BIOLOGICA NYSSANA
7 (1) • September 2016: 47-52
DOI: 10.5281/zenodo.159103

Original Article

Received: 22 January 2016
Revised: 23 February 2016
Accepted: 01 Mart 2016

Genomic DNA from rat blood: A comparison of two extraction methods

Dijana Takić Miladinov[1,2]*

[1]*Department of Molecular Biology, Institute for Biological Research, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia*

[2]*Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia*

* *E-mail: takicdijana@gmail.com*

***Abstract*:**

Takić Miladinov, D.: ***Genomic DNA from rat blood: A comparison of two extraction methods. Biologica Nyssana, 7 (1), September 2016: 47-52.***

In this study, two methods for DNA extraction from fresh rat blood were compared. One is based on the use of cetyltrimethylammonium bromide (CTAB method), while the other one is well-known salting out method. Spectrophotometric analysis was employed to assess yield and purity of isolated DNA, while agarose gel electrophoresis was carried out to evaluate DNA integrity. The results have clearly demonstrated that the extraction method has significantly influenced the quantity and purity of isolated DNA. By using the CTAB method, a larger quantity of high-molecular weight DNA with good purity is obtained which, along with time- and cost-efficiency of the procedure, makes this method more suitable for the extraction of DNA from rat whole blood.

Key words: DNA extraction; CTAB; salting out; rat whole blood

***Apstrakt*:**

Takić Miladinov, D.: ***Genomska DNK iz krvi pacova: Poređenje dveju metoda ekstrakcije. Biologica Nyssana, 7 (1), Septembar 2016: 47-52.***

U ovoj studiji su upoređivane dve metode za ekstrakciju DNK iz sveže krvi pacova. Jedna je zasnovana na upotrebi cetil trimetil amonijum bromida (CTAB metoda), dok je druga dobro poznata metoda isoljavanja. Za procenu prinosa i čistoće izolovane DNK korišćena je spektrofotometrijska analiza dok je agarozna gel elektroforeza korišćena za procenu integriteta DNK. Rezultati su jasno pokazali da je način ekstrakcije značajno uticao na količinu i čistoću izolovane DNK. Korišćenjem CTAB metode dobijena je veća količina DNK velike molekulske težine i dobre čistoće što, zajedno sa efikasnošću u pogledu trajanja i ekonomičnosti procedure, čini ovu metodu pogodnijom za ekstrakciju DNK iz pune krvi pacova.

Key words: DNK ekstrakcija; CTAB; isoljavanje; puna krv pacova

Introduction

Deoxyribonucleic acid (DNA) isolation is a process of extraction of DNA from wide variety of sources. The first DNA was isolated from white blood cells in 1869 by Swiss physician and biologist Friedrich Miescher (Dahm, 2008). Currently, it is a routine procedure in molecular biology, forensic science and medical diagnostics. The preparation of high quality DNA samples from various sources is the first and vital step for subsequent molecular biological techniques such as polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), mutation detection, cloning as well as genotyping (Phillips et al., 2000; Wang et al., 2003; Tanriover et al., 2010). Although in some cases PCR can be performed without previously DNA isolation step (Kovacevic Grujicic et al., 2012), it is usual to employ pure genomic DNA in determination of genetic disorders, epigenetic studies and diagnostic tests (Angelini et al., 2000; Phillips et al., 2000; Wang et al., 2003; Lewis et al., 2005). Various methods for DNA isolation from blood samples have already been established (Milligan, 1998; Angelini et al., 2000) and many DNA isolation kits are commercially available today. The choice of a method for preparing DNA depends on the source as well as quantity, quality and purity of the DNA desired for downstream applications. An ideal extraction technique should optimize yield, purity and integrity of isolated DNA, and also be efficient in terms of cost, time and safety. The cetyltrimethyl ammonium bromide (CTAB) method is successfully used for extraction of high quality genomic DNA from wide variety of sources (Gustincich et al., 1991; Thomas et al., 1997; Desloire et al., 2006; Jasbeer et al., 2009; Chen et al., 2010; Filho & Almeida, 2013). Also, a rapid salting out DNA extraction method is well documented in the literature (Miller et al., 1988; Olerup & Zetterquist, 1992; Aljanabi & Martinez, 1997). The aim of this study was to determine whether these two methods are suitable for isolation of high-molecular weight DNA from fresh rat blood. The two methods were compared in terms of yield, purity and quality of isolated DNA as well as in terms of cost/time efficiency and environmental safety.

Material and methods

DNA extraction

Published protocols with slight modifications were performed for the CTAB (Gustincich et al., 1991) and salting out (Olerup & Zetterquist, 1992) extraction methods. Samples of fresh whole blood were obtained from rat tail vein and aliquoted in 1.5 or 2 ml tubes containing EDTA. For both methods, DNA was extracted from four samples of fresh blood. In the final step of isolation, DNA was resuspended in 50 µl of TE buffer (10 mM Tris-HCl, 1mM EDTA, pH 7.6) and stored at -20 °C until analysis by agarose gel electrophoresis.

CTAB method

Solution I: 8% CTAB, 1.5 M NaCl, 100 mM TRIS pH 8.5, 50 mM EDTA pH 8.
Solution II: 5% CTAB, 0.1 M NaCl
500µl of solution I was pre-warmed at 68 °C, mixed with 250 µl of fresh blood and further incubated at 68°C for 30 min. Afterwards, 750 µl of chloroform was added, inverted several times to mix and samples were centrifuged at 13 000 rpm for 5 min at room temperature (RT). DNA containing upper phases were transferred into a new tubes, with no disturbing of proteins containing medium phase, and theirs volumes (V) were determined. Aqueous phases were mixed with solution II (V/50) and ddH_2O (V) and centrifuged at 13 000 rpm for 5 min at RT. Supernatants were discarded and pellets were resuspended in 250 µl 1.2 M NaCl. To precipitate DNA, 750 µl of ice cold absolute ethanol was added and samples were centrifuged at 13 000 rpm for 5 min at +4 °C. The pellets were washed twice with 750 µl of 70% ethanol, and centrifuged at the above mentioned conditions to remove residual salt. The pellets were air-dried at RT.

Salting out extraction method (SE method)

Red blood cell lysis buffer (RBCL): 0.5 gr of $KHCO_3$ and 4.14 gr of NH_4Cl were diluted in 500 ml of dH_2O.
Digestion buffer: 100 mM NaCl, 10 mM Tris-HCl pH 8, 25 mM EDTA pH 8, 0.3 mg/ml proteinase K, 0.05% Triton X-100. Adjust final volume to 5 ml with dH_2O.
Fresh blood (500 µl) was aliquoted in four sterile 1.5 ml tubes and centrifuged at 3 000 rpm for 10 min at RT. Supernatants were discarded and pellets of the cells were resuspended in 1.5 ml of RBCL buffer. Following incubation for 10 min at RT with occasional inverting of the tubes, the mixtures were centrifuged at the same conditions mentioned above. The lysis step was repeated for 3-4 times until the supernatants were nearly clear and the pellets were mostly white. After lysis, the pellets were resuspended in 100 µl of digestion buffer. Two tubes with samples were digested at 37 °C overnight, while two others were digested at 65 °C for one hour. After digestion was complete, 240 µl of saturated 5M NaCl was added to all samples and, after centrifugation at

Table 1. DNA yields, absorbance ratios, environmental safety, and estimated duration of the two different methods for DNA extraction from fresh rat blood.

	Concentration (ng/µl)	$A_{260/280}$	$A_{260/230}$	Yield (µg) per 100 µl of blood	Enzymes	Chloroform	Duration (h)
	CTAB method				no	yes	~ 1
Sample 1	456	1.77	2.07				
Sample 2	1577	1.8	2.31				
Sample 3	1093	1.81	1.93				
Sample 4	1395	1.82	1.9				
Mean ± S.E.M.	**1130.25 ± 245**	**1.8 ± 0.01**	**2.05 ± 0.09**	**22.6**			
	SE method (digestion for 1 h at 65 °C)				yes	no	~ 3.5
Sample 1	40	1.76	1.57				
Sample 2	38	1.66	2.1				
Mean ± S.E.M.	**39 ± 1**	**1.71 ± 0.05**	**1.83 ± 0.26**	**0.39**			
	(digestion overnight at 37 °C)						~ 16
Sample 3	220.9	1.51	1.12				
Sample 4	252.4	1.41	1.2				
Mean ± S.E.M.	**236.65 ± 15.75**	**1.46 ± 0.05**	**1.16 ± 0.04**	**2.37**			

3 000 rpm for 10 min, precipitated protein pellets were left at the bottom while supernatants containing DNA were transferred to the new tubes. Ice-cold absolute ethanol was added in a double volume and, after centrifugation at 13 000 rpm for 20 min at +4 °C, supernatants were discarded and pellets were washed with 1 ml of 70% ethanol. After final centrifugation under the same conditions, the pellets were air-dried at RT.

Spectrophotometric analysis – concentration and purity determination

Concentration and purity of extracted DNA samples were determined by BioSpectrometer (Eppendorf, Germany). Two microliters of each DNA sample was mixed with 48 µl of 1 × TE buffer and put in a quartz cuvette. Absorbance was measured at a wavelengths of 260, 280 and 230 nm (A_{260}, A_{280} and A_{230}) which are max absorption points for bases in nucleic acids, proteins and polyphenols/polysaccharides, respectively. DNA was quantified by measuring UV absorbance at 260 nm, while the purity of genomic DNA was evaluated on the basis of UV absorption ratios at 260 and 280 nm (A_{260}/A_{280}) and 260 and 230 nm (A_{260}/A_{230}).

Agarose gel electrophoresis – integrity determination

The quality and integrity of all DNA samples were assessed by agarose gel electrophoresis. About 650 ng of the isolated DNA was analyzed on 0.8% agarose gel (Applied Biosystems, USA) in 1 × Tris-Borate-EDTA buffer containing 0.5 µg/ml ethidium bromide, under conditions: 80V and 120 mA for one hour. The bands were visualized using ChemiDoc XRS UV transilluminator (BioRad, USA).

Results

The two different DNA extraction methods were analyzed according to the following criteria: yield, purity, quality, environmental safety, time and cost efficiency.

DNA yield rates and purity

Table 1 summarizes the yield and purity ranges of DNA samples extracted by two different methods. CTAB method resulted in high mean yield of DNA (1130.25 ng/µl; 22.6 µg of DNA per 100 µl of blood). The DNA yields for the SE method were dependent on duration of digestion step. Samples 1 and 2 were incubated in digestion buffer just 1 hour at 65 °C while samples 3 and 4 were digested overnight at 37 °C. Prolonged digestion yielded 236.65 ng/µl DNA (2.37 µg of DNA per 100 µl of blood), while approach with short digestion resulted in approximately six times lower mean DNA yield (39 ng/µl; 0.39 µg of DNA per 100 µl of blood). According to data from the literature, it is common to

extract approximately 2 μg of genomic DNA per 100 μl of rat/mouse whole blood by employing various extraction methods (Hofstetter et al., 1997; Xing et al., 2007; Chacon-Cortes, 2012) and about 6 μg of DNA by using commercial kits (Noeth & Dasovich-Moody, 1997; Chacon-Cortes et al., 2012).

A260/A280 and A260/A230 absorbance ratios were employed to evaluate the purity of extracted DNA samples. According to Sambrook et al. (1989) pure DNA extracts have 1.8 < A260/A280 < 2 and A260/A230 > 2. The data in Table shows that there are some differences in DNA extracts' purity dependent on the method of extraction. The CTAB method produced A260/A280 ratios ranged from 1.77 to 1.82, with a mean value of 1.8. Such results indicate the pure DNA samples with no protein or RNA contaminations. Furthermore, A260/A230 ranged from 1.9 to 2.31, with a mean value of 2.05, indicating the high purity of samples 1 and 2. Samples 3 and 4 had slight lower A260/A230 ratios which means certain contamination with carbohydrates, salts or organic solvents. On the other side, SE method produced DNA samples with poor A260/A280 and A260/A230 ratios. All DNA samples have been contaminated with proteins, salts or organic solvents. Sample 3 was the only one which met criteria A260/A230 > 2, but had a poor A260/A280 ratio of 1.66 indicating protein contamination.

The two techniques were found to be very different in terms of yield and purity of DNA recovered from blood. CTAB method resulted in much higher yield of pure DNA compared to SE method which produced DNA samples of poor yield and purity. Since source effect was reduced by using the same samples, variations in yield and purity can be attributed to the effects of the extraction methods. High DNA yield and good purity obtained by CTAB method were previously described in the literature for a variety of extraction sources (Jasbeer et al., 2009; Chen et al., 2010; Filho & Almeida, 2013). On the contrary, salting out method often results in an insufficient purity of DNA extracts (Filho & Almeida, 2013). To improve purity, Sambrook et al. (1989) suggested taking a special care when dispart supernatant, containing DNA, from the sediment, containing so many contaminants. Also, in a case of contamination of DNA extract with salts, it is suggested to re-precipitate DNA with ethanol.

Gel electrophoresis analysis of isolated DNA

The quality and integrity of genomic DNA samples isolated by two different methods were examined by 0.8% agarose gel electrophoresis. This technique has been widely used for determining the size of DNA samples (Zimmermann et al., 1998). As can be seen in Figure 1, the main bands of DNA were slow-migrating and located high above 3 kb. All samples contained high-molecular weight DNA with no low-molecular weight smear tails. Although all lines should contain the same quantity of DNA (650 ng), the lane 4 in SE method had much lower DNA content on the gel (**Fig. 1**). One possible reason could be over-estimation of DNA concentration in the spectrophotometric analysis. Proteins, RNA, salts, lipids and other contaminants can increase the spectrophotometric estimation of DNA concentration (Haque et al., 2003). Except RNA molecules, the other contaminants can not be visualized on gel by staining with ethidium bromide, but one can notice the lower amount of DNA on a gel in regard to the expected amount. The data from DNA agarose gel electrophoresis supplement the data from absorbance readings indicating compatibility of the two techniques.

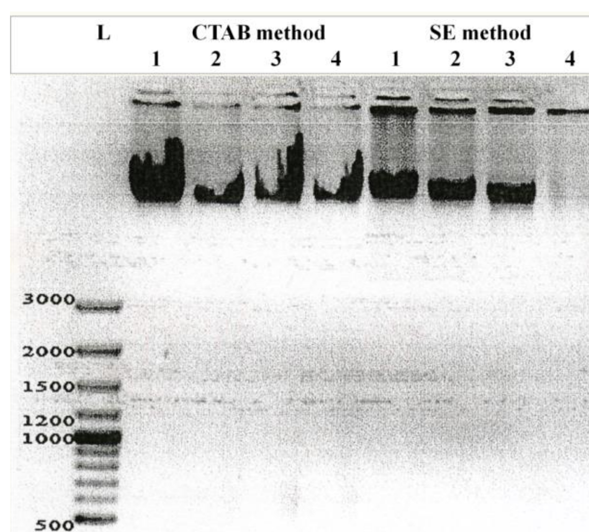

Fig. 1. Agarose gel electrophoresis of total genomic DNA samples isolated from rat fresh blood by two different extraction methods. L – 100 bp DNA ladder (Fermentas).

Environmental safety, time and cost efficiency

The CTAB method required approximately 1 h compared to 3.5 h or overnight incubation required for the SE method. Moreover, the use of proteinase K makes SE method less cost-effective than CTAB method. Despite time and cost efficiency, CTAB method employs chloroform, well-known hazardous organic solvent.

Conclusion

Yield, purity and quality of DNA extracted from rat whole blood depend greatly on the extraction

method. CTAB method provided a high yield of pure DNA and, according to time and cost efficiency, this method is suitable for simultaneous processing of a large number of samples. Salting out extraction method yielded several times lower amount of DNA of unsatisfactory purity and along with special care needed during execution procedure to avoid contamination, this method is much less efficient compared to CTAB method.

Acknowledgements. This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant OI 173020.

References

Aljanabi, S.M., Martinez, I. 1997: Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research*, 25 (22): 4692-4693.

Angelini, A., Di Febbo, C., Rullo, A., Di Ilio, C., Cuccurullo, F., Porreca, E. 2002: New method for the extraction of DNA from white blood cells for the detection of common genetic variants associated with thrombophilia. *Pathophysiology of Haemostasis and Thrombosis*, 32 (4): 180-183.

Chacon-Cortes, D., Haupt, L., Lea, R., Griffiths, L. 2012: Comparison of genomic DNA extraction techniques from whole blood samples: a time, cost and quality evaluation study. *Molecular Biology Report*s, 39: 5961-5966.

Chen, H., Rangasamy, M., Tan, S.Y., Wang, H., Siegfried, B.D. 2010: Evaluation of five methods for total DNA extraction from Western corn rootworm beetles. *PLoS ONE*, 5 (8): 1-6.

Dahm, R. 2008: Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. *Human Genetics*, 122 (6): 565–81.

Desloire, S., Valiente Moro, C., Chauve, C., Zenner, L. 2006: Comparison of four methods of extracting DNA from *D. gallinae* (Acari: Dermanyssidae). *Veterinary Research*, 37 (5): 725-732.

Filho, E.S., Almeida, T. 2013: Optimized protocols for extraction of DNA in plant and blood tissues. *International Journal of Scientific Research*, 2 (11): 57-58.

Gustincich, S., Manfioletti, G., Del Sal, G., Schneider, C., Carninci, P. 1991: A fast method for high-quality genomic DNA extraction from whole human blood. *BioTechniques*, 11 (3): 298-301.

Haque, K.A., Pfeiffer, R.M., Beerman, M.B., Struewing, J.P., Chanock, S. J., Bergen, A.W. 2003: Performance of high-throughput DNA quantification methods. *BMC Biotechnology*, 3: 20.

Hofstetter, J.R., Zhang, A., Mayeda, A.R., Guscar, T., Nurnberger, J.I., Lahiri, D.K. 1997: Genomic DNA from Mice: A comparison of recovery methods and tissue sources. *Biochemical and molecular medicine*, 62: 197-202.

Jasbeer, K., Son, R., Ghazali, M.F., Cheah, Y.K. 2009: Real-time PCR evaluation of seven DNA extraction methods for the purpose of GMO analysis. *International Food Research Journal*, 16: 329-341.

Kovačević Grujičić, N., Davidović, S., Takić, D., Mojsin, M., Stevanović, M. 2012: Direct PCR amplification of the HVSI region in mitochondrial DNA from buccal cell swabs. *Archives of Biological Sciences*, 64 (3): 851-858.

Lewis, C.M., Cler, L.R., Bu, D.W., Zöchbauer-Müller, S., Milchgrub, S., Naftalis, E.Z., Leitch, A.M., Minna, J.D., Euhus, D.M. 2005: Promoter hypermethylation in benign breast epithelium in relation to predicted breast cancer risk. *Clinical Cancer Research*, 11 (1): 166-172.

Miller, S.A., Dykes, D.D., Polesky, H.F. 1988: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, 16 (3): 1215.

Milligan, B.G. 1998. Total DNA isolation. In: Hoelzel, A.R. (ed.), Molecular Genetic Analysis of Population: A Practical Approach 2: 29–64, Oxford, New York, Tokyo: Oxford University Press.

Noeth, L., Dasovich-Moody, M. 1997: Use of the DNA Isolation Kit for Mammalian Blood for the Purification of Genomic DNA. *Biochemica*, 1: 25-27.

Olerup, O., Zetterquist, H. 1992: HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSCP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens*, 39 (5): 225-235.

Phillips, H.A., Howard, G.C.W., Miller, W.R. 2000: P53 Mutations as a marker of malignancy in bladder washing samples from patients with bladder cancer. *British Journal of Cancer*, 82 (1): 136-141.

Sambrook, J., Fritsch E.F., Maniatis T. 1989: In molecular cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Tanriover, M.D., Tatar, G.B., Uluturk, T.D., Erden, D.D., Tanriover, A., Kilicarslan, A., Oz, S.G., Yurter, H.E., Sozen, T., Guven, G.S. 2010: Evaluation of the effects of vitamin D receptor and estrogen receptor 1 gene polymorphisms on bone mineral density in postmenopausal women. *Clinical Rheumatology*, 29: 1285-1293.

Thomas, J.C., Khoury, R., Neeley, C.K., Akroush, A.M., Davies, E.C. 1997: A Fast CTAB Method of Human DNA Isolation for Polymerase Chain Reaction Applications. *Biochemical Education*, 25 (4): 233-235.

Wang, S.S., Thornton, K., Kuhn, A.M., Nadeau, J.G., Hellyer, T.J. 2003: Homogeneous real-time detection of single-nucleotide polymorphisms by strand displacement amplification on the BD ProbeTec ET System. *Clinical Chemistry*, 49 (10): 1599-1607.

Xing, C., Dafu, C., Changchun, L., Hui, L., Weixing, Z. 2007: Sample pretreatment microfluidic chip for DNA extraction from rat peripheral blood. *Frontiers of Chemistry in China*, 2 (1): 74-78.

Zimmermann, A., Lüthy, J., Pauli, U. 1998: Quantitative and qualitative evaluation of nine different extraction methods for nucleic acids on soy bean food samples. *Zeitschrift fur Lebensmitteluntersuchung und - Forschung A*, 207: 81-90.