J Biochem Tech (2012) 3(4): 384-388
ISSN: 0974-2328

Instrument-free, automation and multi-platform ready unmodified iron Oxide based DNA isolation system

Rajani Kanth Vangala*, Sridevi G, Veeresh Hugar

Received: 27 October 2011 / Received in revised form: 21 February 2012, Accepted: 22 March 2012, Published online: 27 October 2012

Abstract

The majority of molecular genetic tests start with the extraction of DNA, indicating the great importance of a suitable and reliable method for this purpose. Next to many in-house standard operating procedures (SOPs), a lot of commercial extraction kits have been developed. These methods are generally accepted and have proven their validity with good results. However, it is obvious that the automation and simplification of the extraction procedure could have major benefits; i.e. a higher throughput, more reliable and reproducible processing of the samples, improved traceability and possible streamlining of the entire DNA extraction procedure. To make the process of DNA isolation robust and possible for automation on any automation machine available (example, automated liquid handler), there are several roadblocks like centrifugation. Therefore, we have developed a simple, yet robust isolation technology which can be used on any automation machine and also can be used outside the lab environment at the point of sample collection by anyone.

Keywords: DNA isolation, magnetic nanoparticles, iron oxide, automation.

Introduction

Completion of human genome sequencing marked the end first phase of the genomics revolution. The second phase that has already begun, involves evaluating genetic sequences for genotyping (single-nucleotide polymorphism analysis) and complete genetic profile of each individual.

Rajani Kanth Vangala, Sridevi G, Veeresh Hugar

Institute of Bioinformatics and Applied Biotechnology
Electronics City, Bangalore

Tel: 0091 9880568987, Fax:0091 80 27835302
*E-mail: rajani@triindia.org.in

This requires next generation capabilities for DNA purification, PCR (Polymerase Chain Reaction) and sequencing. In addition to DNA analysis, the rapidly growing field of molecular diagnostics needs a quick, simple, robust, and high throughput procedure for extraction of DNA from diverse organisms and tissues. The process of genomic DNA isolation and purification has evolved considerably within the last decade. During recent years, use of the magnetic techniques using magnetic particles coated with different kinds of polymers (e.g., agarose, silica) has increased in the molecular biology field [Sayed etal 2003 and Safarik et al 2002].
The purification of genomic and plasmid DNA using magnetizable support (beads or matrix) has already been attempted from different biological sources [Davies M.J et al 1997, Prodelalova J et al 2004, Xie X et al 2004, Nagy M et al 2005, Chiang C L et al 2005]. Furthermore, carboxyl coated magnetic particles have been used as adsorbent for DNA purification under high-salt conditions [Hawkins T.L et al 1994]. All the above mentioned extraction procedures have used coated magnetic particles, which means only the magnetic property of the particles was used to achieve quick separation. However, the use of naked (uncoated) magnetic nanoparticles (Fe_2O_3) permits to exploit also its property to reversibly bind DNA under specific conditions. Additionally, there are several inherent advantages of the use of naked particles; where molecules are directly linked to the magnetic support. Due to the absence of any polymer coating the size of the particle is small; this provides larger surface area (on a weight basis) for the binding of the biological molecules.

Our present discovery relates to developing an instrument-free technology for DNA isolation which can be performed anywhere by any one and does not require any toxic chemicals or alcohol. We have developed metal oxide nanoparticles, which at specific molar concentrations of different salts bind to DNA with very high affinity. Once the DNA bound to the nanoparticles, it is eluted using cation replacement method by molar excess of the same salts. The novelty of our technology is that unlike many other similar technologies which use modified iron oxide particles, we used unmodified particles and from diverse array of tissue sources. This method is very advantageous as it reduces the manual intervention and cost and to perform DNA isolation at the point of collection itself.

Materials and Methods

Preparation of iron oxide particles

Iron oxide particles were synthesized as described by Dallas et al., (2007) with slight modification. Briefly, 1.25 g of $FeSO_4.7H_2O$ and 0.6 g KOH (Merck, India) was dissolved in 30 ml H_2O of prefixed temperature (50°C). The mixture was refluxed for 1 h. The as-prepared magnetic solids were insoluble in aqueous or organic media. They were isolated by centrifugation, washing several times with water and drying. The particles thus obtained exhibited a strong magnetic response.

Genomic DNA isolation from different sources using iron oxide particles

Iron oxide particles with strong magnetic response, prepared by the above mentioned method were used for the isolation of genomic DNA from different sources such as bacteria, plant, blood and saliva etc. After the isolation, 5µl of each sample was run on 0.8% agarose gel electrophoresis for visualization.

Genomic DNA isolation from saliva

20 mg of iron oxide particles were mixed with 50 mM sodium acetate buffer, pH 6.0, 250 mM Guanidium thiocyanate, 250 mM Lithium chloride and 200 µl of saliva. The mixture was incubated at RT (25°C) for 20 min. The magnetic pellet was immobilized by application of an external magnet and the supernatant was removed. The magnetic pellet was washed once with 1x concentration of phosphate buffered saline by gently inverting the tube and the wash was removed by immobilizing the magnetic pellet using external magnet. The magnetic pellet was finally resuspended in 50 µl of 10 mM Tris buffer, pH 8.0 containing 50 mM NaCl and incubated at RT (25°C) for 20 min.

Genomic DNA isolation from bacteria

20 mg of iron oxide particles were mixed with 500 µl of 20 mM Tris buffer, pH 8.0 and 200 µl overnight grown bacterial culture (O.D at 600 nm is 1.0). The mixture was incubated at RT (25°C) for 20 min. The magnetic pellet was immobilized by application of an external magnet and the supernatant was removed. The magnetic pellet was washed once with 1x concentration of phosphate buffered saline by gently inverting the tube and the wash was removed by immobilizing the magnetic pellet using external magnet. The magnetic pellet was finally resuspended in 50 µl of 10 mM Tris buffer, pH 8.0 containing 50 mM NaCl and the resuspended mixture was incubated at RT (25°C) for 30 min.

Genomic DNA isolation from plant

Plant leaves approx. 0.5-1 g was macerated with 1 ml of extraction buffer (100 mM Tris buffer, pH 8.0, 50 mM EDTA, pH 8.0, 500 mM sodium chloride, 10 mM beta meracptoethanol, 0.5% SDS). The mixture was allowed to stand at RT (25°C) for 15 min. To the clear supernatant, 20 mg of iron oxide nanoparticles were added and incubated at room temperature for 10 min. The magnetic pellet was immobilized by application of an external magnet and the supernatant was removed. The magnetic pellet was washed once with 1x concentration of phosphate buffered saline by gently inverting the tube and the wash was removed by immobilizing the magnetic pellet using external magnet. The magnetic pellet was finally resuspended in 50 µl of of 10 mM Tris buffer, pH 8.0 containing 50 mM NaCl and incubated at RT (25°C) for 20 min.

Genomic DNA isolation from blood

To the whole blood sample (50 µl), 3 % SDS was added and incubated at RT (25°C) for 15 min. To the above mixture, 0.5 ml of 100 mM Tris buffer, pH 8.0, 250 mM Guanidium thiocyanate, 250 mM Lithium chloride and 20 mg of iron oxide particles were added and incubated once again for 5 min. The magnetic pellet was immobilized by application of an external magnet and the supernatant was removed. The magnetic pellet was washed once with 1x concentration of phosphate buffered saline by gently inverting the tube and the wash was removed by immobilizing the magnetic pellet using external magnet. The magnetic pellet was finally resuspended in 50 µl of 10 mM Tris buffer, pH 8.0 containing 50 mM NaCl and incubated at RT (25°C) for 20 min.

PCR amplification

The quality of the genomic DNA isolated from saliva using iron oxide particles was analyzed using PCR amplification. Beta globulin and Glucose-6-Phosphate Dehydrogenase (G6PD) genes from human genome was amplified. All PCRs were performed in a 25 μl reaction volume containing 100 ng of template, ten picomoles concentration of gene specific primers for the amplification of different genes. Sequence of the different primers is given in Table 1. PCR was performed on a thermal cycler PCR system (Biorad, USA). Thermal cycling was performed at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1.0 min, and a final extension step of 72 °C for 10 min. The PCR products were analyzed on 1% agarose gel stained with ethidium bromide.

Table 1: Primers for human G6PD and beta globulin genes

Primers	Sequence
G6PD forward	5`ATCTTGGCTCACTGCAACCT3`
G6PD reverse	5`ACAGCGCGTTGTTCTATGTG3`
β-globulin forward	5-GCCCTCTGCTAACAAGTCCTAC-3
β-globulin reverse	5-CCCTAAAAAGAAAATCGCCAATC-3’

DNA Sequencing

Human DNA isolated from saliva sample using iron oxide particles and the isolated DNA was used for the amplification of beta globulin gene. Sequence of the beta globulin primers was forward (5-GCCCTCTGCTAACAAGTCCTAC-3) and reverse (5-GCCCTAAAAAGAAAATCGCCAATC-3). Thermal cycling was performed at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 1.0 min, and a final extension step of 72 °C for 10 min. The PCR products were analyzed on 1% agarose gel stained with ethidium bromide. The PCR product was purified using High Pure PCR product purification kit (Roche). Purified PCR product (35 ng/ul) was sent for sequencing (Eurofins genomics).

Results and Discussion

Higher pH enhances the binding capacity of DNA to iron oxide

It is understood that Fe2O3 has a isoelectric potential (IEP) close to 5.19 [Micheal J etal 2009]. It is also known that as the pH increases to acidic environment, the agglomeration of particles also increase. However, as stated earlier that decrease in the particle size would be more advantageous for creating higher surface area and thus higher yield of DNA. We tested the binding of the DNA to Fe2O3 at different pH ranges from 2 to 10 (figure 1a). We found that as the pH increases, there is higher binding of DNA and thus higher yield

of DNA. Therefore, we concluded that, the binding of DNA to iron oxide is best done at any pH above 7 and we selected pH 8 for all the further DNA isolations.

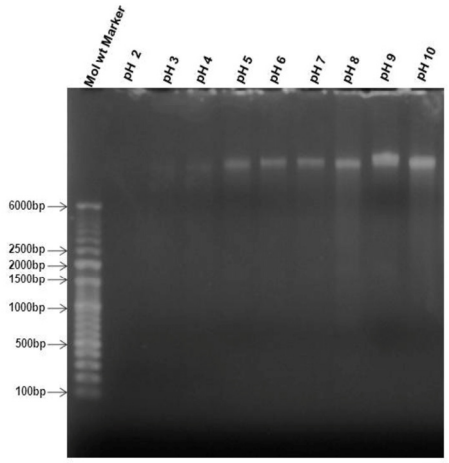


Figure1a: Influence of pH of binding buffer on binding capacity of the DNA to iron oxide: Increase in pH of the buffer for lysis increases the surface area of iron oxide particles leading to increase in DNA yield

Guanidinium and Lithium salts increase the binding of DNA to iron oxide

It was earlier reported that presence of Lithium Chloride can make the lysis of the samples and also increase the amount of DNA eluted in the process of isolation [Yong-Ki Hong et al 1995].

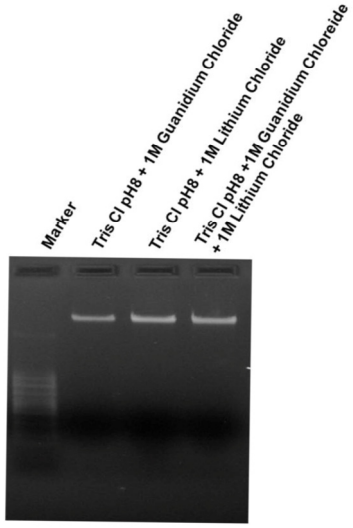


Figure1b: Influence of salts on DNA binding to iron oxide: The basic buffer of TrisCl was used in all DNA isolations from saliva with LiCl2 and / or GnHCL.

In many cases the amount of sample is very limiting and enhancing the lysis and DNA yield is very important. Therefore, we used two different salts Lithium Chloride (LiCl2) and Gaunidinium Chloride (GcHCl) individually and together in combination to check the enhancement of DNA yield. We observed (figure 1b) that use of LiCl2 alone or GcHCl and LiCl2 in combination results in better quality DNA (no streaking of DNA on the gel) and higher yield also as observed in the gel. This experiment suggests that LiCl2 and GcHCl can play an important role in lysis of the cells and enhance DNA recovery.

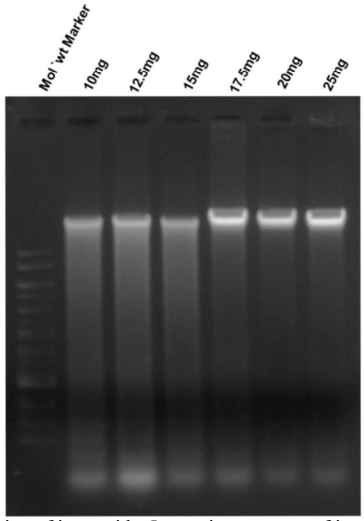


Figure 2a: Titration of iron oxide: Increasing amounts of iron oxide was used from 10mg to 25mg and DNA was isolated from saliva.

Titration of Iron Oxide for better DNA isolation

To check the best concentration of iron oxide which can give best yield of DNA from a fixed amount of the sample, we used varying amounts of iron oxide for DNA isolation from saliva. We have used 10 to 25mg of iron oxide for DNA isolation from 100microlitre of saliva samples. We observed that the best concentration of DNA was eluted using 20mg of iron oxide (Figure 2a).

Sample concentration standardization

In our next experiment, we wanted to check the saturation point of the 20mg of iron oxide particles for best yield of DNA from saliva. Therefore, we used varying amounts of saliva keeping the iron oxide concentration same. We observed that (Figure 2b) 100-200µl of saliva sample would give best yield of DNA.

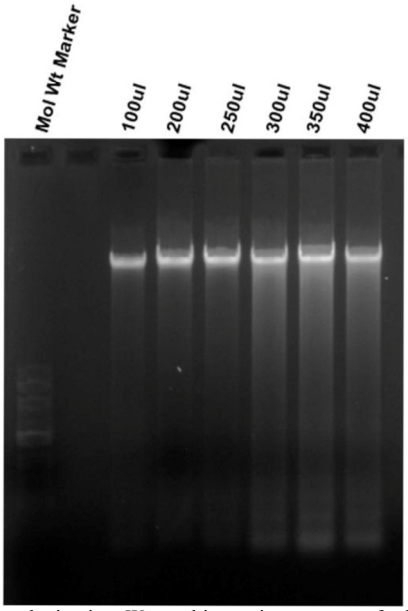


Figure 2b: Sample titration: We used increasing amounts of saliva from 100 µl to 400µl of saliva during the DNA isolation.

Isolation of DNA from various sources

Primarily all the experiments were performed using saliva as saliva is a very useful and easily accessible samples material. However, in order to check if this technology will be useful for isolating DNA from various other sources, we have performed the same from Blood (using Urea and Sodium Dodecyl Sulfate in the lysis buffer), bacteria and plant leaves. As shown in the figure 3, we could observe a good isolation of DNA in all these conditions.

Application of DNA isolation

Further to the isolation of DNA using the naked iron oxide particles, the usability of the DNA was also checked. We performed PCR and also direct sequencing of the isolated genomic DNA from saliva.

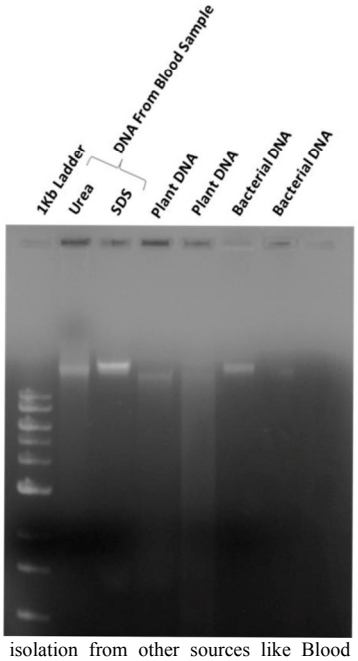


Figure 3: DNA isolation from other sources like Blood (lane1,2), Plant (lane3,4) and bacteria (lane5,6)

DNA amplification and sequencing

We have performed PCR for G6PD gene using genomic DNA isolated from saliva samples and we observed that the PCR resulted in a good amplification without any noise (Figure 4a). Furthermore, we have performed PCR for Beta globin gene and sequenced the PCR product (Figure 4b).

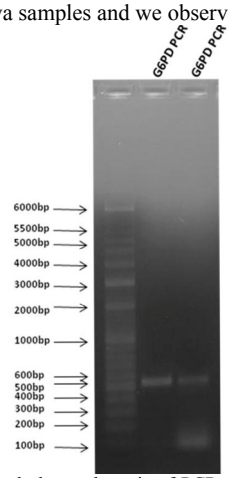
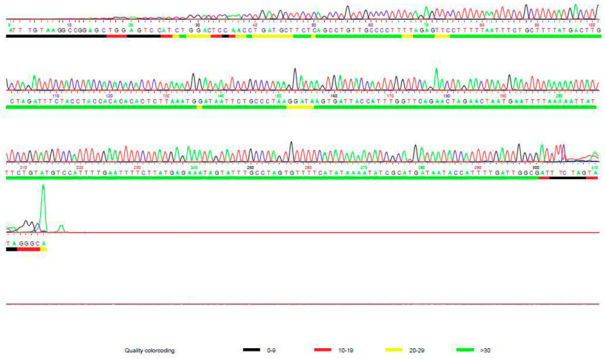
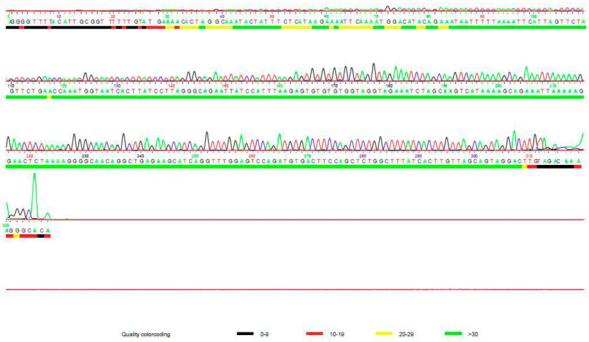


Figure 4a: Agarose gel electrophoresis of PCR product of G6PD gene from genomic DNA isolated from saliva. Lane 2 and 3 are from different isolations

Forward primer



Reverse primer



Sequencing results

Forward:

>TCTGGACTCCAACCTGATGCTTCTCAGCCTGTTGCCCCTT
TTAGAGTTCCTTTTTAATTTCTGCTTTTATGACTTGCTAGA
TTTCTACCTACCACACACACTCTTAAATGGATAATTCTGCC
CTAAGGATAAGTGATTACCATTTGGTTCAGAACTAGAACT
AATGAATTTTAAAAATTATTTCTGTATGTCCATTTTGAATT
TTCTTATGAGAAATAGTATTTGCCTAGTGTTTTCATATAAA
ATATCGCATGATAATACCATTTTGATTGGC.

Reverse:

>AAAACACTAGGCAAATACTATTTCTCATAAGAAAATTCA
AAATGGACATACAGAAATAATTTTTAAAATTCATTAGTTC
TAGTTCTGAACCAAATGGTAATCACTTATCCTTAGGGCAG
AATTATCCATTTAAGAGTGTGTGTGGTAGGTAGAAATCTA
GCAAGTCATAAAAGCAGAAATTAAAAAGGAACTCTAAAA
GGGGCAACAGGCTGAGAAGCATCAGGTTTGGAGTCCAGA
TGTGACTTCCAGCTCTGGCTTTATCACTTGTTAGCAGTAGG
AC

Figure 4b: Sequencing data of the Beta globin gene from the PCR product from saliva genomic DNA. The sequences (330 bp) were analyzed for similar sequences using nucleotide blast programme in NCBI. It was 100% matching for partial beta globulin gene (serum globulin) from human.

In summary our present work, has shown that naked iron oxide particles can be used for DNA isolation from any tissue and in doing so we have eliminated the use of any extra instrument like centrifuge etc. This clearly suggests that we can use this technology for automation and yields are in required amount for performing other assays post DNA isolation like PCR and sequencing. Many such technologies [Saiyed Z.M 2007] inadvertently have used an extra instruments resulting further modifications to the technology development and not so useful for automation. Apart from that, our

technology has a time advantage of performing the entire procedure in very short span of ~30minutes. Furthermore, the entire procedure can be performed in single tube making it more amenable for automation.

Conclusions

In conclusion, we present a modified technique of using Iron oxide particles use to isolation of genomic DNA. Our procedure is more user friendly and is less time consuming and can be performed without any training. It is also noteworthy that the iron oxide particles are not modified and can used directly after synthesis unlike many other techniques where the particles are coated with specific chemicals. We have also enhanced the yield by identifying a mixture of divalent metal salts (Lithium and Gaunidinium salts. We believe that our technique can be used by anyone outside the lab, thus making technologies reach the wider populations without much of scientific knowledge.

Acknowledgement

This work was supported by Department of Information Technology, Government of India to Institute of Bioinformatics and Applied Biotechnology (IBAB), Bangalore, India. Authors also thank faculty at IBAB who have supported and helped during the project.

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