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ISOLATION AND DETERMINATION OF DNA CONCENTRATION FROM HAIR

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ABSTRACT

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA). The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences. To isolate DNA from hair, compare their DNA concentration. The yield of DNA obtained from hair root is highly compared to hair shaft.

KEYWORDS: DNA, Hair, Isolation, adenine (A), guanine (G), cytosine (C), and thymine (T).

INTRODUCTION

DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder.

An important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell.

Biologist in the 1940s had difficulty in accepting DNA as the genic material because of the apparent simplicity of its chemistry. DNA was known to be a long polymer composed of only four types of subunits, which resemble one another chemically. Early in the 1950s, DNA first examined by x -ray diffraction analysis, a technique for determining the three dimensional atomic structure of a molecule. The early x-ray diffraction results indicated that DNA was composed of two strands of the polymer wound into a helix. The observation that DNA was double stranded was of crucial significance and only when this model was proposed did DNAs potential for replication and of the DNA molecule and explain in general terms how it is able to store hereditary information.

DNA is a double helix formed by base pairs attached to a sugar-phosphate back bone. DNA is a polynucleotide. The constituent units are coupled by means of 3'-5'phosphodiester bonds. The nature properties and function of the nucleic acids depend on the exact order of the purine and pyrimidine bases in the molecule.

Isolation and Determine DNA Concentration of Hair

Hair is made of a tough protein called keratin. A hair follicle anchors each hair into the skin. The hair bulb forms the base of the hair follicle. In the hair bulb, living cells divide and grow to build the hair shaft. Hair is made up of 2 parts Hair shaft and hair follicle.

Hair Follicle

The hair follicle is the point from which the hair grows.The terminal part of the hair follicle within the skin is called a hair bulb. The hair bulb is the structure formed by actively growing the cells.The cells produce the long,fine and cylindrically shaped hair fibres. The hair bulb has special cells which producing it re known as melanocyte, androgen which hormone located on the cells of this structure.

Hair Shaft

The hair shaft is the part of the hair that sticks out of the skin. The hair shaft is connected to the follicle. There are many components that make up hair structure make it the fastest growing tissue in the body. Hair can be found on many parts of the body,with the most evident being The hair one's head, where the hair shaft is much longer than other parts of the body. Hair shaft damage can cause hair loss and other hair issues.This disease is often caused by genetic problem.

The mammalian hair follicle represents a unique, highly regenerative neuroectoderma mesodermal interaction system that contains numerous stem cells. It is the only organ in the mammalian organism that undergoes life-long cycles of rapid growth (anagen), regression (catagen), and resting periods (telogen).

These transformations are controlled by changes in the local signaling milieu, based on changes in expression/activity of a constantly growing number of cytokines, hormones, neurotransmitters, and their cognate receptors as well as of transcription factors and enzymes that have become recognized as key mediators of hair follicle cycling.

Transplantation experiments have shown that the Transplantation experiments have shown that the driving force of cycling, the "hair cycle clock," is located in the hair follicle itself.However, the exact underlying molecular mechanisms that drive this oscillator system remain unclearthe exact underlying molecular mechanisms that drive this oscillator system remain unclear.

The Hair Cycle

Hair cycling is the rhythmic change of the hair folliclethrough phases of growth (anagen), regression (catagen), and (telogen). *Synchronized* hair follicle cycling (inmammals) prepares the hair coat for seasonal changes in habitat conditions as well as recreationl activities. The purpose of haircycling in mammals with individual (*asynchronous*) follicle waves (eg, humans) is not as obvious, but may include cleaningthe skin surface of debris and parasites, and excretion ofdeleterious chemicals by encapsulation within trichocytes.In addition, follicle cycling might serve as a regulator of paracrineor even endocrine secretion of hormones and growthmodulators produced within the follicle and secreted into theskin or circulation. Finally, hair follicle cycling may act as a Safe-guarding system against malignant degeneration by protecting rapidly dividing keratinocytes from oxidative damage by deletion during catagen.Anagen (the growth phase of the hair cycle) is divided into 6 different stages defined by specific morphologic criteria. The recurrent formation of the hair follicle displays morphologic and molecular analogies to fetal hair follicle morphogenesis.Many molecular key regulators of hair biology (members of the transforming growth factor (TGF)-_/BMP family, andneurotrophils) not only activate morphogenesis but also regulate anageninduction and duration. During anagen, epithelial stem cells differentiate into at least 8 different cell lines, forming the ORS, companion layer, Henle's layer, Huxley's layer, cuticle of the IRS, cuticle of the hair shaft,shaftcortex, and shaft medulla. The ORS probably is established by the downward migration of the regenerating epithelium. IRS and hair shaft are tied together by their interlocked cuticle structures.

After regression, the hair follicle enters telogen, a phase of relative quiescence regarding proliferation and biochemical activity. The follicle remains in this stage until it is reactivated by intrafollicular and extrafollicularsignals. Theunpigmented club hair often remains stuck in the hair canal. In mice, this process takes place mainly in anagenfollicles. Chronobiology of the hair follicle. Every hair follicle is controlled by different timing devices. 1, morphogenesis clock; 2,cycling inducer; 3, hair cycle clock; 4, desynchronizer. The timing devices could be connected with each other and share molecular timing mechanisms (for example the hair cycle clock, which could be "set" already during morphogenesis and therefore incorporate parts of the morphogenesis clock). APM, arrectorpili muscle; CTS,connective tissue sheath; DP, dermal papilla; IRS, inner root sheath;ORS, outer root sheath; SG, sebaceous gland; POD, programmed organ deletion (modified after Paus and coworkers, 1999.

Basic Data

The human scalp, eyebrows, and lashes consist of long, thick medsullated and pigmented terminal hair shafts, whereas the body is covered with short, thin and often unpigmentedvellushairs. Each of us displays an estimated total number of 5million hair follicles, of which 80,000 to 150,000 are locatedon the scalp. The hair length is defined by the duration of anagen, which lasts for 2 to 6 years. Approximately 85% to90% of all scalp hairs are within anagen follicles. Catagen lasts only for a few weeks, followed by the phase,which lasts 2 to 4 months. The usual growth of scalp hairfollicles the rate of hair shaft elongation lies between 0.3and 0.5 mm per day and is dependent on proliferation and subsequent follicular-type differentiation of the matrix keratinocytesin the hair bulb. The thickness of the hair shaft isrelated to the size of the hair bulb, which in turn is dictated by of the hair follicle's mesenchymal.

Forensic Examination of Hair

Microscopy is the main technique used in the forensic examination of hair. The first step is to determine that the sample is really hair. This is carried out by a quick microscopic examination for the presence of cuticle scals, medulla, and pigment granules.

The next step is to determine whether it is of human origin or from some other species. Determination of human origin is based on the relative size and appearance of the scales, and the diameter of the hair shaft.most

animal hairs have smaller diameter and larger medullary index than human hairs.the medullary index is the ratio of the medulla to that of the overall hair shaft which for human hairs isabout one third but one half or greater most for animals.the medulla of most animal hairs is more regular or geometrically patterned than human hair,which tends to have no regular structure. the tips of human hair may be blunt from cutting and styling process,where animal tends to be more pointed.cuticle scals that extend from the shaft of animal hair tends to be more uniform in shape and size and are arranged in more regular patterns than of human hairs.

MATERIALS AND METHODS

Sample Collection and Procedure

Hair samples [three hair each] from the five subjects were washed by immersing them in fresh water to remove the surface dirt and other contamination.the hair samples were picked with clean forceps, washed with 500µl70% ethanol in a 1.5ml microcentrifuge tube, and then kept in a tube containing sterile, deionized water.The hair samples were examined further under a magnifying glass for removing any body fluids if present. The hair were cut off 5-10 mm 5gmof the proximal [root] and shaft end for digestion.

DNA Lysis from Hair Sample

Hair Shaft

DNA was isolated from hair shafts using modified versions of the microscopic glass grinding and organic solvent extraction protocol.these protocols expose the specimen to increased risks of decontamination, the present study has replaced the tedious physical digestion method with a smooth chemical dithiotheritol [DTT] [Hi media] as it is a strong reducing agent with relatively high salt content and also anionic detergent. digestion buffer [500µl; 10Mm TrisHcl, 10Mm EDTA, 50mM Nacl, 20% SDS, PH 7.5] was added to a 1.5ml microcentrifuge tube, along with 40 µl of 1M DTT [to a final concentration of 0.3 mg/ml;Himedia]. Hair sample was added to this solution beforevortexing and incubating for 2hours at 56 C. After 2hours of incubation, the sample tube was vortexed again, and an additional 40µl of 1 M DTT and 15µl of 10mg\ml proteinase K were added, followed by gentle mixing and incubation at 60 C for 2 more hours or until hair was dissolved completely.

Table 1: DNA Lysis from Hair Sample.

BUFFER	10ml	5ml	2.5ml
TrisHcl	12.5mg	6.05mg	2.42mg
EDTA	29.2mg	14.6mg	5.84mg
NaCl	25mg	12.5mg	5mg
SDS	40 µl	20 µl	10 µl

Extraction of Hair Shaft

The DNA was extracted from each Sample with an equal volume of phenol;chloroform;isoamylalcohol(25:24:1) alcohol and mixed gently by inverting the tube for a few minutes. the sample were centrifuged [eppendorf 5415R] for 10 minutes with 10,000g [4 C], followed by transferring the upper aquous layer into fresh,sterilizedmicrocentrifuge tube. The RNAase [10 l of 10mg /ml;fermentas, thermo scientific]was added and kept incubation at 37 C at 30 mintues. An equal volume of chloroform;isoamyl alcohol was added, and the tube was centrifuged [eppendorf 5415R]again at 10,000g [4C] For 10 minutes.the upper aqueous layer was transferred into a fresh, sterilized microcentriuge tube before double volume of chilled isopropanol and 1-10 volume of 3M sodium acetate were added. The sample was chilled at 20 C for 1 hours for the DNA precipitation to occur. the sample was centrifuged [eppendorf 5415R] at 10,000g [4 C] for 10minutes.the supernatant was discarded,250 µl 70% ethanol was added, and the pellet was tapped gently before further centrifugation [eppendorf 5415R] at 10,000 rpm for 10 minutes.the supernatant was discarded, and the pellet was air dried in laminar air flow.theresuspended 5oµl nuclease free water or 1 free buffer, and frozen at 20 C or 80 C storage.

Lysis of Hair Root

Table 2: Lysis of Hair root.

BUFFER	10ml	5ml	2.5ml
TrisHcl	12.5mg	6.05mg	2.42mg
EDTA	29.2mg	14.6mg	5.84mg
NaCl	25mg	12.5mg	5mg
SDS	40µl	20µl	10µl

DNA was isolated from hair root using modified versions of the microscopic glass grinding and organic solvent extraction protocol. These protocols expose the specimen to increased risks of contamination, the present study has replaced the tedious physical digestion method with a smooth chemical dithiothreitol [DTT] [Hi media] as it is a strong reducing agent with relatively high salt content and also anionic detergent. digestion buffer [500µl; 10Mm TrisHcl, 10Mm EDTA, 50mM Nacl, 20% SDS, PH 7.5] was added to a 1.5ml microcentrifuge tube, along with 40µl of 1M DTT [to a final concentration of 0.3 mg/ml; Himedia]. Hair sample was added to this solution beforevortexing and incubating for 2hours at 5µl6 C. After 2hours of incubation, the sample tube was vortexed again, and an additional 40 µl of 1 M DTT and 15µl of 10mg\ml proteinase K were added, followed by gentle mixing and incubation at 60 C for 2 more hours or until hair was dissolved completely.

Extraction Method of Hair Root

The DNA was extracted from each Sample with an equal volume of (25:24:1) phenol;chloroform;isoamyl alcohol and mixed gently by inverting the tube for a few minutes. The sample were centrifuged [eppendorf 5415R] for 10 minutes with 10,000g [4 C], followed by transferring the upper aquous layer into fresh,sterilizedmicrocentrifuge tube. The RNAase [10 l of 10mg /ml; fermentas, thermo scientific] was added and kept incubation at 37 C at 30 mintues. An equal volume of chloroform;isoamyl alcohol

was added, an the tube was centrifuged [eppendorf 5415R]again at 10,000g [4 C]For 10 minutes.the upper aqueous layer was transferred into a fresh, sterilized microcentriuge tube before double volume of chilled isopropanol and 1-10 µlvolume of 3M sodium acetate were added. The sample was chilled at 20 C for 1 hours for the DNA precipitation to occur. thesample was centrifuged [eppendorf 5415R] at 10,000 [4 C] for 10minutes. The supernatant was discarded,250µl 70% ethanol was added, and the pellet was tapped gently before further centrifugation [eppendorf 5415R] at 10,000 rpm for 10 minutes. the supernatant was discarded, and the pellet was air dried in laminar air flow.theresuspended 50µl nuclease free water or 1 free buffer, and frozen at 20 C or 80 C storage.

Concentration and Purity Determination

A quantitative spectrophotometric assay of DNA was performed using a cary 60 UVvisible spectrophotometer [Agilent technologies, santaclara, CA,USA].absorbance was measured at wavelengths of 260 and 280 [A 260 and A 280, Respectively] nm.Theabsorbance quotient [OD 260 / OD 280] provides an estimate of DNA purity. 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.

RESULT AND DISSCUTION

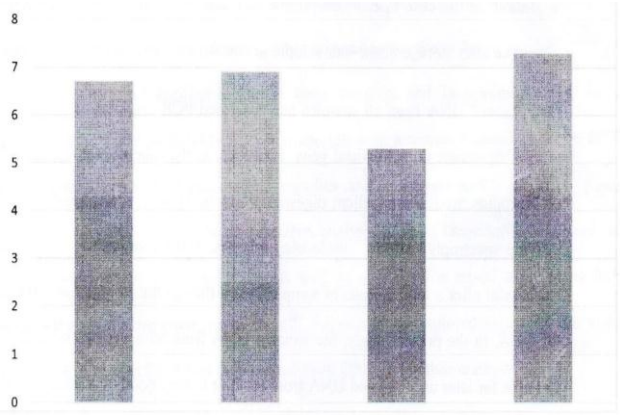
In the present study, we demonstrated a rapid, reliable,and robust method for obtaining spectroscopy ready genomic DNA from human hair samples, demanding very low sample volume with an isolation amplification time that is, at least, a facter of compared two different lysis methods by modifying the conventional phenol –chloroform method, we also successfully developed and demonstrated a reliable protocol that is rapid, cost effective,and readily implemented for the isolation of DNA with optimal concentration and purity.

Yield and purity

The yield of the extracted DNA from the two different hair sample sources was evaluated using a UV spectrophotometer The DNA concentration of extracted by two lysis method and the result showed that the better first steplysis method when they gave the higher DNA concentration of hair root 28 ng/ µland 22ng/ µl compare to hair shaft 20ng/ µland 14ng/ / µl method, although the DNA concentration is low.

Table 3: Difference between hair shaft and hair root.

Hair shaft	Hair root	Hair shaft	Hair root
20mg/ µl	28mg/ µl	14mg/ µl	20mg/ µl



Hair sht hair root hair shaft hair root
20ng/µl 22ng/ µl 14ng/ µl 28ng/µl

Graph 1: Difference between hair shaft and hair root.

DISCUSSION

The yield of DNA obtained from hair root is highly compared to hair shaft. The expected yield using this protocol is hair root 28 ng\ µl and 22mg/ µl hair shaft 20ng\and 14mg/ µl collection which is, at least,a factor of one higher when compared with the conventional methods.the yield and purity of isolated DNA are also dependent on the researcher's handling procedure. A decrease in DNA quality and quantity was observed when the material was not placed immediately in cell lysis buffer for further processing. The whereas degradation in hair sample is not observed, probably as a result of the nature of the sample and the extent of nuclease enzyme concentration in sample before digestion. In the case of hair samples, a high quality of DNA can be obtained for later use, even after storage of the hair sample in ethanol for more than two months at -20 C.

The isolated DNA from all samples has generated of products of a similar base pairs size of the target mitochondrial gene. However, in the case of restriction digestion, hair produced excellent digested products. Hence, we can hair samples instead for the spectrophotometric molecular analysis. Have reported the amplification was successful after a long storage of samples, even though the storage has reduced the yield of DNA. In the present study, the isolated DNA from all samples stored at -20 C was suitable for later use. Isolated DNA from the hair is very good on the basis of the stability of DNA for storage at frozen at -20 C for further analysis.

CONCLUSION

The successful sample collection and the extraction of genomic DNA from hair are noninvasive and reliable alternatives to the sample collectors. We have demonstrated here a simple and novel method of the sample collection and DNA extraction, which is cost effective, easy and rapid, providing a sufficient quantity and quality of DNA for spectrophotometric based analysis. Comparison of the extraction procedure show

that the simple phenol chloroform method is the most suitable for DNA extraction from hair samples. under appropriate storage conditions, DNA isolated from hair can be successfully used to perform to assays. The DTT, high salt, anionic detergent solution mitochondrialDNAamplication success rate of the standard glass organic solvent extraction tecniquescurrently use many forensic laboratories. This relatively lesser number of steps used in this method facilitates shorter time duration and in addition, result in a significantly reduced probability of contamination with a minimal sample loss. The DTT chemical digestion method uses reagent, supplies, and equipment readily available in any basic laboratory. Its case will help in mitochondrialDNA analysis in those laboratories that have yet to undertake forensic mitochondrialDNA testing, as well as a population based study using hair samples. However, important questions still Remain to be explored regarding the yield and quality of human DNA can be obtained from DNA extraction method.

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