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COMPARISON OF DNA EXTRACTION'S METHODS FROM PERIPHERAL BLOOD USING SIMPLE LABORATORY REAGENTS

Abstract

The aim of this study was to compare efficiency and cost of genomic DNA isolation from peripheral blood. In our study we used common methods like: chloroform/phenol and high salt method and their modifications where to cell lysis proteinase K and laundry detergent were used. For comparison DNA from same blood was isolated by commercial kit. Yield and purity of isolated DNA was measured by spectrophotometer. Chloroform/ethanol method with proteinase K gave best results, most contaminated samples were from method using NaCl and proteinase K. Chloroform/phenol isolation was the cheapest one but use commercial kit was fastest way to isolate DNA from peripheral blood.

Key words: *DNA isolation, chloroform/phenol, NaCl*

Extraction of pure DNA is most important part of many procedures in molecular biology like PCR (polymerase chain reaction), hybridization or genomic library construction. Many different methods have already been described for isolation of the genomic DNA – some of them are very simple and easy like commercial kits other need time e.g. to prepare many buffers. Many protocols for isolation of DNA from small amount of tissues or blood, where final product must be DNA without contamination, low degraded and good quality, very often involve detergents (e.g. SDS) or enzymes (e.g. proteinase), whose preparation is time-consuming and often very expensive. All known methods of DNA extraction are very similar to itself, and the aim of these methods is obtain DNA of high- purity and low-degraded. Initial step in all methods is lysis of blood cells, next remove all proteins components till we get pure, biological active, and at the lowest pitch damaged DNA. The aim of this study was comparison few methods of DNA isolation with their modifications and compare quality of DNA with commercial extraction kit. Moreover we tried to define isolation's cost of one sample of blood using those methods.

In this research peripheral blood obtained from nutria (Myocastor coypus) were used. For DNA isolation were used methods described and used in some protocols, very important was availability and price of detergents and enzymes used in isolation :

-> chloroform/phenol method (Sambrook and Russell 2001)
-> high salt method(sodium chloride) (Drabek and Petrek 2002)
-> commercial DNA extraction kit -> DNA Extractor (Nuscana, Poland)

and modification of this methods, where we add to lysis buffer laundry detergent *Persil* (Bahl and Pfenninger 1996) and proteinase K. Every method were repeated five times.

25 Eppendorf tubes each 1,5 ml by volume were prepared. To 20 of them were added 40 µl of blood and 400 µl of lysis buffer (NaCl 100mM, TRIS 10 nM, EDTA 10 mM, SDS 1,5%), afterwards to ten tubes were added 3 µl of proteinase K (20 mg/ml), and 50 µl of 20% laundry detergent solution. Capped tubes were mixed by inverting and incubated overnight at 56^{o}C. After incubation to ten tubes with lysate (five tubes with proteinase K and five with laundry detergent) were added 400 µl of phenol, tubes were capped and mixed by inverting and centrifuged at 12000 rpm for 8 minutes. After that supernatant were carefully transferred to new tubes. Next step was adding 500 µl of chloroform - isoalymalcohol (24:1) mi, tubes were well mixed and centrifuged at 12000 rpm for 8 minutes. Supernatant were aspirated to new tube, 400 µl of isopropanol (-20^{o}C) were added, capped tubes were gently mixed and centrifuged at 4000 rpm for 1 minute. Upper, liquid phase was carefully removed and pellet was washed in 200 µl of 70% ethanol. After centrifuged at 4000 rpm for 1 minute, upper phase were poured out and pellet was dried in room temperature. After ethanol's dry off , precipitate were dissolved in 200 µl of deionized water. Isolation by salting out using NaCl (about 6M) was carry through last ten tubes with lysate (five with proteinase K and five with laundry detergent). After overnight incubation to every single tube was added 20 µl of saturated NaCl solution, which was half volume of blood taken to isolation. This solution was mixed and centrifuged at 2000 rpm for 10 minutes. After this supernatant was poured to new tubes (1,5 ml) with 2 volume of ice-cold 96% ethanol. Tubes were gently mixed till DNA precipitation and stored in -20^{o}C for one hour. After that tubes were centrifuged at 3000 rpm for 10 minutes. Supernatant was removed once again and 200 µl of ice-cold 70% ethanol were added. Tubes were centrifuged again under same condition like before and supernatant was poured out after centrifuged. Pellet was air dried and dissolve in 0,5 ml of deionized water. Isolation by commercial DNA kit was made according to procedure provided by producer. Five samples were prepared and reagents obtained in DNA extraction kit (lysis buffer, proteinase K, buffers for DNA purification) were used.

Concentration and purity of isolated DNA was checked by spectrophotometer with use of Optical Density (OD) for 260 and 280 nm wavelength (OD equal 1 correspond to 50 µg/ml concentration of double strains DNA) (Sambrook and Russel 2001). As blind sample deionized water was used. DNA can be consider as pure and biological active if OD 260/280 is between 1,8 and 2,0. Ratio lower then 1,8 is contaminated by proteins and higher then 2,0 is contaminated by RNA. (Sambrook and Russel 2001) Statistical analysis were performed using SAS, in estimation of differences Scheffe's test were used.

Results of researches and conclusions

Highest purity DNA was isolated using chloroform/phenol method with their modifications. Most contaminated DNA was isolated from NaCL and proteinase K (tab.1). Yield of DNA isolated in our study was between 1,45 µg/ml and 35,80 µg/ml (tab.2).

Table.1

OD 260/280 value of DNA isolated by different methods

DNA Isolation Method		N	Mean	Min	Max	SD
Nuscana kit		5	1,47	0,85	1,83	0,39
Proteinase K	chloroform/phenol	5	1,70	1,55	1,97	0,16
Laundry	chloroform/phenol	5	1,43	1,15	2,13	0,41
Proteinase K	NaCl	5	1,3	1,01	1,67	0,24
Laundry	NaCl	5	1,32	1,02	2,19	0,49

SD – standard deviations

Table.2

Concentration of DNA isolated by different methods

DNA Isolation method		N	Mean	Min	Max	SD
Nuscana kit		5	$2,42^{B}$	1,45	4,88	1,44
Proteinase K	chloroform/phenol	5	$8,77^{B}$	4,92	13,70	3,27
Laundry	chloroform/phenol	5	$22,76^{A}$	16,47	30,88	6,09
Proteinase K	NaCl	5	$3,34^{B}$	0,98	7,30	2,35
Laundry	NaCl	5	$24,87^{A}$	15,72	35,80	8,69

SD – standard deviations

A,B – means were significant different ($p>0,01$)

Despite information regarding using laundry detergent (Drabek and Petrek 2002) and NaCl (Bahl and Pfenninger 1996) DNA purification in our laboratory using this methods wasn't as good as expected. Samples were less or more contaminated by proteins, their use for further procedures will need additional purification what will increase costs and time required for whole isolation. High repeatability of chloroform/phenol +proteinase K method made certain in using this method as reliable source of DNA of good yield and purity. Costs of isolation were calculated as price of all buffers and reagents used for purification of DNA in one tube. It shown that methods using simple and available reagents are 60-200 times cheaper than costs of isolation one tube by commercial kit (chloroform/phenol+ proteinase K – 0,35 zl/tube; chloroform/phenol + laundry detergent – 0,10 zl/tube; NaCl + laundry detergent – 0,10 zl/tube; NaCl + proteinase K – 0,35 zl /tube; commercial kit – 20 zl/tube). However time required for isolation was considerably different – counting in time needed for incubation. Time required only for incubation in methods using chloroform/phenol and NaCl was very long -12h- compared to lysis time by reagents from commercial kit. Whole isolation by commercial kit was made in about 1,5h it was 10 times shorter than time needed in other methods (15h).

For small laboratory, like ours, this method is economical of use thanks to low costs of reagents and their availability. Moreover this reagents and not harmful as some reagents from commercial kit

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Стаття надійшла до редакції 20.04.2010