Study on the feasibility of applying DNA molecular marking technology to anti-counterfeiting of artifacts and artworks

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Abstract. Artifacts and artworks record the wisdom of human activities and civilization. They not only trace an era’s history or reflect the aesthetics of contemporary art and different lifestyles, but they also enable us to understand the codes of wisdom passed down from our ancestors and explore history. Therefore, determining artworks’ authenticity is increasingly important. The objective of study was to investigate the feasibility of applying DNA molecular marking technology to anti-counterfeiting of artifacts and artworks. The DNA sequence used for testing in this research came from the team’s previous studies. A genetic substance was embedded in the artwork to test the DNA’s feasibility, repeatability, and reproducibility, as well as the difference in the deterioration conditions of the DNA embedded artwork. Our results showed that about 70% to 90% of the DNA fragments remained intact. Therefore, we conclude that embedding the extracted DNA directly into a painting and detecting whether the DNA fragments persist after reconstitution could help us to determine the authenticity of a painting.

1. Introduction

Molecular markers, often referred to as DNA markers, have seen extensive application. Molecular markers are different among individuals or ethnic groups (polymorphic issues facing artifacts and artwork with molecular markers and their technirphism); therefore, experimenters have used them as the basis for identification and in experimental applications [1]. Given the markers’ distinctions, it is possible to find a close correlation between a suitable molecular marker and the target trait, and to resolve the practical characteristics. Along with the rise of Taiwan’s cultural education level and economic status, art collecting has become a fashionable trend and an aspiration pursued by all types of collectors. However, as the number of authentic and valuable artifacts and artworks is limited, a greater demand than supply has increased market prices sharply. In Taiwan, collectors have sought not only local works of art but also valuable Chinese artifacts circulating in the global market. The short supply phenomenon has triggered numerous counterfeit incidents in Taiwan and internationally[2]. Counterfeit artwork circulates commonly in Taiwan’s art market. For example, works by senior Taiwanese painters like Liao Chi-Chun, Chen Cheng-Po, Li Mei-Shu, Li Shih-Chiao, Kuo Po-Chuan, and Chu Ming have all been implicated in counterfeiting disputes. Counterfeit artworks also appear in Western art markets, where collectors describe these counterfeit artworks as an incurable plague on the art market [3]. Counterfeiting techniques are also advancing with the times; the relationship between works of art and counterfeits can be described as a pair of twins who are inseparable. So long as art is with us, fakes and forgeries will be, too [4]. However, not all counterfeiting incidents can be clearly dealt with by the law—indeed, the majority cannot. A number of disputes fell into discussions at cross purposes or were left unresolved due to
insufficient and limited scientific detection technology. In some cases, the evidence provided by relevant parties was not strong enough to support the party’s claims, or else commercial interests between galleries or auction houses implicated in disputes rendered these disputes unresolvable [5]. Many fraud detection techniques derive from scientific and technological advances. Art history style analysis and scientific detection analysis are the two most common methods for artifact detection. Art history style analysis refers to the observation of appearance, texture, structure, production technology, technique, brushstroke, style, signature, and genuineness, which provide the basis for determining authenticity. Scientific detection refers to a series of quantitative analyses, such as material analysis and dating analysis, using scientific detection instruments to infer an object’s age and material. Scientific detection methods are passive means of testing artworks’ authenticity, and these methods fail to achieve anti-counterfeiting. Therefore, we should take anti-counterfeiting into overall consideration to resolve artwork authenticity issues more effectively.

Among scientific detection instruments, non-destructive testing (NDT) remains preferable. NDT examples include X-ray fluorescence (XRF) spectrometers, used on inorganic materials, and Fourier-transform infrared spectroscopies (FT-IR), used on organic materials. Related components are detected, and graphic analysis determines the obtained data’s composition, permitting speculation on components’ ingredients. Researchers ascertaining a certain object’s age may use the carbon-14 dating protocol, a destructive detection method. The carbon-14 dating protocol is a method using the radioactive isotope carbon-14, which exists widely in nature, as a marker to determine the age of formerly living animals or plants. As a carbon isotope, carbon-14 exists throughout the atmosphere and ocean, and in all living tissues. When a plant or animal dies, the amount of carbon-14 in the dead plant or animal continues to decay (half-life 5,730 ± 40 years) as the plant or animal stops bringing in new carbon-14. Therefore, by measuring the amount of carbon-14 remaining in dead samples, we can estimate the animal or plant’s time of death [6]. These methods are used to detect whether the composition of an art object matches materials used by the object’s contemporaries or by current material sources, although these methods will not feasibly prevent artwork forgeries, nor can these methods identify an object’s authenticity. However, molecular (DNA) evolution has, in recent years, advanced rapidly. Molecular DNA studies are applied in the study of life barcodes, species identification, phylogenetic structure, gene transfer, biogeography, and genetic relationship. Methods include allozymes, mitochondrial DNA, restricted fragment length polymorphism (RFLP), and microsatellites. The structure of mitochondria in animal cells is composed of 22 transfer ribonucleic acid (tRNA), 13 protein-coding genes, 2 ribosomal ribonucleic acid (rRNA) genes, and a control region (D-loop). The control region (D-loop) is less conservative and has the highest variation, making this region vulnerable to insertion and deletion events [7][8]. Related studies on the application of DNA in identification and anti-counterfeiting include those by Hayward (2007), who used DNA to prevent counterfeiting and product diversion[9]; Yang (2006), who studied PCR-RFLP mitochondrial gene analysis on the identification of species and related cephalopod species products[10]; Wang et al. (2008), who applied DNA gene polymorphism to identify animal hair fibers[11]; Gao and Song (2009), who used DNA as a new anti-counterfeiting measure[12]; Liao et al. (2010), who applied DNA barcodes in anti-counterfeiting ink[13]; Cai (2010), who used DNA barcodes to study leptocephali around Taiwan[14]; and Hsiao et al. (2010), who used life barcoding to develop identification technology for oyster species and ascertained differences between oyster species in Taiwan and in other regions[15]. Hayward and Meraglia (2011) applied DNA marking and authentication to the electronics industry[16]; Wei (2012) used DNA barcodes to identify larval fish species and their genetic relationship[17]; Lu et al. (2012) found a novel method to identify yak fiber in textiles[18]; and Chang and Zhong (2012) converted DNA sequences into the barcode of life data system to establish plant IDs. Some scholars [20][21] used gene sequence fragments to explore genetic relationships, and these scholars’ experiments yielded strong outcomes.

In view of the above, the rapid development of molecular biology has helped in the expansion of its application. DNA is a suitable molecular marker for species identification, product anti-counterfeiting, and certification. To date, however, relevant studies on embedding DNA into works of art are rare. Based on DNA’s characteristics of stability, diversity, and continuity, as well as on the ability to produce gene variations of DNA’s genetic substance, this study attempts to apply DNA marking in artifacts and artworks for the purpose of anti-counterfeiting and to prove that the characteristics of genetic markers
shall provide sufficient reference for the prevention of art forgery. This study aims to accomplish the following objectives: (1) Test and evaluate the feasibility of DNA embedment; (2) Test the feasibility of embedding DNA fragments into artworks and the repeatability and reproducibility of the DNA; (3) Test the difference in the deterioration conditions of DNA embedded artworks.

2. Research methods
Forged artworks or swapped items persist in the market. Scientific methods are now used to assist with the identification of artworks and obtain relevant information such as material or production date. Yet these methods remain at the verification stage; the methods are not used proactively as preventative measures to avoid genuine items being forged or swapped. DNA is a unique biological genetic substance that can scarcely be reproduced. It also has a proven track record of good research outcomes in various fields. Applying DNA characteristics to anti-counterfeiting provides stronger evidence for the authenticity of artifacts and artworks. Therefore, this study takes DNA as its research topic, first using existing short DNA sequence fragments (about 200 bp) as materials for the experiment and embedding them in the art object as molecular markers, then testing whether the DNA fragments can be re-extracted. Figure 1 shows the research method’s concept.

![Figure 1](image-url)

Figure 1. The research method’s concept.

This study used the DNA samples (rice) obtained from previous studies as the test material. The operating procedures for DNA extraction were primarily based on the Kit. To confirm the quality of the DNA product, we took about 5μl of DNA extraction solution and added 1μl of dye before loading the solution onto the 1.2% agarose gel. An electrophoresis experiment was conducted at a voltage of 100 volts for about 35 minutes in a TBE buffer; we then dyed the extraction with Ethidium Bromide (EtBr) solution for 30 minutes. We photographed the extraction in a UV light box to confirm the size of the DNA fragment.
Next, we carried out the polymerase chain reaction (PCR) technology to copy the DNA fragments. The PCR reaction condition was done in the mixed solution with the total volume of 50μl per tube. The mixed solution contains 1μl DNA extraction sample; 5μl of 10X PCR buffer; 0.4μl of 0.2mM dNTP (containing 0.2mM of dATP, dGTP, dCTP, dTTP each); 1μl each for the primers; and 1.5mM MgCl₂, 0.4μl of 2.5 unit super Taq DNA polymerase. The solution was filled with sterile water. It was sent into a thermal cycler (Perkin Elmer-Cetus 9600) for the PCR reaction. The temperature settings are as follows: the solution was first heated to 94℃ for 1 minute to melt the double-stranded DNA into separate strands (denaturation); cooled to 45℃ for 1 minute, as a low temperature would promote binding of the primer and DNA (annealing); and the temperature was increased to 72°C for 1.5 minutes to allow the extension of the hybridized primers (extension). The steps of denaturation, annealing, and extension constitute one cycle. A total of 35 amplified reaction cycles were conducted.

3. Results and discussion

3.1. Detect whether the DNA product still exists in the petri dish
We used specific DNA gene fragments (from Rice A and Rice B) to detect the specific DNA gene fragments in the petri dish, which was first left to air-dry. We then used a TE buffer to reconstitute the DNA fragments (Figure 2). Subsequently, we used a spectrophotometer (Nano Drop) to detect changes in concentration of the DNA and followed the agarose gel electrophoresis protocol to detect the integrity of the DNA. The results show that the concentration of specific DNA gene fragments was recovered almost completely (98.9 ng/μl → 95.5ng/μl) (Table 1), and the electrophoresis gel results also indicate that the DNA was intact and without degradation (Figure 3).

Table 1. Original compared to air-dried and reconstituted concentrations of the specific DNA.

| Fragment     | Original | Air-dried and Reconstituted |
|--------------|----------|-----------------------------|
| DNA fragment | 98.9     | 95.5                        |

*The rice DNA samples were obtained from the team’s previous studies

3.2. Detect whether the DNA product remains on the canvas
We dropped two specific DNA fragments on a canvas sample painted with four color pigments (white, blue, black and green) (Figure 4), and left it to air dry at room temperature before using TE buffer to reconstitute the DNA fragments. Next, we used a spectrophotometer (NanoDrop) to detect the changes in DNA concentration and followed the agarose gel electrophoresis protocol to detect the product’s DNA integrity. The results show that the concentration of specific DNA gene fragments could be
recovered with a rate ranging from 70% to 90% (Table 2). The electrophoresis gel results also indicate that the DNA was effectively intact with no sign of degradation (Figure 5). We also found the trace of DNA on all four of the air-dried and reconstituted color fabrics, which allowed us to conduct and complete the subsequent PCR experiment. Figure 6 shows the program settings for the PCR experiment.

### Table 2. Comparison of the original and the air-dried/reconstituted concentrations of specific DNA fragment A on the canvas painted in different colors.

| Item                              | Concentration of the original DNA fragment | Concentration of the reconstituted DNA fragment |
|-----------------------------------|--------------------------------------------|------------------------------------------------|
| Canvas painted with white pigments| 180.6                                      | 132.6                                          |
| Canvas painted with blue pigments | 104.2                                      | 98.3                                           |
| Canvas painted with black pigments| 99.4                                       | 96.6                                           |
| Canvas painted with black pigments| 133.5                                      | 100.8                                          |

Figure 4. Rice A DNA on the canvas.
4. Conclusion
By dropping specific rice DNA fragments in a petri dish and conducting concentration detection and agarose gel electrophoresis experiment after air drying and reconstitution, we found that the DNA fragments in the reconstituted buffer solution remained intact. We then dropped the same DNA fragments on the canvas and followed the same steps to conduct another experiment. The results showed that about 70% to 90% of the DNA fragments remained intact. Therefore, we conclude that embedding the extracted DNA directly into a painting and detecting whether the DNA fragments persist after reconstitution could help us to determine the authenticity of a painting. Embedded DNA could therefore be feasible as an anti-counterfeiting tool.

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