Degradation of Extracted DNA from Human Male Sperm Cells by UV Radiations and Exposure Time

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ABSTRACT

Deoxyribonucleic acid (DNA) degrades rapidly when exposed to environmental factors like free radicals, high temperature, different types of radiation, and relative humidity). These studies are being conducted to investigate the trend of DNA degradation when known whole semen samples are exposed to UVA, UVB, and UVC radiation, respectively for different periods of exposure. Forensic techniques utilized included DNA extraction from human male sperm cells through organic methods, genomic human DNA quantitation using Quantifiler®Y Human Male DNA Quantification Kit on ABI 7500 Real-Time PCR. Human DNA was amplified by using AmpFlSTR®Yfiler® Kit as per manufacturer- recommended protocol and electrophoretic separation of amplified products by the ABI PRISM® 310 Genetic Analyzer through capillary electrophoresis. GeneMapper® ID v3.2.1 is used to analyze data with a peak detection threshold of 100 relative fluorescence units. Genetic analysis data was used to monitor the level of DNA degradation by analyzing 17 Short Tandem Repeats (STR). The data collected from the samples exposed to natural UV radiation showed that 100 minutes of exposure resulted in no viable DNA remaining. The data from the 365 nm (UV A) artificial exposure indicated no notable loss or damage of DNA. The data collected after exposure to 254 and 302 nm (UV B&C) of artificial UV radiation was inconclusive and is currently undergoing further testing. Final results of this study can be helpful to establish the limitations of human identification utilizing Y-STR markers.

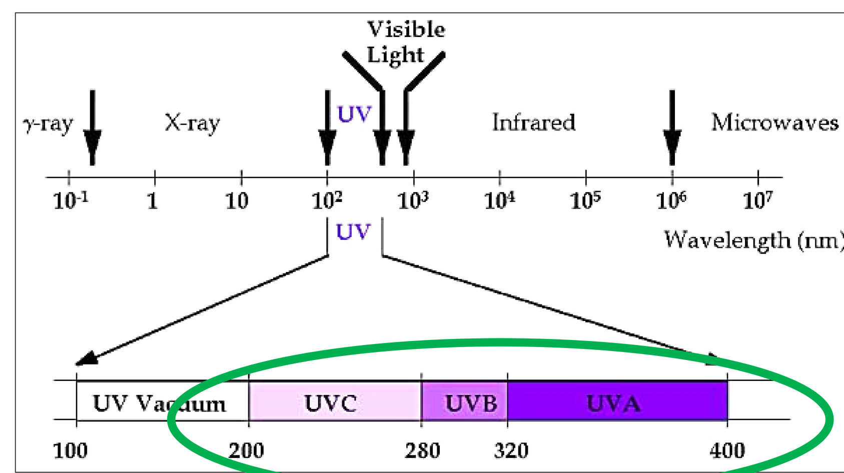
INTRODUCTION

It is known that Ultraviolet (UV) radiation has a progressive degrading effect on DNA. In the case of sperm cells (haploid) it is unclear the time of exposure or the intensity of light rays required to render the extracted DNA no longer useful for determination of DNA profiles. Haploid cells contain only one complete set of chromosomes which in reproduction combine with one complete set of chromosomes from the other parent. In cases involving human semen, such as sexual assaults, it is assumed that such frequencies of light waves will have similar effects on DNA extracted from sperm cells. Particularly in the field of forensic science, this information will be useful in determining the extent of damage done over the time semen is exposed to UV radiation.

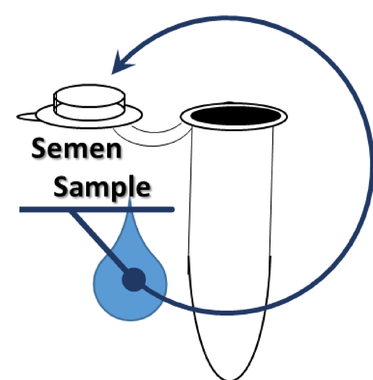
UV radiation can cause cyclobutane pyrimidine dimers to form or (6-4) photoproducts to occur, where a pair of thymine or cytosine bases become covalently bonded within a DNA strand, which causes the base pairs to be misconstrued during analysis. In order for polymerase chain reaction (PCR) amplification to occur, the DNA must be intact where the two primers bind as well as between the primers so that full extension can occur. The short tandem repeat (STR) region serves as a template strand; if there is a break in the DNA strand that are found between these regions the PCR primer extension will be unsuccessful.

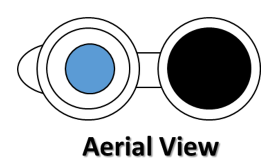
Differential extraction can be used to separate male and female DNA, but is often not able to completely separate the male and female components. Y-STR testing is most commonly used in sexual assault cases to detect and amplify specifically the male portion of the sample in question. AmpFlSTR®Yfiler® Kit used in PCR amplification allows only the male fraction to be amplified while in the presence of female DNA. Capillary electrophoresis is lastly used for identification of male STRs (17 markers) within DNA sample.

Comparison of whole human semen and extracted DNA from human sperm cells of the same donor will show an applicable linear slope of degradation when exposed to both natural UV light and synthetic UV light. In theory, when haploid DNA is bound to histone protamines in its chromosome packaging, there will be less opportunity for fragmentation of DNA or introduction of pyrimidine dimers. The highly specialized nuclear proteins in sperm create a chromatin structure that is at least six times denser than histone bound (diploid) DNA, and is highly compacted by the replacement of histones with sperm-specific low molecular weight protamines.



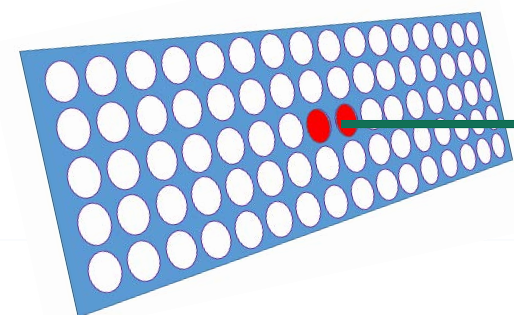
MATERIALS AND METHODS





Whole semen was used to make two sets of exposure types; 1) dried on the cap of the tube and 2) on FTA paper 3mm punch, both using 10 µl of semen from a single donor. Each exposure type was broken down into groups by the wavelength pre-exposure, and again by the time of exposure. Once exposure time was complete, samples were removed from the UV source and placed in dark storage.

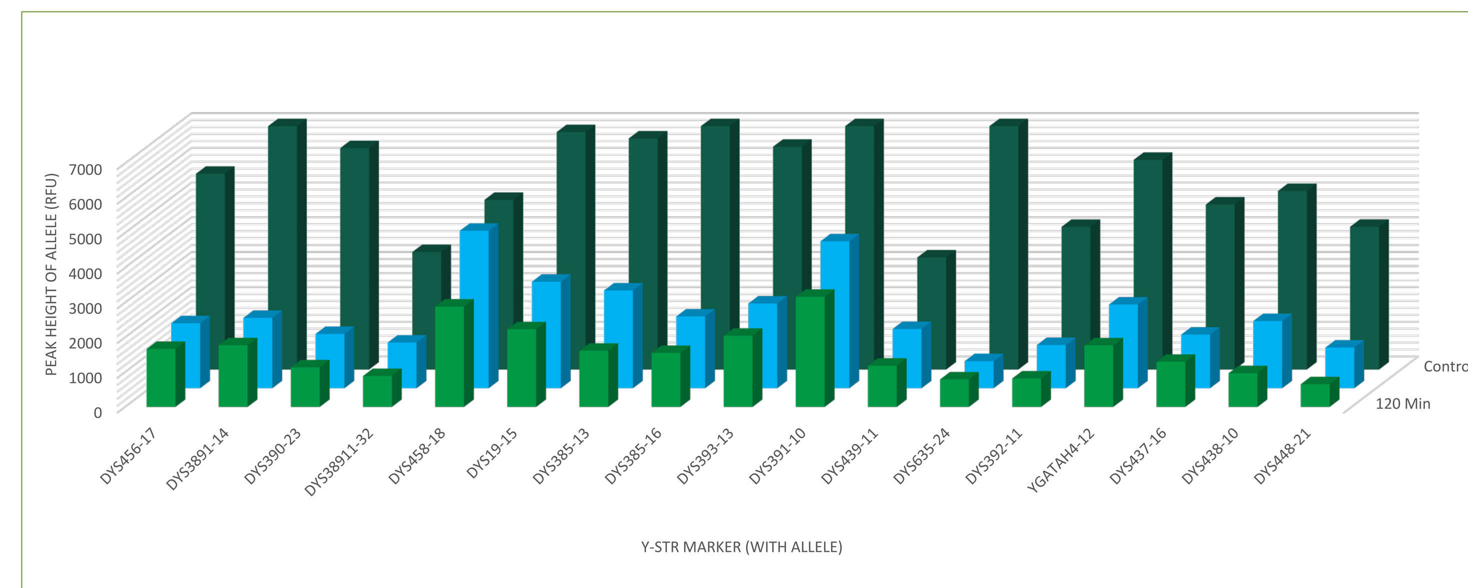
The samples were quantified after organic extraction from whole semen exposed samples using Quantifiler®Y Human Male DNA Quantification Kit on ABI 7500 Real-Time Polymerase Chain Reaction (PCR) mechanism to calculate the concentration of male DNA in each sample. Once the concentration was determined the samples were diluted to 0.1 ng/µL before moving the samples on to amplification via AmpFlSTR®Yfiler® Kit processed on the BIORAD iCycler™ Thermal cycler. Following amplification, the samples were ran on the 310 Genetic Analyzer which used capillary electrophoresis to produce the genetic profile of the DNA contributor. Quality Controls were ran along with these samples to ensure there was no concern of contamination.



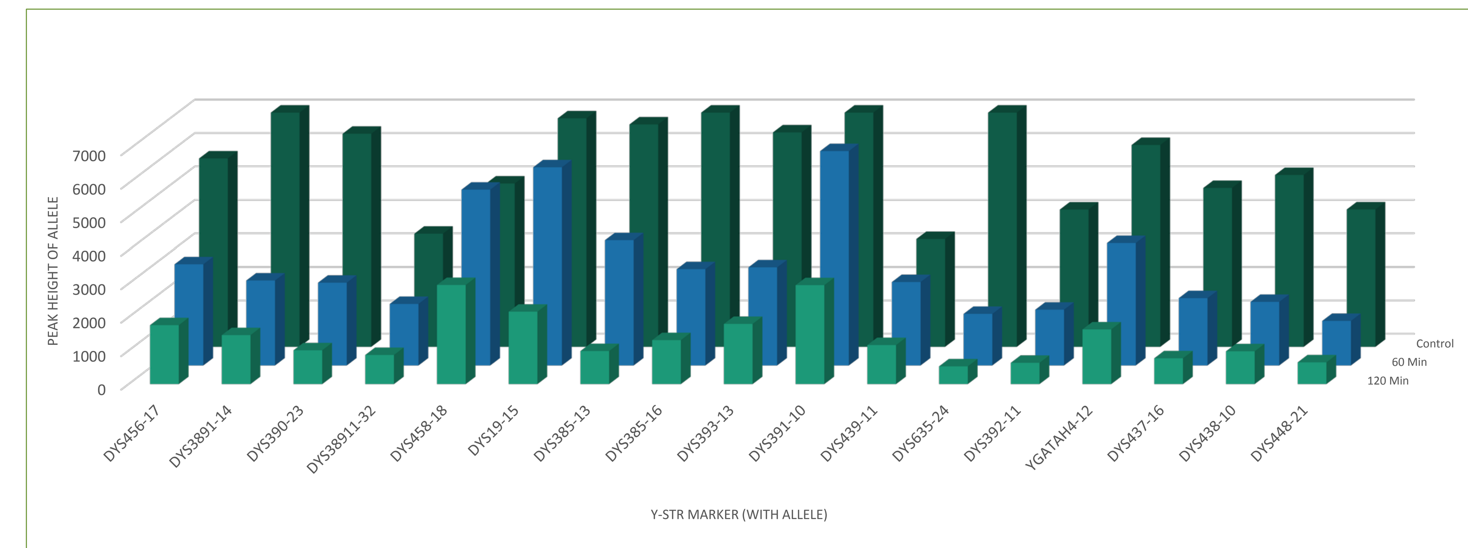
Sample tubes centered on rack through exposure to insure maximum strength exposure.

RESULTS

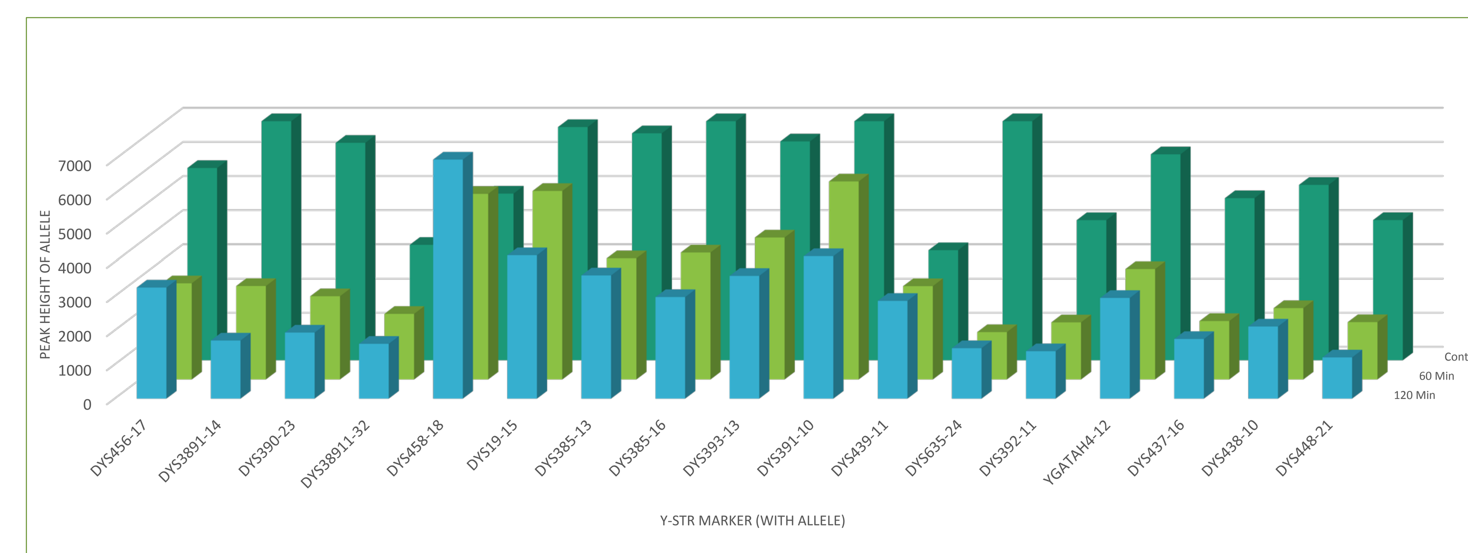
The height of each of the alleles between time variations at increments of 20 minutes are compared to a positive control sample (for the whole semen exposure). Signs of fragmentation and other forms of DNA mutation caused by UV radiation are noted in peak height changes and fluctuation of concentration of DNA in quantitative results. Such information was charted and analyzed to produce statistical data showing the correlation between time exposed and amount of mutation/degradation that affected the quantitative and qualitative results.



Artificial UV Exposure (254 NM): Y-STR markers (labeled on the X-axis) show significant loss of peak height (Y-axis) over the two hour time period (120 Min) of exposure when compared to the control sample which did not receive any sort of UV treatment and was stored in a dark room.



Artificial UV Exposure (302 nm): Y-STR markers (labeled on X-axis) show a significant decrease in peak height between the control samples (0 min) and the two hour (120 min) samples after exposure. One of the highest degrees of degradation can be seen in DYS635 marker that is greatly depleted after exposure.

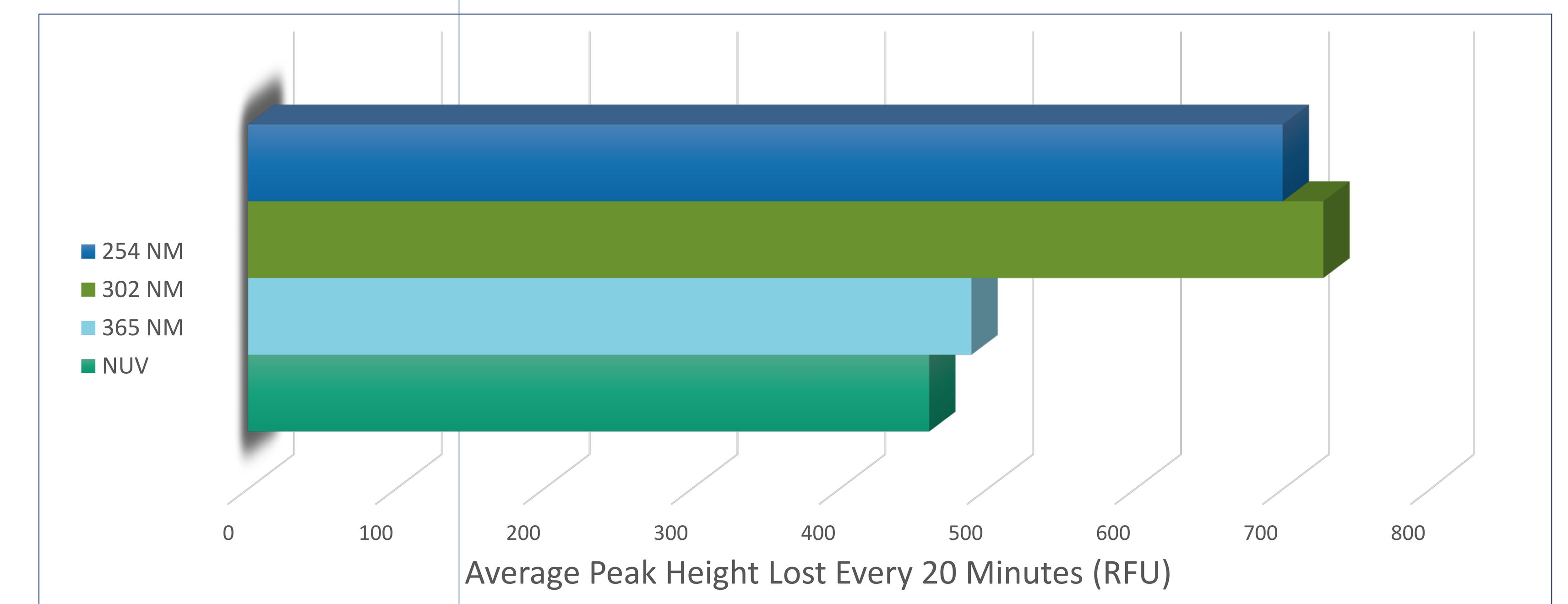


Artificial UV Exposure (365 nm): The least amount of damage done via artificial UV light in this group is from the 365 NM exposure. Of the three categories of UV light seen this wavelength is most common at the earth's surface.

Natural UV Exposure: The Y-axis depicts the peak height of the allele while the X-axis depicts the marker (sequence) represented. After exposure genetic analysis shown above includes peak heights recorded after a specific length of exposure to UV radiation (Time listed on the far right).

Not pictured is the negative control and reagent blank that were included in the testing for quality assurance, each sample gave the expected result of no DNA being detected.

ANALYSIS



Rate of Loss for Wavelengths/Exposure: The measured loss values are calculated by comparing decrease in value of peak heights per twenty minute intervals for each wavelength and for natural exposure. As shown above the greatest average loss was found to be in samples exposed to 254 & 302 nm or UVC and UVB wavelengths. Loss within the 365 nm and Natural UV Exposure (NUV) are seen to be comparable, this is mainly due to the 365 nm wavelengths' ability to reach the Earth's Surface and accounts for a portion of the natural ultraviolet light.

CONCLUSIONS

- The quantitative and qualitative results of the samples, both in concentration and peak height, show a reasonable and expected amount of degradation after exposure.
- Analysis of the peak height for each allele when compared to the unexposed sample (control) was able to define a rate of loss for the specific exposure type.
- Each exposure type or wavelength seen to cause different variations in amounts of damage, the most of which is 302 nm, followed by 254 nm, both are known to rarely reach the Earth's surface and are filtered by the ozone in the atmosphere. Thus accounting for the expected result that the two would have little correlation with the damage done in the natural UV exposure.
- Genetic analysis of the extracted (pre-exposure) samples will be done in the near future to gather a better understanding of the hypothesized "protective" effect of the packaging of haploid DNA to such things as ultraviolet light. This will allow for comparison of the rate of loss DNA (from the same donor) can differ depending on its origin from the human body.
- The data collection throughout these processes are to be analyzed as well to define the limitations of the technology.

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