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DNA isolation method from human blood with MasterPure DNA Purification Kit™ – review article

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ABSTRACT

The main element of genomic molecular analysis is isolation of DNA from biological material. DNA isolation is an important process of PCR methods. Efficency of PCR processes depends of purness and concentration of DNA. Many methods allows to izolate good quality DNA. There are many Purification Kits and methods of extraction genomic material. We show available techniques and methods of increasing efficiency of the process in our studies. Previous data shows that operating with kits needs optimization, whilst protocols do not have all information about isolation process. We concentrate on Epicentre MasterPure DNA Purification Kit, which is cheap, fast and allows to purification nucleic acids from various materials. We show detailed advantages and disadvantages of this set. Our main aim is to develop protocol to get optimal concentration (50-100 ng/μL) and pureness (A 260/280; 1.6-1.9) of DNA. We also should optimize the process of DNA isolation to obtain efficiency of about 100%. Optimized methodology of DNA isolation, with using MasterPure DNA Purification Kit allows to get good quality material in laboratory without specialistic equipment. This survey contains our assumptions of ways how we can influent on purification process according to previous data.

***Keywords*:** DNA, isolation, optimization, human, whole blood, review

1. INTRODUCTION

The 1869 is one of the most significant date in history of molecular biology. In that year Swiss chemist Friedrich Miescher as the first researcher isolated DNA [1]. Today deoxyribonucleic acid isolation is common procedure for which we have many different methods and protocols, mostly commercially available, and often automated [2].

Extraction is crucial step in many molecular aplications becouse good quality, pure DNA is required. Mistakes can leads to further problems such as inhibition of polymerase chain reaction PCR caused by both extrinsic (e.g., traces of sodium dodecyl sulfate SDS, phenol, anticoagulants) and intracellular factors (e.g., porphyrin ring of haem) [3].

There are two general categories of isolation methods and all of them have some advantages and disadvantages. Which of them should be used has to be decided based on a number of criteria such as costs, available equipment, knowledge, experience and so on. The most important thing is it should deliver good quality and quantity DNA [2,4].

Solution base methods based on the difference of solubility. The phenol-chloroform type of extraction use sodium dodecyl sulfate for cell lysis and phenol for inactivation of cellular enzymes. Removal of unwanted components is accomplished by use of phenol-chloroform. Lastly DNA is percipitated in propan-2-ol [4,5].

Salting out is second liquid-liquid type isolation, and one developed into commercial set for example Epicentre MasterPure™ DNA Purification Kit. This method use proteinase K or loundry powder for denaturation of nucleoproteins and high concentration of sodium chloride to purify [5,6].

Second and more recent group of purification methods are solid-phase DNA extraction [2,4]. They are definitly much faster and less labor intensive and also are developed into commercial kits . All of them have similar principles. After cell lysis DNA is bound to solid phase, then contaminants are removed and lastly DNA is released. Each of the above steps usually needs, different pH and centrifugal force [2,4]. Te most popular and widely used solid phase are silica matrices. They are positvley charged and have affinity to negatively charged nuclein [2,4]. Similar to silica are anion exchange resins. They can react with negatively charged DNA backbone [2,4].

Magnetics beads are also commonly used [2,4]. Made of mineral like magnetite or maghemite and coated with silica. Together with deoxyribonucleic acid they are immobilized with magnet to wasched away contaminants. After that DNA is diluted and beads are removed [7].

The sources of DNA in our studies is peripheral human blood. Collecting blood is more invasive and painful, then e.g. buccal cell collection, however in most diagnostics procedures blood is colected anyway. Compared to buccal swabbing not required special training or supervision, and DNA has higher yeld and quality [8].

2. THE AIM OF STUDY

The aim of our research is to develop a protocol that will allow conducting efficient isolation of DNA from human whole blood. The development of method is based on the orginal Epicentre protocol. We are committed to obtain the DNA concentration in the range 50-100 ng/μL [12]. We need a high concentration of DNA to be able to perform more

procedures during our research. Also we want to get purness (A 260/280) in the range 1.6-1.9 [11]. During the development of the methods we will try to get a protocol, that allows efficient (approx. 100%) isolation of DNA.

3. EPICENTRE

The Epicentre The MasterPure™ DNA Purification Kit contains all of the reagents necessary for recovery of DNA from different biological sources. This kit uses a rapid desalination process to remove impurities macromolecules, avoiding the toxic organic solvents. The purified DNA can then be used in many applications, including hybridization, restriction enzyme digestion and PCR amplification.

MasterPure DNA Purification Kit is tested by purifying DNA from *E. coli*. DNA quality and performance are tested by agarose gel electrophoresis, spectrophotometry, fluorimetry, and used as a template for PCR. The kit isolates DNA from a variety of sources including: bovine liver, human HL-60 tissue culture cells, paraffin-embedded breast tumor tissue, human whole blood and plasma, saliva, mouse tail, corn and geranium leaf, *E. coli*, and lambda phage. Tissues other than those listed here may be consistent with a set with some optimization [14].

Devices that were used in the optimization methods: centrifugate (Eppendorf), laboratory incubator (Poll CL-60), vortex (Eppendorf), Nanodrop (Eppendorf), pipette variable capacitance (Eppendorf).

3. 1. Advantages

1. Sample size: can purify nucleic acid from samples of various sizes by proportionately adjusting the amount of reagents to the amount of starting material.
2. Many tissue sources: bovine liver, human HL-60 tissue culture cells, paraffin-embedded breast tumor tissue, human whole blood and plasma, saliva, mouse tail, corn and geranium leaf, *E. coli*, and lambda phage.
3. Financial cost – favorable price when buying the set for more DNA.
4. Equipment and tools – can use a kit with basic laboratory equipment such as a centrifuge, pipettes or incubator.

3. 2. Disadvantages

These disadvantages are mainly due to incorrect use of the kit or improper storage. The key is the experience of the person conducting the isolation.

1. Little or no DNA was suspended in TE buffer.
2. Avoid contamination by exogenous or endogenous nucleases.
3. Ensure that DNA remains following isopropanol precipitation.
4. A_{260}/A_{280} ratio is too low or too high.
5. DNA rehydrates slowly.
6. Residual RNA in DNA preparations.

4. PREVIOUS TRIALS OF DNA EXTRACTION WITH VARIOUS COMMERCIALLY AVAILABLE KITS OR MODIFIED PROCEDURES BASED ON AVAILABLE LITERATURE

The effect of DNA extraction depends on basal material from which DNA is extracted as well as on extraction technique. Ghatak et al. (2013) compared concentrations and purity of DNA extracted from different kinds of material, also blood samples. Their procedure started from adding appropriate volume of Red Blood Cells Lysis Buffer. After vortexing and iverting as well as centrifugation the most of supernatant was threw away. Then authors again added RBC Lysis Buffer to obtained pellet and repeated vortexing, inverting and centrifugation a few times to get clear white pellet. It was suspended in PBS with addition of Cell Lysis Buffer and Proteinase K. After vortexing the pellet undergo dissolution and was incubated for about 2 hours (56 °C). In further critical steps authors added phenol. After mixing and centrifugation obtained aqueous layer was added to the mixture of phenol, chloroform and isoamyl alcohol. After another inverting and centrifugation they added RNase to the supernatant. After half an hour incubation (37 °C) mixture of chloroform and isoamyl alcohol was added. In following stages authors added absolute alcohol to the supernatant, repeated inverting, chilled the tube in –20 °C and centrifuge (20 min., 4 °C). Finally threw the supernatant away and added 70% ethanol to the pellet. Repeating centrifugation after this step authors finally decanted the supernatant and dried the pellet which was subsequently suspended in TE Buffer. In the procedure processed by Ghatak et al. mixing, vortexing and centrifugation were highly underlined as well as adding phenol-chloroform in specific proportions which was a significant innovation. Based on spectrophotometric analysis authors estimated the concentration of DNA extracted from 50 μL of blood in the range of 57-94 ng/μL and the purity (A260/280) in the range of 1.76-1.86 [13-17].

Kuchler et al. (2012) introduced a procedure of DNA extraction from saliva without using of phenol-chloroform. After obtaining the pellet authors added extraction solution (mixture of Tris-HCl, EDTA and SDS) with K-proteinase. The tubes were incubated overnight and then treated with ammonium acetate to remove protein contaminants. Then inverting, centrifugation and finally DNA precipitation with isopropanol (30 min., –20 °C). In the next step authors centrifuged the tubes (20 min., 4 °C), threw away the supernatant and achieved the pellet which was rinsed with 70% ethanol. In final step (when the ethanol dried out) DNA was resuspended in TE Buffer. The results were little diversified according to time from saliva gaining to DNA extraction (samples immediately extracted, extracted after receiving ethanol and 4 days incubation at room temperature as well as extracted after receiving ethanol and 8 days incubation at room temperature) [9].

El Bali et al. (2014) compared the efficiency of DNA isolation from urine with seven different commercially available isolation kits. Only one extraction kit (QIAamp® Viral RNA Mini Kit) was certified as acceptable. The results obtained with this kit regarding to DNA concentration was over 900 ng/mL urine for male and female urine based on NanoDrop measurement while over 400 ng/mL for female urine and 200 ng/mL for male urine based on Quant-iTTM PicoGreen® assay.

In case of DNA purity mean A260/280 ratio was 3.33+/-0.12 for woman and 3.39+/-0.06 for man. Authors underlined that these results were too high regarding to acceptable purity norm (1.8-2.0) but they speculated that the spectrophotometric measurement might be falsified by presence of RNA carrier and sodium azide in wash buffer.

Based on qPCR analysis authors affirmed almost complete lack of PCR inhibitors in extracted DNA. Similarly DNA integrity was estimated as acceptable (relatively high proportion of long DNA fragments) and relatively low concentrations of bacterial DNA in particular samples was affirmed [10].

Ghantous et al. (2014) compared efficiency of several lysis-based commercially available kits in DNA extraction from neonatal dried blood spots. Authors introduced modifications to tested protocols to achieve optimal extraction effect (DNA yield and purity) They combined reagents from different kits in certain steps of the procedure or exchanged one reagent to another. E.g., changing of DNA precipitation buffer to ethanol reduced the need of thorough vortexing to dissolve precipitates but did not result in improvement in DNA integrity. In turn ethanol in place of precipitation buffer increased DNA yield in certain protocols.

Finally authors selected the optimal procedure by combination of the reagents from GenSolveTM kit – in phase I of DNA extraction – and QIAamp® – in phase II [11]. Phase I included blood extraction, blood cell lysis to release DNA into solution and enzymatic digestion of cellular proteins, while phase II: DNA precipitation, DNA binding silica-gel columns followed by purification and cleanup as well as DNA elution and heating. Results that authors achieved with GQ procedure were: DNA quantity 808+/-376 ng and DNA purity (A260/280) 1.83+/-0.14. Comparing to other tested kits GQ extraction did not result in the highest DNA yield (still it was sufficient) but provided the best DNA purity. Furthermore, DNA was relatively integral with GQ (less fragmented that in other tested protocols). Nevertheless, DNA extracted with practically all tested kits was effective in PCR analysis (results in detectable PCR bands) but GQ appeared to be the best regarding to quantity and quality parameters of DNA [11].

Rajatileka et al. (2013) analysed the quantity and quality of DNA extracted from three different sources: newborn dried blood spots, whole blood and umbilical cord tissue. Dried blood spots samples were additionally diversified regarding to different storage time (from 3-22 years). Obtained concentrations of genomic DNA were measured based on spectrophotometry at 260 nm. According to these results, the most abundant source of DNA was umbilical cord (117.3+/-112.9 ng/μL). Whole blood appeared to be less abundant (40.3+/-10.9 ng/μL).

In case of dried blood spots time of storage did not influenced on DNA concentrations (average concentration for period of 3-22 years 8.3+/-10.7 ng/μL). Both DNA extracted from whole blood as well as from umbilical cord provided relatively good range of purity (A260/280: 1.8 and 1.9 accordingly). Authors noted that in case of both groups of samples (whole blood and umbilical cord) time of storage was shorter than 1 month. On the other hand purity of DNA isolated from dried blood spots varied due to storage time. Shorter storage (one month) provided purity at about 1.7 while 3-5 years: 2.0.

DNA extracted from dried blood spots stored longer than 5 years (6-22 years) exhibited unacceptable purity beyond the range of 1.7-2.0 (6-10 years: 2.2; 11-15 years: 2.5; 15-22 years: 2.4). In result of electrophoresis DNA extracted from whole blood and umbilical cord created single high-molecular-weight band. DNA from dried blood spots oppositely created a smear of low-molecular-weight fragmented bands (possible DNA degradation). However authors affirmed that DNA extracted from all samples might be effectively used in PCR amplification [12].

5. SUMMARY AND CONCLUSIONS

Based on available literature we may summarize that achieving of best DNA quantity and quality due to extraction depends on many factors. The first one is the choice of source of DNA. Rajatileka et al. (2013) affirmed that DNA extracted from umbilical cord tissue provided the best quantity (117.3+/-112.9 ng/µL) and quality (A260/280 1.9). Ghatak et al. (2013) achieved different yield and purity of extracted DNA according to the basal material. Every source of DNA has advantages and disadvantages. El Bali et al. [10] underlined that blood still remains the most common source but certain disadvantages – requirement of professional equipment and perssonel during sample collection, infectious risk, presence of certain interfering proteins and PCR inhibitors in blood – cause that other sources of DNA like urine or buccal swabs gaining popularity. The main advantage of urine as well as saliva samples is their noninvasive collection and less risk for pathogenic infections [9,10].

The time of storage is equally important. Rajatileka et al. (2013) demonstrated that long term storage (3-22 years) may worsen the purity of DNA extracted from dried blood spots. Finally the choice of extraction kit appear to be crucial. Ghantous et al. (2014) tested several kits regard to quantity and quality of extracted DNA. Authors affirmed that combination of two kits (based on silica-gel columns) provided the best results for extraction from dry blood spots [11]. Analysed literature also showed that optimal results are often achieved in the way of optimization and introducing modifications to the basal procedure.

MasterPure DNA Purification Kit^{TM} is popular, cheap and usefull for many materials. This protocol (which is not based on silica columns) need to be optimized to obtain best possible results since particular procedures vary due to available source of DNA (protocols available on www.epicentre.com). In our research, we want to test DNA isolation from human whole blood. In previous studies researchers introduced changes in procedure due tu materials as a urine, saliva and blood. The authors used doubble dose of RBC Lysis Buffer [11], cold isopropanol and incubation in the temperature of –20 °C [9], precipitation in ethanol [11], This informations shows that DNA isolation with useing this kit should be improved to get good quality DNA for further studies.

We use concentration and pureness of DNA isolated with using the orginal Epicentre protocol in our investigations. If effects will be not good enough, we want to introduce changes which were presented in previous studies. In our studies, firstly we want to concentrate on adding RBC Lysis Buffer. According to Ghatak et al. studies (2013), if our pellet will be still polluted by erythrocytes we want to use double dose of this buffer. In next step we want to use cold isopropanol. If our procedure will not effect in concentration DNA we willstart useing chilled alcohol and incubate probes in the temperature of –20 °C [9]. We expect that efects of isolation based on orginal Epicentre protocol will be unsatisfactory, but we want to confront orginal Epicentre protocol to optimized procedure to show how significant are the differences.

DNA isolation is an important process of PCR methods. Efficency of PCR processes depends of purness and concentration of DNA. Many methods allows to izolate good quality DNA. Optimized methodology of DNA isolation, with useing MasterPure DNA Purification Kit allows to get good quality material in laboratory without specialistic equipment.

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