

2014

DNA Extraction from Experimental Samples



Institute of
Biochemistry and
Biotechnology
UVAS, Lahore



Nexus Academic Publishers (NAP)
Lahore Pakistan

DNA Extraction from Experimental Samples

DNA Extraction from Experimental Samples

Institute of Biochemistry and Biotechnology,
University of Veterinary and Animal Sciences, Lahore



Nexus Academic Publishers (NAP)
Lahore Pakistan

Edited by

Ihsan Ullah
PhD Research Scholar,
Institute of Biochemistry and Biotechnology,
University of Veterinary and Animal Sciences, Lahore

Dr. Muhammad Yasir Zahoor
Assistant Professor,
Institute of Biochemistry and Biotechnology,
University of Veterinary and Animal Sciences, Lahore

Dr. Muhammad Imran
Assistant Professor,
Institute of Biochemistry and Biotechnology,
University of Veterinary and Animal Sciences, Lahore

Dr. Muhammad Nauman Zahid
Assistant Professor,
Quality Operations Laboratory,
University of Veterinary and Animal Sciences, Lahore

First Published in Pakistan in November 2014

The first edition published in 2014 by

Nexus Academic Publishers (NAP)

1-B Opposite School -4, Gate # 2, Old Officer Colony Zarar Shaheed Road, Sadar Lahore Cantt. Lahore, Pakistan

email: info@nexusacademicpublishers.com

All rights reserved No part of Publication may be reproduced graphically or electronically, including storage or retrieval system without prior written permission of the Publisher

Whilst the advice and information in this book are believed to be true and accurate at the date of going to press, neither the author(s) nor the publisher can accept any legal responsibility or liability for any errors or omissions that may be made. In particular (but without limiting the generality of the preceding disclaimer) every effort has been made to check information; however it is still possible that errors have been missed.

Nexus Academic Library Cataloguing in Publication Data

A catalogue record for this book is available for the Nexus Academic Publishers

Typeset in 8.5 Californian FB by Nexus Academic Publishers, Punjab, Lahore Cantt (Pakistan)

Printed and bound in Pakistan

What do you think about this book? Or any other Hodder Arnold title? Please send your comments to info@nexusacademicpublishers.com

Acknowledgement

This manual has been dedicated to the all students of University of Veterinary and Animal Sciences Lahore, Pakistan.

Contents

CHAPTER 1.....	1
Personal and General Laboratory Safety.....	1
DNA Extraction.....	1
DNA/RNA Extraction from Tissue via Trizol	3
DNA/RNA Extraction from Saliva	3
CHAPTER 2.....	4
Function of Different Reagents used for DNA Extraction from Blood	4
DNA/RNA Quantification by NanoDrop Spectrophotometer	4
Quantification of DNA by Gel Electrophoresis.....	4
Solutions Preparation.....	4

CHAPTER

1

Personal and General Laboratory Safety

- Never eat, drink, or smoke while working in the laboratory.
- Read labels carefully.
- Do not use any equipment unless you are trained and approved as a user by your supervisor.
- Wear safety glasses or face shields when working with hazardous materials and/or equipment.
- Wear gloves when using any hazardous or toxic agent.
- Clothing: When handling dangerous substances, wear gloves, laboratory coats, and safety shield or glasses. Shorts and sandals should not be worn in the lab at any time. Shoes are required when working in the machine shops.
- If you have long hair or loose clothes, make sure it is tied back or confined.
- Keep the work area clear of all materials except those needed for your work. Coats should be hung in the hall or placed in a locker. Extra books, purses, etc. should be kept away from equipment, which requires air flow or ventilation to prevent overheating.
- Disposal – Students are responsible for the proper disposal of used material, if any, in appropriate containers.
- Equipment Failure – If a piece of equipment fails while being used, report it immediately to your lab assistant or tutor. Never try to fix the problem yourself because you could harm yourself and others.
- If leaving a lab unattended, turn off all ignition sources and lock the doors.
- Never pipette anything by mouth.
- Clean up your work area before leaving.
- Wash hands before leaving the lab and before eating.

DNA Extraction

For the genomic DNA extraction either inorganic or organic method of DNA extraction is generally used, and the DNA extracted by using these methods can be PCR-amplified. Below are given protocols for both of these DNA extraction methods.

Inorganic Method of DNA Extraction from Blood

- Five mL blood samples are thawed, if frozen, either at room temperature or in water bath at 37 °C.
- Then 35 mL of chilled DNA lysis buffer (Tris-HCl 10 mM, EDTA 2 mM, pH 8.0) is added and mixed by inverting and vortexing the samples. 50 mL falcon tubes can be used as containers for this purpose.
- After this samples are centrifuged at 3750 rpm for 15–20 min at 4 °C.
- Then supernatant containing RBCs and other blood contents is discarded and the pellet containing white blood cells is broken by tapping and vortexing. And again lysis buffer is added up to 35

mL and mixed by inverting and vortexing the samples.

- Step number 3 and 4 are repeated until the white color of pellet is observed.
- After washing white pellet is broken by tapping and vortexing. Then 100 μ L of 10% SDS, 70 μ L proteinase K and 1 mL buffer A (Tris-HCl 10 mM, EDTA 2 mM, NaCl 40 mM, pH 8.0) are added and mixed by shaking. After shaking samples are incubated in water bath for overnight at 45 °C for the complete digestion of histones and other cellular proteins.
- After the overnight incubation 1 mL of 6M NaCl is added and mixed well in the samples which are then placed at a low temperature.
- Then these samples are centrifuged at 3750 rpm for 15 min at 4 °C and three layers appear from which two appear as more prominent and visible. The upper clear aqueous layer contains DNA and lower layer contains impurities and debris.
- Carefully supernatant is taken out with pipette in new tube with same label and pellet is discarded. Then equal volume of isopropanol is added in the tube and the mixture is inverted gently until DNA threads become visible. These DNA containing samples are kept at either room temperature or 4 °C for 20 min.
- Then centrifugation at 3750 rpm for 10 min at 4 °C is carried out and supernatant is discarded carefully.
- After that up to 5 mL of 70% ethanol is added to wash DNA pellet and samples are placed for 30 min to 1 h in chemistry shaker at room temperature. Then centrifugation is done at 3750 for 15 min at 4 °C.
- Then ethanol is discarded.
- The DNA pallet is air-dried at room temperature until the smell of ethanol disappears.
- Double distilled 500 uL water is used to dissolve DNA first by short spin then by keeping at room temperature or in shaker at 37 °C for 30 min.
- Then heat-shock is given in water bath at 70 °C for 30 min to inactivate nucleases.
- DNA is preserved in cryo vials labeled with specific code name and stored at -20 °C in refrigerator.

Organic Method of DNA Extraction from Blood

- Five mL blood samples are thawed, if frozen, either at room temperature or in water bath at 37 °C.

- Then 35 mL of chilled TE lysis buffer (Tris-HCl 10 mM, EDTA 2 mM, pH 8.0) is added and mixed by inverting and vortexing the samples. 50 mL falcon tubes can be used as containers for this purpose.
- After this samples are centrifuged at 3750 rpm for 15-20 min at 4 °C.
- Then supernatant containing RCBs and other blood contents is discarded and the pellet containing white blood cells is broken by tapping and vortexing. And again lysis buffer is added up to 35 mL and mixed by inverting and vortexing the samples.
- Step number 3 and 4 are repeated until the white color of pellet is observed.
- After washing white pellet is broken by tapping and vortexing. Then 100 μ L of 10% SDS, 70 μ L proteinase K and 1 mL TNE buffer (Tris-HCl 10 mM, EDTA 2 mM, NaCl 400 mM, pH 8.0) are added and mixed by shaking. After shaking samples are incubated in water bath for overnight at 45 °C for the complete digestion of histones and other cellular proteins.
- After incubation, equal volume of PCI (phenol, chloroform, isoamyl alcohol in a ratio of 25:24:1) and mixed gently.
- Then these samples are centrifuged at 3750 rpm for 15 min at 4 °C and three layers appear. The upper clear aqueous layer contains DNA, the middle layer proteins and the lower layer cellular debris and impurities.
- Carefully the upper layer is taken out with pipette in new tube with same label and the remaining two layers are discarded. Then equal volume of chilled isopropanol is added in the tube and the mixture is inverted gently until DNA threads become visible. These DNA containing samples are kept at either room temperature or 4 °C for 20 min.
- Then centrifugation at 3750 rpm for 10 min at 4 °C is carried out and supernatant is discarded carefully.
- After that up to 5 mL of 70% ethanol is added to wash DNA pellet and samples are placed for 30 min to 1 h in chemistry shaker at room temperature. Then centrifugation is done at 3750 for 15 min at 4 °C.
- Then ethanol is discarded.
- The DNA pallet is air-dried at room temperature until the smell of ethanol disappears.

- Double distilled 500 uL water is used to dissolve DNA first by short spin then by keeping at room temperature or in shaker at 37 °C for 30 min.
- Then heat-shock is given in water bath at 70 °C for 30 min to inactivate nucleases.
- DNA is preserved in cryo vials labeled with specific code name and stored at -20 °C in refrigerator.

DNA/RNA Extraction from Tissue via Trizol

- About 10–20 mg of fresh or ethanol-preserved tissue is cut with a calm scalpel or surgical blade.
- The tissue is grounded well in 1 mL liquid nitrogen (-191 °C). After grinding 1 mL of Trizol is added, vortexed and mixed until lysate becomes homogeneous. The lysate is then kept for 15 min at room temperature.
- After this centrifugation is done at 13500 rpm for 15 min at 4 °C and the supernatant is taken.
- Then centrifugation is done at 13500 rpm for 15 min at 4 °C after adding 250 uL chloroform. Three layers form: The upper layer contains RNA, the middle layer DNA and the lower layer proteins. For DNA the middle milkish layer is taken very carefully in another labeled eppendorf.
- For DNA isolation 100 uL of ethanol is poured, and centrifugation is then done at 13500 rpm for 15 min at 4 °C and the supernatant is discarded keeping the pellet which is air-dried on clean place for overnight so that ethanol is completely vaporized. The pellet is then dissolved in low TE buffer or double distilled water.
- For RNA isolation 500 uL or equal volume of isopropanol is poured, and centrifugation is then done at 13500 rpm for 15 min at 4 °C and the supernatant is discarded keeping the pellet. Then pellet is washed by adding 100 uL of absolute ethanol and centrifuging at 13500 rpm for 15 min at 4 °C. The supernatant is discarded keeping the pellet (RNA) which is air-dried on clean place for overnight so that ethanol is completely vaporized. The pellet is then dissolved in low TE buffer or double distilled water.

DNA/RNA Extraction from Saliva

- 5 mL saliva is taken when the donor has not eaten anything for 2–3 h.
- The saliva samples are kept at room temperature for 10–15 min after adding 5 mL Trizol.
- Then centrifugation is done at 13500 rpm for 15 min at 4 °C, the pellet is taken and supernatant is discarded.
- Then centrifugation is done at 13500 rpm for 15 min at 4 °C after adding 250 uL chloroform. Three layers form: The upper layer contains RNA, the middle layer DNA and the lower layer proteins. For DNA the middle milkish layer is taken very carefully in another labeled eppendorf.
- For DNA isolation 100 uL of ethanol is poured, and centrifugation is then done at 13500 rpm for 15 min at 4 °C and the supernatant is discarded keeping the pellet which is air-dried on clean place for overnight so that ethanol is completely vaporized. The pellet is then dissolved in low TE buffer or double distilled water.

For RNA isolation 500 uL or equal volume of isopropanol is poured, and centrifugation is then done at 13500 rpm for 15 min at 4 °C and the supernatant is discarded keeping the pellet. Then pellet is washed by adding 100 uL of absolute ethanol and centrifuging at 13500 rpm for 15 min at 4 °C. The supernatant is discarded keeping the pellet (RNA) which is air-dried on clean place for overnight so that ethanol is completely vaporized. The pellet is then dissolved in low TE buffer or double distilled water.

CHAPTER

2

Function of Different Reagents used for DNA Extraction from Blood

- Phenol/Chloroform is used to precipitate the proteins present along with nucleic acids in supernatant.
- Proteinase K degrades the proteins during digestion of white blood cells.
- Sodium dodecyl sulphate (SDS) is used to lyse cells and is a detergent by nature.
- Tris-EDTA buffer chelates divalent ions
- Absolute ethanol is used to precipitate DNA
- Main function of Tris-HCl is to adjust the pH of medium for DNA extraction.
- In presence of isopropanol, salts and ions present in DNA solution are removed.
- Isoamyl alcohol acts as antifoaming agent and prevents DNA shearing and damage.

DNA/RNA Quantification by NanoDrop Spectrophotometer

- First the blank reading of water or T.E. in which DNA is diluted is taken. Then 1 μ L from extracted DNA sample is taken with the help of pipette and placed on the pedestal of NanoDrop and concentration value is measured in ng/ μ L by computer at a 260/280 nm ratio of optical density.

Quantification of DNA by Gel Electrophoresis

Agarose gel (0.8%) is used to check DNA quality and quantity. Gel is prepared by following method.

- Agarose (0.8 g) is taken in a conical flask and dissolved in 100 mL of 1 \times TAE buffer. Then, flask is put in the microwave oven for 1 min. The agarose solution is then taken out to let it cool down at room temperature to 45 $^{\circ}$ C. About 12 μ L of ethidium bromide is added in the cooled agarose solution for DNA visibility.
- Agarose is mixed gently with ethidium bromide and poured into gel caster carefully to avoid bubble formation and then gel is allowed to solidify at room temperature. The solidified gel is dipped in TBE buffer contained in electrophoresis tank. 2 μ L DNA samples mixed with 3 μ L bromophenol blue (6 \times) of loading dye are loaded in wells made in agarose gel. Voltage of 120 volts is applied for 30 min after connecting gel apparatus to power supply. After the

run is completed, the DNA bands are seen under UV light by using Bio-Rad gel documentation system. All samples are brought at same concentration by dilutions.

After NanoDrop and gel imaging stock DNA is diluted to 25 ng/ μ L concentration.

Solutions Preparation

Following solutions are required for DNA extraction and are prepared using protocols given below:

- **Lysis buffer**
Dissolve 1 g potassium bicarbonate (KHCO_3), 8.29 g ammonium chloride and 200 μ L 0.5M EDTA in water and make the final volume of 1L.
- **EDTA**
Dissolve 18.62 g EDTA in water to make up the final volume up to 500 mL.
- **10N NaOH**
Dissolve 400 g NaOH in water to make final volume up to 1L.
- **6M Sodium Acetate**
First dissolve 492 g sodium acetate in water to make final volume up to 1L and then adjust the pH at 5.2 with acetic acid.
- **Buffer A**
Mix 5 mL (1M) Tris-HCl, 40 mL (1M) NaCl and 2 mL (0.5M) EDTA and adjust the final volume with water up to 500 mL.



- **Proteinase K (10 mg/ml)**

Dissolve 100 mg of proteinase K in 10 mL water and let it remain for 30 min at room temperature because it is self-digested. Store it at -20°C .

- **TNE Buffer**

Mix 5 mL (1M) Tris-HCl, 40 mL (6M) NaCl and 2 mL (0.5M) EDTA and adjust the final volume with water up to 500 mL.