International Research Journal of Computer Science (IRJCS)
Issue 06, Volume 3 (June 2016)
ISSN: 2393-9842
www.irjcs.com

Re-evaluation of the Current NMI01 STR Sizing System of *Cannabis* DNA

Ryan P. Clarke*
Department of Forensic Science
University of New Haven

Prof. Heather Miller Coyle
Department of Forensic Science
University of New Haven

Abstract— The NMI01 STR region of Cannabis sativa DNA is currently developed for source attribution of seized Cannabis by law enforcement. However, the current system does contain some limitations, mainly the lack of a commercially available allelic ladder in conjunction with the GeneScan 500 internal size standard. As part of an extended study on the stability of plant DNA on evidence collection cards, FTA cards with seized Cannabis from 2009 and 2012 and fresh samples were amplified using CS1F and CS1R primers. Overall, the alleles 2, 16.1, 22.1, 23, 26, 28, and 30.1 were chosen to include in the proposed custom NMI01 allelic ladder. The sizing program Open Source Independent Review and Interpretation System (OSIRIS) was modified for the NMI01 bin sets as it contains its own custom-sizing algorithm using double Gaussian sizing compared to the Local Southern method used in GeneMapper. Although OSIRIS with an allelic ladder and the current system with GeneMapper exhibited similar sizing precision, the sizing software OSIRIS was a significant improvement over the current system with GeneMapper when comparing allele call accuracy. Overall, this research study has shown that modifying the NMI01 system for the automated sizing software OSIRIS along with the use of a further comprehensive allelic ladder may help the current system approach 100% accuracy in correct allele assignment.

Keywords— Cannabis sativa; NMI01; STR; allelic ladder; expert system

I. INTRODUCTION

Cannabis sativa has had an increase in relevance over the past several decades as debates have raged regarding its medicinal use [1]. Throughout this process, twenty states in addition to the District of Columbia have exercised their states' rights in allowing conditional *Cannabis* use for certain medical conditions [2]. Despite this, *Cannabis* is still considered an illegal substance by the federal government through the Controlled Substances Act [2]. Law enforcement, therefore, relies heavily on ways to both quickly identify and individualize *Cannabis* when enforcing the law.

Currently, forensic scientists utilize methods of DNA identification in order to positively identify seized *Cannabis* and provide information regarding its particular strain to law enforcement. A large part of providing this information is related to the stability of the samples on the FTA cards they are stored and transported on until later laboratory analysis. Past research has shown that these cards can be utilized for long-term storage of human DNA [3], [4]. This current research looks to utilize similar stability tests by performing STR analysis on *Cannabis sativa* treated FTA cards from fresh *Cannabis* samples as well as treated cards from three and six years ago [5]. Overall, the results of this research will look to strengthen the use and future application of FTA cards in storing plant DNA for the law enforcement community.

Despite the success the NMI01 region has had for source attribution of *Cannabis* samples, some limitations are present in the current system. When analyzing *Cannabis* DNA at the NMI01 region, there is currently no allelic ladder utilized to ensure the accurate sizing of the amplicons; therefore, this technology is still in it's development phase [6]. Instead, the accurate sizing of a positive control is utilized to ensure that these amplicons are indeed sized correctly as a reliable mobility reference standard per batch of samples. Due to the fact that the NMI01 system is microvariant-like in nature with 1 base pair bin sets, a small shift in sizing precision will drastically affect the allele calls. Therefore, this research will utilize amplified *Cannabis* samples to develop a broad and comprehensive custom allelic ladder for analysis for sizing precision studies.

Currently, the NMI01 sizing system utilizes the GeneScan 500 size standard to accurately size the *Cannabis* amplicons. Some research has indicated that the GeneScan 500 has a "large hole in its sizing ability" at the 250 base pair peak due to the fact that it's temperature dependent, so it is generally excluded from analysis [7]. Subsequent correspondence with Applied Biosystems attributes the temperature dependent nature of the 250 base pair fragment to a hairpin loop formed in the polymer medium. As the GeneScan 500 size standard went from being run in semi-solid phase acrylamide gel in the ABI 377 Genetic Analyzer to a liquid polymer medium in the ABI 310 Genetic Analyzer, this hairpin loop became a problem. Therefore, this gap from 200 to 300 base pairs in fragment sizing is particularly concerning because the NMI01 system has alleles that range from 122 to 344 base pairs in size. Consequently, some research has indicated that the Local Southern method utilized by GeneMapper software may not be the best sizing method with the GeneScan 500 size standard for medium sized alleles [8]. Currently, the sizing software Open Source Independent Review and Interpretation System (OSIRIS) through the National Center for Biotechnology Information (NCBI) is gaining traction as a free alternative to GeneMapper software with its own custom sizing method.

Its custom-sizing algorithm corresponds data from the Internal Lane Standard (ILS) and the allelic ladder using a double Gaussian function to better-fit designated peaks with their corresponding alleles [9]. Therefore, OSIRIS will be examined as a possible alternative to GeneMapper software in an effort to increase the sizing precision and accuracy of the NMI01 system. Beyond the sizing implications, the open source and relatively mobile nature of OSIRIS will further enhance the efforts of law enforcement in tracking illegal *Cannabis* activity, and potentially globally without financial restriction for software purchases. Overall, the focus is to build on the current NMI01 system by improving its overall sizing precision and accuracy in addition to streamlining its analysis for law enforcement agencies.

II. METHODS

A. Sample Analysis

Multiple FTA cards previously treated with suspected *Cannabis* samples as well as a dry Cannabis sample (SD) were obtained from the Drug Enforcement Agency (DEA) through a 2008 Federal grant from the National Marijuana Initiative (NMI) (Award #18PSCP505Z). Tommy LaNier from NMI coordinated the effort and sent the FTA cards and *Cannabis* sample to the University of New Haven in West Haven, CT. In addition, fresh samples were acquired through the UNH campus police. Following initial analysis, the fresh *Cannabis* samples were rubbed for transfer to Whatman FTA cards for this study. These Whatman FTA cards were then stored in secured lockers until further analysis.

B. PCR Amplification

Hole punch samples for processing were taken from each FTA card utilizing a 3mm manual hole puncher. In order to prevent contamination, the hole puncher was sterilized with a SpectroLinker^{TM} XL-1500 UV Crosslinker in an attempt to denature any DNA present from one sample hole punch to another. The Optimal Crosslink mode was utilized which had a pre-set UV energy concentration of 120 mJ/cm^2. Following UV denaturation, the 3mm hole puncher was cleaned with Conflikt spray (VWR) to ensure removal of possible contaminants inside the hole puncher tube followed by cleaning with an alcohol wipe to remove any Conflikt spray residue. Then, each disc followed a set washing procedure with FTA Purification Reagent followed by TE^{-1} Buffer, pH 8, per FTA card manufacturer protocol.

The washed 3mm discs were transferred from 1.5 ml microcentrifuge tubes to 0.2mL Thermowell tubes prior to PCR amplification. The samples were then amplified utilizing the Terra PCR Direct Genotyping Kit in addition to custom CS1F and CS1R primers for the NMI01 region of *Cannabis* DNA. An amplification master mix of 12.5ul Direct Buffer, 1.5ul 10mM CSF1 primer, 1.5ul 10mM CSFR primer, and 0.5ul Taq Polymerase Mix (Terra Direct PCR kit, Clontech) was made in stock solution for the desired number of samples in a 1.5ml microcentrifuge tube. Following this, 16ul of the master mix and 9ul of Molecular Biology Grade water (Thermo Fisher Scientific) were pipetted into each of the 0.2mL Thermowell tubes containing samples. PCR amplification was performed on an Applied Biosystems PCR GeneAmp 9700 thermocycler using a previously defined method with a total of 32 cycles of 94^{O}C denaturation for 60 sec, 55^{O}C annealing for 60 sec, 72^{O}C extension for 60 sec, and one final 72^{O}C extension for 30 min. Following amplification, the amplified samples were then stored at 4^{o}C until further analysis was performed.

C. Fragment Analysis

Following PCR amplification, a master mix of 8.75ul Hi-Di Formamide and 0.25ul of ROX size standard (both from Thermo Fisher Scientific) was prepared for the specific number of reactions performed. Then, 9ul of the master mix was added to each of the wells being utilized on the 96 well plate along with 1ul of either Molecular Biology Grade water for the negative control, 1ul of 4A *Cannabis* sample for the positive control, and 1ul of resultant amplified DNA for the samples being analyzed. The 96 well reaction plates were then placed in the Perfect Spin plate centrifuge for 30 seconds to concentrate sample at the bottom of the plate wells. The samples were then denatured on the Applied Biosystems PCR GeneAmp 9700 thermocycler using the method “denature” with a 94^{o}C denaturation for 3 minutes, immediately followed by a 4^{o}C incubation for 3 minutes. Then, the 96 well plate was loaded onto the ABI 3130 Genetic Analyzer and the individual samples were separated and detected. The resulting data was then analyzed using GeneMapper software with pre-set NMI01 conditions and bin sets. NMI01 amplicons were determined based on a peak height over a 50 RFU analytical threshold.

D. Allelic Ladder Development

The alleles for the custom allelic ladder were chosen from samples previously amplified in this research. Since the template DNA was bound to the FTA cards, the allelic ladder had to be developed from the resulting amplicons from individually amplified samples. The alleles 2, 16.1, 22.1, 23, 26, 28, and 30.1 were chosen for the allelic ladder from the samples 4A, 4AA, Box 17 #083, CPLS09, and SD. As an initial trial experiment, each one of the chosen samples was amplified in a 25ul reaction volume and the resulting amplicons were mixed in several 20ul allelic ladder samples. Despite the ideal nature of these preliminary allelic ladders, a 100ul stock solution of the allelic ladder needed to be generated for further sizing precision experiments. Therefore, the amplification master mix was increased to a total volume of 50ul by doubling the amplification reagents.

Then, 3mm hole punch samples from 4A, 4AA, Box 17 #083, CPLS09, and SD were amplified in the 50ul amplification master mix. The resulting amplicons for each sample were separated and detected on the Applied Biosystems 3130xl Genetic Analyzer and analyzed via GeneMapper software.

Overall, a 100ul stock solution, termed AL1A, was generated by combining 20 ul of amplicons from the sample Box 17 #083 in addition to 20 ul of 1:2 dilution amplicons from the sample 4A, 4AA, CPLS09, and SD (Table 1). Due to the increase in amplification volume, several low level peaks occurred near 217 and 264 bp in the custom allelic ladder sample. However, these alleles were not selected for scoring in fragment analysis due to the fact that they were low level and not peaks that appeared consistently above baseline noise.

E. OSIRIS Calibration

In collaboration with Dr. Riley and Dr. Goor at the National Center for Biotechnology and Information (NCBI), the bin sets and settings for NMI01 were transferred to the Open Source Independent Review and Interpretation System (OSIRIS) software utilizing the software's user guide [32]. The settings for the NMI01 system were calibrated relative to the custom allelic ladder generated for the previous sizing precision experiments. In an effort to mimic real-life casework, previously treated FTA cards were chosen for extraction and amplification following the previously outlined protocol. The 100ul stock allelic ladder solution was loaded into the 96-well plate along with a negative control and an amplified sample of 4A as a positive control in addition to the questioned *Cannabis* samples.

F. OSIRIS versus GeneMapper

Following sequencing of the previously outlined samples, several of the samples were chosen for further analysis. Particularly, the samples Box 21 #22, Box 21 #25, 6F, 4AA, Box 17 #084, and Box 20 #088 were chosen for the sizing precision experiment. Each sample was loaded a total of twelve times within each module along with two allelic ladder samples, one negative control and one positive control (Table 2). After all of the samples had been sequenced, the resulting data was then analyzed with both OSIRIS and GeneMapper software.

After the samples had been properly sized, the average fragment size for each allele was determined along with its corresponding standard deviation (SD) and standard error (SE). Additionally, the total number of allele calls and correct allele calls were calculated for both OSIRIS and GeneMapper software. From this, the allele call accuracy in percentage form was calculated by dividing the number of correct allele calls by the total number of allele calls. Hypothesis testing for the difference between proportions was then calculated for the allele call accuracy data comparing proportions analyzed with OSIRIS and GeneMapper software. A p-value of 0.05 was utilized to determine the significance of the results.

III. **RESULTS**

A. Analyze NMI01 STR Profiles

All of the *Cannabis* treated FTA cards from 2009 that were chosen exhibited a profile for the NMI01 STR region of *Cannabis* DNA (Table 1A). Of particular interest, the sample RW09 exhibited a tri-allelic profile with the alleles 1.5, 17.1, and 30.1. In addition, the sample CPMS09 had the allele 16 with a peak height of 71 RFU due to uneven transfer of *Cannabis* DNA to the FTA card. When analyzing the *Cannabis* treated FTA cards from 2012, all of the samples exhibited a profile for the NMI01 STR region of *Cannabis* DNA (Table 1B).

A

Sample ('09)	Allele	Size (bp)	Height (RFU)
RW09	1.5	127.42	496
	17.1	218.56	580
	30	296.09	451
CPMS09	16	211.69	71
CPLS09	30.1	296.59	8193

B

Sample ('12)	Allele	Size (bp)	Height (RFU)
Box 21 #22	16.1	212.94	3350
	22	248.33	2074
Box 21 #25	16.1	212.74	5138
	22	248.15	2790
Box 17 #083	22.1	248.52	3555
	23	253.99	4074
Box 17 #084	11.2	183.83	7446
	26	271.82	2541
Box 20 #088	23	253.69	2544
	26	271.72	2470
Box 18 #086	16.1	212.75	1456
	26	271.66	975
Box 16 #080	16.1	212.72	2124
	25.5	271.41	1374

C

Sample (Fresh)	Allele	Size (bp)	Height (RFU)
4AA	26	272.23	6215
4A	16.1	212.99	7575
	28	283.52	5099
SD	2	127.79	8084
	22.1	248.63	2415
6F	25.5	271.21	881
	27.4	282.19	1028

Table 1. Profiles from Cannabis treated FTA cards from 2009 (A), 2012 (B), and fresh samples (C) utilizing the GeneScan 500 for fragment sizing.

When analyzing the fresh *Cannabis* treated FTA cards, all of the samples exhibited a profile for the NMI01 STR region of *Cannabis* DNA (Table 1C). Of particular importance, the sample SD obtained a profile, which was interesting as it was a dry *Cannabis* sample from 2005. This result shows that *Cannabis* samples can be transferred to FTA cards to stabilize the DNA even after long-term storage in evidence lockers. Also, in this sample, there was a peak at allele 22.1, which appeared at a much lower peak height than allele 2 (Table 1C). This result was expected, as older samples will exhibit degradation in alleles with a greater number of base pairs.

B. Allelic Ladder Development

Following the analysis of the *Cannabis* samples, each resulting profile was analyzed for its potential inclusion in the custom allelic ladder sample. Overall, the alleles were chosen based on amplification repeatability and peak morphology. In addition, an effort was made to make a fairly uniform ladder where the chosen alleles would span the spectrum of the NMI01 bin sets. The 100ul stock allelic solution AL1A had relatively uniform peak heights that ranged from 1200 to 2000 RFUs as well as sufficient peak morphology (Figure 1). Despite these positives, the allelic ladder solution did contain several smaller degradation peaks at 161 and 274 base pairs. Overall, the peaks at these locations were excluded from analysis and only the alleles 2, 16.1, 22.1, 23, 26, 28, and 30.1 were considered for analysis.

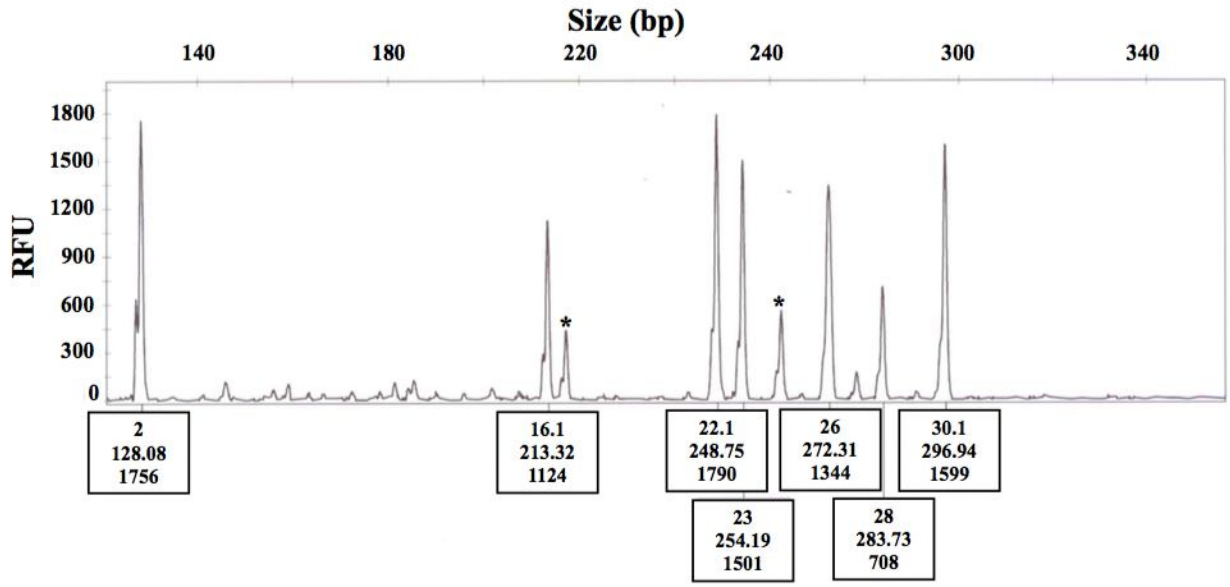

Fig 1. Electropherogram of 100ul stock NMI01 custom allelic ladder, AL1A. Asterix (*) indicates peaks excluded from analysis.

C. OSIRIS Casework

The custom "*Cannabis*" kit definition for OSIRIS was utilized to analyze a 96-well plate module with two allelic ladder AL1A samples, one negative control, one positive control, nine *Cannabis* samples, and two blank FTA punches. The samples Box 18 #083 and Box 16 #080 from MAVMIT were included in the *Cannabis* samples in an effort to mimic real-life casework samples. The samples took approximately several seconds to analyze and the OSIRIS editing software was easy to traverse. Overall, the allelic ladder samples were accurately recognized and analyzed by the OSIRIS software and the positive control was accurately called. All of the *Cannabis* samples exhibited NMI01 profiles as expected and, additionally, the MAVMIT samples both obtained a NMI01 profile of 16.1, 22.1. In real world case-work, these results would indicate common origin between the samples Box 21 #22 and Box 21 #25 and provide an investigative lead for law enforcement.

D. Expert Systems: Sizing Precision and Accuracy

By having standard deviations less than 0.10, GeneMapper (Table 2B) and OSIRIS (Table 2A) software's both exhibited acceptable fragment sizing precision. This precision is particularly important for the NMI01 STR system due to its microvariant nature and the 1base pair bin sets.

A

Sample	Box 21 #22		Box 21 #25		6F		4AA	Box 17 #084		Box 20 #088	
Data Points	11		12		12		12	12		12	
Allele(s)	22.1	16.1	22.1	16.1	26	28	26	11.1	26	23	26
Average Size	212.97	248.99	212.96	248.97	272.36	283.97	271.96	182.57	271.92	254.03	271.88
SD	0.0548	0.0935	0.0835	0.1288	0.0791	0.0612	0.0494	0.0565	0.0724	0.0791	0.0849
SE	0.0165	0.0282	0.0241	0.0372	0.0228	0.0177	0.0143	0.0163	0.0209	0.0228	0.0245

B

Sample	Box 21 #22		Box 21 #25		6F		4AA	Box 17 #084		Box 20 #088	
Data Points	12		12		12		12	12		12	
Allele(s)	22.1	16	22.1	16	26	27.5	26	11.2	26	23	26
Average Size	212.71	248.18	212.61	248.06	271.96	283.01	271.65	183.52	271.59	253.59	271.52
SD	0.0762	0.0652	0.0427	0.0567	0.0766	0.0850	0.0748	0.1085	0.0761	0.0792	0.0679
SE	0.0220	0.0188	0.0123	0.0164	0.0221	0.0245	0.0216	0.0313	0.0220	0.0229	0.0196

Table 2. Sizing precision for samples analyzed with OSIRIS using the custom allelic ladder (A) and GeneMapper (B).

Further, when analyzing *Cannabis* samples, the correct allele call was obtained 98.5% of the time with OSIRIS using the custom allelic ladder, while the current system under GeneMapper had 92.4 % accuracy (Table 3). The OSIRIS software only failed to call the 11.1 allele in sample Box 17 #084 accurately most likely due to the fact that this allele is not present in the custom allelic ladder. When performing a hypothesis test for the difference between proportions, the value of z was determined to be 3.628 indicating a p-value of 0.0001 (Table 3). Therefore, there was a significant difference between the allele call accuracy of the current system with GeneMapper and utilizing OSIRIS with a custom allelic ladder.

Sizing Software	Correct Allele Calls	Total Allele Calls	Allele Call Accuracy (%)	P-value
OSIRIS w/ Allelic Ladder	128	130	98.5	P<0.001
GeneMapper	122	132	92.4	

Table 3. Allele call accuracy for samples analyzed with both GeneMapper and OSIRIS.

IV. DISCUSSION

A. Analyze NMI01 STR Profiles

The effectiveness of FTA cards in long-term storage of DNA has been shown in human biological samples [4], [5]. These research experiments were able to store treated FTA cards over long periods of time, up to 17.5 year, as well as at different climates and still obtain a DNA profile. The results of this current study indicate that FTA cards can be utilized in storing *Cannabis* DNA at least up to a period of six years in room temperature locker storage while still providing a complete NMI01 profile (source of samples, 2008 Federal grant from the National Marijuana Initiative (NMI) (Award #18PSCP505Z).. This research builds on past research of long-term storage of DNA from potato leaves on FTA cards [10]. Here, FTA cards were shown to amplify low copy number genes even after ten months of storage at room temperature (fresh sample study, UNH Police Department). The fact that FTA cards can be stored at room temperature for long-periods of time as well as being easily transportable makes them extremely useful to law enforcement agencies. Future research can re-amplify these samples at a later date to determine the limitations of long-term storage beyond six years.

B. Allelic Ladder Development

The custom allelic ladder in this study was developed to contain as many alleles as possible in order to be thoroughly representative. Previously, many research groups separated allelic fragments through gel electrophoresis and combined them in solution for subsequent amplification [11], [12]. Due to lack of legally available fresh *Cannabis* samples, the allelic ladder in this study could not be generated in this method. The majority of the *Cannabis* samples in this study were in the form of treated FTA cards where the template DNA was permanently bound to FTA card hole punches (NMI Award #18PSCP505Z). The allelic ladder in this study was generated by combining amplicons from various samples into a stock solution. One of the drawbacks of this method was that several artifact peaks appeared in the stock allelic ladder solution. Specifically, two peaks appeared in the allelic ladder solution at 217 bp and 264 bp. As these artifact peaks were not seen in any blank FTA punches, it is believed these fragments were the result of over-amplification issues as a direct result of increasing the amplification volume from 25ul to 50ul.

C. *OSIRIS Casework*

In this current study, the sizing software OSIRIS was able to effectively size NMI01 amplicons from the positive control as well as casework simulated *Cannabis* samples. In addition, the "*Cannabis*" method was able to successfully label and interpret the custom allelic ladder, AL1A. Overall, OSIRIS has been shown to effectively work with a variety of reagent kits both from Promega and Applied Biosystems [9]. Therefore, the fact that OSIRIS was successfully applied to the NMI01 *Cannabis* sizing system was as expected.

D. *OSIRIS versus GeneMapper Sizing Precision and Accuracy*

OSIRIS and GeneMapper software programs both exhibited sizing precision within 0.10 bp for the *Cannabis* NMI01 amplicons analyzed. These results agree with other research that has shown OSIRIS software can typically obtain sizing precision within 0.10 base pair [9]. Despite similar sizing precisions, OSIRIS software exhibited better allele call accuracy at 98.5% compared to 92.4% accuracy for the current system under GeneMapper software. The p-value of 0.0001 when comparing the allele call accuracies of GeneMapper and OSIRIS indicated there was a significant difference in allele call accuracy between them. Overall, the allele 11.1 was the only allele improperly called by OSIRIS most likely due to the fact that this allele isn't contained within the allelic ladder. For custom allelic ladders, every effort is made to ensure that every possible allele is contained within the allelic ladder to ensure accurate sizing [11], [12]. A future direction is to expand the allelic ladder to contain all common alleles analogous to human identification systems.

Despite the improvement of OSIRIS with an allelic ladder over the current system with GeneMapper software, in the future, it would be interesting to calibrate GeneMapper software to the custom allelic ladder, AL1A. By modifying the GeneMapper software, a direct comparison between the different sizing methods utilized by both OSIRIS and GeneMapper software could be made. Of particular interest, the Local Southern method utilized by GeneMapper software might struggle with the 200-300bp sizing gap present in the GeneScan 500 size standard when compared to the custom sizing algorithm used by OSIRIS.

V. CONCLUSIONS

Due to the effectiveness of STR analysis, there has been a large push to identify additional STR regions in *Cannabis* DNA in an effort to improve identification accuracy [13], [14]. One group in particular has taken the initiative to develop a 13 STR multiplex system for *Cannabis sativa* in order to further distinguish samples that have undergone clonal propagation with an anticipated success for highly inbred but not identical breeding lines [14]. Particularly, they created an allelic ladder solution as well as generating population statistics in an effort to abide by the International Society of Forensic Genetics (ISFG) guidelines for STR analysis of DNA samples. In the future, more research needs to be focused on further improving *Cannabis sativa* genetic identification techniques while following the necessary guidelines set forth by SWGDAM for STR analysis of DNA samples [15].

The majority of people who utilize the NMI01 STR system are park rangers, narcotics task forces and border patrol agents who need quick and reliable investigative leads. In the future, this research can help apply the current NMI01 STR system to rapid PCR DNA technology and simplify the overall process of *Cannabis* seizure identification. In order for this application to happen, however, every effort must be made to follow SWGDAM guidelines to optimize the reliability of the current system [15]. By deferring the cost with an open source sizing program like OSIRIS, law enforcement agencies can more easily implement the expensive rapid DNA technology into the field using portable FTA collection cards and hand-held PCR instrumentation.

Overall, this research study has shown that modifying the NMI01 system for the automated sizing software OSIRIS, which uses a double Gaussian algorithm, along with the use of a more comprehensive allelic ladder will help the current system approach 100% accuracy in correct allele assignment.

ACKNOWLEDGMENT

Sincerest thanks to Professor Heather Coyle, Professor Claire Glynn, and Professor Elaine Pagliaro for their patience and support. Special thanks also to Dr. George Riley and Dr. Robert Goor for their help with modifying Open Source Independent Review and Interpretation Software for the NMI01 system; and with appreciation to the University of New Haven Graduate School.

REFERENCES

[1] A. C. Society, "Marijuana and Cancer," 2015. [Online]. Available: http://www.cancer.org/treatment/treatmentsandsideeffects/physicalsideeffects/chemotherapyeffects/marijuana-and-cancer.

[2] O. of N. D. P. Control, "Marijuana Resource Center: State Laws Related to Marijuana," 2013. [Online]. Available: https://www.whitehouse.gov/ondcp/state-laws-related-to-marijuana.

[3] E. I. Schwartz, S. E. Khalchitsky, R. C. Eisensmith, and S. L. C. Woo, "Polymerase chain reaction amplification from dried blood spots on Guthrie cards," *Lancet*, vol. 336, no. 8715, pp. 639–640, Sep. 1990.

[4] L. C. Harty, M. Garcia-Closas, N. Rothman, Y. A. Reid, M. A. Tucker, and P. Hartge, "Collection of Buccal Cell DNA Using Treated Cards," *Cancer Epidemiol. Biomarkers Prev.*, vol. 9, no. 5, pp. 501–506, May 2000.
[5] A. Agostino, A. Gervason, A. Squassina, and J. Mushling, "Direct PCR Amplification of Buccal Samples on the NUCLEIC-CARD™ COLdentifier® Direct PCR Amplification Kit," *Forensic News*, 2013. [Online]. Available: http://www.lifetechnologies.com/forensicnews.
[6] N. Shirley, L. Allgeier, T. Lanier, and H. M. Coyle, "Analysis of the NMI01 marker for a population database of cannabis seeds.," *J. Forensic Sci.*, vol. 58 Suppl 1, pp. S176–82, Jan. 2013.
[7] J. Butler and B. McCord, "Capillary Electrophoresis in DNA Analysis," *Northeastern Association of Forensic Scientists Conference Proceedings*, 2004. [Online]. Available: http://www.cstl.nist.gov/strbase/pub_pres/NEAFS_DataInterpretation.pdf.
[8] S. Klein, J. Wallin, and M. Buoncristiani, "Addressing Ambient Temperature Variation Effects on Sizing Precision of AmpFlSTR® Profiler Plus™ Alleles Detected on the ABI Prism® 310 Genetic Analyzer," *Forensic Sci. Commun.*, vol. 5, no. 1, pp. 1–3, 2003.
[9] R. M. Goor, L. F. Neall, D. Hoffman, and S. T. Sherry, "A Mathematical Approach to the Analysis of Multiplex DNA Profiles," *Bull. Math. Biol.*, vol. 73, no. 8, pp. 1909–1931, 2011.
[10] R. Karle, F. Holder, and M. Smith, "Application of FTA -based Technology for Sample Collection, Transport, Purification, and Storage of PCR-ready Plant DNA," *Whatman*, 2003. [Online]. Available: http://www.laboplus.pl/images/stories/Whatman/pdf/fta/fta_zastosowanie_rolinne dna.pdf.
[11] F. S. Baechtel, J. B. Smerick, K. W. Presley, and B. Budowle, "Multigenerational amplification of a reference ladder for alleles at locus D1S80," *J Forensic Sci*, vol. 38, no. 5, pp. 1176–1182, 1993.
[12] R. A. L. Griffiths, M. D. Barber, P. E. Johnson, S. M. Gillbard, M. D. Haywood, C. D. Smith, J. Arnold, T. Burke, A. J. Urquhart, and P. Gill, "New reference allelic ladders to improve allelic designation in a multiplex STR system," *Int. J. Legal Med.*, vol. 111, no. 5, pp. 267–272, 1998.
[13] C. Howard, S. Gilmore, J. Robertson, and R. Peakall, "Developmental validation of a *Cannabis sativa* STR multiplex system for forensic analysis.," *J. Forensic Sci.*, vol. 53, no. 5, pp. 1061–7, Sep. 2008.
[14] R. Houston, M. Birck, S. Hughes-Stamm, and D. Gangitano, "Evaluation of a 13-loci STR multiplex system for *Cannabis sativa* genetic identification," *Int. J. Legal Med.*, pp. 1–13, 2015.
[15]. Scientific Working Group on DNA Analysis Methods, "SWGDAM Interpretation Guidelines for Autosomal STR Typing," *Forensic Science Communications*, 2014. [Online]. Available: https://www.fbi.gov/about-us/lab/biometric-analysis/codis/swgdam.pdf.