ANALYSIS OF DNA ISOLATION EFFICIENCY DEPENDING ON THE TYPE OF BIOLOGICAL MATERIAL COLLECTED FROM RABBITS

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Abstract

The aim of the study was to examine the efficiency of DNA isolation in Termond White rabbits depending on the type of collected biological material. The experiment was conducted in the Experimental Station of the Department of Genetics, Animal Breeding and Ethology of the University of Agriculture in Kraków. The research material was blood, hair, urine and saliva collected from six male Termond White rabbits. Saliva and hair were collected ante-mortem from animals at 84 days. Blood and urine were collected during post-mortem treatment. DNA isolation was done using the commercial GeneMATRIX Tissue DNA Purification Kit (EURx). The isolated DNA was evaluated using a UV-VIS spectrophotometer at a wave-length of 260 and 280 nm. The purity and content of the tested DNA were determined. Studies have shown that the type of biological material affects the content and purity of DNA. The highest content of DNA was in samples isolated from blood. The highest purity was detected in DNA preparations isolated from urine.

Keywords: rabbit, DNA, isolation

Introduction

DNA isolation and purification is a key stage in most procedures used in molecular biology. These stages are designed to produce high-quality and high-purity biological material to be used for further analysis (Stryer, 1999). The first method of nucleic acid isolation is the procedure for 'nuclein' isolation developed by Miescher in 1869. It is divided into two steps of cell preparation and isolation, preceded by the extraction of cell nuclei (Gabryelska et al., 2009). Nowadays, there are many methods of nucleic acid isolation, with new and more sophisticated procedures being constantly developed. Also, efforts are underway to perform as many analyses as possible at the same time (Kapińska and Szczerkowska, 2004; Słomski, 2008).

Currently, DNA isolation methods can be divided into three groups. In the first group, a phenol-chloroform mixture is used to deproteinise preparations for DNA isolation. In the second group, a salting-out procedure is used on proteins from the resulting cell lysates. The third group of methods uses a suitable carrier to bind DNA and then remove any contamination and release the bound DNA. Many scholars, including Ryszard Słomski, the author of a book entitled *DNA Analysis* (2008), believe that the phenol-chloroform method continues to be one of the most effective ways to isolate DNA. It produces high-purity preparations that can be successfully used for further analysis, cloning or PCR reactions.

Pursuant to the Act of 15 January 2015 (Journal of Laws, 2015) on the protection of animals used for scientific or educational purposes, Local Ethics Committee approval is not required for scientific experiments involving "activities that, according to veterinary art, do not cause the animal pain, suffering, distress or permanent body damage equal to or worse than that caused by a needle prick."

The law restricts the range of procedures that can be performed on animals. The resulting problem is that biological material samples cannot be collected from live animals. Efforts are underway to find alternative methods of sourcing research material. Therefore, the objective of this study was to investigate the efficiency of DNA isolation from Termonde White rabbits, using various types of biological material samples.

Material and methods

The experiment was conducted at the Experimental Station of the Department of Animal Genetics, Husbandry and Ethology of the University of Agriculture in Kraków. The experiment material consisting of blood, hair, urine and saliva was collected from six male Termode White rabbits. For the first 35 days of life, the rabbits were housed with their mothers in metal cages equipped with a nesting box. The hall where the animals were housed had access to drinking water (nipple drinkers) and lighting (14L:10D) as well as forced ventilation. From weaning on day 35 until day 84, the animals were kept in the same hall in litterless metal battery cages designed for commercial rabbit rearing. Before and after separation from their mothers, the rabbits were fed *ad libitum* with a full-ration commercial pelleted feed, containing 10.2 MJ of metabolic energy, 16.5% total protein and 14% crude fibre. Such feeding covered the nutritional requirements of the animals as specified in the dietary standards (Gugołek et al., 2011).

On the day of slaughter, a saliva sample was taken from each rabbit by swabbing its mouth with a wooden swab stick (Polnet, Poznań). Samples of hairs with follicles were also collected from the same animals. Both saliva and hair samples were placed in plastic centrifuge tubes.

The rabbits were slaughtered and subjected to post-slaughter processing at 12 weeks of age, at a body weight of approximately 2.6 kg, after 24 hours of starvation with constant access to drinking water. The animals were stunned, bled, skinned and eviscerated. At the time of bleeding, blood was drawn from the rabbits into 2 ml Eppendorf tubes to which ethylenediaminetetraacetic acid (EDTA) had previously been added. After skinning, but before evisceration of the carcasses, urine was collected from the rabbits' urinary bladders using 2 ml disposable syringes and then also placed in 2 ml Eppendorf tubes. All the samples were packed into a collective bag and then frozen for 72 hours at -18°C.

DNA isolation from tissue samples was carried out, using the GeneMATRIX Tissue DNA Purification Kit (EURx).

DNA isolation from blood

From each sample collected, 200 µl of blood was measured out and transferred to 1.5 ml Eppendorf tubes. After a five-minute incubation at room temperature, 10 µl of proteinase K was added to the blood.

DNA isolation from saliva

The cotton wool was carefully separated from the wooden part of the swab stick and then transferred to a 1.5 ml Eppendorf tube. To the samples, 350 µl of lysis buffer T and 20 µl of proteinase K were added. Samples were placed on a heating block set at 56°C and incubated for approximately one hour.

DNA isolation from urine

Approximately 1 ml of urine from each collected sample was measured out and transferred to 1.5 ml Eppendorf tubes. The samples were then centrifuged and the resulting supernatant was separated from the pellet. To the separated cells, 350 µl of lysis buffer T and 10 µl of proteinase K were added. The prepared samples were incubated at room temperature.

DNA isolation from hair

Follicles were separated from the hairs and transferred to 1.5 ml Eppendorf tubes. To the prepared hairs, 350 µl of lysis buffer T, 20 µl of proteinase K and 20 µl of 1M DTT were added. The samples were incubated for approximately one hour at 56℃.

After the prior preparation of the samples, buffer T solution was added to each tube and then incubated for 10 minutes at 70℃. After incubation, 96% ethanol was added to the tubes, centrifuged and then successively transferred to a silica bed column. Multiple column centrifugations were performed, alternating with washing the silica beds with wash buffers to purify the genomic DNA from previously used chemicals such as ethanol. Purified DNA was eluted using an elution buffer.

The isolated DNA was assessed using a UV-VIS spectrophotometer at wavelengths of 260 and 280 nm. The absorbance ratios of A260 to A280 allowed determining DNA purity and content.

Statistical analysis was performed using the SAS statistical package (2014). A single-factor analysis of variance was performed, with the type of collected biological material as the experimental factor. The significance of differences between the resulting averages was tested using the Tukey test.

Results

As shown in Table 1, the studied biological materials differ in the amount of DNA. Significant differences in the amount of DNA were found between samples isolated from blood and urine, urine and hair, and hair and saliva. Blood and hair did not differ in the amount of isolated DNA. When using the GeneMATRIX Tissue DNA Purification Kit (EURx), the highest values for the amount of DNA can be obtained for DNA isolated from blood. The lowest values were obtained for DNA isolated from urine and saliva.

The low values were due to the low cellular content of the two studied biological fluids. Saliva is largely composed of water (94-99.5%) and a solid remainder, with the main organic components being protein substances such as enzymes, blood serum proteins, mucins, immunoglobulins, blood group substances, kallikrein and lactoferrin. None of these substances contain genetic material, so DNA can only be extracted from exfoliated oral epithelial cells (Szydlarska et al., 2008). A similar difficulty arises when isolating DNA from urine. The organic substances found in urine include albumin, urea, ammonia, creatinine and glucose. As with saliva, none of the above substances carry genetic material. Therefore, in the case of physiological urine, only the exfoliated epithelial cells in the urinary tract can be a source of nucleic acids (Okrągła et al., 2014).

Table 1. The content of isolated DNA depending on the type of biological material tested

Materiał biologiczny Biological material	Ilość DNA (ng/µl) Amount of DNA (ng/µl)		
	Średnia Mean	SD	Min-Max
Krew Blood	55.86 [a]	66.00	14.25 – 192.62
Mocz Urine	5.35[b]	2.24	3.05 – 10.20
Ślina Saliva	8.37[b]	3.99	4.12 – 14.42
Włosy Hair	37.37[a]	21.54	8.72 – 61.06

Explanation: a, b – means marked with different letters are significantly different (P≤0.05)

Large specimen-level differences were observed in the amount of DNA extracted from each type of tissue. The greatest differences emerged in DNA isolated from blood, with the highest DNA content index standing at an exorbitant 192.62 ng/µl and the lowest value only 15.66 ng/µl. The most homogeneous results can be reported for the group with DNA isolated from urine and saliva. After analysing the results for different biological materials in terms of DNA quantity, none of the tested rabbits was found to show the highest or lowest DNA concentration in all the collected sample materials. More reliable results could be produced by repeating the experiment with a larger test group, as this could rule out any individual factors. Repeated analyses based on other tissues (for example, liver or kidney) could provide a great deal of new information to help confirm or rule out the findings of this paper.

Statistical analysis showed that the type of sampled biological material significantly influenced DNA purity. Statistically significant differences in that regard were found between blood and urine, urine and hair, urine and saliva as well as hair and saliva. Statistical analysis showed no significant differences between blood and hair or blood and saliva.

The spectroscopic properties of macromolecules allow determining the approximate purity of nucleic acid preparations. Purity is determined by making calculations based on the absorbance ratio values at wavelengths of 260 nm and 280 nm (A260/A280). Contamination-free double-stranded DNA (dsDNA) has an A260/A280 ratio value of 1.8. A value greater than 1.8 indicates RNA contamination, while a value less than 1.8 suggests DNA contamination with proteins (biochigen.slam.katowice.pl/praktikum/013.pdf).

Table 2 shows that the mean value closest to 1.8 was obtained by isolating DNA from urine. Also in this group, the greatest differences showed up between the results (lowest value - 1.20, highest value - 2.44). Comparable discrepancies were seen in the group where DNA was isolated from blood. The heterogeneity of the results for the 260/A280 absorbance ratio in both groups may be due to the poorly selected DNA isolation kit. The GeneMATRIX Tissue DNA Purification Kit (EURx) is not designed for use on fresh material and performs better when DNA is isolated from stains of biological origin, such as blood or semen stains.

Table 2. Purity of isolated DNA depending on the type of biological material tested

Materiał biologiczny Biological material	Stosunek absorbancji A260/A280 Absorbance ratio A260/A280		
	Średnia Mean	SD	Min-Max
Krew Blood	1.28[a]	0.28	0.93 – 1.66
Mocz Urine	1.68[b]	0.46	1.20 – 2.44
Ślina Saliva	1.37[b]	0.14	1.24 – 1.64
Włosy Hair	1.18[a]	0.21	1.02 – 1.60

Explanation: a, b – means marked with different letters are significantly different (P≤0.05)

The most reproducible results were obtained for the group with DNA isolated from saliva, but the A260/A280 absorbance ratio values were nowhere near 1.8. They averaged at 1.37, which may indicate contamination of the preparations with protein. Also in the case of DNA isolated from hair, it can be seen that it has been contaminated, with an average ratio value of 1.18.

Discussion

No research could be traced in the Polish or global literature regarding a comparison of differences in the efficiency of methods of DNA isolation from rabbits for different types of biological material samples. Therefore, it is impossible to offer any comparison of the findings of this study with any other results for that particular animal species.

Genomic DNA isolation methods were addressed by Ghatak et al. (2013). The researchers analysed the preparations obtained by DNA isolation from human blood, saliva, urine and hair. In their experiment, the following average DNA contents were reported for each sample group: blood - 86 ng/µl, saliva 83 ng/µl, urine - 31 ng/µl, hair - 49 ng/µl. As in our own study, the lowest DNA content was obtained by isolating DNA from urine. All the values reported by the cited authors were higher than those for isolated rabbit DNA. In a study by Prośniak et al. (2006), the QuantiBlot method was used to compare the efficiency of different methods of DNA isolation from stains of human semen, blood and saliva. Various isolation methods were used in the experiment, including commercial nucleic acid isolation kits (Fast DNA, Sherlock, DNeasy, Wizard Genomic Purification Kit-Promega). DNA was isolated from human blood with average amounts of 11.25 ng/µl, 27.00 ng/µl, 90.00 ng/µl, 2.44 ng/µl, respectively. The Sherlock kit and Wizard Genomic Purification kit were used to isolate DNA from human saliva. The average values obtained were: 2.06 ng/µl and 0.37 ng/µl. In comparison with our own results, the contents of DNA isolated from blood were similar, whereas the amount of DNA obtained from saliva in the experiment by Prośniak et al. (2006) was significantly lower. Małodobra et al. (2011) used an automated Janus station to test the performance of three commercial kits for DNA and RNA isolation from varied clinical material and physical evidence. In their experiment, DNA was isolated from frozen whole human blood and had an average concentration of 14.7 ng/µl. In our own study, higher average DNA amounts were obtained. Di Pietro et al. (2011) carried out an experiment in which they isolated genomic DNA from whole human blood stored at -20°C. DNA was isolated in two ways: using a phenol-chloroform mixture and using silica bed columns. The average DNA content isolated by the column method stood at 10.85 ng/µl and was lower than in our own study.

In their research on methods of isolating human genomic DNA, Ghatak et al. (2013) also determined the quality of the deoxyribonucleic acid obtained. In their study, they analysed DNA samples from blood, hair, urine and saliva. The average absorbance values were 1.86; 1.72; 1.48 and 1.57, respectively. In contrast to the results in our own experiment, DNA obtained from urine had the lowest purity. DNA isolated from hair had a high absorbance ratio value, while DNA preparations from rabbit hair were characterised by low purity.

When investigating the efficiency of isolation of genomic DNA from human whole blood stored at -20°C by the phenol-chloroform method and using silica bed columns, Di Pietro et al. (2011) analysed the purity of the DNA. The A260/A280 absorbance ratio for DNA isolated by the column method averaged at 1.98. The DNA in the study by Di Pietro et al. (2011) was less contaminated and qualitatively superior to DNA from rabbit blood.

In Salazar et al. (1998), a comparison was made between the quality of DNA isolated from fresh blood with EDTA, clotted blood and cryopreserved human blood. Blood with the addition of EDTA had an average absorbance ratio of 1.96. Compared to isolated rabbit DNA, DNA isolated from human blood showed better quality and purity.

The experiment conducted by Małodobra et al. (2011) to test the efficiency of three commercial DNA and RNA isolation kits showed that the A260/A280 absorbance ratio for frozen human whole blood averaged at 1.04. The result is not close to the ideal A260/A280 value, but is comparable to the results obtained in our own study.

Summary

Under the above-mentioned law on the protection of animals used for scientific or educational purposes, any activity causing pain, suffering, distress or permanent bodily damage to animals, equivalent to or worse than a needle prick require approval from the Local Ethics Committee. Our own research has confirmed that DNA preparations obtained from alternative biological materials have a lower DNA content than those isolated from blood, which is the typical biological fluid commonly used in scientific research.

Despite the existing possibility of obtaining nucleic acids from unconventional biological materials that do not affect the welfare of the animal when collected, biological material obtained by an invasive method (in this case, blood) offers the best yield.

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