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Extraction, Quantitation and Evaluation of Genomic DNA from Two Rat Tissues

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Background:Genomic DNA extraction for genotyping of rat is a common procedure in animal facilities. A suitable method of obtaining this DNA must not cause undue distress to the animal. Tail tip appears to be the most common sampling method for DNA extraction, both in young and adult rats. The aim of this study was to compare the quantity and quality of DNA extracted from tail tip and blood samples obtained from rats.Methods:Samples were collected from the tail and blood from seven rats aged 3.5 months and weighting 150-200 gram. DNA was isolated using commercial kits and concentrations and purity were determined by NanoDropLite spectrophotometry. The integrity of DNA was evaluated by agarose-gel electrophoresis.Results:DNA in all samples was extracted successfully but the intensities of bands after electrophoresis were heterogeneous. In general, DNA obtained from tail tip was more than that obtained from blood, with differences being not statistically significant (55.5± 26.40 ng/μL for tail tips; 46.6± 21.74ng/μL for blood; P= 0.2). The DNA purity (OD260/OD280) of DNA obtained from blood samples was slightly better than that obtained from tail tips, with differences being not statistically significant (1.87±0.09 for blood; 1.81±0.05 for tail tip; P= 0.1).Conclusions:Adequate amount and high-quality of DNA were obtained from blood and tail tissue of rats. These results support the previous recommendations for collection of minimal lengths of tail tissue from rats, making this method more suitable for the extraction of DNA from rat.

Keywords: DNA extraction, Rat tail,Whole blood, Genomic DNA,QIAamp DNA Blood Mini Kits.

1. Introduction

Over the last decade, breakthrough technologies in transgenic animal technology and functional genomics have contributed dramatically to important molecular, genetic and disease-related discoveries that might not otherwise have been possible and played an essential role in the explosive growth of rodent modeling and in scientific invention. The objective of medical research is to improve human health and to find treatments and drugs for diseases that cause suffering to many people (Schaefer et al., 2010 and Castelhano-Carlos et al., 2010). To determine whether rodents are of the suitable genotype for research studies, a sufficient number of cells or a piece of tissue must be sampled from tissue of rodents in order to isolate genomic DNA (Hankenson et al., 2008, and Cinelli et al., 2007). A large amount of genomic DNA is not required, it is therefore not substantial to perform large surgical operations, such as partial hepatectomy, to secure tissue samples. (Al-Griw et al., 2017 ,1993, and Pereira et al., 2016). The quality and quantity of the DNA obtained from blood samples is usually good, but sampling requires a certain kind of procedures may cause pain, some tissue damage and/or discomfort to the animals. The most widely used methods for obtaining the living tissue from animals are commonly collected by invasive methods, such as cutting a small piece of the tip of the tail, ear, or toe biopsies. These methods of tissue collection are standard around the world, and are used because they are considered to be the best for the animal and for the purposes of research. The method of taking tissue samples in this way has been evaluated many times, and is recommended by European organisations for laboratory animal science as causing the least suffering to the animals. (Picazo et al., 2015, Schaefer et al., 2010 , Castelhano-Carlos et al., 2010, and Shirota et al., 2017). Obvious guidelines have not been confirmed for the minimal length of tail tissue to cutting and ideal age of animal to sample in order to maximize DNA quantification and minimize adverse physiologic effect. Depending on the length of tail tissue removed, the animal's age at the time of biopsy and its genetic background, this procedure may carry a chance for acute and chronic pain (Hankenson et al., 2008, Murphy ,1993, Morales et al., 2009, and Picazo et al., 2015). According to the National Institutes of Health, “Obtaining tissue from a mouse or rat for DNA analysis by tail biopsy (tail snip) procedure is a safe, effective and humane procedure that provides an adequate tissue sample with minimal or transient pain and distress when performed properly (Hankenson FC et al., 2008 and Picazo M. G..et al., 2015). In this work, we collected samples from the tail tip and sampled blood of the rats. We extracted DNA using commercial kits (QIAamp DNA Blood Mini Kits). We have determined and compared the genomic DNA concentrations and purity by Nanodrop spectrophotometry. The integrity of DNA was evaluated by agarose gel electrophoresis. The aim of present study was to compare the outcomes of DNA extraction from samples obtained from blood and tail tip of the ratsand looking for an efficient way with low health risks.

2. Material and methods:

Animals:All experiments were performed in accordance with the guidelines of the National Institutes of Health and The International Association for the Study of Pain and were approved by the Animal Experimentation Committees of National Center for Medical Research in Zawia

- (Zawia, Libya). All efforts were made to fulfil the ethical experimentation standards such as minimizing the pain during animal handling and experiments as well as reducing the number of animals used. Seven male albino rats, with an age range of three to four months and weight range of 150 to 200 g, were used in this study. They were bred in the animal house of the National Center for Medical Research in Zawia-(Zawia, Libya), and housed in a controlled environment .

The temperature in the room ranged from 20°C to 24°C, and the light/dark cycle was 12/12 h and 55 ± 5% relative humidity. During this period food and water were available ad libitum. At the age of three to four months, tail tips were performed. In the tail-biopsied rats, a segment from the tip of the tail was removed using a razor blade

Sampling: All samples were taken from rats at the same time. Rats were restrained by a caretaker, who held them firmly by the back of the neck. Then, the researcher collected samples sequentially, as follows: For each tail biopsy, a piece less than 1cm in length was cut from the tail tip with sharp surgical scissors into small pieces (~ 1gm) and stored immediately in sterile Eppendorf tubes at -20°C without using any preservative. Blood samples were obtained from rats by venipuncture (n=7) in 1.5 or 2 ml tubes containing EDTA and stored at -20 °C.

Genomic DNA extraction: All samples were stored at -20°C until analysis.

- Extraction of DNA from rat blood

DNA was isolated from animal blood using the commercial kits (QIAamp DNA Blood Mini Kits), according to manufacturer instructions. In this method proteinase K (20µL) and Buffer AL (200µL) were added to blood samples (200µL) and incubated at 56°C for 10min. Absolute ethanol (200µL) was added, and the mixtures were transferred to the QIAamp spin columns. These were centrifuged at 6,000g for 1 min, and then the waste tube was discarded. The columns were washed with 500µL of buffer AW1 and then again with 500µL of AW2. The purified DNA was eluted from the spin columns with 200µL of buffer AE into clean sterile microfuge tubes and stored at -20°C until further analysis.

- Extraction of DNA from rat tails

DNA was isolated from rat tail tip using the commercial kits (QIAamp DNA Blood Mini Kits), according to manufacturer instructions. In this method we were cut up to 25 mg of tail and placed in a microfuge tube, then Buffer ATL (180µL) and proteinase K (20µL) were added and incubated at 56°C for overnight while being constantly rotated until the tissue is completely lysed. Buffer AL (200µL) were added to samples (200µL) and incubated at 70°C for 10min.Absolute ethanol (200µL) was added, and the mixtures were transferred to the QIAamp spin columns. These were centrifuged at 6,000g for 1 min, and then the waste tube was discarded. The columns were washed with 500µL of buffer AW1 and then again The purified DNA was eluted from the spin columns with 200µL of buffer AE into clean sterile microfuge tubes and stored at -20°C until further analysis.

Evaluation of DNA Extraction

- Concentration and Purity Determination

The amounts of DNA isolated from the various samples were assessed using the Thermo Scientific™ NanoDrop Lite Spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE, USA). Absorbance was measured at wavelengths of 260 and 280 (A260 and A280, respectively) nm, the concentration of the sample was determined from the 260 nm absorbance reading and the purity from the 260/280 nm ratio. An absorbance quotient value of 1.8 < ratio (R) < 2.0 was considered to be good, purified DNA. A ratio of <1.8 is indicative of protein contamination, where as a ratio of >2.0 indicates RNA contamination.

- DNA Integrity determination

The quality and integrity of all genomic DNA samples were assessed by agarose gel by electrophoresis apparatus (Micro – Bio -Tec, Horisontal agarose).Each DNA sample was analyzed on 1% agarose gel in 1 × Tris Acetate-EDTA buffer containing 2µl SYBR® Gold Nucleic Acid Gel Stain, under conditions: 70V and 120 mA for one hour. The bands were visualized using MultiDoc-It™ Imaging System by UVP (Cambridge, UK). Each DNA sample was graded, according to the electrophoretic migration of sample DNA compared with a known molecular weight marker (Gelpilot 100 bp Ladder, QIAGEN), and the images were captured.

Statistical analysis:Means of amounts of DNA and ratios were compared by Paired t-Test: Two Sample for Means. P-values of less than 0.05 were considered evidence of statistical significance.

3. Results

Efficacy of DNA-extraction procedures

The extraction of DNA from blood samples took 40 min. By contrast, the isolation of DNA from the tail samples took 24h, including an overnight incubation. The final elution volume was 200 µL in the case of DNA extracted from the tail and blood samples. Detectable amounts of DNA were obtained from all samples (Table 1). In rats, the largest amounts were obtained from tail samples (55.5± 26.40 ng/µL; n = 7) and less amounts were obtained from blood samples (46.6± 21.74ng/µL; mean ± S.D. n = 7) as shown in (Fig. 1). Results for tail samples were not significantly different to those obtained from blood samples (P = 0.2). In general, samples from tail tip samples yielded more DNA than those from blood samples.

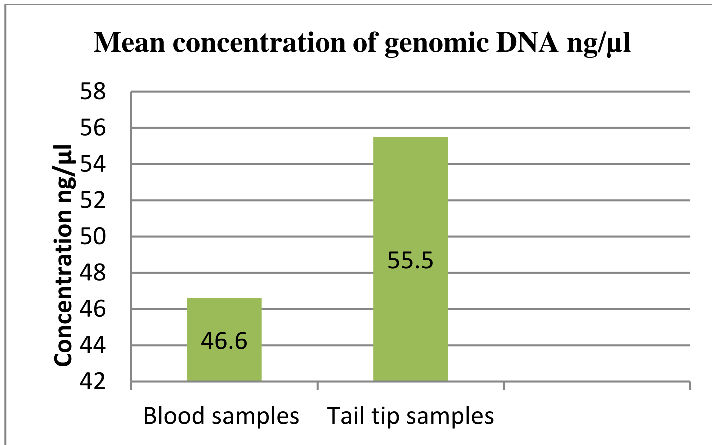

Fig.1. Histogram showing the mean concentrations of DNA obtained from samples from adult rats, as indicated. Values are means ± S.D. of results from 7 adult rats.

Pure DNA (A260/A280 ≈ 1.8) was obtained from samples of the tail and blood from rats. The purity of the DNA obtained from tail tip and from blood samples of rats was the highest (1.81 ± 0.05and 1.87 ± 0.09; n = 7 and n = 7, respectively) with no statistically significant difference for samples (P = 0.1) as shown in (Fig. 2).

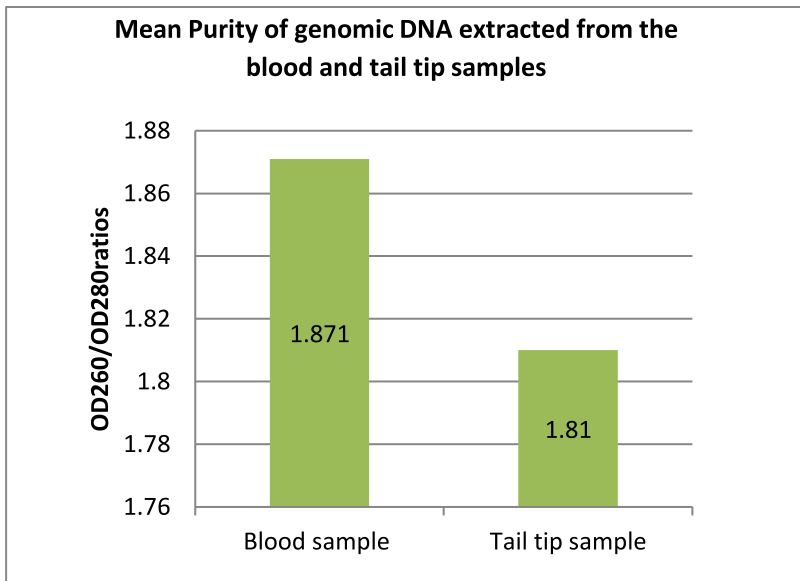

Fig.2. Histogram showing the mean purity of DNA obtained from samples from adult rats, as indicated. Values are means ± S.D. of results from 7 adult rats.

The following results indicate that high concentration and purity of DNA extracted from the methods reported as in table 1:

Table1:- The DNA concentration and purity was determined as:-

DNA Sample	Concentration ng/µl Blood sample	OD260/ OD280	Concentration ng/µl Tail tip sample	OD260/ OD280
1	81.8	1.96	93.9	1.79
2	49.5	1.90	34.5	1.81
3	63.2	2.00	49.6	1.74
4	14.3	1.80	36.6	1.82
5	47.1	1.92	31.8	1.78
6	35.8	1.75	91.4	1.87
7	34.2	1.79	50.6	1.87
Mean ±SD	($46.6\pm 21.7 ng\cdot\mu L^{-1}$)	(1.87 ± 0.09)	($55.5\pm26.4 ng\cdot\mu L^{-1}$)	(1.81 ± 0.05)

Integrity of the extracted DNA was assessed by agarose gel electrophoresis (Fig. 3). Gel electrophoresis revealed that most intense bands with high-molecular-weight non-degraded genomic DNA were observed in the case of DNA from tail. The less intense bands with high-molecular-weight degraded genomic DNA were observed in the case of DNA from blood.

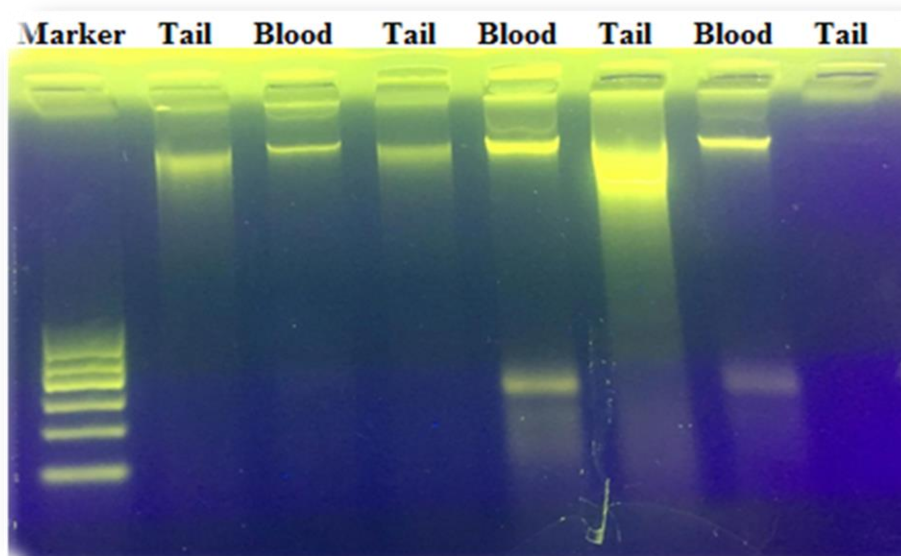

Fig. 3. Photographs of agarose gels showing the results of genomic DNA extraction obtained from samples from adult rats:. Lanes 1: 100-bp DNA ladder; lanes 2, 4, 6: samples from tail tips; lanes 3, 5, 7: samples from blood

4. Discussion

The decision as to the most appropriate method of sampling tissues for DNA extraction of rats will be achieved by choosing that method which has minimal impact on the animal and is suited to the scientific aims because most methods are invasive.However, non-invasive methods have also been described lately. Moreover, inadequate quantity and/or poor quality of DNA extracted for genotyping can be a problem if large amounts are desired and if animals

have to be subjected to repeat analyses (Picazo et al., 2015 and Benavides F et al., 2020),with many studies have recommended the use of mice or rats for genotyping, in particular if biopsies are taken from the tail or phalanx (Bonaparte et al., 2013 and Picazo et al., 2015).In a previous study, it has been showed that tail biopsy appears to be the most common sampling method for DNA extraction, both in young and adult rats (Bonaparte et al., 2013).

This study was designed to take the rat samples in different sampling methods, thus assuming different tissue states such as tail tips and blood, where samples can then be store at -20°c.Two sampling protocols have been compared in this study for DNA extraction from the animal tissues in respect to the quantity, quality and time consumed for the extraction. The Blood protocol required less time (40 to 60min), compared to the tail tip protocol, which required 24 hrs.Our results support the recommendations of previous studies since in general, biopsies from tail tip resulted in larger amounts of pure DNA in all cases (55.5± 26.40 ng/µL; n = 7). Indeed, commercial kit allows efficient isolation of good-quality DNA from small and varied samples. The major disadvantage of the tail tip is that it does not allow animals have to be subjected to repeat sampling for analyses.Extraction of DNA from blood samples from adult rats is quick and effective and provides high quality DNA (46.6± 21.74ng/µL; mean ± S.D. n = 7). This method has the advantage of being repeatable.However, proper training is required to avoid inaccurate puncture and/or hemorrhages. In the present study, the procedure appeared to be the most painful and lead to mortality for rats and the most uncomfortable for the researcher and results of this study revealed gradual degradation of intact nuclear DNA in the blood .Such results could be explained by the ability of the tail to hold intact DNA longer than the blood cells.

5. Conclusion

In conclusion, the results of the present study do support the use of tail tip methods for sampling to DNA extraction. The use of tail tissue samples showed the highest efficiency for DNA extraction with, possibly, the minimum pain and stress for animals.

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