Biotechnology Reports 12 (2016) 33–39

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Nucleic acid protocols: Extraction and optimization

Saeed El-Ashram[a,b,c,d,*], Ibrahim Al Nasr[e,f], Xun Suo[a,b,c,*]

[a] State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100193, China
[b] National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing 100193, China
[c] Key Laboratory of Animal Epidemiology and Zoonosis of Ministry of Agriculture, Beijing 100193, China
[d] Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt
[e] College of Science and Arts in Unaizah, Qassim University, Unaizah, Saudi Arabia
[f] College of Applied Health Sciences in Ar Rass, Qassim University, Ar Rass 51921, Saudi Arabia

ARTICLE INFO

Article history:
Received 11 May 2016
Received in revised form 25 September 2016
Accepted 3 October 2016
Available online 5 October 2016

Keywords:
Prokaryotic and eukaryotic sources
DNA
RNA
DNase
RNase

ABSTRACT

Yield and quality are fundamental features for any researchers during nucleic acid extraction. Here, we describe a simplified, semi-unified, effective, and toxic material free protocol for extracting DNA and RNA from different prokaryotic and eukaryotic sources exploiting the physical and chemical properties of nucleic acids. Furthermore, this protocol showed that DNA and RNA are under triple protection (i.e. EDTA, SDS and NaCl) during lysis step, and this environment is improper for RNase to have DNA liberated of RNA and even for DNase to degrade the DNA. Therefore, the complete removal of RNA under RNase influence is achieved when RNase is added after DNA extraction, which gives optimal quality with any protocols. Similarly, DNA contamination in an isolated RNA is degraded by DNase to obtain high-quality RNA. Our protocol is the protocol of choice in terms of simplicity, recovery time, environmental safety, amount, purity, PCR and RT-PCR applicability.

1. Introduction

Biomolecule extraction, such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from a variety of starting biological materials to be used in downstream applications and other analytical or preparative purposes, is the most important first step in the molecular biology. The widely employed nucleic acid isolation methods can be divided into organic extraction method (phenol/chloroform), inorganic extraction method (salting out) and solid phase extraction method (solid matrix); moreover, four indispensable steps are generally required for successful nucleic acid purification:

1. Cell lysis through disruption of the cellular membranes, cyst wall or egg wall
2. Dehydration and precipitation of the cellular proteins (protein denaturation)
3. Separation of cellular proteins and other cellular components out of the nucleic acid
4. Precipitation and dissolving the nucleic acid

The routinely practised cell lysis step can be divided into three types to cope with different tissues, thereby achieving optimum nucleic acid yield:

1. Grinding in liquid nitrogen (mortar and pestle), such as different animal and plant tissues
2. Glass-bead grinding, for example, oocysts (e.g. *Eimeria* spp.), metacercariae (e.g. *Fasciola* spp.) and nematodes' eggs (e.g. eggs of *Haemonchus contortus*)
3. Repetitive pipetting, notable examples of it are animal cells and zoites of apicomplexan parasites, such as sporozoites, merozoites, tahyzoites and bradyzoites, and trypanosomal forms of *Trypanosoma* spp. and *Leishmania* spp., for example, trypomastigote, promastigote, amastigote and epimastigote.

In recent years, the development of molecular techniques has created a need for establishing simple and efficient novel methods of DNA and RNA extraction for PCR amplification and other related techniques. Carbohydrates, tannins, polyphenols and proteins in addition to hazardous organic solvents, such as phenol and chloroform are the major enemies of the embattled researchers. No existence for DNA or RNA extraction method that is suitable for all prokaryotic and eukaryotic organisms.

* Corresponding authors at: State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100193, China.
E-mail addresses: saeed_elashram@yahoo.com (S. El-Ashram), suoxun@cau.edu.cn (X. Suo).

http://dx.doi.org/10.1016/j.btre.2016.10.001

Furthermore, there is an urgent need to address the insufficiency of reasonable environment for RNase to have DNA free of RNA and even for DNase to degrade the DNA.

2. Materials and methods

2.1. Reagents

Proteinase K, 100% Ethanol, 70% Ethanol, Double distilled (DD) water, Ethylene diamine tetra acetic acid (EDTA), RNase, DNase, Pyrex beads, Agarose, Deoxyribonucleic acid (DNA) Marker, 2 × EasyPfu PCR SuperMix, 10% Sodium dodecyl sulfate (SDS), Glacial acetic acid (CH_3COOH), Hydrochloric acid (HCl) and Sodium hydroxide (NaOH).

2.2. Equipments

Mortar, Pestle, PCR machine, Microscope, Refrigerated Benchtop centrifuge (MIKRO200R, Germany), Weighing scale, Pipettes (20, 100, and 1000 μl), 15 and 50 ml falcon tubes, 50 ml centrifuge tubes and Disposable Polypropylene micro-centrifuge tubes

2.3. Reagent setup

Tris buffer, Tris-EDTA (TE), DEPC-treated water, Saturated salt solution (NaCl), Neutral saturated salt solution, Acidic saturated salt solution and Lysis buffer:1X STE buffer (50 mM NaCl, 50 mM Tris-HCl and 100 mM EDTA; PH 8.0)

2.4. Procedure

2.4.1. Grinding in liquid nitrogen (Mortar and pestle)

2.4.1.1. DNA extraction protocol. Hepatic DNA extraction from mouse can be divided into six steps. These are:

2.4.1.1.1. Homogenization. 1 g of the liver was taken and cut into pieces then ground using a porcelain mortar and pestle in 3 ml of lysis buffer containing 900 μl of 10% SDS. The emulsion was transferred to micro-centrifuge tubes and 100 μg proteinase K was added per ml of emulsion solution, and incubated for 1 h at 50 °C.

2.4.1.1.2. Phase separation. 350 μl of neutral saturated salt solution (NaCl) per ml was added to the previous emulsion, the micro-centrifuge tube was capped and shaken gently by hand for 15 s, and then incubated at room temperature for 10 min. The micro-centrifuge tube was centrifuged at 590 × *g* for 15 min at room temperature with DNA remaining exclusively in the aqueous phase (see Fig. 1A for illustration).

2.4.1.1.3. DNA precipitation. The resulting aqueous phase was transferred into another micro-centrifuge tube, and mixed with two volumes of room temperature absolute ethyl alcohol. Then the micro-centrifuge tube was inverted several times for 10 s.

2.4.1.1.4. DNA wash. The supernatant was removed; the DNA pellet was washed once with 75% ethanol, and the DNA was precipitated out of the solution by centrifugation at 9500 × *g* for 5 min.

2.4.1.1.5. DNA dissolving. The DNA pellet was allowed to dry for 5 min, and dissolved in DD water. Then the DNA was quantified and aliquoted to be stored at −20 °C.

2.4.1.1.6. Removal of RNA from DNA preparation. 50 μg per ml RNase was added and the mixture was incubated for 1 h at 37 °C.

- Critical step: *The treatment of DNA with RNase should be done in Tris buffer at the end of the extraction protocol. Salting out step can be repeated as before according to the protocol to obtain DNA with highest quality. The DNA can be precipitated and washed with 70% ethanol, and then the pellet can be dissolved in Tris-EDTA (TE) for DNA protection from degradation by metal dependent nucleases during storage.*

2.4.1.2. RNA extraction protocol. Hepatic RNA extraction method from mouse can be listed as follows:

2.4.1.2.1. Homogenization. 1 g of the liver was taken and cut into pieces then ground using a porcelain mortar and pestle in 3 ml of lysis buffer containing 900 μl of 10% SDS. The emulsion was transferred to micro-centrifuge tubes.

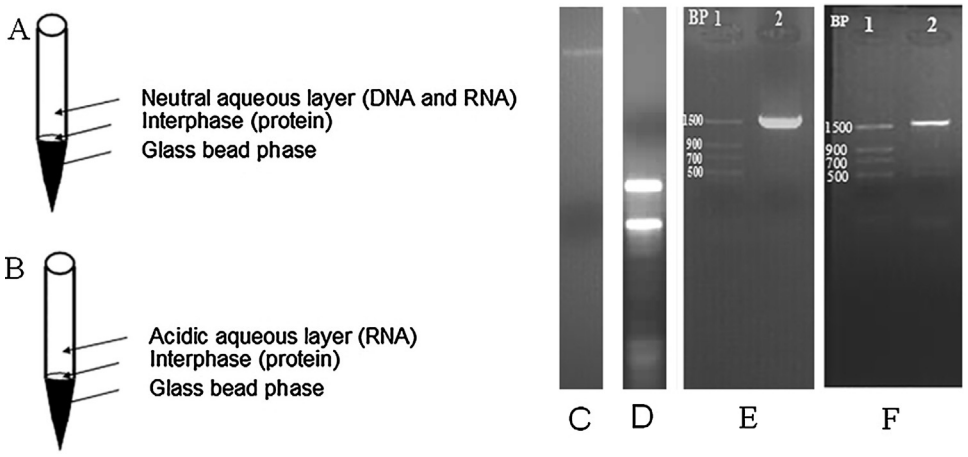

Fig. 1. Nucleic acid extraction and downstream application.
A. Neutral salting out (DNA extraction).
B. Acidic salting out (RNA extraction).
C. GoldView™ Nucleic Acid Stained 1.5% Agarose gel demonstrating the integrity of total DNA extracted from *Eimeria tenella.*
D. GoldView™ Nucleic Acid Stained 1.5% Agarose gel of total RNA of *E. tenella.*
E. Standard PCR amplification of MICII of *E. tenella.*
F. GoldView™ Nucleic Acid Stained 1.5% Agarose gel of EtMIC-2 RT-PCR amplified product of *E. tenella* using Finnzymes phusion™ High-Fidelity DNA Polymerase.