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Design of a new rapid and efficient kit for extracting DNA from blood sample

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Abstract

Extraction of DNA with a pure and high and product is a main goal for researchers in the field of molecular biology or forensic science. Over recent years, isolation of pure, intact, and highly concentrated genomic DNA has been a prerequisite for successful and reliable genetic analysis. The classical DNA extraction method involves the use of organic and inorganic reagents such as chloroform andphenol, which have a toxic effect on humans. Many of the current modern technologies rely on physical extraction, which has contributed greatly to the development of methods for dealing with DNA, such as extraction based on magnetic beads or extraction based on cellulose paper.Based on the wide range of options available in automated DNA extraction protocols, it would be ideal to select those that offer the best cost-effectiveness and time-efficiency performance. The aim of this research was to design a new set to extract DNA from whole blood samples in an easy and highly efficient manner in terms of the amount of DNA extracted and the time required to perform the extraction. In addition, it is economically suitable in terms of cost, based on the physical method of DNA isolation using silica-coated magnetic beads. On this basis, the main conclusion of this work, it can be drawn that a new kit of DNA extraction has been prepared with good properties. The new kit is easy to use, less expensive, safe and offers a good yield.

Keywodes : DNA extraction, DNA concentration, gelelectrophorysis, Nanodrop.

1. Introduction

Deoxyribonucleic acid (DNA) contains all the genetic information for all living things and many viruses. DNA extraction techniques work to isolate and purify DNA from cell membranes, proteins and other cellular components with good quality and quantity[1]. Scientists have made great progress in designing DNA extraction methods with high reliability, ease and speed in performance, less cost and more returns, since the first DNA extraction conducted by Friedrich Miescherin 1869.[2].In the fields of ancient DNA and forensic genetics, the samples investigated are often highly degraded and the DNA present at a low copy number, so extraction is the most important step for any DNA analysis. DNA can be extracted from various clinical samples such asfine needle aspirates of body fluids and biopsy samples; forensic samples such as dried blood spots, buccal swabs and fingerprints; to soil, insects,plant and animal tissue, protozoa, bacteria and yeast[3]. DNA extraction methods are based on some common procedures that aim to efficiently rupture cells, denature cell proteins, inhibit nucleases and other enzymes, and remove biological and chemical contaminants. Finally, DNA precipitation includes basic steps such as the use of organic solvents and the use of centrifugation[4],[5]. Optimizing the DNA extraction process and increasing the sensitivity of DNA kits allowed laboratories to slowly reduce the amount of starting material needed for the extraction process, allowing the destruction of skeletal materials to a minimum [6].DNA isolated from different biological samples can be used for a wide range of different end applications, such as DNA sequencing, polymerase chain reaction (PCR), preparation for genomic libraries as well as amplified fragment length polymorphism (AFLP),quantitative PCR (qPCR), southern blotting, random amplification

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of polymorphic DNA (RAPD), short tandem repeat polymorphism (STRP), restriction fragment length polymorphism (RFLP), single nucleotide polymorphism (SNP) and variable number of tandem repeat (VNTR) applications[7]. These subsequent applications can be the basis for a diagnosis genetic diseases or identifying carrier status, gene therapy, pharmacogenomics [8].Whole blood is one of the available sources for genomic DNA extraction, which has been widely used in this field around the world. There are many methods and protocols available today for extracting DNA from blood samples that must be considered and carefully selected because they subsequently affect the performance and success of any technique applied to DNA[9],[10]. In recent years, with advances in technology, some of these protocols have been adapted to micro-devices to develop complete miniature chemical analysis systems or microfluidic genetic analysis microchips[11]. In fact, DNA is extracted from fresh blood. In addition, DNA profiles are routinely obtained from other biological sources such as saliva, hair, semen, any source whose cells contain a nucleus, even cellular debris on the body that has been touched[12]. The ability to prepare human genomic DNA from whole blood with high purity and in sufficient quantities from fresh trace blood is important both in basic science research and in the clinical setting , it has become an important source of genetic DNA due to the presence of white blood cells that contain a nucleus and DNA so that blood has become an integral part of biochemistry, hematology, clinical studies and forensic investigations.[13],[14]. DNA extraction requires fast and economical procedures with minimal co-extraction of inhibitors that affect subsequent downstream processes that are applied to the DNA. Furthermore, it needs to be flexible enough to apply to frozen and clotted samples. Moreover, it should recover high amounts of pure and integral gDNA. Unfortunately, there is no general DNA extraction protocol to meet all these criteria[15].

2. Experimental part

There are 3 basic steps involved in DNA extraction, that is, lysis, precipitation and purification. In lysis, the nucleus and the cell are broken open, thus releasing DNA. This process involves mechanical disruption and uses enzymes and detergents like Proteinase K to dissolve the cellular proteins and free DNA.

1-Red blood cell (RBC) lysis buffer (R1): (For 50 mL)
0.56 M NH_4Cl (1.5 g)
0.014 M $NaHCO_3$ (0.56 g)
0.5 M NaCl (1.461 g)
0.13 M EDTA (2.6 g)
Mix these ingredients in sterile distilled water up to 50 ml at 7.6 pH, then put it in an autoclave for sterilization, then wait until it reaches room temperature and then add 0.5% from SDS (0.250 g).

2- White blood cell (WBC) lysis buffer (R2): (For 50 mL)
1M Tris (6.05 g)
0.5 M EDTA $(Na)_2$ (9.3 g)
0.5 M NaCl (1.46 g)
Mix these ingredients in sterile distilled water up to 50 ml at 7.6 pH, then put it in an autoclave for sterilization, then wait until it reaches room temperature and then add 10% from SDS (5 g).

3-Precipitation solution: 5M sodium acetate, pH 5.5, Ethanol absolute precipitation is a commonly used technique for concentrating and de-salting nucleic acid (DNA or RNA) preparations in an aqueous solution.

4-Purification solution: Mix (0.135 M,7ml) FeCl3 and (0.135 M) FeSO4 then we add a little water to the previous mixture with constant stirring, then add 100 ml of ammonia through a separating funnel and drip continuously for an hour. We separate the excess ammonia using a magnet. We take 100 ml of the filtered solution, add 1 ml of 0.1 M $NaSiO_3$ to it, add a quantity of water to it, and leave it in a Teflon-coated container for 8-10 hours. Then we notice a change in its colour from black to grey.

5-Washing buffer solution: (For 50 mL)
0.02 M NaCL (0.058 g)
0.002 M Tris (0.012 g)
80% Ethanol (50 mL)

6-DNA Hydration (Elution buffer):
0.0005 M EDTA $(Na)_2$
0.01 M Tris (0.06 g)
Mix these ingredients in sterile distilled water up to 50 ml at 9 pH, then put it in an autoclave for sterilization.

7- DNA extraction protocol:
1-Add 300 µl of blood in a capacity of 1.5 ml Eppendorf and add 200 µl of R1 and 200 µl of R2 and 20 µl of proteinase K.
2-Doing Vortex for 20 seconds, this helps to give a good amount of DNA.

3- then the sample is incubated at a temperature of 60 ° C for 10 minutes.
4-Add 10 µl NaOAC solution, 300µl of absolute ethanol and 100 µl of SCM (silica coated magnetic)solution and work Vortex for 10 seconds.
5-Transferring Eppendorf to magnetic rack and isolating the filter from the particals SCM and neglecting the filter.
6-Add 600 µl of the washing solution and work Vortex for 10 seconds, Then separate the liquid from the magnetic particles by magnetic isolation. We repeat this step again.
7-Dry the pellets SCM from the remaining wash solution, then add 50 µl of the elution solution buffer and is incubated at a temperature of 65 °C for 6 minutes until the DNA is dissolved. Then we separate the soluble DNA from the magnetic particles by magnetic isolation and freeze it at a low temperature until use.
Storage Conditions: Components of the blood extraction kit may be stored at room temperature (20-25 °C), Proteinase K stored at deep freeze.

3-Results and discussion: The results of electrophoresis of the samples extracted after several attempts to succeed the prepared kit showed clear bundles under the influence of ultraviolet rays, which is the beginning of the path towards improving the prepared kit:

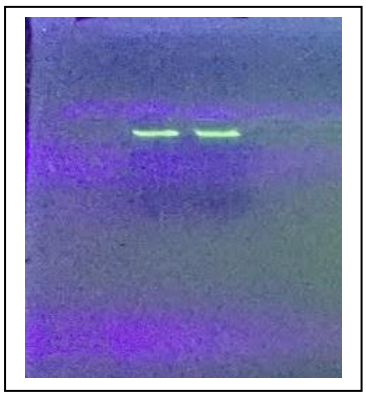

Fig. 1. Shows the result of the electrophoresis of the samples that were extracted with the prepared new kit.
The next step is to determine the productivity and purity of the DNA by measuring its absorbance. The concentration of the extracted DNA was measured at 260 nm, and its purity was confirmed by measuring the absorbance at 260/280 nm, which gave the following results.

No.	Concentration ng / µl	Purity at 260/280 nm	DNA yieldµg
S1*	78.5	1.89	0.0039
S2*	57.3	1.81	0.0028
S1	65.7	1.90	0.0032
S2	85.4	2.1	0.0042

Table 1: shows the comparison in purity and concentration results between samples extracted with the new prepared kit and samples extracted with the standard kit. (S*= sample that extracted by new kit, S=sample that extracted by commercial kit)
In comparison with the results of samples extracted by commercial kits, it was found that there is a large convergence with the general specifications of the extracted DNA in terms of quantity and purity.
Where the value of the concentration needed for subsequent applications of DNA ranges between 50-100 ng / µland the absorbance ratio ranges at 260/280 nm between 1.8-2.0 and this indicates the purity of the DNA from the alcohol used, as well as the remaining proteins after extraction, where the percentage less than 1.8 indicates the presence of alcohol A ratio more than 2 indicates the presence of the remaining proteins as contaminants

4-Conclusion:
In line with the great development taking place in the science of molecular biology and the huge revolution in this field, it has become necessary to search for more advanced, safer, faster, and less expensive DNA extraction methods that can be prepared simultaneously in the laboratory with primary components that are somewhat available and easily obtainable, in addition to unrestricted storage conditions. The method of preparing this new kit was free from volatile solvents and toxic substances, and this was the desired goalto obtain a safer kit. The results of DNA extraction with the laboratory prepared kit showed good results of purity, concentration and quantity within the normal range compared to the commercial kit. On this basis, the main conclusion of this work is that it can be drawn that a new kit of DNA extraction has been prepared with good properties. The new kit is easy to use, less expensive, safe and offers a good yield.

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6-References

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