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Isolation of Genomic DNA from Mammalian Cells and Fixed Tissue

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Isolation of Genomic DNA from Mammalian Cells and Fixed Tissue

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Examination of DNA variation is central to understanding the function of mammalian cells, tissues, and whole bodies. Extraction of high-quality DNA from cells and tissues is necessary for innumerable different experiments. We present protocols for the extraction of DNA from both fresh samples and formalin-fixed tissue. Methods for extracting DNA have been standardized and streamlined over the past couple of decades and many extraction kits are available for a reasonable cost. In addition, many of the extraction procedures can also be automated for even higher throughput sample preparation. © 2023 The Authors. Current Protocols published by Wiley Periodicals LLC.

Basic Protocol 1: DNA isolation from whole blood, tissue, and cultured cells
Alternate Protocol: DNA extraction using automated machines
Basic Protocol 2: DNA isolation from saliva and buccal swabs
Basic Protocol 3: DNA isolation from formalin-fixed, paraffin-embedded tissue

Keywords: blood • DNA • formalin-fixed paraffin-embedded • human tissue • saliva

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INTRODUCTION

Obtaining DNA for genetic studies has become an essential component of virtually all biomedical research. This unit provides an update for simple, cost-effective preparations of DNA from whole blood, human tissue, cultured cells, and saliva/buccal swab samples. When working with fresh or frozen samples, high-molecular-weight DNA suitable for downstream assays, such as genotyping arrays or next generation sequencing, can be routinely isolated. DNA can also be isolated from formalin-fixed, paraffin-embedded (FFPE) tissues, though it is more challenging and there are limitations in the downstream applications for which this DNA is suitable. Over the last couple of decades, the availability and decreased cost of commercial kits has made their use prominent in the field. There are many vendors offering similar types of extraction kits which are a cost-effective option for obtaining high quality DNA from human specimens. Most commercially available extraction methods no longer require the use of toxic chemicals, such as phenol and chloroform, also making them a safer option for researchers. Commercially available kits generally perform as well as manual methods in terms of yield and quality of DNA (Chacon-Cortes & Griffiths, 2014; Guha et al., 2018). Basic Protocol 1 describes the

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isolation of DNA from fresh or frozen human blood, tissue, or cultured cells. The Alternate Protocol describes how automated machines could be utilized for high throughput sample processing. Basic Protocol 2 describes methods for obtaining and extracting high quality DNA from saliva or buccal swabs collected from inside of the cheek. Basic Protocol 3 outlines how to extract DNA from formalin-fixed paraffin-embedded (FFPE) tissues. For more detailed information about some of the extraction procedures below, a recent book chapter is a useful source of information (Mullegama et al., 2019).

CAUTION: When working with human samples, it is essential to utilize safety precautions for biohazardous samples and bloodborne pathogens. These include proper personal protective equipment, waste disposal, and decontamination procedures. Please consult with your institution's Environmental Health and Safety department to ensure you are complying with all guidelines.

NOTE: All protocols involving animals must be reviewed and approved by the appropriate Animal Care and Use Committee and must follow regulations for the care and use of laboratory animals. Appropriate informed consent is necessary for obtaining and use of human study material.

BASIC PROTOCOL 1

DNA ISOLATION FROM WHOLE BLOOD, TISSUE, AND CULTURED CELLS

Most commercially available extraction kits can be used to extract DNA from multiple types of human samples, such as blood, tissue, and cultured cells. In addition, there are kits that are designed to handle varying amounts of starting material. The yield of DNA is mainly dictated by the amount and type of starting material used as input for the extraction process. The main components of the extraction kits and their purposes are described. It is crucial to follow the specific instructions that come with each kit. Basic Protocol 1 describes the extraction of DNA from human blood, tissue, or cultured cells using a column-based kit such as the QIAamp DNA Blood Mini Kit or the DNeasy Blood and Tissue Kit. Though far from an exhaustive list, Table 1 provides information about most of the commonly used DNA extraction kits in research laboratories.

Materials

Whole blood, cultured cells, or human tissue (fresh or frozen and thawed)
DNA extraction kit containing:
- Lysis buffer
- Proteinase K digestion enzyme
- Wash buffer(s)
- Elution buffer

Water bath
Additional reagents and equipment for quantitation of DNA by absorption spectroscopy (Gallagher & Desjardins, 2007)

1. Add a mixture of lysis buffer and proteinase K enzyme to an aliquot of whole blood, cultured cells, or tissue. The lysis buffer contains detergents that will disrupt cellular and nuclear membranes, releasing the cell contents, including the DNA from the nuclei. Incubate the sample at 56°C for a length of time required to digest the proteins in the sample.

   The length of the incubation depends on the sample; most human tissue samples will likely require an overnight incubation to achieve complete digestion.

2. After digestion, add the cell lysate mixture to a resin-containing column. The resin in the column is positively charged, allowing the negatively charged DNA to bind to the

Table 1 Commercial Kits Available to Extract DNA from Blood, Cells, or Tissue

Kit name	Manufacturer	Starting material(s)	# preps	Starting vol (µl) or amt (mg)	Yield (µg)	Special notes
DNeasy Blood & Tissue Kit	Qiagen	Cells, blood, or tissue	50, 250	2×10^6 cells, 100 µl blood, 25 mg tissue	3-30	
QIAwave DNA Blood & Tissue Kit	Qiagen	Cells, blood, or tissue	250	2×10^6 cells, 100 µl blood, 25 mg tissue	3-30	Eco-friendly reagents
QIAamp DNA Blood Mini Kit	Qiagen	Cells, blood, or tissue	50, 250	1×10^6 cells, 25 mg tissue, 200 µl blood	4-50	
Monarch Genomic DNA Purification Kit	NEB	Cells, blood, or tissue	50, 150	Up to 5×10^6 cells, 100 µl blood, 25 mg tissue, buccal swabs, 500 µl saliva	2.5-9	Works on fatty, fibrous tissue
Monarch HMW DNA Extraction Kit for Cells and Blood	NEB	Cells or blood	5, 50	Up to 10×10^6 cells, 2 ml blood	125	Best for long-range seq applications
Monarch HMW DNA Extraction Kit for Tissue	NEB	Tissue	5, 50	10-25 mg	60	Best for long-range seq applications
DNAzol Reagent	ThermoFisher	Cells, blood, or tissue	100	1-3 $\times 10^7$ cells, 100 µl blood, 25-50 mg tissue	25-250	10-30 min protocol
PureLink Genomic DNA Mini Kit	ThermoFisher	Cells, blood, or tissue	50	Up to 5×10^6 cells, 1 ml blood, 25 mg tissue, buccal swabs, 500 µl saliva	3-30	
Quick-DNA Miniprep Plus Kit	Zymo	Cells, blood, or tissue	10, 200	1×10^6 cells, 1 mg tissue, 100 µl blood	1-7	20 min protocol, low elution volume
GenElute Blood Genomic DNA Kit	Millipore Sigma	Blood	70, 350	200 µl	10	
GenElute Mammalian Genomic DNA Miniprep Kit	Millipore Sigma	Cells or tissue	10, 350	2×10^6 cells, 25 mg tissue	25-30	

resin. The remaining biomolecules and debris will flow through the column when it is centrifuged.

Some kits make use of magnetic beads to capture the DNA. This procedure would require a magnetic plate to bind the beads during the subsequent wash steps.

3. Wash the column with the wash buffers that contain ethanol and various salts to wash away any contaminants or unwanted biomolecules that may have bound to the column.

After the final wash, spin the column for an extra-long spin (e.g., 3 min instead of 1 min) to be sure any residual wash buffer is removed and the ethanol has been dried, as it could interfere with downstream applications.

4. Elute the DNA from the resin in the column by adding an elution buffer in which the DNA is highly soluble.

DNA may be stored for several years at 4°C if storage vials are tightly sealed. For long-term storage, DNA can be frozen at −20°C or −80°C, though DNA degradation could occur if the DNA is subjected to many freeze-thaw cycles.

5. Quantify DNA concentration using UV or fluorescence absorption spectroscopy. Taking a measurement at 260 and 280 nm and comparing the ratio of these numbers (260/280) will give you an indication of the quality of the DNA as well.

DNA can also be run out on an agarose gel at this point to confirm the presence of high molecular weight fragments and minimal degradation.

ALTERNATE PROTOCOL

DNA EXTRACTION USING AUTOMATED MACHINES

Many of the extraction kits and protocols can be modified or run using automated machines. Table 2 lists many of the commonly used automated machines that are

Table 2 Automated Systems for DNA Extraction

Machine name	Manufacturer	Special notes	Associated extraction kits
QIAcube Connect	Qiagen	Spin-column based, 12 samples at a time	QIAamp DNA Mini QIAcube Kit
QIAcube Connect HT	Qiagen	96 samples at a time	QIAamp 96 DNA QIAcube HT Kit
QIASymphony	Qiagen	24 samples at a time; load up to 4 batches	QIAsymphony DSP DNA Mini/Midi
KingFisher Duo Prime	ThermoFisher	12 samples at a time; load up to 2 batches	MagMAX DNA Multi-Sample Ultra Kit
KingFisher Flex	ThermoFisher	24 or 96 samples at a time	MagMAX DNA Multi-Sample Ultra Kit
epMotion 5073	Eppendorf	24 samples at a time	NucleoMag DNA Blood, Tissue; other companies' kits
epMotion 5075	Eppendorf	96 samples at a time	NucleoMag DNA Blood, Tissue; other companies' kits
Maxwell RSC	Promega	16 samples at a time; includes quantification capability	Maxwell RSC Blood DNA Kit, Tissue DNA Kit
Maxwell RSC 48	Promega	48 samples at a time; includes quantification capability	Maxwell RSC Blood DNA Kit, Tissue DNA Kit
Chemagic Prepito Instrument	PerkinElmer	12 samples at a time	Prepito DNA Blood Kit, Tissue Kit

available for use in research labs. Of note, these machines are generally quite expensive to purchase and require service contracts for preventative maintenance. It might only be a cost-effective choice for labs that routinely extract DNA from hundreds or thousands of samples per year. This Alternate Protocol describes extraction using a QIASymphony machine.

Materials

Whole blood (fresh or frozen and thawed)
Reagent cartridge containing all lysis, wash, and elution buffers
Pipet tips compatible with extraction machine
Tubes for final elution

Water bath
Additional reagents and equipment for quantitation of DNA by absorption spectroscopy (Gallagher & Desjardins, 2007)

1. Thaw blood quickly in 37°C water bath or aliquot appropriate volume of blood as specified in the machine and kit manual of choice.

   Most extractions machines also have kits available for extraction of DNA from cell pellets and tissues as well. These may require some upstream processing to prepare for the lysis step before loading onto the machine.

2. Load the samples, pipet tips, the reagent cartridge, and tubes for elution into the extraction machine.

3. Choose the appropriate extraction protocol for the appropriate sample type and volume, then run the extraction procedure.

   As an example, to extract DNA from 24 1-ml aliquots of whole blood, the QIASymphony machine takes about 1.5 hr to run, and other batches can be immediately loaded on the machine as soon as the first run has finished.

4. Run any clean up or maintenance procedures as necessary on the machine.

   It is also important to keep up with preventative maintenance and calibration of the machine as covered by the service contract.

5. Quantify DNA concentration using UV or fluorescence absorption spectroscopy.

DNA ISOLATION FROM SALIVA AND BUCCAL SWABS

BASIC PROTOCOL 2

Saliva and oral/buccal samples (cheek swabs and brushes) have become increasingly valuable sources of genetic material for clinical applications due to ease of access (non-invasive sample collection), as well as convenient storage and transport procedures that result in adequate DNA yield and quality for genetic studies. Multiple reports indicate that saliva samples provide better quality DNA than buccal samples (Rethmeyer et al., 2013; Rogers et al., 2007). Saliva-derived DNA is now a common alternative to blood-derived DNA, and there are several commercially available saliva collection kits that act to stabilize the samples at ambient temperatures for extended periods prior to DNA extraction. Basic Protocol 2 describes the extraction of DNA from saliva collected using the Oragene-DX Saliva Collection Kit. Table 3 provides information about additional saliva and buccal swab sample collection and DNA extraction kits.

Materials

Saliva, 0.5 to 2 ml
1.5-ml microcentrifuge tubes, sterile and nuclease-free
Oragene-DX saliva collection kit (DNA Genotek, cat. no. OGR-600)

Table 3 Commercial Kits Available for Extraction of Buccal and Saliva Samples

Kit name	Manufacturer	Starting material(s)	# preps	Starting vol (µl) or amt (mg)	Yield (µg)	Special notes
Monarch Genomic DNA Purification Kit	NEB	Saliva, buccal	50, 150	500 µl	Varies	
PrepIT-L2P-5	DNA Genotek Inc.	Saliva	200	0.5-2.0 ml	Varies	
Saliva DNA Isolation kit	BioChain Inst. Inc.	Saliva		50-200 µl	0.25-4.0	
GeneFix Saliva-prep DNA Isolation kit	Diagnostica Longwood	Saliva	12, 50	0.5-2 ml	Up to 100	
Saliva DNA Isolation kit	Norgen Biotek Corp.	Saliva	50	0.25-0.5 ml	3-7	
ChargeSwitch gDNA Buccal Cell Kit	ThermoFisher	Buccal swabs	50, 960	1 swab	6	
GenElute Mammalian Genomic DNA Miniprep Kit	MilliporeSigma	Buccal swabs	10, 70, 350	1 swab	Varies	
Buccalyse DNA Isolation Kit	Boca Scientific Inc.	Buccal swabs	3, 50	1 swab	2-4	No centrifugation required
BuccalFix Plus DNA Isolation Kit	Boca Scientific Inc.	Buccal swabs	50	1 swab	Varies	Precipitation-based with buccal swabs already stored in BuccalFix buffer
Buccal-Prep Plus DNA Isolation Kit	Boca Scientific Inc.	Buccal swabs	3, 50	1 swab	Varies	Precipation-based with all buffers included
HigherPurity Buccal Swab Genomic DNA Extraction Kit	Canvax	Buccal swabs	20, 50, 250	1 swab	0.3-3	
QIAmp DNA Mini Kit	Qiagen	Saliva, buccal	50	1 swab	Varies	

Saliva samples can be exposed to a wide range of temperatures during collection and transport (−20°C to 50°C) and can be stored at room temperature (15 to 25°C) for up to a year before extraction of DNA.

PrepIT-L2P DNA extraction kit (DNA Genotek, cat. no. PT-L2P)
Ethanol, 100% (200 proof)
Ethanol, 70%
TE buffer, nuclease-free

Water bath
Ice bucket
Vortex
Additional reagents and equipment for quantitation of DNA by absorption spectroscopy (Gallagher & Desjardins, 2007)

1. Mix saliva sample (invert and gently shake) in the Oragene-DX saliva collection tube for a few seconds.
2. Incubate the sample at 50°C for a minimum of 2 hr, or overnight if it is more convenient.

   Heat treatment is required to ensure that DNA is adequately released, and nucleases permanently inactivated. At this stage, the sample can be stored at room temperature or frozen (−20°C) for up to 1 year.

3. Invert the saliva sample 10 times, then transfer 500 µl of mixed sample to a sterile 1.5-ml micro-centrifuge tube.
4. Add 20 µl prep-IT-L2P to the tube and vortex 10 sec.

   Sample will become turbid as impurities and inhibitors are precipitated.

5. Incubate on ice for 10 min.
6. Centrifuge 10 min at 15,000 × *g*, room temperature.
7. Carefully transfer 500 µl of the supernatant to a new microcentrifuge tube.

   Pellet may be accidentally disturbed during transfer. If so re-centrifuge tube for 5 min at 15,000 × g, room temperature before attempting to repeat supernatant transfer step.

8. Add 600 µl of 100% ethanol (200 proof) to the tube and gently invert 10 times.

   The DNA may appear as a clot of DNA fibers, or as a fine precipitate, depending on amount of DNA in the sample. DNA will still be recovered even if no clot is visible.

9. Incubate the sample for 10 min at room temperature.

   Allows DNA to fully precipitate.

10. Centrifuge 2 min at 15,000 × *g*, room temperature. with hinge portion of micro-centrifuge tube pointing out from center of rotor.

    If DNA pellet position cannot be seen after centrifugation, it will be located on the wall of the tube below hinge.

11. Carefully remove the supernatant by pipetting; discard supernatant.
12. Add 250 µl of 70% ethanol.
13. Incubate the sample for 1 min at room temperature.

    If pellet becomes detached, re-centrifuge for 5 min at 15,000 × g, room temperature.

14. Pulse-spin the tube and remove any residual ethanol with 200 µl pipettor.

Removal of all ethanol from the tube is important as ethanol carryover may impact downstream assays. If available, the tubes can be run in a vacuum centrifuge for 1 to 2 min to ensure all ethanol is removed.

15. Add 50 µl TE buffer, vortex for 15 sec to dissolve DNA pellet.
16. Incubate overnight at room temperature or incubate sample at 50°C for 1 hr, vortexing every 15 min.
17. Give sample tube a final vortex and store DNA at −20°C or −80°C for long term storage.
18. Quantify DNA concentration using UV or fluorescence absorption spectroscopy.

BASIC PROTOCOL 3

DNA ISOLATION FROM FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE

There is a potential wealth of information present in tissue samples taken from patients. Almost all tissues biopsied or surgically removed due to a condition or disease involve pathological testing. The most common pathological analysis of tissues involves fixation with formalin followed by embedding the fixed material in a paraffin block. Thin slices of the block can then be made and mounted on slides for staining and examination by a pathologist. Over the last one or 2 decades, there has been a great deal of interest in examining the sequence of the DNA present in these human tissue samples. However, purification of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue presents several challenges. To purify the DNA, the paraffin must be removed from the sample by volatile chemicals such as xylene. When the tissue is fixed in formalin it creates tangled crosslinked structures of proteins and DNA in the cells of the tissue. It can be difficult to achieve adequate digestion of cellular proteins to purify the DNA. In addition, the resulting DNA that can be isolated consists of short fragments that are not suitable for some applications, such as long-range sequencing. Library preparation techniques have been developed using the polymerase chain reaction (PCR) to amplify short regions of this DNA for use in some next-generation sequencing protocols. Basic Protocol 3 describes the extraction of DNA from FFPE tissue using the QIAamp DSP DNA FFPE Tissue Kit. Table 4 provides information about other common commercially available DNA extraction kits for FFPE tissue. Kresse et al. (2018) compared several FFPE DNA extraction kits and found that most produced comparable sequencing libraries and sequencing data.

CAUTION: Chemical substances such as xylene should be used in chemical fume hood and proper chemical waste disposal procedures.

Materials

FFPE blocks of tissue, cut into sections ∼5 µm thick
The sections can be mounted (unstained) onto glass slides for ease of transport, or formed into curls or scrolls and put into microcentrifuge tubes. Alternatively, a pellet or section can be punched from the block (∼2 mm wide × 3 to 5 mm deep) and then flattened into a thin section using a mortar and pestle. Using a piece of weigh paper in the pestle (under the pellet) helps with transporting the flattened sample into a microcentrifuge tube.
Scalpel, sterile and disposable
Microcentrifuge tubes, sterile and nuclease-free
DNA extraction kit compatable with FFPE tissue, containing digestion buffer, wash buffers, elution buffer, and spin columns
Xylene
Ethanol, 100% (200 proof)

Fume hood
Vortex

Table 4 Commercial Kits Available for Extraction from Formalin-Fixed Paraffin Embedded Tissue

Kit name	Manufacturer	Section thickness (μm)	# of sections	# preps	Yield (μg)	Special notes
Ion AmpliSeq Direct FFPE DNA Kit	ThermoFisher	5-10	1	8, 96	Varies	Used in conjunction with library prep kit
RecoverAll Total Nucleic Acid Isolation Kit for FFPE	ThermoFisher	20	4	40	24	
MagMAX FFPE DNA/RNA Ultra Kit	ThermoFisher	20	2	20	Varies	Can be automated
PureLink Genomic DNA Mini Kit	ThermoFisher	5-15	1-8	50, 250	Varies	
QIAamp DNA FFPE Tissue Kit	Qiagen	10	3-8	50	Varies	Can be automated
EX-WAX Paraffin-embedded DNA Extraction Kit	Millipore Sigma	5	3-5	20	Varies	
NucleoMag DNA FFPE Extraction Kit	Takara/Macherey-Nagel	3-20	3-10	96, 384	Varies	High throughput
Maxwell FFPE Plus DNA Kit	Promega	5-10	4-10	48	Varies	Used with Maxwell instrument
Prepito FFPE Kit	PerkinElmer	10	4	1-12	Varies	Used with chemagic Prepito instrument

Water bath or heat block
Additional reagents and equipment for quantitation of DNA (Gallagher & Desjardins, 2007)

1. Scrape the tissue from the slides using a sterile scalpel, or transfer the scroll or flattened pellet/section to a sterile 1.5-ml microcentrifuge tube.
2. Add 1 ml xylene to the sample.

   All steps using xylene must be performed in a safety fume hood.
3. Pulse-vortex to remove paraffin from the tissue.

   Usually requires 10 to 25 sec, depending on the amount of paraffin.
4. Centrifuge sample for 2 minutes at maximum speed, room temperature. Remove supernatant carefully with pipette, leaving pellet in the tube.

   Xylene waste should be disposed in a designated hazardous waste bottle.
5. Add 1 ml 100% ethanol (200 proof) to sample, pulse vortex 10 sec, and remove supernatant by pipetting.
6. Re-spin tube briefly to bring down residual ethanol and remove it by pipetting.
7. Leave sample pellet to air-dry at room temperature.

   Safety fume hood is no longer required for the remaining steps.
8. Add 180 µl Buffer ATL (digestion buffer) and 20 µl proteinase K solution, vortex for 15 sec. Incubate mixture for 1 hr at 56°C.

   Vortex 2 to 3 times per hour during incubation period to disperse the sample. No residual particles should be visible at end of incubation. It may be possible to increase the yield by allowing the digestion to go for as long as 7 days with a daily spike-in of 50 to 100 µl fresh Buffer ATL/proteinase K mixture.
9. Incubate sample for 1 hr at 90°C.
10. Pulse-centrifuge the tube to remove drops of liquid from inside the lid.
11. Add 200 µl Buffer AL, vortex for 15 sec.
12. Add 200 µl 100% ethanol (200 proof).
13. Vortex 15 sec and repeat step 10.
14. Apply mixture carefully to a QIAamp spin column in a 2-ml collection tube, without wetting the rim.
15. Close cap of spin column and centrifuge 1 min at 6000 × *g*, room temperature.
16. Transfer spin column to a new collection tube and discard the tube containing the filtrate.
17. Add 500 µl Buffer AW2 to the spin column.
18. Close the cap and centrifuge for 1 min at 6000 × *g*, room temperature.
19. Repeat step 16.
20. Close the cap of the spin column and centrifuge for 2 min at max speed (approximately 20,000 × *g*), at room temperature.

   Ensure all buffer is removed from the column as any ethanol present can interfere with downstream applications.

21. Transfer spin column to a 1.5-ml microcentrifuge tube and discard the collection tube containing the filtrate.
22. Add 200 µl Buffer ATE to spin column and incubate sample for 5 min at room temperature.
23. Centrifuge for 1 min at max speed (approximately 20,000 × *g*), room temperature.
24. Quantify DNA concentration using UV or fluorescence absorption spectroscopy.

Store eluate containing DNA at 4°C. For long-term storage, store DNA at −20°C or −80°C.

COMMENTARY

Background Information

Since the discovery that DNA is the genetic material for most organisms, there has been much interest in studying the sequence of DNA to better understand biological processes that determine phenotype expression. In order to study DNA structure and function, there first had to be a method to isolate this biomolecule in a relatively pure and intact state. Early methods for DNA isolation were very time-consuming and used harsh chemicals, such as phenol and chloroform. Over the last 2 decades, the methods for DNA extraction have been improved immensely and are now much more streamlined and safer for the person performing the extraction. There has also been the development of automated extraction machines to help facilitate high-throughput isolation of DNA from tens to hundreds of samples simultaneously. In most clinical, research, and forensic laboratories, commercially available kits are being utilized to isolate DNA from human samples, such as blood, tissue, saliva, and even formalin-fixed paraffin-embedded (FFPE) tissue that has been collected for pathological analysis. Commercial kits are cost-effective and result in consistent extraction of high-quality DNA for use in many downstream applications such as genotyping or next-generation sequencing.

Critical Parameters and Troubleshooting

The amount and quality of DNA obtained from human samples can vary widely depending on the state and type of the starting material. In general, best laboratory practices would involve minimizing the amount of time between the removal of the bodily fluid or tissue from the human body (or cell culture dish) and storage at a very low temperature (−80°C or liquid nitrogen storage highly recommended). For FFPE tissues, it is critical to optimize the fixation conditions of each tissue type to not over-fix the tissue in the formalin. This will make downstream DNA isolation much more difficult and result in lower yield. Most pathology labs have standardized operating procedures for fixing various tissues. When using a kit for DNA extraction, is important to pay attention to the expiration dates of the kits. Many of the wash buffers contain ethanol, which can evaporate and react with water, and result in less-effective washing. Before using DNA in downstream assays, it is highly recommended that the DNA concentration be determined using a fluorometric assay, as this is the most accurate method. In addition, DNA quality should be determined by running DNA on a 1% agarose gel or E-Gel (Invitrogen) to ensure that the DNA is not degraded.

Understanding Results

Again, the amount of DNA can vary depending on the starting material. From whole blood, we can routinely isolate about 50 µg of DNA from 3 ml. Saliva tends to have more variation. We have isolated an average yield of 16 µg per 0.5 ml of saliva with a range of 0.8 to 97 µg. The DNA obtained from fresh/frozen human samples is suitable for most downstream applications including PCR, genotyping, or next-generation sequencing. FFPE tissues have the most variability as anywhere from 1 to 50 µg of DNA may be obtained. The DNA obtained from FFPE tissue would be suitable for PCR and most short-read NGS applications, but not for long-range sequencing.

Time Considerations

The column-based kits for DNA extraction are straightforward. It takes approximately 2 hr of hands-on time to extract 12 whole-blood samples and approximately 3 hr to extract 24 whole-blood samples. For an automated extraction machine, such as the QIASymphony, the setup takes approximately 30 min of hands-on time, then the run takes

approximately 1.5 hr for 24 samples. Saliva extraction using the Oragene kit is also straightforward. There are a few incubation steps, so it is approximately 1 hr of hands-on time for 12 samples, then an overnight resuspension of the DNA is recommended. The time constraints for extracting DNA from tissue (fresh or FFPE) depend on the proteinase K digestion step. Often, this is an overnight digestion of approximately 16 hr or more. After that, the column-based extraction steps are straightforward, the same as for whole-blood.

Author Contributions

Kristy Miskimen: Methodology, resources, writing original draft, writing review, and editing. **Penelope Miron:** Methodology, resources, writing original draft, writing review, and editing.

Conflict of Interest

The authors have no conflicts of interest to disclose.

Data Availability Statement

Any data is available upon request to the corresponding author.

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