Original Article

Increasing Efficiency of Short-Tandem-Repeat Genotyping for a Single Hair by Improving DNA Extraction: A Practical Study

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Abstract:

Background: Although DNA isolation using the commercial chelex 100 is inexpensive and fast, the DNA gained by this method shows a low concentration and is of poor quality in certain materials, such as hairs; high amount of DNA will also inhibit the efficiency of the PCR amplification.

Material and Methods: In the present study, we have improved a hair DNA isolation approach based on chelex 100, where ammonium acetate was added to precipitate proteins, ethanol was used to pellet out DNA, and glycogen was applied as a carrier of successful DNA recovery.

Result: As a result, we obtained high quality of DNA, which can be successfully used for authentic STR analysis from one single hair. We also noticed that the DNA dilution from 25 to 125 fold presented very good quality for short-tandem-repeat (STR) genotyping by only chelex 100 extraction, demonstrating the efficiency for STR genotyping can be improved by dilution of PCR inhibitors or other unknown inhibitors.

Conclusion: Taken together, our improved extraction and dilution of DNA would be used as an option for the application of hair STR analysis in judicial authentication and forensic sciences. This method may be usable for other samples, such as, eyelashes, eyebrows, tissues, sperms and blood samples in human and animals for STR genotype or DNA fingerprinting, and genetics diagnosis including retinal diseases.

Keywords: Short-tandem-repeat (STR); Genotype; Hair; DNA extraction; Genetics.

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Introduction

Short-tandem-repeat (STR) is 2~6 base pairs of repeats of small DNA fragments in both coding and non-coding regions scattering throughout the entire human genome, accounting for ~ 3% [1, 2]. STR markers were first used in the early 1990s and were quickly adopted for the purposes of individual authentication and forensic sciences [3-5]. STR or DNA fingerprinting technology is now used to characterize genetic material identity in human beings [6-9], plants [10, 11], animals [12], and humans or cultured cell lines [13]. The use of poor quality DNA is a common challenge for the STR analysis [14, 15]. To better apply this technology, a high quality DNA is necessary, especially from low-template DNA samples [16]. Small biological samples such as a strand of hair, eyelash, or eyebrow can play an important role in both precision medicine and forensics. In precision medicine, small biological samples such as hair, skin, or blood can be used to gather information about a person's genetic makeup, which can then be used to tailor medical treatment specifically to that individual. This can help to improve the effectiveness of treatments and reduce the risk of side effects. For example, a small sample of a person's hair can be used to analyze their DNA and determine if they carry any genetic mutations that might indicate a higher risk of developing certain diseases. In forensics, small biological samples can be used to identify individuals and determine their involvement in criminal activities. For example, a strand of hair or eyelash found at a crime scene can be used to extract DNA, which can then be compared to a database of known individuals or to a sample from a suspect. If the DNA from the sample match with the suspect, it can provide strong evidence linking that individual to the crime. Similarly, eyelashes or eyebrows can contain trace amounts of DNA that can be used for forensic analysis.

Thus, extracting DNA from small biological samples, such as a strand of hair, eyelash, or eyebrow, can be important for a variety of reasons. In forensic Science: DNA analysis is a powerful tool for forensic scientists in criminal investigations. Samples of hair, eyelash, or eyebrow can contain enough DNA for analysis, even if other biological samples, such as blood or saliva, are not available. This type of analysis can be used to link a suspect to a crime scene or to exclude someone from suspicion. MoreOver in paternity Testing: Extracting DNA from hair, eyelash, or eyebrow can be used to determine paternity or other family relationships. This type of testing can be especially useful when a biological sample from one of the individuals cannot be obtained. Furthermore, in medical Research: Researchers may use small biological samples, such as hair or eyelashes, to study the genetics of a particular disease or condition. This can provide insight into the causes of the disease and help develop new treatments. Finally, in ancestry Testing: DNA extracted from small biological samples can be used to trace ancestry and genealogy. This type of testing can provide information about a person's ethnic background and potential health risks based on their genetic makeup.

Although gDNA isolation using the commercial chelex 100 is fast and rapid, DNA gained by this method shows a low concentration and poor quality from certain materials, such as hair, resulting in the inhibition of the PCR amplification. High amount of DNA will also inhibit the efficiency of the PCR amplification. Hairs are often used for DNA analysis in criminal investigations, judicial authentications and forensic sciences. However, there are some problems with the hair

DNA extraction: (1) the small DNA quantity is highly degraded; (2) the PCR inhibitors are always existed; (3) particular hair pigments, such as melanin, sebum, and cornifin, are known to suppress PCR amplification [17]. In addition, hairs are exposed to sunlight and partly processed chemical oxidation, making even more difficult for STR genotyping.

To increase the qualitative STR genotyping from human hair materials, a small amount but high quality DNA must be successfully isolated and the inhibitors have to been removed or neutralized well. Therefore we present an improved DNA extraction method for increasing efficiency of STR genotyping.

Materials and methods

Reagents and buffers

Chelex 100 was purchased from BioRad (Cat. #: 143-2832). DTT (dithiothreitol) was purchased from Solarbio (Cat. #: D8220, Beijing, China). EDTA (Cat. #: E9884-500G) and isopropanol (Cat. #: I9516-500ML) were purchased from Sigma-Aldrich, ammonium acetate, ethanol from Merck (K45420483412), and glycogen from Thermo Fisher Scientific (Cat. #: R0561). Other reagents were purchased with an analytic grade. 5% chelex 100 suspension (pH 8.0) was prepared in ddH_2O.

Hair DNA extractions by chelex 100

After the informed consent was obtained from the hair donor, one hair strand from a healthy male was collected with root and cut into 1cm in length and placed it into a 500µL Eppendorf tube after the informed consent was obtained from the hair donor. It was rinsed with 500µL of pure water once, centrifuged at 13,000 rpm for 1 min, and removed the supernatant. Then 50µL of 5% chelex 100, 2µL of proteinase K and 2µL of DTT (1M) were added, vortexed and centrifuged shortly. After incubated in water bath at 56°C for at least 2 hr, the sample was boiled at 100°C for 8 min, cooled and centrifuged at 13000 rpm at room temperature for 4 min. The supernatant was transferred into another tube. The DNA quality and quantity were measured by a NanoDrop spectrophotometer and evaluated in a 0.8% agarose gel electrophoresis and visualized by GoldView staining of the samples [18]. Images were documented using the machine ChemiDoc XR (Bio-Rad, USA). And the samples were stored in the refrigerator at -20°C for a long-term storage, or directly used to carry out the next experiment after 5 fold serial dilutions, or further purification.

Protein precipitation

Ammonium acetate solution (7.5 M) was applied to add into the supernatant that was collected after extracting DNA chelex 100, making the ammonium acetate final concentration in solution is 2.5 M. The solution was mixed and vortexed for 5 sec. The supernatant was then centrifuged at 12000 rpm for 10 min. Then we collected the clear supernatant which contained gDNA.

DNA precipitation

Glycogen and absolute ethanol were added to the supernatant containing genomic DNA, vortexed for 5 seconds, and kept at -80 °C for 30 minutes. Then the sample was centrifuged at 15000 g for 15 min at 4 °C. The supernatant was discarded and the pellet was washed twice by using 75% ice-cold ethanol. Each washing step is correct was followed by centrifugation at 15000 rpm at 4 °C for 5 min. The pellet was in air for dry. After air-drying the pellet, 25 µL of 1× TE or deionized water (ddH2O) was added to it and incubated at 55°C for 10

min to ensure DNA solubilization. The quality and quantity of DNA were measured using a NanoDrop spectrophotometer. The DNA was also ran by electrophoresis on a 0.8% agarose gel. The assays for hair DNA extractions by chelex 100, protein precipitation and DNA precipitation were triplicated.

PCR amplification and STR genotyping

STR genotyping was followed to the relevant provisions of the Specification for Parentage Testing by China (GB/T 37223-2018). The AGCU Expressmarker 22 kit (EX22 kit) was purchased from the Wuxi AGCU ScienTech Incorporation (HuiShan, Wuxi, China) [8].

PCR reactions were performed with slightly modification as descried previously [19, 20]. After PCR amplification, 3 μL of product was mixed with 10 μL sample mixture, including AGCU Marker SIZE-500 30 μL and deionized formamide 1000 μL, for Capillary Electrophoresis (CE) with 3500 DX Genetic Analyzer. Genotyping for STR profiles was performed by using our previously described GeneMapper ID-X software [21, 22].

Data analysis

To measure the amplification efficiency, STR values of electropherograms at different DNA dilutions were calculated, and plotted into curves.

Results

Electropherograms of STR genotypes from one hair strand with different dilution folds by chelex 100 extraction are showed in Figure 1. From this figure, we noticed that the DNA dilution from 25 to 125 folds present very good quality of electropherograms for STR genotypes. Without dilution, even though have higher peaks, higher noises in some loci, for example, D3S1358 and D21S11, were noticed (Fig. 1a, red bar), and uneven amplifications in alleles of some loci, for example, D7S820, D2S441, vWA, D6S1043 and D12S391, were also found (Fig. 1a, orange bar). However, highest dilution at 625 fold, showed very low amplification, except no peak at locus D13S317, due to small amount of DNA sample (Fig. 1e). The results were repeated and showed similar quality of electropherograms. In addition, the profiles were consistent with that from his blood (data not shown), demonstrating that the STR genotypes are correct even with different dilutions.

We then plotted relative fluorescent unit (RFU)[23] peak values in D19S433 as a curve with different diluted folds, and found that the PCR amplified efficiency is not positively correlated with the DNA amounts (Fig. 2). The fluorescent signal intensity is positively correlated with the number of labelled DNA fragments. Using the STR genotyping generated by the Genemapper ID-X software, the corresponding alleles can be selected and RFU values obtained. We used the peak height of the allele in locus D19S433 in Figure 1a as the standard value for RFU 1. Lower concentration or small amount of DNA showed higher PCR amplifying efficiency (Fig. 2, blue curve). Consistent with electropherograms for STR genotypes in Figure 1, 25 to 125 folds DNA dilutions presented highest PCR amplifying efficiency with the good quality of electropherogram (Fig. 2). This happened probably due to the presence of certain PCR inhibitors, not due to the high DNA concentration because we only used one hair strand. We plotted the curves by other STR markers and found similar results like locus D19S433 (data not shown).

Then we purified DNA from the chelex 100 extraction products by ammonium acetate and ethanol (Fig. 3). With chelex 100 extraction

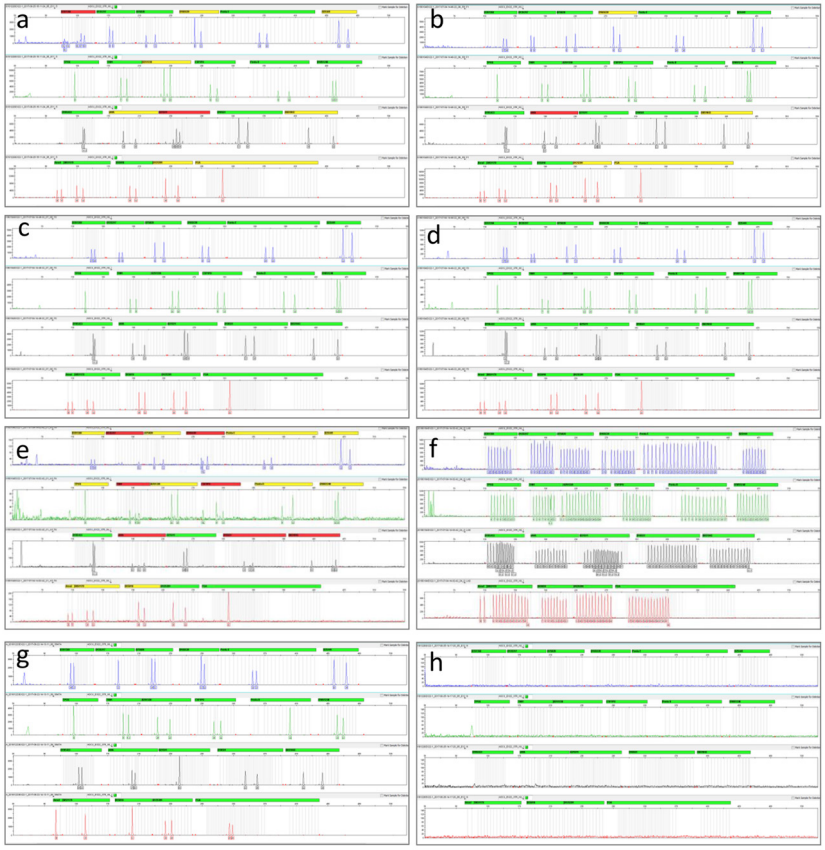

Figure 1: Electropherograms of STR genotypes from one hair strand with different dilution conditions. a. No dilution. b. 5 fold dilutions. c. 25 fold dilutions. d. 125 fold dilutions. e. 625 fold dilutions. f. An EX22 ladder. g. A positive control 9947A. h. A negative control without any DNA template

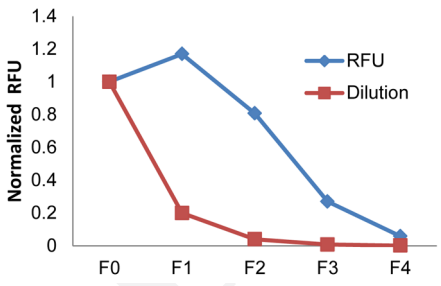

Figure 2: The plots of relative PCR amplifying efficiency. Blue curve, the relative PCR amplifying efficiency. Red curve, series of 5 folds DNA dilutions from F0 to F4. F0, no dilution. RLU, relative fluorescent unit

only, the DNA quality was very poor measured by NanoDrop spectrophotometer and agarose gel electrophoresis. From Figure 3, we noticed that the highest peak is not located at wavelength 260nm, but 245nm (Fig. 3a, arrow), while with purification, the highest peak was exactly located at wavelength 260nm (Fig. 3b, arrow). The ratio of 260/280 for DNA was less than 1.8 (1.73) without purification (Fig. 3a), but after purification, the ratio of 260/280 reached 1.96 (>1.8) (Fig. 3b). Furthermore, we found that the 260/230 ratio for DNA was 0.79 (<2.00) without purification, whereas our modified method yielded an increase in quality (260/230 ratio was 2.00), demonstrating that some chemical inhibitors were successful removed after purification (Fig. 3a&b). More importantly, with the chelex 100 extraction, DNA concentration reached 570ng/μL (average 512.5ng/μL), which false positive, by NanoDrop spectrophotometer, while by agarose gel electrophoresis, the signals on the agarose were similar with purified DNA when

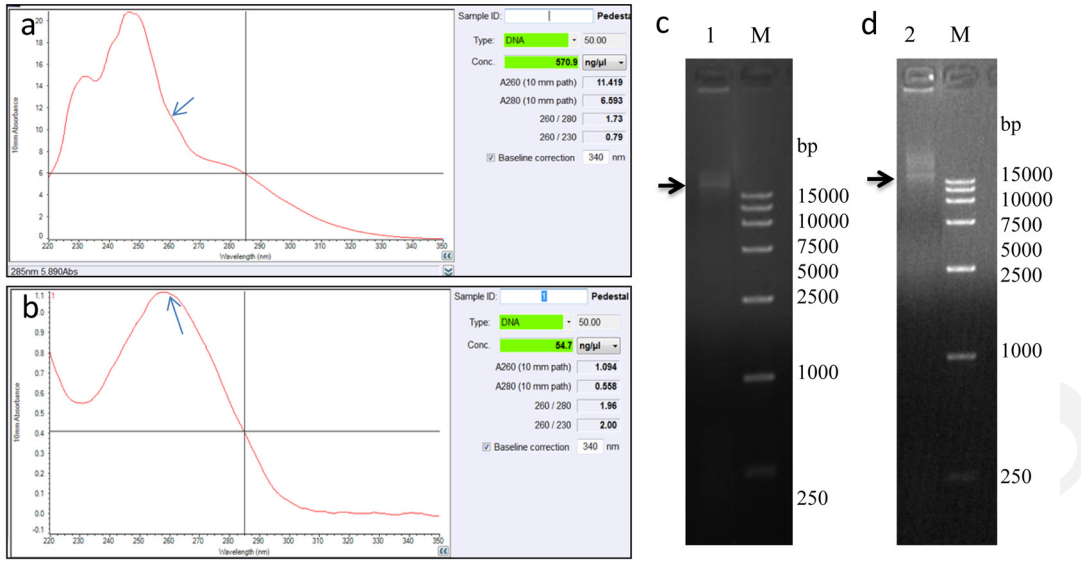

Figure 3: The DNA quality and quantity measurement. The quality and quantity of DNA were measured by a NanoDrop spectrophotometer (a, b) and agarose gel electrophoresis (c, d). a. With chelex 100 extraction but without purification. b. With both chelex 100 extraction and ammonium acetate purification. c. With chelex 100 extraction but without purification. "1", DNA with chelex 100 extraction but without purification. "M", DNA molecular weight maker with size (bp). d. With both chelex 100 extraction and ammonium acetate purification. "2", DNA with both chelex 100 extraction and ammonium acetate purification

we loaded same volume samples (Fig. 3 c & d, compare to the same loads on of the marker). The DNA concentration by ammonium acetate purification was only 54.7ng/µL (average 41ng/µL) by NanoDrop spectrophotometer, which demonstrated that DNA concentration with chelex 100 extraction might show 10 fold higher values than the actual concentration as measured by NanoDrop spectrophotometer measurement. In addition, DNA purification procedures did not affect genome stability (comparing Fig. 3 c to Fig. 3 d). The purified steps were repeated and showed similar results. With 5 folds' series of dilution using purified DNA as a template, the electropherograms of STR genotypes are shown in Figure 4. The quality of STR genotyping was increased with the purified DNA. Specifically, highest concentration, i.e, no or lower dilution, showed slightly better electropherograms (Fig. 4a&b vs Fig. 1a&b) as well as increased RFU values (8000 in Fig. 4a vs 3000 in Fig. 1a of Y axis). Meanwhile, lowest concentration, i.e, 625 fold dilutions, also showed slightly better electropherograms, due to high DNA quality (Fig. 4e vs Fig. 1e). However, we pointed out, even increased the PCR amplification efficiency, poor quality in some electropherograms was still existed.

Then, we plotted the curves by D19S433 RFU values with different dilutions, and found that the PCR amplifying efficiency is positively correlated with the DNA amounts (Figure 5). And we plotted the curves by other STR markers showed similar results like D19S433 (data not shown).

Discussion

The extraction of DNA from small biological samples has a wide range of applications and can provide valuable information in various fields. The use of hair, eyelash, or eyebrow

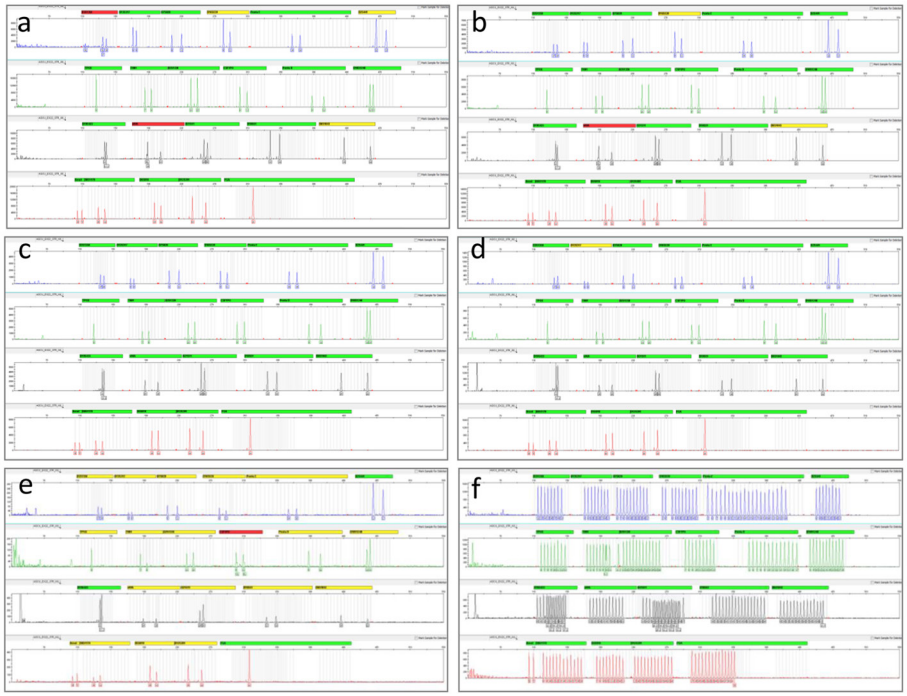

Figure 4: Electropherograms of STR genotypes from one hair strand with different dilutions after purification. a. No dilution. b. 5 fold dilutions. c. 25 fold dilutions. d. 125 fold dilutions. e. 625 fold dilutions. f. An EX22 ladder

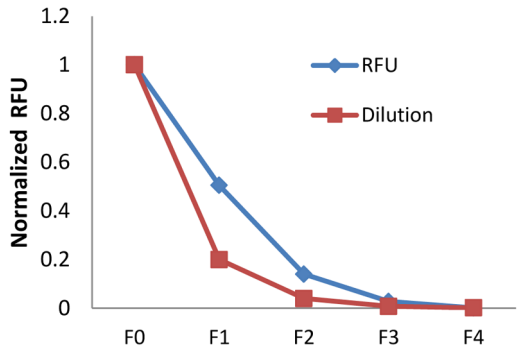

Figure 5: The plots of relative PCR amplifying efficiency after purification. Blue cure, the relative PCR amplifying efficiency. Red curve, series of 5 fold DNA dilutions from F0 to F4. F0, no dilution. RLU, relative fluorescent unit

samples can be particularly useful in situations where other traditional biological samples are not available or feasible to obtain. The ability to extract DNA from small biological samples has helped to advance many areas, including precision medicine, forensics, paternity testing, medical research, and ancestry testing. The advancements in this field have also led to improved methods for DNA extraction and analysis, making it possible to obtain reliable results from smaller and less invasive samples. Overall, the ability to extract DNA from small biological samples has been instrumental in advancing the fields of forensic science, pathophysiology, genetics and more, and has helped to provide important information for many individuals and communities.

Chelex 100 as a medium for DNA extraction from different biological samples has been developed since 1991 [24, 25], and became the standard protocol in forensic medicine in some countries including China, requiring the rapid extraction of DNA from traces of forensic materials. However, the drawback of using Chelex 100 for this is the contamination that can inhibit PCR amplifications [17], such as, organic debris in the form of proteins, including

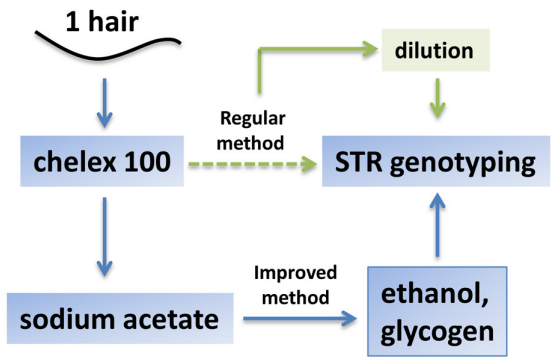

Figure 6: Schematic workflows of DNA extraction and STR genotyping. Regular method with dilution and improved method with purification by sodium acetate, ethanol and glycogen may be useful for STR genotyping. Blue arrows indicate schematic workflow for DNA chelex 100 extraction/purification; solid green arrows indicate schematic workflow for DNA chelex 100 extraction with dilution, whereas dashed arrows indicate schematic workflow for DNA chelex 100 extraction without dilution

haemoglobin in blood, and other compounds such as immunoglobin G, lactoferrin, and myoglobin [26, 27]. Moreover, by using hairs, some problems have been encountered in judicial authentications and forensic sciences, including the small and degraded DNA quantity, PCR inhibitors, particular hair pigment, chemical oxidation by exposure of sunlight, even makes more difficult STR analysis. Developing an improved DNA extraction method is thus necessary in our practical applications [14, 27, 28]. Recently the efficiency of DNA extraction from samples coated with fingerprint powders was evaluated, which highlighted the importance of screening the DNA of the fingerprint powder [29]. In addition, high amount of DNA will inhibit the efficiency of the PCR amplification. In our study, we extracted high quality DNA for STR analysis, through successfully removing the PCR inhibitors using ammonium acetate for protein depletion and ethanol for DNA precipitation. It is also helpful in the precipitation of DNA by ammonium acetate [30]. Based on this study, we used ammonium acetate as an important part of our approach. We also added glycogen as a carrier for the successful DNA recovery, especially for trace DNA, which is much better than that of tRNA or sonicated genomic salmon sperm DNA. Because tRNA or genomic salmon sperm DNA as a carrier will hinder PCR amplification. By comparing these two methods (regular vs improved method), we found that the 260/230 ratio for DNA is 0.79 and the DNA concentration was false positive after multiple iterations of the previously standard Chelex method, whereas our modified method yielded an increase in quality (260 and 230 nm ratio of 2.00), demonstrating that some chemical inhibitors were successfully removed. 260/280 ratios were also increased from 1.79 to 1.96, demonstrating that proteins were successfully removed. Our method also yielded an increase in the quantity of DNA with a minimum amount of sample (single hair). Thus the DNA yield and success rate of STR analysis should be increased by this modified method. This method was repeated and showed very similar results. Thus, the method might also be useful for other samples, such as eyelashes, eyebrows, tissues, sperms and blood of human and animals for STR genotyping or DNA fingerprinting, and gene diagnosis.

Moreover, we also noticed that the DNA dilution from 25 to 125 folds presented very good quality for STR genotyping, demonstrating efficiency can be improved through dilution of PCR inhibitors and other unknown inhibitors. We may dilute the DNA if only chelex 100 was used for extraction as standard protocol in the practical applications when sampling hair(s). The two methods

or strategies for DNA extraction and STR genotyping are summarized in Figure 6. Of course, a hair from a man may not enough. Genders and ages may have influences on STR analysis. In forensic practice, we should comprehensively consider those issues.

Taken together, our improving DNA extraction and DNA dilution might be useful for STR analysis in our judicial authentications and forensic sciences. Chelex 100 extraction with dilution is an optional method for STR genotyping.

Conclusions

We have successfully authenticated and identified STR analysis from one hair strand by improved DNA extraction. We noticed that the DNA dilution with appropriate folds presents better quality of STR genotyping. Thus our improved extraction and dilution of DNA can be used as an option for the application of hair STR analysis in judicial authentication and forensic sciences. This method may also be useful other than hair samples, such as eyelashes, eyebrows, tissues, sperms and blood samples of human and animals for STR or DNA fingerprinting. In addition of forensic sciences, it may also have applications in the gene diagnostics such as pathogenic DNA and retinal diseases. In conclusion, the ability to extract DNA from small biological samples is an important tool for various fields, as it allows for the analysis of DNA when larger or more traditional samples are not available. Thus, our work helps to improve extracting DNA from small biological samples such as a strand of hair, eyelash or eyebrow.

Ethics approval and consent to participate

This study was ethical approved by the Southwest Medical University. Written informed consent for hair sample collection was obtained.

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.