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# Effect of visible laser irradiation on DNA examination in crime scene investigation

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**ABSTRACT** The laser appearing technique of 445nm and 532nm visible bands is widely used in the field of crime scene investigation and physical evidence identification. The discovery of the remaining biological specimens on scene is one of the important functions for laser visualization technique. The combination of laser appearing technique and DNA inspection technique has greatly improved the on-scene efficiency. The purpose of this paper is to study the influence of visible light laser irradiation on DNA test results. Firstly, the absorption spectrum of DNA in the ultraviolet visible band was measured by using the calf thymus DNA (ctDNA) as the object of study. The results showed that DNA was not absorbed in 445nm and 532nm bands, while DNA showed strong absorption in short wave ultraviolet band. Secondly, the DNA standard products were irradiated under the extreme experimental conditions which based on DNA standard material (AmpFLSTR® Control DNA 9947A) as the experimental object, using 445nm, 532nm laser and 254nm light source, combined with the actual situation of the scene, irradiation of the DNA standard in the experimental conditions are extreme. And the irradiated DNA standard material was tested. The experimental results showed that the irradiation of 445nm and 532nm laser would not affect the detection of DNA standard grade point, while 254nm short wave UV had obvious influence on DNA test. It can be concluded that the irradiation of 445nm and 532nm laser will not significantly affect the DNA test results. While the 254nm short wave ultraviolet light can easily destroy the structure of DNA molecules during irradiation, thus affecting the DNA test results, because of the high photon energy. Although the visible laser irradiation does not significantly affect the DNA test results, considering the object and inspection materials is complicated, high power laser irradiation on the object and the long time to sample damage and may even damage the DNA. It is suggested that low power laser should be used as far as possible to reduce the damage of laser to the object and DNA specimen when using laser to investigate the scene.

***KEY WORDS*** Crime scene investigation; Laser; UV-visible absorption spectrum; DNA test; Forensic science.

## 1. INTRODUCTION

Laser is a strong coherent light, showing monochromatic, directional and high brightness characteristics. It exhibits its unique advantages in exciting fluorescence, which is unparalleled in other conventional light sources. For a strong coherent light, with its excellent characteristics, the laser in the scene search and traces of the show has played a huge role [1-4]. Especially with the laser miniaturization and portability to further improve, the laser has become evidence identification and scene investigation process indispensable identification of exploration equipment. It is one of the main functions of the laser to use the laser to search for the biological samples that are left on the scene. This mainly includes the search and display of traces of left handprints, blood, saliva, fine spots and other biological samples. After the discovery of various types of biological samples, it often needs to carry out its

DNA test. At present, in the actual physical evidence identification and scene investigation commonly used 445nm and 532nm visible band laser. These two bands of laser on a variety of objects on the sweat, grease fingerprints, and various types of biological samples have a very good effect [4-6]. In this paper, DNA standard was used as the experimental object, combined with the actual scene investigation, the appropriate method to exclude the object factor, the use of 445nm and 532nm laser DNA standards for a long time irradiation. The effect of visible band laser irradiation on DNA test has been studied and compared with 254nm shortwave UV.

## 2. MATERIALS & METHODS

### 2.1 Experimental subjects

Calf thymus DNA (ctDNA, Sigma, USA); DNA standard materials (AmpFLSTR® Control DNA 9947A, Thermo Fisher, USA).

### 2.2 Experimental equipment

Dual-beam UV-visible spectrophotometer (TU-1901, Beijing Purkinje General Instrument Co.,Ltd., China）; 445nm laser biological discovery instrument (AD-III, Hubei Anda Security Equipment Co., Ltd., China); 532nm laser material investigation instrument (XS-L-MINI-G8, Suzhou Xiaosong Technology Development Co., Ltd., China)； 254nm UV light source (EF-160C/12, Spectronics, USA); Quartz cuvette with the optical path of 1cm (Nanjing Chenghua, China); Quartz cuvette with the optical path of 0.cm (Nanjing Chenghua, China); ProFlexTM PCR System (Thermo Fisher, USA); 3500XL Genetic Analyzer (Thermo Fisher, USA).

### 2.3 Experimental methods and procedures

Experiment with DNA as the experimental object. The UV - Vis absorption spectra of DNA were measured by using cortis DNA (ctDNA), and the DNA standard (AmpF STR Control 9947A) was used for laser and short - wave ultraviolet irradiation.

The experimental steps are as follows:

1) A certain amount of ctDNA was dissolved in secondary distilled water, allowed to stand at 4 ° C for 24 hours, was sufficiently dissolved and placed in a quartz cuvette having an optical path length of 1 cm. The ctDNA solution was measured by a two-beam ultraviolet-visible spectrophotometer Of the UV - Vis absorption spectra;

2) A solution of four 100 μL, 0.1 ng / μLDNA standard (AmpFLSTR® Control DNA 9947A);

3) Two equal amounts of the DNA standard solution were placed in a quartz cuvette with an optical path length of 0.2 cm, and the height of the DNA standard solution in the cuvette was less than 1 cm, respectively, using 445 nm and 532 nm laser irradiation , Irradiation time is 20 minutes, irradiation distance is 10cm;

4) The solution of the same amount of DNA standard was placed in a quartz cuvette with an optical path length of 0.2cm, and the solution was irradiated with 254nm shortwave ultraviolet light for 20 minutes and the irradiation distance was 10cm.

5) After the end of the illumination, 200 μL of ultrapure water was added to the cuvette, and the turbulence was fully vortexed. The PCR was carried out with 4 μL solution as template. The amplification system was 10 μL, , MIX 4 μL, primer 2 μL, according to 95℃ × 11min → (94℃× 20sec → 59℃× 3 min) × (28-29) cycle → 60℃× 10min → 4℃ insulation, the program for PCR expansion The amplification products were detected by 3500XL Genetic Analyzer, the loading voltage was 10KV, the loading time was 3s. The results were analyzed by Gene Mapper IDX software, and the results were analyzed and compared.

The standard solution was placed in a quartz cuvette. In order to make the laser and short-wave ultraviolet light and DNA standard solution full effect, the use of quartz cuvette optical path is only 0.2cm, the direction of irradiation perpendicular to the cuvette transparent surface. In the actual scene investigation search traces when the laser irradiation time is generally not very long and the laser output from the traces of the distance. In the experiment, more extreme experimental conditions were considered, the irradiation time was set to 20 minutes, and the irradiation distance was 10 cm. In the case of laser irradiation, the spot diameter at 10 cm from the laser output was adjusted to about 2.5 cm. The height of the DNA standard solution in the cuvette is less than 1 cm and is located at the center of the laser spot. The laser spot completely covers the DNA standard solution. Laser power adjustment for the maximum power, 445nm laser maximum output power of 8W, 532nm laser maximum output power of 8W.254nm. Short-wave UV light source output side of the larger, when the irradiation of 254nm short-wave ultraviolet light completely covered DNA standard solution.

## 3. RESULTS

The UV-visible absorption spectra of the ctDNA solution are shown in Fig.1. The absorbance of ctDNA solution at 260nm is A260 = 1.891, the absorbance at 280nm is A280 = 1.09, the ratio is A260 / A280 = 1.73, and the content of protein in the prepared ctDNA solution is very small in the range of 1.6 to 2.0.

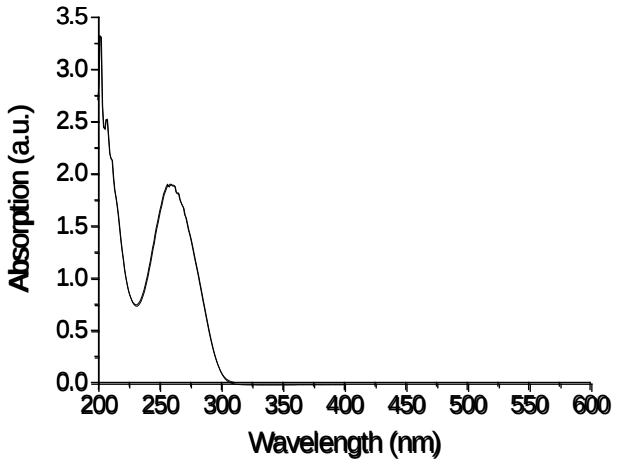


Fig.1 UV-visible absorption spectrum of the ctDNA solution

According to formula [7, 8]

$$C(\text{ctDNA}) = A_{260}/6600\ \text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1},$$

where L is the thickness of the cuvette used is 1cm, the concentration of ctDNA solution can be 2.865 × 10-5 mol / L.

It can be seen from Fig. 1 that the ctDNA solution is hardly absorbed in the visible region and absorbs in the short-wave ultraviolet region with a wavelength less than 300 nm, and increases with the shortening of the wavelength, an absorption peak occurs at 260 nm [9].

Figure 2 is a DNA test result obtained by testing the untreated DNA standard solution. Figure 3 and 4 shows the 445nm and 532nm laser irradiation after the DNA standard solution obtained test results. Figure 5 for the 254nm short-wave ultraviolet radiation DNA standard solution obtained after the DNA test results.

It can be seen from Fig. 2, Fig. 3 and Fig. 4 that the 445 nm laser and 532 nm laser irradiation did not affect the sites in the DNA assay, although the amplitude at the different sites was slightly different. And DNA standard solution in 254nm short-wave ultraviolet radiation, has been unable to detect the various sites, loss of DNA test value, as shown in Figure 5.

## 4. DISCUSSION

In order to accurately obtain the effect of laser irradiation on DNA test, and other factors to exclude the object of the experimental results of the interference, the experiment only use DNA as the experimental object. In the scene investigation encountered in the paper, clothes, leather and other objects a wide range, including DNA blood, saliva, fine spot and other biological samples are also varied. In order to study the effect of visible laser irradiation on DNA test, to avoid interference between objects and other substances in biological samples, the human DNA standard solution was selected as the experimental object and placed in quartz cuvette. The aqueous solution in the DNA standard does not absorb visible light and shortwave UV. Quartz cuvette on the visible light transmittance is of 90% or more, the shortwave UV transmittance of more than 80% [10]. The aqueous solution and the cuvette do not affect the results of DNA testing. The maximum power of the 445 nm and 532 nm lasers was used during the irradiation, and the distance between the laser output port and the cuvette was set to 10 cm and the irradiation time was set to 20 minutes. In practical applications, in order to improve the search efficiency, in the search will often set a larger laser beam divergence angle, while increasing the distance between the laser output and the target to expand the search area. Even when the suspicious traces are displayed, the maximum laser power is rarely used and the irradiation time is not long. In the use of laser for scene search, it can be determined that, the unit area for the material received by the laser energy is much smaller than the experimental exposure to DNA standards on the energy. The UV-visible absorption spectra of the ctDNA solution in Figure 1 show that there is little absorption of 445 nm and 532 nm light, and no significant energy transfer occurs between the laser photon and the DNA molecule when the laser is irradiated. DNA peaks were tested for DNA standards that had not been irradiated with laser light, and 26 peaks were detected (Fig. 2). The same sites were detected by the DNA standard after 445 nm and 532 nm laser irradiation, as shown in Fig. 3 and Fig. Compared with Fig. 2, Fig. 3 and Fig. 4, it can be seen that there is no significant difference in the DNA test results given by the laser irradiation and the irradiated DNA standard, so that it can be inferred that the irradiation of the visible band laser does not significantly affect the DNA test results. While the 254nm shortwave UV irradiation showed a significant difference. The DNA test results of DNA standard after 254 nm shortwave ultraviolet irradiation are shown in Fig. 5. After prolonged exposure to 254 nm short-wave UV, the DNA test did not get the information of each locus. At this time, the DNA standard solution lost the value of DNA test. Significant difference in UV wavelengths of 254 nm was related to the strong absorption of DNA molecules on 254 nm shortwave UV absorption. The photon of the shortwave ultraviolet band has a high energy that destroys the structure of the DNA molecule during the interaction with the DNA molecule and thus has a significant effect on the DNA test results [11-14].

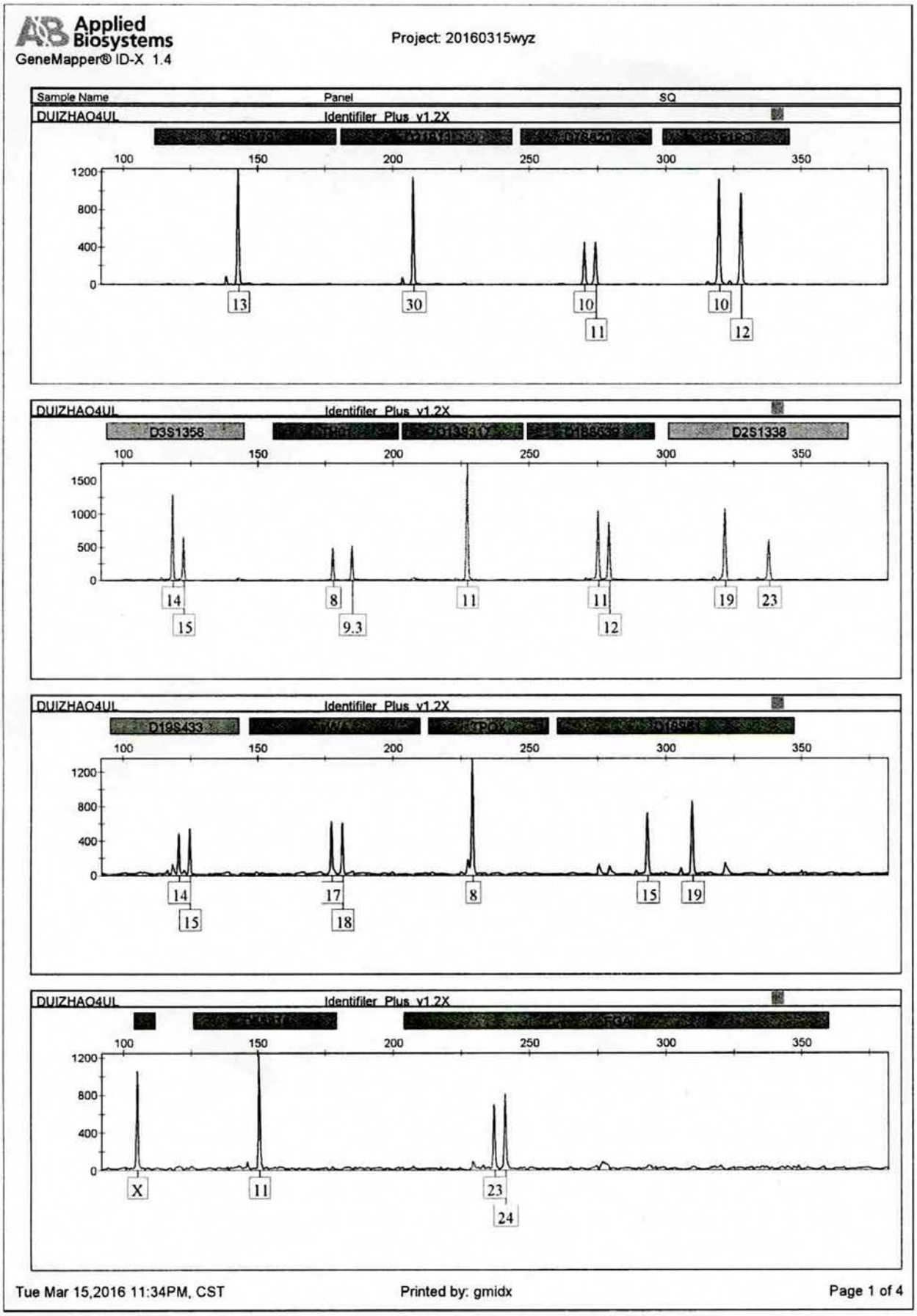


Fig.2 The result of the DNA examination of the DNA standard solution

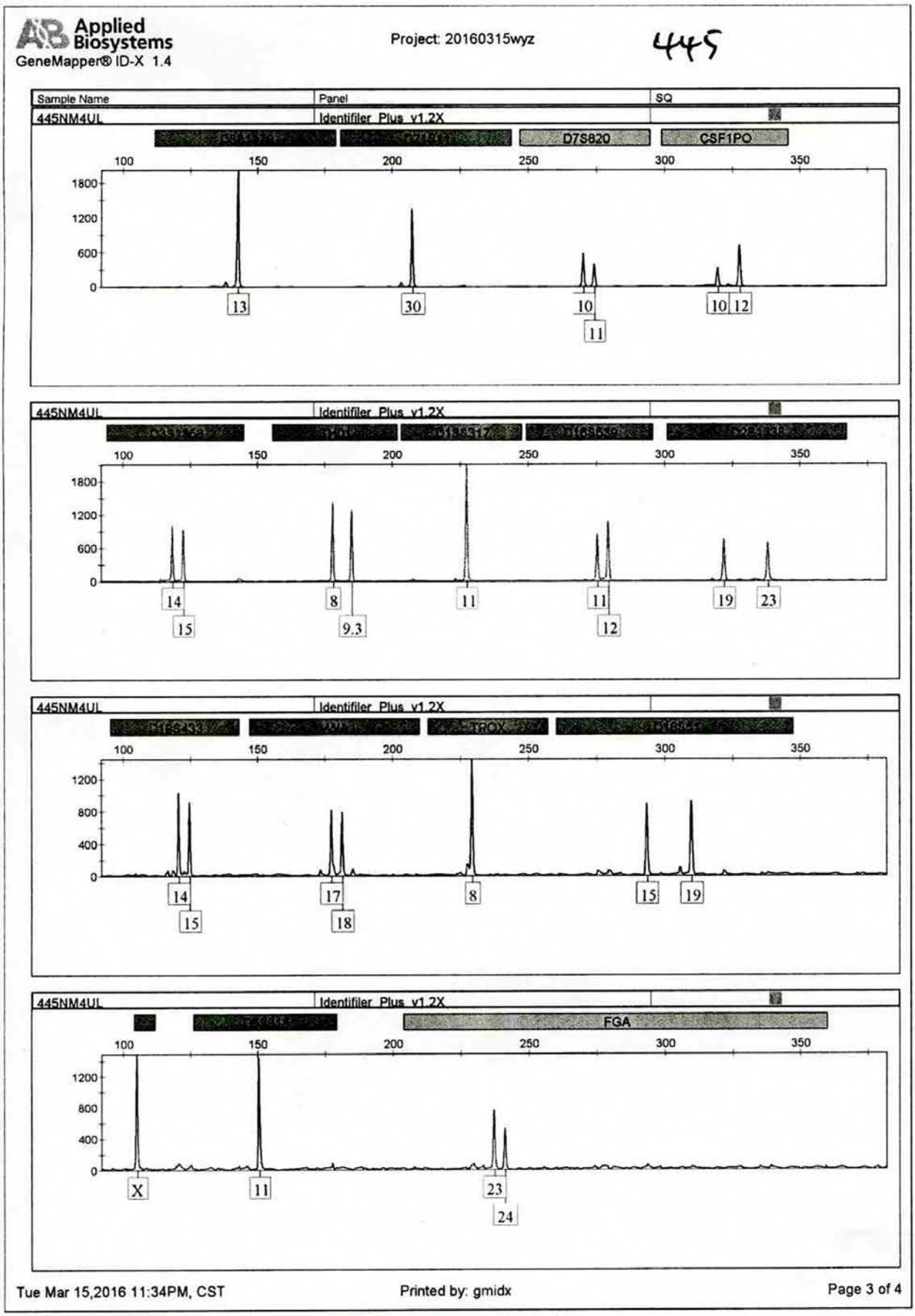


Fig.3 The result of the DNA examination of the DNA standard solution after irradiation with 445nm laser

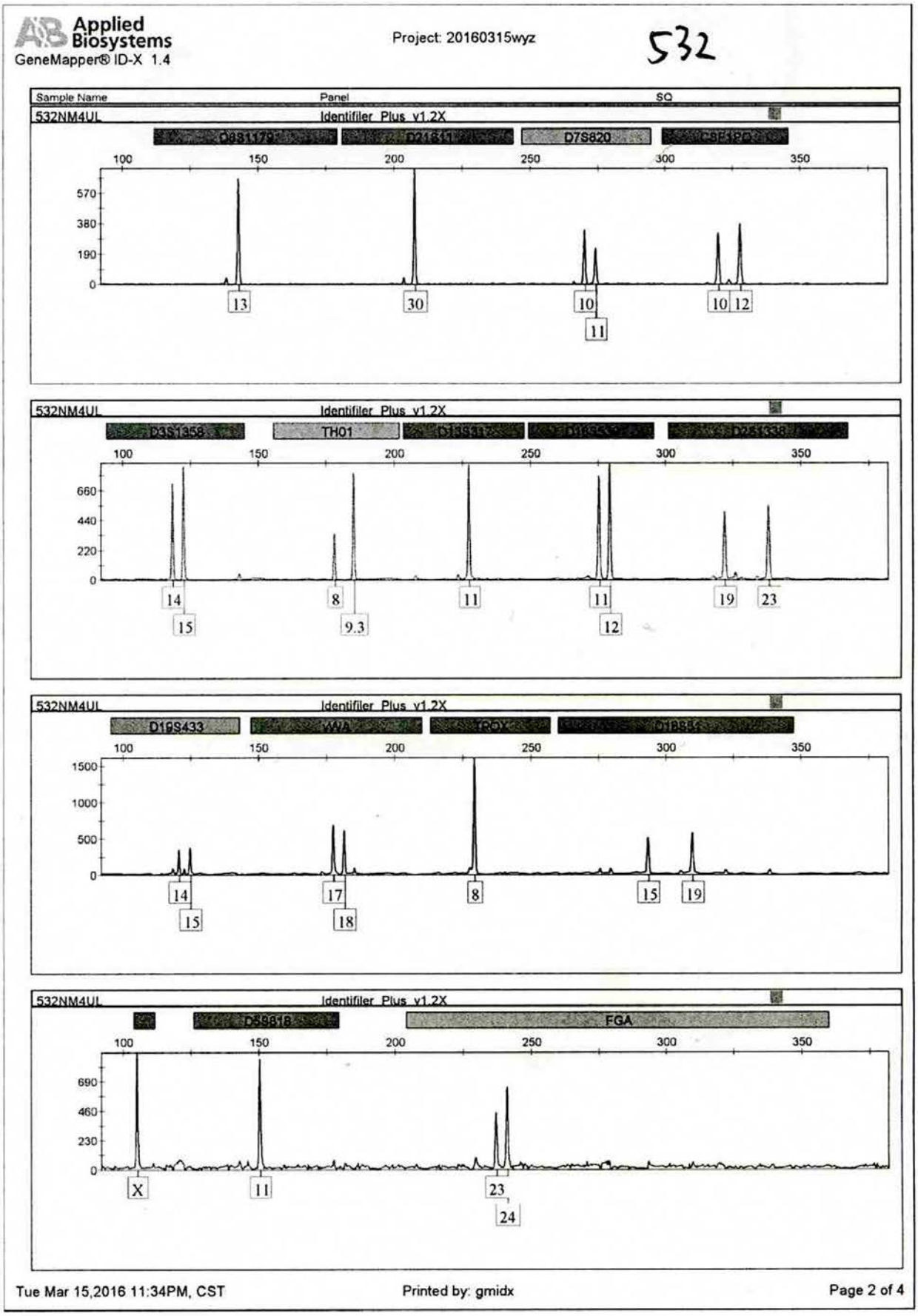


Fig.4 The result of the DNA examination of the DNA standard solution after irradiation with 532nm laser

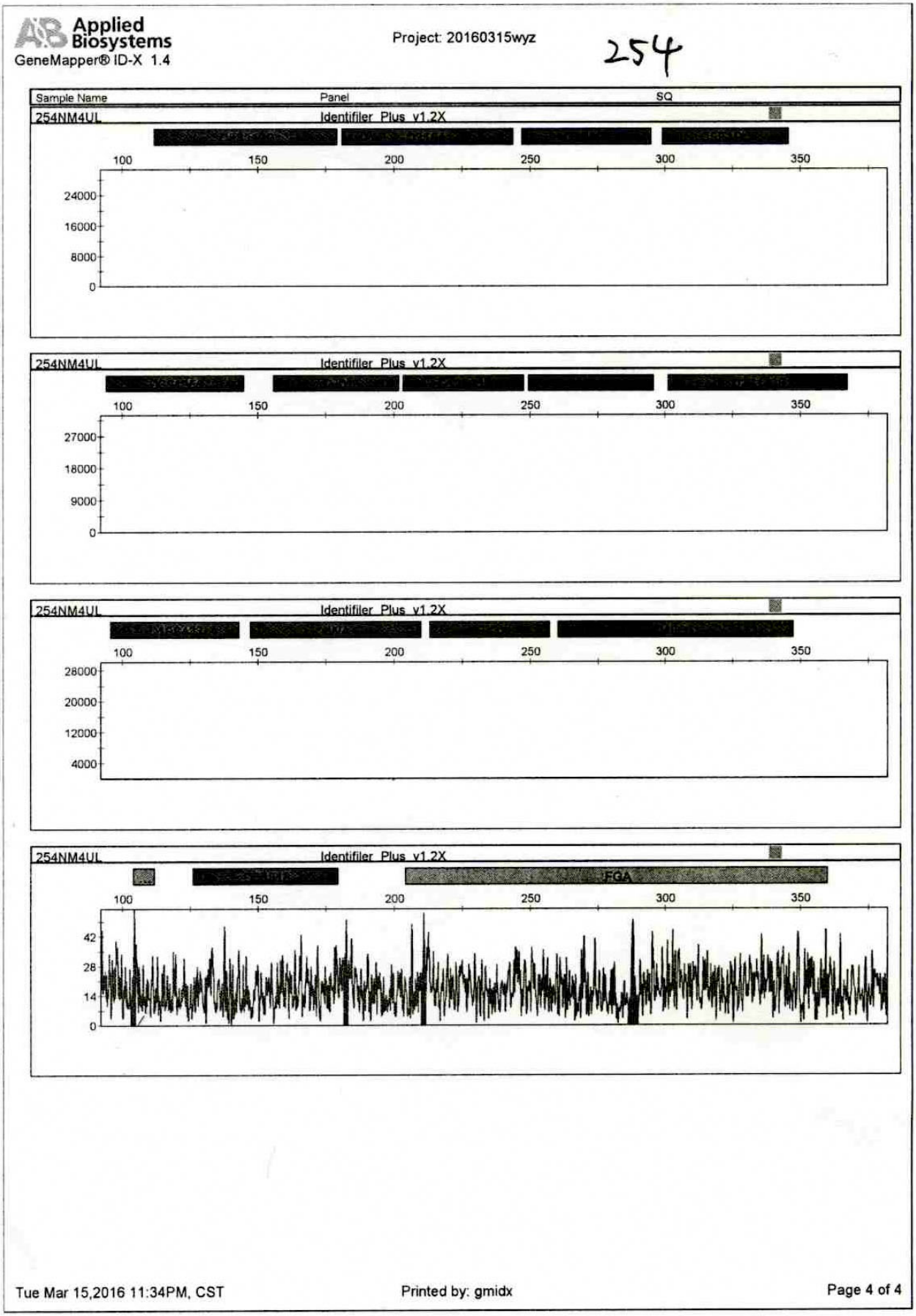


Fig.5 The result of the DNA examination of the DNA standard solution after irradiation with 254nm UV light

It uses laser to search scene traces of evidence, mainly through the laser excitation fluorescence to achieve the presence of traces of the scene. On the scene, the atoms or molecules in the fluorescent substance absorb the laser energy under the excitation of the laser to form the excited state, and the atoms or molecules in the excited state migrate to the ground state and emit a certain wavelength of fluorescence. However, DNA molecules can hardly absorb visible light and cannot fluoresce under visible laser excitation, and the endogenous fluorescence of DNA is very weak [15], and cannot be detected by conventional detection equipment. In the crime scene DNA molecules do not exist independently, but included in a variety of biological samples. The fluorescence observed under laser excitation in the actual scene investigation is not the fluorescence emitted by the DNA itself. Fluorescence signal mainly comes from the two parts, one is a variety of components in the sample issued by the fluorescence, such as fine spot, in the laser excitation of the fluorescence signal is mainly semen in the protein, amino acids, Component of the fluorescence emitted [15]; the other is the object of the object issued by the fluorescence, such as blood footprint where the cement floor, cement flooring in the laser excitation issued under the strong fluorescence, and blood show the dark traces and cement floor The emitted fluorescence is clearly contrasted. Although the visible band of laser irradiation does not destroy the DNA molecules, a variety of biological samples and the object on the visible band of laser often have strong absorption. Laser energy is absorbed by the specimen or object, part of the conversion into fluorescence, the other part into heat. The heat generated by this energy conversion is very easy to damage the DNA molecules, especially dark objects such as dark clothing, leather, etc., in high-power laser irradiation is easy to produce high temperature caused by damage to the test material, and even lead to DNA Molecular damage. Therefore, in the scene investigation process, in achieving the effect of the premise, it should use low-power laser and reduce the irradiation time, to avoid the laser on the object and the damage. 254nm shortwave ultraviolet light can also stimulate a variety of biological samples and produce fluorescence, because of its high photon energy and most of the material on the 254nm showed strong absorption. However, 254 nm shortwave UV has a significant destructive effect on the molecular structure of DNA, and requires careful caution when searching and traces using 254 nm shortwave UV.

## 5. CONCLUSIONS

DNA molecules in the visible band are almost no absorption. In scene investigation, the commonly used 445nm, 532nm visible band laser on the DNA molecules will not produce significant damage, almost no impact on the current forensic DNA test. However, due to the complexity of the objects and samples in the crime scene, the laser on the different objects and the role of different materials, 445nm and 532nm laser in the course of the use of laser attention to the object and the damage, try to use low power And reduce the exposure time, to avoid the laser object and the destruction of the material. Laser display technology in the scene investigation has played a significant role, has become an indispensable exploration scene investigation means. In the process of using laser to carry out exploration, we need to take full account of the characteristics of laser and the actual scene, take the appropriate technology and methods and combined with a variety of other investigation techniques, give full play to the role and advantages of laser in order to improve the efficiency of investigation.

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