Chapter 10
DNA and RNA Extractions from Mammalian Samples

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Abstract DNA and RNA isolation is the initial step for most molecular genetic analysis testing. Genomic DNA and total RNA can be extracted from many sources, including peripheral blood, bone marrow, body fluid, and fresh or frozen tissues. For DNA isolation, two mechanistically different methodologies that are typically used are silica spin column and phenol–chloroform. RNA isolation methods can be divided into three groups: isopycnic gradient centrifugation method, phenol–chloroform extraction method, and silica spin column absorption method, although isopycnic gradient centrifugation method is rarely used nowadays. In this chapter, we present representative methods to isolate DNA and RNA and discuss general considerations. We also discuss nucleic acid extraction from formalin-fixed paraffin-embedded (FFPE) tissue samples. The nucleic acid isolated from FFPE is fragmented, but, because FFPE has been a method of choice for histologic and pathologic examination, FFPE tissue samples are widely used for research and clinical molecular assays. Nucleic acid extraction from FFPE tissue specimens is based on deparaffinization, protein digestion, and nucleic acid purification.

Keywords DNA isolation • RNA isolation • FFPE (formalin-fixed paraffin-embedded) tissue • Spin column • Monophasic lysis reagent

1 Introduction

DNA and RNA isolation is the initial step for most molecular genetic analysis testing in basic and applied biomedical sciences. For molecular diagnosis, DNA is utilized for the detection of particular genes, analysis of sequence variation. RNA is utilized for the analysis of gene expression, by reverse transcription, RT-PCR, or Northern blot. RNA can be also used to detect fusion gene products that result from chromosome rearrangement such as *BCR-ABL1*. Furthermore, DNA or RNA can be

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used to detect microorganisms, especially clinically significant viruses such as HIV and HCV.

Genomic DNA and total RNA can be extracted from many sources, including peripheral blood, bone marrow, body fluid, and fresh or frozen tissues. DNA can also be extracted from buccal swab, nail clip, or, in forensic cases, even from bone, tooth, or skeletal remains. Two mechanistically different methodologies that are typically used are silica spin column and phenol–chloroform. Currently, most commonly used is silica-based spin column, although phenol–chloroform method is still widely used [1].

Nucleic acid can also be isolated from formalin-fixed paraffin-embedded (FFPE) tissue samples, although the nucleic acid isolated is fragmented. However, because formalin fixation and paraffin embedding has been a method of choice for histologic and pathologic examination, FFPE tissue samples are widely used for research and clinical molecular assays. Furthermore, this method allows us to distinguish target tissues (e.g., tumor vs. normal tissue) microscopically and isolate pure DNA/RNA exclusively from target parts of a mixed tissue specimen using Hematoxylin and Eosin stained slides as a guide. Nucleic acid extraction from FFPE tissue specimens is based on deparaffinization, protein digestion, and nucleic acid purification [2].

Nucleic acids from microorganisms can also be isolated from plasma. For this purpose, automated extraction systems are used widely. Automated or semi-automated nucleic acid extraction platforms can offer less hands-on time, shorter turnaround time, and improved assay performance [3]. Several nucleic acid extraction systems are commercially available (Table 10.1). Most of the automated extraction is based on lysis of cells and/or pathogens, inactivation of cellular nucleases, capture of nucleic acids through binding to the silica surface of magnetic particles, purification, and elution of the purified nucleic acids.

2 DNA Extraction from Blood, Bone Marrow, and Tissue Using Silica-Gel Column

Total genomic DNA is purified from whole blood, bone marrow, or fresh or frozen tissue after cell lysis with protease and guanidine hydrochloride. The DNA is then adsorbed onto a silica-gel membrane [4, 5]. Salt and pH conditions in the lysate ensure that protein and other contaminants, which can inhibit polymerase chain reaction (PCR), are not retained on the membrane. DNA bound to the membrane is washed with two different wash buffers and purified DNA is eluted from the membrane with elution buffer. RNA will be co-purified with DNA if present in the sample. RNA will not inhibit PCR and therefore does not need to be removed from specimens for PCR testing.

Table 10.1 Comparison of automated nucleic acid extraction instruments

Product (manufacture)	Genomic DNA from blood[a]	Total RNA	Viral and bacterial NA	Sample number	Input volume (μL)	Elution volume (μL)	Run time (min)	Dimensions (cm)	Mechanism
QIAsymphony (Qiagen)	Yes (FFPE)	Yes	Yes	1–96 (24 × 4)	100–2000	50–400	60	130 × 75 × 103	Magnetic particles chemistry
MagNaPure 96 (Roche)	Yes (FFPE)	Yes	Yes	1–96	50–1000	50–200	50–60	136 × 81.5 × 100	Magnetic particle chemistry
NucliSense easyMAG (Biomerieux)	Yes	Yes	Yes	1–24	10–1000	25–110	40–60	100 × 65 × 53	Magnetic silica particle chemistry
Maxwell 16 (Promega)	Yes (FFPE, swab)	Yes	Yes	1–16	300	50–400	30–50	33 × 44 × 33	Para magnetic particles chemistry
EZ1 Advanced XL (Qiagen)	Yes	Yes	Yes	1–14	50–1000	50–200	20	51 × 51 × 57	Magnetic particles chemistry
Arrow (Autogen)	Yes (FFPE, swab, stool)	Yes	Yes	1–12	200–500	100	30	44.2 × 44.5 × 46.5	Magnetic beads
AutoMate Express (Life Technologies)	Yes (bone, tooth)			1–13	5–1000	50	30	50.8 × 55.9 × 57.2	PrepFilter LySep Column

[a]Additional sample types are listed in parenthesis [1, 3, 6, 7]

2.1 Specimen Types

(a) Whole blood or bone marrow collected in ethylenediaminetetraacetic acid (EDTA) (lavender top tube). Store at 2–8 °C for no more than 2 days before extraction. Avoid the tube with heparin if PCR is to be performed on the specimen, as heparin may inhibit PCR reactions.
(b) Cultured cells (monolayer or suspension).
(c) Fresh or frozen tissue.
(d) Formalin-fixed paraffin-embedded (FFPE) samples (see Sect. 4 for detail).

2.2 Stepwise Protocol (See Fig. 10.1)

Please note all procedures are carried out at room temperature unless specifically stated.

(a) Mix 200 µl specimen with 180 µl lysis buffer (5.25 M guanidine thiocyanate, 50 mM Tris–HCl, pH 6.4, 20 mM EDTA, 1.3 % (w/v) Triton 100) and 20 µl of 20 mg/ml proteinase K, and vortex briefly.

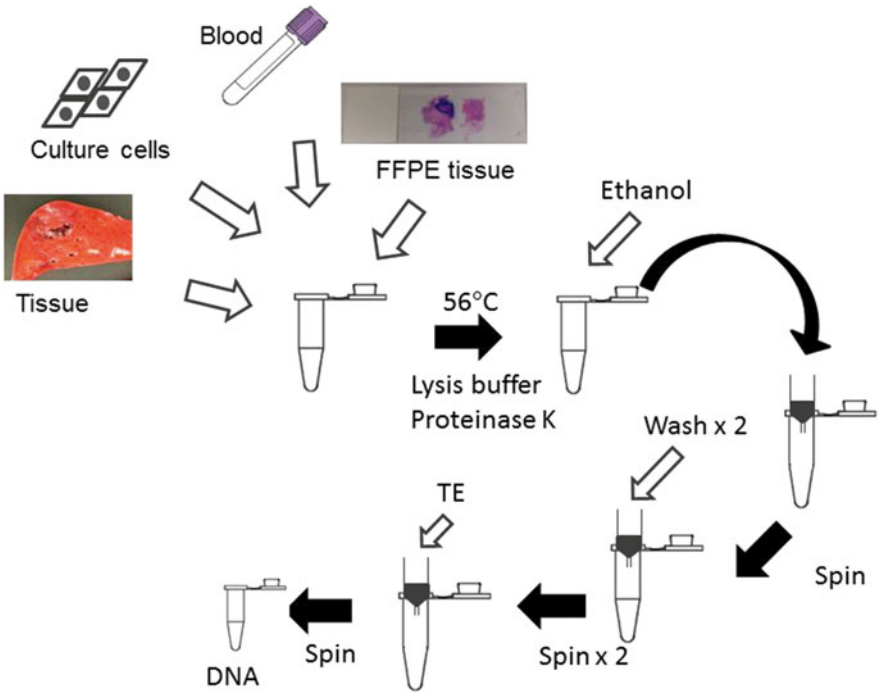

Fig. 10.1 DNA isolation workflow

(b) Incubate at 56 °C for 10 min up to overnight until the solution becomes clear, then add 200 µl ethanol (100 %) and vortex for 15 s.
(c) Spin columns with silica gel membrane are commercially available (e.g., Qiagen, Valencia, California; Promega Corp., Madison, Wisconsin). Place a spin column in collection tube (microfuge tube) and apply the lysate onto the column, then microcentrifuge at 6000 × *g* (8000 rpm) for 1 min and discard the flow-through.
(d) Add 1 mL of wash buffer I (5.25 M guanidine thiocyanate in 50 mM Tris–HCl, pH 6.4) to the column, microcentrifuge at 6000 × *g* for 1 min, and then discard the flow-through.
(e) Add 1 mL of wash buffer II (20 mM Tris–HCl, pH 7.4, 1 mM EDTA, 50 mM NaCl, and 70 % ethanol) to the column, microcentrifuge at 20,000 × *g* (14,000 rpm) for 3 min, and then discard the flow-through. Perform an additional 1 min spin if the lysate has not completely passed through.
(f) Place the column in a new collection tube and add 50–200 µl TE, pH 8.0 (10 mM Tris–HCl, pH 8.0, 0.5 mM EDTA) to the column, and incubate for 10 min. Microcentrifuge at 6000 × *g* (8000 rpm) for 60 s. Keep the eluate in a DNase/RNase-free tube and store at 4 °C for short term or −20 °C for long term.

3 DNA Extraction from Blood, Bone Marrow, or Tissue Using Phenol–Chloroform Extraction

Phenol–chloroform extraction is the standard method for DNA extraction. This separation method is based on the difference in solubility of nucleic acids, proteins and lipids in these organic solvents. Following centrifugation, phenol–chloroform mixture separates into three layers. *An upper aqueous phase contains nucleic acid*, *whereas interphase and lower organic phase contains proteins*. Then, extracted DNA in aqueous phase is precipitated with alcohol. The advantage of this method is being able to obtain DNA with *higher recovery and higher purity*. However, it is time-consuming and cumbersome, and employs toxic components [1, 2].

3.1 Stepwise Protocol

(a) Mix 200 µl of specimen with 180 µl extraction buffer (10 mM Tris–HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 2 % SDS), and mix well.
(b) Add 20 µl of 20 mg/ml proteinase K and incubate at 56–60 °C for 10 min up to overnight until the solution becomes clear and homogeneous.
(c) Add 800 µl of phenol–chloroform–isoamyl alcohol (25:24:1) and mix vigorously, then microcentrifuge for 1 min at 11,000 × *g* to separate aqueous and organic phases.
(d) Transfer the upper aqueous phase to a new tube. Add 1:1 volume of isopropanol, vortex and microcentrifuge for 15–20 min at 14,000 × *g* (13,000 rpm).

(e) Discard the supernatant, and add 500 µl of 70 % ethanol and vortex. Microcentrifuge for 15–20 min at 14,000 × *g*.
(f) Remove as much liquid as possible without disturbing the pellet using a fine tip transfer pipette. Air-dry the pellet, add 200 µl TE or H_2O and let the pellet to dissolve.

4 DNA Extraction from Formalin-Fixed Paraffin-Embedded (FFPE) Tissue

The first step of the most of the methods involves dissolution of the wax in xylene and ethanol. Generally, xylene solubilizes the majority of chemicals used in glues or paraffin. After deparaffinization, proteins and residual cellular components are lysed with proteinase K, SDS, and guanidine hydrochloride, and the released DNA is adsorbed onto a silica-gel membrane, as described in Sect. 2.

The quality of DNA isolated from tissue sections is affected by (1) the fixative used, (2) duration of the fixation, and (3) the age of the paraffin block [8, 9]. *Since DNA extracted from FFPE tissue is fragmented, it is useful mainly for PCR amplification of short, up to 200–300 bp amplicons* [6].

4.1 Specimens

Paraffin block containing embedded tissue or unstained sections on slides (see box).

> Hematoxylin and eosin (H&E or HE) stain had been believed to inhibit the DNA amplification reaction. However, Morikawa et al. [10] showed histochemical stains do not interfere downstream applications such as PCR. However, precaution has to be taken since eosin has been shown to produce an artificial peak at 71 bp on capillary electrophoresis [11].

4.2 Specimen Preparation

4.2.1 Scrape from Slides

From a tissue block, prepare five to ten unstained slides of the tissue of interest (7–10 µm thickness, on uncharged slides) and one H&E stained slide. Using H&E stained slide as a reference, scrape the tissue from the slides with a scalpel and transfer to the tube containing 1200 µl of xylene by rinsing the scalpel in the xylene. Mix vigorously to let paraffin dissolve.

4.2.2 Section from a Tissue Block

Cut 5–10 consecutive sections with 7–10 μm thickness by microtome or punch 3–5 × 1 mm cores using disposable biopsy punch with plunger, and put them in microfuge tube. Add 1200 μl of xylene and mix vigorously to let paraffin dissolve.

> Make sure to resuspend at least 10× excess xylene. Sometimes it required longer incubation time to dissolve paraffin. Deparaffinized tissue is opaque and fluffy in appearance.

4.3 Deparaffinization Protocol

(a) Microcentrifuge at 12,000 rpm (13,000 × *g*) for 5 min, and carefully remove the supernatant.
(b) Add 1200 μl of 96–100 % ethanol to pellet to remove residual xylene and mix gently by vortex. Microcentrifuge at 12,000 rpm for 5 min, and carefully remove the supernatant.
(c) Repeat step b one more time. Remove residual ethanol. Incubate at 37 °C for 15–25 min or until ethanol has evaporated.
(d) Go to Sect. 2.2 and follow the protocol for DNA isolation.

4.4 Alternative Methods for Dissection

Scraping the exact area from the slide is sometimes difficult. There are reagents that aid in isolating areas of interest from a selected microscopic area on a slide-mounted tissue section. PinPoint Slide DNA Isolation System (Zymo Research, Irvine, California) does not require any special equipment, such as microdissection system, and makes more precise dissection (macro-dissection) possible [12]. The method increases the accuracy to target the areas of interest on the slide, and changes the tissue of interest into a thin blue film, which is easy to peel off the slide. Please refer the manufacture's manual for detailed protocol.

Alternative is semi-automated dissection system, such as MesoDissection System (AvanSci Bio LLC, Salt Lake City, Utah), which allows automated transfer and alignment of the aria of the interest to the slide dissecting with better precision [13].

4.5 Other Considerations

Formaldehyde, the active component of formalin, is a strong cross-linker, which forms protein-protein and protein-nucleic acid cross-links [14, 15]. Therefore, the nucleic acids extracted from FFPE tissue typically consists of shorter fragments.

Avoid fixatives containing acids or metals such as Bouin's solution or B5 solution because the acidic environment denatures nucleic acids [14]. Other factors, such as pre-fixation time, duration in the fixative, pH, and temperature, also affect quality and yield of the isolated nucleic acid [8, 9, 14].

The first deparaffinization step involves dissolution of the wax using xylene and washing in a descending concentration series of ethanol solutions. Since xylene is toxic, xylene-free alternatives such as Citrisolv (Fisher, Pittsburgh, Pennsylvania), Q-solution (TrimGen, Sparks, MD, USA) or Deparaffinization solution (Qiagen) are also available.

Due to rapidly increasing demand of genomic analysis of FFPE samples, automated system is needed to decrease hands-on time. Recent publication demonstrated that DNA isolation using the EZ1 DNA Tissue Kit on BioRobot EZ1 workstation (Qiagen) decreases hands-on time significantly (45–10 min) with equivalent or better quality and yield of DNA compared to QIAmp FFPE Tissue Kit [16].

5 RNA Extraction

5.1 RNA-Specific Considerations

In contrast to DNA isolation, it is critical to protect the RNA from ribonucleases (RNases) during RNA isolation. RNases are ubiquitously present in all cells and very stable and resistant to traditional denaturation methods, such as prolonged boiling or incubation with mild denaturants. Two reagents are frequently used to inactivate RNases. Diethylpyrocarbonate (DEPC) is a highly reactive alkylating reagent and is used to inactivate possible RNases contamination in solution, on glassware and plasticware. Guanidinium salts are chaotropic agents, which can destroy the three-dimensional structure of proteins, include RNases. Guanidine isothiocyanate (GITC), used in the presence of a reducing agent, such as β-mercaptoethanol, can break the intrachain disulfide bonds of RNases and inactivate them.

RNA isolation methods can be divided into three groups: isopycnic gradient centrifugation method, phenol–chloroform extraction method, and silica spin column absorption method. Isopycnic gradient centrifugation, although historically important, is rarely used nowadays, due to its requirement of ultracentrifuge with *g*-force >100,000 for overnight. Phenol–chloroform RNA isolation [17] has been widely used, especially after Chomczynski and Sacchi [18] developed the single step method by combining all extraction steps into a single one using a reagent composed of GITC, phenol, and chloroform (monophasic lysis reagent: MLR). It requires only microcentrifuge (no ultracentrifugation), decreased both the time requirements and the sample size needed, and increased the method throughput and minimizing the loss of RNA. Because of these advantages, the MLR method has been the method of choice for RNA isolation since it was developed. Another method is using silica spin columns, which is similar to DNA isolation and com-

mercially available. After total RNA extraction, mRNA can be further purified from this product with oligo(dT) or oligo(dU)-cellulose.

5.2 RNA Extraction with Monophasic Lysis Reagent (MLR)

This method is based on lysing cells with a MLR of GITC, phenol, and chloroform [19]. The mixture is separated into three layers by centrifugation, similar to DNA extraction. However, *when thepHis acidic*, *phosphate groups of DNA are preferentially neutralized and go to the organic phase*, *allowing RNA to be selectively extracted in the aqueous phase*. The total RNA is then precipitated from the aqueous phase with isopropanol and washed by ethanol.

Many MLRs with different formulations and trade names are commercially available. The detailed composition of MLR, such as TRIzol Reagent (Life Technologies), has not been published. All of these reagents contain GITC, phenol, and a solubilizing reagent such as glycerol.

5.2.1 Sample Types

(a) Plasma, serum or whole blood. These samples should be collected in EDTA (lavender top tube). Store at 2–8 °C for no more than 16–18 h before extraction unless they are mixed in RNA*later* solutions (Life Technologies).
(b) Cultured cells (monolayer or suspension).
(c) Fresh or frozen tissue (see the box).
(d) Formalin-fixed paraffin-embedded (FFPE) samples. Deparaffinization is necessary. See Sect. 4 for detail.

5.2.2 Stepwise Protocol

(a) *Homogenizing Samples*: Mix 0.25 ml of sample and 0.75 ml of MLR in 1.5 ml microfuge tube and vortex and invert the tube to be sure the solution is homogenous. Homogenize with micropestle or homogenizer if necessary. Incubate the homogenates for 5 min.

For the tissue with high content of fat, protein, polysaccharides, or extracellular matrix, microcentrifuge at full speed for 1 min and remove the top fat/protein layer and the pellet containing polysaccharides and extracellular matrix. For the tissues rich in RNases (e.g., pancreas, gastrointestinal tract), cut the tissue into small pieces, freeze immediately in liquid nitrogen and proceed to homogenizing step.

(b) *Phase Separation*: Add 0.2 ml of chloroform to the tube, shake vigorously for 15 sec, and incubate for 2–3 min at room temperature.
(c) Microcentrifuge at full speed for 15 min at 4 °C. Without disturbing the interphase, immediately transfer the aqueous phase (top layer) to a new RNase-free tube. It is crucial not to transfer the interphase or organic phase. Leave some of the aqueous phase behind to avoid the risk of contaminating the sample.
(d) *RNA precipitation and wash*: Add 0.5 ml of isopropanol and mix thoroughly by inverting several times, and incubate at room temperature for 10 min.
(e) Microcentrifuge at full speed for 10 min at 4 °C. The RNA will appear as a gel-like white pellet on the side and bottom of the tube.
(f) Carefully aspirate the supernatant, and add 1 ml of 75 % ethanol, vortex the sample, and incubate 10 min at room temperature to dissolve the pellet.
(g) Microcentrifuge at full speed for 5 min at 4 °C, and carefully aspirate the supernatant. Air-dry the RNA pellet for about 5 min.
(h) *RNA resuspension*: Resuspend the pellet in 20–50 μl of RNase-free water (orDEPC-treated water).
(i) Incubate at 55–60 °C for 10 min to completely dissolve the RNA pellet. RNA sample can be used immediately or stored at −20 °C for 1 month or at −70 °C for longer period.

Contaminated DNA can be removed from the purified RNA by DNase I digestion, then extract RNA again by Phenol–chloroform–isoamyl alcohol (25:24:1) and precipitation by ethanol.

5.3 Isolation of RNA with QIAamp RNA Blood Kit

There are several different RNA isolation spin column systems commercially available and the principle of the isolation system is similar among the kits and the readers can refer the manufacturer's instruction manuals. We introduce one of these methods, QIAmp RNA blood kit, as an example, which is designed to prepare total cellular RNA from whole blood or bone marrow [20]. The kit enriches for RNAs larger than 200 nucleotides since small RNAs do not bind in quantity to the column under the conditions used.

5.3.1 Stepwise Protocol

(a) Add 7.5 ml Buffer EL into 1.5 ml blood or bone marrow in a 15 ml conical and. Mix by vortex and incubate on ice for 10 min. Mix by vortex twice during this incubation. The cloudy suspension becomes translucent, indicating lysis of RBCs. If the suspension is still cloudy, incubate another 10 min.
(b) Centrifuge at 400 × *g* for 10 min at 4 °C and completely remove the supernatant. If pellet is red, incubate for an additional 5–10 min on ice after addition of Buffer EL in step c.

(c) Add 3 ml Buffer EL and mix by vortex, and then centrifuge at 400 × *g* for 10 min at 4 °C. Completely remove the supernatant.
(d) Add 600 μL Buffer RLT (with 2-mercaptoethanol added) to the cell pellet, and mix by vortex. No cell clumps should be visible before next step.
(e) Pipette sample into a QIAshredder spin column in a 2 ml collection tube. Ensure that the lysate can be added to the column in a single step by adjusting pipette to greater than 750 μl. If the lysate is very viscous, then too many cells have been used. Divide the sample into two aliquots and use two columns. Centrifuge for 2 min at maximum speed. Discard the column and save the homogenized lysate.
(f) Add 600 μl of 70 % ethanol to the lysate and mix by pipetting.
(g) Pipette approximately 600 μl of the sample, including any precipitate, into a QIAamp spin column in a 2 ml collection tube and centrifuge for 15 sec at maximum speed.
(h) Place the QIAamp spin column in a new 2-ml collection tube and load the remaining lysate onto the QIAamp spin column. Repeat centrifugation.
(i) Transfer the QIAamp spin column to a new 2-ml collection tube and add 700 μL Buffer RW1. Centrifuge for 15 sec at maximum speed.
(j) Transfer the QIAamp spin column in a new 2-ml collection tube and add 500 μL Buffer RPE (with ethanol added). Centrifuge for 15 sec at maximum speed. Repeat this step one more time.
(k) Transfer the spin column to a new 2-ml collection tube and centrifuge for 1 min at maximum speed.
(l) Transfer the QIAamp spin column into a 1.5 ml microcentrifuge tube and pipet 40 μl of RNase-free water directly onto the QIAamp membrane. Centrifuge for 1 min at maximum speed to elute binding RNA. Store RNA at −70 °C.

6 Quantitation of DNA/RNA

Many labs use UV spectrometer (e.g., NanoDrop, Thermo Scientific) to quantitate DNA/RNA concentration. An A_{260} reading of 1 equals to 50 μg/mL of double-stranded DNA, 33 μg/mL of single-stranded DNA and 40 μg/mL of single stranded RNA. Pure DNA has an A_{260}/A_{280} ratio between 1.6 and 2.0. A lower ratio suggests possible contamination of proteins. A high A_{260}/A_{280} ratio (above 2.0) indicates a high level of residual RNA. RNase treatment can be performed if RNA interfere further steps. In contrast, pure RNA has an A_{260}/A_{280} ratio between 1.8 and 2.0. A lower ratio suggests possible contamination of DNA or proteins.

Several fluorescent dyes can bind to DNA (e.g., PicoGreen, Molecular Probes) or RNA (e.g., RiboGreen, Molecular Probe) and emit fluorescent signal to be quantitated by a fluorometer (e.g., Qubit, Life Technologies). The advantage of the fluorometer is that the dyes are specific for intact DNA or RNA. Therefore, it gives more reliable results when the specimen has a large amount of fragmented DNA or RNA, such as nucleic acids isolated from FFPE tissues.

7 General Considerations

7.1 Cross-Contamination

If isolating DNA for an amplification process, it is important to avoid cross contamination between specimens as well as contamination from post PCR products. Separate pre-amplification area and post-amplification area and never bring amplified PCR products into the pre-amplification/specimen preparation area (unidirectional work flow). Post-amplification area should be under negative air pressure and all other work areas should be under positive pressure. Reagent preparation and specimen preparation activities must be performed in segregated areas. Supplies and equipment must be dedicated to each activity and not moved between areas. Gloves and lab coat must be worn in each area and must be changed before leaving that area. Change gloves as often as needed. Change pipette tips between all liquid transfers. Always use aerosol-barrier tips.

Before and after specimen processing, clean the work area and pipettes with 0.5 % sodium hypochlorite (prepared fresh daily), or with Dispatch® cleaner with bleach. Then follow by 70 % denatured alcohol or CiDehol® to remove the bleach residue. After each specimen processing run, expose the hood and pipettes to UV light for 10 min.

In order to assure no cross-contamination and optimal assay condition, include necessary positive and negative controls. PCR amplification of housekeeping genes in the extracted specimens demonstrates that performance is acceptable and no amplified product from no-template control after amplification with primers targeted for universally expressed genes, such as human beta-globin, assures no contamination.

7.2 RNase

For RNA isolation, the first important practice is to inhibit both environmental and endogenous RNases. Two reagents are frequently used to inactivate RNases during RNA isolation; DEPC and GITC. 0.1 % DEPC H_2O is used to inactivate RNases in solution, on glassware and plasticware. GITC is used as a component of lysis solution to inactivate endogenous RNase.

In addition, designate an isolated bench, area or hood for RNA isolation only and use dedicated pipettes, sectioning equipment, or microcentrifuge. Clean the area with an RNase decontamination solution (e.g., RNaseZap solution from Ambion). Skin is a major source of contaminating RNase. Always wear a lab coat and gloves. Change gloves as frequently as needed to avoid “finger RNase”.

Table 10.2 Typical DNA and RNA yields from various specimen sources

Specimen sources	Specimen amount	Expected DNA yield (μg)	Expected RNA yield (μg)
Blood	1 mL	20–60	1–10
Liver	1 mg	3–4	6–10
Kidney	1 mg	3–4	3–4
Skeletal muscles and brain	1 mg	2–3	1–1.5
Placenta	1 mg	2–3	1–4
Epithelial cells	1×10^6 cells	5–7	8–15
Fibroblasts	1×10^6 cells	5–7	5–7

7.3 Troubleshooting

Typical expected yields of DNA and RNA are shown in Table 10.2 [20]. In case of a low nucleic acid yield, repeat the isolation with a new sample. Be sure to mix the sample with lysis buffer vigorously and use freshly prepared proteinase K. Extend the incubation with proteinase K to make sure all proteins are digested.

In addition, the quality of nucleic acid can be determined by internal control for PCR amplification. If there is no amplification, the possibilities are, (1) too much or too little crude DNA/RNA, (2) DNA/RNA is degraded, (3) PCR inhibitors in the sample, and (4) incomplete inactivation of proteinase K when no further purification step is used after Pin Point (Sect. 4.4).

Degradation of RNA by RNase is the major problem during RNA isolation. It can be best avoided by working quickly and keeping everything cold until lysis buffer with GITC is added to the cytoplasmic extract. For tissues that contains large amount of RNases (e.g., liver, pancreas), it is recommended to snap freeze the tissue in liquid nitrogen or immerse the tissue into preserve agent (e.g., RNA*later*, Life Technologies) as soon as possible.

Since nucleic acid extracted from FFPE tissue is fragmented, typically the yield is low and the quality is poor. Increasing the time of Proteinase K digestion may improve the yields. It is important to design the primers to keep the amplicon size small for successful assay.

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