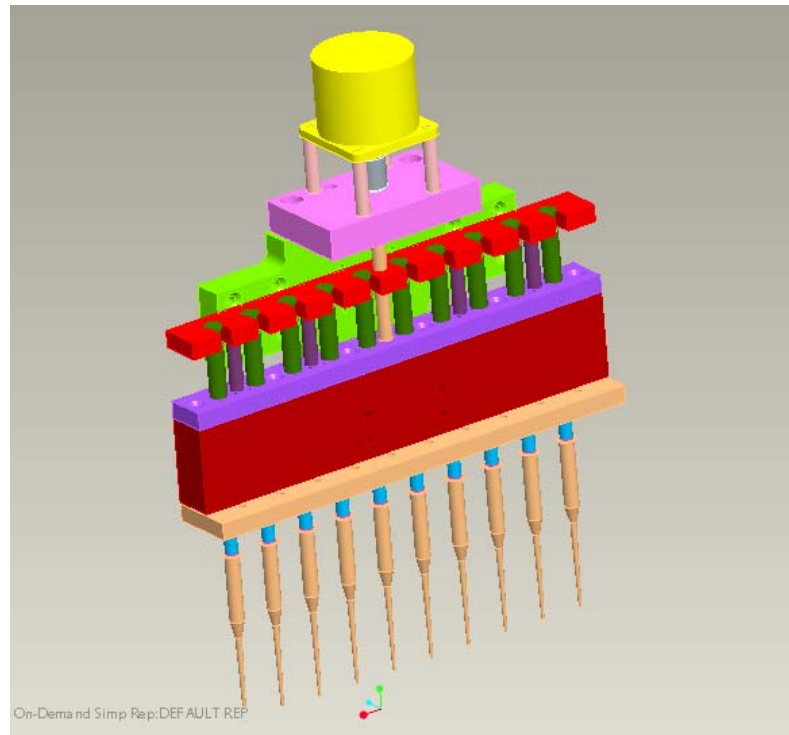
Figure 4.4. View of syringe and nozzle system design

When the piston moves upward, low pressure will occur in the cylinder and will result in a pressure lower then the atmospheric pressure in the pippetting volume. Thus, the chemical mixture is forced to flow up inside the reaction chamber.

4.1.5. Design of Pipette Nozzle

Pipette nozzle must be designed to ensure a sealed volume in the pipette tip. To place a suitable diameter o-ring between the nozzle and the pipette tip can over come the sealing problem. The same geometry and dimensions that hand pipetting tools have can be used when designing the pipette tip (Figure 4.5). Figure 4.5 represents a

commonly used pipette nozzle with an o-ring. The diameter of the pipette nozzle must be decided according to the inner diameter of the pipette tip ensuring a tight mounting.

Figure 4.5 Pippette nozzle

4.1.6. Design of Magnetic Seperator

It is expected from the magnetic separator to create a magnetic field in the intermediate section of the pipette tips. Magnetic field can be created in two different ways; electromagnetically or by permanent magnets.

If electromagnets are being used, magnetic field is created electromagnetically. Electromagnets are composed of a core (usually iron) and a coil wrapped around with ends of applied voltage. When there is no current on the coil, tiny magnets are randomly polarized in the core, and they neutralize each other. When there is current in the coil, the electromagnetic field of the coil positions the tiny magnets to a form parallel to the magnetic field, thus the core behaves like a magnet. The microbeads can be affected by the magnetic forces.

The magnetic force needed can also be created by using a pemanent magnet. The commercially available type of the magnets that have the highest magnetic force are neodium permanent magnets. It is available in desired dimensions. In the following

figure use of a permanent magnet and its effect on the microbeads in the solution can be seen (Figure 4.6).
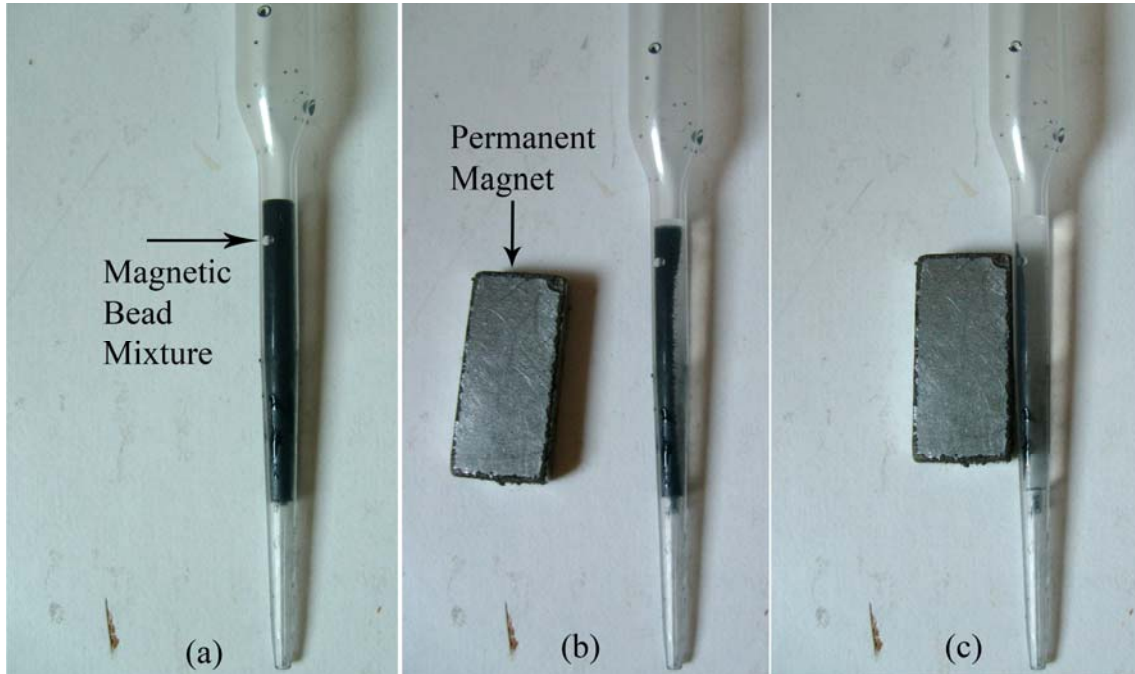
Figure 4.6. View of a pipette with magnetic particle solution, (a) in no magnetic field, (b) affected by magnet, and (c) when the magnet is in contact.

If it is desired to use electromagnet for the system, extra electrical power will be required to feed the system, which results in choosing a higher capacity power supply. When permanent magnets are used, the problem will be the permanent magnetic field next to the pipette tips. Whether it is a permanent magnet or an electromagnet, after application of magnetic force, the magnet system have to stay in a distance for easy movement of the pipette tips.

So the magnet mechanism has to be capable of moving towards the pipette tips and back to a certain position. This can be achieved by either using a DC motor or a stepper motor as an actuator. When a DC motor is used, limit switches and a DC motor driver to maintain the desired positions of the magnetic separator should be used. When using stepper actuation system, a stepper motor driver should be used in combination with the stepper motor. Altought using a servo motor is another alternative; it is not a

cost-effective solution for moving the magnets. These two are the possible alternatives for the magnetic separator mechanism.

It has been experimentally analyzed that, when the distance between the magnet and the chemical mixture including magnetic beads, is 30 mm, the microbeads are not effected by the magnetic field (Figure 4.6 (a)).

4.1.7. Design of Pipette Dropper

Another expected property for the system is to be able to put on and pull out the pipettes from the syringe nozzle system. Putting on the pipette tip is relatively easy. Positioning the pipette nozzles over the pipette tips and lowering it slowly mounting the pipette tips flanges will result engaging the pipettes by the nozzles, due to the dimensional tolerances between pipette nozzle and pipette tip flanges diameter (as it is in the manual pipetting equipment). However pulling out the pipettes is more complicated. As the pipettes will be stucked on the nozzles, we need an opposite directional force to be applied to pull the pipette tips out. This problem can be solved like the mechanism of the hand pipeting tools with a moving plate that pushes the pipettes out. The dropper plate can be moved by two linear actuators placed on the two sides of the syringe pumps. Once actuators are turned on, the moving rod pushes the dropper plate causing pipettes sliding from the nozzles. Actuator system of pipette dropper is schematically represented in Figure 4.7. These kinds of linear actuators are commercially present on the market, for wide range of applications. Basicly there are two applicable types:

- Stepper motor actuated
- Solenoid (electromagnet) actuated.

A stepper linear actuator has very smooth movement, silent but relatively slow. A solenoid actuator needs more current but cheaper. A stepper actuator needs a stepper motor driver, but a solenoid actuator can simply driven by a relay. A motor mobilizing the syringes pistons is already available in the system, which move in the same axis with the foce needed to pull out the pipettes. If this movement is transferred to the dropper plate without preventing the syringe systems capabilities, this would be a

logical solution for pulling out the pipettes. The major problem here is to combine the movement of the dropper plate with the pistons. Figure 4.7 represents the conceptual design of the mechanism. This design will be further discussed in the final design section.
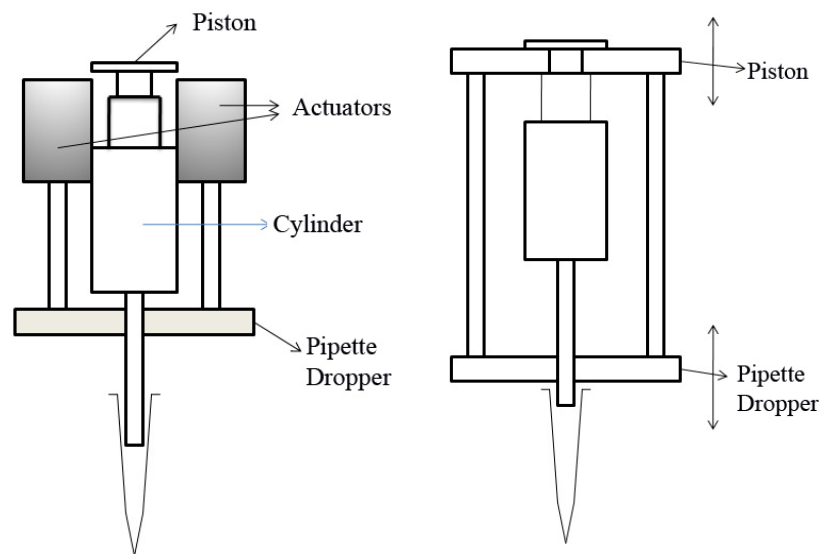
Figure 4.7 View of pipette dropper conceptual designs

4.1.8. Actuators

There are two possible types of power for axis drives:
- Stepper motor
- Servo motor (either AC or DC)

Either of these types of motor can drive the axes through leadscrews (plain- or ball-nut), belts, chains, or rack and pinion. The mechanical drive method will determine the speed and torque required from the motors and hence any gearing required between the motor and machine.

Properties of a stepper motor drive include:
- Low cost
- Simple 4-wire connection to motor
- Low maintenance

Motor speed limited to about 1000 rpm. Getting the maximum speed depends on running the motor or the drive electronics at their maximum permitted voltage. Getting the maximum torque depends on running the motor at its maximum permitted current (A).

For practical purposes, steppers need to be driven by a chopped micro-stepping controller to ensure smooth operation at any speed with reasonable efficiency. The properties of this controller are:

- Provides open loop control, which means it is possible to lose steps under high loading, and this may not immediately be obvious to the device user. In practice, stepper motor drives give satisfactory performance.

  On the other hand, servo motor drive:

- Is relatively expensive (especially if it has an AC motor).

- Needs wiring for both the motor and encoder.

- Requires maintenance of brushes on DC motors.

- Allows motor speed of 4000 rpm or more.

- Provides closed loop control so drive position is always known to be correct (or a fault condition will be raised if an error occurs).

Stepper motors are a digital actuator whose input is in the form of programmed energization of the stator windings and whose output is in the form of discrete angular rotation. It is, therefore, ideally suited for use as an actuator in computer control systems, digital control systems and etc. A stepper motor is an electromechanical device which converts electrical pulses into discrete mechanical movements. The shaft or spindle of a stepper motor rotates in discrete step increments when electrical command pulses are applied to it in the proper sequence. The sequence of the applied pulses is directly related to the direction of motor rotation. The speed of the motor's shaft is directly related to the frequency of the input pulses; and the length of rotation is directly related to the number of input pulses applied.

One of the most significant advantages of a stepper motor is its ability to be accurately controlled in an open loop system. This type of control eliminates the need for expensive sensing and feedback devices such as optical encoders. Its position is known simply by keeping track of the input step pulses. A stepper motor can be a good

choice whenever controlled movement is required. They can be used in applications where rotation angle, speed, position and synchronism need to be controlled. Because of the inherent advantages, stepper motors have found their place in many different applications. Some of these include printers, plotters, high end office equipment, hard disk drives, medical equipment, fax machines, machine tools, automotive devices and many more.There are three basic types of stepping motors:

- Variable-reluctance (VR)
- Permanent magnet (PM)
- Hybrid (HB)

PM motors have a magnetized rotor, while VR motors have toothed soft-iron rotors. HB motors combine aspects of both PM and VR technology. The two most commonly used types of stepper motors are the PM and the HB types. The HB stepper motor is more expensive than the PM stepper motor but provides better performance with respect to step resolution, torque and speed. Typical step angle for the HB stepper motor ranges from 3.6° to 0.9° (100-400 steps per revolution). The HB stepper motor combines the best features of both the PM and VR type stepper motors.

There are three common types of drive modes of stepper motors: Wave Drive, Full Step Drive and Half Step Drive. In wave drive only one winding is energized at any given time. In full step drive two phases should be energized at any given time. Full step mode results in the same angular movement as one-phase-on drive; but, the mechanical position is offset by one half of a full step. Half step drive combines both wave and full step drive modes. Every second step only one phase is energized, and during the other steps two phases are energized. This process results in angular movements that are half of those in one- or two-phase-on drive modes.

Stepper motors have a wide range of applications. Some applications require that a stepper motor should rotate continuously or periodically with a constant speed or a variable speed; some applications also require that it should position a device at the right time to a certain position according to a program, some of them require accelerating or decelerating motions up to a certain speed; some require mixed motions of them and etc. When these different motion types are taken into account, developing a computer program for programming and controlling the stepper motors is practical.

The minimum step with a stepper motor depends on how it is controlled. Many commonly available stepper motors have 200 full steps per revolution, but controllers also permit "micro-stepping". Micro-stepping helps give smooth running over the full range of feed speed and many controllers will allow us to have 10 micro-steps per full step. A 200-step motor with 10 micro-steps per full step would allow 1/2000 of a revolution as the minimum step.This must be viewed with some caution, however. As the number of microsteps per step increases, the torque falls off rapidly. Depending on the load being imposed on the motor, there may not be enough torque to actually move the motor by a single microstep. It may be necessary to make several microsteps before there is sufficient torque. Briefly, microstepping can not be relied on to achieve fine accuracy. The primary benefits of microstepping are reduced mechanical noise, gentle actuation, and reduced resonance problems. Conservatively, it is assumed that the maximum motor speed is 500 rpm. For example 2 mm pitch leadscrew; 500 rpm would give a rapid feed of 1000 mm/minute. This would be satisfactory, although not spectacular. At that speed, the micro-stepping motor drive electronics would need 16,667 pulses per second. (500 rpm ×200 steps per rev×10 microsteps per step/60 seconds per minute). On a 1 GHz PC, Mach3 can generate 35,000 pulses per second simultaneously on each of the six possible axes, which is an acceptable result. The torque values should be to determine the power of the motor.

A servo motor has an encoder to tell its drive electronics where it is. This consists of a slotted disc which will generate four "quadrature" pulses for each slot in the disc. Thus a disc with 300 slots, for example, generates 300 cycles per revolution (CPR). This is fairly low for commercial encoders. The encoder electronics would output 1200 quadrature counts per revo-lution (QCPR) of the motor shaft. The drive electronics for the servo will usually turn the motor by one quadrature count per input step pulse. Some high specification servo electronics can multiply and/or divide the step pulses by a constant (e.g. one step pulse moves by 5 quadrature pulses or 36/17 pulses). This is often called electronic gearing. As the maximum speed of a servo motor is around 4000 rpm, we will certainly need a speed reduction on the mechanical drive. 5:1 would seem sensible. This will also decrease the movement per step. Notice, however, that the speed is limited by the pulse rate from Mach3, not by the motor speed. The limitation would be even worse if the encoder gave more pulses per revolution. It will often be necessary to use servo electronics with electronic gearing to overcome this limitation if we have high count encoders.

4.1.9. Power Transmission Mechanism

Treaded screws and nuts for each axis are predicted to be used. For X (moving the work table) and Y (moving the nozzles) axis 2 mm pitch screws are selected, since the stroke values are higher than the Z and A axis (accurate srynges and magnet). For the Z and A axis using 1.25 mm tread screw and nut is suitable since the positioning resolution needed is higher. Suitable pitch sizes should be used according to the speed and torque values expected.

$$\text{Max Velocity} = \text{Max rpm} \times \text{Pitch of the screw} \qquad (4.1)$$

It is assumed that the stepper motors maximum rotational speed is 500 rpm, due to most of the stepper motors can reach 1000 rpm with a suitable driver. From the equation 4.1 maximum velocity of X and Y axis is calculated as 1000 mm/min at 500 rpm. This result seems acceptable for X and Y axis, since these axes maximum travel is not more than 300 mm, a full stroke time will be under 20 sec.

If the steppers motors are driven in half step mode, for a one full revolution we need 400 pulses. Therefore the positioning resolution will be 2 mm pitch divided by 400.

$$\text{Positioning Resolution} = 2/400 = 0.0005 \text{ mm} \qquad (4.2)$$

From the equations 4.1 and 4.2, 1.25 mm pitch screw maximum velocity is calculated as 625 mm/min, and positioning resolution is calculated as 0.003125 mm.

4.1.10. Design of Axis Motions

Firstly, number of axis has to be decided. According to the basic motions pipette tips must move along the work table in parallel lines(as X axis) and position at different heights(as Y axis), magnetic separator must move next to the pipette tips(as A axis), syringe pistons must suck and purge(as Z axis).

There are two possible options for choosing X and Y axis motions. The work table can move along the X axis and the syringes move along Y axis, or the work table can be static and the syringes can move along both X and Y axis.

The fisrt option is relatively a simplier design, because the motion of two axis is shared between two different parts of the workstation, cable routing is easier due to less moving cables.The second design option needs less space, because the work table is static , but needs more linear force since the actuated mass by the X axis motor is bigger. Figure 4.8 represents the two concepts.
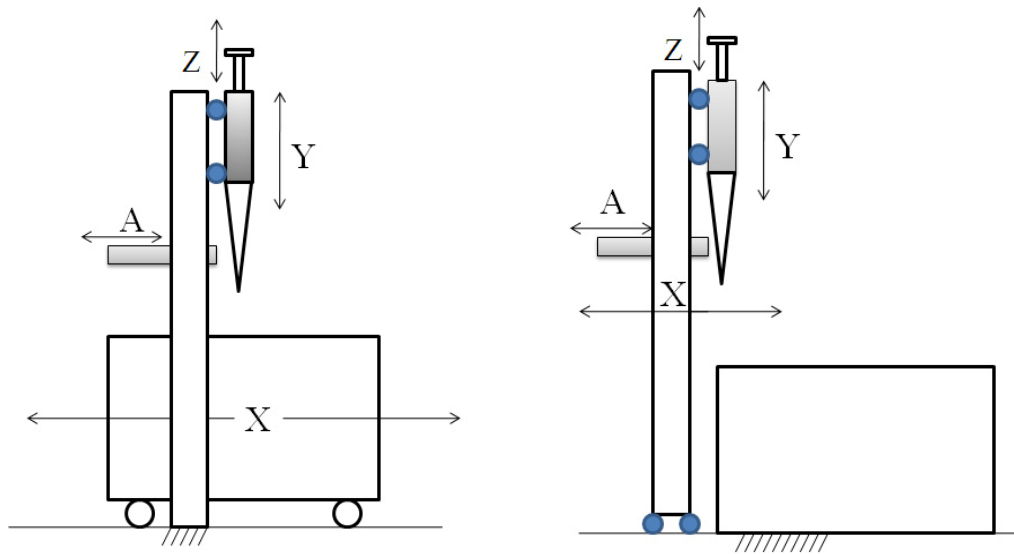
Figure 4.8. Possible axis motions.

4.2. Conceptual Designs

Present status of our design leads us to two conceptual designs. Design alternative A has a moving worktable; the loading of X axis is reduced since the X axis motor does not move the syringes and magnet system. Stepper motors are used for driving each axis which is relatively cheaper. Permanent magnets are selected to create magnetic field around pipette tips, resulting less power consumption, lighter and smaller construction. Pipette tip dropper is actuated by Z axis motor, eliminating the need for extra actuators for pipette tip dropping.

Design Alternative A

- Worktable is driven by motor as X axis
- Stepper motor actuated axis
- Stepper motor actuated permanent magnet
- Pipette tip dropper is actuated by Z axis motor

Design alternative B has a static worktable, this results a relatively smaller construction becouse worktable is one of the biggest components a moving one needs more space. Servo motors have encoders so the motion is almost fault free. Electromagnets create magnetic field around pipette tips. Pipette dropper is moved by solenoid actuators.

Design Alternative B

- Static worktable, syringes move along both X and Y axis
- Servo motor actuated
- Electro magnet
- Selenoid actuated pipette dropper

4.3. Final Design

To compare the two possible designs, the design parameters given in Table 4.1 (cost, accuracy, speed, manufacturing method, power consumption) are being considered with rates according to their importances.

Cost is the most important design parameter, the cost of the sytem should be under the ones currently avalible on the market. Therefore maximum score of cost design parameter is predicted as 40 points. Design A has a greater advantage when cost is being considered. The cost of stepper motor actuation system and permanent magnet is lower than the cost of servo motor actuation and electro magnets.

Accuracy is another significant design parameter. %5 volumetric accuracy is suitable for pipeting volumes. Maximum score of accuracy is predicted as 30 points. Servo motors have great advantage in positioning accuracy but stepper motors also give satisfactory performance when ideal speed and acceleration values are determined.

Speed has low importance as a design parameter; the opretion of the DNA exractor includes pause steps for several minutes, so the maximum rating for this parameter is projected as 15 points. Servo motor system has superior advantages compared with stepper motor systems when speed is considered. For this robotic workstastion stepper motors give enough speed performance.

Manufactoring method is the consequent desing parameter and maximum score is proposed as 10 points. Moving syringes along both X and Y axis is more difficult construction compared with a moving worktable as X axis, since the total moving mass is greater. Using servo motors needs extra wiring for encoders which complicates manufacturing.

Power consumption is the less curial design parameter, maximum score for this parameter is proposed 5 points. More electrical power is required when using electromagnets and servo motors.

Table 4.1. Comparison of the design alternatives of the conceptual design

Design Parameters	Max. score	Design A	Design B
Cost	40	35	20
Accuracy	30	25	30
Speed	15	10	15
Manufacturing Method	10	10	8
Power Consumption	5	5	4
Results	100	85	77

As the final design, design A is the selected one according to the design parameters and their importances. According to that basic decision the final design of the components of the robotic device continues as follows (Appendix B).

The piston diameter is decided as 10 mm. 1 mm move of the piston results as a suction of 78.5 μl. This value must be considered as pipetting amount per unit move. 1200 μl is the maximum pipetting volume, but for possible further applications it is logical do design the maximum pipetting volume as 1400 μl. The piston must move 17.8 mm for this pipetting volume. The calculations are represented in equations 4.3-5

$$\text{Unit travel pipetting volume} = \text{piston area} \times 1 \text{ mm} = 78.5 \text{ μl} \qquad (4.3)$$

$$\text{Total travel} = \text{max pipetting volume/unit travel pipetting volume} \qquad (4.4)$$

$$\text{Total travel} = 1400/78.5 = 17.8 \text{ mm} \qquad (4.5)$$

Since pipette tip dropper is actuated by Z axis motor, Z axis needs 7 mm more travel to be the pipette tips pushed. So, total travel is 24.8 mm which can be taken as 30 mm.

For the X axis the total travel is calculated as 216 mm, corresponding the pipette tips to reach every station on the worktable. The total travel of the X axis can be taken as 230 mm. The Y axis travel is calculated as 136.5 mm to meet the needs. The total travel of the Y axis is considered as 150 mm. The A axis is the one moving the magnetic separator and 30 mm travel is adequate. The total travel of A axis is taken as 40 mm.

The workstations, commercialy available for NA extraction, have a wide range of sample per run capacities (6 to 96 samples per run). Generally the ones up to 16 samples per run is named as low throughput, 16-96 samples per run workstations are named as high throughput. The high throughput ones can do simultaneous operations up to 8 samples. A low throughput design targeting 10 samples per run has been desided. The worktable, reagent kits, syringes, nozzles and magnets must be considered for 10 samples.

For X and Y axis, SCE-12B model SAMICK linear bearings are selected referring to the acceptable dynamic load 410 N and static load 590 N (Appendix A). The properties of these bearings are as follows.

- Maintanence free
- Lubrication needed at installation
- Low coefficient of friction
- Low wear
- High load capacity

For A and Z axis due to the limited space, a plastic bearing solution Iglide® Plastic Plain Bearing by IGUS is selected. The properties of these bearings are as follows.

- Maintenance-free

- Lubrication-free design

- Lightweight

- Low coefficient of friction

- Low wear

- High load capacity

- Corrosion-free

- Vibration dampening

- Resistant to dirt and dust

- Chemical resistant

- Suitable for temperatures up to 482 ºF

- Waterproof

The Figures 4.9 and 4.10 represent the design corresponding the needs.
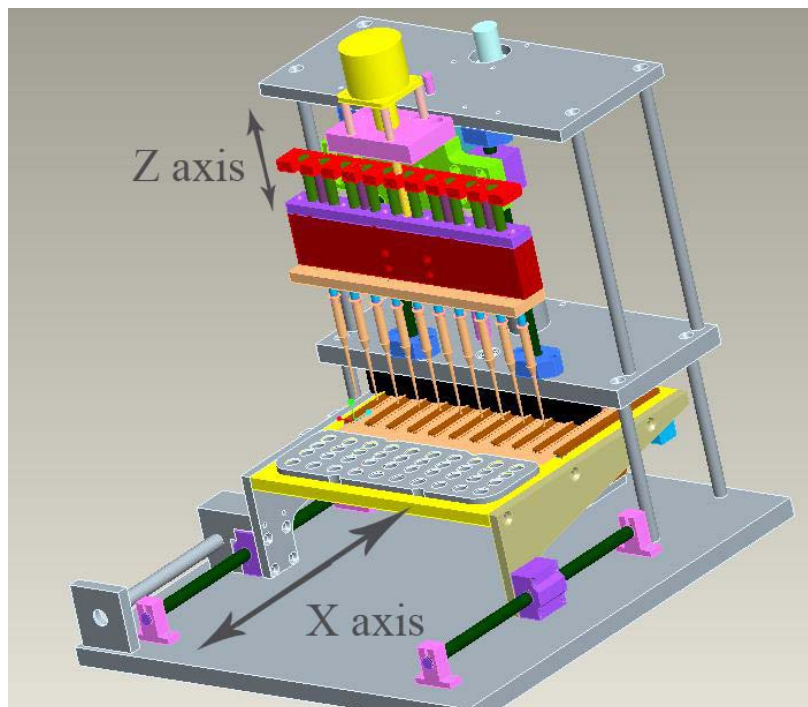
Figure 4.9. General view showing the moving axis of the work station
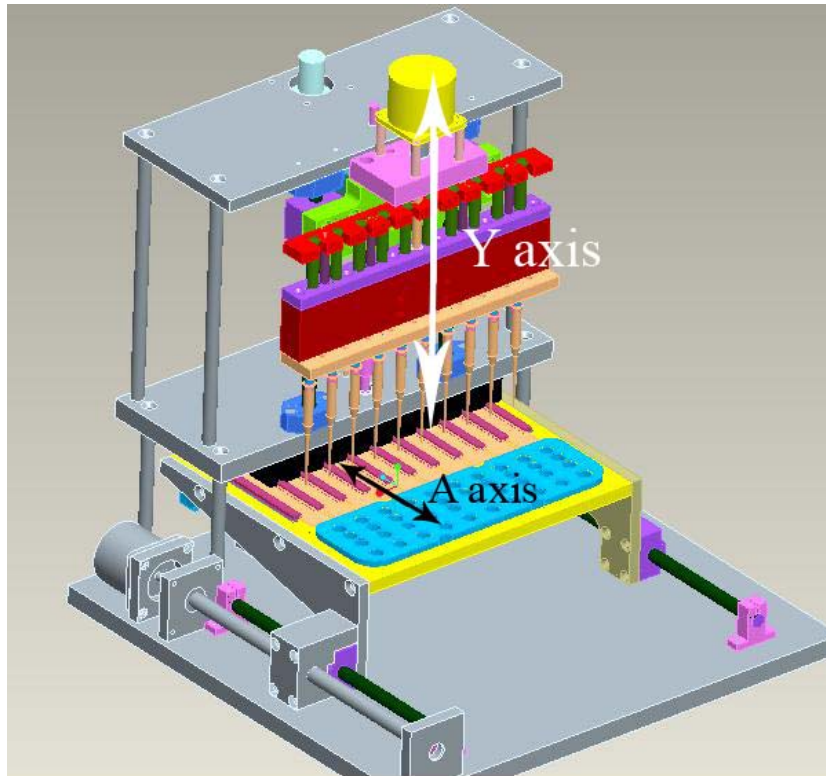
Figure 4.10. General isometric view showing the moving axis of the work station

4.4. Motor Selection

It is decided to use a stepper motor. Due to the avalibility, cost problems and torque and rotational speed values, it is choosen a stepper motor made by MINEBEA Co. Ltd.-ASTROSYN, type 23LM-C355-P6V. This is a hybrid stepper motor as mentioned in the actuators section. Technical specifications are listed in the Table 4.2.

4.5. Force Analysis

Using stepper motors torque values, we can reach the maximum force on each axis. Due to the different pitch screws X, Y and Z, A axis must be calculated separately. X and Y axis have 2 mm pitch screws, which means 1 full turn of the screw positions the axis 2 mm apart. With the motors having 550 g.cm torque, 17.285 kgf can be created with the 2 mm pitch setup. Equation 4.8 represents the calculation.

Table 4.2. Technical specifications of the stepping motor.

Manufacturer	MINEBEA Co. Ltd - ASTROSYN
Type	BipolR Stepping Motor
Model	23LM-C355-P6V
Rated Current/Winding, A	1.5
Winding Resistance, Ohm	2.2
Holding Torque, Nm (kg.cm)	0.614 (6.2)
Inductance, mH	5.5
ROTOR INERTIA, kg.cm^2	0.110
Detent Torque, Nm (g.cm)	0.0540 (550)
Weight, g	450
Coil Resistance, Ohm	1.4
Positioning Accuracy, degree/step	1.8

$$\text{Maximum Force}=2\pi\times\text{Torque/pitch} \qquad (4.8)$$

For Z and A axis with 1.25 mm pitch screw, maximum force is calculated as 27.657 kgf using equation 4.8.

These values are considered to be more than maximum loadings of the each axis. The stepper motors selected are sufficient for the system.

4.6. Manufacturing of the DNA Extractor

Prior to the manufacturing of the DNA extractor, the materials to be used to produce the mechanism were selected based on currently available DNA extractors in the market. Due to the engineering properties like light weight, resistance to corrosion and machining, aluminium was selected as the raw material for the construction of the main parts. Brass was used for construction of the pistons and the nozzles as it has low friction coefficient and proper machining properties. On the lineer bearing guides and screws, steel was used because of high rigidity. The pipette tip and tube carrier was made of delrin which is washable and durable. Stainless steel nuts and screws were used during the assembly.

The design and 3D assembly was made using Pro Engineer CAD program (Appendix B). The CAM data was achived using Power Mill CAM program. The dimensions of the raw material were calculated by the help of the CAD program. The cutting process was made by C-Tek KM-100 D 3-axis machining center and Femco HL-25 CNC Lathe.

The bottom plate was made of 12 mm thick 540 x 475 mm aluminum plate. This plate is used for mounting X axis linear bearings, motor mount, X axis leadscrew mounting bearings and middle plate carrier rods. 6mm holes are drilled at certain places for assembly with M5 and M6 screws (Figure 4.11).

The middle plate was made of 10mm thick 360 x 140 mm aluminum plate. This plate keeps the magnetic separator system components and holes for mounting the upper plate carrier rods. M6 and M5 screws used to assembly the magnetic separator and carrier rods (Figure 4.12). Similarly the top plate was made of 10mm thick 360 x 140 mm aluminum plate which keeps the Y axis motor, support bearings for Y axis leadscrews and the belt drive system for Y axis.

Figure 4.11. Bottom plate
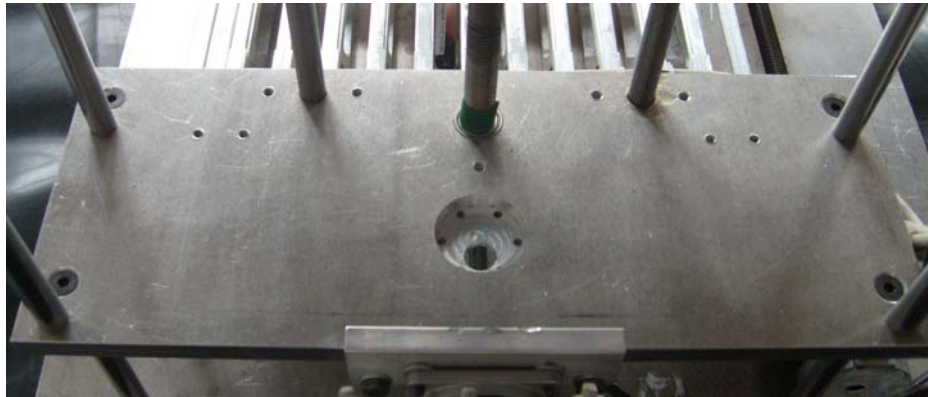
Figure 4.12. Middle plate

The pistons and nozzles were made of 12mm diameter brass rods, using CNC lathe. The nozzles have 1.2 mm diameter holes, o-ring slots, M6 treads for mounting the syringe block and have a 2 degree camber to stuck in the pipettetips (Figure 4.13). The sealing is achived by o-rings between nozzle and pipette tip and sryringe block. The pistons have a smaller diameter at the top to ensure fastening with the piston plate(Figure 4.14). The piston plate and the pipette dropper plate were made of 8 mm thick aluminnum plate and connected to each other with 8mm brass rods. M5 screws were used to attach the plates with the brass rods.

Figure 4.13. Nozzle

Figure 4.14. Piston

The magnetic separator was made of aluminum (Figure 4.15). CNC milling machine was used to machine the raw material. The magnet slots were made by CNC wire electrical discharge machine and magnets were attached using metal epoxy.

The raw material for the kit carrier was selected as 10 mm thick aluminum (Figure 4.16). 11 kit places was produced separately using cnc milling machine and attached on 8 mm thick plate by M5 screws.
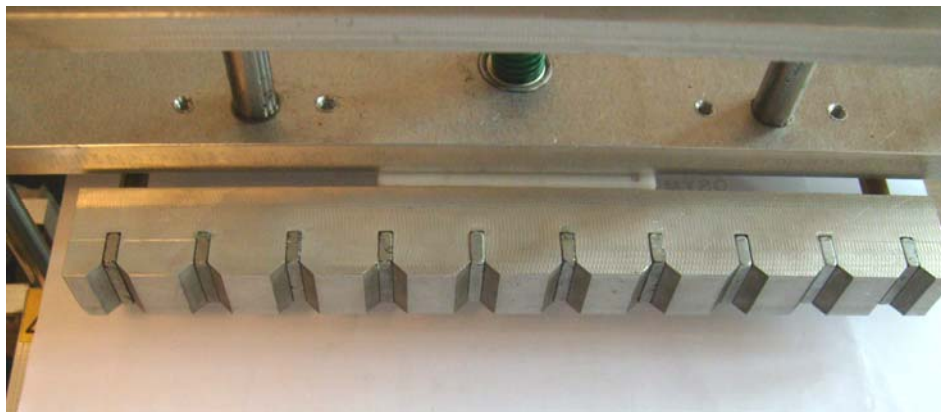
Figure 4.15. Magnetic seperator

The heater block was made of 25x25 mm aluminum rod and machined by CNC milling machine, the resistors attached by M4 screws and silicone gresed to increase the heat conductivity (Figure 4.17). The block attached to the worktable by M5 screws. The worktable and the side supports of the work table was made of 10 mm thick 270 x 285 mm aluminum (Figure 4.18). M6 screws were used for attachments of the side supports.
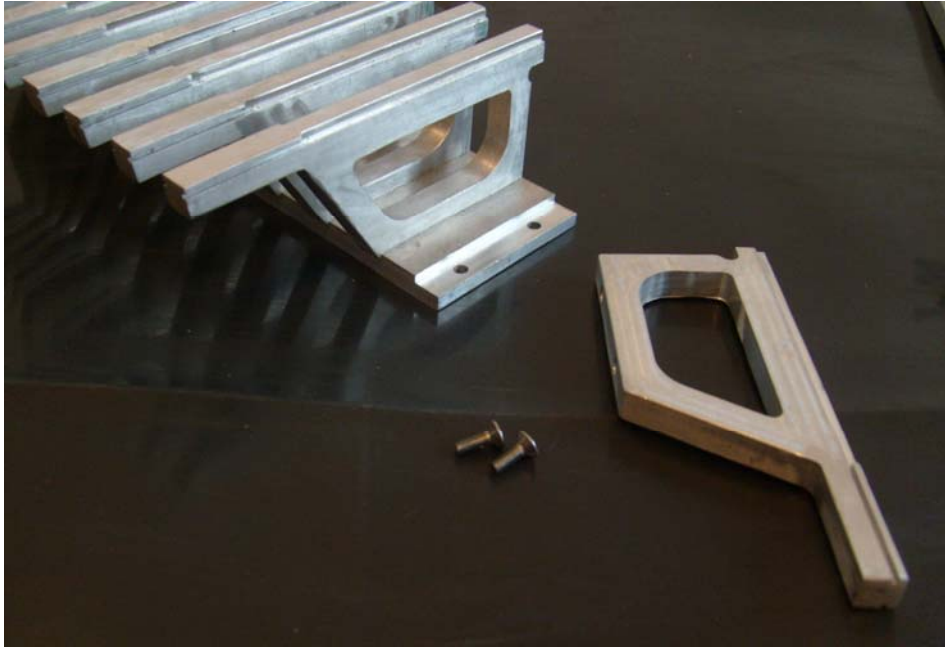
Figure 4.16. Kit carrier

Figure 4.17. Heating block

Figure 4.18. Worktable

The syringe cylinder block was made of 270 x 60 x 25 mm aluminum raw material. 10 mm holes were drilled for syringes and tapped M6 at certain places for mounting the cylinder cap and nozzles (Figure 4.19). 10 x 2.5 mm o-rings were used to ensure sealing between cylinders and pistons.

Aluminum couplings were used between X, Z and A axis motors and leadscrews, couplings fixed on the spindles by M4 set screws. Toothed belt and pulleys with the same tooth numbers were used between Y axis motor and leadscrew ensuring 1:1 drive ratio (Figure 4.20).

Figure 4.19. Syringe cylinder block
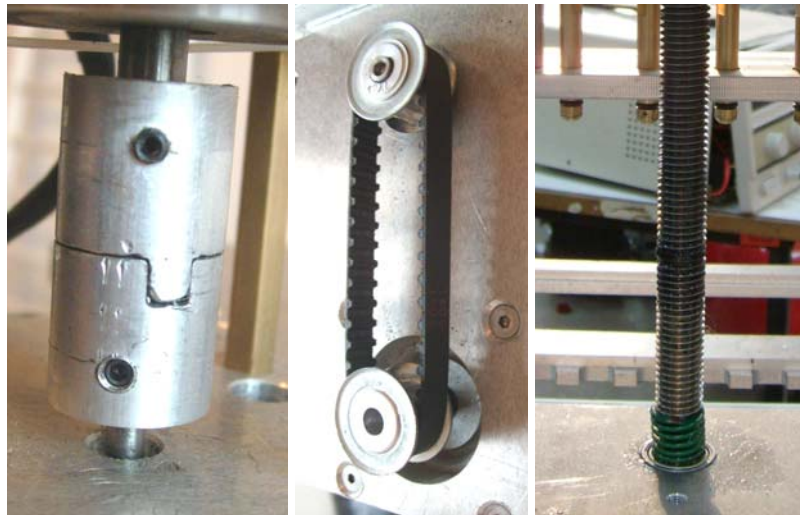
Figure 4.20. (a) Coupling, (b) toothed belt and (c) screw

The pipette tip and tube carrier was made of 10 mm thick, 250 x 15 mm plate delrin material. The holes for placing pipette tips and tubes were drilled by cnc milling machine.
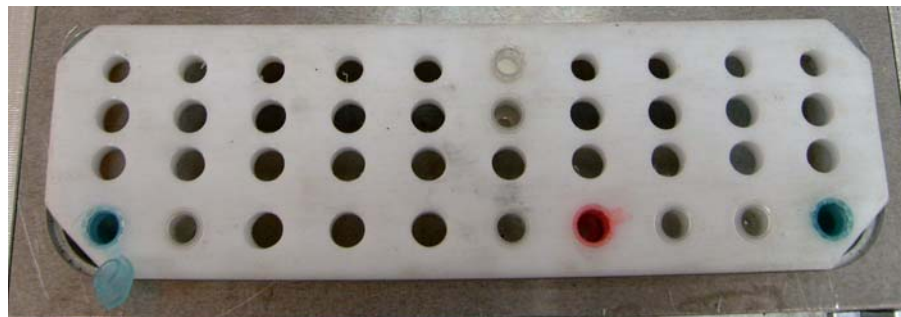
Figure 4.21. Pipette tip and tube carrier

CHAPTER 5

ELECTRONICS AND COMPUTER SOFTWARE

5.1. Electrics, Electronics and Control Circuit

To reach an accomplished sytem, required electrics and electronics components are represented in details in the following sections.

5.1.1. Interface Card

Figure 5.1. Interface card

Interface card is the electronic circuit that combines the PC parallel port with the stepper motor drivers. It processes the PC output data to the way stepper motor drivers can run the motors. The Figure 5.1 represents the interface card that is choosen for the device according to availibility and cost. It is capable of interfacing between 4 motor drivers and PC parallel port. It is produced by TEKNO ELEKTRONİK.

5.1.2. Stepper Motor Drivers

According to the motors, proper stepper motor drivers are needed. The major factor is the stepper motors torque. Since the motors have 6.2 kg.cm torques the expected torque from the driver must be higher than this value. Due to the needs, availability and cost, a stepper motor drive circuit produced by TEKNO ELEKTRONİK is choosen. Properties of stepper motor driver (Figure 5.2) used for the device are:

- For stepper motors up to 6.4 N.cm torque
- Half or full step mode
- Nominal current   : 4 A per winding, max current 4.2 A per winding
- Operating voltage   : 24-35 V
- Control circuit input voltage : 5 V

Figure 5.2. View of the stepper motor driver

Each of the drivers is controlled by 3 wires. Clock signal for the step pulses, direction signal for the direction of rotation and the enable signal. In the following picture, the motor outputs of the 4 wire stepper driver can be seen (Figure 5.3).
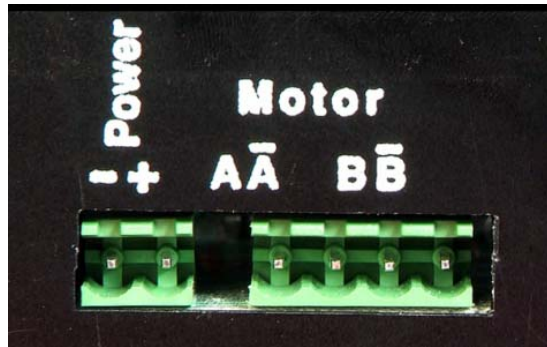
Figure 5.3 View of motor outputs of the 4 wire stepper driver

5.1.3. Heating Unit

As the incubation step needs a tepperature around 55 ^oC there should be a heat transfer to the aluminum block. It is decided to use two 50 W resistors connected in parallel as heating device resulting the total power 100 W. There are several types of temperature control systems commercially available on the market. The capabilities of these temperature control systems vary due to control system. These are on-off, proportional, proportional-integral, proportional-differential and proportional-integral-differential controls. Altough on-off control is the simplest and less expensive one, the accuracy is about 1 ^oC meeting our needs. So we have choosen an on-off temprature control system made by SİSEL ENGINEERING, which is combined with NiCr-Ni termocoupler. The device is shown in Figure 5.4. The specifications of this control unit can be summarized are as follows:

- Keypad   : Micro switch
- Out Led   : 1 red out led
- Display   : 3 digits 14 mm, 7 piece red led
- Dimensions   : 77×34×70
- Weight   : 198 g including box
- Inputvoltage   : 230 Vac+ %10 - %20, 50/60 Hz, 9-30 Vdc
- Input   : NiCr-Ni thermocouple
- Range   : for NiCr-Ni thermocouple (0-600 ^oC)
- Accuracy   : ±%5
- Power consumption : 4 VA

- Outputs : 16 A for resistive load
- Histeresis : 0-20 ^{o}C
- Kontrol method : on-off

Figure 5.4 Temperature control system

Since we have 100 W resistive load and 24 Vdc input voltage the rated current is calculated as 4.17 A using equation 5.1. The temperature control unit has maximum 16 A output responding our needs.

$$P=V \times I \qquad (5.1)$$

5.1.4. Power Supply

As the stepper motor drivers are working with 24-35 V range, it is reasonable to choose a power supply with 24 V. Since the rated current for each stepper motor is 1.5 A, and as four of these motors are used, 6 A will be needed for driving the motors. According to the characteristics of the stepper motor drivers some of the electrical energy will be converted to heat energy, assuming that the efficiency of the motor drivers is 50%, the total amount of current needed is 12 A at maximum power. The total amount of electrical power is calculated as 288 W using Equation 5.1. It will be safer to choose a power supply more than 300 W, due to avalibility 350 W power supply is used for the motor drive system.

A second power supply is needed for the heating unit of the incubation step. The electrical power need for the heating unit is 100 W, thus a 150 W power supply is choosen for safety.

The reason for using two power supplies instead of using one with a bigger capacity is the dynamic loading of the heating unit on the power supply. Since the heating mechanism is an on/off sytem there will be dynamic loading between on and off positions. This dynamicly changing electrical load can create peaks cousing the motors loose steps. Therefore it is planned to use two power supplies.

5.2. Computer Software (Graphical User Interface)

Mach3 is a program designed to control machines such as milling machines, lathes, plasma cutters, and routers. It is decided to use this graphical user interface as control software for DNA exraction. The Mach 3 graphical user interface uses G codes for programming the axis motions (Mach Support 2009). Features of these machines that are used by Mach3 includes connections between the robotic device and the PC running Mach3 are made through the paralel (printer) port(s) of the computer. A simple machine will need only one port; a complex one will need two. Since there are 4 axis controls, operating with one paralel port is enough. Mach3 will control up to six axes simultaneously. The feed rate during these moves is maintained at the value requested by program, subject to limitations of the acceleration and maximum speed of the axes. The axes can be moved with various jogging controls manually.

Mach3 interfaces to the motor drivers and other hardware through PC's paralel port(s). The parallel port connector on the PC is a 25-pin female "D" connector. The connector, as seen from the back of the PC, is shown in Figure 5.5. The arrows give the direction of information flow relative tothe PC. Thus, for example, pin 15, the second pin from the right on the bottom row, is an input to the PC.
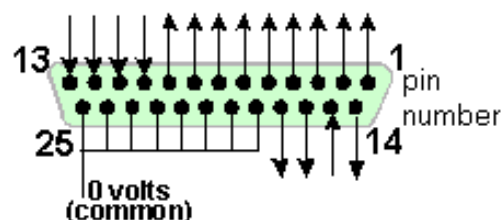
Figure 5.5. Parallel Port Female Connector (seen from back of PC)

All the signals output by Mach3 and input to it are binary digital (i.e. zeros and ones). These signals are voltages supplied by the output pins or supplied to the input pins of the parallel port. These voltages are measured relative to the computer's 0 volt line, which is connected to pins 18 to 25 of the port connector. Finally all data is transfered to the motor drivers after processing of the interface card resulting an angular movement of the motor shaft.

5.2.1. Calculating the Steps per Unit

Mach3 rotates a motor by steps (Mach Support 2009). The number of steps that Mach3 must send to a motor to cause one "unit" of movement (inch or mm) depends on:

- The mechanical drive (e.g. pitch of leadscrew, gearing between the motor and the screw).
- The properties of the stepper motor or the encoder on the servo motor.
- The micro-stepping or electronic gearing in the drive electronics.

5.2.2. Calculating Mechanical Drive

It is needed to calculate the number of revolutions of the motor shaft (*motor revs per unit*) required to move the axis by one unit (Mach Support 2009). For a machine leadscrew and nut, the pitch of the screw (that is, the thread crest-to-crest distance) and the number of starts should be known.

Metric screws are usually specified in terms of pitch, so no conversion calculation is necessary. If the screw is directly driven from the motor (1:1 drive ratio), then this is also the *motor revs per unit*. If motor having a gear, chain, or belt drive to the screw is used with Nm teeth on the motor gear and Ns teeth on the screw gear, Equation 5.2 represents the calculation for revolutions per unit.

$$\text{Motor revs per unit} = \text{screw revs per unit} \times \text{Ns/Nm} \qquad (5.2)$$

5.2.3. Calculations for the Mach3 Software

It is decided to use 1:1 drive ratio between the drive shaft and screw for the entire 4 axis has. X and Y axis have 2 mm pitch screw, which means one revolution of the motor shaft couses a 2 mm displacement. For a unit of 1mm travel, half revolution is needed. The motors have 200 steps/rev, because of the stepper motor drivers are in half step mode, 2 pulses for a full step must be sent. For a one full revolution of the shaft 400 pulses are needed. Targeting 1 mm of displacement 200 pulses must be sent. Unit Travel Pulse Number (UTPN) is represented in Equation 5.3.

UTPN=(motor steps per rev)×(microstepping ratio)/(pitch of the screw)   (5.3)

200 pulses is the input for X andY axis to the Mach 3 parameters.Z and A axis have 1.25 mm pitch screw, UTPN is 320 pulses according to the Equation 5.3.

5.2.4. Graphical User Interface Set Up

The stepper motors can loose steps if affected by more torque than the maximum value of the motor torque. Altough the holding torque of stepper motors are satisfactory, detent torque values are poor compared with the servo motors. Since a stepper motor has no feed back, acceleration and rotational speed values must be mentioned carefully, without permiting to lose a step, resulting false positioning (Mach Support 2009).

The speed values are considered as follows. It is decided during the experimental observation that, under no-load, the stepper motors selected are running unstable over 700 rpm, due to the torque loss as the rotational speed increases. As a result not going over 600 rpm is logical. For X and Y axis those have 2 mm pitch screws, the maximum velocity is calculated as 1200 mm/min using Equation 5.4.

Max. Velocity=Max rpm×Pitch (mm)   (5.4)

During preliminary tests it has been observed that X axis is running smooth at 1200 mm/min, but Y axis is loosing steps before reaching 1200 mm/min value. Loading of Y axis is higher than X axis. Since Y axis is moving along a vertical axis under

ground force affect.The maximum smooth running velocity reached for the Y axis is 900 mm/min targeting 450 rpm. As a result 1200 mm/min maximum velocity for X axis, and 900 mm/min for Y axis is decided. These are movement profile inputs for Mach 3 software as represented in Figure 5.6.
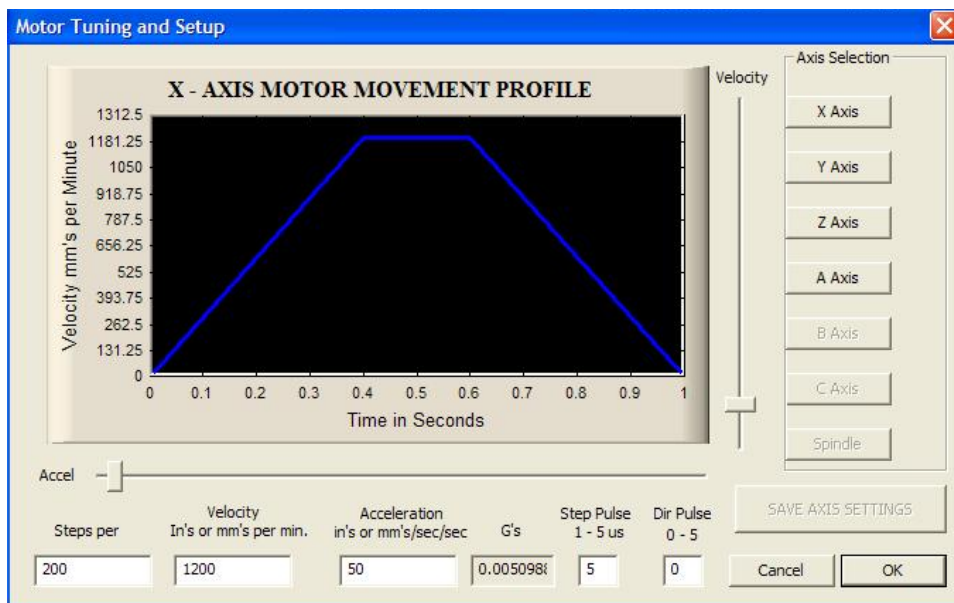
(a)
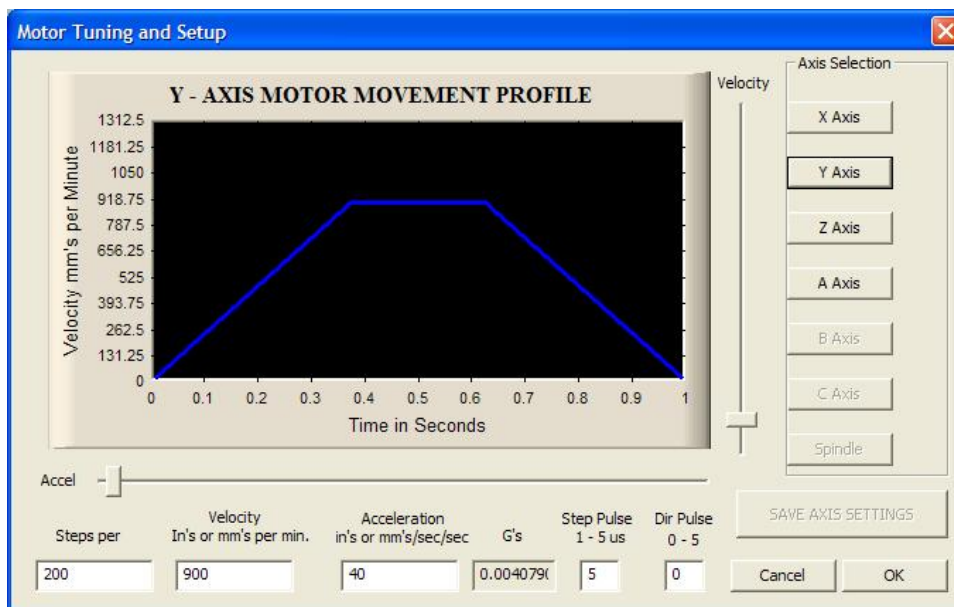
(b)

Figure 5.6. (a) X and (b) Y axis movement profile graphics
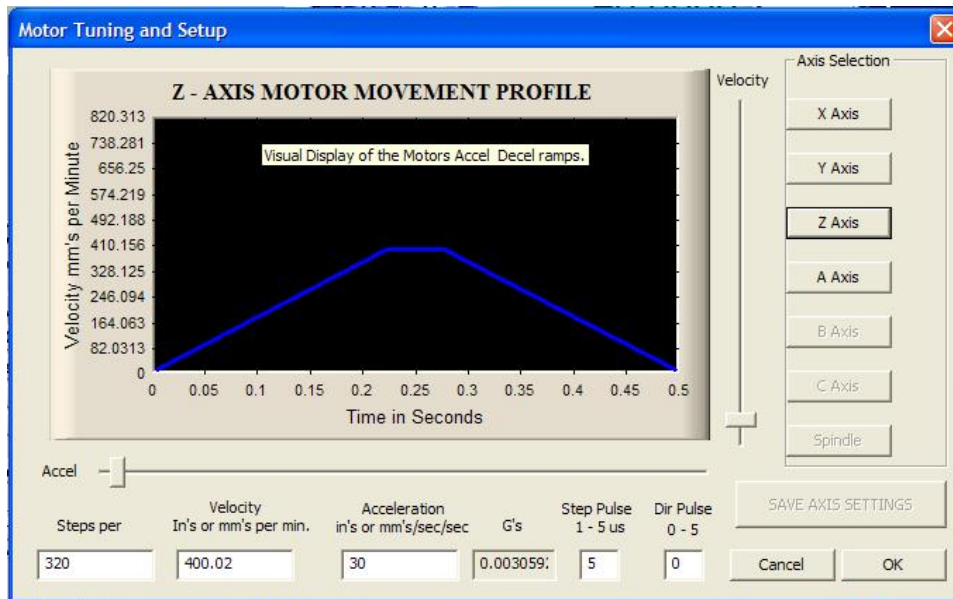
(a)
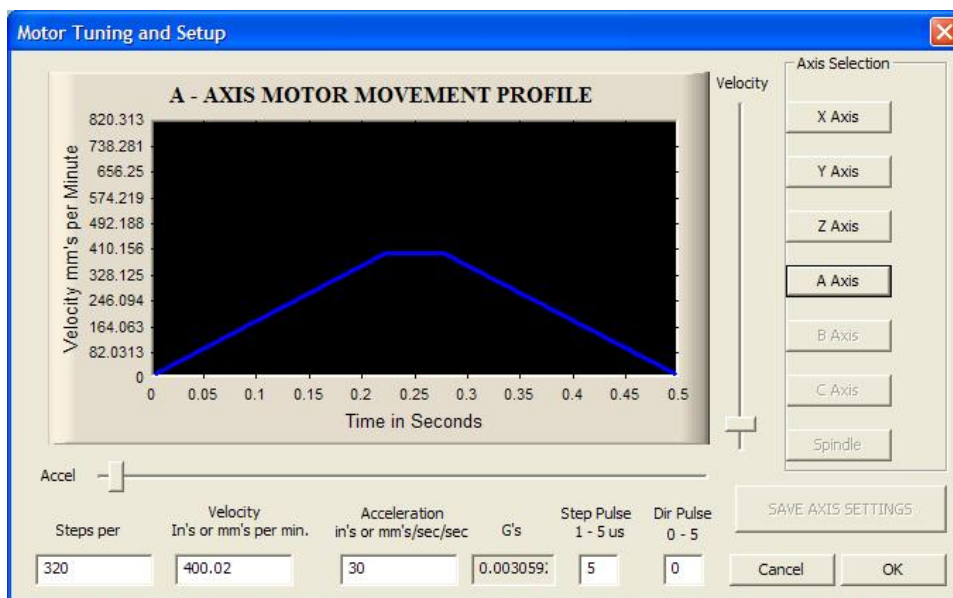
(b)

Figure 5.7. (a) Z and (b) A axis movement profile graphics

The Z axis is expected to have more load than others due to the friction between pistons and o-ring. Also positioning accuracy must be higher comparing with the other axis. Total stroke of Z axis is relatively shorter. According to these cases Z axis maximum velocity must be determined lower than X and Y axis. During tests it has been observed that 400mm/min velocity is optimum for Z axis. These are the movement profile input for Mach 3 software as represented in Figure 5.7. The same parameters are also suitable for A axis, which have the same pitch screw.

The velocity-time graphics of the GUI have trapezoid caracteritics to obtain smooth operation. During tests it is aimed to have a smooth running on each axis without loosing a step. Try and errors (iterations) resulted different values for each axis. The values are listed below:

- X: 50 mm/sec^2
- Y: 40 mm/sec^2
- Z: 30 mm/sec^2
- A: 30 mm/sec^2

Pins and ports setup considerations are shown in Figure 5.8. To drive the stepper motors Mach 3 must send the right signals to the interface card through the parallel port. Each motor driver is commuted by 3 wires: enable, pulse (clock) and direction. The Mach 3 software should be tuned for each driver to send signals to the right pin of the parallel port. The enable signal can be a common pin for every motor driver since the motors are energized at the same time. 2 more pins for each axis for clock and direction signals will be required.

Signal	Enabled	Step Pin#	Dir Pin#	Dir LowActive	Step Low Ac...	Step Port	Dir Port
X Axis	✓	2	3	✗	✗	1	1
Y Axis	✓	8	9	✗	✗	1	1
Z Axis	✓	6	7	✗	✗	1	1
A Axis	✓	4	5	✗	✗	1	1
B Axis	✗	0	0	✗	✗	1	1
C Axis	✗	0	0	✗	✗	0	0
Spindle	✗	0	0	✗	✗	0	0

Figure 5.8. Pins and ports set-up

All the components of the final design are produced as a prototype. The workstation is assembled. The electronics are integrated to the system. The conceptual G codes are written according to the NA extraction procedure. Initial test results are presented in the following chapter.

CHAPTER 6

TEST RESULTS

The robotic platform has been tested to confirm that it fullfills the specifications as a system (Appendix C). In order to check if the system is working in complete coordination, before going into a whole process of DNA isolation from blood, some preliminary tests are performed.

The system is composed of the mechanical, electronic and software subunits. Mechanical system had also been tested before integrating the electronic control by moving the axes manually in all directions. After installing the stepper motors, the same tests had been repeated by giving rotation to the motors. At this level, motor torques and speeds could be observed and measured real time. The final testing stage before a full extraction process is testing the device with the electronic system on. Initially, all axes have been moved to their homes and the range of motion for all four axes has been calculated, tested and marked. Rotation speed and acceleration values had been determined for each individual axis by trial and error and default values are adjusted to the optimum settings.

After all adjustments have been completed, some important reference coordinates were noted by jog control. They are:

- For Y axis: the bottom of the tube, the lowest clearance level for moving around, the pipette tip approaching and wearing, the on magnet level
- For X axis: stations for every chemical, hot plate position, pipette tip, sample tube and elute tube;
- For Z axis: pipette tip neutral, pipette tip off and all levels corresponding to the calculated volumes.
- For A axis: magnet off, magnet near and magnet on positions.

Finally, the Mach3 code for the whole process is run on the device when all chemicals and sample are replaced with water. The test is repeated again with exact volumes and magnetic beads, then with the true chemicals several times. After tens of

rehersals, many minor adjustments are made and the code achieved its eventual form, which is ready to be applied to the sample, itself (Appendix D).

When programming the axis motions manually DNA extraction steps were simulated. The reference points noted for each step were considred and created each station on the worktable. The calculated pipetting volumes per unit move of the pistons were taken into account as well. The flow chart of the G coding steps is represented in the Figure 6.1. The conceptual codings are represented in Appendix D.
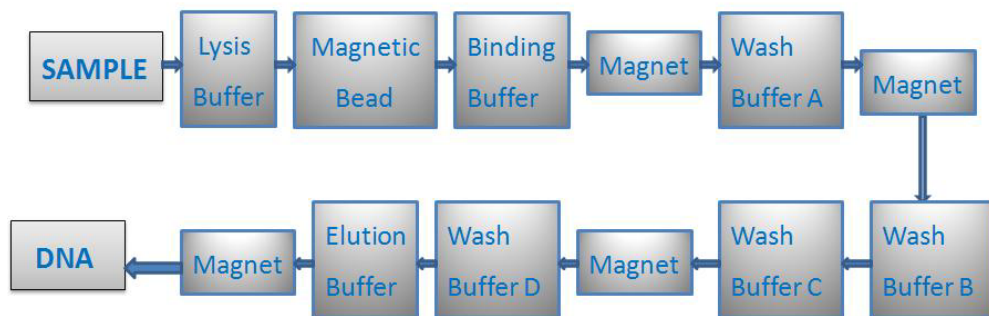
Figure 6.1. Process flow chart

During the preliminary testing of the DNA extractor out some application problems are figured out. The heater did not reach 55 ^{o}C although the temperature control unit was set to 55 ^{o}C. To overcome this problem, the temperature control is set to higher temperature and tested the incubation tubes temperature. These iterations resulted to set the temperature control at 60 ^{o}C in order to keep the incubation tubes at 55 ^{o}C (Figure 6.2).

Figure 6.2. Temperature test of the heating unit

Another problem was the fluid dropping from the pipette tips. After the suction step as the pipette tips move away from the reagents, tiny drops at the pipette tip were observed. This problem is solved by adding a step to the G coding. The idea was to suck a little more volume after the pipette tip left the fluid (Figure 6.3).

Figure 6.3. Fluid drop from pipette tip

Bubles were observed during pipetting. The reason for these bubles in the pipette tip was determined as the acceleration and velocity values of Z axis. These parameters reduced in values to prevent bubling (Figure 6.4).

Figure 6.4. Bubles in the pipette tip.

Also some problems occurred in the magnetic separation step. The magnetic beads did not stay in the right position facing the magnets during magnetic separation. The problem is resolved by positioning the pistons so that the magnetic beads positioned at a certain place in the pipette tip facing the magnets (Figure 6.5).

Over heating of stepper motors and stepper motor drivers was another problem. The acceptable temperature limit of the stepper motors is 80 ^{o}C. During tests it is observed that motors did not reached this temperature level but has exceeded 70 ^{o}C. Altough 70 ^{o}C is under the maximum level, to make it secure 50 ^{o}C is determined as target motor temperature. To limit the temperature at 50 ^{o}C, aluminium cooling plates placed to the back side of the motors. Similarly to overcome the stepper motor drivers overheating problem, 24 V cooling fans placed on each of the drivers cooling block (Figure 6.6).
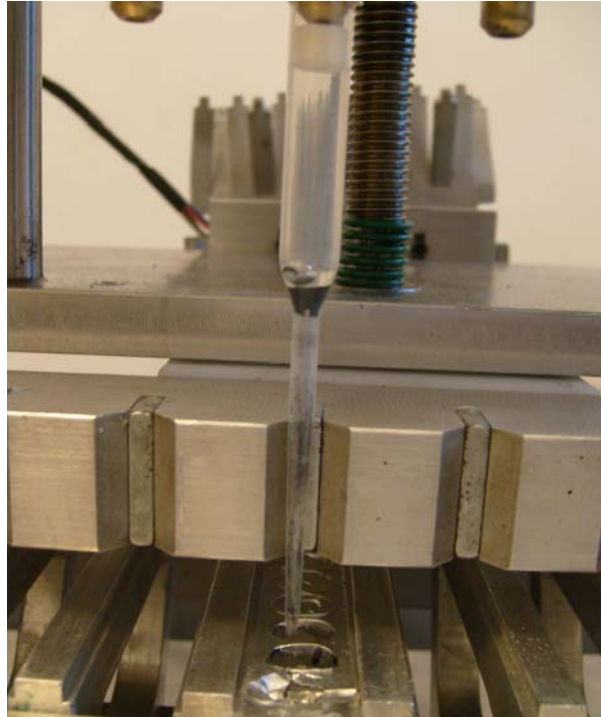
Figure 6.5 Magnetic beads which are not facing the magnets

| (a) | (b) |
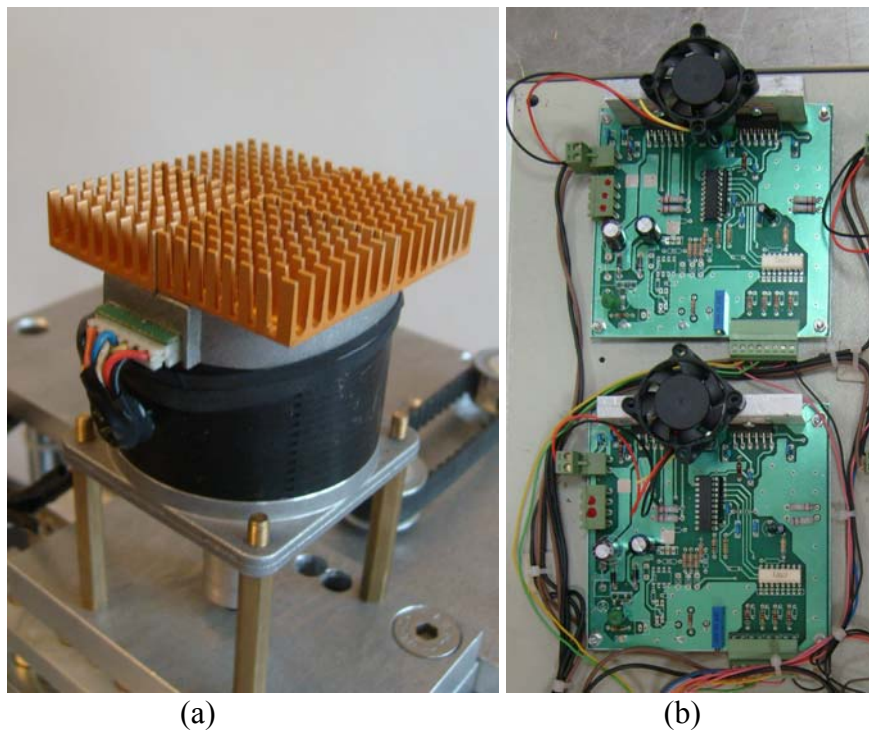
Figure 6.6. (a) Aluminum fins to cool the motor and (b) cooling fans for stepper motor
drivers

After the installation of lead screws, some backlash is figured out from the end bearing housings. This problem was resolved by placing preloaded springs between the end bearing of each axis and leadscrew. This springs applied opposite directional force to leadscrew which eliminates backlash (Figure 6.7).
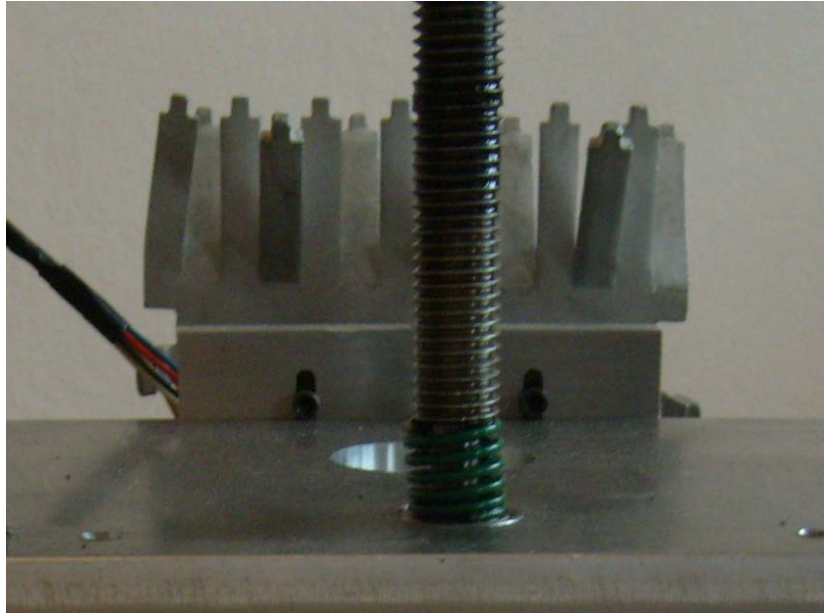
Figure 6.7. Spring preloaded screw

To be able to optimize the process we tried to figure out the exact positions of each tube on the worktable. Finally we managed to extract DNA from 250 µl blood at about 30 minutes.

CHAPTER 7

DISCUSSION AND CONCLUSION

With the variety of DNA extraction methods available to the scientific community, scientists have critical decisions, concerning which methodologies might yield the best results for particular samples. Timesaving NA extraction methods that yield purified samples with high quality NA are crucial for laboratories to meet the rising demands for their services.

Automated NA extraction methods reduce analysis time and allow laboratories to process a high number of samples helping to eliminate backlogs. Large-scale robotic NA extraction instruments are available too, however these systems are often out of reach to smaller laboratories due to their cost or where the number of samples analyzed is too few to justify the purchase of a large robotic platform. Small to medium-sized laboratories can benefit from considerable timesaving and more effective case management without a loss in sample quality by incorporating the robot for NA extractions.

This robotic workstation is designed to extract NA from blood. Automation eliminates operator-to-operator variations, and the system provides protection from infected blood samples. Therefore, this workstation is an ideal extraction system for laboratories which need processing under 100 samples per day, and it is suitable for genetic testing of humans and animals, veterinary diagnostics, biobanking and genetic research.

The processing capacity (1-10 samples/run) enables routine applications for clinical laboratories. The low-throughput of the workstation ensures that priority samples can be handled safely and quickly without decreasing laboratory efficiency.

The mechanical design of the workstation is satisfactory. The motors work well without loosing steps and silent enough for laboratory use. The stepper motor drivers performed satisfactory performance in combination with the stepper motors. The interface card ensured practical connection between PC parallel port and stepper motor drivers with a 25 pin D connector cable. The cable routing between interface card and stepper motor drivers are easily applicable. The Mach 3 software has an advantage of

tuning of the motors' acceleration and speed values in a wide range. The interface screen has jogging controls, manual data input, emergency stop, single block run options which help tuning and programming. The G coding programming is a practical solution for try and errors and rapid interfering can be done directly from PC.

Automation of the method NA isolation using paramagnetic microparticles were succeeded. The main advantage of the method is it is relatively faster. The NA isolation process from ten samples lasted about 30 min.

7.1. Future Works

It has been exprienced during testing period that if the process time decreased by increasing the motors' velocity values, the stepper motors start to loose steps that results as wrong positioning. For a faster workstation motors with higher torque can be used, a lighter construction can be manufactured, bearings with lower friction coefficient and ballscrews can be selected. Also, these motors can help to increase the number of samples per run.

Present system needs more space in labrotary as it is dependent on a PC. If an electronic card is designed that can run the drivers with built in softwares then the system will become standalone which will be free of any PC system can be achieved. Due to the laboratory conditions an open system is not a suitable choice. To prevent the reagents, samples and result fluids contacting with the environment and operator, a stainless steel outher shell with control buttons and load-unload door can be designed and manufactured.

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APPENDIX A

DATASHEET FOR SELECTED LINEAR BEARING

SCW ALUMINUM CASE UNIT LONG

PART NUMBER	L/B	h	D	W	H	G	A	J	E	S×ℓ	S_1	K_w	L_w	BASIC LOAD RATING (DYNAMIC) N	(STATIC) N	WEIGHT (g)
SC8W-B	LM8UU	11	17	34	22	18	6	24	5	M4×8	Ø3.4	42	56	410	800	94
SC10W-B	LM10UU	13	20	40	26	21	8	28	6	M5×10	Ø4.3	46	68	580	1060	147
SC12W-B	LM12UU	15	22	44	30	24.5	8	33	5.5	M5×10	Ø4.3	64	77	650	1180	220
SC13W-B	LM13UU	15	22	44	30	24.5	8	33	5.5	M5×10	Ø4.3	64	77	800	1540	245
SC16W-B	LM16UU	19	25	50	38.5	32.5	9	36	7	M5×12	Ø4.3	79	89	1230	2340	376
SC20W-B	LM20UU	21	27	54	41	35	11	40	7	M6×12	Ø5.2	90	100	1370	2470	476
SC25W-B	LM25UU	26	36	76	51.5	41	12	54	11	M8×18	Ø6.8	119	136	1560	3120	1115
SC30W-B	LM30UU	30	39	78	59.5	49	15	58	10	M8×18	Ø6.8	132	146	2480	5480	1375
SC35W-B	LM35UU	34	45	90	68	54	18	70	10	M8×18	Ø6.8	140	160	2650	6260	2200
SC40W-B	LM40UU	40	51	102	78	62	20	80	11	M10×25	Ø8.6	150	180	3440	8020	3200
SC50W-B	LM50UU	52	61	122	102	80	24	100	11	M10×25	Ø8.6	200	230	6810	15860	6720

Note 1) Dynamic load rating is based on the nominal life of 50km.
In case of 100km, C on the table need to be divided by 1.26
Ex) LM12's 50km basis dynamic load rating C = 410N
LM12's 100km basis dynamic load rating C_{100} = 410 / 1.26 = 325.42N
Note 2) Based on the weight of resin retainer
Note 3) Dimension : mm

SC ALUMINUM CASE UNIT

PART NUMBER	L/B	h	D	W	H	G	A	J	E	S×ℓ	S_1	K	L	BASIC LOAD RATING (DYNAMIC) N	(STATIC) N	WEIGHT (g)
SC8-B	LM8UU	11	17	34	22	18	6	24	5	M4×8	Ø3.4	18	30	260	400	56
SC10-B	LM10UU	13	20	40	26	21	8	28	6	M5×10	Ø4.3	21	35	370	540	90
SC12-B	LM12UU	15	21	42	28	24	7.4	33	5.5	M5×10	Ø4.3	26	39	410	590	112
SC12N-B	LM12UU	15	21	42	28	24	7.4	33	5.5	M5×12	Ø4.3	26	36	410	590	112
SC13-B	LM13UU	15	22	44	30	24.5	8	33	5.5	M5×10	Ø4.3	26	39	500	770	123
SC16-B	LM16UU	19	25	50	36.5	32.5	9	36	7	M5×12	Ø4.3	34	44	770	1070	189
SC20-B	LM20UU	21	27	54	41	35	11	40	7	M6×12	Ø5.2	40	50	860	1370	237
SC25-B	LM25UU	26	36	76	51.5	41	12	54	11	M8×18	Ø6.8	50	67	980	1560	555
SC30-B	LM30UU	30	39	78	59.5	49	15	58	10	M8×18	Ø6.8	58	72	1560	2740	685
SC35-B	LM35UU	34	45	90	68	54	18	70	10	M8×18	Ø6.8	60	80	1660	3330	1100
SC40-B	LM40UU	40	51	102	78	62	20	80	11	M10×25	Ø8.6	60	90	2150	4210	1600
SC50-B	LM50UU	52	61	122	102	80	24	100	11	M10×25	Ø8.6	80	110	3800	7930	3350

Note 1) Dynamic load rating is based on the nominal life of 50km.
In case of 100km, C on the table need to be divided by 1.26
Ex) LM12's 50km basis dynamic load rating C = 410N
LM12's 100km basis dynamic load rating C_{100} = 410 / 1.26 = 325.40N
Note 2) Based on the weight of resin retainer
Note 3) Dimension : mm

APPENDIX B

VIEWS OF THE DNA EXTRACTOR AND THE COMPONENTS
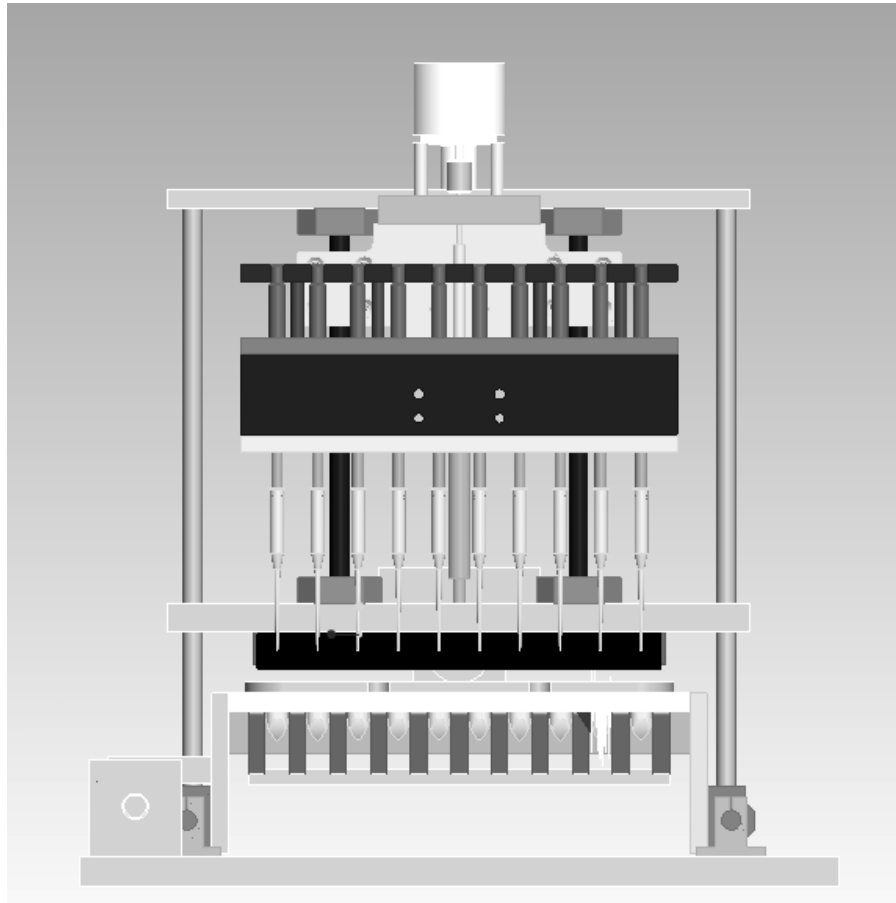
Figure B.1. Front view.
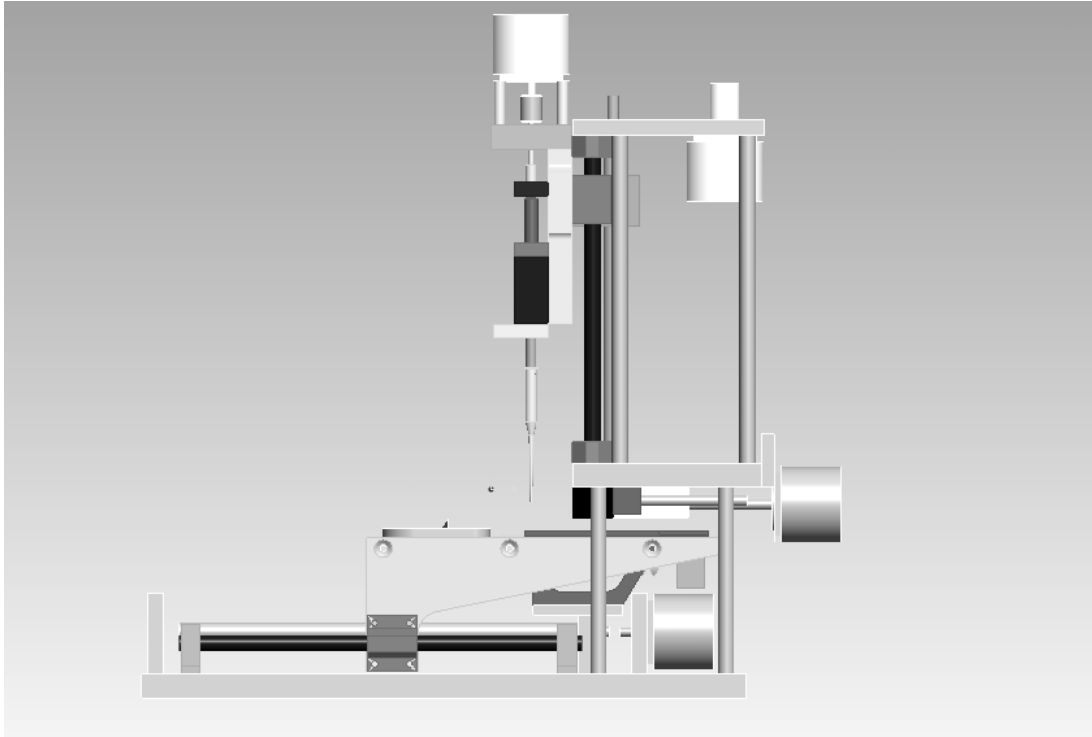
Figure B.2. Right view.
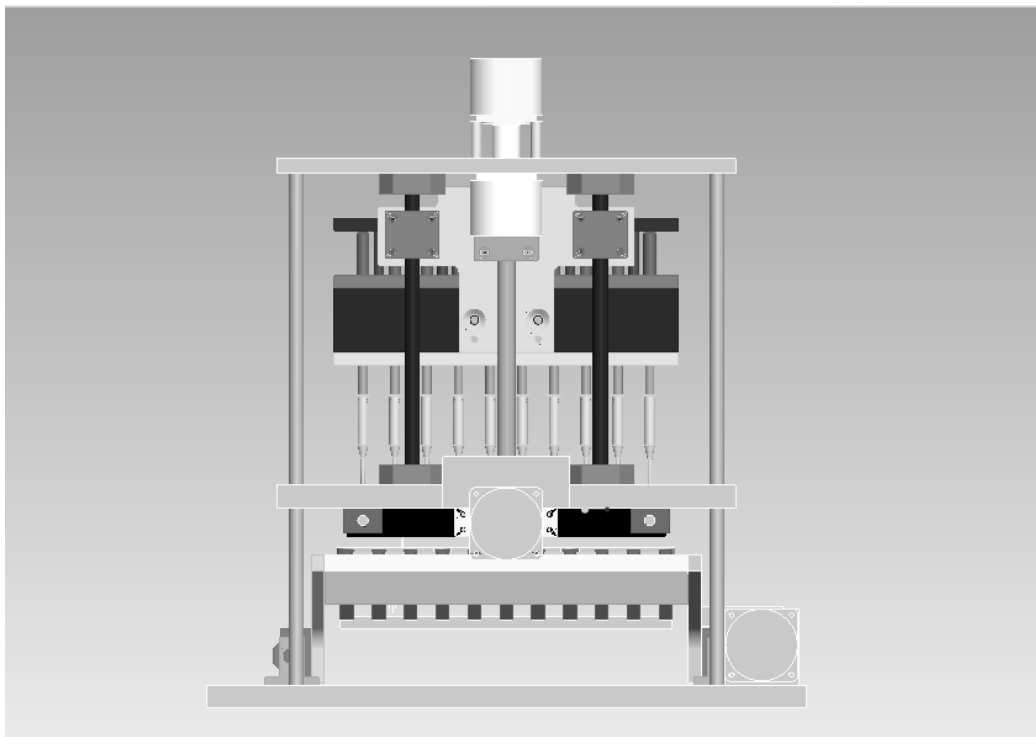
Figure B.3. Back view.
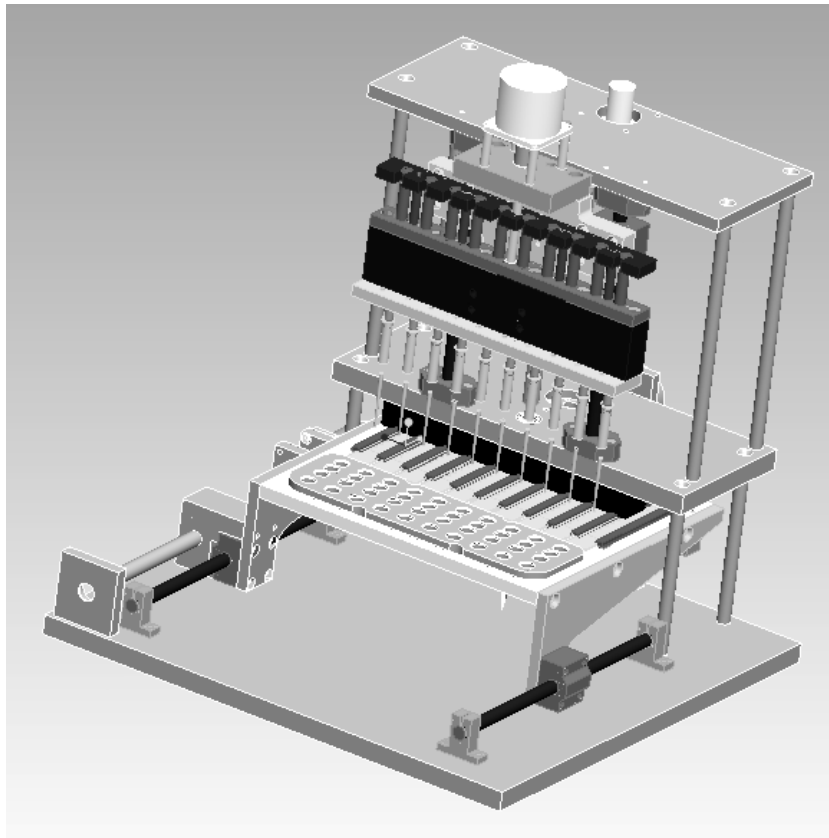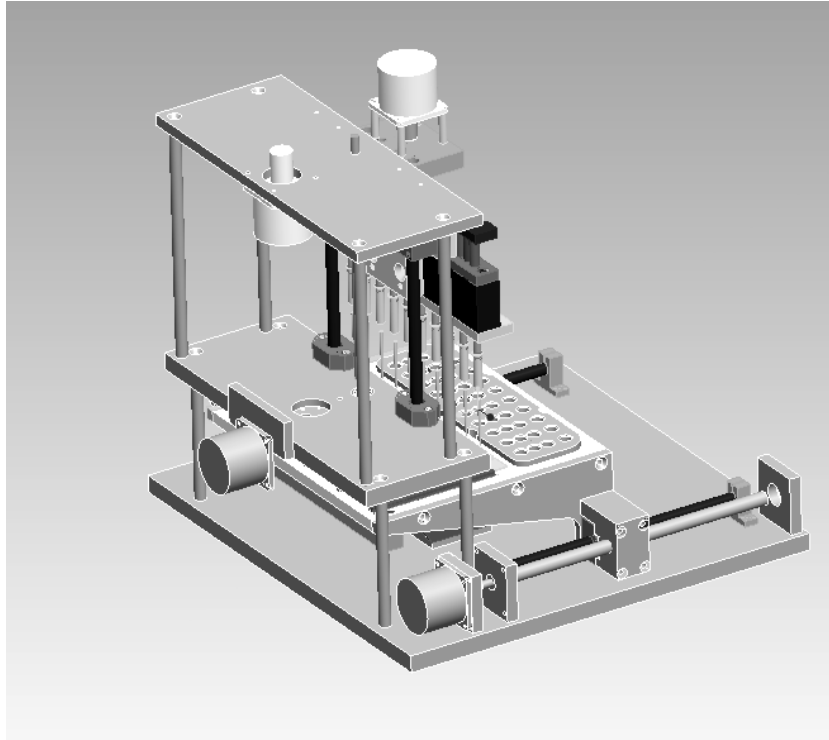
Figure B.4. Isometric views.
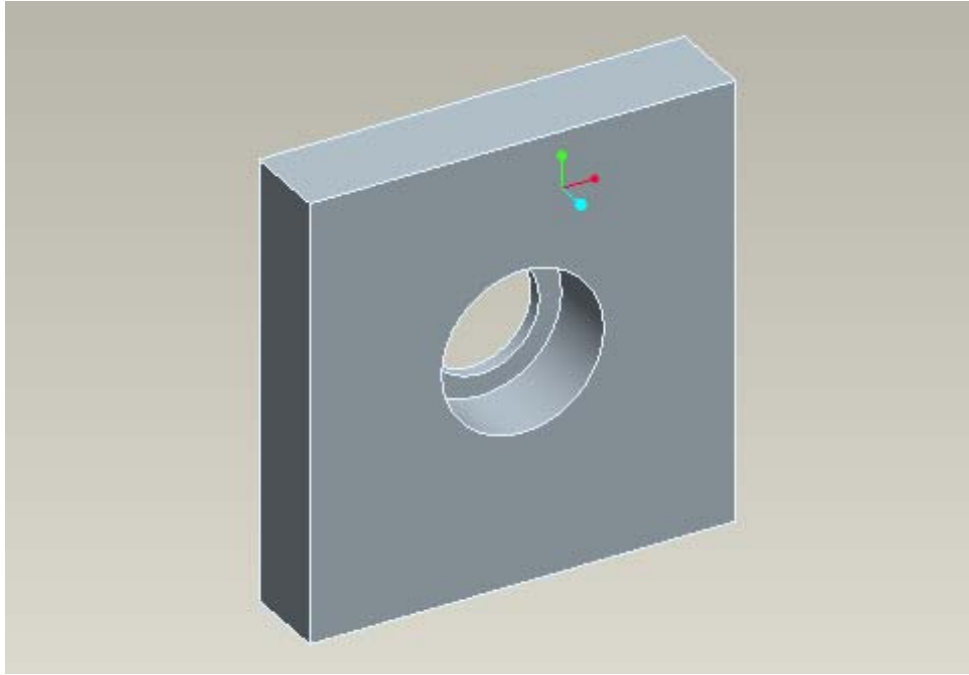
Figure B.5. Screw bearing support
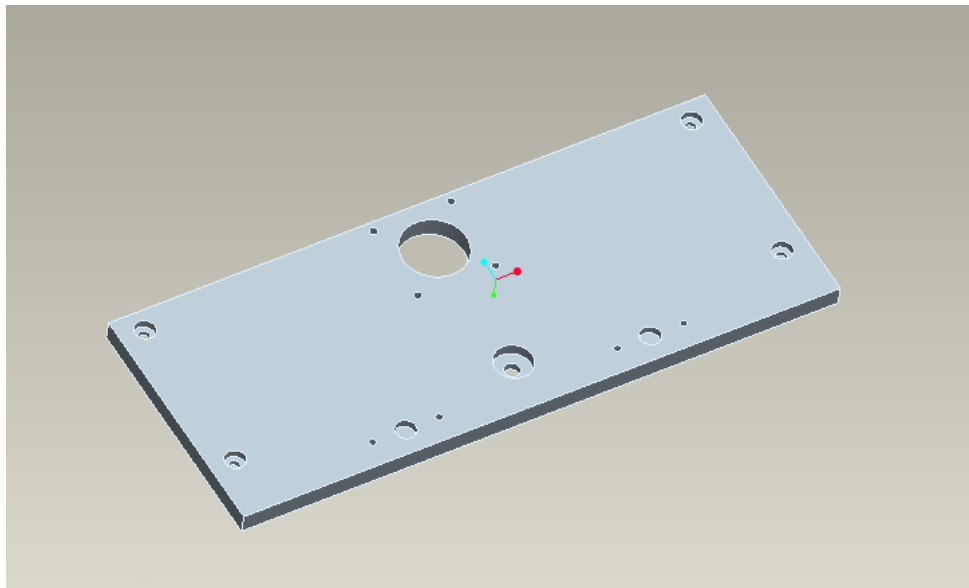
Figure B.6. Upper plate
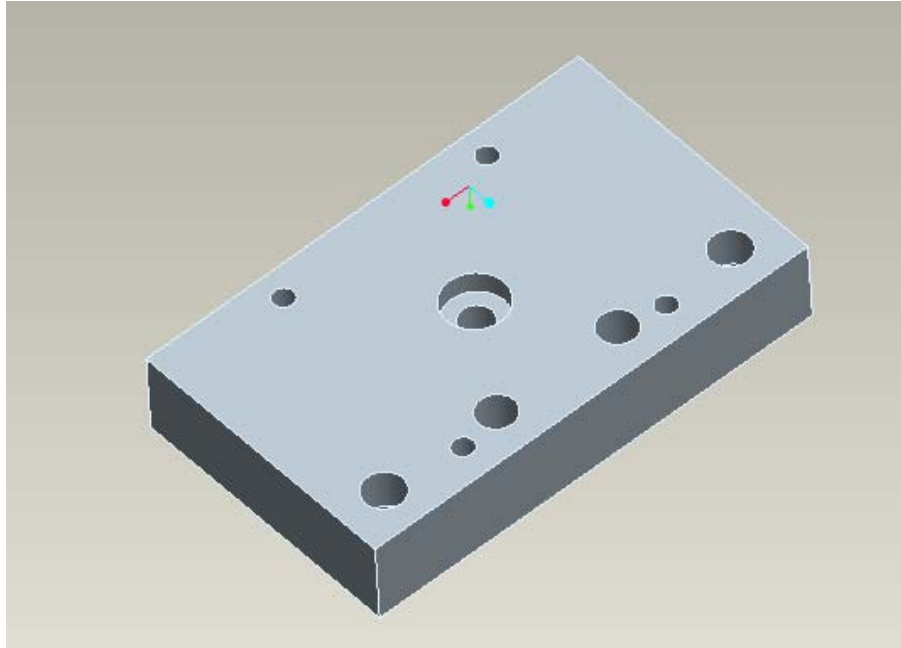
Figure B.7 Z-axis motor mount
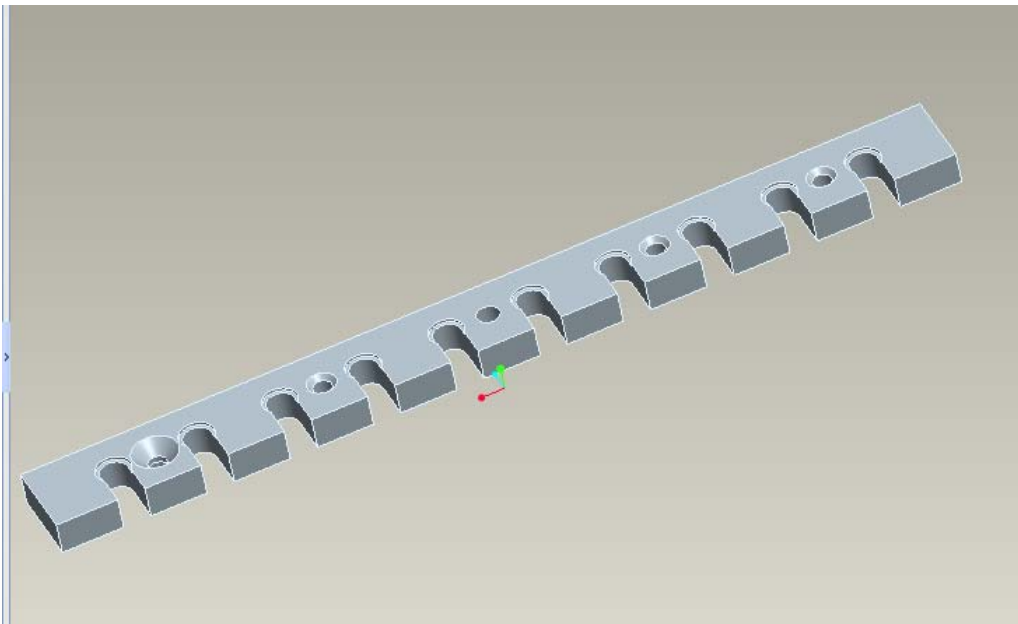
Figure B.8 Piston pusher plate
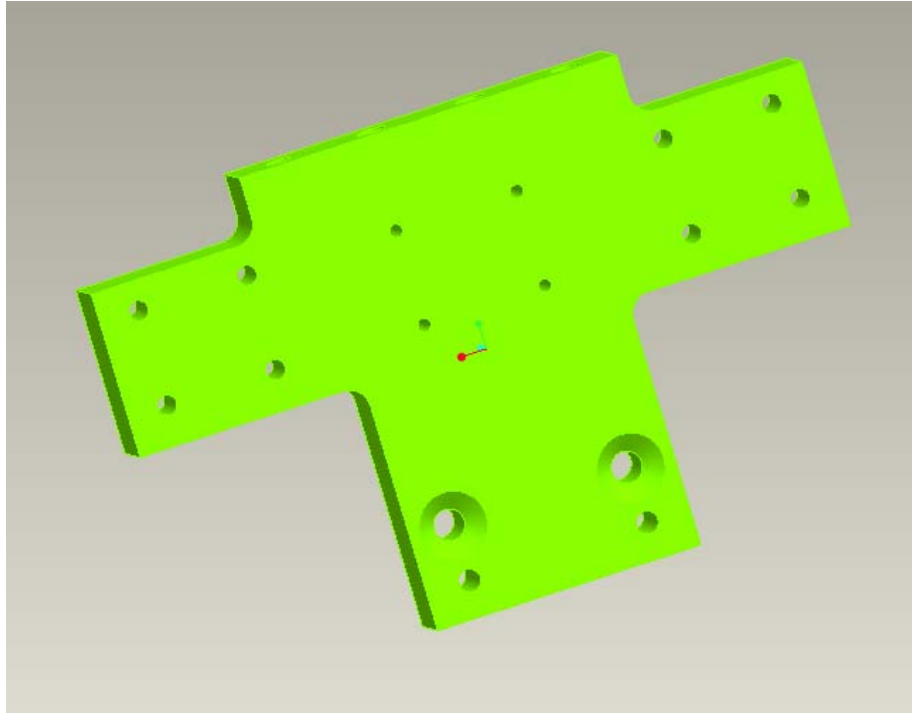
Figure B.9 Z-axis support
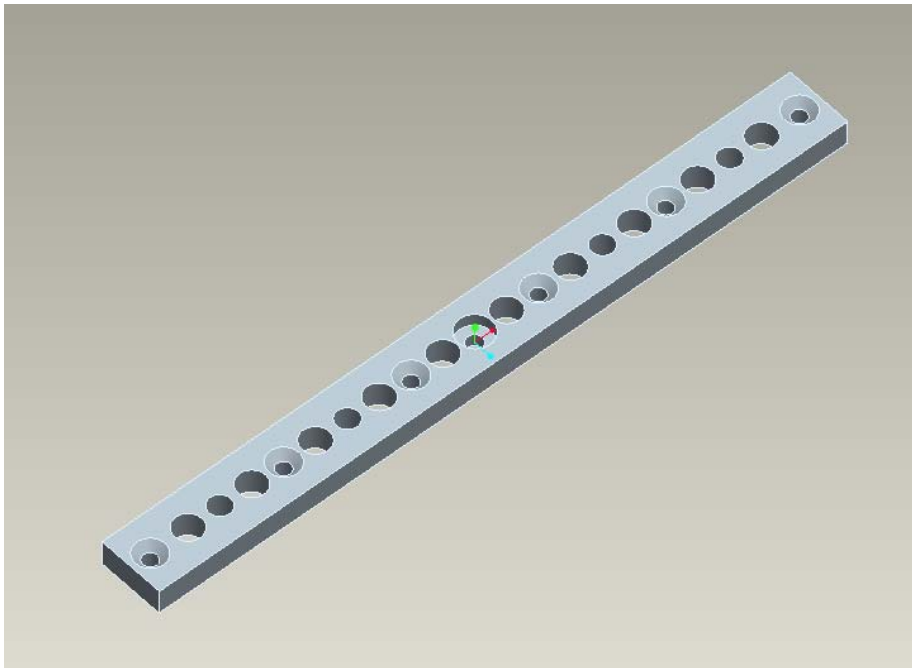
Figure B.10 Syringe cap
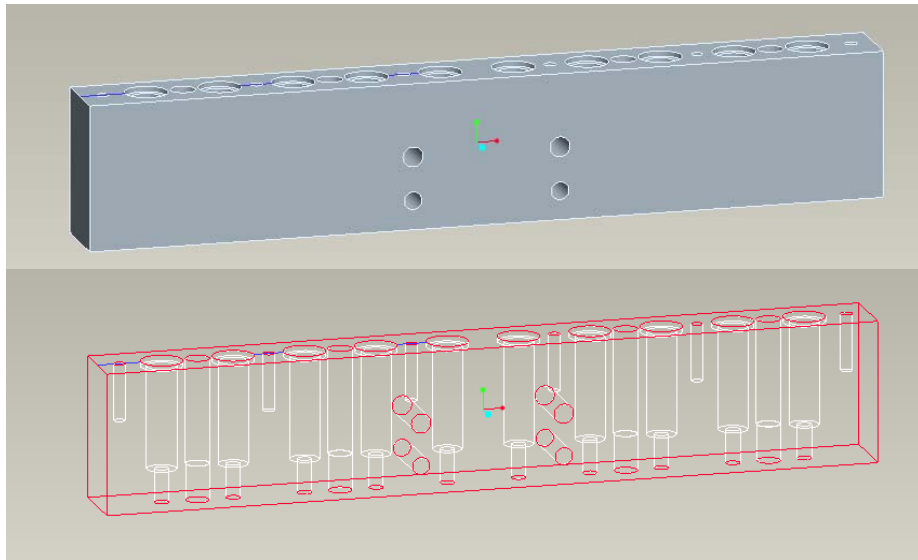
Figure B.11 Syringe block
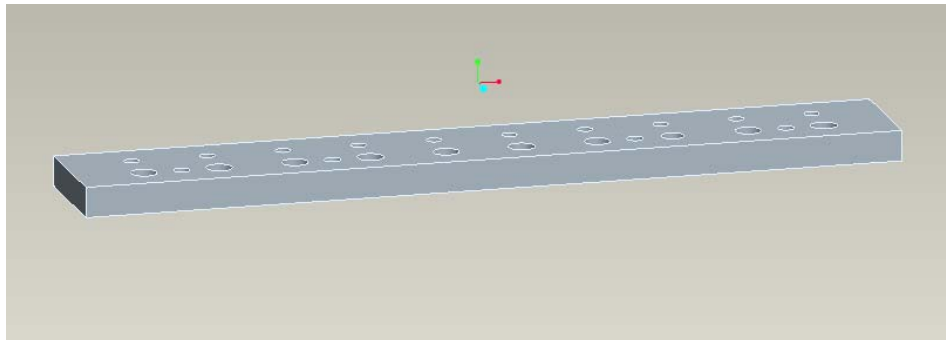
Figure B.12 Pipette tip dropper plate

Figure B.13 Piston
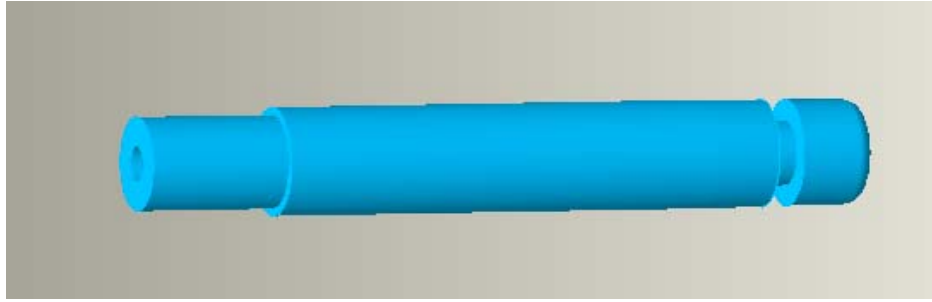
Figure B.14 Nozzle
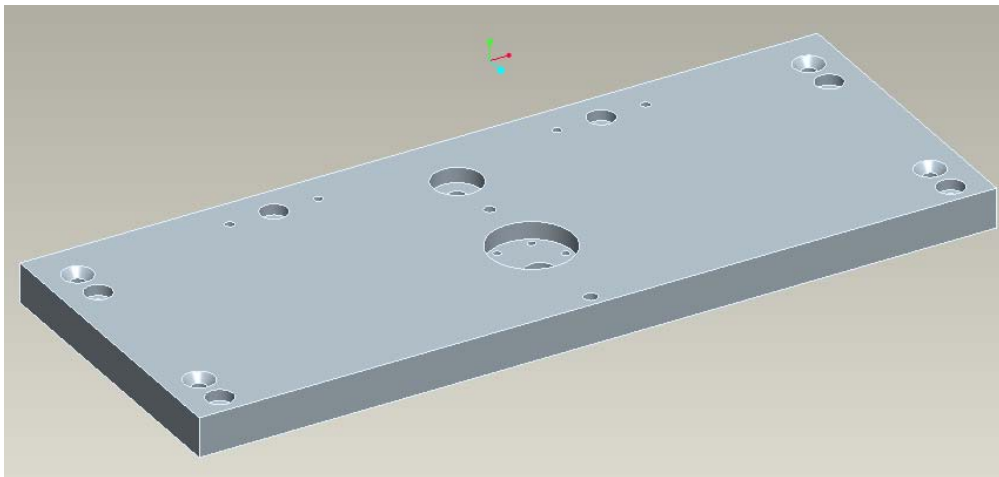
Figure B.15 Middle plate
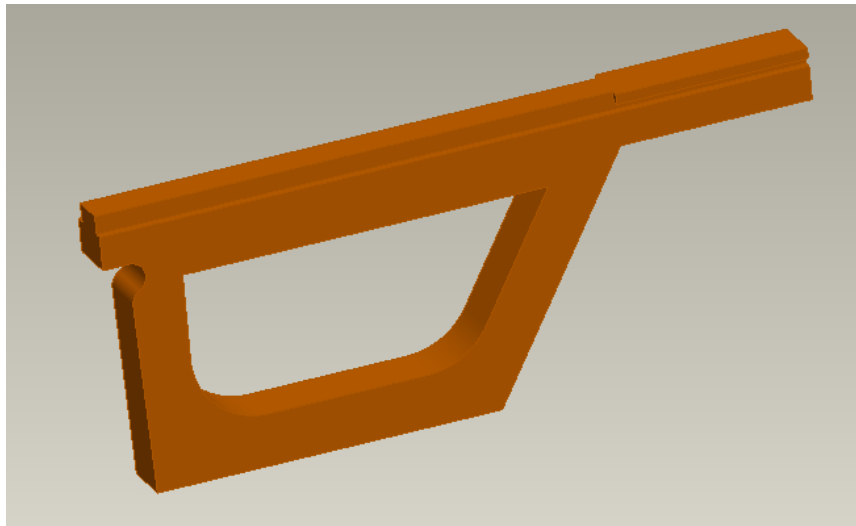
Figure B.16 Kit carrier
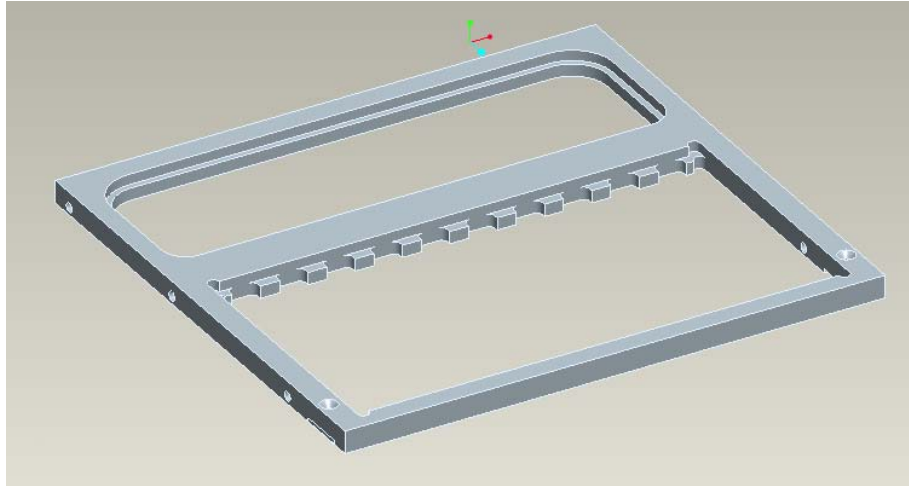
Figure B.17 Worktable
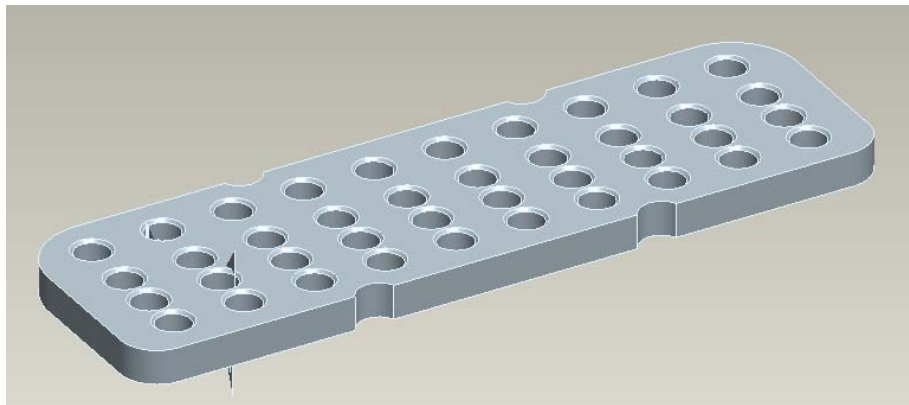
Figure B.18 Pipette tip and tube carrier
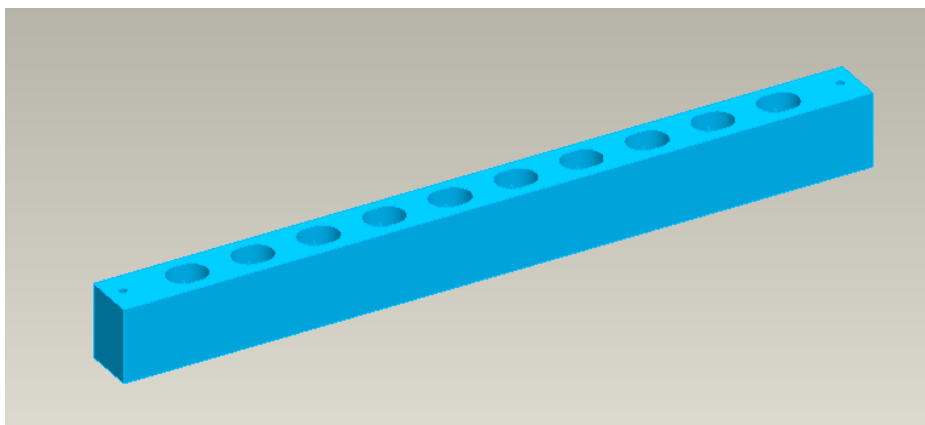
Figure B.19 Heating block
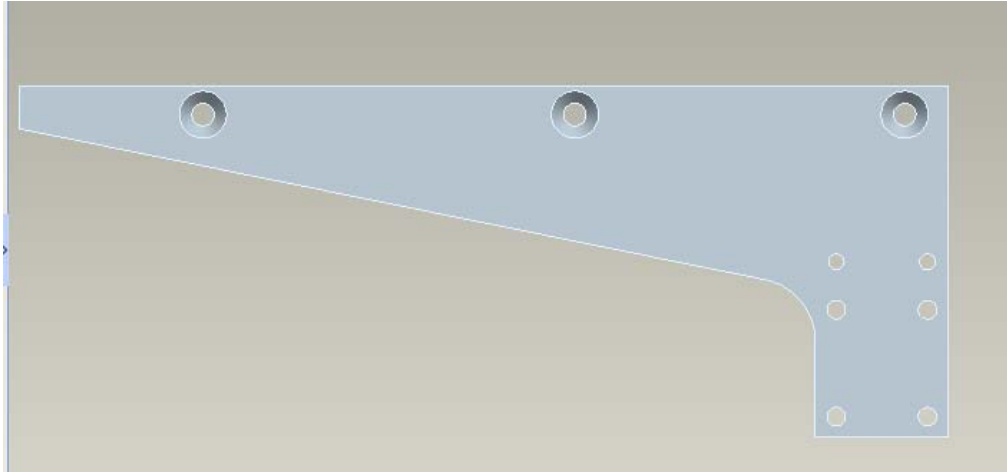
Figure B.20 Worktable support
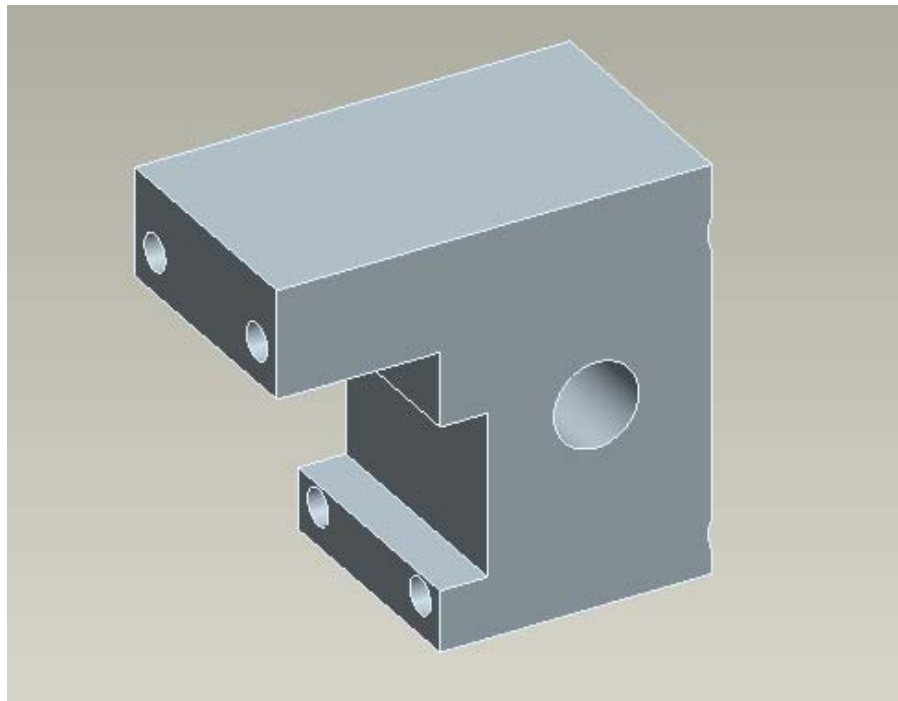
Figure B.21 X-axis screw nut
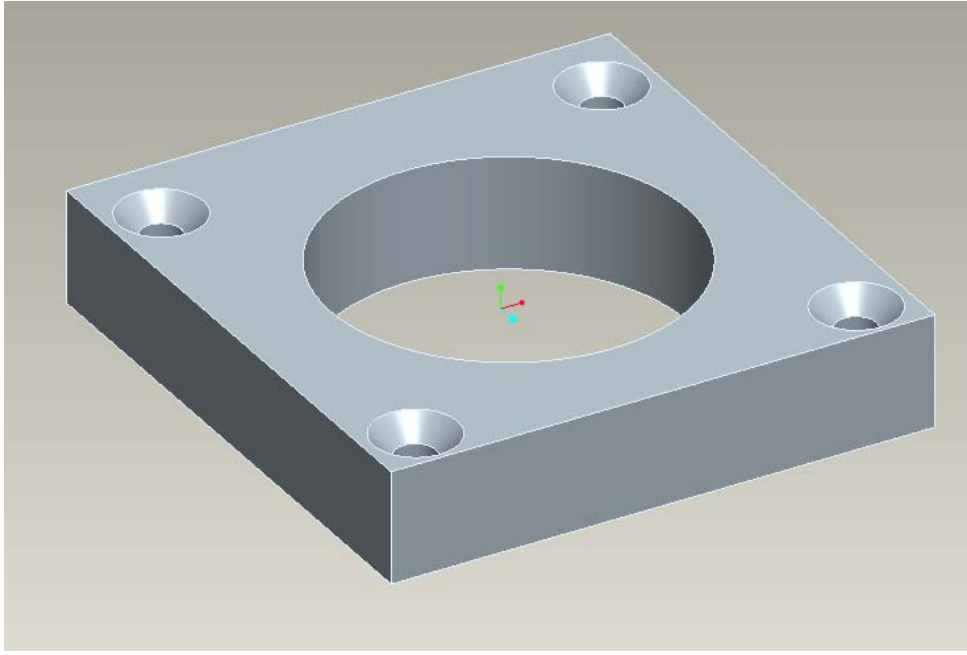
Figure B.22 Motor mount

APPENDIX C

FINAL PRODUCT
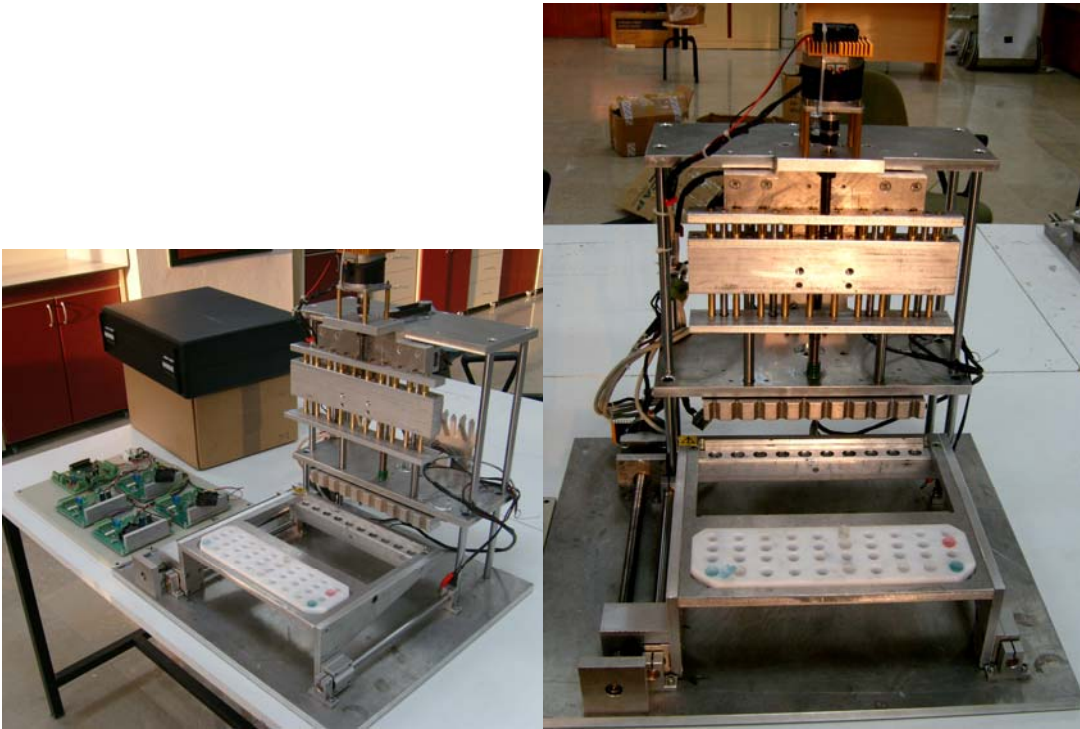
Figure C.1. General view.

Figure C.2. The work table and the heater block.
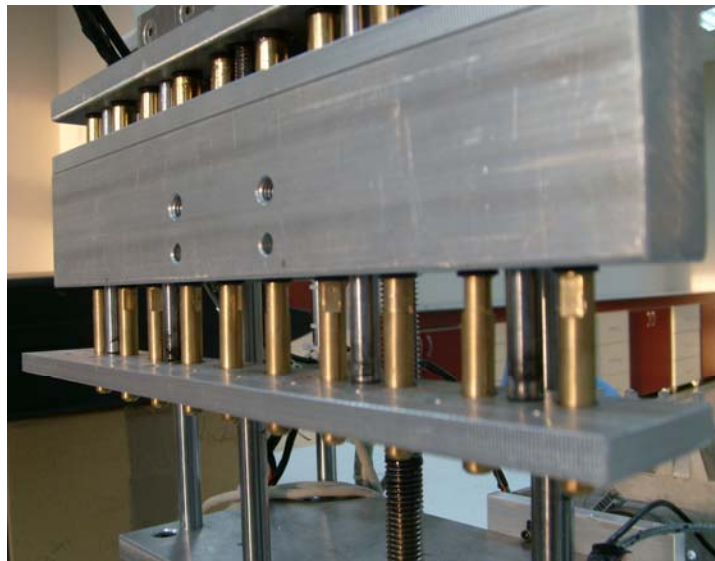
Figure C.3. Syringes.

Figure C.4. Reagent carrier.
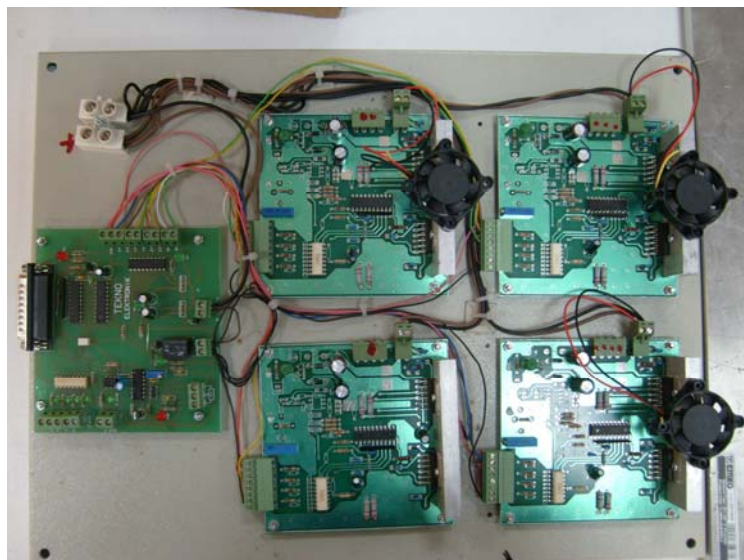
Figure C.5. Interface card and the stepper drivers.
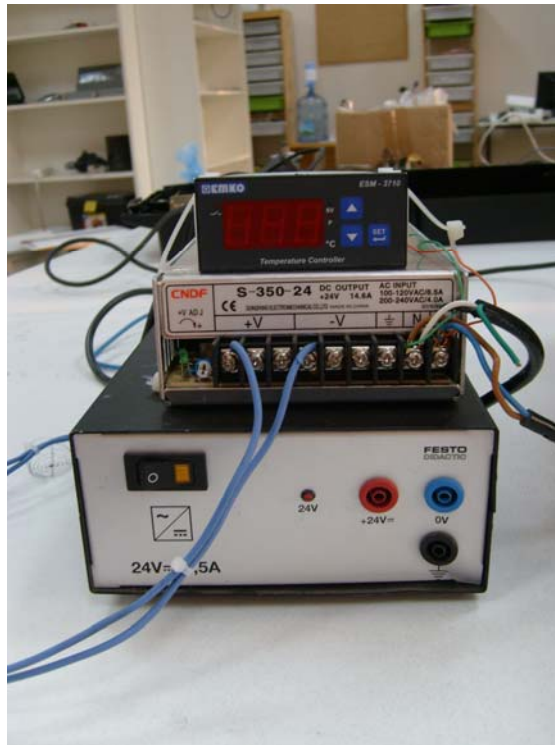
Figure C.6. Power supplies and the temperature controller.
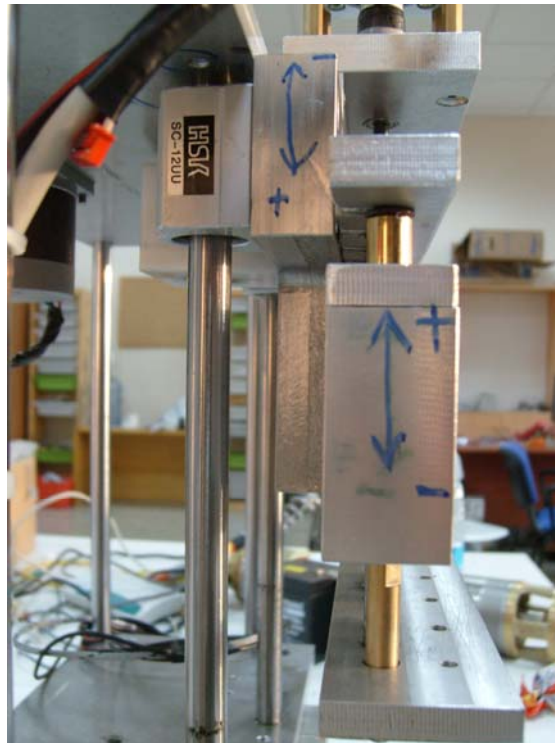
Figure C.7. Linear bearings and y-axis.

Figure C.8. Heater block, resistances and the thermocouple.

APPENDIX D

G CODES

FINAL G CODES

(WEAR PIPET TIP ON)	Z9.85
G0X166.2 (PIPET TIP)	Z2.3
Y125.1 (PIPET TIP HEADING)	Z9.85
G1F300Y136.5 (PIPET TIP ON SLOW)	Z2.3
G0Y35 (MOVE AROUND POSITION FOR Y)	Z9.85 (MIX 10 TIMES)
Z2 (TOLERANCE FOR Z)	Z2.3
	Z9.85
(ASPIRATE SAMPLE)	Z2.3
X181.5 (SAMPLE)	Z9.85
Y80 (SAMPLE TUBE BOTTOM)	Z2.3
Z4.55 (200 UL ASPIRATE)	Z9.85
Y35	Z2.3
	Z9.85
(LYSIS)	Z2.3
X119 (LYSIS BUFFER)	Z9.85
Y75 (TUBE BOTTOM)	Z2.3
Z2.3 (RELEASE SAMPLE NOT ALL)	Z10.89 (ASPIRATE 1000 UL PLUS)
Z8.09 (ASPIRATE NOT ALL)	Y35
Z2.3 (RELEASE NOT ALL)	
Z8.09	(MIX BEADS WITH SAMPLE LYSATE)
Z2.3	X119 (SAMPLE)
Z8.09	Y75
Z2.3	Z2.3 (RELEASE NOT ALL)
Z8.09	Z16 (ASPIRATE NOT ALL)
Z2.3	Z2.3
Z8.09	Z16
Z2.3	Z2.3
Z8.09 (MIX 10 TIMES)	Z16
Z2.3	Z2.3
Z8.09	Z16
Z2.3	Z2.3
Z8.09	Z16
Z2.3	Z2.3
Z8.09	Z16
Z2.3	Z2.3
Z8.09	Z16 (MIX 10 TIMES)
Z2.3	Z2.3
Z8.09	Z16
Z1 (RELEASE AND PURGE)	Z2.3
Y35 (Y REST POSITION)	Z16
Z2 (Z REST POSITION)	Z2.3
G4P20 (WAIT 4 MIN)	Z16
	Z2.3
(MIX MAGNETIC BEADS)	Z16
G0X101 (BEAD BINDING BUFFER)	Z1 (RELEASE AND PURGE)
Y75	Y35 (REST Y)
Z10.15 (ASPIRATE 950 UL)	G4P20 (WAIT 5 MIN)
Y35	G0Y75
X110 (MAGNETIC BEADS)	A-14 (MAGNET NEAR)
Y75	Z6
Z2.3 (RELEASE BINDING BUFFER NOT ALL)	Z2.3
Z9.85 (ASPIRATE NOT ALL)	Z6 (MIX HALF 6 TIMES BEFORE ASPIRATING)
Z2.3	Z2.3
Z9.85	Z6
Z2.3	Z2.3

Z6
Z2.3
Z6
Z2.3
Z16.0 (ASPIRATE BEAD AND SAMPLE LYSATE)
G1F30Z18.2 (ASPIRATE BEAD AND SAMPLE
LYSATE SLOW)

(MAGNETIC SEPARATION 0)
G0Y55 (MAGNET Y LEVEL)
G0A-25 (MAGNET ON)
G4P120 (WAIT 2 MINUTES)
G1F50Z1 (DISCARD SUPERNATANT SLOW)
G0A-14 (MAGNET NEAR)
Y35 (MOVE UP)
Z3 (NO DRIP)

(WASH A)
X92 (WASH BUFFER A)
Y50
Z1 (Z READY)

G0Y75
Z10.15 (ASPIRATE 640 UL)
G0A0 (MAGNET OFF)
Z2.3 (RELEASE NOT ALL)
Z5.7 (ASPIRATE SOME)
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7 (MIX 15 TIMES)
Z2.3
Z9.85 (ASPIRATE MORE NOT ALL)
Z2.3
Z9.85
Z2.3
Z9.85
Z2.3
Z9.85
Z2.3
Z9.85
Z2.3
Z9.85
Z2.3
Z9.85
Z2.3
Z9.85
Z2.3
Z9.85
Z2.3
A-14 (MAGNET NEAR)
Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
Y55 (MAGNET Y LEVEL)
(MAGNETIC SEPARATION A)
Y55 (MAGNET Y LEVEL)
A-25 (MAGNET ON)
G4P60 (WAIT 1 MINUTE)
G1F65Z1 (DISCARD SUPERNATANT)
G0A-14 (MAGNET NEAR)
Y35 (MOVE UP)

Z3 (NO DRIP)
(WASH B)
X83 (WASH BUFFER B)
Y50
Z1 (Z READY)
G0Y75
Z10.15 (ASPIRATE 640 UL)
G0A0 (MAGNET OFF)
Z2.3 (RELEASE SOME)
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7 (MIX 15 TIMES)

Z2.3
Z9.85 (ASPIRATE MORE NOT ALL)
Z2.3
Z9.85
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Z9.85
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Z9.85
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Z9.85
Z2.3
A-14 (MAGNET NEAR)
Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
(MAGNETIC SEPARATION B)
Y55 (MAGNET Y LEVEL)
A-25 (MAGNET ON)
G4P60 (WAIT 1 MINUTE)
G1F65Z1 (DISCARD SUPERNATANT)
G0A-14 (MAGNET NEAR)
Y35 (MOVE UP)
Z3 (NO DRIP)

(WASH C)
X74 (WASH BUFFER C)
Y50
Z1 (Z READY)
G0Y75
Z10.15 (ASPIRATE 640 UL)
G0A0 (MAGNET OFF)
Z2.3 (RELEASE NOT ALL)
Z5.7 (ASPIRATE SOME)
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3

Z2.3	Z2.3
Z5.7 (MIX 15 TIMES)	Z4.7
Z2.3	Z2.3
Z9.85 (ASPIRATE MORE NOT ALL)	Z4.7 (MIX 15 TIMES)
Z2.3	Z2.3
Z9.85	Z4.7
Z2.3	Z2.3
Z9.85	Z4.7
Z2.3	Z2.3
Z9.85	Z4.7
Z2.3	Z2.3
Z9.85	Z4.7
Z2.3	Z2.3
Z9.85	Z4.7
Z2.3	Z2.3
Z9.85	Z4.7
Z2.3	Z2.3
Z9.85	Z4.7
Z2.3	Z2.3
Z9.85	Z4.7
Z2.3	Z2.3
A-14 (MAGNET NEAR)	Z4.7
Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)	Z1 (DISCARD AND PURGE)
(MAGNETIC SEPARATION C)	Y50 (UP REST LOW LEVEL)
Y55 (MAGNET Y LEVEL)	G4P30 (WAIT 30 SECONDS 1)
A-25 (MAGNET ON)	G0Y75
G4P60 (WAIT 1 MINUTE)	Z4.7 (ASPIRATE PLUS)
G1F65Z1 (DISCARD SUPERNATANT)	Z2.3
G0A-14 (MAGNET NEAR)	Z4.7
G0Y50 (MOVE UP)	Z2.3
G1F30Z3 (NO DRIP SLOW)	Z4.7
G0Y35	Z2.3
	Z4.7
(WASH D)	Z2.3
X65 (WASH BUFFER D)	Z4.7 (MIX 7 TIMES)
Y50	Z2.3
Z1 (Z READY)	Z4.7
G0Y55 (MAGNET Y LEVEL)	Z2.3
A-25 (MAGNET ON)	Z4.7
G1F30Z5.7 (ASPIRATE 300 UL SLOW)	Z2.3
G4P60 (WAIT 70 SECONDS)	Z4.7
G1F30Z3 (DISCARD SLOW)	Z1 (DISCARD AND PURGE)
G0Z1 (PURGE FAST)	Y50 (UP REST LOW LEVEL)
G0A-14 (MAGNET NEAR)	G4P30 (WAIT 30 SECONDS 2)
Y35 (MOVE UP)	G0Y75
Z3 (NO DRIP)	Z4.7 (ASPIRATE PLUS)
	Z2.3
(ELUTION)	Z4.7
G0X56 (ELUTION BUFFER)	Z2.3
Y50	Z4.7
Z1 (Z READY)	Z2.3
G0Y75	Z4.7
Z4.7 (ASPIRATE 200 PLUS)	Z2.3
Y35	Z4.7 (MIX 7 TIMES)
X2 (HOT PLATE)	Z2.3
G0A0 (MAGNET OFF)	Z4.7
Y75	Z2.3
Z2.3 (DISCARD NOT ALL)	Z4.7
Z4.7 (ASPIRATE PLUS)	Z2.3
Z2.3	Z4.7
Z4.7	Z1 (DISCARD AND PURGE)
Z2.3	Y50 (UP REST LOW LEVEL)
Z4.7	G4P30 (WAIT 30 SECONDS 3)
Z2.3	G0Y75
Z4.7	Z4.7 (ASPIRATE PLUS)
Z2.3	Z2.3
Z4.7	Z4.7

Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7 (ASPIRATE 200 PLUS)
Z2.3	Y35 (MOVE AROUND POSITION FOR Y)
Z4.7 (MIX 7 TIMES)	
Z2.3	(FINAL ELUTE)
Z4.7	X216 (FINAL ELUTE NUCLEIC ACID TUBE)
Z2.3	Y55 (MAGNET Y LEVEL)
Z4.7	A-25 (MAGNET ON)
Z2.3	G1F50Z5.7 (MOVE OVER 2 TIMES)
Z4.7	Z4.7
	Z5.7
Z1 (DISCARD AND PURGE)	Z4.7
Y50 (UP REST LOW LEVEL)	G4P50 (WAIT 50 SECONDS)
G4P30 (WAIT 30 SECONDS 4)	G1F50Z5.7 (MOVE OVER 2 TIMES)
G0Y75	Z4.7
Z4.7 (ASPIRATE PLUS)	Z5.5
Z2.3	Z4.7
Z4.7	G4P50 (WAIT 50 SECONDS)
Z2.3	G1F30Z1 (DISCARD SUPERNATANT)
Z4.7	G0A-14 (MAGNET NEAR)
Z2.3	Y35
Z4.7	Z3 (NO DRIP)
Z2.3	
Z4.7 (MIX 7 TIMES)	(WASH PIPETTE TIP)
Z2.3	X119 (INTO SAMPLE TUBE)
Z4.7	G0A0 (MAGNET OFF)
Z2.3	Y75
Z4.7	G1F300Z18
Z2.3	Z3
Z4.7	Z15
Z1 (DISCARD AND PURGE)	Z3
Y50 (UP REST LOW LEVEL)	Z15
G4P30 (WAIT 30 SECONDS 5)	Z3
G0Y75	Z15
Z4.7 (ASPIRATE PLUS)	Z3
Z2.3	G0Z10
Z4.7	Z3
Z2.3	Z10
Z4.7	Z3
Z2.3	Z10
Z4.7	Z1
Z2.3	Y35
Z4.7 (MIX 7 TIMES)	
Z2.3	(DISCARD PIPETTE TIP)
Z4.7	X166.2 (PIPETTE TIP)
Z2.3	G0A0 (MAGNET OFF)
Z4.7	Y120.1 (PIPETTE TIP DISCARD Y LEVEL)
Z2.3	Z-5 (DISCARD PIPETTE TIP)
Z4.7	Z0
Z1 (DISCARD AND PURGE)	Y0X0
Y50 (UP REST LOW LEVEL)	M30 (END)
G4P30 (WAIT 30 SECONDS 6)	
G0Y75	
G0A-14 (MAGNET NEAR)	
Z4.7 (ASPIRATE PLUS)	
Z2.3	
Z4.7	
Z2.3	
Z4.7	
Z2.3	
Z4.7	
Z2.3	
Z4.7 (MIX 7 TIMES)	
Z2.3	
Z4.7	

(WEAR PIPET TIP ON)	Z2.3
G0X166.2 (PIPET TIP)	Z9.85
Y125.1 (PIPET TIP HEADING)	Z2.3
G1F300Y136.5 (PIPET TIP ON SLOW)	Z9.85
G0Y35 (MOVE AROUND POSITION FOR Y)	Z2.3
Z2 (TOLERANCE FOR Z)	Z10.89 (ASPIRATE 1000 UL PLUS)
	Y35
(ASPIRATE SAMPLE)	(MIX BEADS WITH SAMPLE LYSATE)
X181.5 (SAMPLE)	X119 (SAMPLE)
Y80 (SAMPLE TUBE BOTTOM)	Y75
Z4.55 (200 UL ASPIRATE)	Z2.3 (RELEASE NOT ALL)
Y35	Z16 (ASPIRATE NOT ALL)
	Z2.3
(LYSIS)	Z16
X119 (LYSIS BUFFER)	Z2.3
Y75 (TUBE BOTTOM)	Z16
Z2.3 (RELEASE SAMPLE NOT ALL)	Z2.3
Z8.09 (ASPIRATE NOT ALL)	Z16
Z2.3 (RELEASE NOT ALL)	Z2.3
Z8.09	Z16
Z2.3	Z2.3
Z8.09	Z16
Z2.3	Z2.3
Z8.09	Z16 (MIX 10 TIMES)
Z2.3	Z2.3
Z8.09	Z16
Z2.3	Z2.3
Z8.09 (MIX 10 TIMES)	Z16
Z2.3	Z2.3
Z8.09	Z16
Z2.3	Z2.3
Z8.09	Z16
Z2.3	Z1 (RELEASE AND PURGE)
Z8.09	Y35 (REST Y)
Z2.3	G4P20 (WAIT 5 MIN)
Z8.09	G0Y75
Z2.3	A-14 (MAGNET NEAR)
Z8.09	Z6
Z1 (RELEASE AND PURGE)	Z2.3
Y35 (Y REST POSITION)	Z6 (MIX HALF 6 TIMES BEFORE ASPIRATING)
Z2 (Z REST POSITION)	Z2.3
G4P20 (WAIT 4 MIN)	Z6
	Z2.3
(MIX MAGNETIC BEADS)	Z6
G0X101 (BEAD BINDING BUFFER)	Z2.3
Y75	Z6
Z10.15 (ASPIRATE 950 UL)	Z2.3
Y35	Z6
X110 (MAGNETIC BEADS)	Z2.3
Y75	Z16.0 (ASPIRATE BEAD AND SAMPLE LYSATE)
Z2.3 (RELEASE BINDING BUFFER NOT ALL)	G1F30Z18.2 (ASPIRATE BEAD AND SAMPLE
Z9.85 (ASPIRATE NOT ALL)	LYSATE SLOW)
Z2.3	
Z9.85	(MAGNETIC SEPARATION 0)
Z2.3	G0Y55 (MAGNET Y LEVEL)
Z9.85	G0A-25 (MAGNET ON)
Z2.3	G4P120 (WAIT 2 MINUTES)
Z9.85	G1F50Z1 (DISCARD SUPERNATANT SLOW)
Z2.3	G0A-14 (MAGNET NEAR)
Z9.85	Y35 (MOVE UP)
Z2.3	Z3 (NO DRIP)
Z9.85 (MIX 10 TIMES)	
Z2.3	(WASH A)
Z9.85	X92 (WASH BUFFER A)
Z2.3	Y50
Z9.85	Z1 (Z READY)
Z2.3	G0Y75
Z9.85	Z10.15 (ASPIRATE 640 UL)

G0A0 (MAGNET OFF)
Z2.3 (RELEASE NOT ALL)
Z5.7 (ASPIRATE SOME)
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7 (MIX 15 TIMES)
Z2.3
Z9.85 (ASPIRATE MORE NOT ALL)
Z2.3
Z9.85
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Z9.85
Z2.3
Z9.85
Z2.3
A-14 (MAGNET NEAR)
Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
Y55 (MAGNET Y LEVEL)
(MAGNETIC SEPARATION A)
Y55 (MAGNET Y LEVEL)
A-25 (MAGNET ON)
G4P60 (WAIT 1 MINUTE)
G1F65Z1 (DISCARD SUPERNATANT)
G0A-14 (MAGNET NEAR)
Y35 (MOVE UP)
Z3 (NO DRIP)

(WASH B)
X83 (WASH BUFFER B)
Y50
Z1 (Z READY)
G0Y75
Z10.15 (ASPIRATE 640 UL)
G0A0 (MAGNET OFF)
Z2.3 (RELEASE SOME)
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7 (MIX 15 TIMES)
Z2.3
Z9.85 (ASPIRATE MORE NOT ALL)
Z2.3
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Z2.3
Z9.85
Z2.3
A-14 (MAGNET NEAR)
Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
(MAGNETIC SEPARATION B)
Y55 (MAGNET Y LEVEL)
A-25 (MAGNET ON)
G4P60 (WAIT 1 MINUTE)
G1F65Z1 (DISCARD SUPERNATANT)
G0A-14 (MAGNET NEAR)
Y35 (MOVE UP)
Z3 (NO DRIP)

(WASH C)
X74 (WASH BUFFER C)
Y50
Z1 (Z READY)
G0Y75
Z10.15 (ASPIRATE 640 UL)
G0A0 (MAGNET OFF)
Z2.3 (RELEASE NOT ALL)
Z5.7 (ASPIRATE SOME)
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7
Z2.3
Z5.7 (MIX 15 TIMES)
Z2.3
Z9.85 (ASPIRATE MORE NOT ALL)
Z2.3
Z9.85
Z2.3
Z9.85
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Z9.85
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Z2.3
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Z2.3
Z9.85
Z2.3
Z9.85
Z2.3
A-14 (MAGNET NEAR)
Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
(MAGNETIC SEPARATION C)
Y55 (MAGNET Y LEVEL)

A-25 (MAGNET ON)	G0Y75
G4P60 (WAIT 1 MINUTE)	Z4.7 (ASPIRATE PLUS)
G1F65Z1 (DISCARD SUPERNATANT)	Z2.3
G0A-14 (MAGNET NEAR)	Z4.7
G0Y50 (MOVE UP)	Z2.3
G1F30Z3 (NO DRIP SLOW)	Z4.7
G0Y35	Z2.3
	Z4.7
(WASH D)	Z2.3
X65 (WASH BUFFER D)	Z4.7 (MIX 7 TIMES)
Y50	Z2.3
Z1 (Z READY)	Z4.7
G0Y55 (MAGNET Y LEVEL)	Z2.3
A-25 (MAGNET ON)	Z4.7
G1F30Z5.7 (ASPIRATE 300 UL SLOW)	Z2.3
G4P60 (WAIT 70 SECONDS)	Z4.7
G1F30Z3 (DISCARD SLOW)	Z1 (DISCARD AND PURGE)
G0Z1 (PURGE FAST)	Y50 (UP REST LOW LEVEL)
G0A-14 (MAGNET NEAR)	G4P30 (WAIT 30 SECONDS 2)
Y35 (MOVE UP)	G0Y75
Z3 (NO DRIP)	Z4.7 (ASPIRATE PLUS)
	Z2.3
(ELUTION)	Z4.7
G0X56 (ELUTION BUFFER)	Z2.3
Y50	Z4.7
Z1 (Z READY)	Z2.3
G0Y75	Z4.7
Z4.7 (ASPIRATE 200 PLUS)	Z2.3
Y35	Z4.7 (MIX 7 TIMES)
X2 (HOT PLATE)	Z2.3
G0A0 (MAGNET OFF)	Z4.7
Y75	Z2.3
Z2.3 (DISCARD NOT ALL)	Z4.7
Z4.7 (ASPIRATE PLUS)	Z2.3
Z2.3	Z4.7
Z4.7	Z1 (DISCARD AND PURGE)
Z2.3	Y50 (UP REST LOW LEVEL)
Z4.7	G4P30 (WAIT 30 SECONDS 3)
Z2.3	G0Y75
Z4.7	Z4.7 (ASPIRATE PLUS)
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7 (MIX 15 TIMES)	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7 (MIX 7 TIMES)
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z1 (DISCARD AND PURGE)
Z4.7	Y50 (UP REST LOW LEVEL)
Z2.3	G4P30 (WAIT 30 SECONDS 4)
Z4.7	G0Y75
Z2.3	Z4.7 (ASPIRATE PLUS)
Z4.7	Z2.3
Z2.3	Z4.7
Z4.7	Z2.3
Z2.3	Z4.7
Z4.7	Z2.3
Z1 (DISCARD AND PURGE)	Z4.7
Y50 (UP REST LOW LEVEL)	Z2.3
G4P30 (WAIT 30 SECONDS 1)	Z4.7 (MIX 7 TIMES)

Z2.3	(WASH PIPETTE TIP)
Z4.7	X119 (INTO SAMPLE TUBE)
Z2.3	G0A0 (MAGNET OFF)
Z4.7	G1F300Z18
Z2.3	Z3
Z4.7	Z15
Z1 (DISCARD AND PURGE)	Z3
Y50 (UP REST LOW LEVEL)	Z15
G4P30 (WAIT 30 SECONDS 5)	Z3
G0Y75	Z15
Z4.7 (ASPIRATE PLUS)	Z3
Z2.3	G0Z10
Z4.7	Z3
Z2.3	Z10
Z4.7	Z3
Z2.3	Z10
Z4.7	Z1
Z2.3	
Z4.7 (MIX 7 TIMES)	(DISCARD PIPETTE TIP)
Z2.3	X166.2 (PIPETTE TIP)
Z4.7	G0A0 (MAGNET OFF)
Z2.3	Y118 (PIPETTE TIP DISCARD Y LEVEL)
Z4.7	Z-7 (DISCARD PIPETTE TIP)
Z2.3	Z0
Z4.7	Y0X0
Z1 (DISCARD AND PURGE)	M30 (END)
Y50 (UP REST LOW LEVEL)	
G4P30 (WAIT 30 SECONDS 6)	
G0Y75	
G0A-14 (MAGNET NEAR)	
Z4.7 (ASPIRATE PLUS)	
Z2.3	
Z4.7	
Z2.3	
Z4.7	
Z2.3	
Z4.7	
Z2.3	
Z4.7 (MIX 7 TIMES)	
Z2.3	
Z4.7	
Z2.3	
Z4.7	
Z2.3	
Z4.7 (ASPIRATE 200 PLUS)	
Y35 (MOVE AROUND POSITION FOR Y)	
(FINAL ELUTE)	
X216 (FINAL ELUTE NUCLEIC ACID TUBE)	
Y55 (MAGNET Y LEVEL)	
A-25 (MAGNET ON)	
G1F80Z5.7 (MOVE OVER 2 TIMES)	
G1F80Z4.7	
G1F80Z5.7	
G1F80Z4.7	
G4P50 (WAIT 50 SECONDS)	
G1F80Z5.7 (MOVE OVER 2 TIMES)	
G1F80Z4.7	
G1F80Z5.5	
G1F80Z4.7	
G4P50 (WAIT 50 SECONDS)	
G1F80Z1 (DISCARD SUPERNATANT)	
G0A-14 (MAGNET NEAR)	
Y35	
Z3 (NO DRIP)	

(WEAR PIPET TIP ON)	Z9.85
G0X166.2 (PIPET TIP)	Z2.3
Y125.1 (PIPET TIP HEADING)	Z10.89 (ASPIRATE 1000 UL PLUS)
G1F300Y136.5 (PIPET TIP ON SLOW)	Y35
G0Y35 (MOVE AROUND POSITION FOR Y)	(MIX BEADS WITH SAMPLE LYSATE)
Z2 (TOLERANCE FOR Z)	X119 (SAMPLE)
(ASPIRATE SAMPLE)	Y75
X181.5 (SAMPLE)	Z2.3 (RELEASE NOT ALL)
Y80 (SAMPLE TUBE BOTTOM)	Z16 (ASPIRATE NOT ALL)
Z4.55 (200 UL ASPIRATE)	Z2.3
Y35	Z16
(LYSIS)	Z2.3
X119 (LYSIS BUFFER)	Z16
Y75 (TUBE BOTTOM)	Z2.3
Z2.3 (RELEASE SAMPLE NOT ALL)	Z16
Z8.09 (ASPIRATE NOT ALL)	Z2.3
Z2.3 (RELEASE NOT ALL)	Z16
Z8.09	Z2.3
Z2.3	Z16
Z8.09	Z2.3
Z2.3	Z16 (MIX 10 TIMES)
Z8.09	Z2.3
Z2.3	Z16
Z8.09	Z2.3
Z2.3	Z16
Z8.09 (MIX 10 TIMES)	Z2.3
Z2.3	Z16
Z8.09	Z2.3
Z2.3	Z16
Z8.09	Z1 (RELEASE AND PURGE)
Z2.3	Y35 (REST Y)
Z8.09	G4P20 (WAIT 5 MIN)
Z2.3	G0Y75
Z8.09	A-14 (MAGNET NEAR)
Z2.3	Z6
Z8.09	Z2.3
Z1 (RELEASE AND PURGE)	Z6 (MIX HALF 6 TIMES BEFORE ASPIRATING)
Y35 (Y REST POSITION)	Z2.3
Z2 (Z REST POSITION)	Z6
G4P20 (WAIT 4 MIN)	Z2.3
(MIX MAGNETIC BEADS)	Z6
G0X101 (BEAD BINDING BUFFER)	Z2.3
Y75	Z6
Z10.15 (ASPIRATE 950 UL)	Z2.3
Y35	Z16.0 (ASPIRATE BEAD AND SAMPLE LYSATE)
X110 (MAGNETIC BEADS)	G1F30Z18.2 (ASPIRATE BEAD AND SAMPLE
Y75	LYSATE SLOW)
Z2.3 (RELEASE BINDING BUFFER NOT ALL)	(MAGNETIC SEPARATION 0)
Z9.85 (ASPIRATE NOT ALL)	G0Y55 (MAGNET Y LEVEL)
Z2.3	G0A-25 (MAGNET ON)
Z9.85	G4P120 (WAIT 2 MINUTES)
Z2.3	G1F30Z1 (DISCARD SUPERNATANT SLOW)
Z9.85	G0A-14 (MAGNET NEAR)
Z2.3	Y35 (MOVE UP)
Z9.85	Z3 (NO DRIP)
Z2.3	(WASH A)
Z9.85 (MIX 10 TIMES)	X92 (WASH BUFFER A)
Z2.3	Y50
Z9.85	Z1 (Z READY)
Z2.3	G0Y75
Z9.85	Z10.15 (ASPIRATE 640 UL)
Z2.3	G0A0 (MAGNET OFF)
Z9.85	Z2.3 (RELEASE NOT ALL)
Z2.3	Z5.7 (ASPIRATE SOME)
Z9.85	Z2.3
Z2.3	Z5.7

Z2.3	Z9.85
Z5.7	Z2.3
Z2.3	Z9.85
Z5.7	Z2.3
Z2.3	Z9.85
Z5.7	Z2.3
Z2.3	Z9.85
Z5.7 (MIX 15 TIMES)	Z2.3
Z2.3	A-14 (MAGNET NEAR)
Z9.85 (ASPIRATE MORE NOT ALL)	Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
Z2.3	(MAGNETIC SEPARATION B)
Z9.85	Y55 (MAGNET Y LEVEL)
Z2.3	A-25 (MAGNET ON)
Z9.85	G4P60 (WAIT 1 MINUTE)
Z2.3	G1F80Z1 (DISCARD SUPERNATANT)
Z9.85	G0A-14 (MAGNET NEAR)
Z2.3	Y35 (MOVE UP)
Z9.85	Z3 (NO DRIP)
Z2.3	(WASH C)
Z9.85	X74 (WASH BUFFER C)
Z2.3	Y50
Z9.85	Z1 (Z READY)
Z2.3	G0Y75
Z9.85	Z10.15 (ASPIRATE 640 UL)
Z2.3	G0A0 (MAGNET OFF)
Z9.85	Z2.3 (RELEASE NOT ALL)
Z2.3	Z5.7 (ASPIRATE SOME)
A-14 (MAGNET NEAR)	Z2.3
Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)	Z5.7
Y55 (MAGNET Y LEVEL)	Z2.3
(MAGNETIC SEPARATION A)	Z5.7
Y55 (MAGNET Y LEVEL)	Z2.3
A-25 (MAGNET ON)	Z5.7
G4P60 (WAIT 1 MINUTE)	Z2.3
G1F80Z1 (DISCARD SUPERNATANT)	Z5.7
G0A-14 (MAGNET NEAR)	Z2.3
Y35 (MOVE UP)	Z5.7 (MIX 15 TIMES)
Z3 (NO DRIP)	Z2.3
(WASH B)	Z9.85 (ASPIRATE MORE NOT ALL)
X83 (WASH BUFFER B)	Z2.3
Y50	Z9.85
Z1 (Z READY)	Z2.3
G0Y75	Z9.85
Z10.15 (ASPIRATE 640 UL)	Z2.3
G0A0 (MAGNET OFF)	Z9.85
Z2.3 (RELEASE SOME)	Z2.3
Z2.3	Z9.85
Z5.7	Z2.3
Z2.3	Z9.85
Z5.7	Z2.3
Z2.3	Z9.85
Z5.7	Z2.3
Z2.3	Z9.85
Z5.7	Z2.3
Z2.3	Z9.85
Z5.7 (MIX 15 TIMES)	Z2.3
Z2.3	A-14 (MAGNET NEAR)
Z9.85 (ASPIRATE MORE NOT ALL)	Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
Z2.3	(MAGNETIC SEPARATION C)
Z9.85	Y55 (MAGNET Y LEVEL)
Z2.3	A-25 (MAGNET ON)
Z9.85	G4P60 (WAIT 1 MINUTE)
Z2.3	G1F80Z1 (DISCARD SUPERNATANT)
Z9.85	G0A-14 (MAGNET NEAR)
Z2.3	Y35 (MOVE UP)
Z9.85	Z3 (NO DRIP)
Z2.3	

(WASH D)	Z2.3
X65 (WASH BUFFER D)	Z4.7
Y50	Z2.3
Z1 (Z READY)	Z4.7
G0Y55 (MAGNET Y LEVEL)	Z2.3
A-25 (MAGNET ON)	Z4.7
G1F30Z5.7 (ASPIRATE 300 UL SLOW)	Z1 (DISCARD AND PURGE)
G4P60 (WAIT 70 SECONDS)	Y50 (UP REST LOW LEVEL)
G1F30Z3 (DISCARD SLOW)	G4P30 (WAIT 30 SECONDS 2)
G0Z1 (PURGE FAST)	G0Y75
G0A-14 (MAGNET NEAR)	Z4.7 (ASPIRATE PLUS)
G0Y50 (MOVE UP)	Z2.3
G1F30Z3 (NO DRIP SLOW)	Z4.7
(ELUTION)	Z2.3
G0X56 (ELUTION BUFFER)	Z4.7
Z1 (Z READY)	Z2.3
G0Y75	Z4.7
Z4.7 (ASPIRATE 200 PLUS)	Z2.3
Y35	Z4.7 (MIX 7 TIMES)
X2 (HOT PLATE)	Z2.3
G0A0 (MAGNET OFF)	Z4.7
Y75	Z2.3
Z2.3 (DISCARD NOT ALL)	Z4.7
Z4.7 (ASPIRATE PLUS)	Z2.3
Z2.3	Z4.7
Z4.7	Z1 (DISCARD AND PURGE)
Z2.3	Y50 (UP REST LOW LEVEL)
Z4.7	G4P30 (WAIT 30 SECONDS 3)
Z2.3	G0Y75
Z4.7	Z4.7 (ASPIRATE PLUS)
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7 (MIX 15 TIMES)	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7 (MIX 7 TIMES)
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z1 (DISCARD AND PURGE)
Z4.7	Y50 (UP REST LOW LEVEL)
Z2.3	G4P30 (WAIT 30 SECONDS 4)
Z4.7	G0Y75
Z2.3	Z4.7 (ASPIRATE PLUS)
Z4.7	Z2.3
Z2.3	Z4.7
Z4.7	Z2.3
Z2.3	Z4.7
Z4.7	Z2.3
Z1 (DISCARD AND PURGE)	Z4.7
Y50 (UP REST LOW LEVEL)	Z2.3
G4P30 (WAIT 30 SECONDS 1)	Z4.7 (MIX 7 TIMES)
G0Y75	Z2.3
Z4.7 (ASPIRATE PLUS)	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z2.3
Z4.7	Z4.7
Z2.3	Z1 (DISCARD AND PURGE)
Z4.7	Y50 (UP REST LOW LEVEL)
Z2.3	G4P30 (WAIT 30 SECONDS 5)
Z4.7 (MIX 7 TIMES)	G0Y75

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Z4.7 (ASPIRATE PLUS)                          Z3
Z2.3                                          Z10
Z4.7                                          Z3
Z2.3                                          Z10
Z4.7                                          Z1
Z2.3
Z4.7                                          (DISCARD PIPETTE TIP)
Z2.3                                          X166.2 (PIPETTE TIP)
Z4.7 (MIX 7 TIMES)                            G0A0 (MAGNET OFF)
Z2.3                                          Y118 (PIPETTE TIP DISCARD Y LEVEL)
Z4.7                                          Z-7 (DISCARD PIPETTE TIP)
Z2.3                                          Z0
Z4.7                                          Y0X0
Z2.3                                          M30 (END)
Z4.7
Z1 (DISCARD AND PURGE)
Y50 (UP REST LOW LEVEL)
G4P30 (WAIT 30 SECONDS 6)
G0Y75
G0A-14 (MAGNET NEAR)
Z4.7 (ASPIRATE PLUS)
Z2.3
Z4.7
Z2.3
Z4.7
Z2.3
Z4.7
Z2.3
Z4.7 (MIX 7 TIMES)
Z2.3
Z4.7
Z2.3
Z4.7
Z2.3
Z4.7 (ASPIRATE 200 PLUS)
Y35 (MOVE AROUND POSITION FOR Y)
(FINAL ELUTE)
X216 (FINAL ELUTE NUCLEIC ACID TUBE)
Y55 (MAGNET Y LEVEL)
A-25 (MAGNET ON)
G1F80Z5.7 (MOVE OVER 2 TIMES)
G1F80Z4.7
G1F80Z5.7
G1F80Z4.7
G4P50 (WAIT 50 SECONDS)
G1F80Z5.7 (MOVE OVER 2 TIMES)
G1F80Z4.7
G1F80Z5.5
G1F80Z4.7
G4P50 (WAIT 50 SECONDS)
G1F80Z1 (DISCARD SUPERNATANT)
G0A-14 (MAGNET NEAR)
Y35
Z3 (NO DRIP)

(WASH PIPETTE TIP)
X119 (INTO SAMPLE TUBE)
G0A0 (MAGNET OFF)
G1F300Z18
Z3
Z15
Z3
Z15
Z3
Z15
Z3
G0Z10
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Conceptual G-Code 3

(WEAR PIPET TIP ON)	Z9.85
G0X166.2 (PIPET TIP)	Z2.3
Y125.1 (PIPET TIP HEADING)	Z10.89 (ASPIRATE 1000 UL PLUS)
G1F300Y136.5 (PIPET TIP ON SLOW)	Y35
G0Y35 (MOVE AROUND POSITION FOR Y)	(MIX BEADS WITH SAMPLE LYSATE)
Z2 (TOLERANCE FOR Z)	X119 (SAMPLE)
(ASPIRATE SAMPLE)	Y75
X181.5 (SAMPLE)	Z2.3 (RELEASE NOT ALL)
Y80 (SAMPLE TUBE BOTTOM)	Z16 (ASPIRATE NOT ALL)
Z4.55 (200 UL ASPIRATE)	Z2.3
Y35	Z16
(LYSIS)	Z2.3
X119 (LYSIS BUFFER)	Z16
Y75 (TUBE BOTTOM)	Z2.3
Z2.3 (RELEASE SAMPLE NOT ALL)	Z16
Z8.09 (ASPIRATE NOT ALL)	Z2.3
Z2.3 (RELEASE NOT ALL)	Z16
Z8.09	Z2.3
Z2.3	Z16
Z8.09	Z2.3
Z2.3	Z16 (MIX 10 TIMES)
Z8.09	Z2.3
Z2.3	Z16
Z8.09	Z2.3
Z2.3	Z16
Z8.09 (MIX 10 TIMES)	Z2.3
Z2.3	Z16
Z8.09	Z2.3
Z2.3	Z16
Z8.09	Z1 (RELEASE AND PURGE)
Z2.3	Y35 (REST Y)
Z8.09	G4P20 (WAIT 5 MIN)
Z2.3	G0Y75
Z8.09	A-14 (MAGNET NEAR)
Z2.3	Z6
Z8.09	Z2.3
Z1 (RELEASE AND PURGE)	Z6 (MIX HALF 6 TIMES BEFORE ASPIRATING)
Y35 (Y REST POSITION)	Z2.3
Z2 (Z REST POSITION)	Z6
G4P20 (WAIT 4 MIN)	Z2.3
(MIX MAGNETIC BEADS)	Z6
G0X101 (BEAD BINDING BUFFER)	Z2.3
Y75	Z6
Z10.15 (ASPIRATE 950 UL)	Z2.3
Y35	Z6
X110 (MAGNETIC BEADS)	Z2.3
Y75	Z18.0 (ASPIRATE BEAD AND SAMPLE LYSATE)
Z2.3 (RELEASE BINDING BUFFER NOT ALL)	(MAGNETIC SEPARATION 0)
Z9.85 (ASPIRATE NOT ALL)	Y55 (MAGNET Y LEVEL)
Z2.3	G0A-25 (MAGNET ON)
Z9.85	G4P120 (WAIT 2 MINUTES)
Z2.3	G1F80Z1 (DISCARD SUPERNATANT SLOW)
Z9.85	G0A-14 (MAGNET NEAR)
Z2.3	Y35 (MOVE UP)
Z9.85	Z3 (NO DRIP)
Z2.3	(WASH A)
Z9.85 (MIX 10 TIMES)	X92 (WASH BUFFER A)
Z2.3	Y50
Z9.85	Z1 (Z READY)
Z2.3	G0Y75
Z9.85	Z10.15 (ASPIRATE 640 UL)
Z2.3	G0A0 (MAGNET OFF)
Z9.85	Z2.3 (RELEASE NOT ALL)
Z2.3	Z9.85 (ASPIRATE NOT ALL)
Z9.85	Z2.3
Z2.3	Z9.85
Z9.85	Z2.3
Z2.3	Z9.85

Z2.3	Z2.3
Z9.85	Z9.85
Z2.3	Z2.3
Z9.85	Z9.85
Z2.3	Z2.3
Z9.85 (MIX 15 TIMES)	Z9.85
Z2.3	Z2.3
Z9.85	A-14 (MAGNET NEAR)
Z2.3	Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
Z9.85	(MAGNETIC SEPARATION B)
Z2.3	Y55 (MAGNET Y LEVEL)
Z9.85	A-25 (MAGNET ON)
Z2.3	G4P60 (WAIT 1 MINUTE)
Z9.85	G1F80Z1 (DISCARD SUPERNATANT)
Z2.3	G0A-14 (MAGNET NEAR)
Z9.85	Y35 (MOVE UP)
Z2.3	Z3 (NO DRIP)
Z9.85	(WASH C)
Z2.3	X74 (WASH BUFFER C)
Z9.85	Y50
Z2.3	Z1 (Z READY)
Z9.85	G0Y75
Z2.3	Z10.15 (ASPIRATE 640 UL)
Z9.85	G0A0 (MAGNET OFF)
Z2.3	Z2.3 (RELEASE NOT ALL)
A-14 (MAGNET NEAR)	Z9.85 (ASPIRATE NOT ALL)
Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)	Z2.3
Y55 (MAGNET Y LEVEL)	Z9.85
(MAGNETIC SEPARATION A)	Z2.3
Y55 (MAGNET Y LEVEL)	Z9.85
A-25 (MAGNET ON)	Z2.3
G4P60 (WAIT 1 MINUTE)	Z9.85
G1F80Z1 (DISCARD SUPERNATANT)	Z2.3
G0A-14 (MAGNET NEAR)	Z9.85
Y35 (MOVE UP)	Z2.3
Z3 (NO DRIP)	Z9.85 (MIX 15 TIMES)
(WASH B)	Z2.3
X83 (WASH BUFFER B)	Z9.85
Y50	Z2.3
Z1 (Z READY)	Z9.85
G0Y75	Z2.3
Z10.15 (ASPIRATE 640 UL)	Z9.85
G0A0 (MAGNET OFF)	Z2.3
Z2.3 (RELEASE NOT ALL)	Z9.85
Z9.85 (ASPIRATE NOT ALL)	Z2.3
Z2.3	Z9.85
Z9.85	Z2.3
Z2.3	Z9.85
Z9.85	Z2.3
Z2.3	Z9.85
Z9.85	Z2.3
Z2.3	Z9.85
Z9.85 (MIX 15 TIMES)	Z2.3
Z2.3	A-14 (MAGNET NEAR)
Z9.85	Z10.85 (ASPIRATE BEAD AND BUFFER PLUS)
Z2.3	(MAGNETIC SEPARATION C)
Z9.85	Y55 (MAGNET Y LEVEL)
Z2.3	A-25 (MAGNET ON)
Z9.85	G4P60 (WAIT 1 MINUTE)
Z2.3	G1F80Z1 (DISCARD SUPERNATANT)
Z9.85	G0A-14 (MAGNET NEAR)
Z2.3	Y35 (MOVE UP)
Z9.85	Z3 (NO DRIP)
Z2.3	(WASH D)
Z9.85	X65 (WASH BUFFER D)

Y50	Z5
Z1 (Z READY)	Z2.3
G0Y55 (MAGNET Y LEVEL)	Z5
A-25 (MAGNET ON)	Z1 (DISCARD AND PURGE)
G1F30Z6.45 (ASPIRATE 300 UL SLOWLY)	Y50 (UP REST LOW LEVEL)
G4P60 (WAIT 70 SECONDS)	G4P30 (WAIT 30 SECONDS 2)
G1F30Z1 (DISCARD AND PURGE SLOWLY)	G0Y75
G0A-14 (MAGNET NEAR)	Z5 (ASPIRATE PLUS)
G0Y35 (MOVE UP)	Z2.3
Z3 (NO DRIP)	Z5
(ELUTION)	Z2.3
X56 (ELUTION BUFFER)	Z5
Z1 (Z READY)	Z2.3
G0Y75	Z5
Z4.7 (ASPIRATE 200 PLUS)	Z2.3
Y35	Z5
X2 (HOT PLATE)	Z2.3
G0A0 (MAGNET OFF)	Z5 (MIX 7 TIMES)
Y75	Z2.3
Z2.3 (DISCARD NOT ALL)	Z5
Z5 (ASPIRATE PLUS)	Z2.3
Z2.3	Z5
Z5	Z2.3
Z2.3	Z5
Z5	Z1 (DISCARD AND PURGE)
Z2.3	Y50 (UP REST LOW LEVEL)
Z5	G4P30 (WAIT 30 SECONDS 3)
Z2.3	G0Y75
Z5	Z5 (ASPIRATE PLUS)
Z2.3	Z2.3
Z5	Z5
Z2.3	Z2.3
Z5	Z5
Z2.3	Z2.3
Z5 (MIX 15 TIMES)	Z5
Z2.3	Z2.3
Z5	Z5 (MIX 7 TIMES)
Z2.3	Z2.3
Z5	Z5
Z2.3	Z2.3
Z5	Z5
Z2.3	Z2.3
Z5	Z5
Z2.3	Z1 (DISCARD AND PURGE)
Z5	Y50 (UP REST LOW LEVEL)
Z2.3	G4P30 (WAIT 30 SECONDS 4)
Z5	G0Y75
Z2.3	Z5 (ASPIRATE PLUS)
Z5	Z2.3
Z2.3	Z5
Z5	Z2.3
Z2.3	Z5
Z5	Z2.3
Z1 (DISCARD AND PURGE)	Z5
Y50 (UP REST LOW LEVEL)	Z2.3
G4P30 (WAIT 30 SECONDS 1)	Z5 (MIX 7 TIMES)
G0Y75	Z2.3
Z5 (ASPIRATE PLUS)	Z5
Z2.3	Z2.3
Z5	Z5
Z2.3	Z2.3
Z5	Z5
Z2.3	Z1 (DISCARD AND PURGE)
Z5	Y50 (UP REST LOW LEVEL)
Z2.3	G4P30 (WAIT 30 SECONDS 5)
Z5 (MIX 7 TIMES)	G0Y75
Z2.3	Z5 (ASPIRATE PLUS)
Z5	Z2.3
Z2.3	Z5

Z2.3 Z5 Z2.3 Z5 Z2.3 Z5 (MIX 7 TIMES) Z2.3 Z5 Z2.3 Z5 Z2.3 Z5 Z1 (DISCARD AND PURGE) Y50 (UP REST LOW LEVEL) G4P30 (WAIT 30 SECONDS 6) G0Y75 G0A-14 (MAGNET NEAR) Z5 (ASPIRATE PLUS) Z2.3 Z5 Z2.3 Z5 Z2.3 Z5 Z2.3 Z5 (MIX 7 TIMES) Z2.3 Z5 Z2.3 Z5 Z2.3 Z4.7 (ASPIRATE 200 PLUS) Y35 (MOVE AROUND POSITION FOR Y) (FINAL ELUTE) X216 (FINAL ELUTE NUCLEIC ACID TUBE) Y55 (MAGNET Y LEVEL) A-25 (MAGNET ON) G1F80Z5.5 (MOVE OVER 2 TIMES) G1F80Z4.7 G1F80Z5.5 G1F80Z4.7 G4P50 (WAIT 50 SECONDS) G1F80Z5.5 (MOVE OVER 2 TIMES) G1F80Z4.7 G1F80Z5.5 G1F80Z4.7 G4P50 (WAIT 50 SECONDS) G1F80Z1 (DISCARD SUPERNATANT) G0A-14 (MAGNET NEAR) Y35 Z3 (NO DRIP) (DISCARD PIPETTE TIP) X166.2 (PIPETTE TIP) G0A0 (MAGNET OFF) Y118 (PIPETTE TIP DISCARD Y LEVEL) Z-7 (DISCARD PIPETTE TIP) Z0 Y0 X0 M30 (END)	