This is to certify that the

thesis entitled

USING STR ANALYSIS TO DETECT
PRIMARY DNA TRANSFER

presented by

Tara Ann Reinholz

has been accepted towards fulfillment
of the requirements for

MS degree in Criminal Justice
with Specialization in
Forensic Science

Major professor

Date 4/3/02

USING STR ANALYSIS TO DETECT PRIMARY DNA TRANSFER

By

Tara Ann Reinholz

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

School of Criminal Justice

2002

ABSTRACT

USING STR ANALYSIS TO DETECT PRIMARY DNA TRANSFER

By

Tara Ann Reinholz

In this study, the primary transfer of DNA from a person's hand to an object was explored. A hammer with an unfinished wood handle was used as a test object, and holding times were varied 1) to determine if a relationship existed between holding time and the amount of DNA recovered and 2) to evaluate the sensitivity of the testing method. An AmpFlSTR® Profiler Plus Amplification Kit and the ABI Prism® 310 Genetic Analyzer, equipped with Genescan® 2.0.2 and Genotyper® 2.0, were used to generate DNA profiles. In addition, two new procedures were developed in an attempt to increase the sensitivity of the testing method. The results show that primary transfer of DNA to this type of surface is common. It is possible to generate complete DNA profiles from holding times as low as 5 seconds, and no apparent connection exists between holding time and the amount of DNA recovered. The new procedures proved useful in increasing allele detection but, occasionally, heterozygote peak imbalances of true alleles were observed.

ACKNOWLEDGMENTS

First, I would like to thank my professor and advisor Dr. Jay A. Siegel. His knowledge, guidance, and commitment to forensic science have shaped me into the scientist that I am today. This project would not have been possible if not for his extraordinary patience and support.

Second, I would like to thank the entire Biology Unit at the Michigan Department of State Police Forensic Laboratory in Northville, Mi. Lynne Helton, Heather Spillane, Leslie Nixon, Dorothy Martus and Guy Nutter all played an instrumental part in my research from the very beginning. Without them, I would not have gained the knowledge or had the resources needed in order to carry out my project. I want to offer a special thanks to Heather Spillane. Heather's suggestions and ideas created the backbone of my project, and her time, knowledge, experience and incredible patience allowed me to successfully carry it out. Without her, and the rest of the unit, none of this would have been possible.

Third, I need to thank all of the individuals who were a part of my sample population. Without the donation of their time, the significance of the results obtained in this study would have been severely compromised.

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LIST OF ABBREVIATIONS

A - letter designation "A" given to an individual who took part in the study

A-5 - individual "A" / 5 second hold

A-15 - individual "A" / 15 second hold

A-30 - individual "A" / 30 second hold

ASCLD - American Society of Crime Laboratory Directors

CE - capillary electrophoresis

DNA - doxyribonucleic acid

FBI - Federal Bureau of Investigation

ILC - internal laboratory control

LCN - low copy number

MSP - Michigan Department of State Police

ng - nanogram

PCR - polymerase chain reaction

pg - picogram

QA - quality assurance

QB - quantiblot

QC - quality control

RFU - relative fluorescent unit

STR - short tandem repeat

SWGDAM - Scientific working group on DNA analysis methods

TAE - tris/acetate/EDTA

TE - tris/EDTA

TWGDAM - Technical working group on DNA analysis methods

ul - microliter

INTRODUCTION

DNA (Deoxyribonucleic acid) represents one's genetic makeup. Located in the nucleus of cells, it is responsible for how the human body functions, as well as its physical appearance. Approximately 99.5% of the DNA code is identical in all human beings [13]. Forensically, it is this 0.5% difference that is important. Today, technology makes it possible to detect these differences, which allows one individual to be differentiated from another.

Common sources of nuclear DNA include white blood cells, spermatozoa, tissues and hair roots. Since these biological constituents are commonly encountered at crime scenes, DNA has proven to be an extremely valuable tool for the criminal justice system.

In this study, the human hand was evaluated as a DNA source. A hammer with an unfinished wood handle was held by an individual for a specific amount of time, and a sample was collected from the handle. This type of transfer, DNA from ones hand to an object, is referred to as a primary transfer. Before the next individual held onto the handle, it was decontaminated in order to eliminate any DNA left over from the previous holder. Choosing to decontaminate before each hold eliminated the of

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need to use separate hammers for each participant. An unfinished wood surface was specifically chosen for two reasons. One, this substrate is found on many common household items that could be used as weapons such as knives, baseball bats, etc. Two, unfinished wood is a rougher surface which could possibly promote the occurrence of DNA transfer. A DNA profile was generated from the sample and compared to the individuals known DNA type. If the two profiles matched, this would prove that the primary transfer of DNA to this type of substrate is possible.

This research has the potential to open up a whole new area of forensic evidence. For example, a knife found at a crime scene exhibiting only a partial fingerprint that could not be identified, would not usually be processed any further. By incorporating the procedures proposed in this study, the knife could become a very probative piece of evidence. The appearance of the partial print proves that an individual did, in fact, touch the handle of the knife. By collecting a sample from the handle, a DNA profile could be generated and a possible suspect could be identified.

In addition to opening a new area of forensic evidence, this study explores the need to address the issue of contamination. The possibility of detecting primary DNA transfers reinforces the importance of wearing gloves during the collection and handling of all evidence. The forensic science community needs to realize the potential for contamination to occur. With the increased sensitivity of the current testing methods, law enforcement agencies and laboratories need to follow the highest level of care in order to insure the reliability and accuracy of the testing performed.

Polymerase Chain Reaction (PCR)

DNA is a double stranded molecule that resides in the nucleus of the cell. It is composed of 4 different bases - Adenine (A), Thymine (T), Guanine (G), and Cytosine (C). It is the specific order of these bases along the DNA strand that determines one's genetic makeup. As seen in Figure 1, two of these bases specifically attach to one another to form a base pair [19]. A binds with T and G binds with C. It is estimated that the human genome consists of over three billion of these base pairs [15].

As mentioned earlier, approximately 0.5% of the 3 billion base pairs differ from one person to the next. These differences are called polymorphisms, and one type of polymorphism is a short tandem repeat, or STR. STRs are 2-7 base pair repeats that occur along a DNA strand [4].

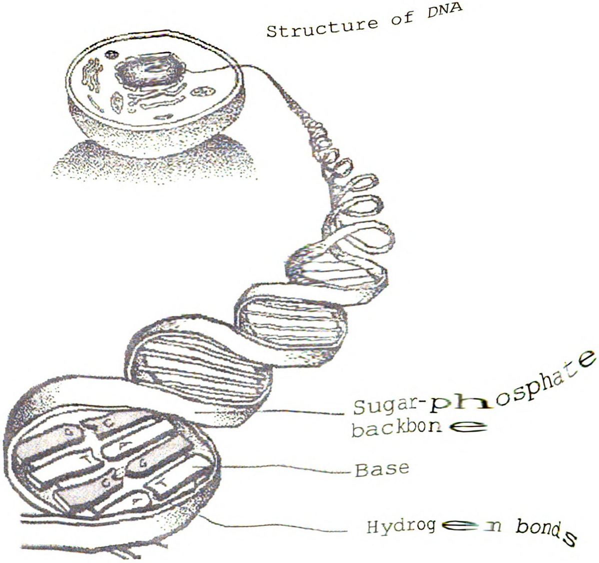


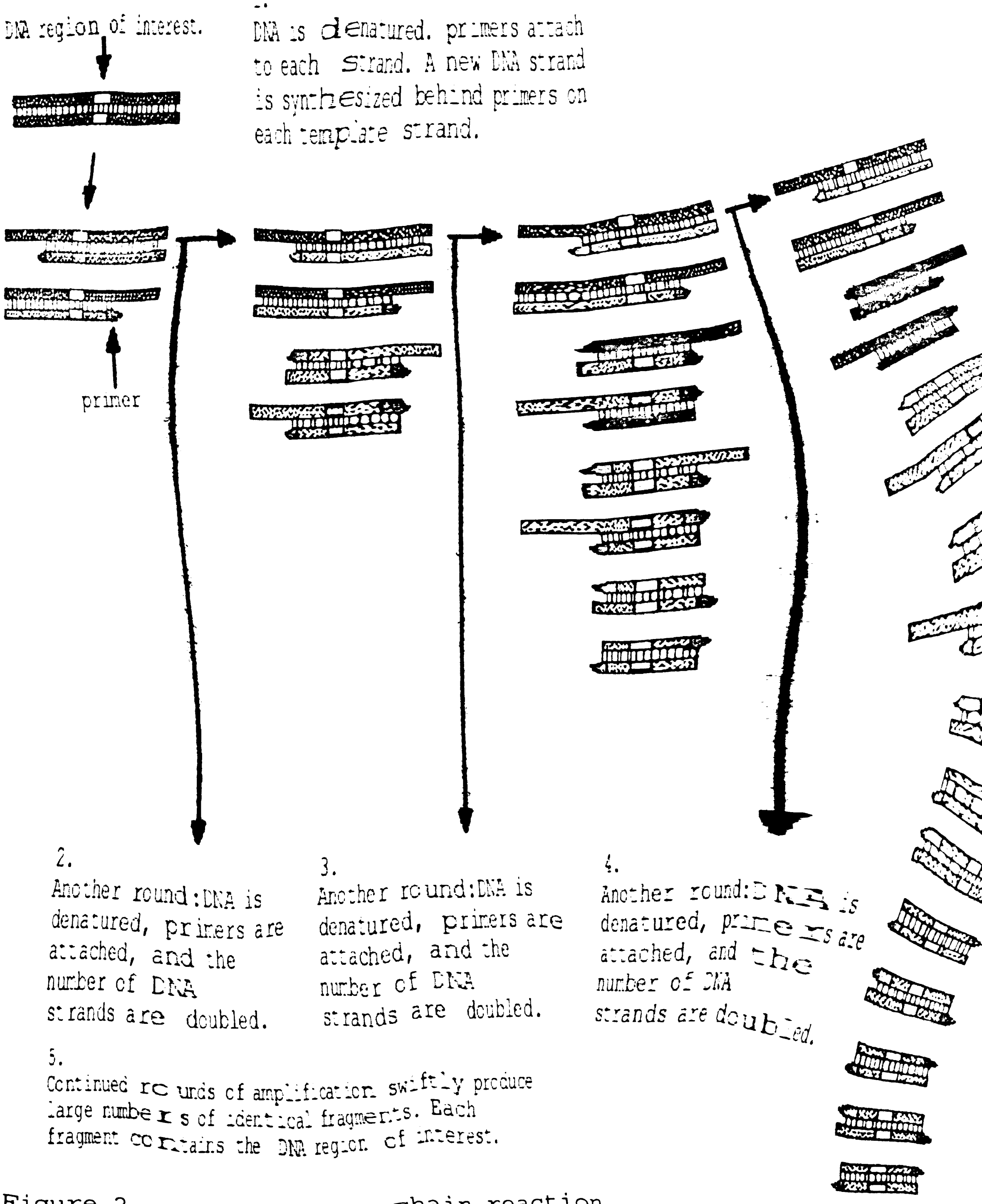
Figure 1 - DNA structure.

Forensically, the tetranucleotide repeats are the current areas of interest. For example, at one location, or locus, on a DNA strand, an STR could exist with a core repeat unit of TAGC. Different forms, or alleles, exist amongst the population, thus allowing for one individual to be differentiated from another. One person may possess the 30 allele (TAGC repeated 30 times) while the next has the 40 allele (TAGC repeated 40 times). Since one allele is longer than the other, this type of polymorphism is referred to as a length polymorphism.

The polymerase chain reaction has two purposes in forensic DNA analysis. One, is to amplify, or make millions of copies of the particular STR of interes doing this, DNA that has become decomposed due to e to environmental insults has the possibility of bec viable sample. Also, very small samples can be enh Two, is to attach a fluorescent label to the STR for detection by a laser. By using primers tagged with different colored labels, many STR locations, or loci, o DNA strand can be amplified at one time, thus creating a much higher power of discrimination. Figure 2 illustrates the process of PCR [22].

Capillary Electrophoresis (CE)

Polymerase Chain Reaction



1\.
DNA is denatured, primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.

2\.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

3\.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

4\.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

5\.
Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.

Figure 2 — Polymerase chain reaction.

Electrophoresis is defined as a technique used for the separation of molecules by means of electric current [1]. Molecules that have similar charges but differ in size can be separated using this technique. Since DNA is negatively charged and the STRs vary in length from one person to another, this makes capillary electrophoresis a perfect technique to use for STR analysis.

As diagrammed in Figure 3, a sample is pulled up into the capillary and the applied electric current allows the negatively charged DNA fragments to travel from the negatively charged cathode to the positively charged anode [6]. As the fragments travel through the capillary they are separated by size. The smaller sized STRs reach the anode first, followed by the larger fragments. When referring to the example given earlier, the 30 allele would reach the anode in front of the 40 allele because it is a smaller fragment. As they pass through a detector window, a laser excites the fluorescent tag that was attached to he STR during PCR, and a CCD camera collects the emitted fluorescence. Computer programs analyze the data and a genetic profile is generated for that particular sample.

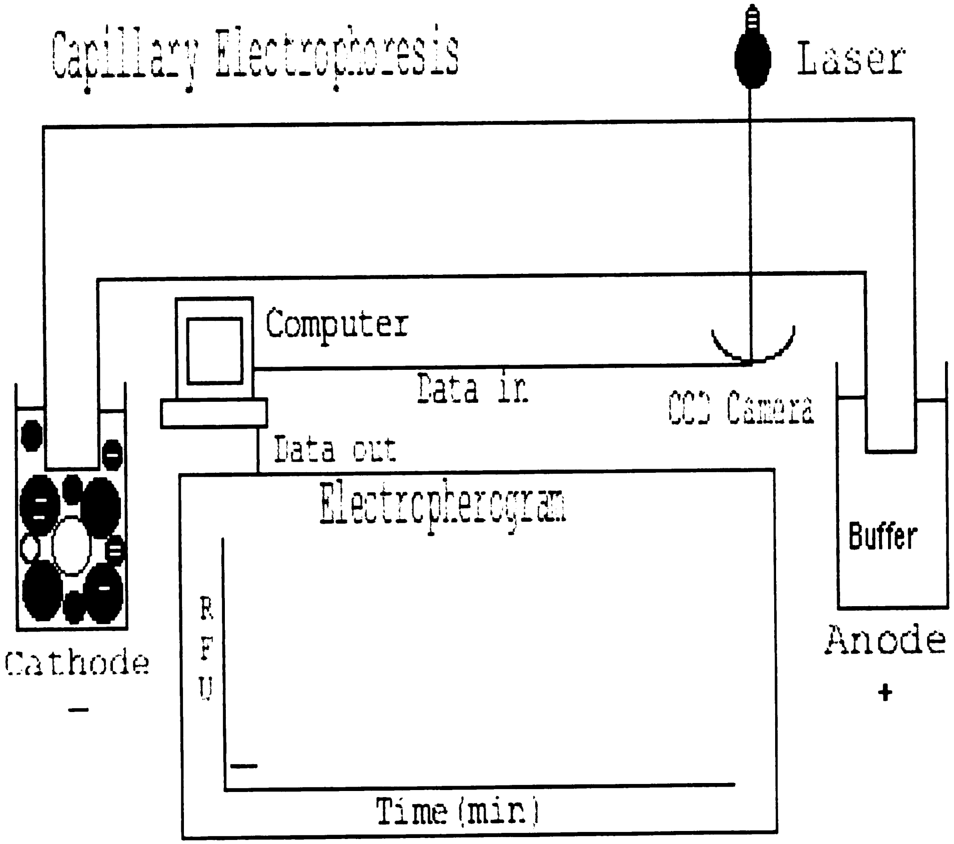


Figure 3 - Capillary electrophoresis.

REVIEW OF THE LITERATURE

In 1997, Roland A.H. Oorschot and Maxwell K. Jones were the first to explore the possibility of using PCR/STR technology to detect primary and secondary DNA transfers [24]. Although the holding times were lengthy (up to 20 minutes), it was proven that the possibility to transfer DNA from ones hand to an object (primary transfer), as well as from an object to ones hand (secondary transfer) does exist. The substrates tested included leather, plastic, and glass. The human hand itself also proved to be a successful substrate. After a one minute handshake, the palm of one participant revealed both DNA profiles. Oorschot and Jones's study set the stage for all future research in this area.

Colleagues at the Connecticut State Police Forensic Laboratory duplicated the Oorschot/Jones study, but concentrated more on secondary transfers [16]. Skin to skin to object and skin to object to skin were the two modes of secondary transfer that were explored. In contrast to Oorschot and Jones study, the handling times were greatly decreased, thus resulting in significantly lower yields of DNA. The results of their study indicate that primary DNA transfer is possible but not always detectable, and, unlike Oorschot and Jones study, secondary transfers were not able to be detected.

In research conducted by R.A. Wickenheiser of the Royal Canadian Mounted Police (RCMP), PCR/STR technology detected the transfer of DNA to over 15 different substrates [28]. Because of these findings, this approach was implemented into everyday casework, and a recent murder case was solved. The handle of a knife recovered at a crime scene revealed the DNA profile of the suspect [27].

P. Wiegand and M. Kleiber of the Martin-Luther-Universitat in Germany used PCR/STR technology to detect the transfer of DNA from the hands of the suspect onto the neck of the victim in cases of strangulation [29]. 16 suspect/victim combinations were used and the success rate was over 70% for the three STR loci tested.

Colleagues at the University of Gent in Belgium performed DNA profiling on physical fingerprints left on glass and wooden plates [23]. Good results were obtained from both substrates when at least 5 physical fingerprints were present.

At the 1999 proceedings of the National Commission on the Future of DNA evidence, Lynne Fereday of the Forensic Science Service in England offered a summary of the past research in this area [7]. In this summary, many of the studies conducted by the authors mentioned above were discussed. In addition, the Forensic Science Service is currently conducting their own DNA transfer studies. These studies were briefly described, but have yet to be published. One experiment involved determining the most recent driver of a vehicle by detecting DNA transferred to the steering wheel by the driver's hands.

MATERIALS AND METHODS

Decontamination Testing

Before an individual was to touch the handle of the hammer, it was decontaminated in order to insure that DNA had not been left over from a previous holder. The handle was soaked in a bleach/distilled water solution for 20 minutes, wiped off with a paper towel, rinsed in distilled water, wiped off with a paper towel, placed in a heated oven to dry, and allowed to come to room temperature. Both a 10% and 20% bleach/distilled water solution were tested and the 20% solution proved to be the most effective.

This entire procedure was extensively tested in order to insure that no DNA carry over was taking place. A male, who consistently transferred DNA, held onto the handle of the hammer for 2 minutes. The handle was decontaminated, followed by a 2 minute hold by a female. Procedures, that will be explained in more detail shortly, were followed in order to generated DNA profiles from the female samples. The absence of a male/female mixture in the female samples will assure that the decontamination process is working properly.

First Round of Sample Collection

Initially, samples were collected from 32 Caucasian males and females, 16 years and older. In order to give a true representation of primary DNA transfer occurring in this type of population, the hands of the individuals were not controlled in any way. Not controlling the hands refers to not requiring any special procedures, such as washing the hands or rubbing them together, before coming into contact with the handle. By encouraging normal, everyday behavior by the test subject, evaluating the rate at which primary transfer occurs can be more accurately determined.

After decontamination, an individual held onto the handle of the hammer for 2 minutes. Forcefully gripping onto the handle, swinging the hammer around, and switching hands were all encouraged, thus mimicking actions that could occur during the commission of a crime with this type of weapon. After 2 minutes, a sample was collected from the handle by using a technique known as the double swab technique [20]. First, a sterile cotton tipped swab was dipped into distilled H20 and rubbed over the entire handle of the hammer. Second, a dry swab was used to do the same. Both swabs were allowed to air dry before placed in a storage tube. This storage tube was given a letter designation in order to identify the individual who contributed the sample. All samples were stored in a -20°C freezer until time to be extracted.

Organic Extraction

For each sample, a scalpel was used to remove the cotton tip from each swab and both were placed in a single extraction tube. Six-hundred microliters of stain extraction buffer (10 mM Tris-HCL, 10 mM EDTA, 50 mM NaCl, 2% SDS, pH 7.5) was added and the tube was placed in a centrifuge and spun down (5 minutes/15,000 g). Thirty microliters of Protinase K (10 mg/ml) were then added. The tube was vortexed to mix, spun down(5 minutes/15,000 g) and placed in a 56°C oven for an overnight incubation.

The next day, the cotton tips were removed from the extraction solution and placed in a basket insert. The basket was inserted into the original extraction tube, and and this was spun down (5 minutes/15,000 g). The basket insert was removed from the extraction tube and thrown away. Five-hundred microliters of a Phenol /Chloroform / Isoamyl Alcohol solution (25:24:1) were added and the tube was vortexed to mix for 5 - 10 seconds. After being spun down (5 minutes /15,000 g), the DNA extract (top layer), was removed and placed into a new microcentrifge tube.

Next, Amicon® centricon concentrators were used to purify the DNA extract. Five-hundred microliters of Tris/EDTA (TE) Buffer (10 mM Tris-HCL, 0.1 mM EDTA, pH 8.0) were added to the centricon filter and it was spun down (500 g/10 minutes). The rinse filtrate in the centricon reservoir was discarded. One milliliter of TE Buffer and the entire DNA extract were added to the centricon filter and spun down (500 g/30 minutes). This procedure was performed three times. After the third wash, the centricon filter was removed from the reservoir and inverted into a vial. This was spun down (3 minutes/1000 g) and the filtrate was removed from the vial and placed into a new microcentrifuge tube. The purified DNA extract (~ 30 ul) was stored at -20°C until further testing.

Yield Gel

A yield gel can be used to determine the quality of DNA and determine how much DNA is present in an extract. A submarine gel electrophoresis unit was used to carry out this procedure. The running buffer, 25 ul of ethidium bromide (0.5 ug/ml) in 250 ml of Tris/acetate/EDTA (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.3), was poured into the apparatus, and a 1% agarose gel (1.3 grams agarose/130 ml TAE buffer), containing two columns of 14 wells, was placed into the buffer.

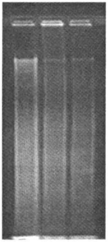
Wells # 1 - 7 in the first column were reserved for standards to which the samples are compared. Three microliters of Lambda Hind III/Eco R1, a mixture of standards, were added to well #1 in columns 1 and 2, and 6 ul of the following DNA standards were added to wells #2-7 in column 1: 500 ng, 250 ng, 125 ng, 63 ng, 31 ng, and 15 ng. For each sample, 4 ul of the purified DNA extract were added to 2 ul of 6x loading buffer (bromophenyl blue/glycerol) and this 6 ul mixture was added to a well. Electrophoresis was carried out for 12 minutes at 175 volts.

An ultraviolet (UV) light box was used to obtain a photograph of the gel. The ethidium bromide that was added to the running buffer binds to the DNA strand and fluoresces in the presence of UV light. Therefore, the DNA in the gel can be visualized when exposed to ultraviolet light. The picture was then used to evaluate the DNA sample.

When interpreting a yield gel (Figure 4), the samples are compared to standards in order to determine the quality and quantity of the DNA sample [2,17]. A solid band represents high molecular weight, or good quality, DNA and a smear represents degraded, or poor quality, DNA. Quantity

Solid, bright band = large quantity of high molecular weight DNA

Solid, light band = small quantity of high molecular weight DNA



Smear = degraded DNA

Figure 4 - Yield gel interpretation.

is determined by picking a standard that most closely resembles the intensity of the sample band. The brightness of the band is directly proportional to the quantity of DNA. If a band is not seen, a more sensitive quantitation method will need to be performed.

Applied Biosystems QuantiBlot® Human DNA Quantitation Kit

A QuantiBlot® kit was used as a more sensitive way to quantitate the purified DNA extract (Table 1). Yield gels detect from 125 ng of DNA/ul to 3.75 ng of DNA/ul, while the QuantiBlot® detects from 2 ng of DNA/ul to 0.03125 ng of DNA/ul. This test is based on the hybridization of a biotinylated, primate-specific probe (D17Z1 locus) to DNA samples immobilized on a nylon membrane and chemiluminescent detection of the bound probe. The procedures for hybridization, chemiluminescent detection, and interpretation of results printed in the product insert were followed [5].

Just as in a yield gel, the samples were compared to standards (10 ng/5 ul to 0.15 ng/5 ul) to in order to determine the concentration of DNA present in the samples. When interpreting the quantiblot (Figure 5), the size and intensity of the bands on the X-Ray film were evaluated [3].

Table 1 - Applied Biosystems QuantiBlot® Human DNA Quantitation Kit contents.

Reagent	**Volume**	**Description**
QuantiBlot® D17Z1 Probe	220 ul	1 vial containing 1pmole/ul biotinylated DNA oligonucleotide probe of the following sequence: 5'-biotin-TAGAAGCATTCTCAGAAACTA CTTTGTGATGATTGCATTC-3'.
Enzyme Conjugate: HRP-SA	2.0 ml	1 bottle containing Horseradish Peroxidase-Streptavidin (HRP-SA) conjugate supplied in buffer with preservative.
Bromothymol Blue Solution	200 ul	1 vial containing 0.04% Bromothymol Blue in water.
QuantiBlot® DNA Standard A	250 ul	1 vial containing 2 ng/ul human genomic DNA in TE buffer.
QuantiBlot® DNA Calibrator 1	100 ul	1 vial containing 0.7 ng/ul human genomic DNA in TE buffer.
QuantiBlot® DNA Calibrator 2	100 ul	1 vial containing0.1 ng/ul human genomic DNA in TE buffer.

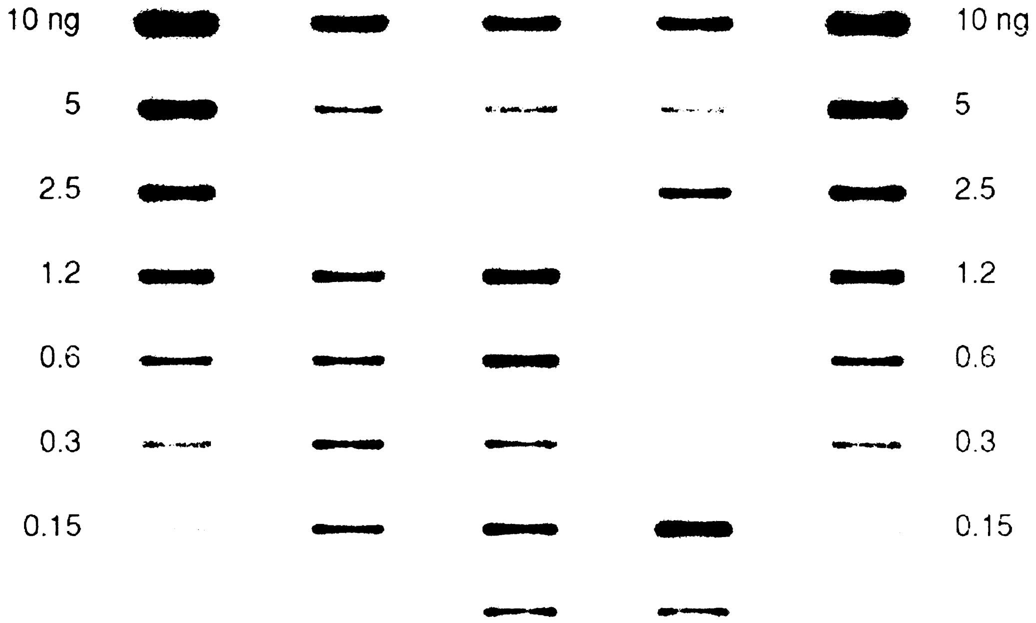


Figure 5 - QuantiBlot® interpretation

Second Round of Sample Collection

The results generated from the quantiblot procedure allowed the samples to be put into groups according to the amount of DNA present in the purified DNA extract. Because of time and resource limitations, approximately half of the individuals from each group were randomly chosen to take part in the second round of sample collection. Three samples, one at 30 seconds, 15 seconds and 5 seconds, were collected from each person. A buccal sample (cells from the inside of the mouth) was also collected from each participating individual so a known DNA profile could be generated. These samples were exposed to all of the same procedures mentioned above. The letter designations previously given to the individuals were used to identify the buccal samples. The letter designation followed by a dash and the handling time in seconds, was used to identify the timed samples.

Applied Biosystems AmpFlSTR® Profiler Plus™ PCR Amplification Kit

PCR was carried out using the Profiler Plus™ Kit (Table 2) and the Applied Biosystems GeneAmp® 2400 PCR Instrument System [3]. This kit will co-amplify nine STR loci and a segment of the amelogenin gene for gender

Table 2 - Applied Biosystems AmpFlSTR® Profiler Plus™ PCR Amplification Kit contents.

Kit Component	**Volume**	**Description**
AmpFlSTR® PCR Reaction Mix	1.1 ml/tube	Two tubes each containing $MgCl_2$, deosynucleoside triphosphates (dATP, dCTP, dGTP, dTTP), bovine serum albumin (BSA), and 0.05% sodium azide (NaN_3) in buffer and salt
AmpFlSTR® Profiler Plus™ Primer Set	1.1 ml	One tube of locus-specific 5-FAM-, JOE-, and NED-labeled and unlabeled primers in buffer to amplify the STR loci D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820, and the gender marker amelogenin
AmpliTaq Gold® DNA Polymerase	50 ul/tube	Two tubes of enzyme with an activity of 5 U/ul
AmpFlSTR® Control DNA 9947A	0.3 ml	One tube containing 0.10 ng/ul human cell line DNA in 0.05% NaN_3 and buffer. The genotype of this female DNA is D3S1358 14,15; vWA 17,18; FGA 23,24; D8S1179 13,13; D21S11 30,30; D18S51 15,19; D5S818 11,11; D13S317 11,11 and D7S820 10,11
Mineral oil	5 ml	One dropper bottle
AmpFlSTR® Blue Allelic Ladder	25 ul	One tube of AmpFlSTR® Blue Allelic Ladder containing the following amplified 5-FAM-labeled alleles: D3S1358 12-19, vWA 11-21, FGA 18-30 (including 26.2)
AmpFlSTR® Green II Allelic Ladder	25 ul	One tube of AmpFlSTR® Green II Allelic Ladder containing the following amplified JOE-labeled alleles: amelogenin X and Y, D8S1179 8-19, D21S11 24.2-38 (including 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35.2), D18S51 9-26 (including 10.2, 13.2, 14.2)
AmpFlSTR® Yellow Allelic Ladder	25 ul	One tube of AmpFlSTR® Yellow Allelic Ladder containing the following amplified NED-labeled alleles: D5S818 7-16, D13S317 8-15, and D7S820 6-15

identification (Table 3). Prior to amplification, the DNA samples were diluted to the appropriate concentration. According to studies done by the Michigan Department of State Police (MSP), approximately 1.0 ng of DNA is required for optimum amplification to occur. The purified DNA extract was diluted in Milli-Q® water to a final concentration of 0.1 ng/ul, and 10 ul of this DNA stock solution would be added to the PCR reaction. If the DNA extract did not contain a high enough concentration of DNA, 10 ul of straight extract was used.

Each PCR reaction required 10.5 ul of reaction mixture, 5.5 ul of primers, and 0.5 ul of AmpliTaq® Gold DNA polymerase. After the number of samples to be amplified was determined, a master mix of these reagents was prepared in a separate microcentrifuge tube. Fifteen microliters of this master mix was added to a PCR reaction tube, followed by 10 ul of the 0.1 ng/ul DNA stock solution prepared earlier. This gave a final reaction volume of 25 ul. PCR was then carried out using the following parameters [3]:

1. Initial Denaturation⇒⇒ 95°C/11 minutes
2. Step Cycles⇒⇒⇒⇒⇒⇒⇒ (28 cycles)
   Denature = 94°C/1 minute
   Anneal = 59°C/1 minute
   Extend = 72°C/1 minute

Table 3 - Applied Biosystems AmpFlSTR® Profiler Plus™ loci.

Locus Designation	**Chromosome Location**	**Common Sequence Motif**	**Size Range (bp)**[a]	**Dye Label**
D3S1358	3p	TCTA(TCTG)$_{1-3}$ (TCTA)$_n$	114-142	5-FAM
VWA	12p12-pter	TCTA(TCTG)$_{3-4}$ (TCTA)$_n$	157-197	5-FAM
FGA	4q28	(TTTC)$_3$TTTTTTCT (CTTT)$_n$CTCC(TTCC)$_2$	219-267	5-FAM
Amelogenin	X: p22.1-22.3 Y: p11.2	- -	107 113	JOE
D8S1179[b]	8	(TCTR)$_2$ [c]	128-168	JOE
D21S11	21	(TCTA)$_n$(TCTG)$_n$[(TCTA)$_3$TA(TCTA)$_3$TCA(TCTA)$_2$TCCATA] (TCTA)$_n$	189-243	JOE
D18S51	18q21.3	(AGAA)$_n$	273-341	JOE
D5S818	5q21-31	(AGAT)$_n$	135-171	NED
D13S317	13q22-31	(GATA)$_n$	206-234	NED
D7S820	7q11.21-22	(GATA)$_n$	258-294	NED

a. The size range is the actual base pair size of sequenced alleles contained in the AmpFlSTR® Profiler Plus™ Allelic Ladders. The sizes in the table include the 3′ A nucleotide addition.

b. In some literature references, this locus is designated as D6S502

c. R can represent either an A or G nucleotide.

3. Final Extension⇒⇒⇒⇒⇒ 60°C/45 minutes

4. Final Step⇒⇒⇒⇒⇒⇒⇒⇒ 25°C/forever

ABI Prism® 310 Genetic Analyzer

The 310 Genetic Analyzer was used to perform capillary electrophoresis on the amplified samples. First, a capillary electrophoresis reaction mixture was prepared. This was composed of 24 ul of deionized formamide and 1 ul of GeneScan-500 [ROX] internal lane size standard for each sample. Just as in PCR, the number of samples to be run on the instrument was determined and a master mix was prepared in a separate microcentrifuge tube. Twenty-five microliters of master mix were added to a 310 sample tube, followed by 1 ul of amplified product. The ladder sample was prepared by adding 3 ul of AmpFlSTR® Profiler Plus™ allelic ladder to 25 ul of the master mix. This ladder consists of DNA fragments of known sizes to which the unknown samples will be compared to. Samples were then incubated at 96°C for three minutes and cooled in a benchtop cooler for three minutes.

After appropriate electrophoresis parameters were selected, the samples were loaded onto the instrument and the run was started (Table 4). Following separation, Genescan® 2.0.2 and Genotyper® 2.0 software were used to

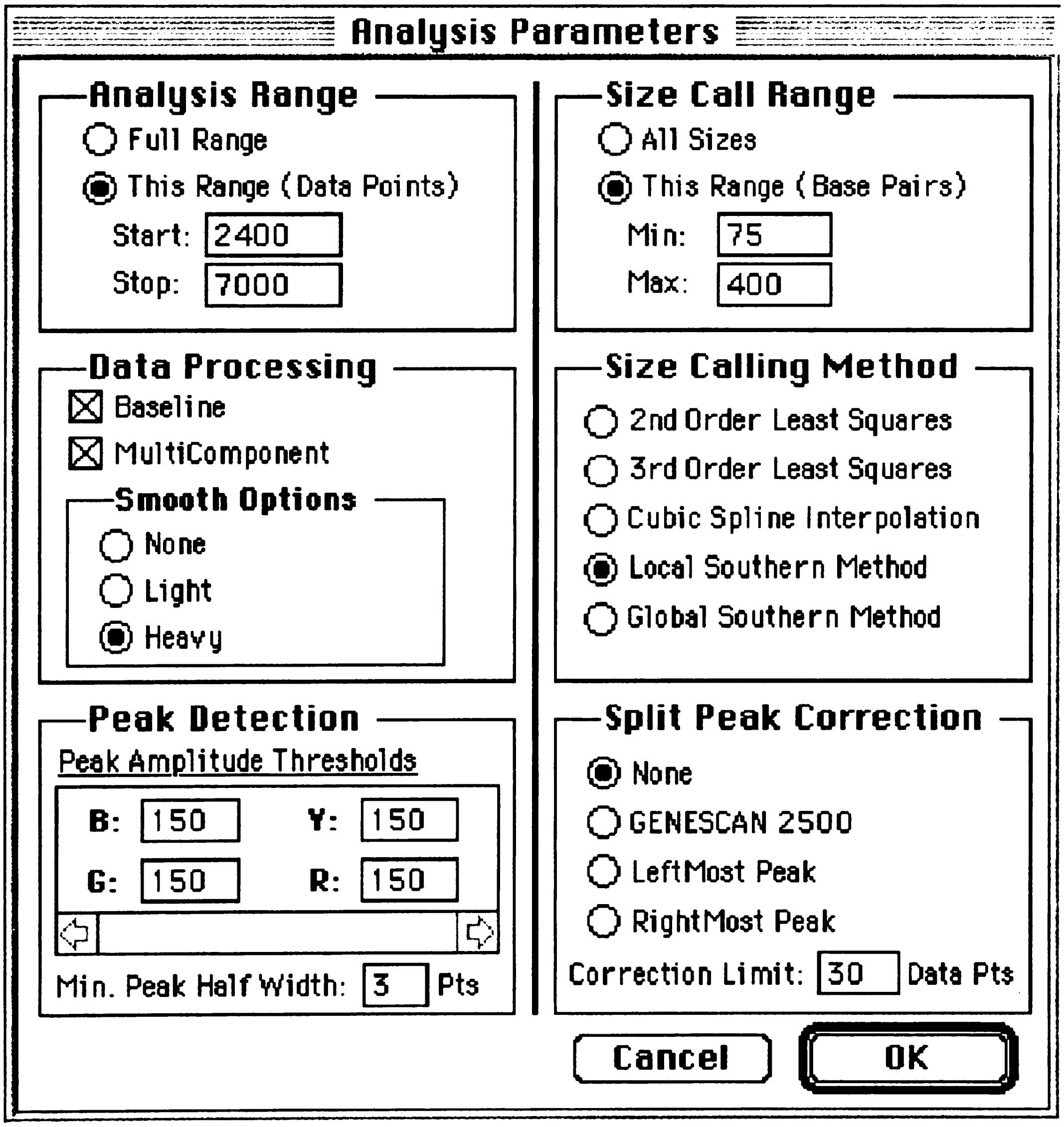
Table 4 - 310 run parameters.

Injection Time	5 seconds
Injection kV.	15.0
Run kV.	15.0
Run Temperature	60°C
Run Time	24 minutes
Matrix File	Profiler plus
Size Standard	Rox GS 500
Instrument Configuration	Pop-4™ polymer with 1-ml syringe
Instrument Module	GS STR POP4 (1ml) F

analyze the raw data and electropherograms, or genetic profiles, were generated. Figure 6 illustrates Applied Biosystems recommended Genescan® analysis parameters [3]. For this study, the peak amplitude thresholds illustrated in Figure 6 were changed from 150 to 50 RFU's (relative fluorescent units) in an attempt to detect more alleles.

When interpreting the genetic profiles, the following guidelines were followed:

1. Alleles of a genetic profile with an RFU value of 150 to 4500 are declared true alleles [3].
2. Alleles of a genetic profile with an RFU value of 50 to 149 are declared active.
3. Alleles of a genetic profile that fall below 50 RFU's are declared undetectable.
4. For alleles from a heterozygous individual at a particular locus, heterozygote peak ratios are determined by dividing the peak height of the allele with the lowest RFU value, by the peak height of the allele with the largest RFU value and multiplying this value by 100 to obtain a percentage. This value must be $\geq$ 70% for a heterozygote to be declared. Any ratios below 70% should be interpreted with caution.
5. A sample can be considered to have originated from a single source if 1) only one or two alleles are present at all loci examined, and 2) the peak height ratios of heterozygous individuals at a locus are within the expected range.
6. A sample can be considered to have originated from multiple (two or more) sources if 1) more than two alleles are present at two or more loci,



Analysis Parameters

Analysis Range

○ Full Range
● This Range (Data Points)
Start: 2400
Stop: 7000

Size Call Range

○ All Sizes
● This Range (Base Pairs)
Min: 75
Max: 400

Data Processing

☒ Baseline
☒ MultiComponent

Smooth Options

○ None
○ Light
● Heavy

Size Calling Method

○ 2nd Order Least Squares
○ 3rd Order Least Squares
○ Cubic Spline Interpolation
● Local Southern Method
○ Global Southern Method

Peak Detection

Peak Amplitude Thresholds
B: 150
Y: 150
G: 150
R: 150
Min. Peak Half Width: 3 Pts

Split Peak Correction

● None
○ GENESCAN 2500
○ LeftMost Peak
○ RightMost Peak
Correction Limit: 30 Data Pts

Figure 6 - Genescan® analysis parameters.

and/or 2) the peak height ratios for heterozygotes fall outside the expected range.

7. Stutter is an expected minor product peak that is four base pairs, or one repeat unit, shorter than the main allele [26]. Table 5 was used as a guideline in determining the maximum % stutter allowed at a particular locus. Peaks in the stutter position that exceed the maximum % stutter value may be designated as a true allele.

8. A complete genetic profile is declared if all 10 loci exhibit alleles with an RFU value of 150 to 4500, and the heterozygote peak ratios are acceptable.

9. A partial genetic profile is declared if at least two out of the ten loci exhibit alleles with an RFU value of 150 to 4500, and the heterozygote peak ratios are acceptable.

10. An active genetic profile is declared if 1) only one out of the ten loci exhibits alleles with an RFU value of 150 to 4500 and the heterozygote peak ratios are acceptable, and/or 2) loci exhibit alleles with an RFU value of 50 to 149.

11. An undetectable profile is declared when all alleles fall below 50 RFU's.

New Procedures

In an attempt to increase the percentage of complete genetic profiles, new procedures, not previously utilized by the manufacturer, were developed.

One of the new procedures developed was to add more PCR product to the capillary electrophoresis reaction mixture. Instead of adding 1ul of PCR product to the 25 ul of formamide/ROX solution, 3 ul were added. Another

Table 5 - Stutter percentages.

DYE	COLOR	LOCUS	CHROMOSOME	% STUTTER*
5-FAM	Blue	D3S1358	3	15
5-FAM	Blue	VWA	12	15
5-FAM	Blue	FGA	4	15
JOE	Green	D8S1179	8	12
JOE	Green	D21S11	21	15
JOE	Green	D18S51	18	18
NED	Yellow	D5S818	5	12
NED	Yellow	D13S317	13	12
NED	Yellow	D7S820	7	12

*Acceptable values established by Applied Biosystems [3] and confirmed by Michigan State Police validation studies.

procedure developed was using Millipore microcon® centrifugal filter devices to concentrate the PCR product. First, the filter was washed by adding 100 ul of TE buffer and spinning down (500 g/15 minutes). All of the PCR product was then added to the filter and spun down (500 g/6 minutes). The filter was removed from the tube, inverted, placed into a new tube, and spun down (1000 g/3 minutes). This tube now contained the concentrated PCR product. Three microliters of this concentrated product were then added to the 25 ul of formamide/ROX solution.

Both procedures developed involve direct manipulation of the PCR product generated from the Profiler Plus™ kit. Since I chose to use the reagents supplied in this kit and the protocols established by the Michigan State Police laboratory, manipulating the actual PCR reaction in hopes of generating more PCR product was not an option. If it were an option, varying reagent concentrations, manipulating PCR cycles, and developing a nested PCR reaction [21] would have all been explored.

Quality Control (QC)/Quality Assurance(QA)

In 1988, the Federal Bureau of Investigation (FBI) formed the technical working group on DNA analysis (TWGDAM). Currently known as SWGDAM (scientific working group on DNA analysis), the function of this group was to provide a forum to discuss various issues concerning forensic DNA laboratories. Specifically, QA/QC issues were addressed and guidelines for a quality assurance program for DNA testing laboratories was established. In 1994, Congress passed the DNA Identification Act which created the DNA Advisory Board (DAB). This group, comprised of members appointed by the Federal Bureau of Investigation, was also created to develop standards for DNA testing and quality assurance. Together, both groups have established guidelines which, if followed, can assure the reliability of forensic DNA testing methods in producing accurate and precise results.

The American Society of Crime Laboratory Directors (ASCLD) is an agency responsible for assessing a laboratories compliance with guidelines established by SWGDAM and the DAB. The Michigan Department of State Police Crime Laboratory in Northville, Michigan, was found to be in full compliance with all established guidelines and has been awarded accreditation by the ASCLD Laboratory Accreditation Board. In order to assure continuous compliance with ASCLD guidelines, the laboratory is re-inspected every four to five years. In regards to this research, ASCLD standards for procedures, equipment and physical plant were all followed in order to assure the reliability of the results. Specifically, laboratory design, validation of the testing method, technique of the scientist, and incorporation of appropriate QC samples will be discussed in more detail to illustrate their importance in this particular study.

Laboratory design is critical when performing forensic DNA analysis. In order to prevent contamination of unamplified DNA (pre-PCR) with amplified DNA (post-PCR), Applied Biosystems recommends designating the following work areas: evidence handling, DNA extraction, PCR setup and amplified DNA [3]. The first three work areas are pre-PCR rooms while the fourth is a post-PCR room. It is required that pre- and post- rooms are physically isolated from one another and contain designate equipment and supplies that never leave their assigned work area (ie. lab coats, pipets, pipet tips, reagents, etc). Figure 7 illustrates this type of laboratory design [3]. The Michigan State Police forensic biology unit in Northville, Michigan, has incorporated these recommendations into their own laboratory design.

Both the Applied Biosystems AmpFlSTR® Profiler Plus™ PCR Amplification Kit and the ABI Prism® 310 Genetic

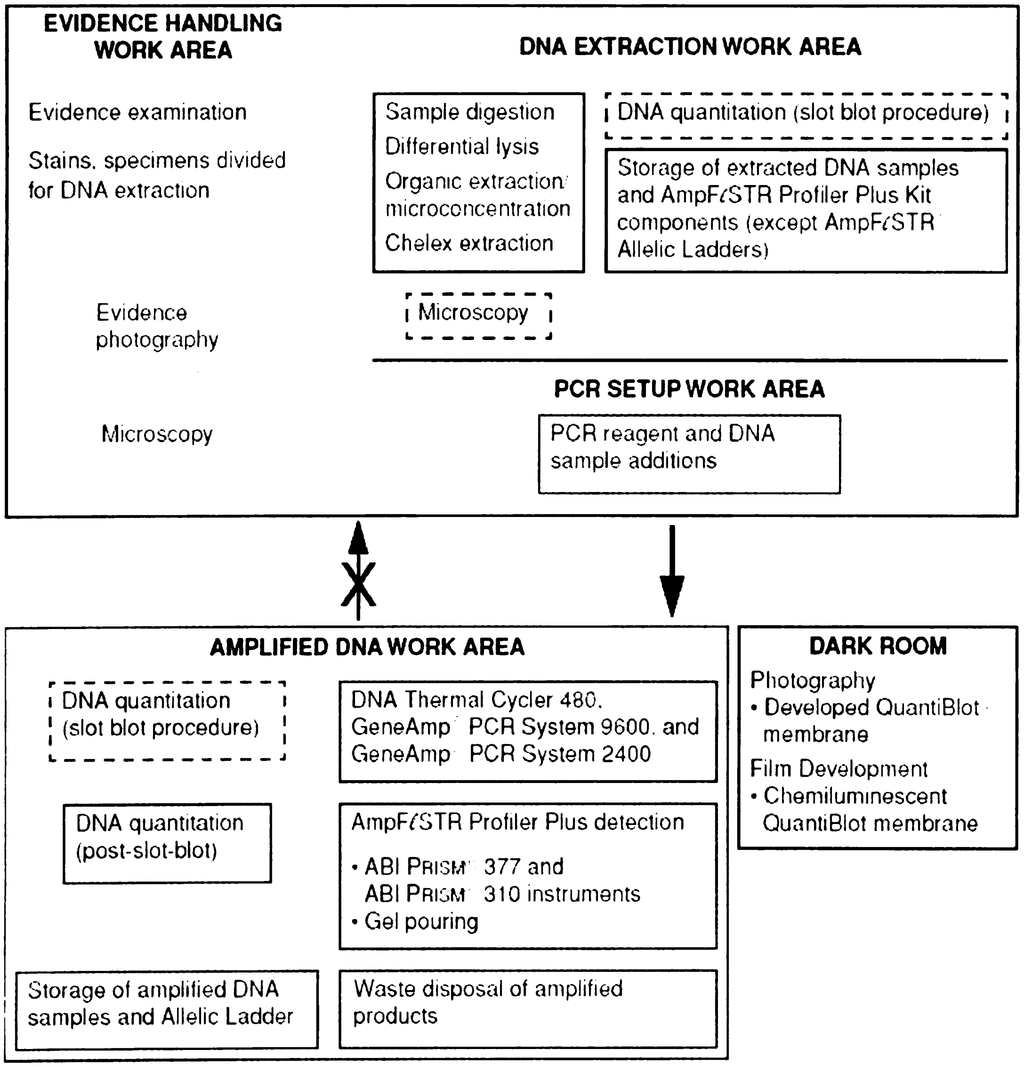


Figure 7 - Laboratory design.

Analyzer have been subjected to rigorous validation studies. These studies, designed by TWGDAM and the DAB, are necessary in order to prove the testing method reliable validation studies on both the kit and the instrumentation. for forensic DNA casework [12]. This re-inforces the fact that manipulating the PCR reaction in forensic casework is not an option. The current cycling parameters and reagent concentrations have been established by TWGDAM and the DAB and must be upheld. Changing any of these established parameters and concentrations is a lengthy and time consuming process which requires a consensus of the forensic scientific community employing the particular method. In addition, the Michigan State Police laboratory has conducted their own validation studies on both the kit and the instrumentation. As a result of these studies, the Michigan State Police has documented specific procedures that must be followed when using this kit and instrumentation in forensic DNA casework. All of these procedures were followed when conducting this research.

The technique of the scientist performing the testing also plays a critical role in preventing contamination. The following is a list of precautions that Applied Biosystems would like all practicing forensic DNA analysts to consider [3]:

1. Examine and sample from pieces of evidence at a separate time from the handling and sampling from known samples.

2. Use disposable gloves at all times and change them frequently.

3. Always use a clean cutting surface and clean scissors when collecting evidence samples.

4. Perform DNA extraction from samples containing high levels of DNA separately from samples containing low levels of DNA.

5. Perform the DNA extraction of evidence samples at a separate time from the DNA extraction of known samples.

6. If possible, PCR reactions should be setup in a dedicated PCR hood equip with a UV light source.

7. Use sterile, disposable, hydrophobic filter-plugged pipet tips and microcentrifuge tubes.

8. Always change pipet tips between handling each sample.

9. Store reagents as small aliquots to minimize the number of times a given tube of reagent is opened.

10. Cap all tubes before beginning the addition of DNA. Only open the tube to which DNA is being added.

11. Do not store reagents close to samples containing high levels of DNA.

All of these recommendations were followed in order to minimize the possibility of contamination.

Running appropriate quality control samples is also very important in assuring that contamination is not occurring and to guarantee the testing methods ability to produce accurate and precise results. In this study, QC samples, positive and negative controls, were introduced into the testing method at three very distinct points of analysis.

First, was during the extraction process. An internal laboratory control (ILC) and a substrate control were both extracted in parallel with research samples. The ILC has a known DNA profile and is used by the Michigan State Police as a positive control. The substrate control was used as a negative control. This sample was collected randomly after the handle was disinfected but before the next individual came into contact with it. A negative result for this sample assures that the disinfection process is working properly to prevent DNA carryover from occurring.

Second, was during the PCR setup process. Again, both a positive and negative control were setup in parallel with research samples. AmpFlSTR® Control DNA 9947A, provided in the Profiler Plus™ kit, was used as a positive control. This control DNA has a known DNA profile, as established by Applied Biosystems. The negative control was PCR master mix without the addition of DNA.

Third, was during the capillary electrophoresis setup process. An additional negative control was run in parallel with research samples. This sample consisted of 310 master mix without the addition of PCR product.

A total of two positive controls and three negative controls were run with every batch of samples that went through analysis. If unexplainable discrepancies exist with any one of the control samples, results generated from that particular batch of research samples are deemed invalid and need to be re-tested.

RESULTS AND DISCUSSION

Decontamination Testing

QuantiBlot® results confirmed that the male consistently transferred DNA to the handle of the hammer. Since male/female mixtures were not seen in any of the DNA profiles generated from the female samples, the decontamination process was working effectively.

First Round of Sample Collection

Yield Gel Results

No visible bands were observed in any of the 32 sample lanes, which represents a concentration of DNA less than 3.75 ng/ul (15 ng/4ul). All results obtained from QC samples were acceptable.

QuantiBlot® Results

DNA concentrations ranged from 0.125 ng/ul to 0.03125 ng/ul, and the results generated from QC samples were acceptable (Table 6). Most important, the substrate control sample did not show a visible band in its slot. For quality control purposes, this sample was randomly collected after the handle had been decontaminated but before the next individual

Table 6 - First round quantiblot results.

Quantiblot Results (ng of DNA/ul)	**Sample**
0.125	HH
	DD*
	ILC (2)
0.0625	G
	N*
	X*
	Y
	CC
0.03125	C
	D
	K
	L*
	W*
	AA*
	EE
	FF*
< 0.03125	A
	B
	E
	F*
	H*
	I
	J*
	M*
	O*
	P
	Q*
	T
	U*
	V
	Z
	BB*
	II
	Substrate Control

*The individuals who supplied these samples were chosen to take part in the second round of sample collection.

held onto it. This was used to assure that the decontamination process was working properly to prevent DNA carryover from occurring.

Second Round of Sample Collection

Yield Gel Results

No visible bands were observed in any of the 5, 15, or 30 second sample lanes, and all results generated from QC samples were acceptable. The known buccal samples showed solid, bright bands in their respective lanes.

QuantiBlot® Results

For all 5, 15, and 30 second samples, the highest amount of DNA recovered was 0.0625 ng/ul (Table 7), and the known buccal samples yielded between 0.25 and 0.125 ng of DNA/ul. Results obtained from QC samples were all acceptable. It should be noted that in both rounds of sample collection, a relationship did not appear to exist between the amount of DNA quantatied and the gender of the individual who supplied the sample.

Not only does this data show the possibility to quantitate DNA from holds as low as 5 seconds, it also

Table 7 - Second round quantiblot results.

Quantiblot Results (ng/ul of DNA)	**Sample**	**Amount of DNA to be amplified**
0.25	FF-KNOWN M-KNOWN U-KNOWN DD-KNOWN	1.0 ng*
0.125	AA-KNOWN J-KNOWN O-KNOWN Q-KNOWN BB-KNOWN ILC N-KNOWN X-KNOWN	1.0 ng
0.0625	Q-30	0.625 ng
0.03125	FF-5,15 L-30 J-15,30 F-15,30 H-15	0.3125 ng
<0.03125	AA-5,15,30 FF-30 L-5,15 J-5 F-5 M-5,15,30 0-5,15,30 U-5,15,30 Q-5,15 BB-5,15,30 H-5,30 DD-5,15,30 N-5,15,30 X-5,15,30 W-5,15,30 Substrate Control	<0.3125 ng

* Amount required for optimum amplification to occur, as determined by Michigan State Police validation studies.

shows that there is no apparent relationship between handling time and the concentration of DNA recovered from the handle. For a true proportional relationship to exist, there should be an increase in the amount of DNA recovered from the 5 second hold up to the 30 second hold. The data generated in this study does not show this to exist, as one might expect.

For example, samples FF-5 and FF-15 each gave 0.03125 ng of DNA/ul but FF-30 gave < 0.03125 ng of DNA/ul. Also, sample H-5 gave < 0.03125 ng of DNA/ul, H-15 went up to 0.03125 ng of DNA/ul, and H-30 dropped back down to < 0.03125 ng of DNA/ul. Data from the initial 2 minute holds also plays a part in evaluating this relationship. After 2 minutes, individual Q transferred < 0.03125 ng of DNA/ul to the handle. At a later date, a sample was collected from individual Q after only 30 seconds. From this sample, 0.0625 ng of DNA/ul were recovered.

Since time does not seem to be related to the amount of DNA recovered, the condition of the test subjects hands appears to be an important factor. DNA from skin cells shed directly from the hands (epithelial cells), can contribute to the DNA recovered from the surface of the handle. One previous study suggested that DNA transfer is highly dependent on the individual handling the object [14]. Those that shed skin cells more readily than others were categorized as "sloughers". Natural human variation can account for one being categorized as a "slougher" or a "non-slougher. For example, those who naturally have dryer hands might shed skin cells at different rates than those who do not.

Cells shed directly from the surface of the hands can contribute to the amount of DNA transferred to the handle, but it does not necessarily represent the entire amount. DNA transferred to the hands from other parts of the body can also contribute. Before coming in to contact with the hammer, the subject could have coughed or sneezed into their hands, scratched their head, rubbed their eyes, or touched any other part of their body that contained large amounts of nucleated cells. Each of these instances can transfer many nucleated cells to the hands which in turn could be transferred to the handle. In this particular study, this was a possibility because the hands of the individuals were not controlled in any way prior to coming into contact with the handle.

To get a true representation of primary DNA transfer occurring in the sample population, it is essential that the hands not be controlled. In many instances, violent criminal activity is an extremely random event. Controlling the hands would not produce "true-to-life" results, thus compromising the significance of the results. This lack of control over the individuals hands, along with natural human variation, is what accounts for the reason why some samples were able to be quantitated while others were not.

STR Results

All known DNA profiles generated from the buccal samples were complete and consistent with originating from a single source (Table 8).

Table 9 shows the genetic profiles generated from each 5, 15 and 30 second sample. Out of the 45 total samples, alleles were detected in 44. This shows that primary DNA transfer to this type of surface is very common in this particular population.

After each profile was subjected to the interpretation guidelines listed previously, its DNA profile type was determined (Table 10). The majority of the samples, 20 out of the 45, produced partial DNA

Table 8 - Known DNA profiles.

Sample ID	**D3S1358**	**VWA**	**FGA**	**XY**	**D8S1179**	**D21S11**	**D18S51**	**D5S818**	**D13S317**	**D7S820**
Known DD	15,16	16,16	21,23	X,Y	13,14	28,29	13,14	11,11	11,12	8,10
Known N	16,16	18,18	23,26	X,X	14,15	28,30	12,17	11,12	11,11	10,12
Known X	16,18	16,18	19,28	X,Y	10,13	29,30.2	13,17	11,12	11,12	9,10
Known W	16,18	16,18	22,23	X,Y	14,15	28,29	12,15	9,13	10,11	10,10
Known AA	15,16	17,18	23,24	X,Y	14,15	28,30	16,17	11,11	9,11	10,12
Known FF	15,18	18,18	24,24	X,Y	12,14	27,28	15,15	10,13	11,12	11,12
Known J	14,16	17,19	21,21	X,X	10,15	28,28	12,14	11,14	11,12	11,11
Known M	16,16	17,19	22,23	X,X	11,14	30,30	12,13	11,13	11,12	10,11
Known O	15,16	18,18	24,26	X,X	8,14	28,31	12,15	9,12	11,12	12,12
Known U	14,17	15,17	21,26	X,Y	13,15	28,31.2	18,21	11,12	8,11	9,11
Known Q	15,18	14,17	24,25	X,X	15,15	30,31	13,19	11,13	8,12	10,11
Known BB	16,16	18,18	24,26	X,Y	14,15	28,30	15,16	11,12	11,11	12,12
*Known F***	15,16	18,19	22,24	X,X	14,14	27,30	13,15	11,13	11,11	11,11
*Known L***	16,16	18,19	22,23	X,X	8,11	29,30	12,13	12,13	11,12	9,13
*Known H***	15,16	17,18	22,24	X,X	11,13	30,30.2	12,14	10,11	11,13	8,10
*Known P***	16,17	14,16	19,22.2	X,X	13,14	30,30	17,18	9,11	10,11	10,12

**Known DNA profiles were previously established

Table 9 - 5,15,and 30 second samples.

Sample ID	D3S1358	VWA	FGA	XY	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
DD-5	15,16	16,16	21,23	X,Y	13,14	28,29	13,14	11,11	11,12	8,10
DD-15	15,16	16,16	21,23	X,Y	13,14	28,29	13,14	11,11	11,12	8,10
DD-30	15^{A},16*	16,16	21^{A}	X,Y	$13^{A},14^{A}$*	$28^{A},29^{A}$	13^{A}	11,11	$11^{A},12^{A}$*	▨
N-5**										
N-15	16^{A}	▨	▨	▨	▨	▨	▨	▨	▨	▨
N-30**										
X-5	16,18	$16,18^{A}$*	19,28	X,Y	10,13	$29,30.2^{A}$	$13^{A},17^{A}$*	$11^{A},12^{A}$	$11^{A},12^{A}$	$9^{A},10^{A}$
X-15	16,18	16^{A},18*	$19^{A},28^{A}$	X,Y	10,13	$29^{A},30.2^{A}$	$13^{A},17^{A}$*	$11^{A},12^{A}$	12^{A}	10^{A}
X-30	16,18	16,18	19,28	X,Y	10,13*	$29,30.2^{A}$*	13^{A},17	11,12	$11^{A},12^{A}$	$9^{A},10^{A}$
W-5	$16^{A},18^{A}$	$16^{A},18^{A}$	$22^{A},23^{A}$	X,Y	$14^{A},15^{A}$	$28^{A},29^{A}$	12^{A}	$9^{A},13^{A}$*	11^{A}	10^{A}
W-15	16,18	$16^{A},18^{A}$	$22,23^{A}$*	X,Y	14,15	28,29	$12^{A},15^{A}$*	$9^{A},13^{A}$	$10^{A},11^{A}$	10^{A}
W-30	16,18	16,18	22,23	X,Y	14,15	28,29	12,15	9,13	10,11	10,10
AA-5	$15^{A},16^{A}$*	17^{A}	▨	X^{A},Y^{A}	14^{A}	28^{A}	▨	11^{A}	▨	▨
AA-15	16^{A}	18^{A}	▨	X^{A},Y^{A}	14^{A}	▨	▨	▨	▨	▨
AA-30	15,16	$17^{A},18^{A}$	$23^{A},24^{A}$*	X,Y	14,15	$28,30^{A}$*	$16^{A},17^{A}$	11,11	$9^{A},11^{A}$	10^{A}
FF-5	15,18	18,18	24,24	X,Y	12,14	27,28	15,15	10,13	11,12	11,12
FF-15	15,18	18,18	24,24	X,Y	12,14	27,28	15,15	10,13	11,12	11,12
FF-30	15,18	18,18	24,24	X,Y	12,14	$27^{A},28^{A}$	15,15	$10^{A},13^{A}$	$11^{A},12^{A}$	$11^{A},12^{A}$
J-5	14,16	$17,19^{A}$	21,21	X,X	$10^{A},15^{A}$	28,28	$12^{A},14^{A}$	$11^{A},14^{A}$	$11^{A},12^{A}$	11^{A}
J-15	14,16	17,19	21,21	X,X	10,15	28,28	12,14	11,14	11,12	11,11
J-30	14,16	17,19	21,21	X,X	10,15	28,28	12,14	11,14	11,12	11,11
M-5	▨	▨	▨	X^{A}	▨	▨	▨	▨	▨	▨

* Heterozygote ratio fell below 70%

^{A} Active alleles (50-149 rfu's)

** Samples showed evidence of a mixture

Bold samples were exposed to new procedures

Table 9 (cont'd).

Sample ID	**D3S1358**	**VWA**	**FGA**	**XY**	**D8S1179**	**D21S11**	**D18S51**	**D5S818**	**D13S317**	**D7S820**
M-15	16,16	17^A,19*	22^A,23^A	X,X	11,14	30,30	13^A	11^A,13^A	11^A,12^A	10^A,11^A
M-30**										
O-5				X^A						
O-15	15^A	18^A		X^A	8^A			12^A		
O-30	15^A,16^A	18,18	24^A,26^A	X,X	8^A,14^A	28^A,31^A	12^A	12^A		
U-5										
U-15	14^A,17^A*	15^A	21^A	X	13^A	28^A		12^A		
U-30	14^A,17^A	17^A		X^A	13^A,15^A*					
Q-5	15,18	14^A,17^A	24^A,25	X,X	15,15	30,31	13^A,19^A*	11^A,13^A	8^A,12^A	10^A,11^A
Q-15	15,18	14^A,17^A	24^A,25^A	X,X	15,15	30^A,31^A	13^A,19^A	11,13^A	8^A,12^A	10^A,11^A
Q-30	15,18	14,17	24,25	X,X	15,15	30,31	13,19	11,13	8,12	10,11^A
BB-5	16,16	18,18	24^A,26^A	X,Y	14^A,15^A	28^A,30^A		11^A,12^A*	11^A	12^A
BB-15	16,16	18,18	24^A,26^A*	X,Y	14,15	28,30		11^A,12	11,11	12^A
BB-30	16,16	18,18	24,26^A	X,Y	14,15	28,30	15^A,16^A	11,12	11,11	12^A
F-5	15,16	18^A,19^A*	22^A,24^A	X,X	14,14	27^A,30^A	13^A,15^A*	11,13^A	11,11	11^A
F-15	15,16	18,19	22,24	X,X	14,14	27,30	13,15	11,13^A	11,11	11,11
F-30**										
L-5**										
L-15	16,16	18,19	22,23^A*	X,X	8,11	29^A,30	12^A,13^A	12,13	11^A,12^A	9^A,13^A*
L-30**										
H-5	15,16	17^A,18^A*	22^A,24^A	X,X	11,13^A*	30^A,30.2^A	12^A	10^A,11^A		
H-15	15,16	17,18	22,24	X,X	11,13	30,30.2	12,14	10,11	11,13	8,10
H-30**										

* Heterozygote ratio fell below 70%

^{A} Active alleles (50-149 rfu's)

** Sample showed evidence of a mixture

Bold samples were exposed to new procedures

Table 10 - 5,15,and 30 second profile types.

Type of DNA profile	**5 seconds**	**15 seconds**	**30 seconds**
Complete	DD-5 FF-5	FF-15 H-15 DD-15 J-15	W-30 J-30
Partial	X-5 J-5 Q-5 BB-5 F-5 H-5	X-15 W-15 M-15 Q-15 BB-15 F-15 L-15	DD-30 X-30 AA-30 FF-30 O-30 Q-30 BB-30
Active	W-5 AA-5 M-5 0-5	N-15 AA-15 O-15 U-15	U-30
Undetectable	U-5		
Mixture	N-5 L-5		N-30 M-30 F-30 L-30 H-30

profiles. Of the remaining 25 samples, nine gave active profiles, eight gave complete profiles, one profile was undetectable and seven profiles showed evidence of a mixture. In mixtures, alleles belonging to the primary handler are present, but additional alleles, not belonging to the handler, are also detected. Again, it should be noted that a relationship did not appear to exist between the type of DNA profile generated and the gender of the individual who supplied the sample.

These results are extremely significant when evaluating the sensitivity of the testing method. With one exception, all of the extracts contained DNA concentrations $\leq$ 0.03125 ng/ul. Therefore, the DNA amounts introduced into the amplification reactions were $\leq$ 0.3125 ng (312.5 pg). This is much lower than the 1.0-1.25 ng recommended by the manufacturer [3]. Generating complete and partial DNA profiles from samples containing such low copy numbers (LCN) of target DNA, as this study did, illustrates the extreme sensitivity of PCR/STR technology.

As the DNA profiles were being interpreted, two issues surfaced which need to be addressed. The first issue is the occurrence of heterozygote peak imbalances. The STR interpretation guidelines, based on results generated from Michigan State Police validation studies, state that the heterozygote peak ratios must be ≥ 70% for a heterozygote to be declared at a particular locus. When looking at the 38 samples that did not show evidence of a mixture, imbalances (ratios <70%) were seen in 45% of them. Of this 45%, 27 separate instances occurred with the majority seen at the VWA and D18S51 loci.

Of the 27 instances, only one instance involved heterozygote alleles ≥ 150 RFU's. The Michigan State Police interpretation guidelines states that true alleles are declared if they fall between 150 and 4500 RFU's [3]. In this study, a new guideline declared active alleles if they fell between 50 and 150 RFU's. Without this guideline in place, imbalances would have occurred in only 3% of the samples.

Explanations as to why these imbalances occurred include primer binding site mutations and low copy number DNA [25]. Given that this study directly involved the analysis of low copy number DNA, this appears to be the reason why imbalances occurred. With low levels of input DNA, it is possible that two alleles of a heterozygote will amplify unequally.

This phenomena is known as stochastic fluctuation [13].

It should be noted that not all laboratories use the same heterozygote peak ratio guideline. In studies conducted by the Federal Bureau of Investigation, ratios ≥ 60% were deemed acceptable [18]. If this guideline were used to interpret the DNA profiles generated in this study, only 16% of the samples would have shown imbalances.

The second issue involves the seven samples (16%) that showed evidence of a mixture. No more than three loci in any one sample showed additional alleles, and no more than one additional allele was seen per loci. Again, it should be recognized that if the active allele guideline was not incorporated into this study, only one sample would have showed evidence of a mixture.

Previous studies have suggested that when dealing with low copy number DNA, mixtures are commonly encountered [8]. There are two explanations as to why the mixtures could have occurred. One is DNA contamination and the other is artifacts produced by the electrophoretic and/or PCR system itself. In regards to DNA contamination, heterozygote peak imbalances at the Amelogenin loci (X:Y) are typical of a male/female mixtures [9]. Since this imbalance was seen in three of the mixed samples, DNA carryover, laboratory contamination and secondary transfer all need to considered as possible sources of DNA contamination.

If the procedure explained earlier to decontaminate the handle of the hammer was not working efficiently, this would cause DNA carryover to occur. This would result in a mixture of alleles belonging to the primary handler and the individual who handled the hammer just before. Since the decontamination process was extensively tested prior to beginning the study, DNA carryover does not seem to be the likely reason as to why mixtures were seen.

Research conducted by Peter Gill of the Forensic Science Service in the United Kingdom has shown that low copy number DNA amplification is prone to sporadic contamination [8]. Just as in this study, strict QA/QC guidelines were followed in order to prevent contamination from occurring, but additional alleles were still detected [8,11]. Possible sources of contamination include extraneous DNA, cross contamination from other samples processed in the laboratory and plastic-ware contamination by the manufacturer [8]. In Gills research, contaminants were typically associated with low molecular weight loci which included Amelogenin, D3S158 and D8S1179 [11]. In this study, 67% of the additional alleles were seen at these loci.

The occurrence of a secondary transfer is another possible source of DNA contamination [16,24]. As explained earlier, the hands of the individuals were not controlled in any way prior to coming into contact with the handle of the hammer. Therefore, the mixtures seen in this study offer proof that it could be possible for one individual to transfer another individuals' DNA to an object and have it be detected. When explained in terms of criminal activity, it could be possible for a criminal to transfer an innocent person's DNA onto a weapon of this type, thus connecting him/her to the crime. More extensive research needs to be conducted in this area in order to prove this possibility to be true.

Artifacts produced by the electrophoretic and/or PCR system itself is the second explanation as to why the mixtures could have occurred. One type of artifact is stutter. Stutter peaks are one repeat unit shorter than the main allele and are caused by slippage of the DNA polymerase enzyme during PCR [26]. Studies conducted by Applied Biosystems and confirmed by the Michigan State Police showed that stutter peak heights are typically 12% to 18% of the main allele. This range exists because different loci show different intensities of stutter. When low copy numbers of DNA are introduced into the PCR reaction, stutter peaks can actually be equivalent to the size of the main allele [9]. In this study, four samples contained additional alleles that were in the stutter position.

Non-specific artifacts can also be produced by the electrophoretic and/or PCR system. This type of artifact occurs as a result of non-specific priming from fragments of bacterial or degraded human DNA [9,10].

The results of Peter Gill's studies show that appropriate guidelines need to be established for interpreting DNA profiles generated from low copy number DNA [8,11]. As recommended by Gill, an allele should only be reported if a duplicate result can be obtained from two or more replicate samples [11].

Only 4 out of 1225 comparisons showed the same spurious allele when replicates were compared. This "duplication guideline" was applied to a statistical theory which proved this to be a reasonable approach to interpreting low copy number DNA profiles. Unfortunately, the results of Gill's studies were not acquired until after the research had been completed in this study. Otherwise, an honest attempt to replicate all samples showing evidence of a mixture would have been made.

Tables 11 and 12 show genetic profiles generated from 15 samples after exposure to the two newly developed procedures. Profile types were determined and compared to each other, and to those generated from the samples exposed to the original procedure (Table 13).

When using 3 ul of PCR product in the 310 reaction, two active profiles became partial, while the remaining four showed increased activity but remained active. One partial profile became complete, while eight showed increased activity but remained partial. Two out of the nine partial profiles would have been declared complete if heterozygote peak ratios would have fallen within the

Table 11 - 3ul of PCR product.

Sample ID	D3S1358	VWA	FGA	XY	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
N-15	16,16	18^{A}		X,X	14^{A}			11^{A}	11^{A}	
X-5	16,18	16,18*	19,28	X,Y	10,13	29,30.2	13,17	11,12	11,12	9,10
X-15	16,18	16,18*	19,28	X,Y	10,13	29,30.2	13,17^{A}*	11,12	11^{A},12	9^{A},10^{A}*
W-15	16,18*	16,18	22,23*	X,Y	14,15	28,29	12,15*	9,13	10,11	10,10
AA-5	15,16	17,18^{A}*	23^{A},24^{A}	X,Y^{A}	14^{A},15^{A}	28,30^{A}*	16^{A},17^{A}*	11,11	9^{A},11^{A}*	12^{A}
AA-30	15,16	17^{A},18^{A}	23^{A},24^{A}	X,Y	14^{A},15	28^{A},30^{A}*	17^{A}	11,11	11^{A}	10^{A}
FF-30	15,18	18,18	24,24	X,Y	12,14	27,28	15,15	10,13	11,12	11,12^{A}
J-5	14,16	17,19	21,21	X,X	10,15	28,28	12,14^{A}	11^{A},14^{A}	11,12^{A}	11,11
M-5				X^{A}	11^{A},14^{A}		12^{A}		12^{A}	
O-5				X^{A}						
U-15	14,17^{A}*	15,17^{A}*	21	X,Y^{A}	13,15^{A}*	28,31.2^{A}*		11^{A},12^{A}*	11^{A}	
U-30	14^{A},17^{A}	17^{A}	21^{A},26^{A}	X,Y^{A}	13^{A},15^{A}*	28^{A}		11^{A}		
Q-15	15,18	14,17	24,25	X,X	15,15	30,31	13,19	11,13	8,12	10,11
BB-5	16,16	18,18	24^{A},26^{A}	X,Y	14,15	28,30	15^{A},16^{A}*	11,12	11,11	12,12
L-15	16,16	18,19	22,23	X,X	8,11	29,30	12,13	12,13	11,12	9,13^{A}*

* Heterozygote ratio fell below 70%

^{A} Active alleles (50-149 rfu's)

Table 12 - 3ul of concentrated PCR product.

Sample ID	**D3S1358**	**VWA**	**FGA**	**XY**	**D8S1179**	**D21S11**	**D18S51**	**D5S818**	**D13S317**	**D7S820**
N-15	16,16	18^{A}	23^{A},26^{A}	X,X	14,15^{A}*	28^{A},30^{A}*	12^{A},17^{A}	11	11	▨
X-5	16,18	16,18*	19,28	X,Y	10,13	29,30.2	13,17	11,12	11,12	9,10
X-15	16,18	16,18*	19,28	X,Y	10,13	29,30.2	13,17*	11,12	11,12	9,10
W-15	16,18*	16,18	22,23*	X,Y	14,15	28,29	12,15*	9,13	10,11	10,10
AA-5	15,16*	17,18*	23,24	X,Y	14,15*	28,30*	16,17	11,11	9,11*	10^{A},12*
AA-30	15,16	17,18	23,24	X,Y	14,15	28,30	16,17	11,11	9,11	10,12
FF-30	15,18	18,18	24,24	X,Y	12,14	27,28	15,15	10,13	11,12	11,12
J-5	14,16	17,19	21,21	X,X	10,15	28,28	12,14	11,14	11,12	11,11
M-5	▨	17^{A},19^{A}	23,23	X,X	11,14	30,30	12,13^{A}*	11^{A},13^{A}	11,12	10^{A}
O-5**										
U-15**										
U-30**										
Q-15	15,18	14,17	24,25	X,X	15,15	30,31	13,19	11,13	8,12	10,11
BB-5	16,16	18,18	24,26	X,Y	14,15	28,30	15,16*	11,12	11,11	12,12
L-15	16,16	18,19	22,23	X,X	8,11	29,30	12,13	12,13	11,12	9,13

* Heterozygote ratio fell below 70%

^{A} Active alleles (50-149 rfu's)

** Samples showed evidence of a mixture

Table 13 - New procedure profile types.

Sample ID	**Quantiblot results (ng of DNA/ul)**	**Original Protocol (1ul of amplified product)**	**3ul of amplified product**	**3ul of concentrated amplified product**
N-15	< 0.03125	Active	Partial	Partial
AA-5	< 0.03125	Active	Partial	Partial
M-5	< 0.03125	Active	Active	Partial
O-5	< 0.03125	Active	Active	Mixture
U-15	< 0.03125	Active	Active	Mixture
U-30	< 0.03125	Active	Active	Mixture
Q-15	< 0.03125	Partial	Complete	Complete
X-5	< 0.03125	Partial	Partial*	Partial*
W-15	< 0.03125	Partial	Partial*	Partial*
X-15	< 0.03125	Partial	Partial	Partial*
AA-30	< 0.03125	Partial	Partial	Complete
BB-5	< 0.03125	Partial	Partial	Partial*
L-15	< 0.03125	Partial	Partial	Complete
FF-30	< 0.03125	Partial	Partial	Complete
J-5	< 0.03125	Partial	Partial	Complete

*All 10 loci exhibited alleles with an RFU value of 150 to 4500, but the heterozygote peak ratios at some loci were below 70%.

acceptable range.

Heterozygote peak imbalances were seen in 60% of the samples. Of this 60%, 20 separate instances occurred with the majority seen at the VWA, D21S11 and D18S51 loci. In comparison to the same samples from the original procedure (1 ul of PCR product), the same nine samples showed imbalances but six more instances were seen. Originally, no more than two loci in any one sample showed imbalances. Adding 3 ul of PCR product generated DNA profiles with up to five loci exhibiting imbalances.

When comparing DNA profiles generated from the original procedure to those generated in this procedure, two major differences were seen. One is the peak heights of the alleles showing imbalances. Of the original 15 samples, only active alleles (50-149 RFU's) were involved. When more PCR product was introduced into the 310 reaction, 25% of the instances involved alleles with peak heights in the acceptable range (150-4500 RFU's). Another difference is the severity of the imbalances. Originally, the majority of the imbalances involved alleles with peak height ratios that fell between 60% and 70%. With this procedure, the majority of the ratios fell between 40% and 60%.

When using 3ul of concentrated PCR product in the 310 reaction, three out of the six active profiles became partial, but the remaining three showed evidence of a mixture. Five partial profiles became complete, while the remaining four would have been declared complete if the heterozygote peak ratios had fallen within the acceptable range.

Heterozygote peak imbalances were seen in 58% of the samples that did not show evidence of a mixture. Of this 58%, 16 separate instances occurred with the majority seen at the VWA and D18S51 loci. Up to six loci in one sample showed imbalances and one sample that did not exhibit imbalances with the original procedure showed imbalances at two loci. The majority of the peak height ratios fell between 50% and 70%, and 75% of the instances involved alleles with peak heights in the acceptable range. When compared to the 3 ul of PCR product that was not concentrated, the ratio imbalances were less severe but the percentage of instances involving peak heights in the acceptable range increased by 50%.

Adding 3 ul of concentrated PCR product to the 310 reaction generated three DNA profiles that showed evidence of a mixture. A total of 32 additional alleles were seen. Up to eight loci in one sample exhibited additional alleles and up to four additional alleles were seen at a single locus. Of the alleles belonging to the primary handler, 84% exhibited peak heights within the acceptable range. In contrast, alleles not belonging to the primary handler were in the acceptable range only 16% of the time.

Both procedures were successful in increasing the ability to detect alleles, but adding 3ul of concentrated PCR product to the 310 reaction generated more complete profiles. Unfortunately, results from both procedures need to be interpreted with extreme caution. In regards to the original 15 samples, all heterozygote peak ratio imbalances involved active alleles and mixtures were not seen. The new procedures produced true alleles (150-4500 RFU's) with imbalances and three samples showed evidence of a mixture. Explanations as to why imbalances and mixed samples occurred are the same as those explained earlier.

In addition to the two procedures developed to increase allele detection, interpretation guidelines established for low copy number DNA analysis could have been implemented into this study and evaluated as a more reliable method [8, 9, 10, 11]. Unfortunately, since the results of these studies were acquired after the research was completed, the guidelines could not be used to interpret DNA profiles. This is due to the fact that duplicates were not run on samples exhibiting imbalances and/or evidence of a mixture.

QA/AC Results

Acceptable results were obtained from all quality control samples. This assures that all results generated from test samples are accurate.

CONCLUSIONS

In a population of Caucasian males and females over the age of 15, the following conclusions can be made regarding primary DNA transfer to an unfinished wood surface:

1. Primary transfer is extremely common.
2. No apparent relationship exists between handling time and the amount of DNA recovered from the substrate.
3. Complete DNA profiles can be generated from holds as low as 5 seconds where < 0.3125ng of DNA is amplified.
4. Adding 3ul of PCR product to the 310 reaction slightly increases the ability to detect alleles, but imbalances in heterozygote peak ratios of true alleles can occur.
5. Adding 3ul of concentrated PCR product to the 310 reaction significantly increases the ability to detect alleles, but imbalances in heterozygote peak ratios of true alleles can occur.

SUGGESTIONS FOR FURTHER RESEARCH

Since DNA transfer has only recently become a topic of interest, conducting further research is going to be critical in fully understanding its forensic significance. It is crucial that this significance be determined in order to assess just how much of an impact it will have on the criminal justice system. In parallel with this study, the following is a list of variables that could be changed in order to gain additional knowledge in the area of DNA transfer:

1. Use a test object made of a different substrate (plastic, glass, finished wood, etc.).
2. After the object is handled for the allotted time, expose it to a variety of storage conditions before collecting the sample in order to determine if the transferred DNA will be able to withstand its environment.
3. Allow the test subjects hands to come into contact with another individual before handling the object. By doing this, the ability to detect secondary transfers can be further studied.
4. Attempt to recover the amount of DNA required for optimum amplification by using Millipore microcon® centrifugal filter devices to concentrate the DNA extract. This could eliminate the need to concentrate the PCR product and allow for less heterozygote peak imbalances.

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REFERENCES

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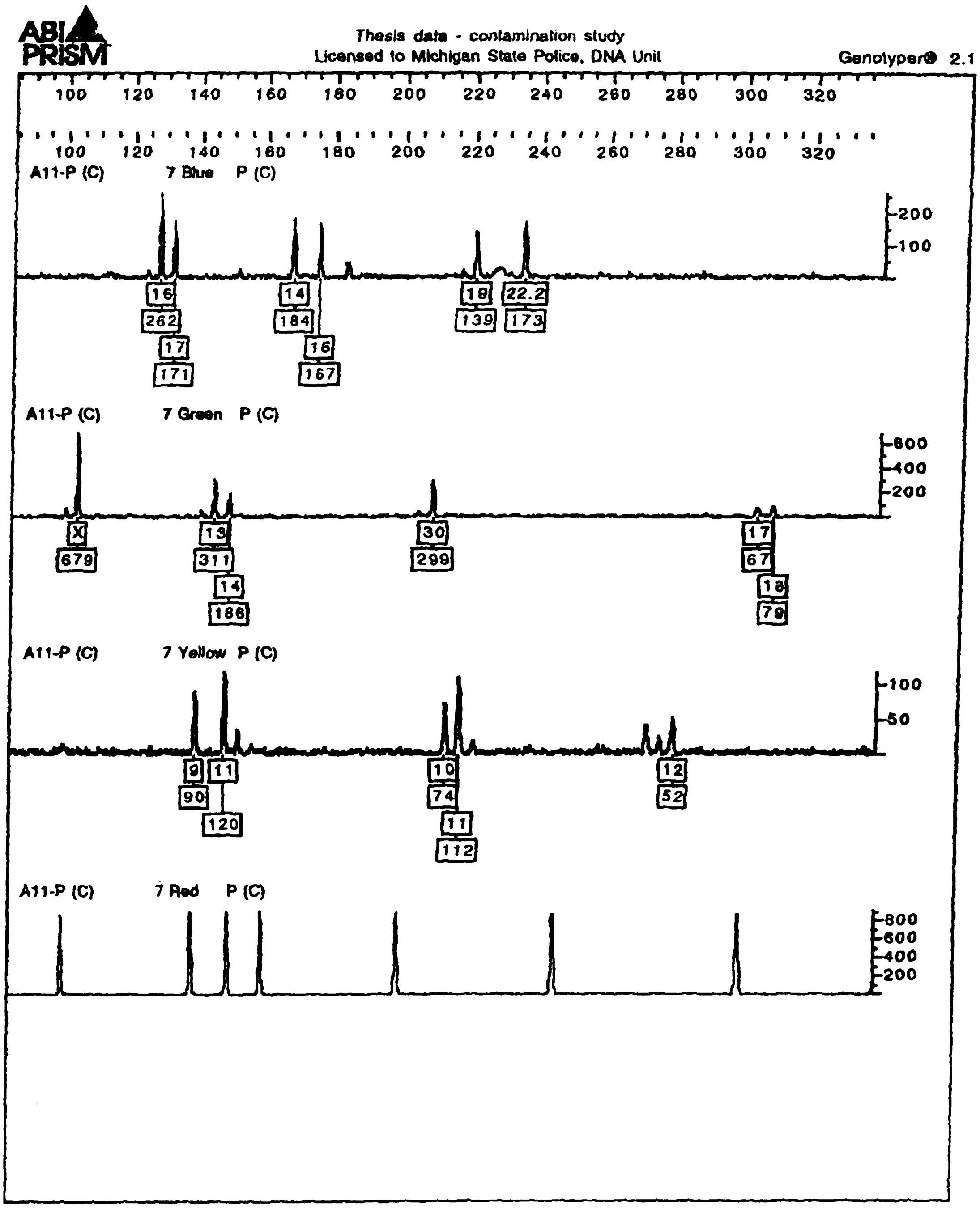
APPENDICES

APPENDIX A

Electropherograms generated from decontamination testing.


ABI PRISM
Thesis data - contamination study
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
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A11-P (C) 7 Blue P (C)
200
100
16
262
17
171
14
184
16
167
19
139
22.2
173
A11-P (C) 7 Green P (C)
600
400
200
X
679
13
311
14
186
30
299
17
67
18
79
A11-P (C) 7 Yellow P (C)
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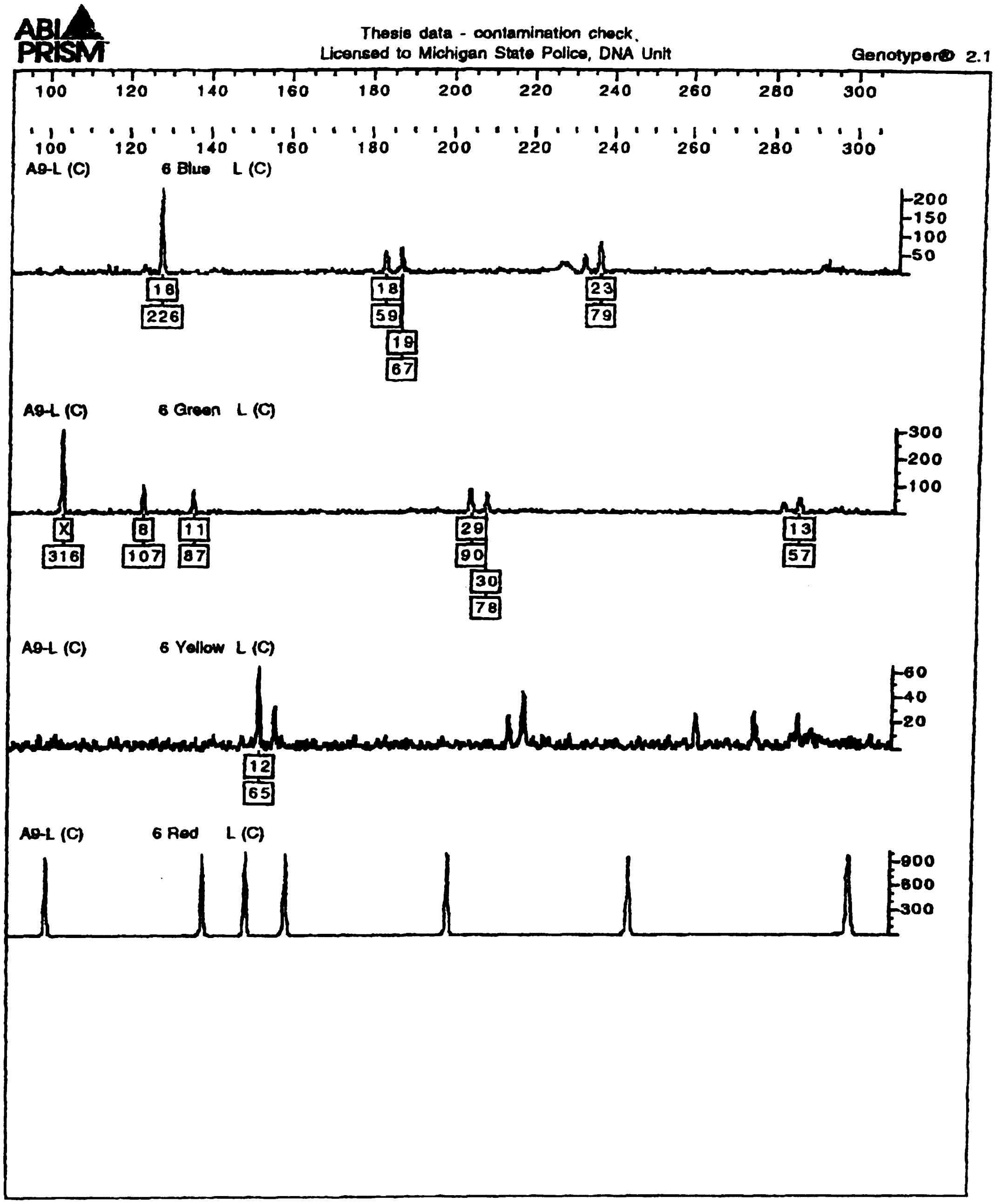
ABI PRISM
Thesis data - contamination check.
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
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100 120 140 160 180 200 220 240 260 280 300
A9-L (C) 6 Blue L (C)
200
150
100
50
18
226
18
59
19
67
23
79
A9-L (C) 6 Green L (C)
300
200
100
X
316
8
107
11
87
29
90
30
78
13
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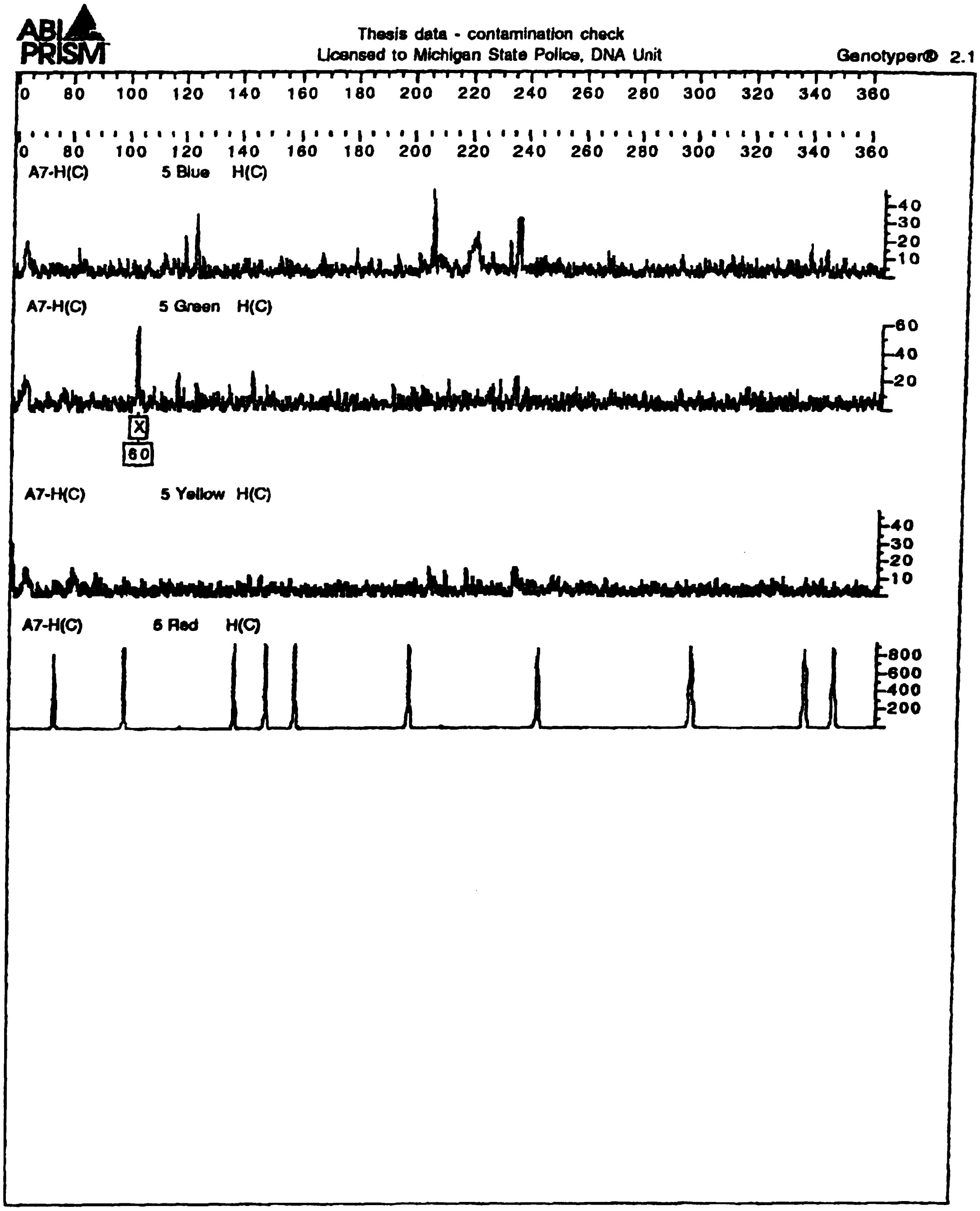
900
600
300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Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

APPENDIX B

Electropherograms generated from known DNA samples.

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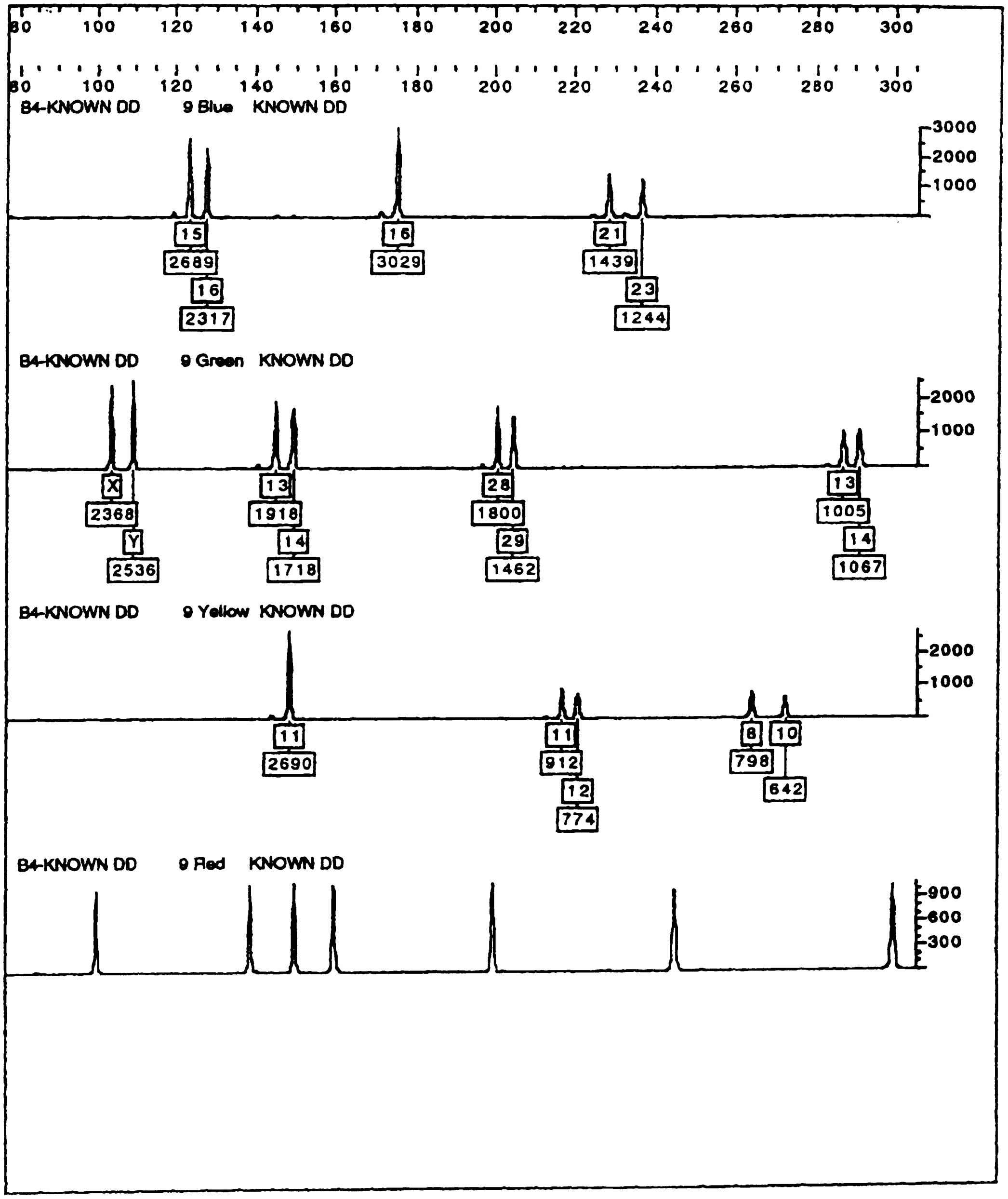
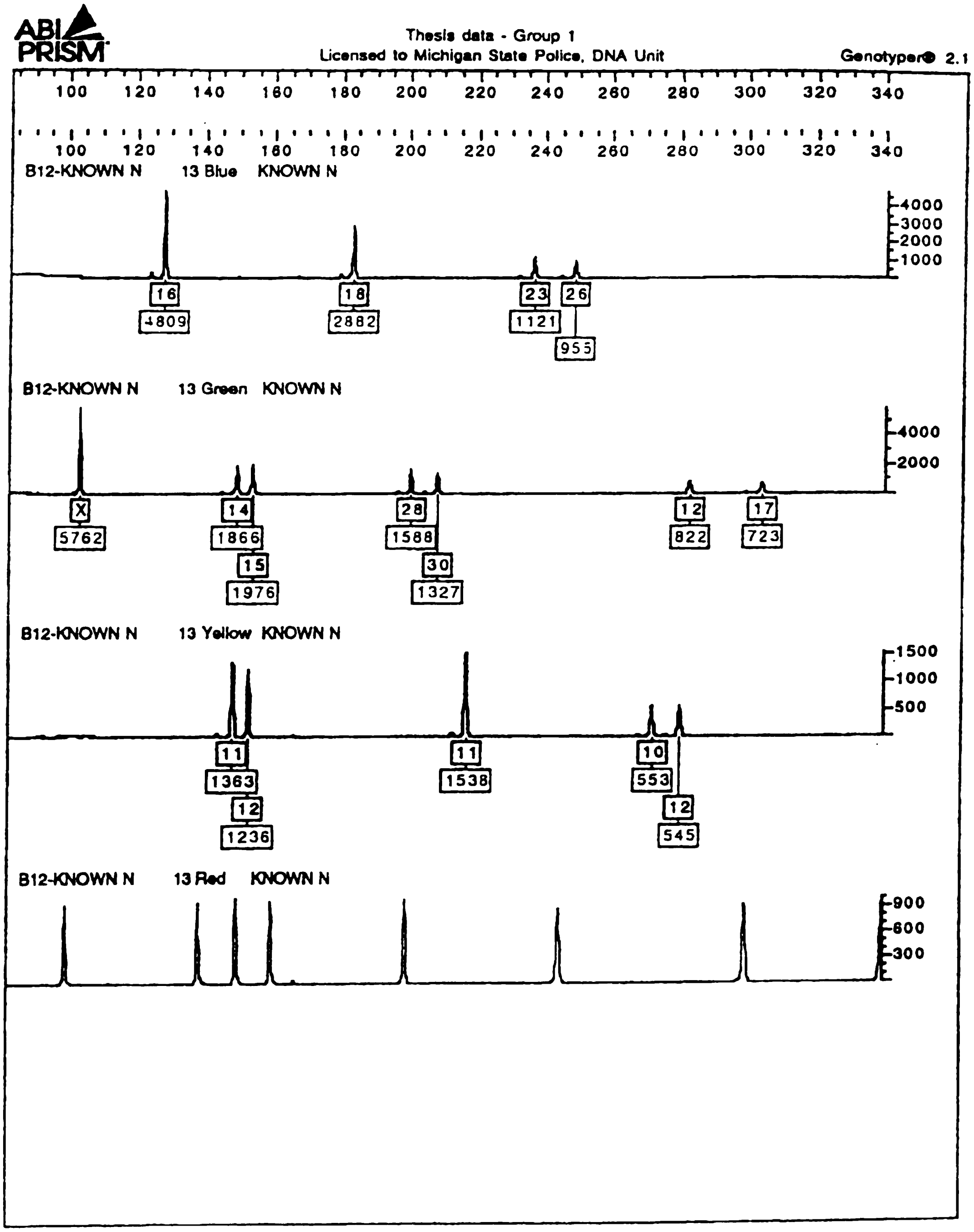
Genotyper® 2.1

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Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
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4000
3000
2000
1000
16
4809
18
2882
23
1121
26
955
B12-KNOWN N 13 Green KNOWN N
4000
2000
X
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1500
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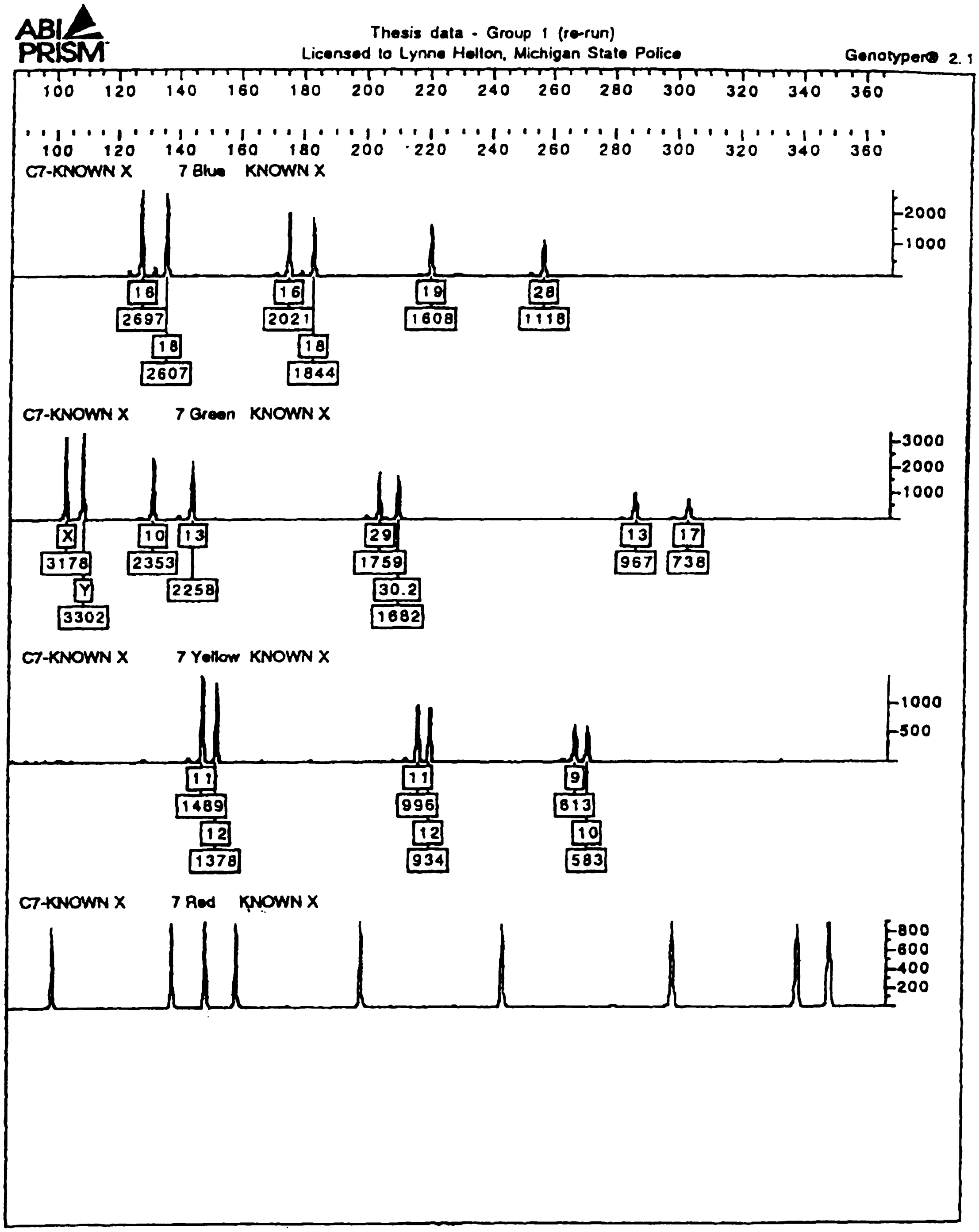
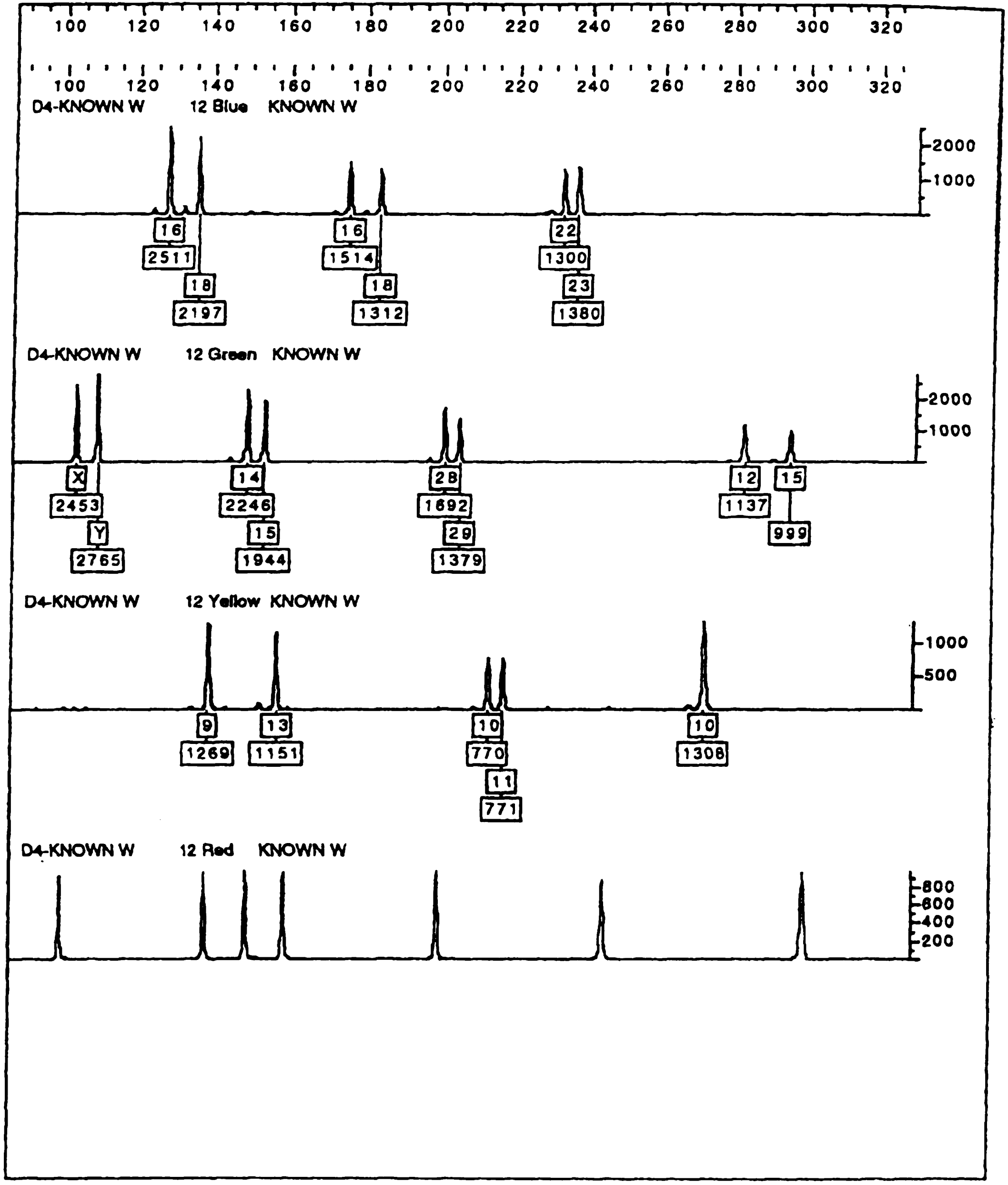
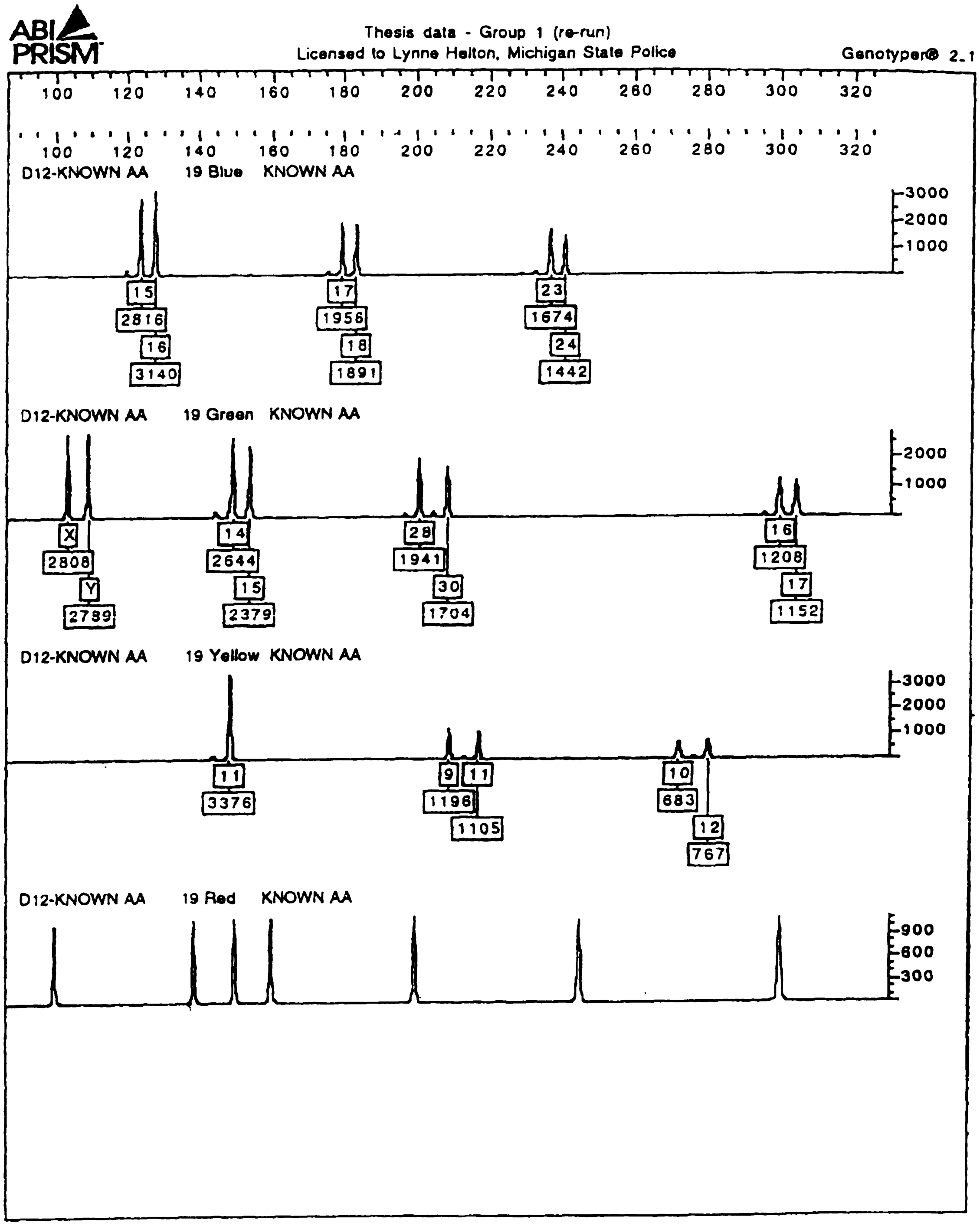


Thesis data - Group 1 (re-run)
Licensed to Lynne Helton, Michigan State Police
Genotyper® 2.1

Thesis data - Group 1 (re-run)
Licensed to Lynne Helton, Michigan State Police
Genotyper® 2.1




ABI PRISM
Thesis data - Group 1 (re-run)
Licensed to Lynne Helton, Michigan State Police
Genotyper® 2.1
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3000
2000
1000
15
2816
16
3140
17
1956
18
1891
23
1674
24
1442
D12-KNOWN AA 19 Green KNOWN AA
2000
1000
X
2808
Y
2789
14
2644
15
2379
28
1941
30
1704
16
1208
17
1152
D12-KNOWN AA 19 Yellow KNOWN AA
3000
2000
1000
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9
1198
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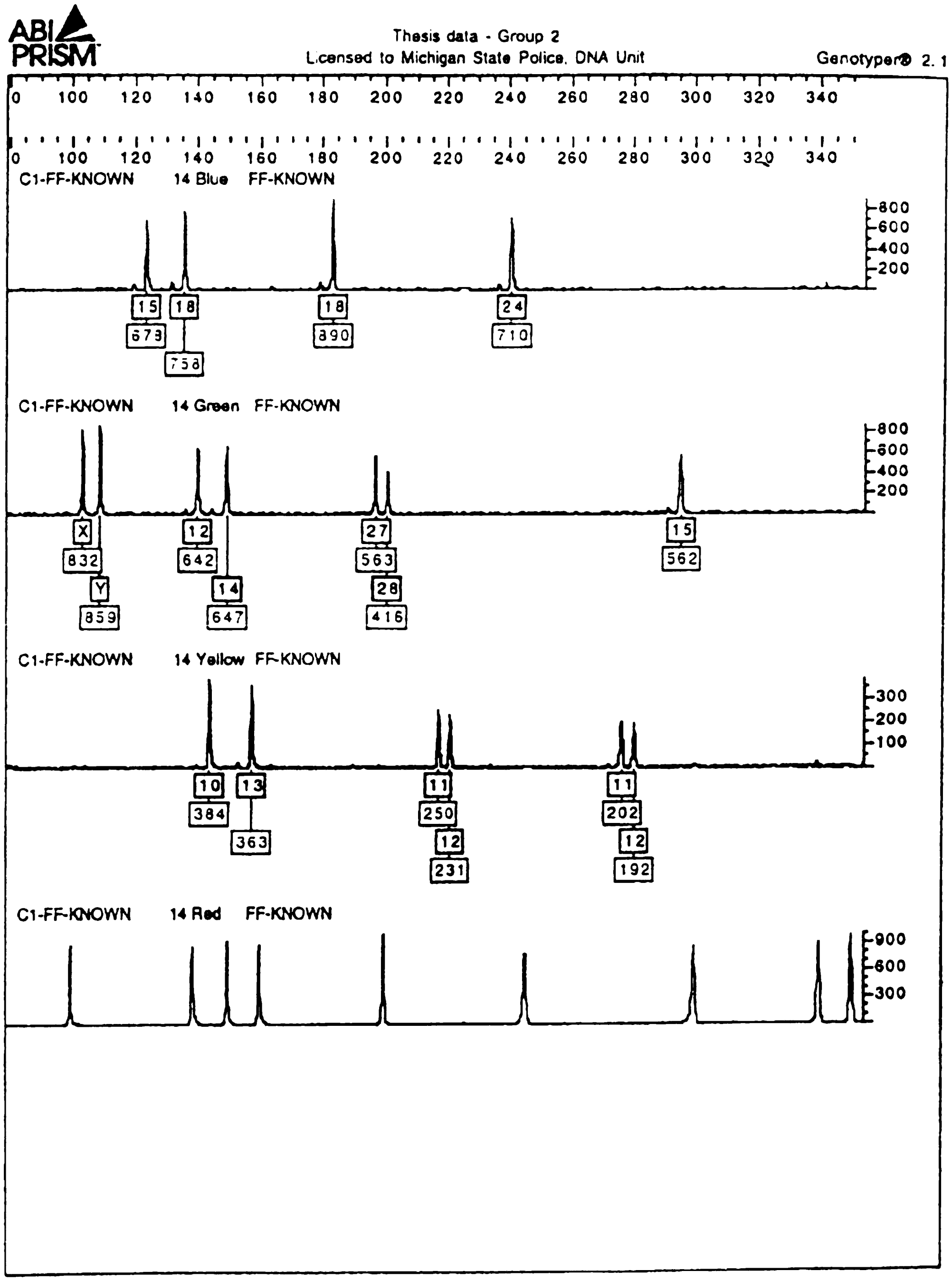
1105
10
683
12
767
D12-KNOWN AA 19 Red KNOWN AA
900
600
300


ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
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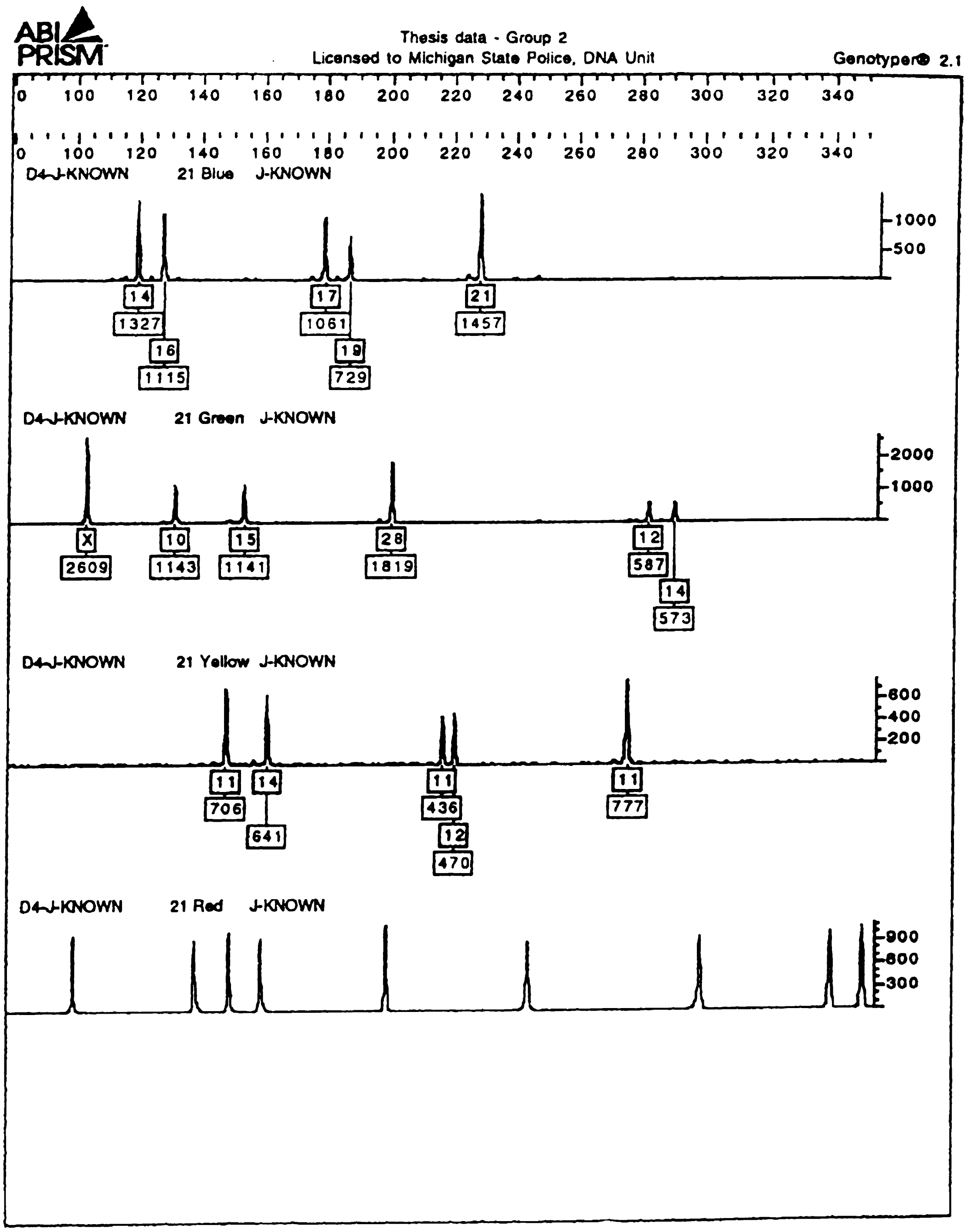
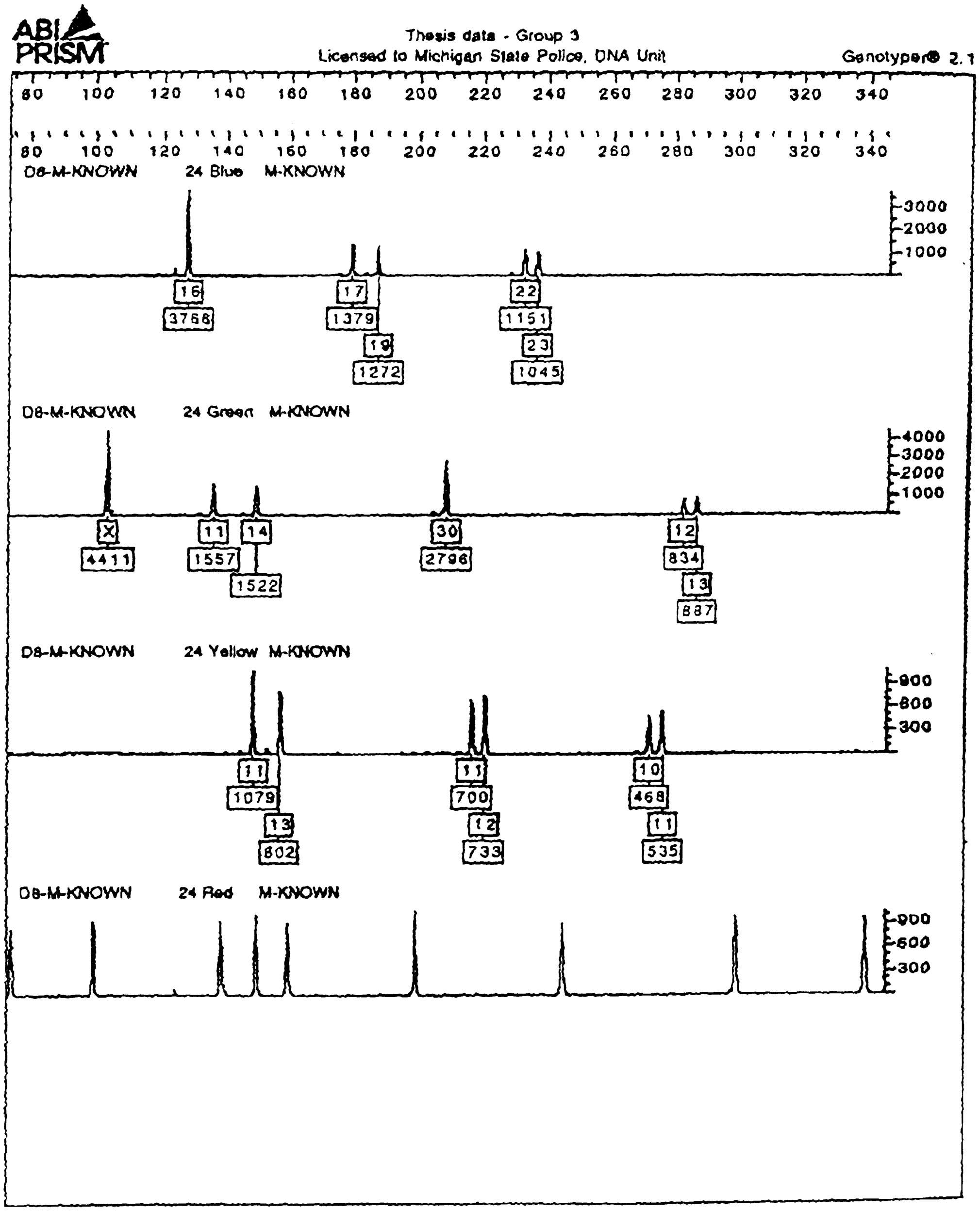
C1-FF-KNOWN 14 Blue FF-KNOWN
800 600 400 200
15 673
18 758
18 890
24 710
C1-FF-KNOWN 14 Green FF-KNOWN
800 600 400 200
X 832
Y 859
12 642
14 647
27 563
28 416
15 562
C1-FF-KNOWN 14 Yellow FF-KNOWN
300 200 100
10 384
13 363
11 250
12 231
11 202
12 192
C1-FF-KNOWN 14 Red FF-KNOWN
900 600 300


ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340
0 100 120 140 160 180 200 220 240 260 280 300 320 340
D4-J-KNOWN 21 Blue J-KNOWN
1000
500
14
1327
16
1115
17
1061
19
729
21
1457
D4-J-KNOWN 21 Green J-KNOWN
2000
1000
X
2609
10
1143
15
1141
28
1819
12
587
14
573
D4-J-KNOWN 21 Yellow J-KNOWN
600
400
200
11
706
14
641
11
436
12
470
11
777
D4-J-KNOWN 21 Red J-KNOWN
900
600
300


ABI PRISM
Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
80 100 120 140 160 180 200 220 240 260 280 300 320 340
80 100 120 140 160 180 200 220 240 260 280 300 320 340
D8-M-KNOWN 24 Blue M-KNOWN
3000
2000
1000
16
3766
17
1379
19
1272
22
1151
23
1045
D8-M-KNOWN 24 Green M-KNOWN
4000
3000
2000
1000
X
4411
11
1557
14
1522
30
2796
12
834
13
887
D8-M-KNOWN 24 Yellow M-KNOWN
900
600
300
11
1079
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802
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700
12
733
10
468
11
535
D8-M-KNOWN 24 Red M-KNOWN
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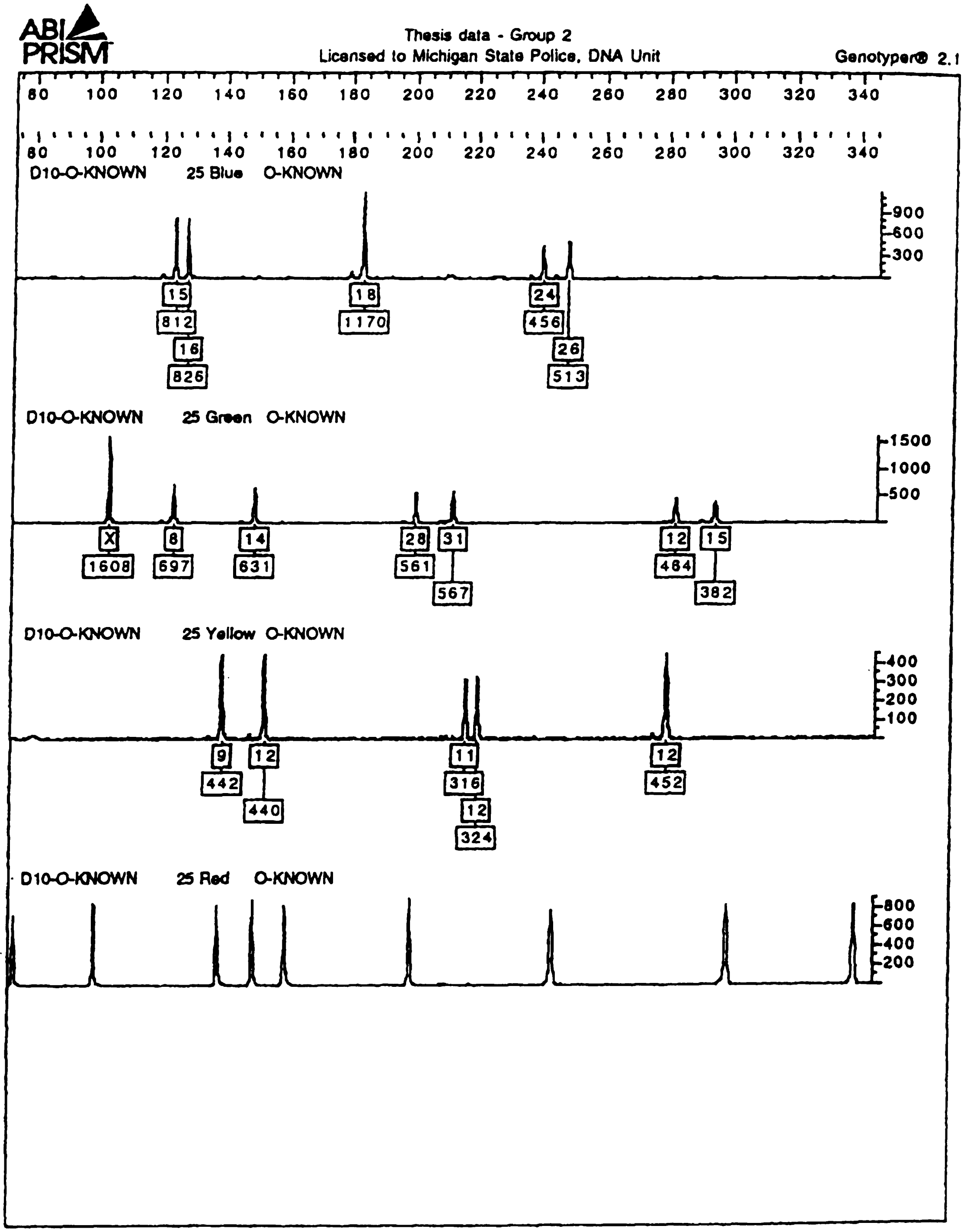
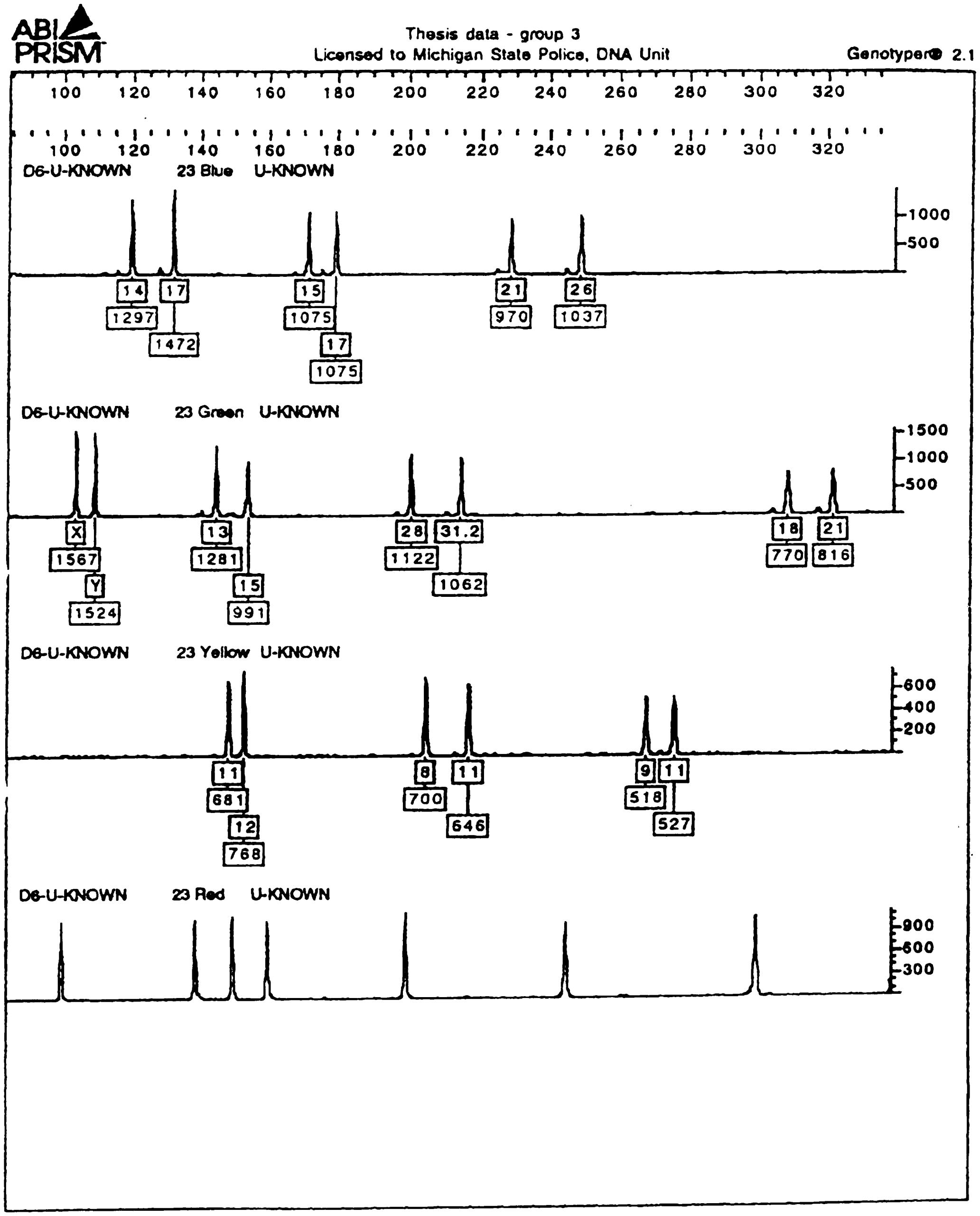
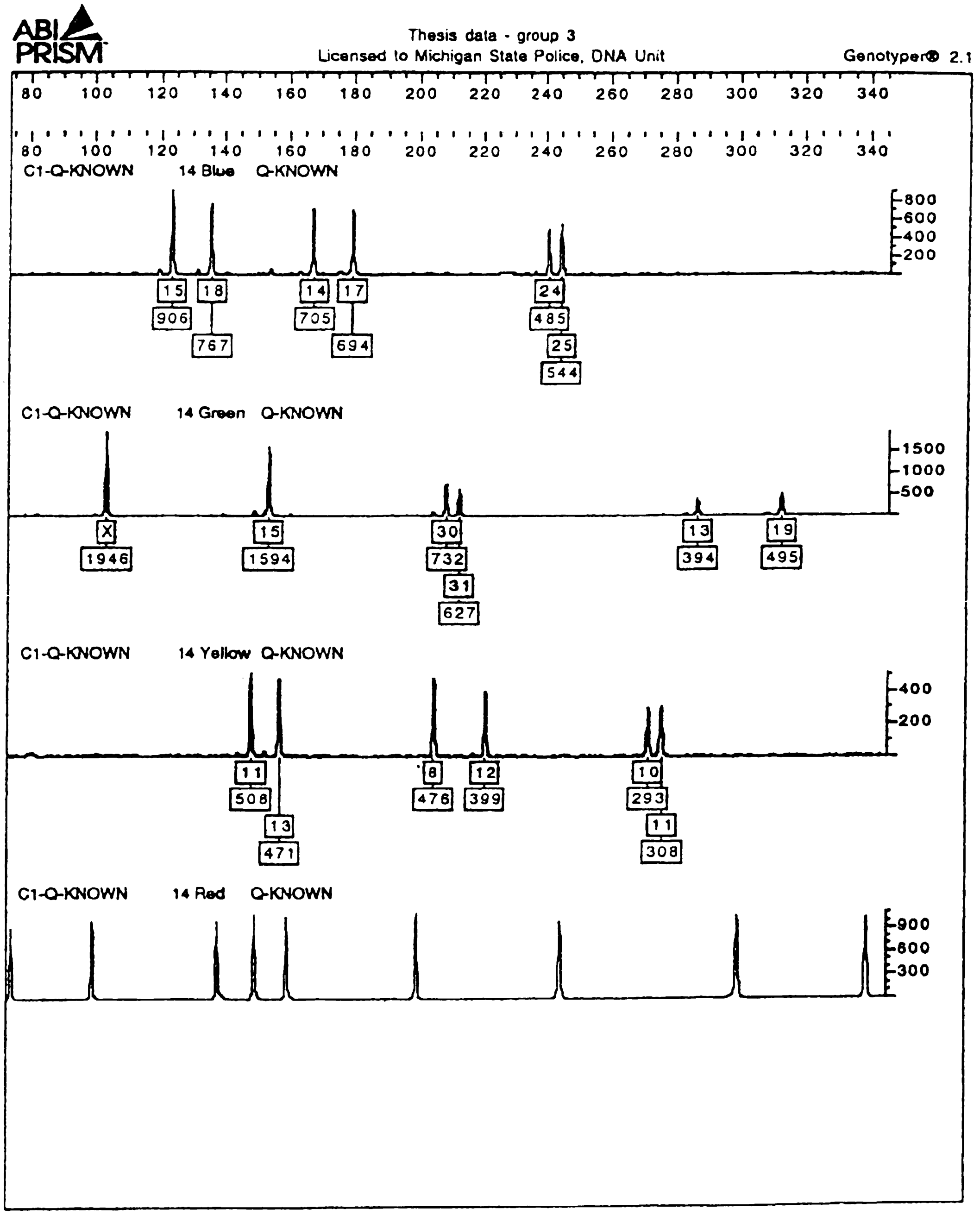
600
300

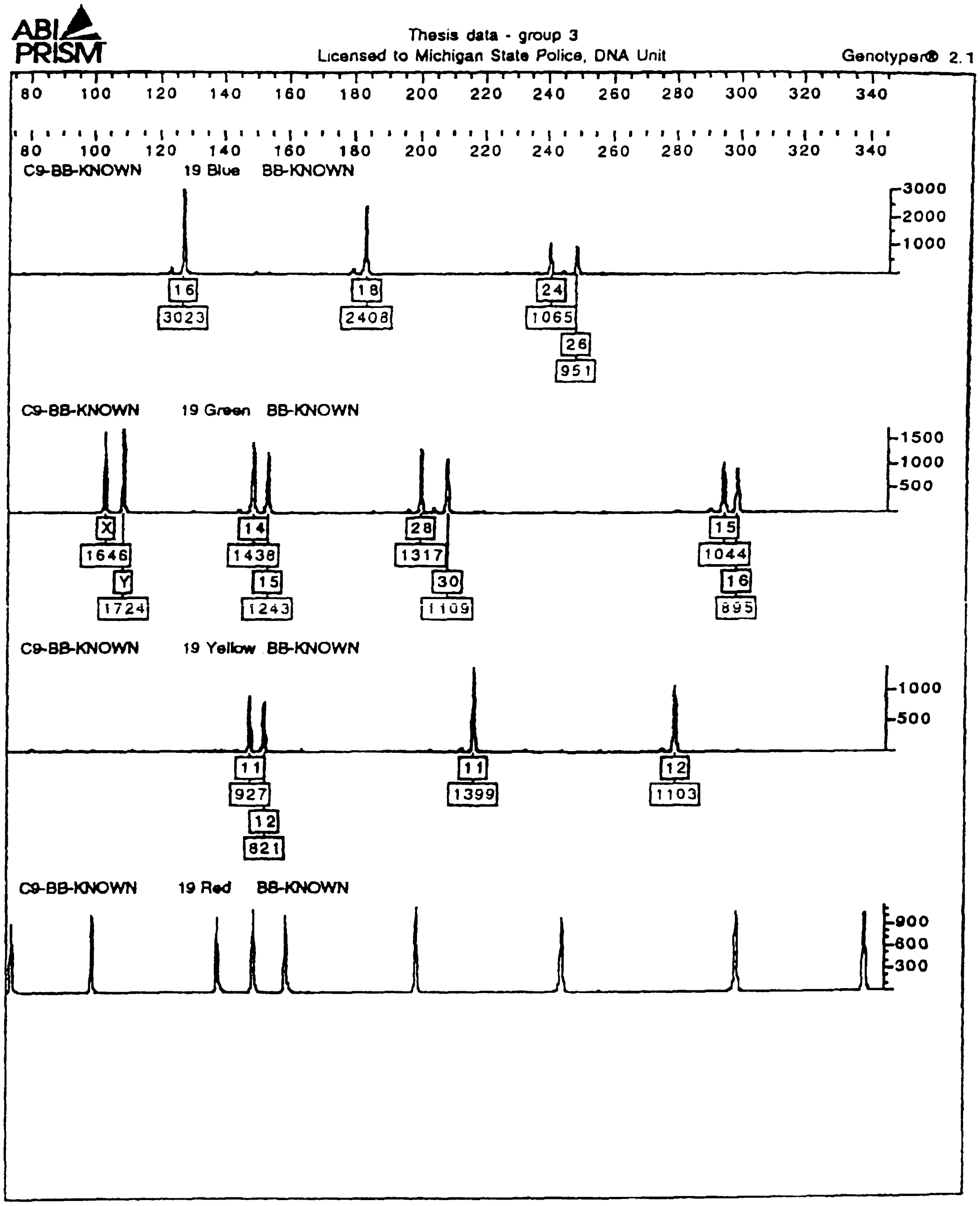

ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
80 100 120 140 160 180 200 220 240 260 280 300 320 340
80 100 120 140 160 180 200 220 240 260 280 300 320 340
D10-O-KNOWN 25 Blue O-KNOWN
900
600
300
15
812
16
826
18
1170
24
456
26
513
D10-O-KNOWN 25 Green O-KNOWN
1500
1000
500
X
1608
8
697
14
631
28
561
31
567
12
484
15
382
D10-O-KNOWN 25 Yellow O-KNOWN
400
300
200
100
9
442
12
440
11
316
12
324
12
452
D10-O-KNOWN 25 Red O-KNOWN
800
600
400
200



Thesis data - group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1



Thesis data - group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1


ABI PRISM
Thesis data - group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
80 100 120 140 160 180 200 220 240 260 280 300 320 340
C9-BB-KNOWN 19 Blue BB-KNOWN
3000
2000
1000
16
3023
18
2408
24
1065
26
951
C9-BB-KNOWN 19 Green BB-KNOWN
1500
1000
500
X
1646
Y
1724
14
1436
15
1243
28
1317
30
1109
15
1044
16
895
C9-BB-KNOWN 19 Yellow BB-KNOWN
1000
500
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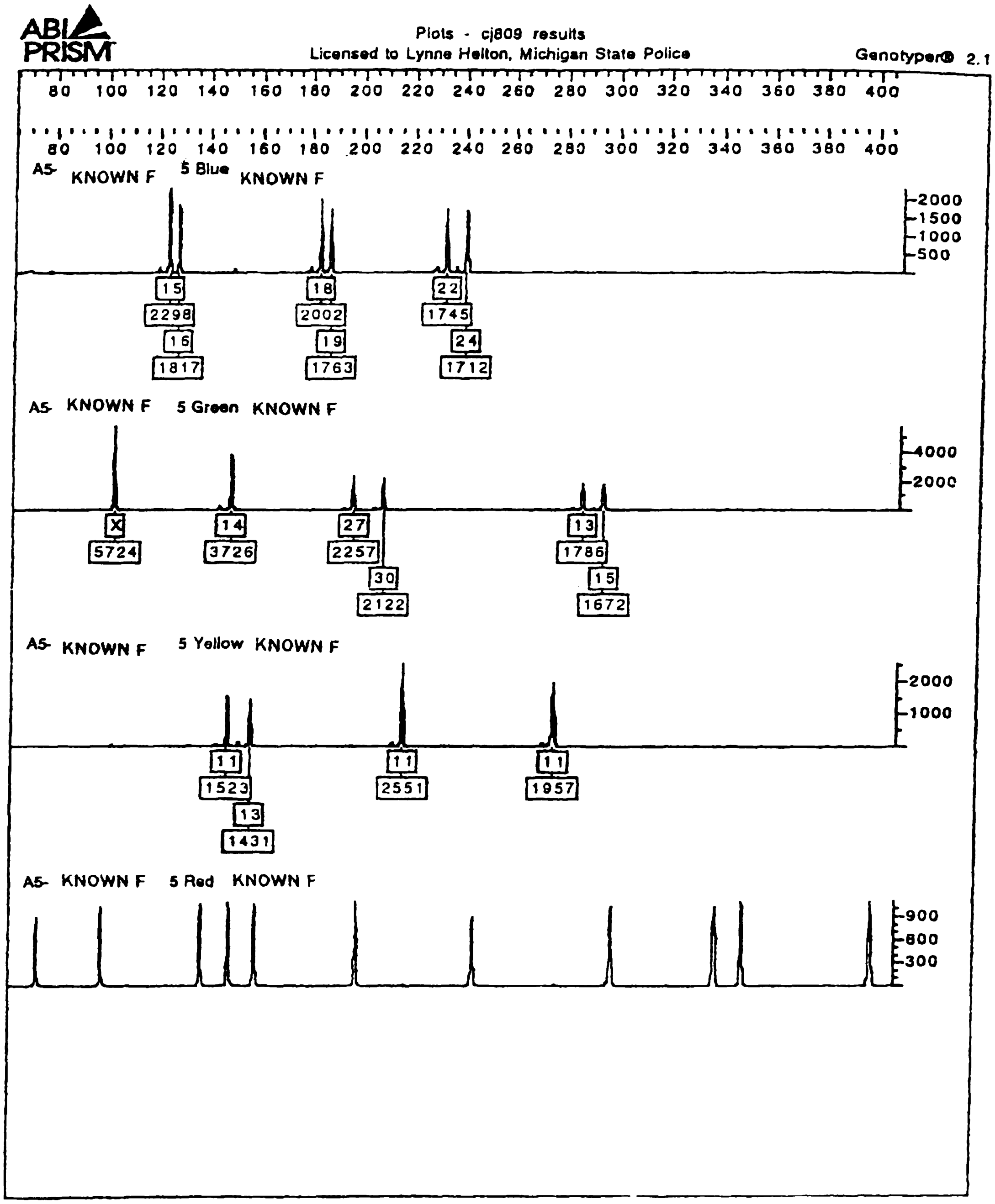
927
12
821
11
1399
12
1103
C9-BB-KNOWN 19 Red BB-KNOWN
900
600
300



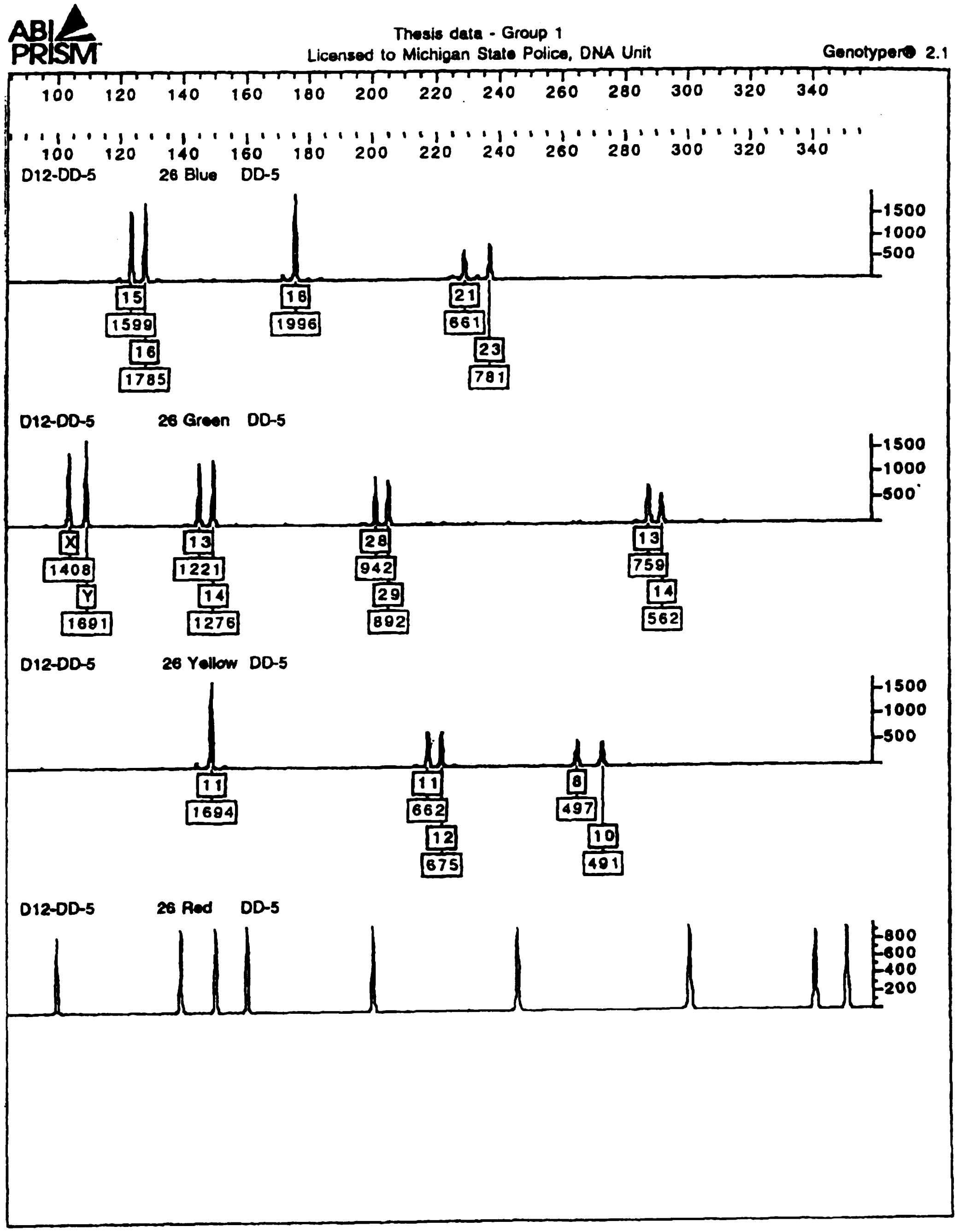
Plots - cj809 results

Licensed to Lynne Helton, Michigan State Police

Genotyper® 2.1

APPENDIX C

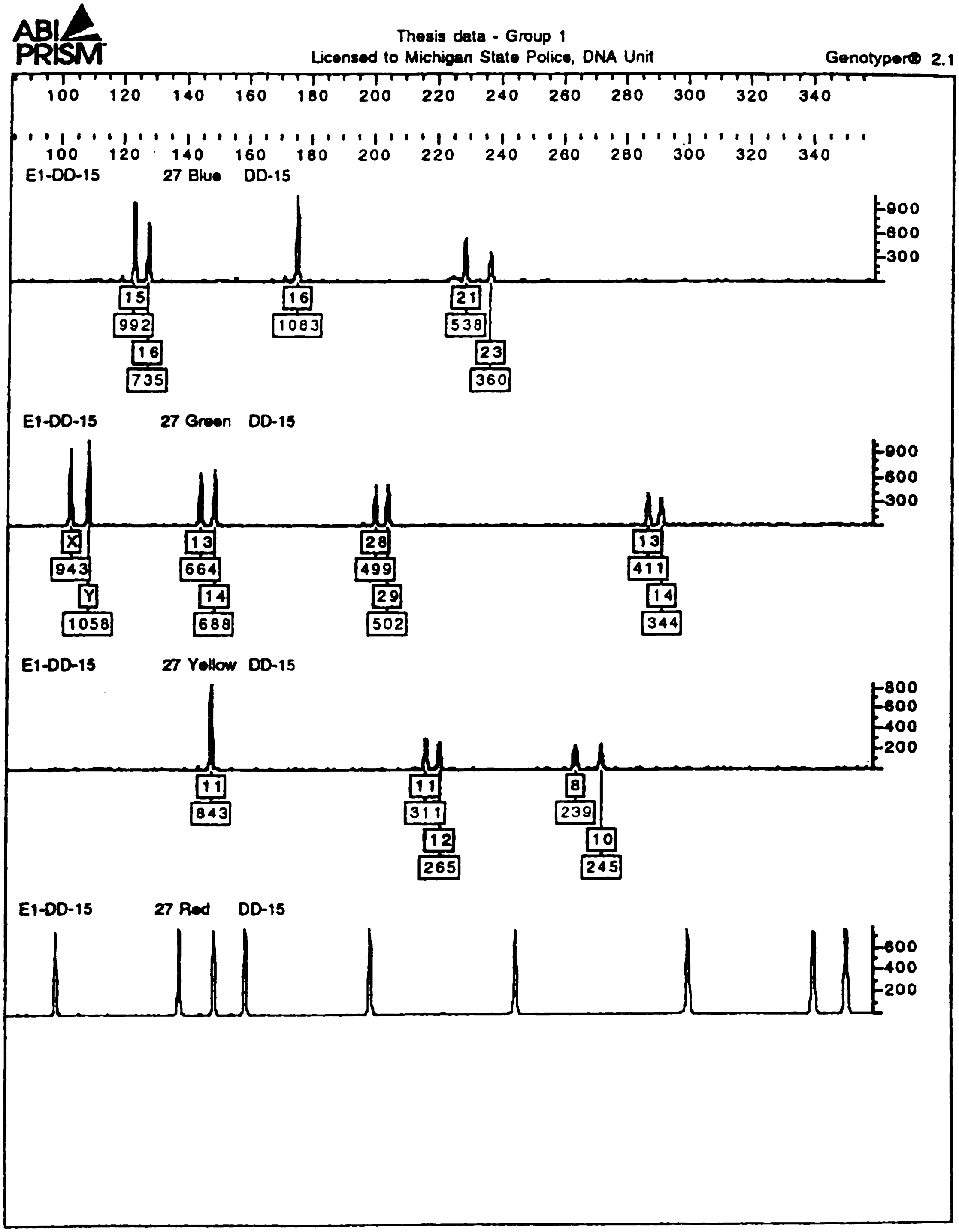
Electropherograms generated from 5, 15, and 30 second samples.

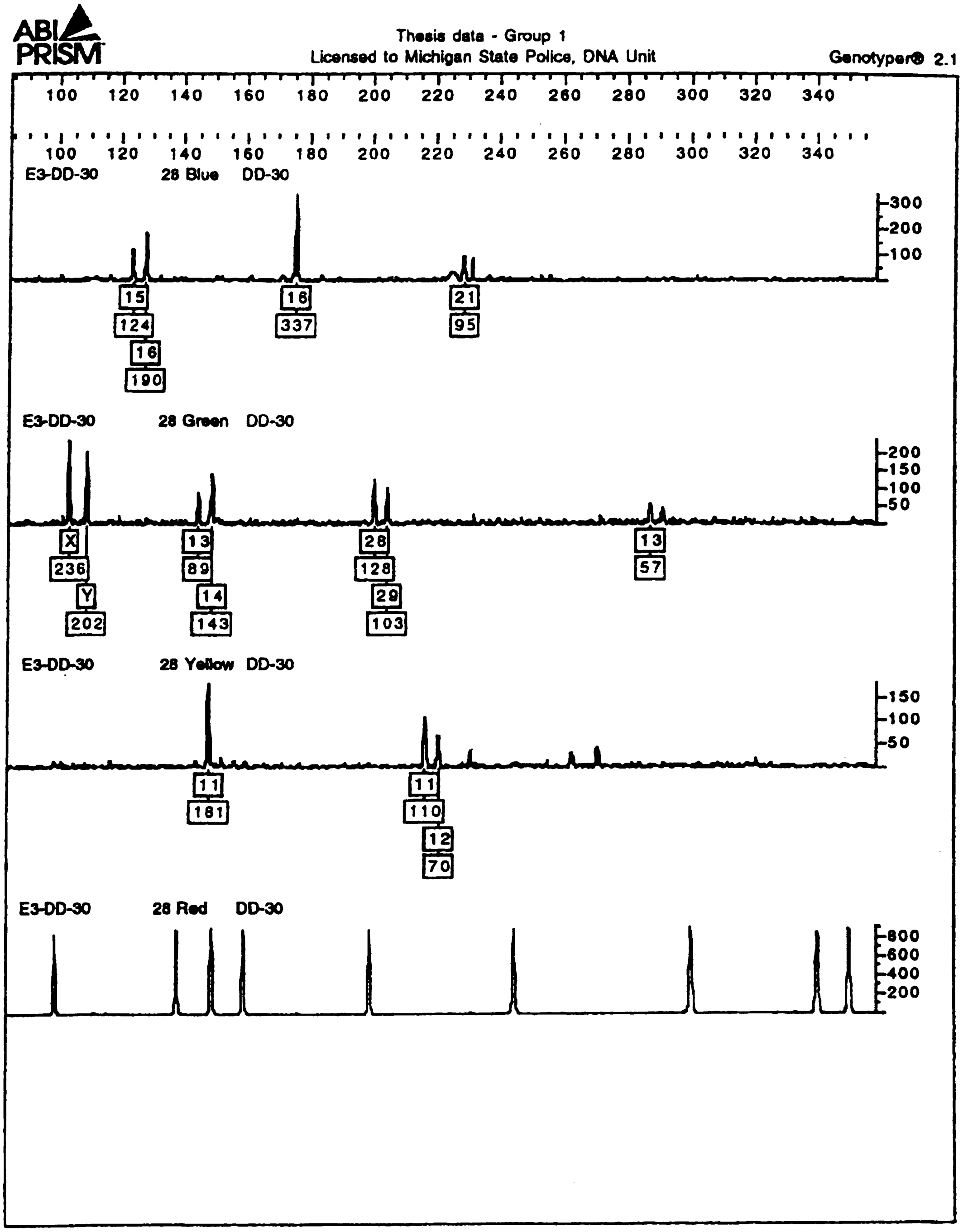


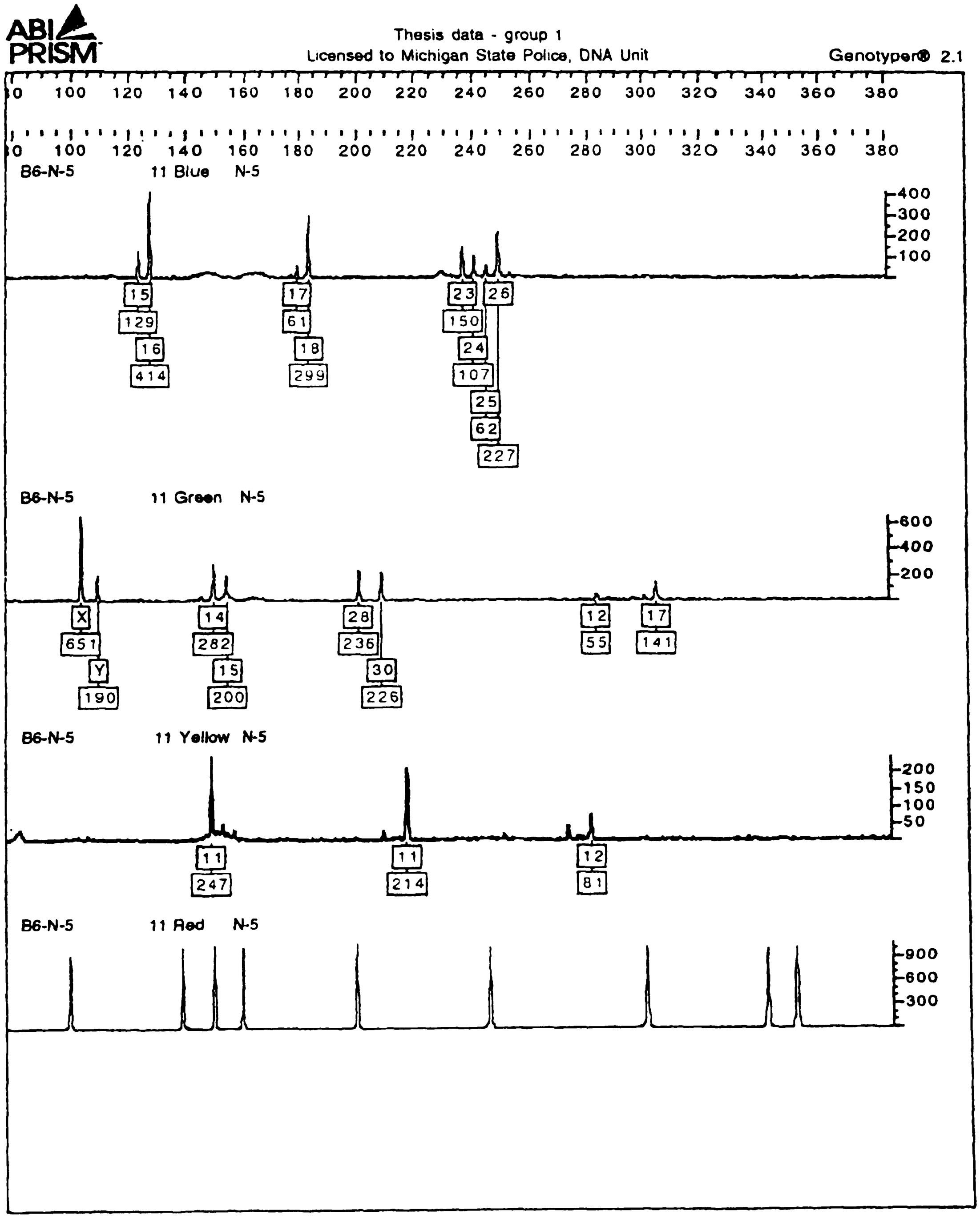
Thesis data - Group 1

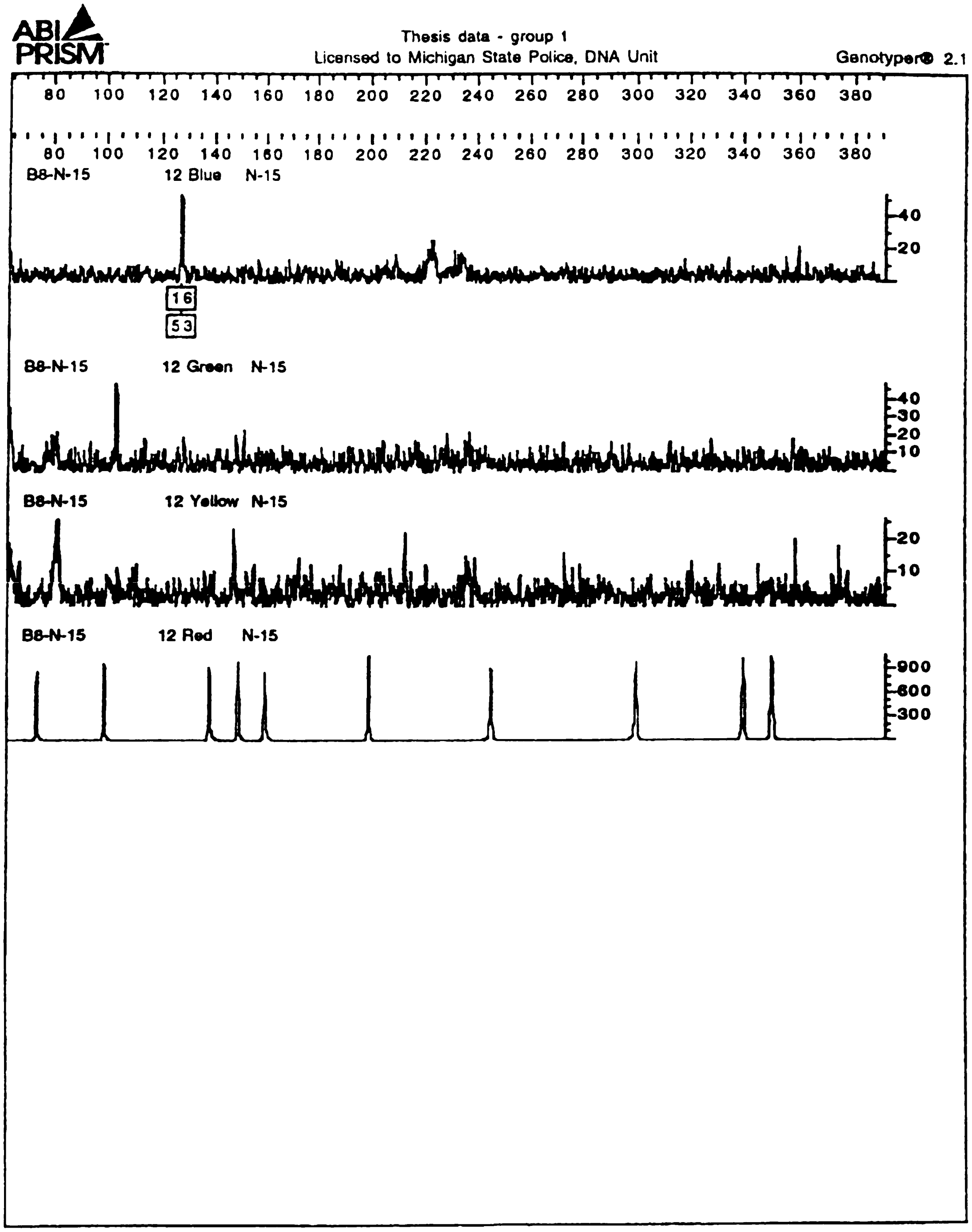
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1


ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
100 120 140 160 180 200 220 240 260 280 300 320 340
E1-DD-15 27 Blue DD-15
900
600
300
15
992
16
735
16
1083
21
538
23
360
E1-DD-15 27 Green DD-15
900
600
300
X
943
Y
1058
13
664
14
688
28
499
29
502
13
411
14
344
E1-DD-15 27 Yellow DD-15
800
600
400
200
11
843
11
311
12
265
8
239
10
245
E1-DD-15 27 Red DD-15
600
400
200


Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

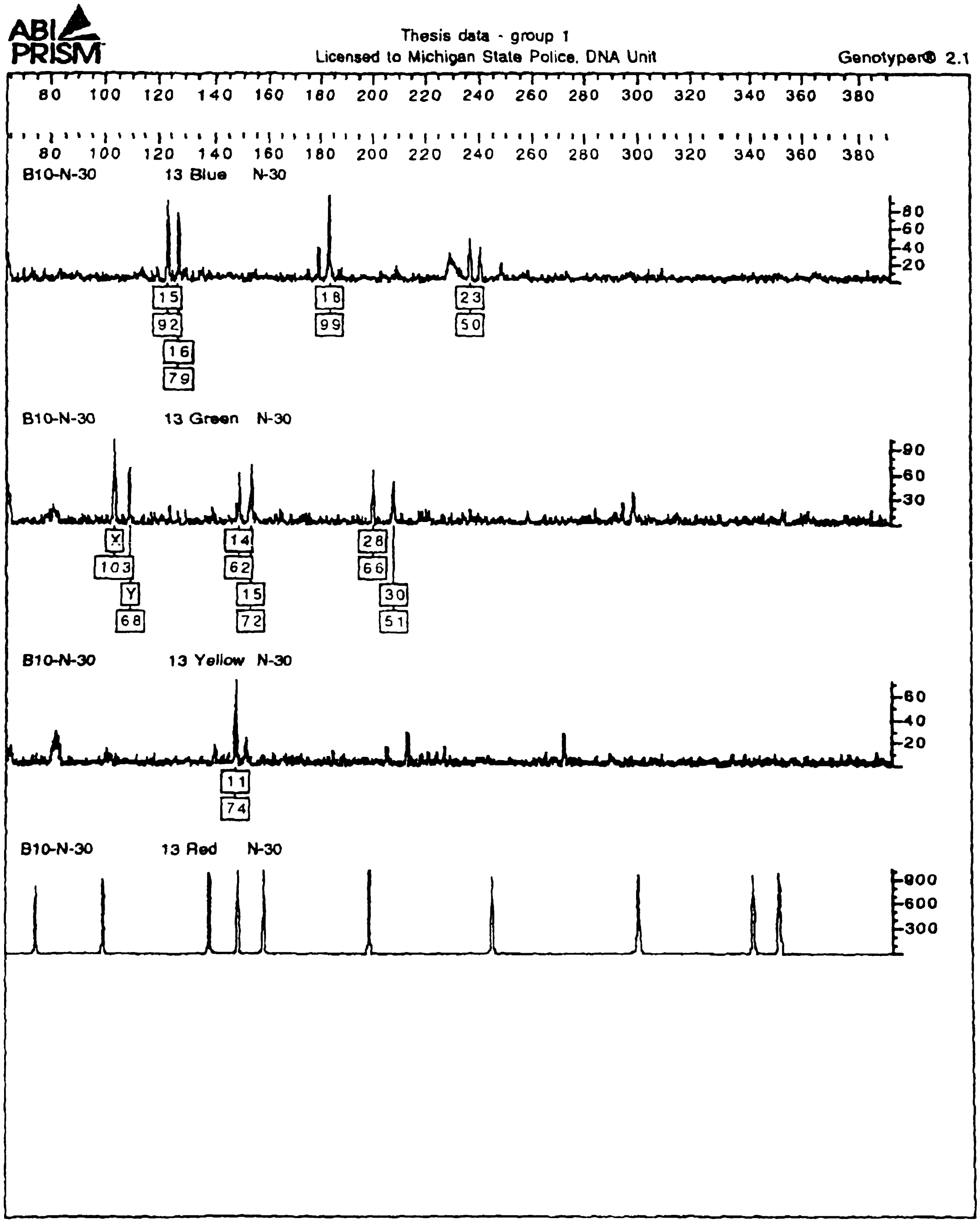

ABI PRISM
Thesis data - group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
B6-N-5 11 Blue N-5
400
300
200
100
15
129
16
414
17
61
18
299
23
150
24
107
25
62
26
227
B6-N-5 11 Green N-5
600
400
200
X
651
Y
190
14
282
15
200
28
236
30
226
12
55
17
141
B6-N-5 11 Yellow N-5
200
150
100
50
11
247
11
214
12
81
B6-N-5 11 Red N-5
900
600
300

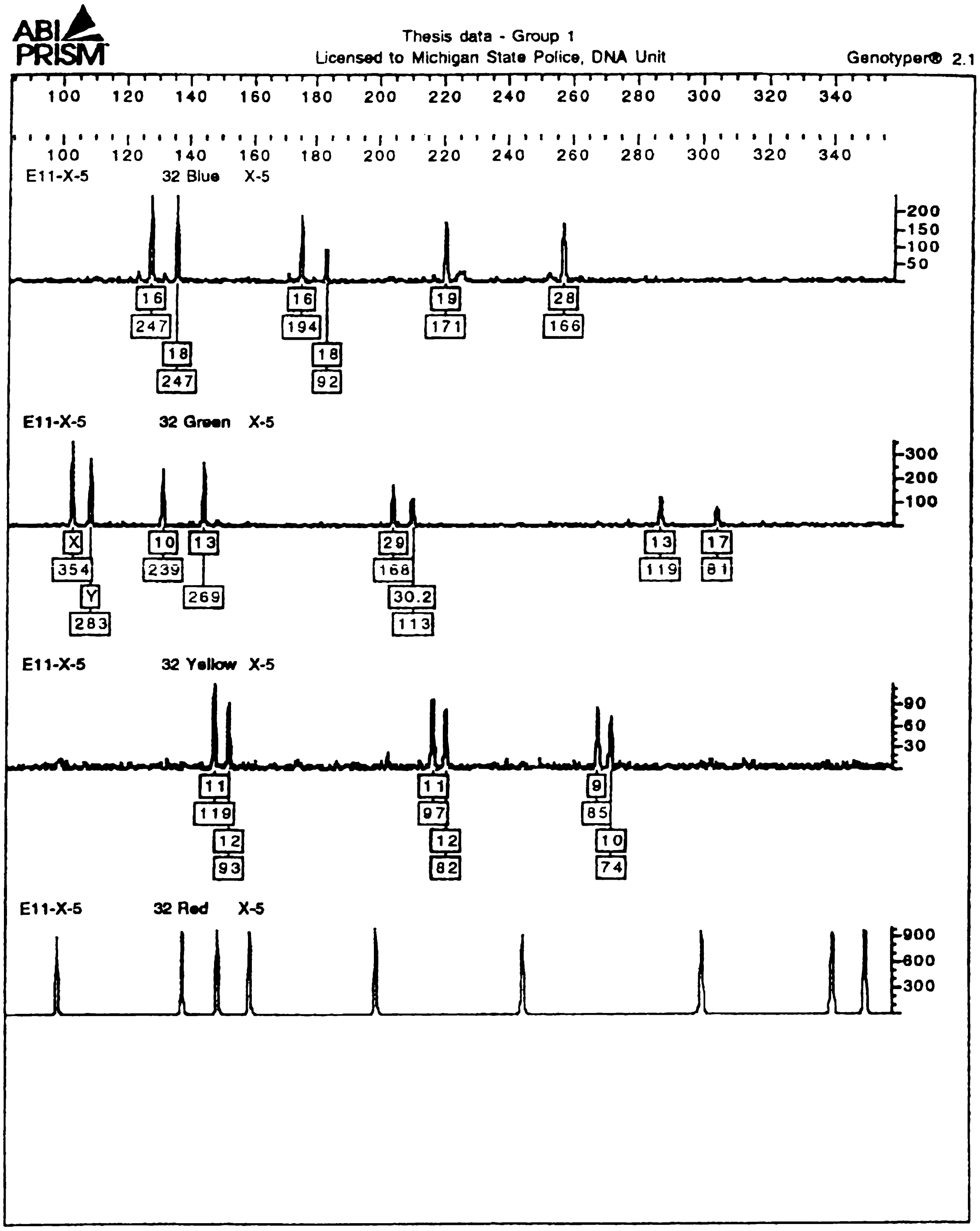


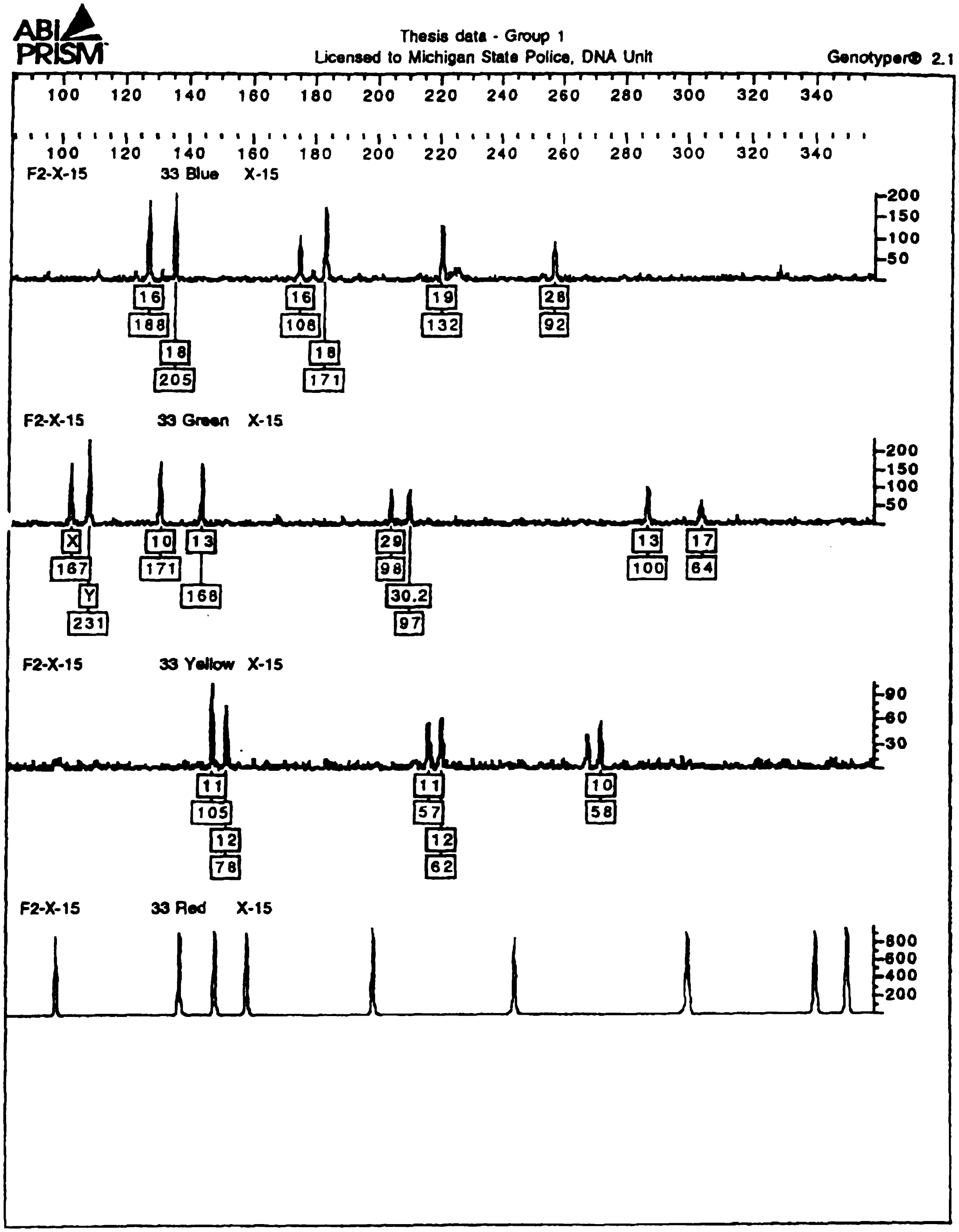
Thesis data - group 1

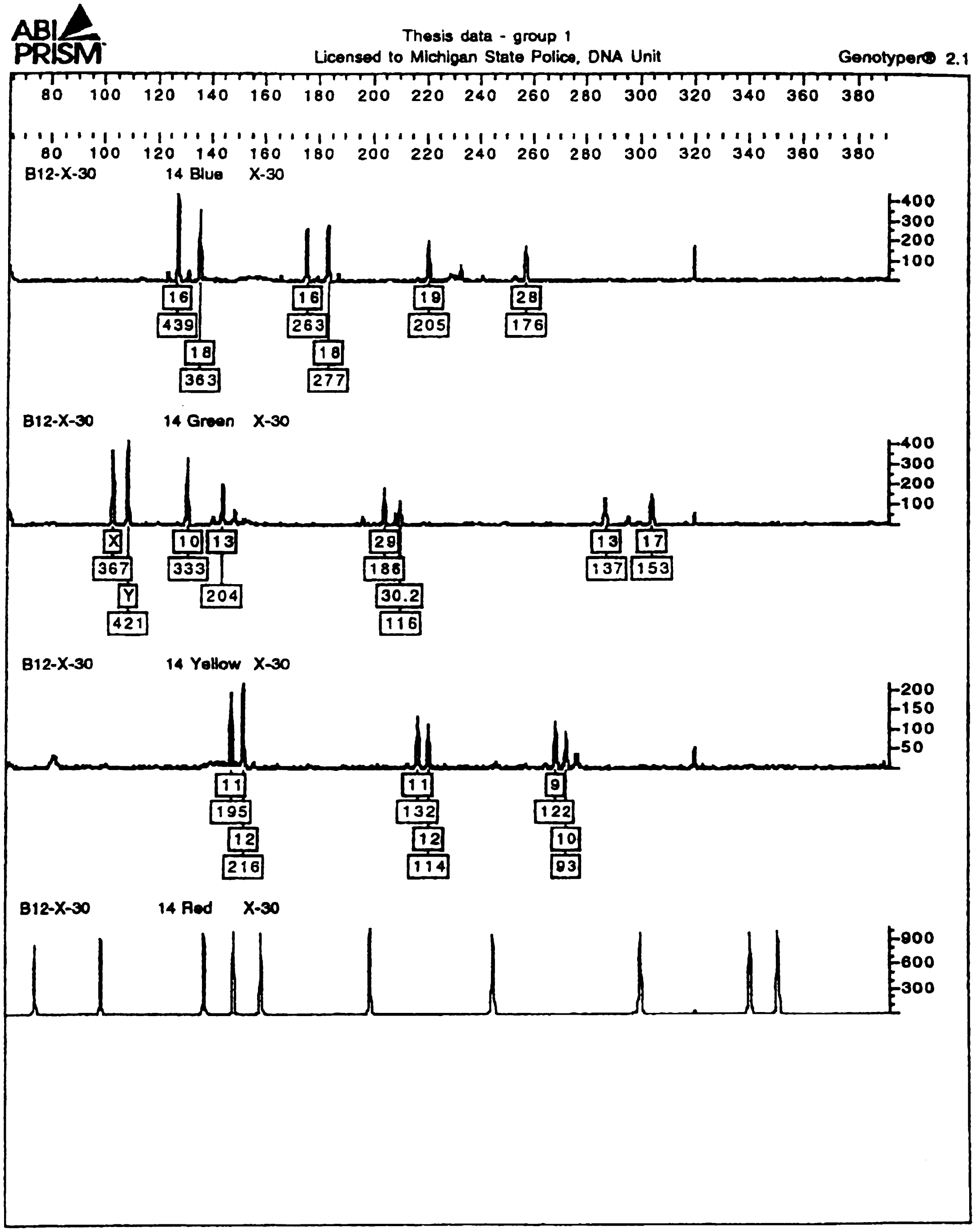
Licensed to Michigan State Police, DNA Unit

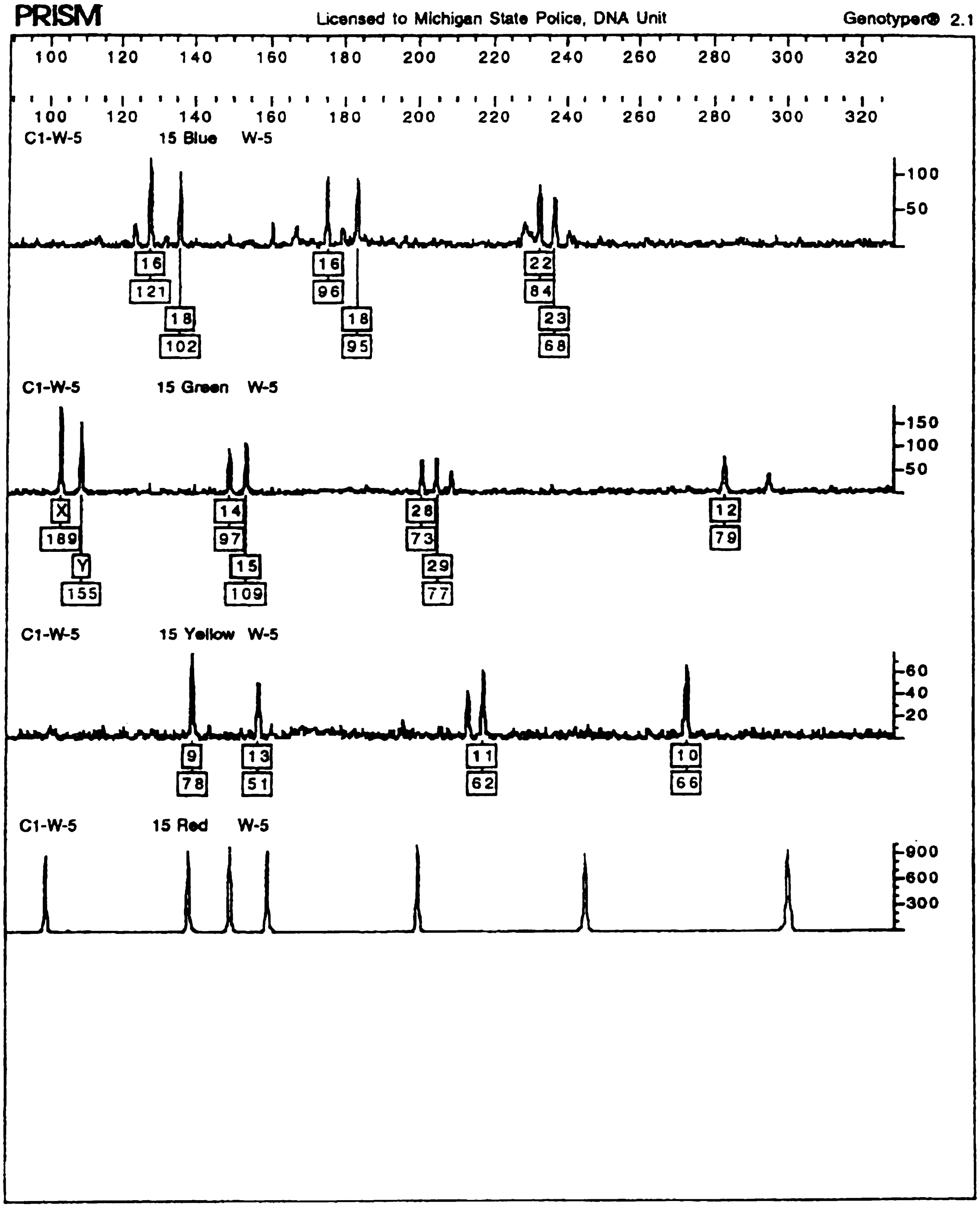
Genotyper® 2.1

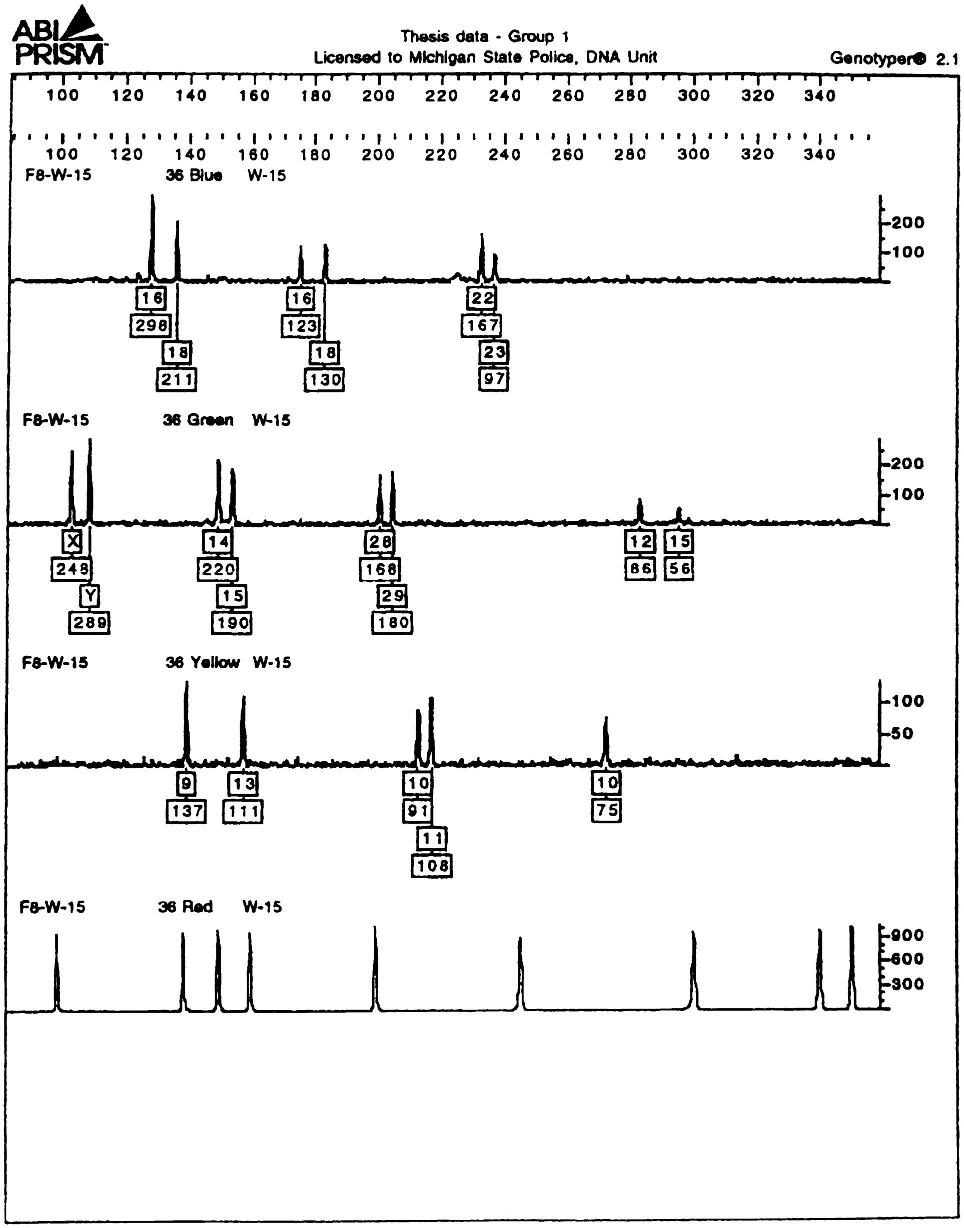

ABI PRISM
Thesis data - group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
B10-N-30 13 Blue N-30
80
60
40
20
15
92
16
79
18
99
23
50
B10-N-30 13 Green N-30
90
60
30
X
103
Y
68
14
62
15
72
28
66
30
51
B10-N-30 13 Yellow N-30
60
40
20
11
74
B10-N-30 13 Red N-30
900
600
300


ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
E11-X-5 32 Blue X-5
200 150 100 50
16 247
18 247
16 194
18 92
19 171
28 166
E11-X-5 32 Green X-5
300 200 100
X 354
Y 283
10 239
13 269
29 168
30.2 113
13 119
17 81
E11-X-5 32 Yellow X-5
90 60 30
11 119
12 93
11 97
12 82
9 85
10 74
E11-X-5 32 Red X-5
900 600 300


ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
F2-X-15 33 Blue X-15
200 150 100 50
16 188
18 205
16 108
18 171
19 132
28 92
F2-X-15 33 Green X-15
200 150 100 50
X 167
Y 231
10 171
13 168
29 98
30.2 97
13 100
17 64
F2-X-15 33 Yellow X-15
90 60 30
11 105
12 78
11 57
12 62
10 58
F2-X-15 33 Red X-15
800 600 400 200


ABI PRISM
Thesis data - group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
B12-X-30 14 Blue X-30
400
300
200
100
16
439
18
363
16
263
18
277
19
205
28
176
B12-X-30 14 Green X-30
400
300
200
100
X
367
Y
421
10
333
13
204
29
186
30.2
116
13
137
17
153
B12-X-30 14 Yellow X-30
200
150
100
50
11
195
12
216
11
132
12
114
9
122
10
93
B12-X-30 14 Red X-30
900
600
300


PRISM
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
C1-W-5 15 Blue W-5
100
50
16
121
18
102
16
96
18
95
22
84
23
68
C1-W-5 15 Green W-5
150
100
50
X
189
Y
155
14
97
15
109
28
73
29
77
12
79
C1-W-5 15 Yellow W-5
60
40
20
9
78
13
51
11
62
10
66
C1-W-5 15 Red W-5
900
600
300


ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
F8-W-15 36 Blue W-15
200
100
16
298
18
211
16
123
18
130
22
167
23
97
F8-W-15 36 Green W-15
200
100
X
248
Y
289
14
220
15
190
28
168
29
180
12
86
15
56
F8-W-15 36 Yellow W-15
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111
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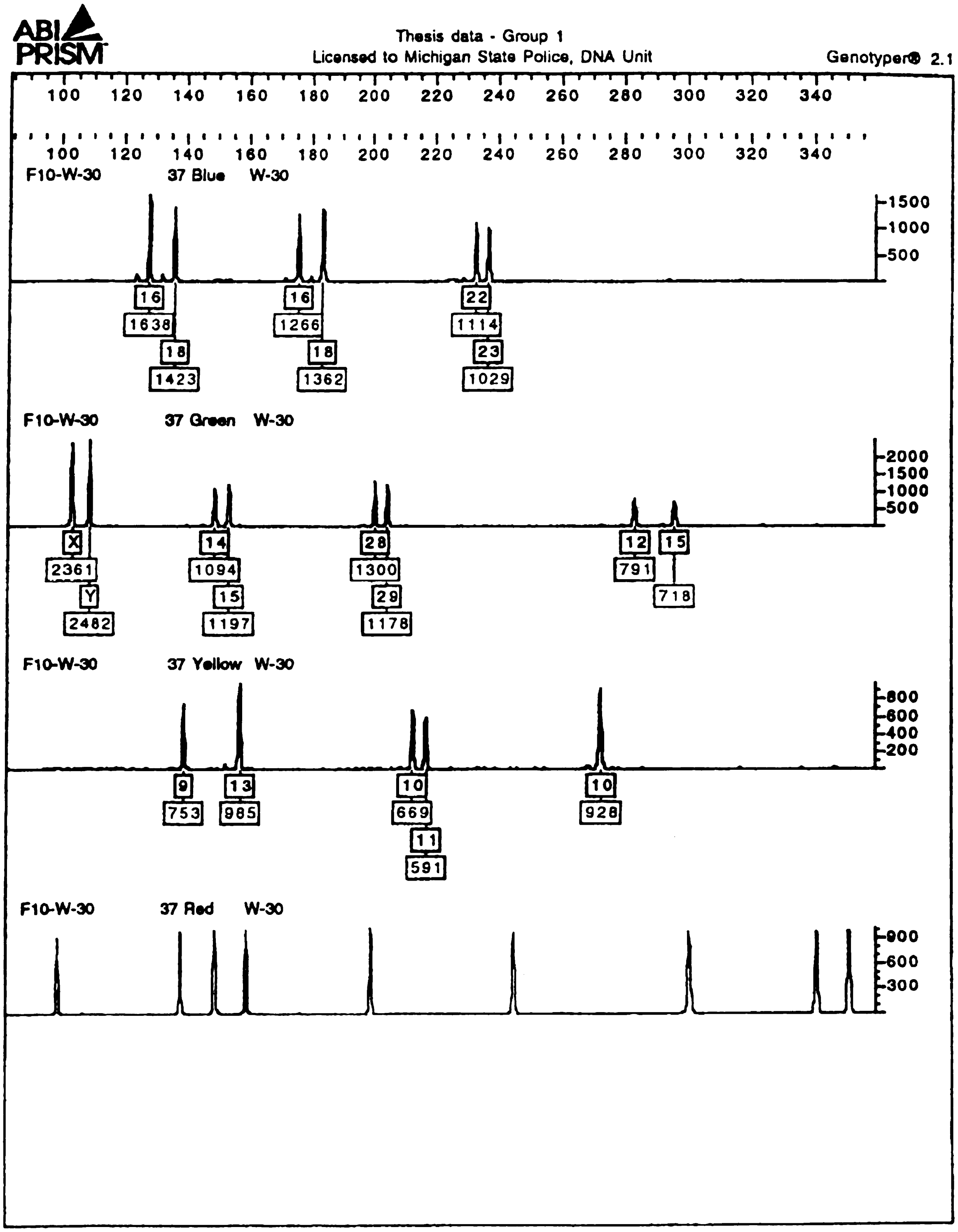
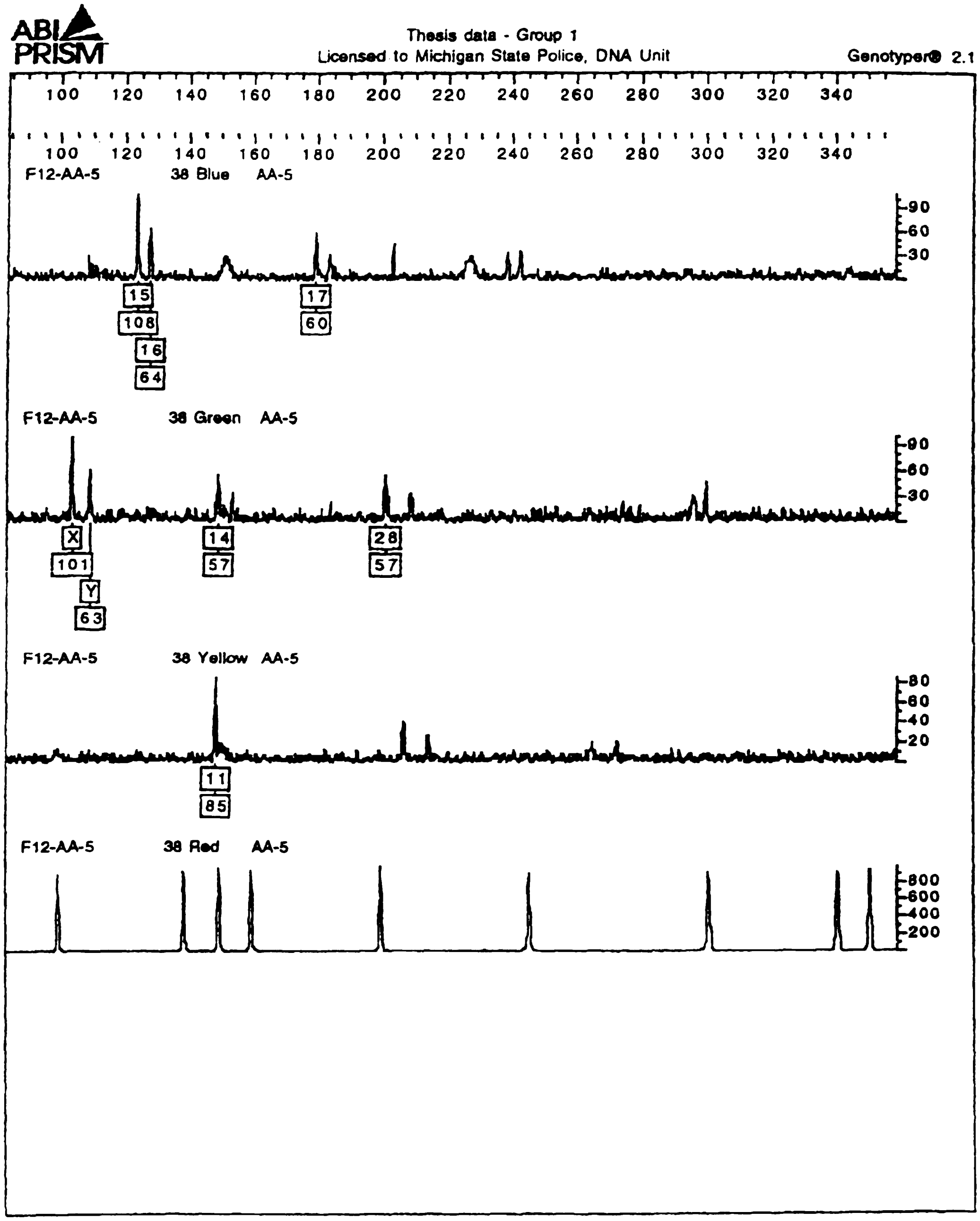
91
11
108
10
75
F8-W-15 36 Red W-15
900
600
300



ABI PRISM

Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1


ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
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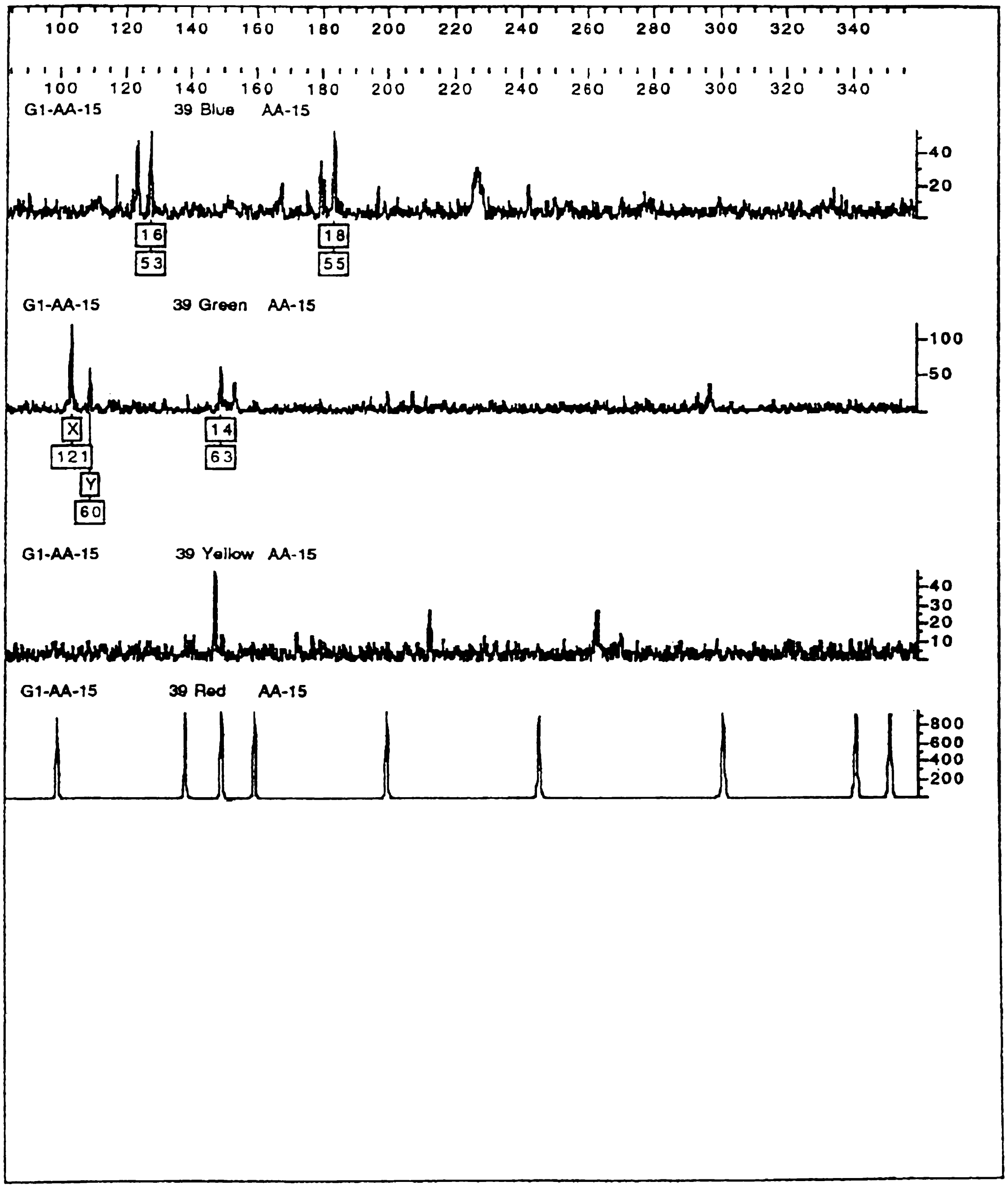
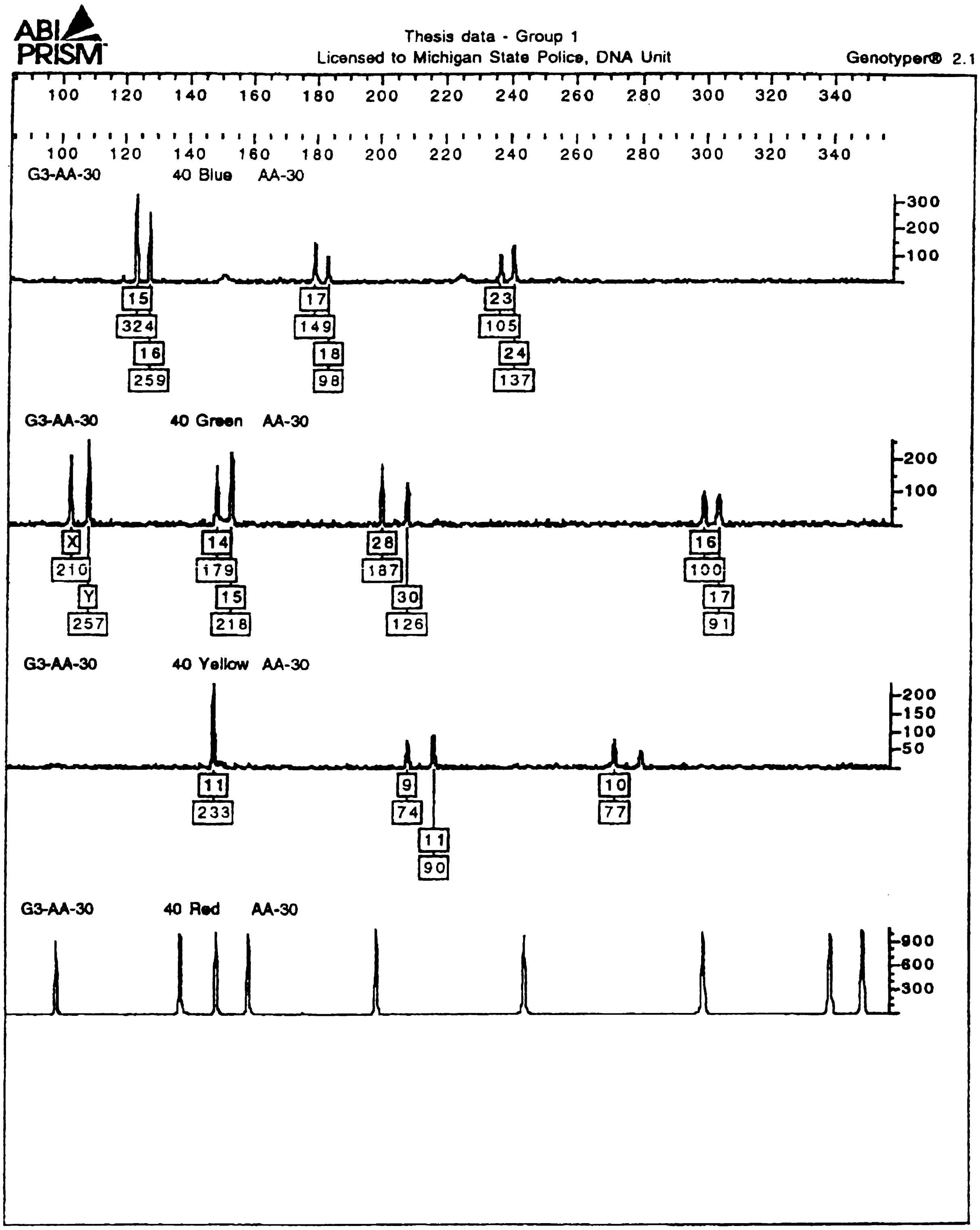
100 120 140 160 180 200 220 240 260 280 300 320 340
F12-AA-5 38 Blue AA-5
90 60 30
15
108
16
64
17
60
F12-AA-5 38 Green AA-5
90 60 30
X
101
Y
63
14
57
28
57
F12-AA-5 38 Yellow AA-5
80 60 40 20
11
85
F12-AA-5 38 Red AA-5
800 600 400 200

Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1




ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
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100 120 140 160 180 200 220 240 260 280 300 320 340
G3-AA-30 40 Blue AA-30
300
200
100
15
324
16
259
17
149
18
98
23
105
24
137
G3-AA-30 40 Green AA-30
200
100
X
210
Y
257
14
179
15
218
28
187
30
126
16
100
17
91
G3-AA-30 40 Yellow AA-30
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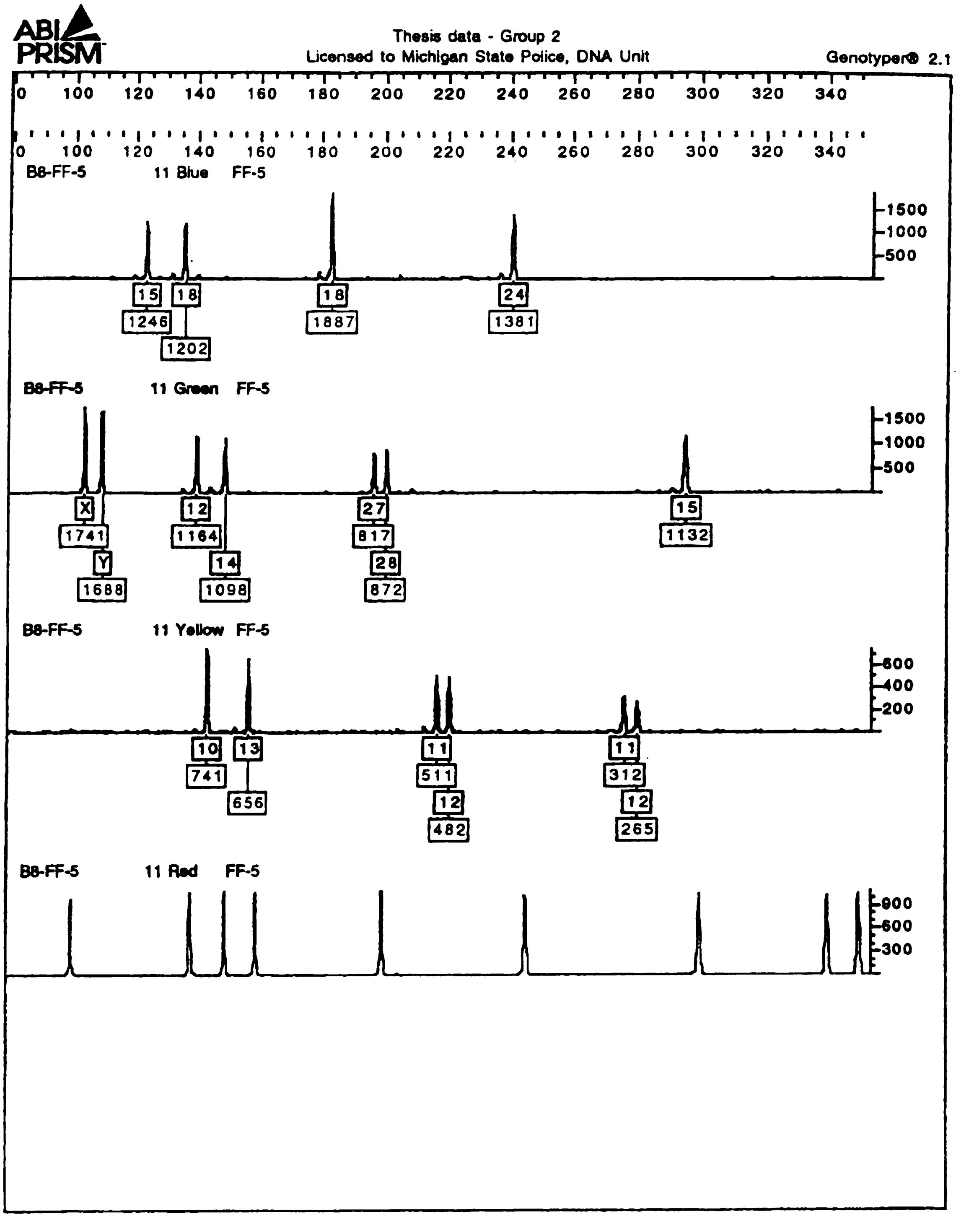
G3-AA-30 40 Red AA-30
900
600
300


ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340
0 100 120 140 160 180 200 220 240 260 280 300 320 340
B8-FF-5 11 Blue FF-5
1500
1000
500
15
1246
18
1202
18
1887
24
1381
B8-FF-5 11 Green FF-5
1500
1000
500
X
1741
Y
1688
12
1164
14
1098
27
817
28
872
15
1132
B8-FF-5 11 Yellow FF-5
600
400
200
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741
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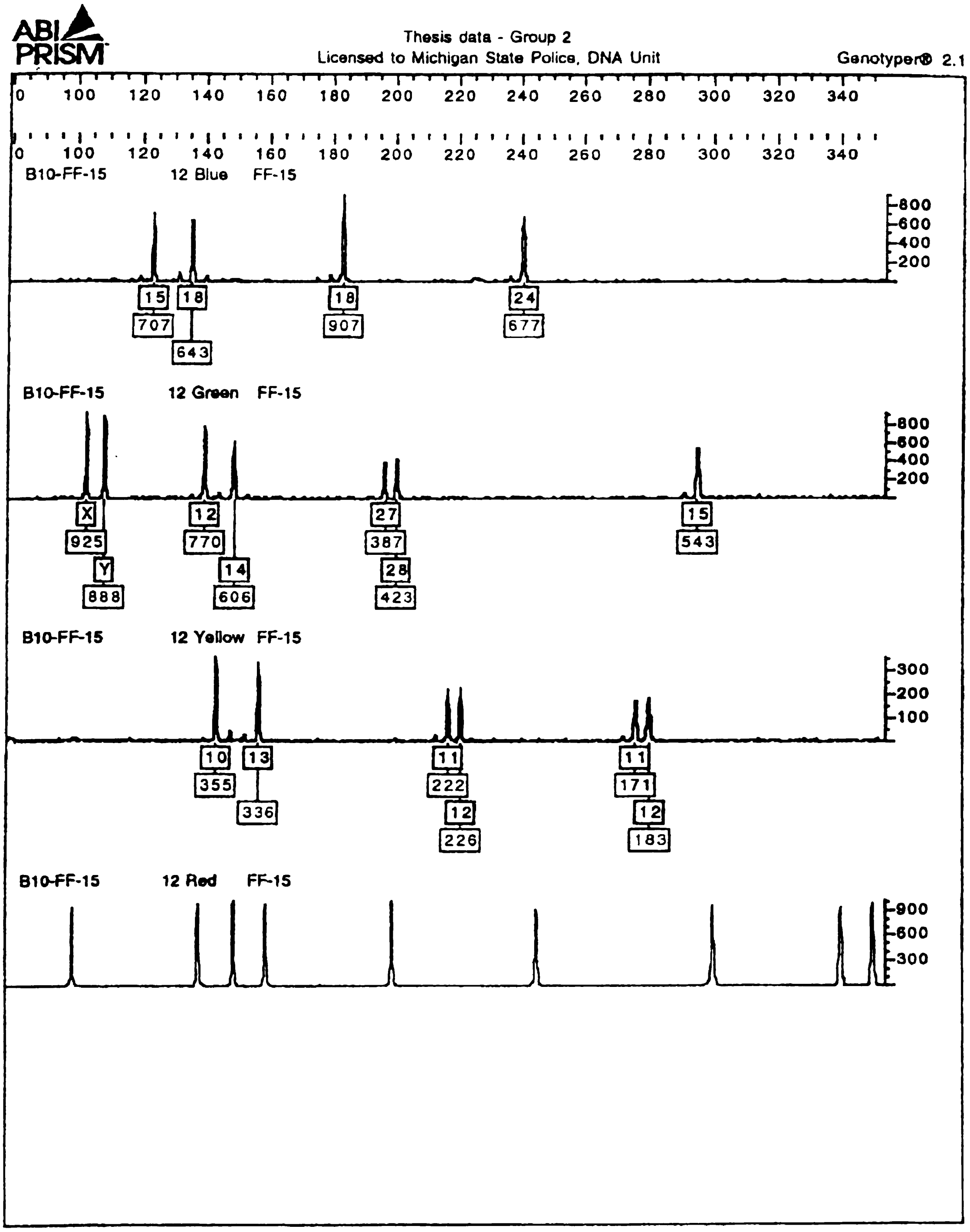
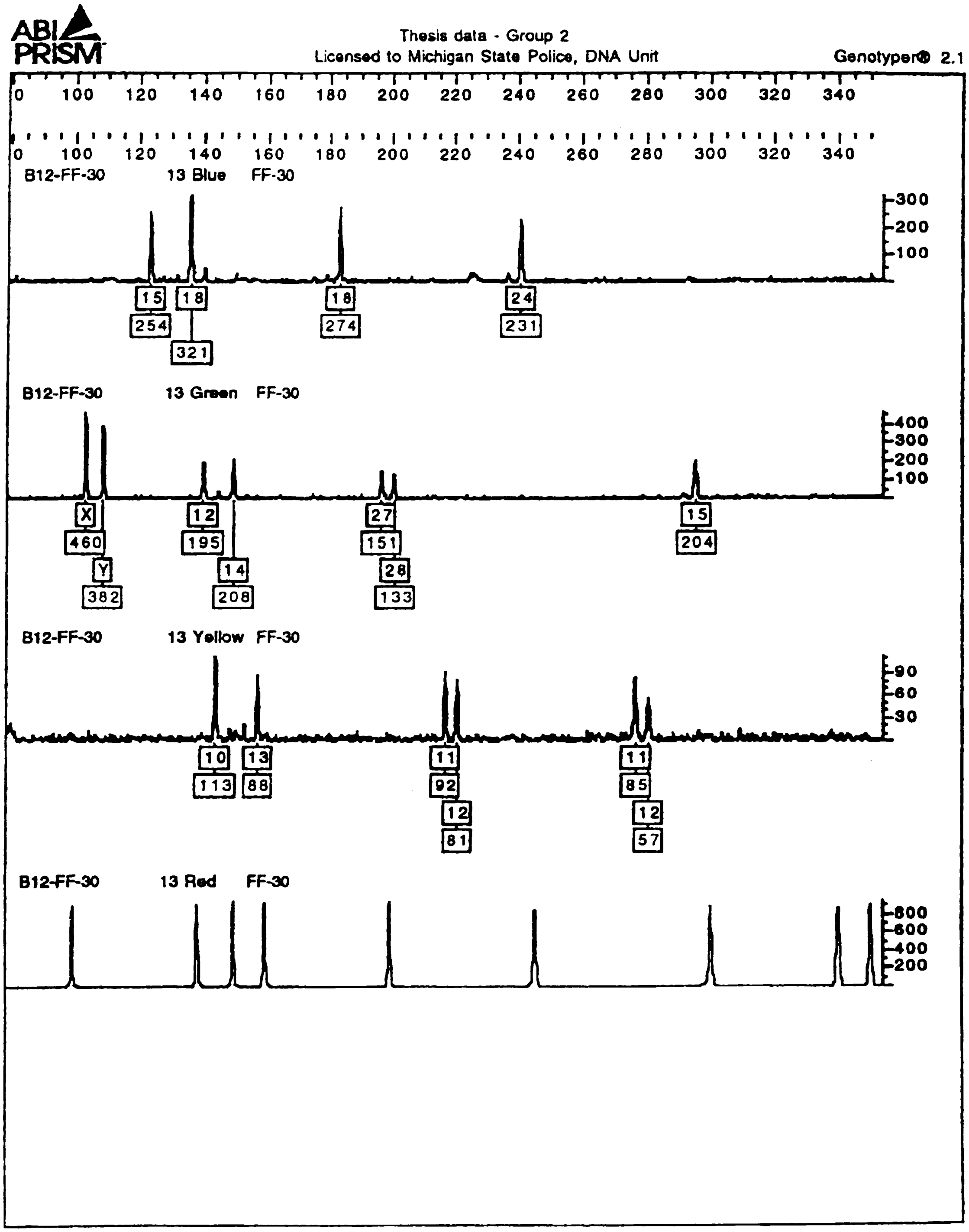
656
11
511
12
482
11
312
12
265
B8-FF-5 11 Red FF-5
900
600
300

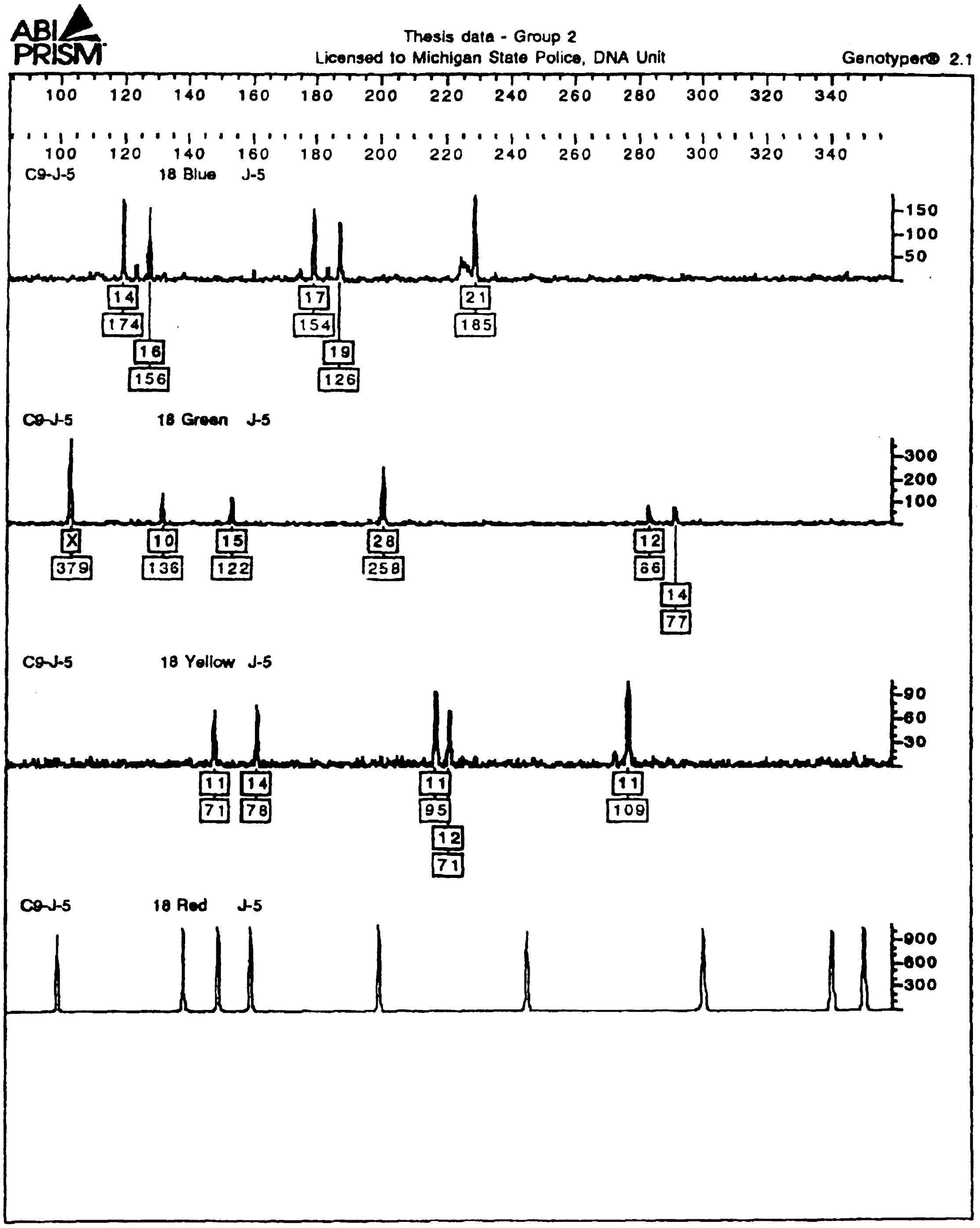


Thesis data - Group 2

Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1


ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340
0 100 120 140 160 180 200 220 240 260 280 300 320 340
B12-FF-30 13 Blue FF-30
300
200
100
15
254
18
321
18
274
24
231
B12-FF-30 13 Green FF-30
400
300
200
100
X
460
Y
382
12
195
14
208
27
151
28
133
15
204
B12-FF-30 13 Yellow FF-30
90
60
30
10
113
13
88
11
92
12
81
11
85
12
57
B12-FF-30 13 Red FF-30
800
600
400
200


ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
100 120 140 160 180 200 220 240 260 280 300 320 340
C9-J-5 18 Blue J-5
150
100
50
14
174
16
156
17
154
19
126
21
185
C9-J-5 18 Green J-5
300
200
100
X
379
10
136
15
122
28
258
12
66
14
77
C9-J-5 18 Yellow J-5
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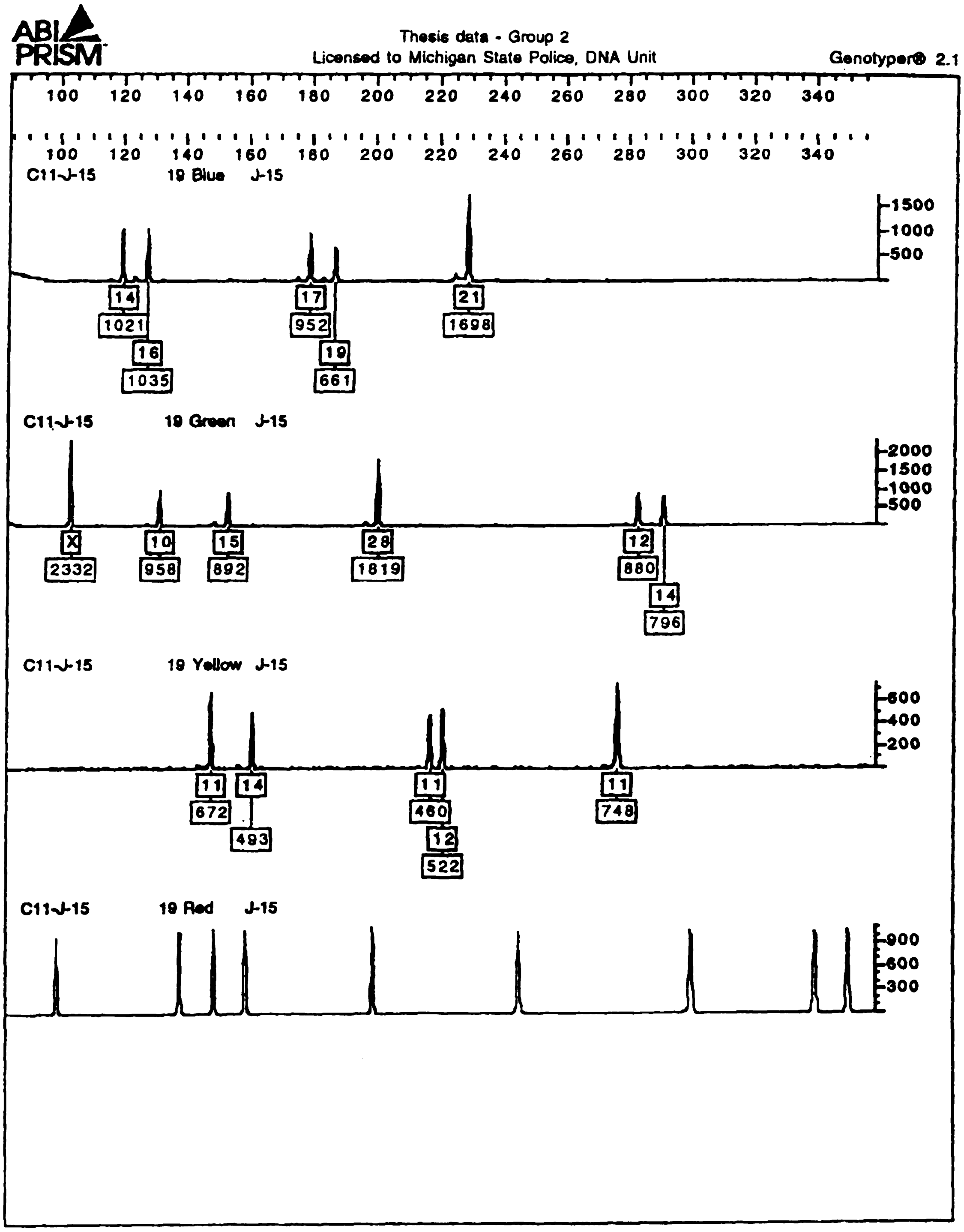
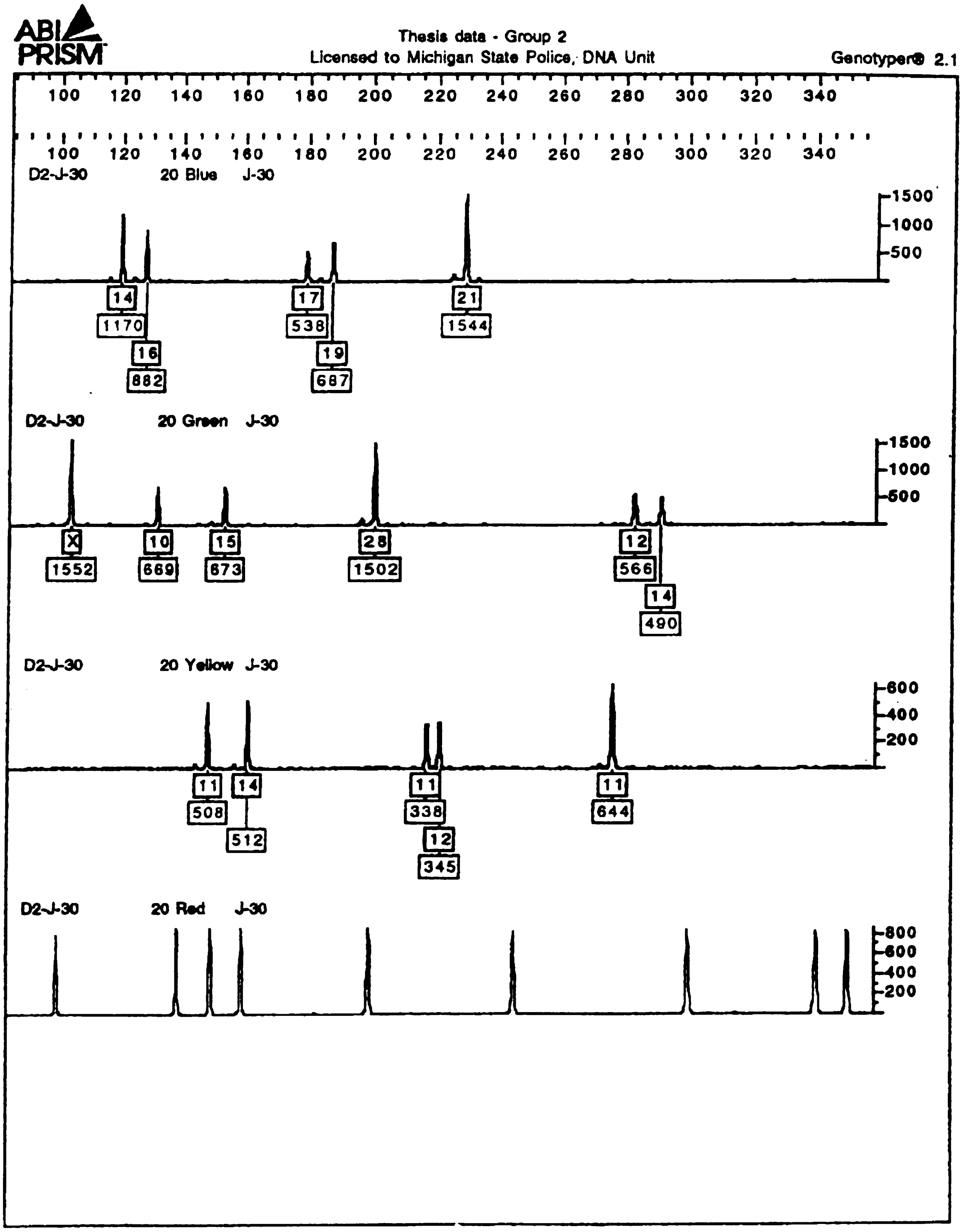
C9-J-5 18 Red J-5
900
600
300

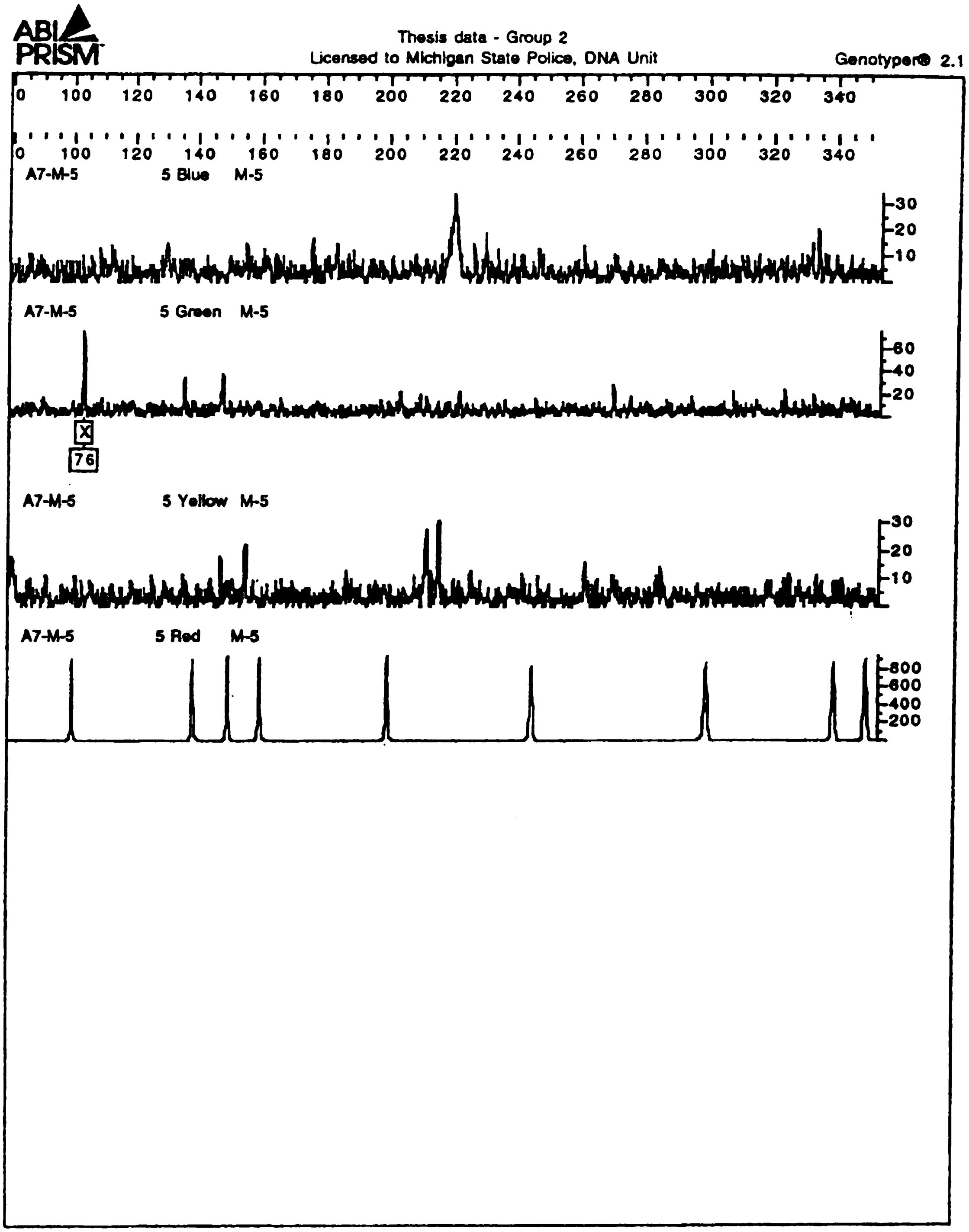


Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1



Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1


ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
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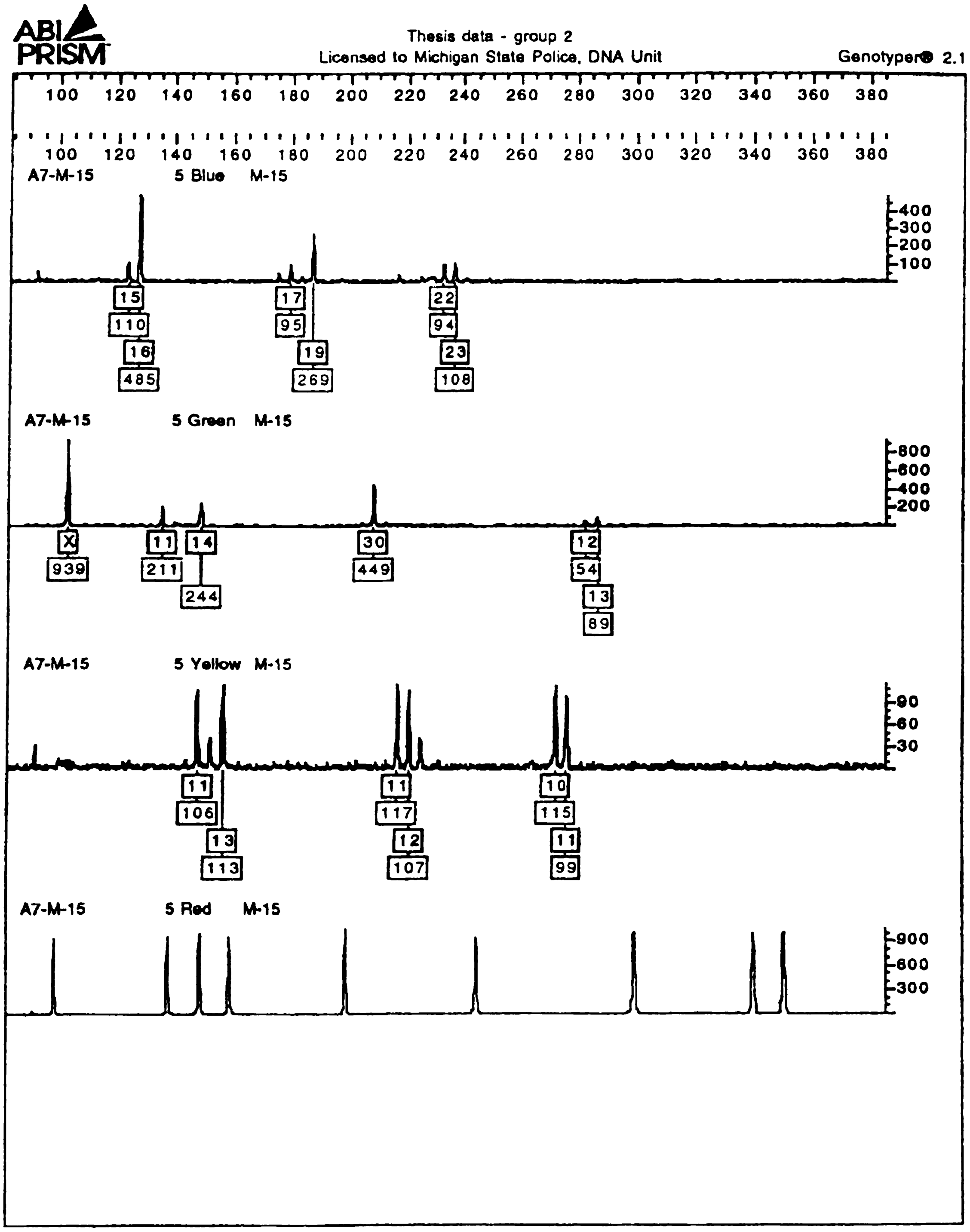
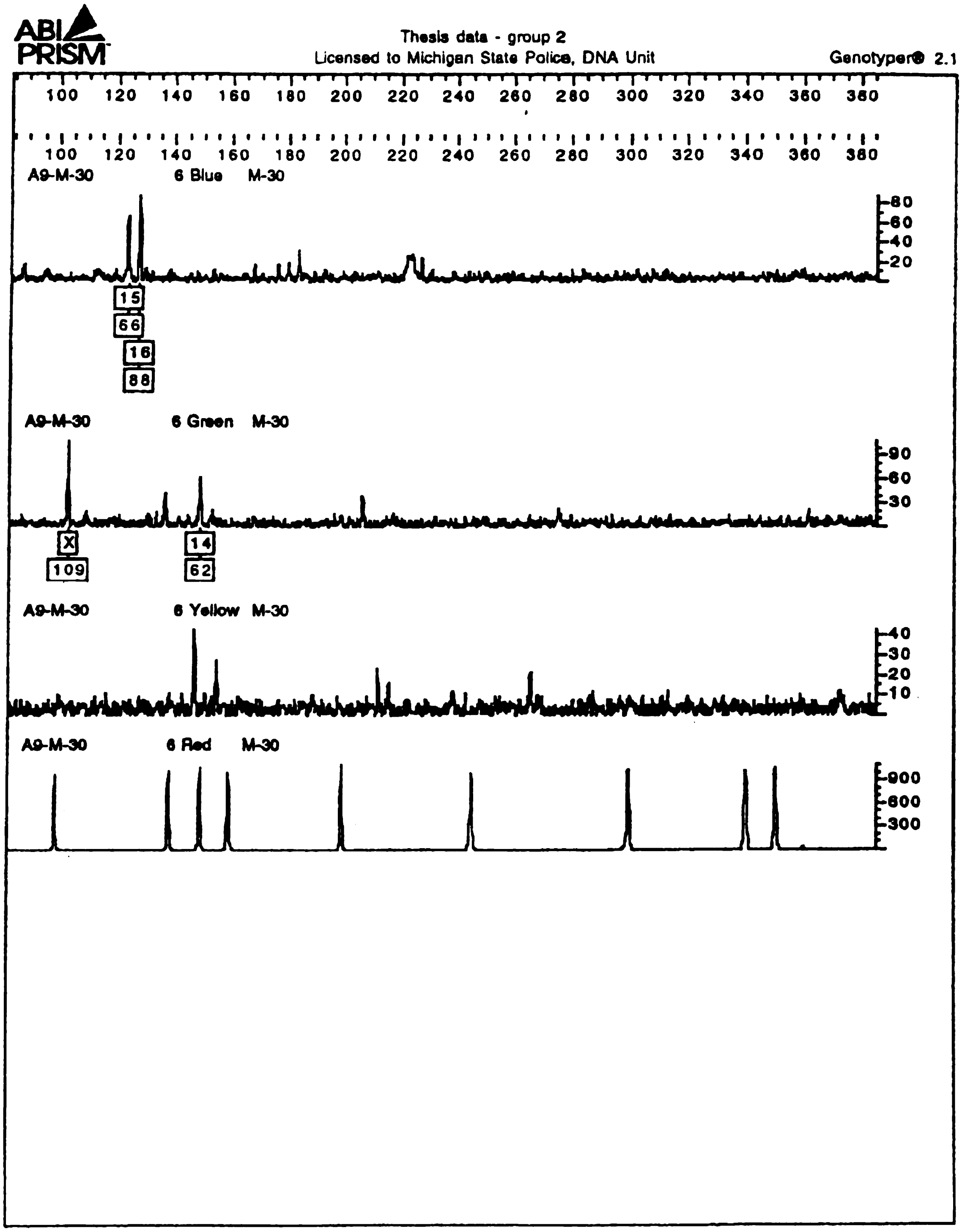
0 100 120 140 160 180 200 220 240 260 280 300 320 340
A7-M-5 5 Blue M-5
30
20
10
A7-M-5 5 Green M-5
60
40
20
X
76
A7-M-5 5 Yellow M-5
30
20
10
A7-M-5 5 Red M-5
800
600
400
200


ABI PRISM
Thesis data - group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
A7-M-15 5 Blue M-15
400 300 200 100
15
110
16
485
17
95
19
269
22
94
23
108
A7-M-15 5 Green M-15
800 600 400 200
X
939
11
211
14
244
30
449
12
54
13
89
A7-M-15 5 Yellow M-15
90 60 30
11
106
13
113
11
117
12
107
10
115
11
99
A7-M-15 5 Red M-15
900 600 300

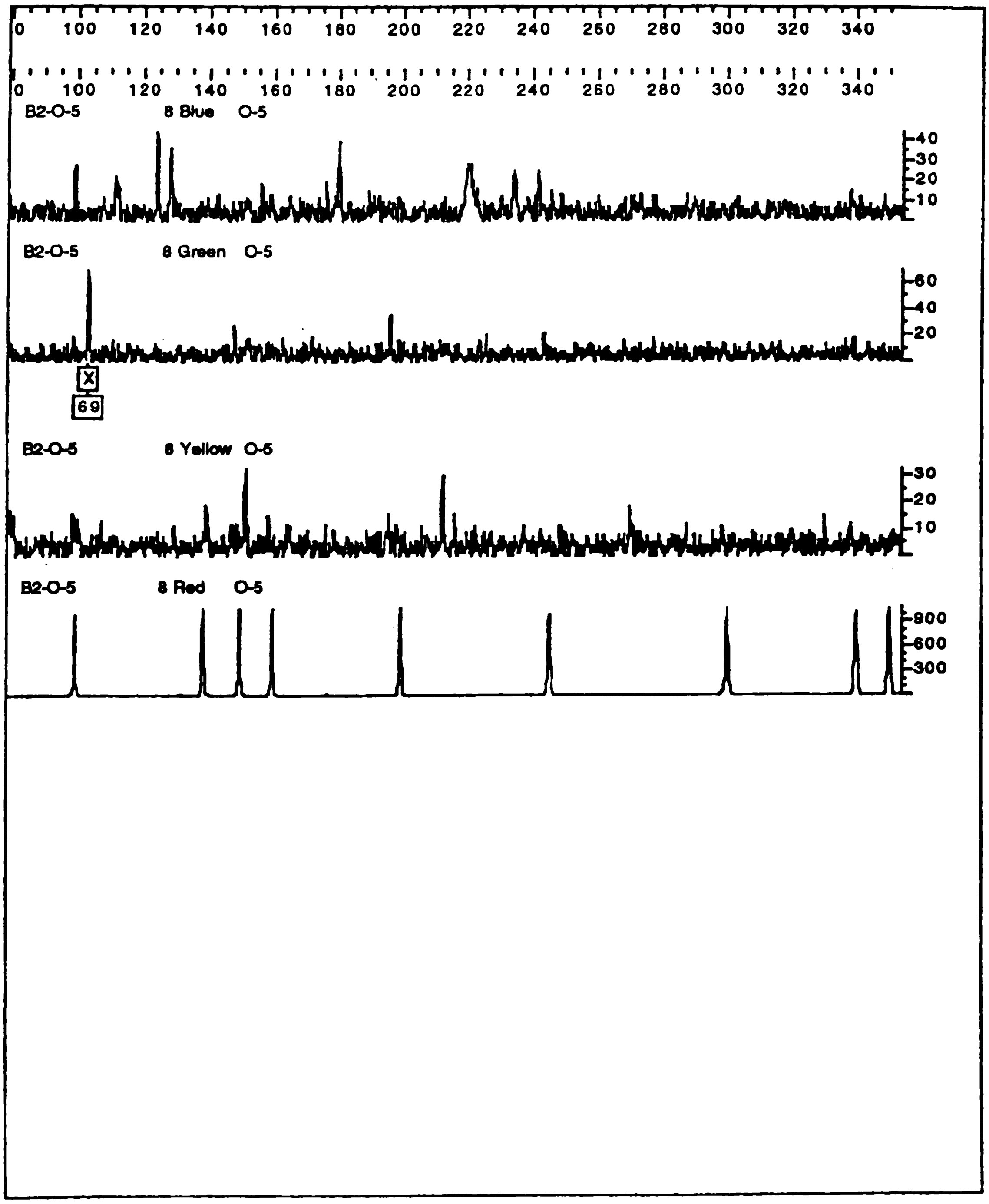


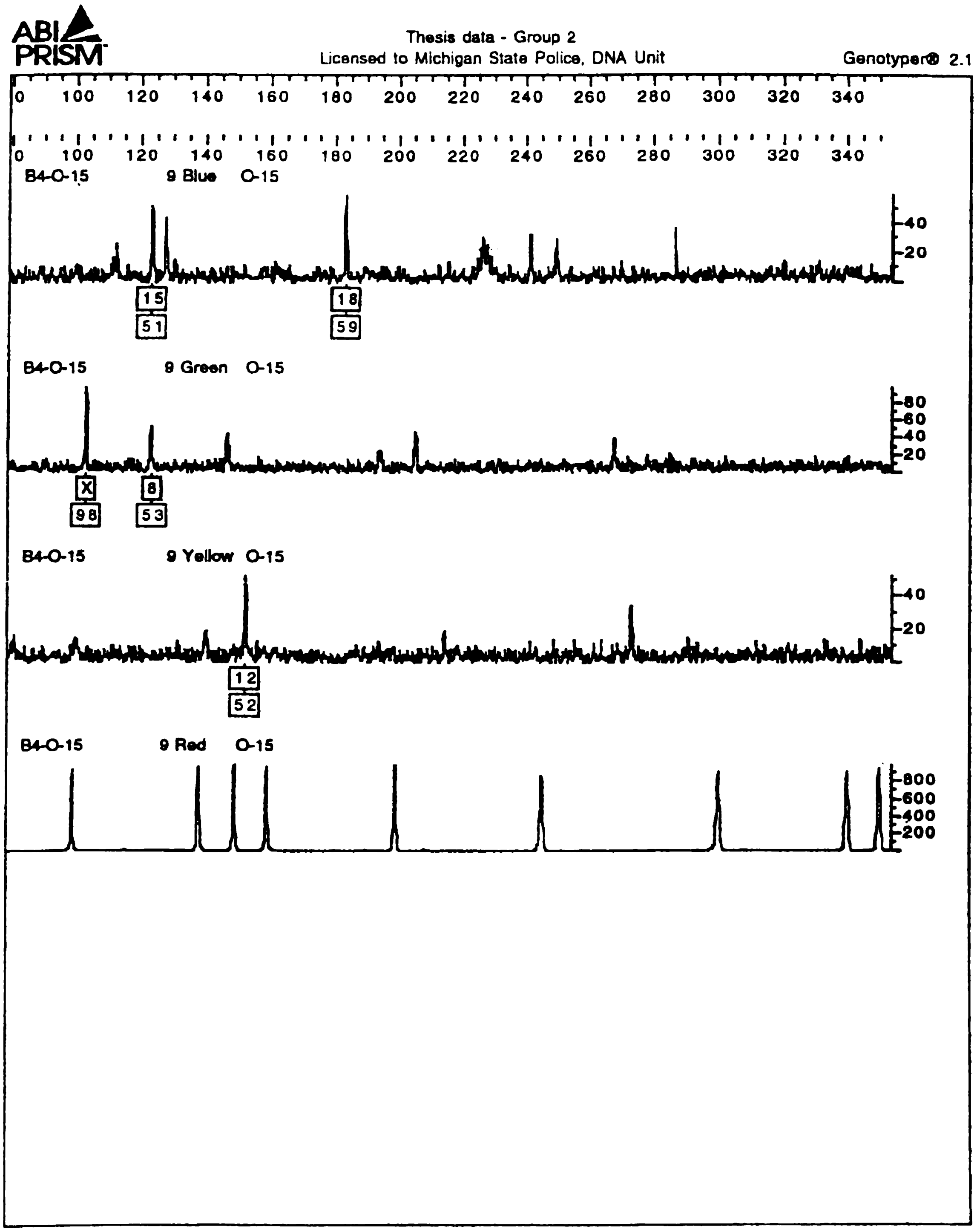
Thesis data - group 2

Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1

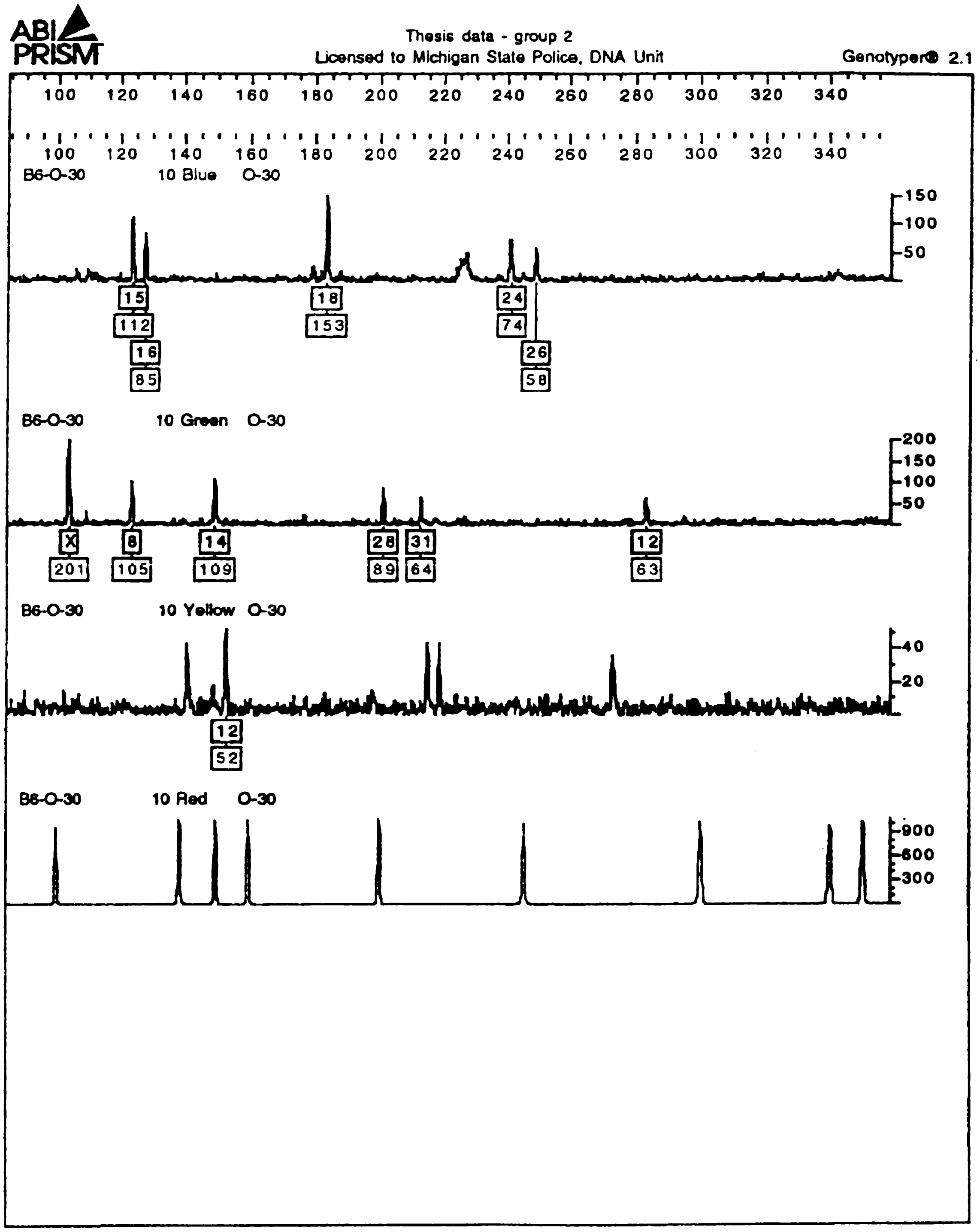
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1



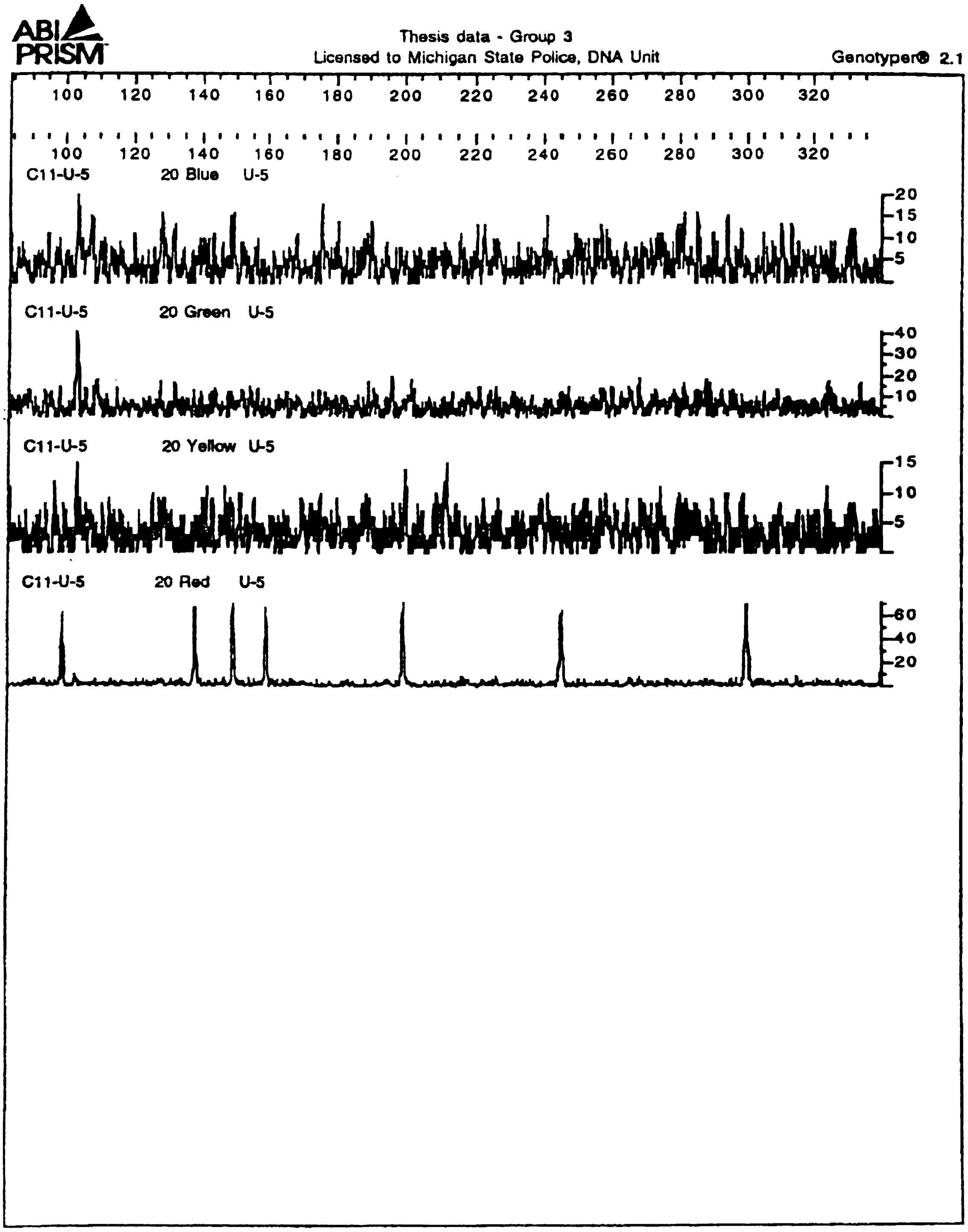


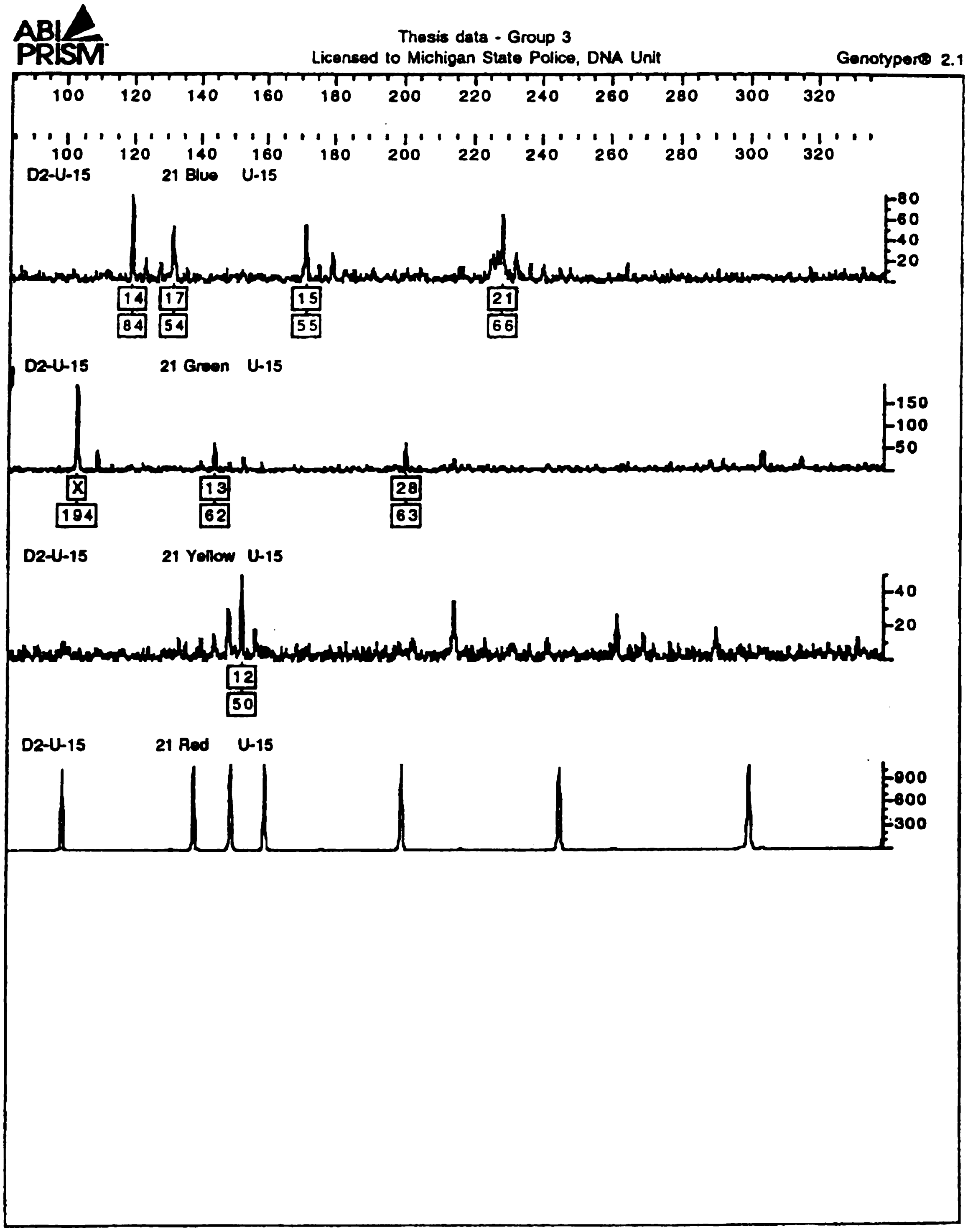
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit

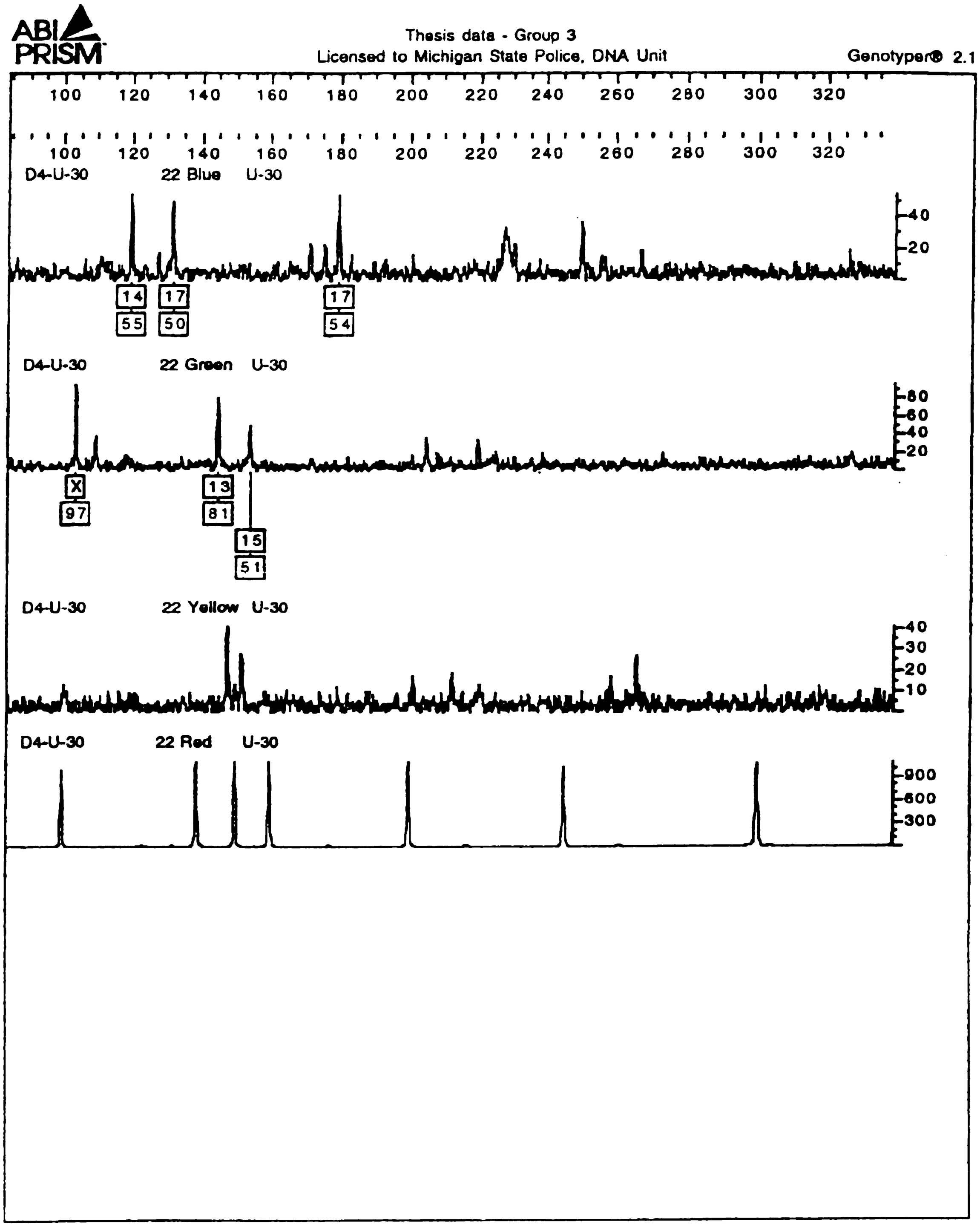
Genotyper® 2.1



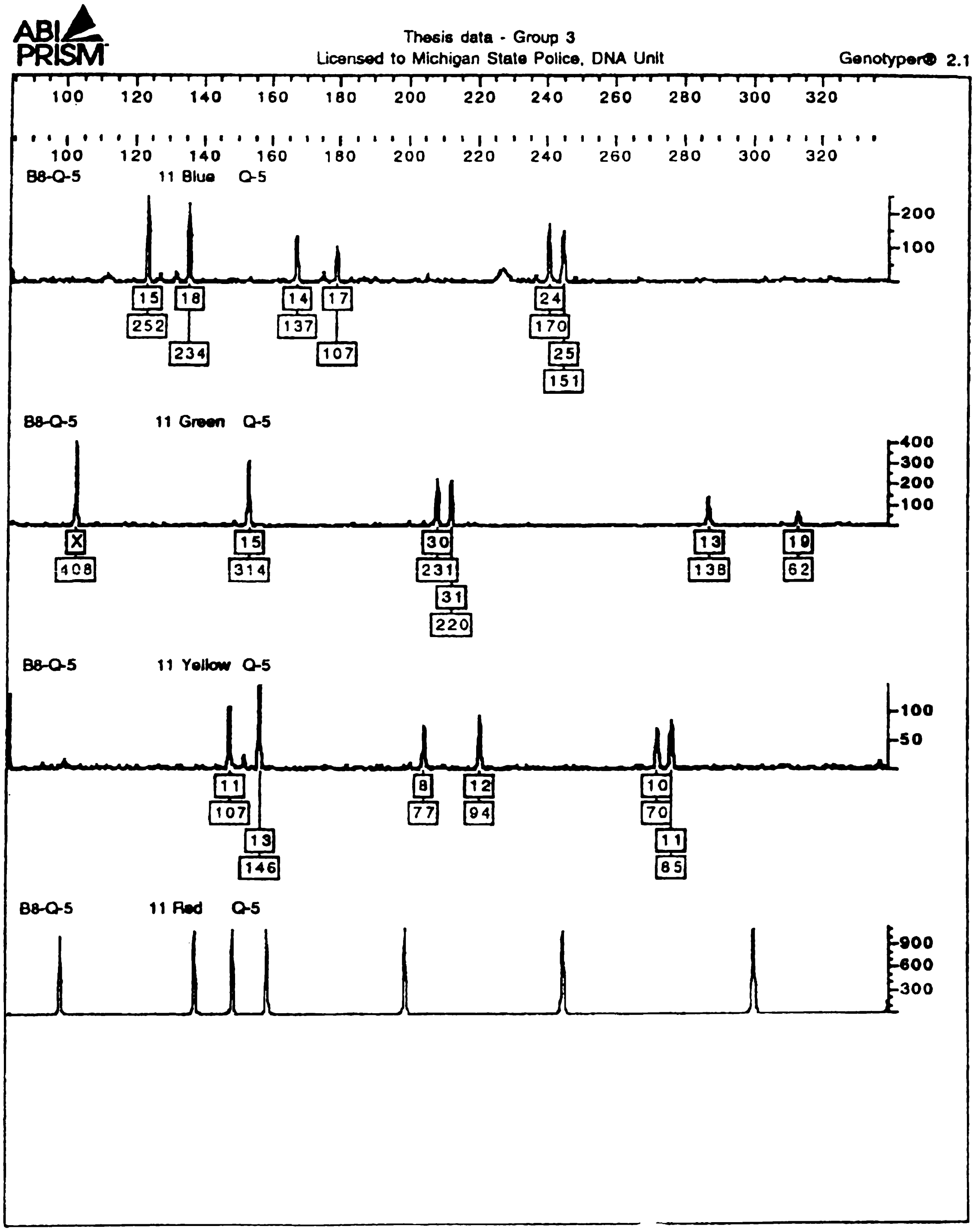
Thesis data - group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1


ABI PRISM
Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
C11-U-5 20 Blue U-5
20 15 10 5
C11-U-5 20 Green U-5
40 30 20 10
C11-U-5 20 Yellow U-5
15 10 5
C11-U-5 20 Red U-5
60 40 20

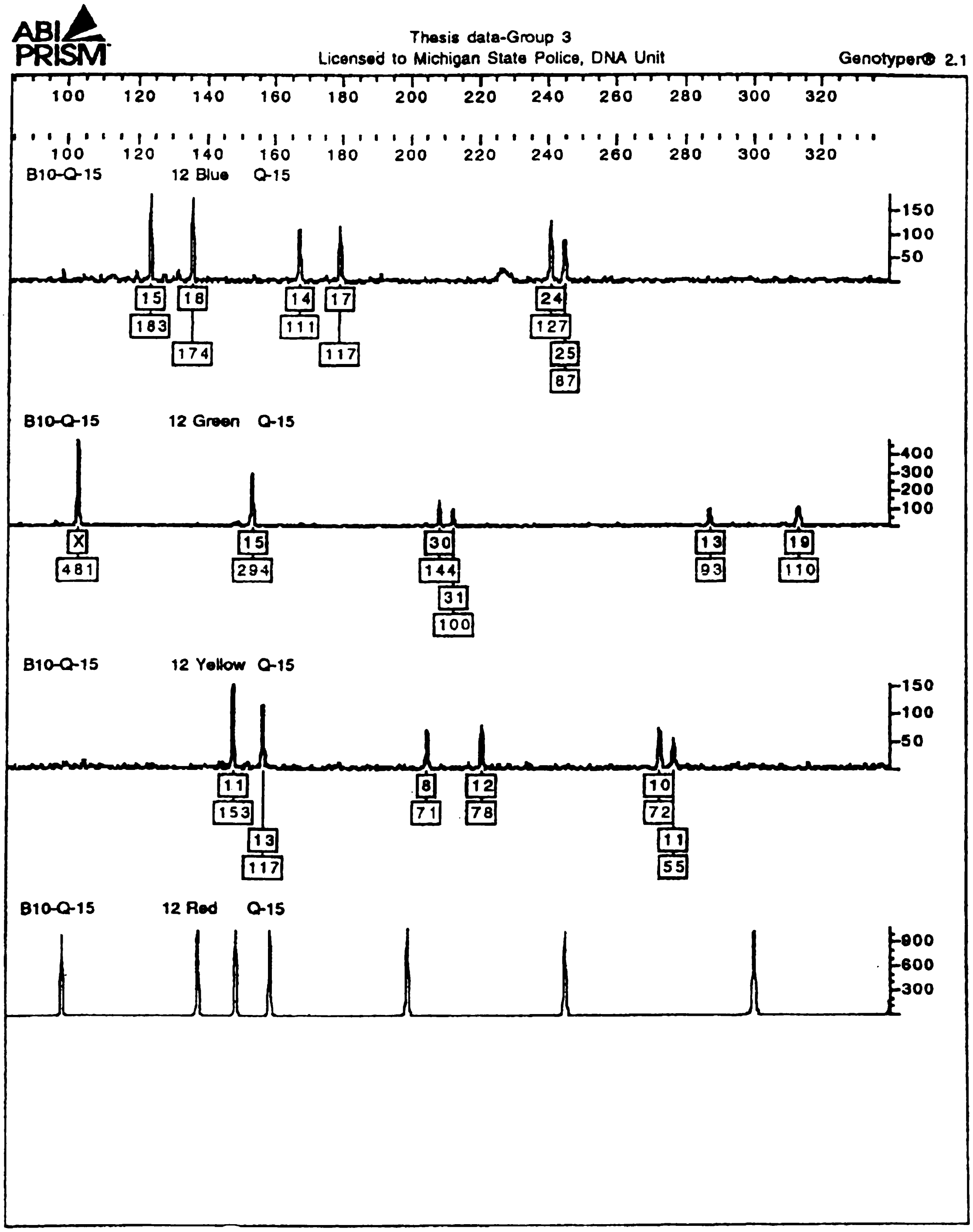

ABI PRISM
Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
D2-U-15 21 Blue U-15
80
60
40
20
14
84
17
54
15
55
21
66
D2-U-15 21 Green U-15
150
100
50
X
194
13
62
28
63
D2-U-15 21 Yellow U-15
40
20
12
50
D2-U-15 21 Red U-15
900
600
300

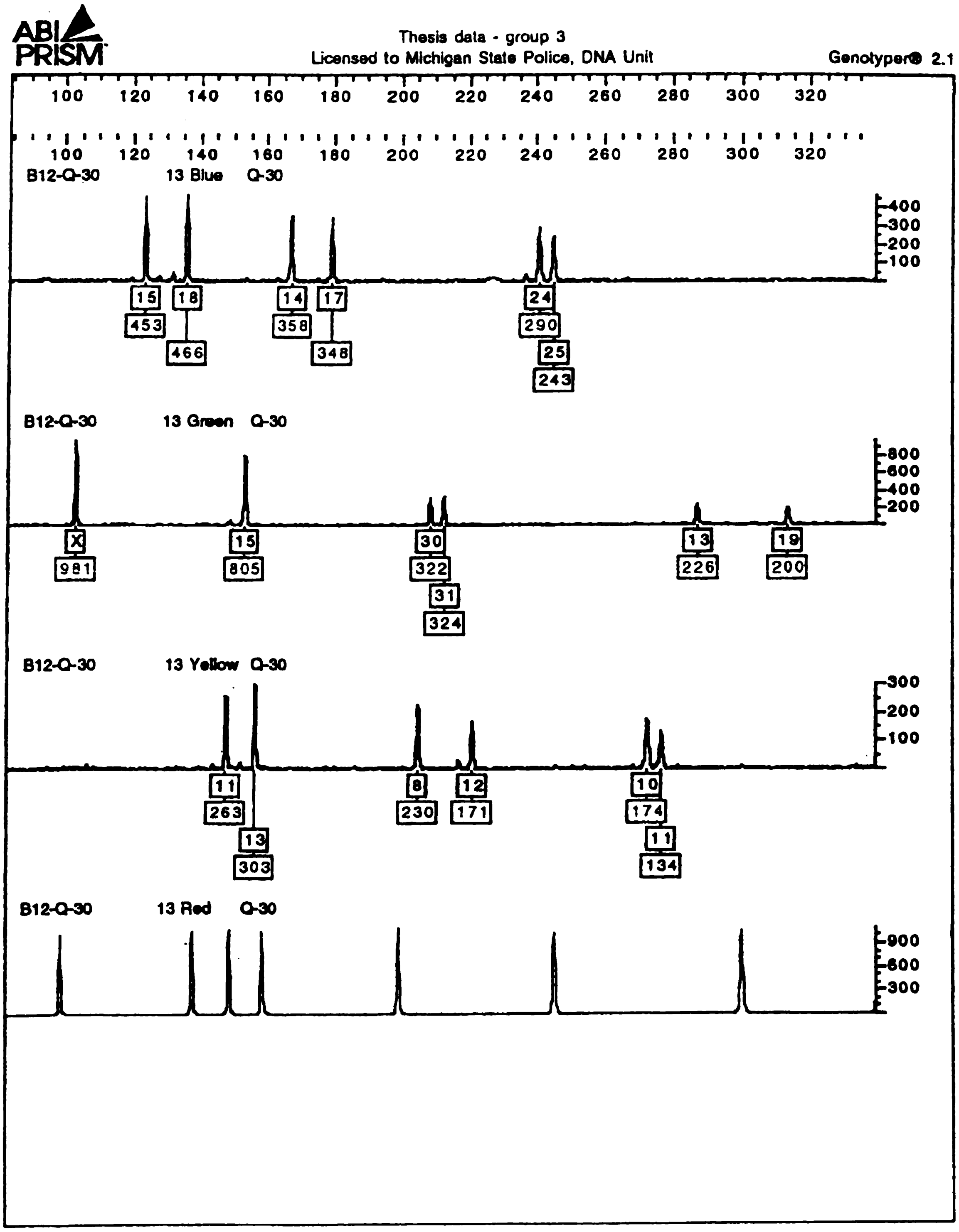


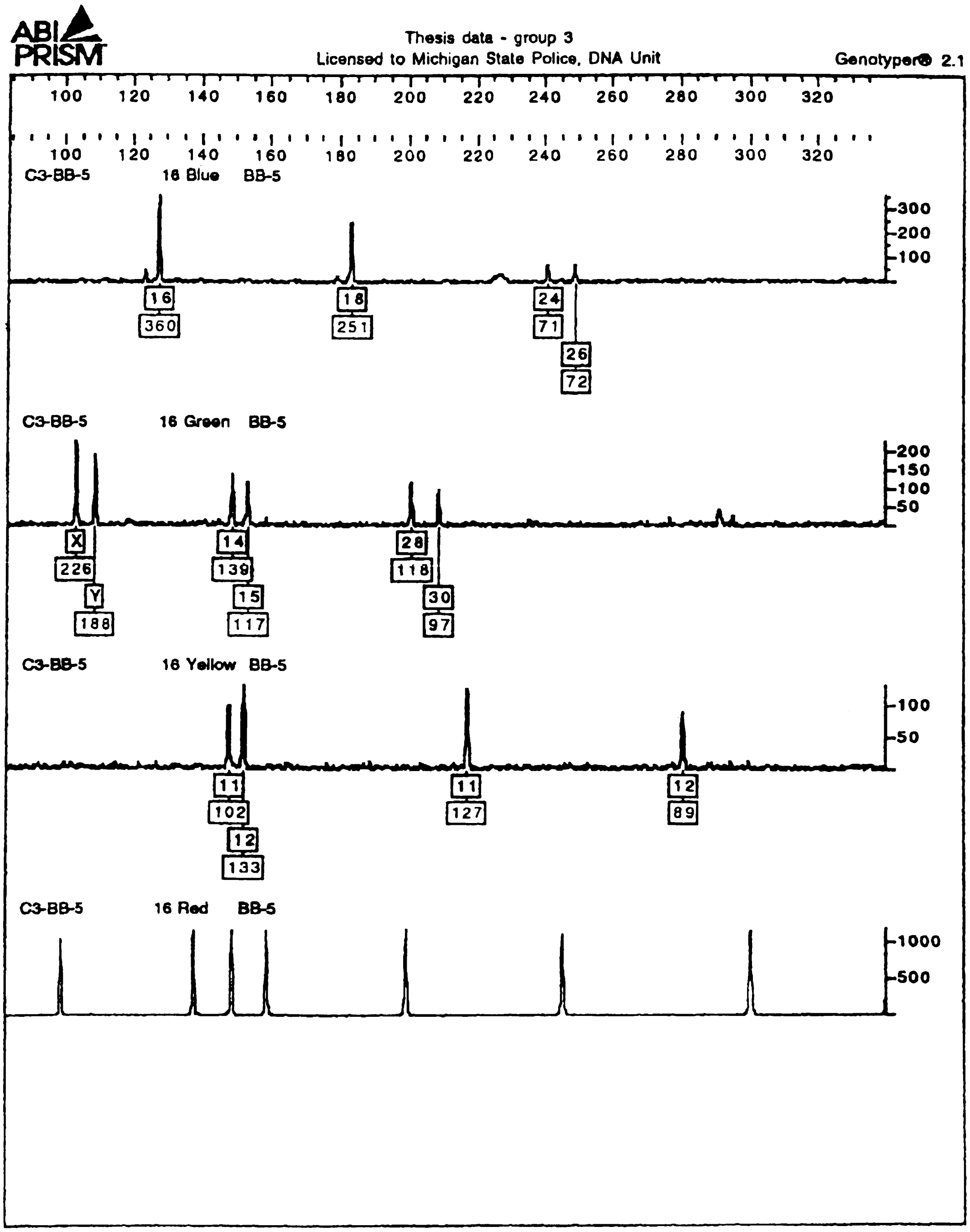
Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

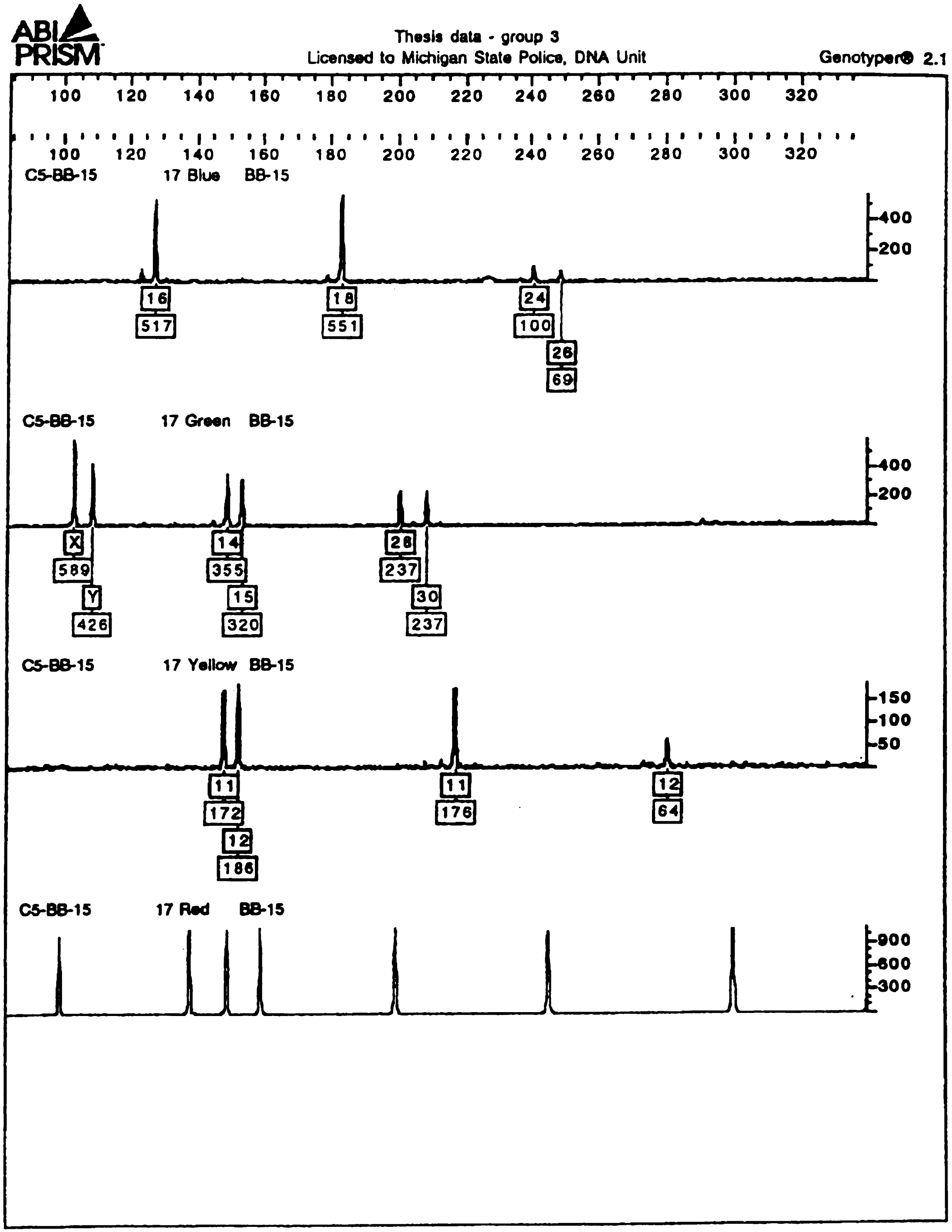


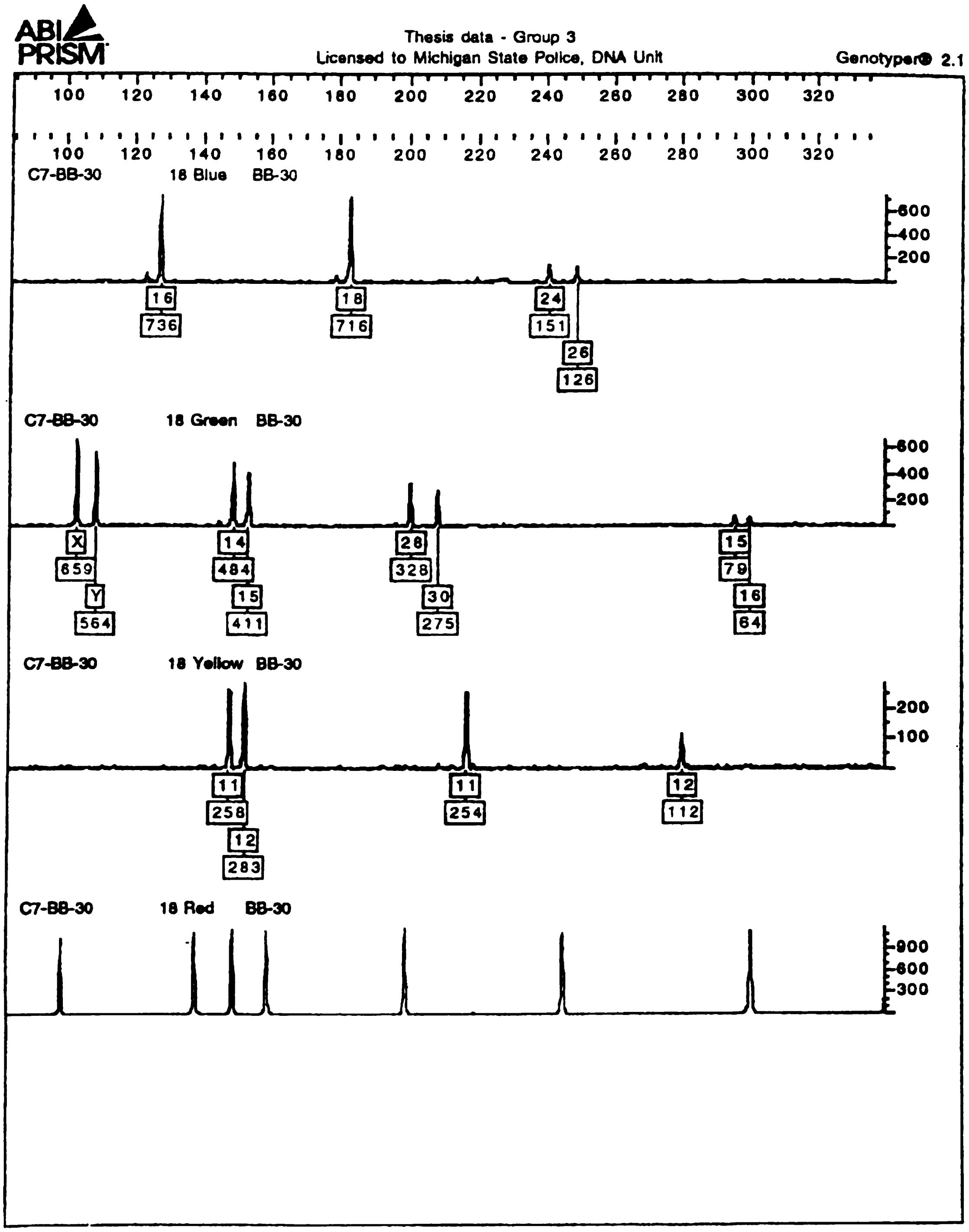
Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1


ABI PRISM
Thesis data-Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
B10-Q-15 12 Blue Q-15
150
100
50
15
183
16
174
14
111
17
117
24
127
25
87
B10-Q-15 12 Green Q-15
400
300
200
100
X
481
15
294
30
144
31
100
13
93
19
110
B10-Q-15 12 Yellow Q-15
150
100
50
11
153
13
117
8
71
12
78
10
72
11
55
B10-Q-15 12 Red Q-15
900
600
300


ABI PRISM
Thesis data - group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
B12-Q-30 13 Blue Q-30
400
300
200
100
15
453
18
466
14
358
17
348
24
290
25
243
B12-Q-30 13 Green Q-30
800
600
400
200
X
981
15
805
30
322
31
324
13
226
19
200
B12-Q-30 13 Yellow Q-30
300
200
100
11
263
13
303
8
230
12
171
10
174
11
134
B12-Q-30 13 Red Q-30
900
600
300


ABI PRISM
Thesis data - group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
C3-BB-5 16 Blue BB-5
300
200
100
16
360
18
251
24
71
26
72
C3-BB-5 16 Green BB-5
200
150
100
50
X
226
Y
188
14
139
15
117
28
118
30
97
C3-BB-5 16 Yellow BB-5
100
50
11
102
12
133
11
127
12
89
C3-BB-5 16 Red BB-5
1000
500

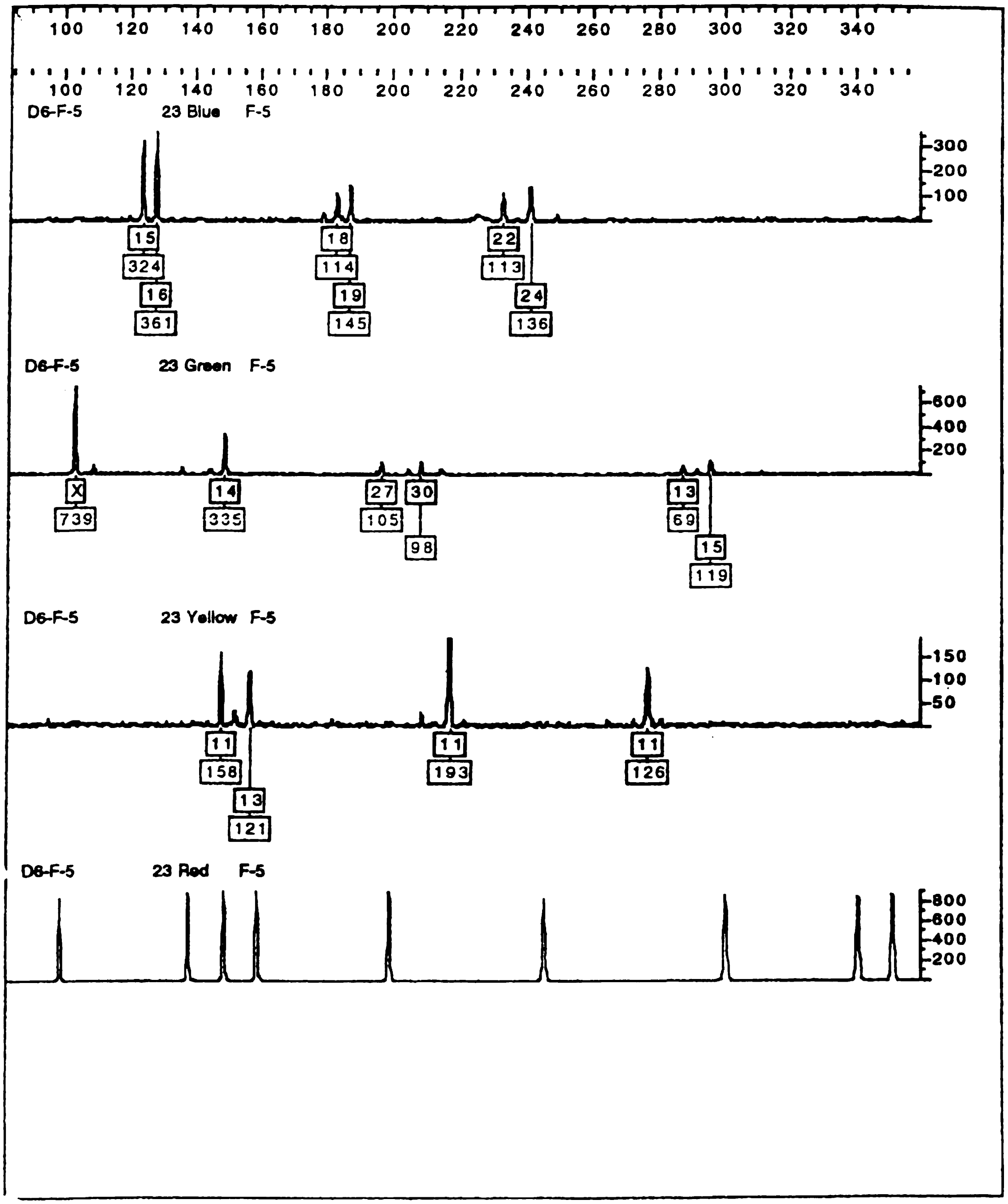

ABI PRISM
Thesis data - group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
C5-BB-15 17 Blue BB-15
400
200
16
517
18
551
24
100
26
69
C5-BB-15 17 Green BB-15
400
200
X
589
Y
426
14
355
15
320
28
237
30
237
C5-BB-15 17 Yellow BB-15
150
100
50
11
172
12
186
11
176
12
64
C5-BB-15 17 Red BB-15
900
600
300

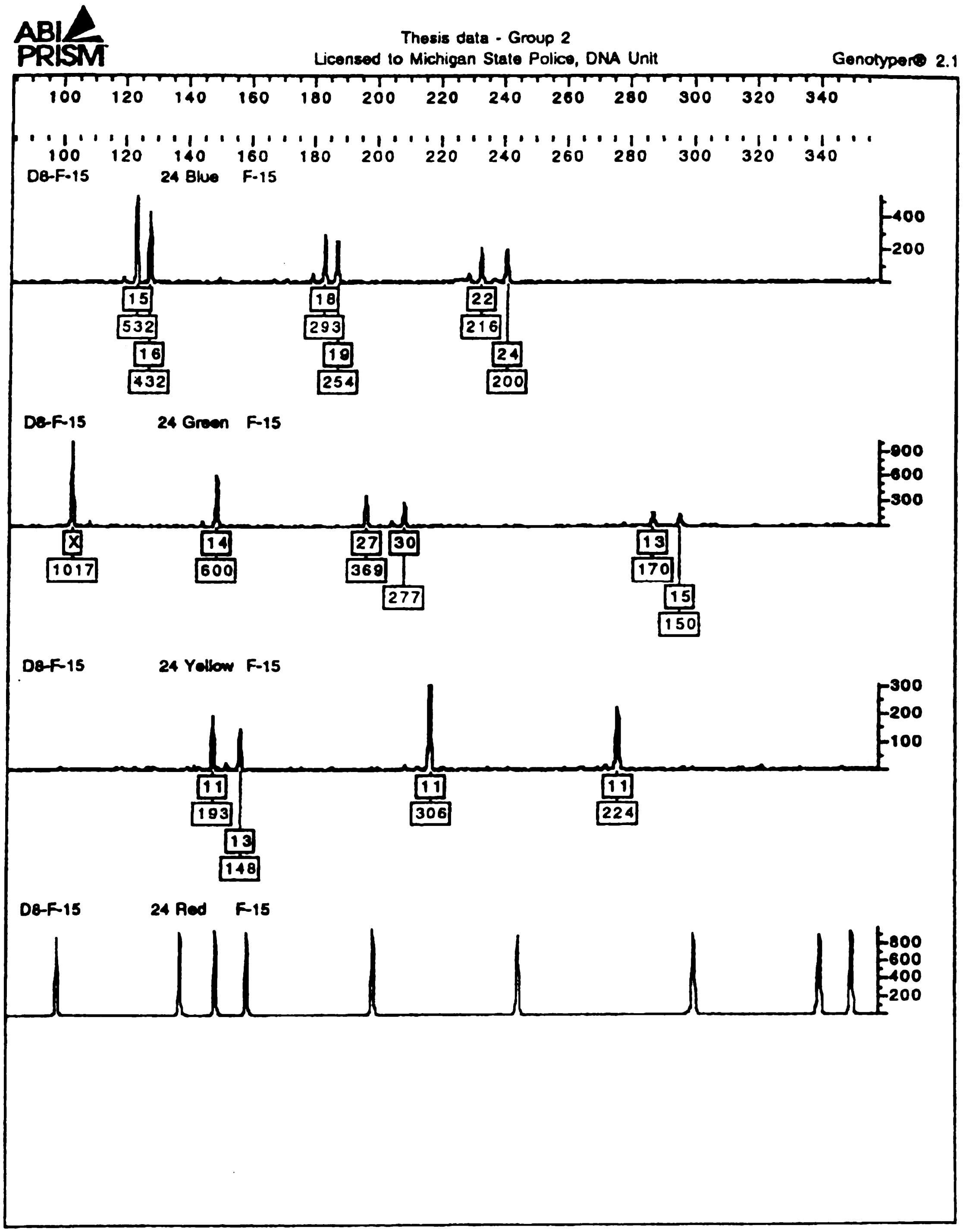

ABI PRISM
Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
C7-BB-30 18 Blue BB-30
600
400
200
16
736
18
716
24
151
26
126
C7-BB-30 18 Green BB-30
600
400
200
X
659
Y
564
14
484
15
411
28
328
30
275
15
79
16
64
C7-BB-30 18 Yellow BB-30
200
100
11
258
12
283
11
254
12
112
C7-BB-30 18 Red BB-30
900
600
300

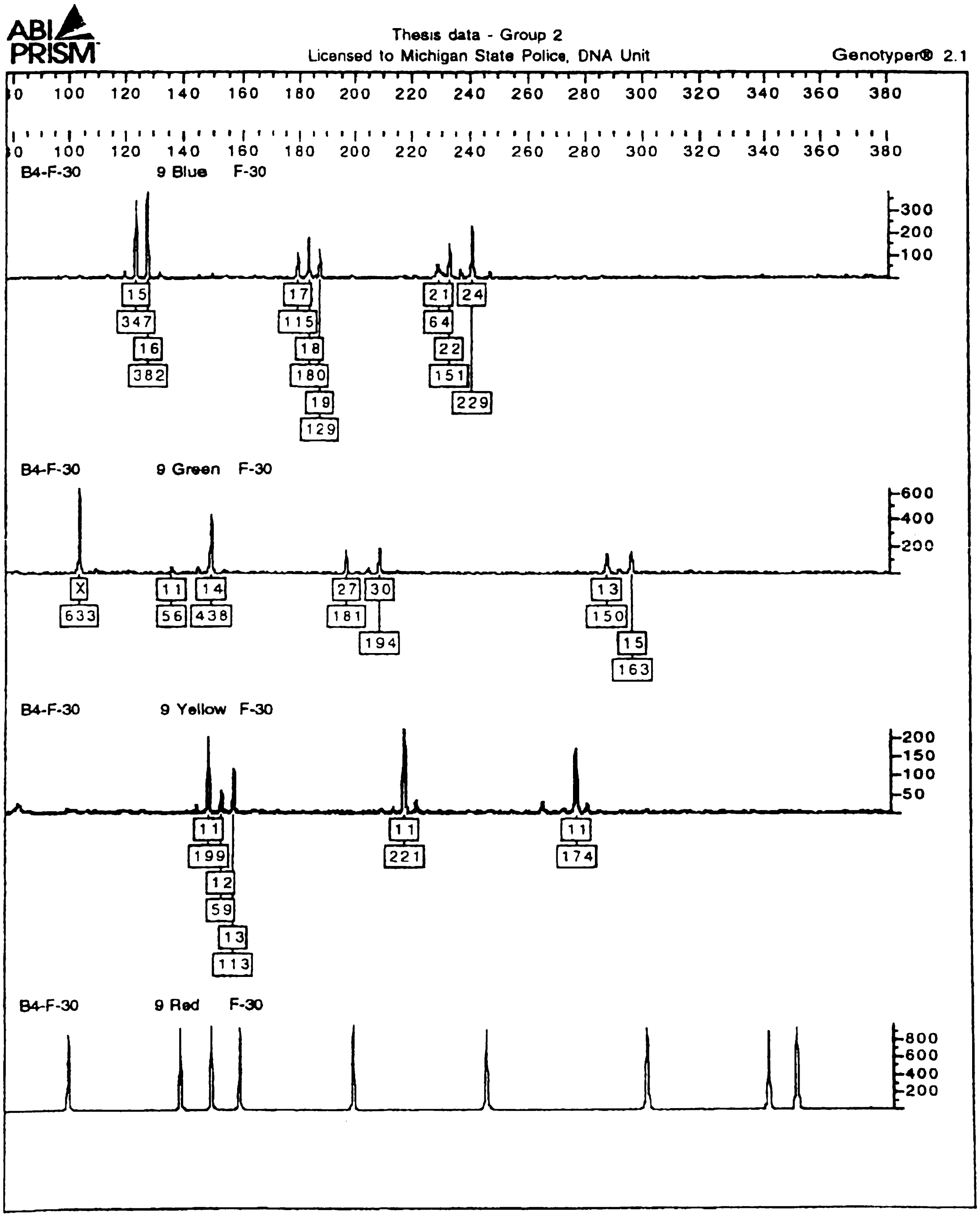
ABI PRISM

Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1



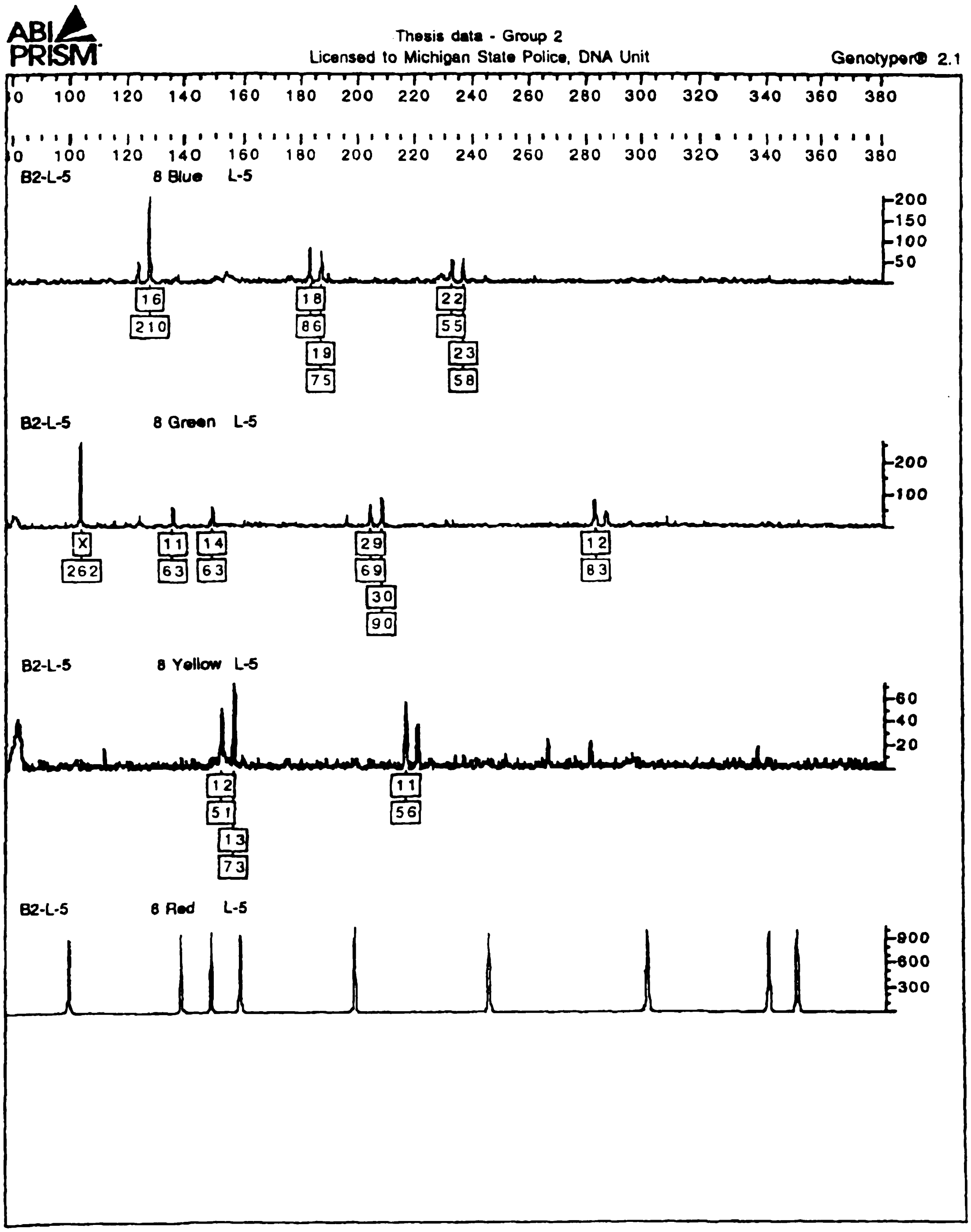

ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
100 120 140 160 180 200 220 240 260 280 300 320 340
D8-F-15 24 Blue F-15
400
200
15
532
16
432
18
293
19
254
22
216
24
200
D8-F-15 24 Green F-15
900
600
300
X
1017
14
600
27
369
30
277
13
170
15
150
D8-F-15 24 Yellow F-15
300
200
100
11
193
13
148
11
306
11
224
D8-F-15 24 Red F-15
800
600
400
200

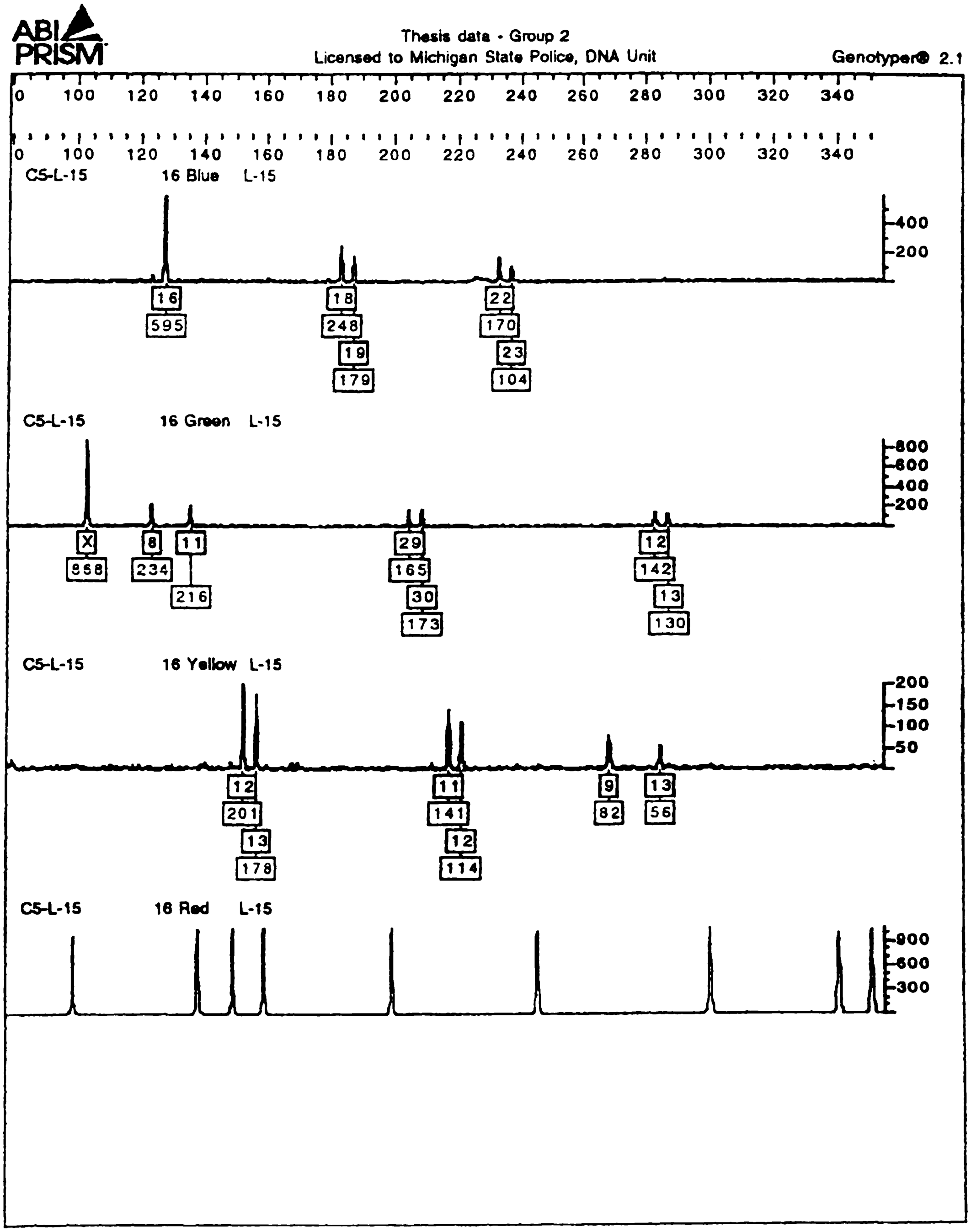


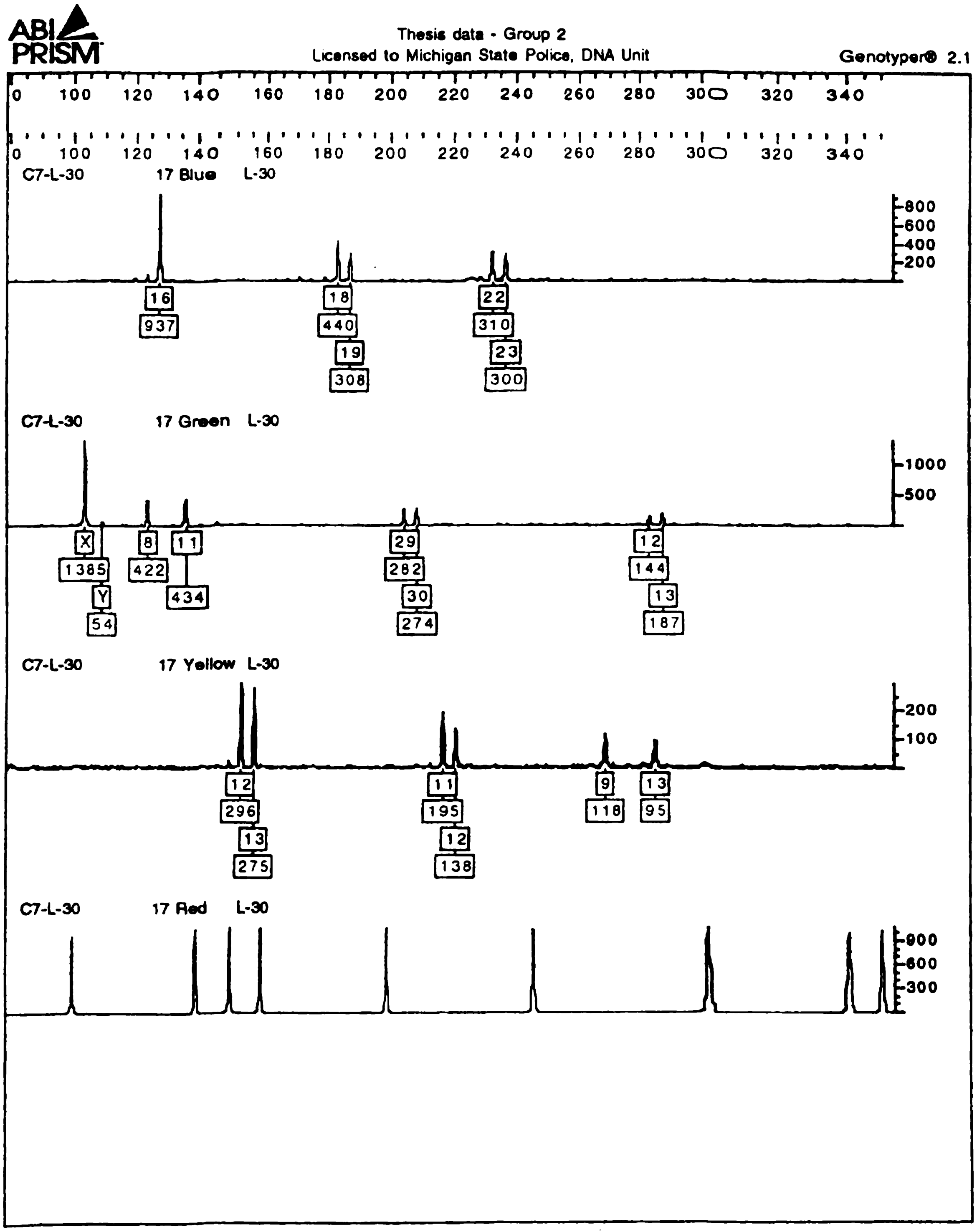
Thesis data - Group 2

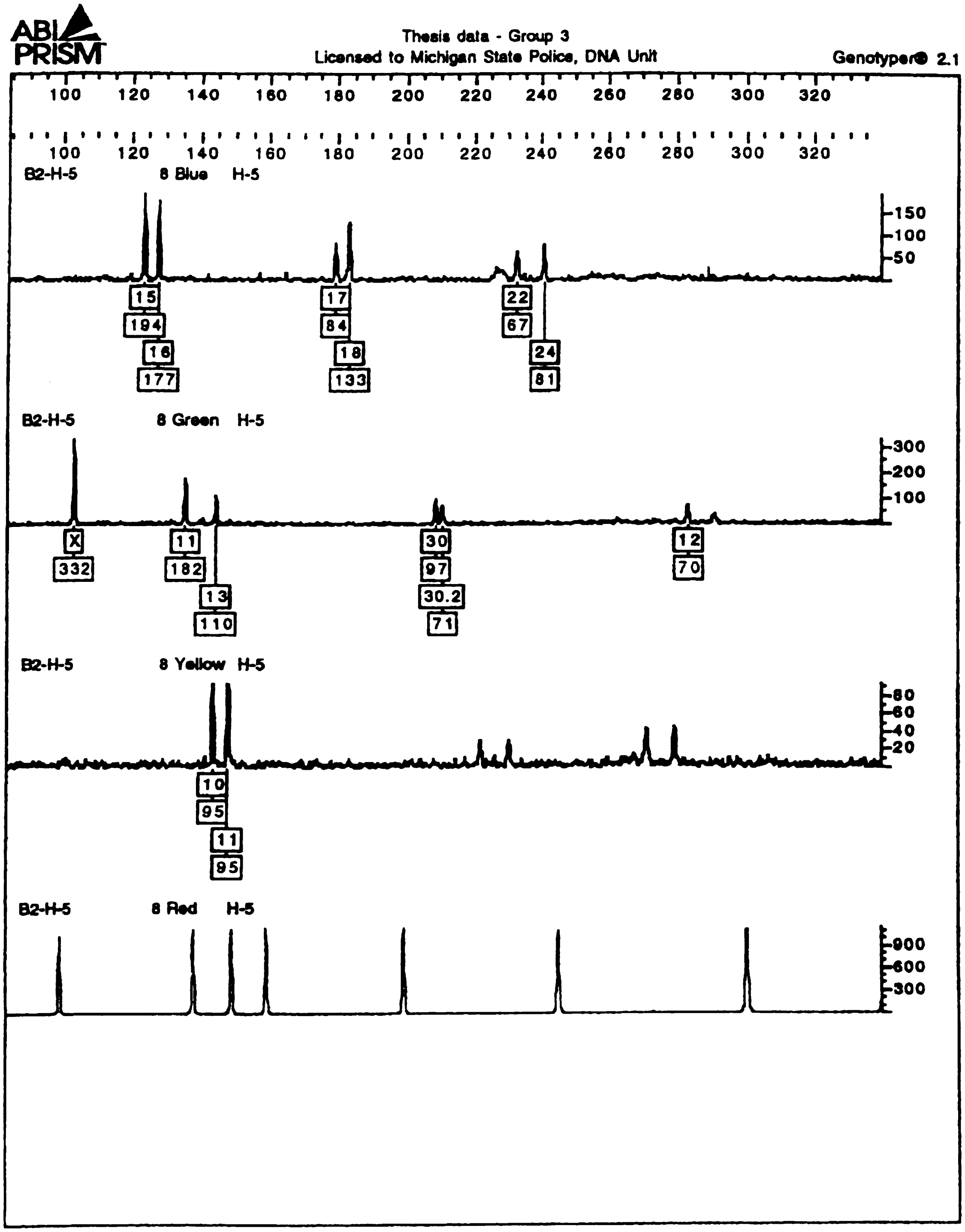
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1


ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
0 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
B2-L-5 8 Blue L-5
200
150
100
50
16
210
18
86
19
75
22
55
23
58
B2-L-5 8 Green L-5
200
100
X
262
11
63
14
63
29
69
30
90
12
83
B2-L-5 8 Yellow L-5
60
40
20
12
51
13
73
11
56
B2-L-5 8 Red L-5
900
600
300


ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340
C5-L-15 16 Blue L-15
400
200
16
595
18
248
19
179
22
170
23
104
C5-L-15 16 Green L-15
800
600
400
200
X
858
8
234
11
216
29
165
30
173
12
142
13
130
C5-L-15 16 Yellow L-15
200
150
100
50
12
201
13
178
11
141
12
114
9
82
13
56
C5-L-15 16 Red L-15
900
600
300

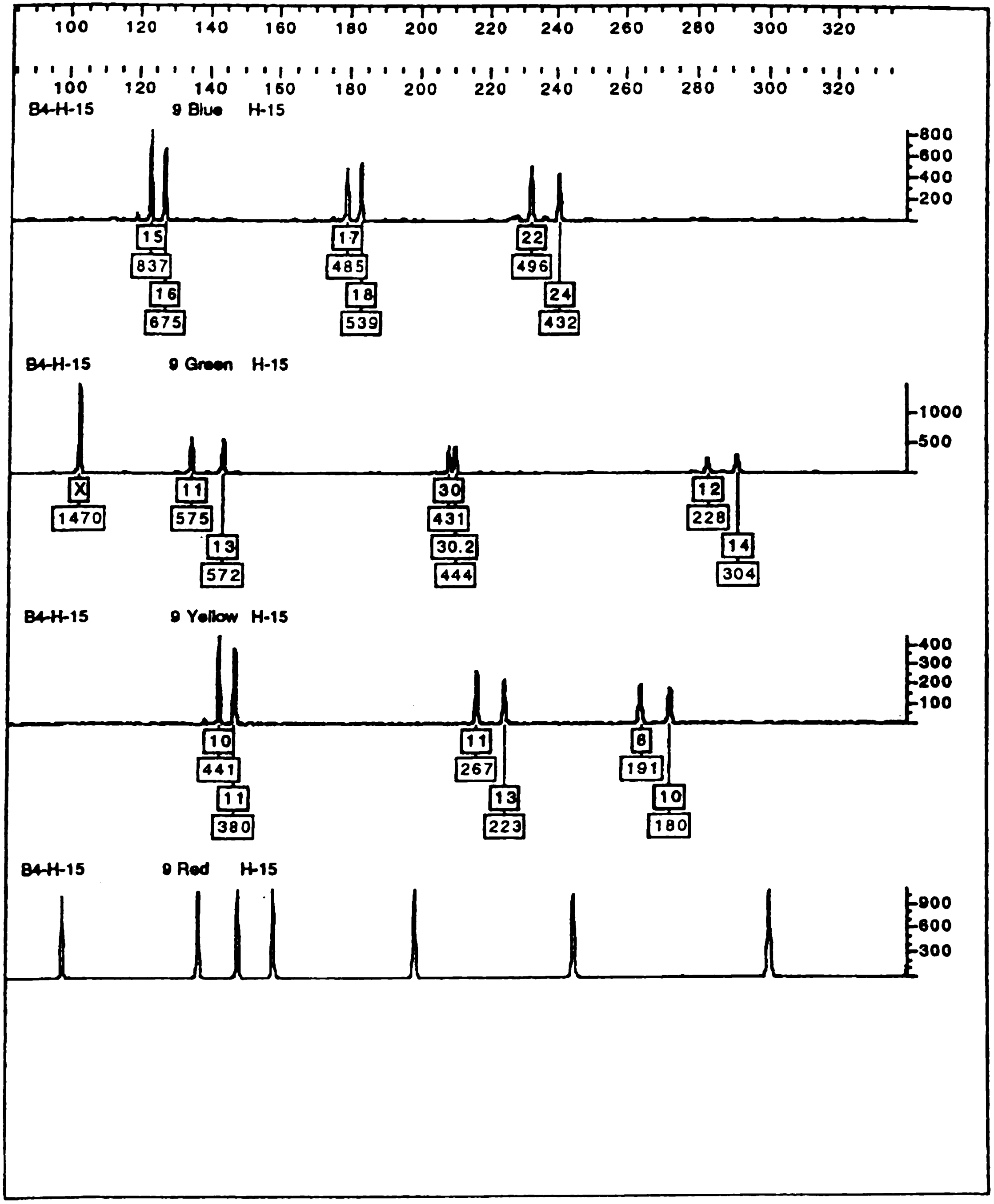

ABI PRISM
Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340
C7-L-30 17 Blue L-30
800
600
400
200
16
937
18
440
19
308
22
310
23
300
C7-L-30 17 Green L-30
1000
500
X
1385
Y
54
8
422
11
434
29
282
30
274
12
144
13
187
C7-L-30 17 Yellow L-30
200
100
12
296
13
275
11
195
12
138
9
118
13
95
C7-L-30 17 Red L-30
900
600
300

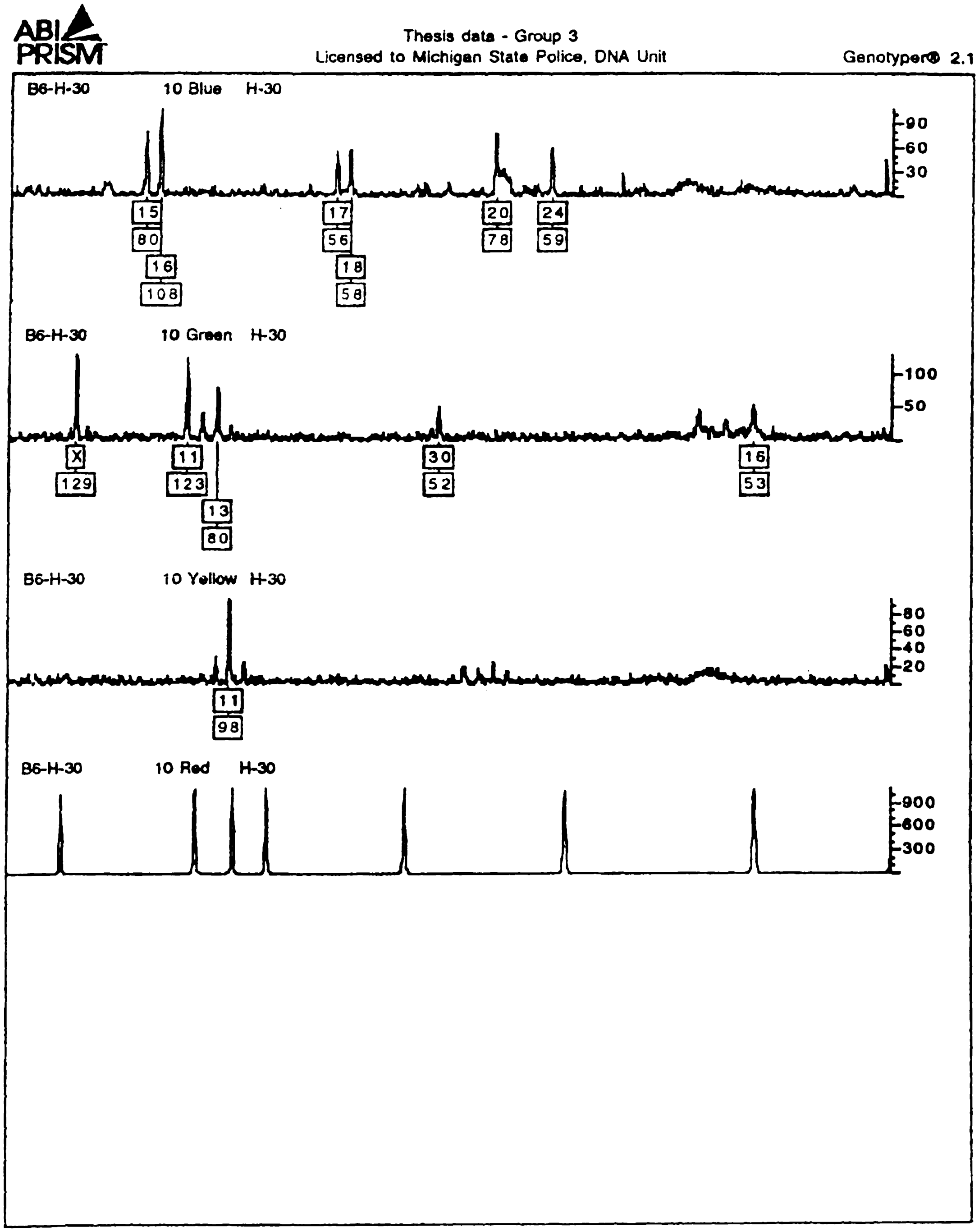

ABI PRISM
Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320
100 120 140 160 180 200 220 240 260 280 300 320
B2-H-5 8 Blue H-5
150
100
50
15
194
16
177
17
84
18
133
22
67
24
81
B2-H-5 8 Green H-5
300
200
100
X
332
11
182
13
110
30
97
30.2
71
12
70
B2-H-5 8 Yellow H-5
80
60
40
20
10
95
11
95
B2-H-5 8 Red H-5
900
600
300

ABI PRISM

Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit

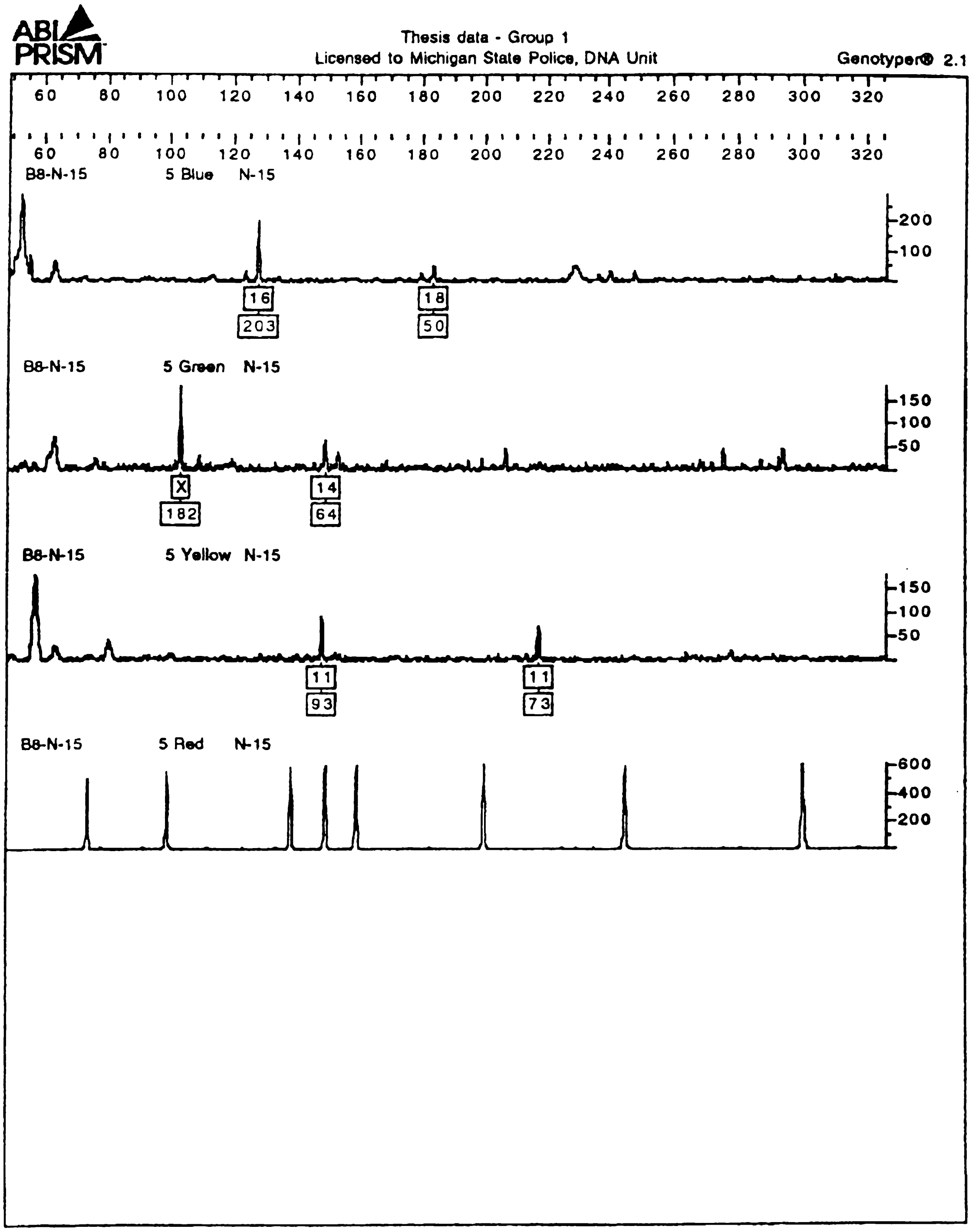
Genotyper® 2.1

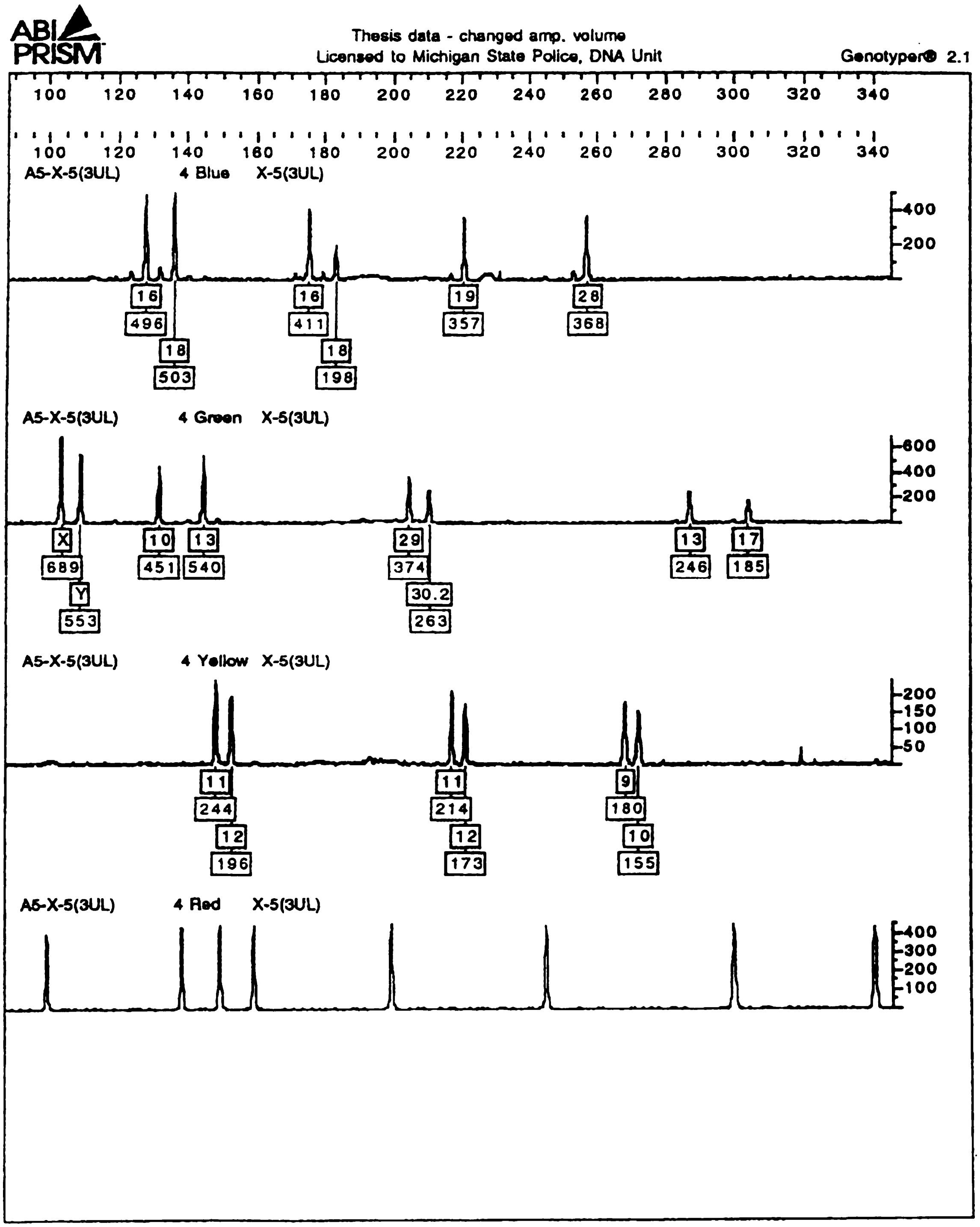


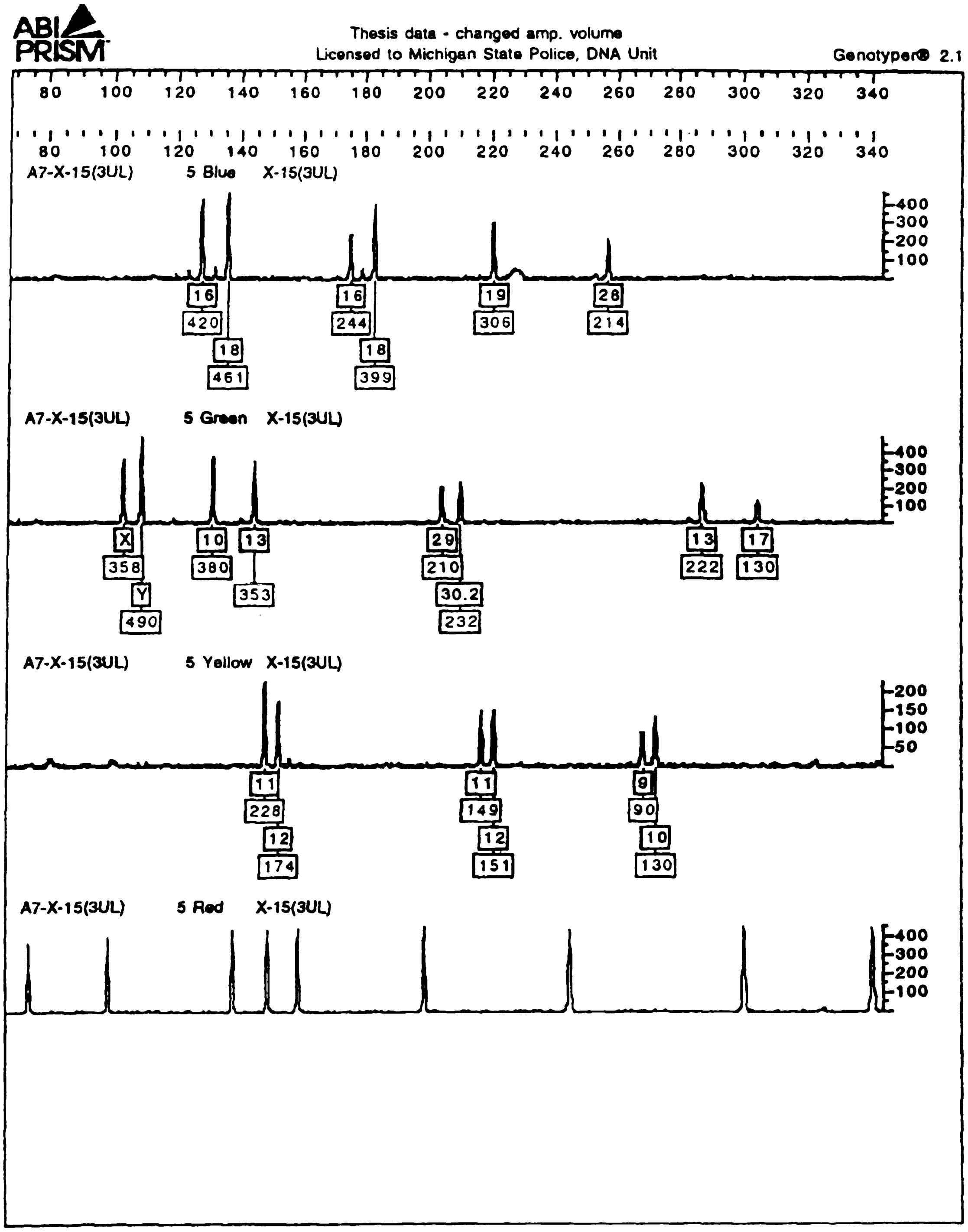

ABI PRISM
Thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
B6-H-30
10 Blue
H-30
90
60
30
15
80
16
108
17
56
18
58
20
78
24
59
B6-H-30
10 Green
H-30
100
50
X
129
11
123
13
80
30
52
16
53
B6-H-30
10 Yellow
H-30
80
60
40
20
11
98
B6-H-30
10 Red
H-30
900
600
300

APPENDIX D

Electropherograms generated from samples containing 3ul of PCR product.


ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
60 80 100 120 140 160 180 200 220 240 260 280 300 320
60 80 100 120 140 160 180 200 220 240 260 280 300 320
B8-N-15 5 Blue N-15
200
100
16
203
18
50
B8-N-15 5 Green N-15
150
100
50
X
182
14
64
B8-N-15 5 Yellow N-15
150
100
50
11
93
11
73
B8-N-15 5 Red N-15
600
400
200


ABI PRISM
Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
100 120 140 160 180 200 220 240 260 280 300 320 340
A5-X-5(3UL) 4 Blue X-5(3UL)
400
200
16
496
18
503
16
411
18
198
19
357
28
368
A5-X-5(3UL) 4 Green X-5(3UL)
600
400
200
X
689
Y
553
10
451
13
540
29
374
30.2
263
13
246
17
185
A5-X-5(3UL) 4 Yellow X-5(3UL)
200
150
100
50
11
244
12
196
11
214
12
173
9
180
10
155
A5-X-5(3UL) 4 Red X-5(3UL)
400
300
200
100


Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1

A7-X-15(3UL) 5 Blue X-15(3UL)

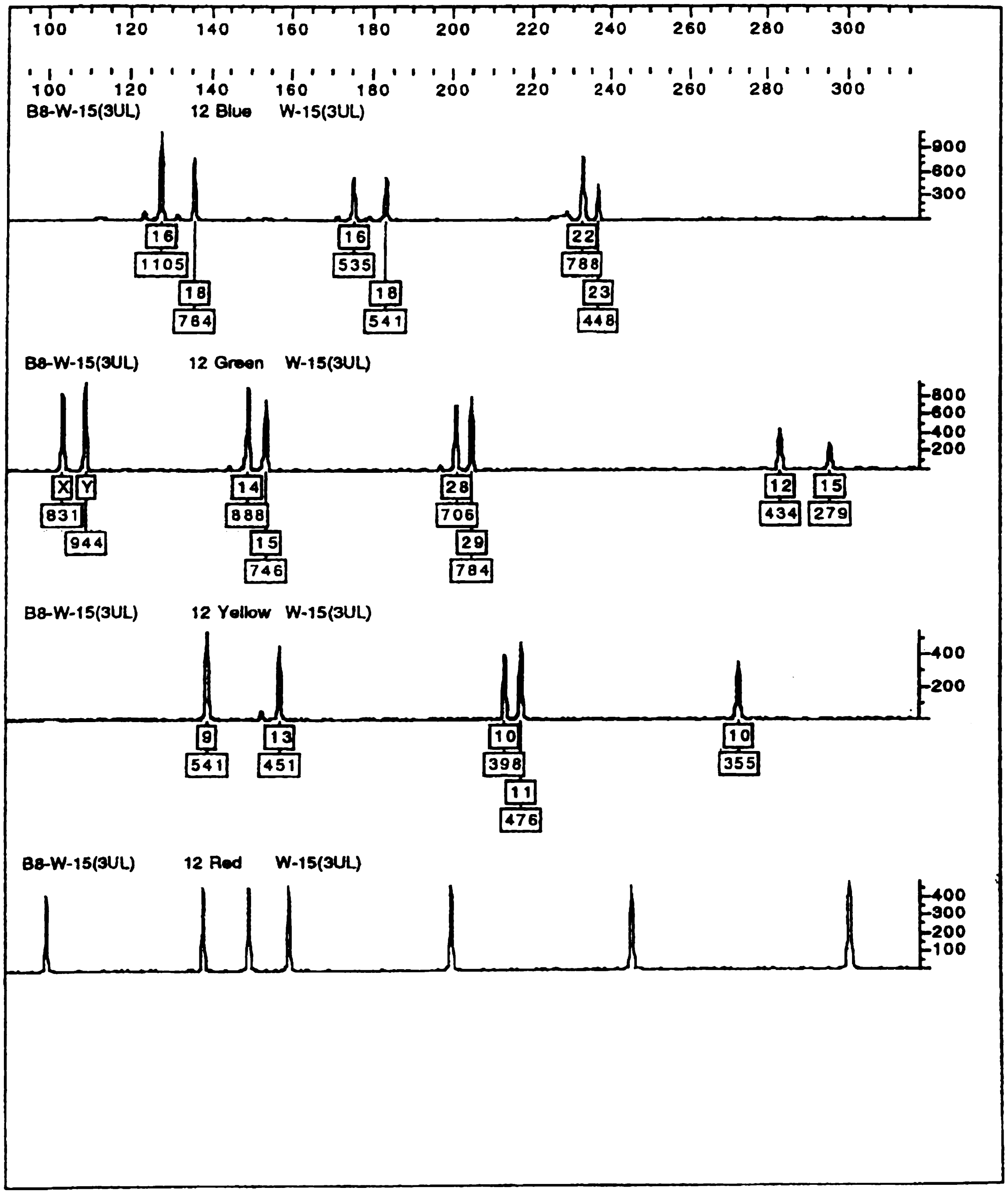
A7-X-15(3UL) 5 Green X-15(3UL)

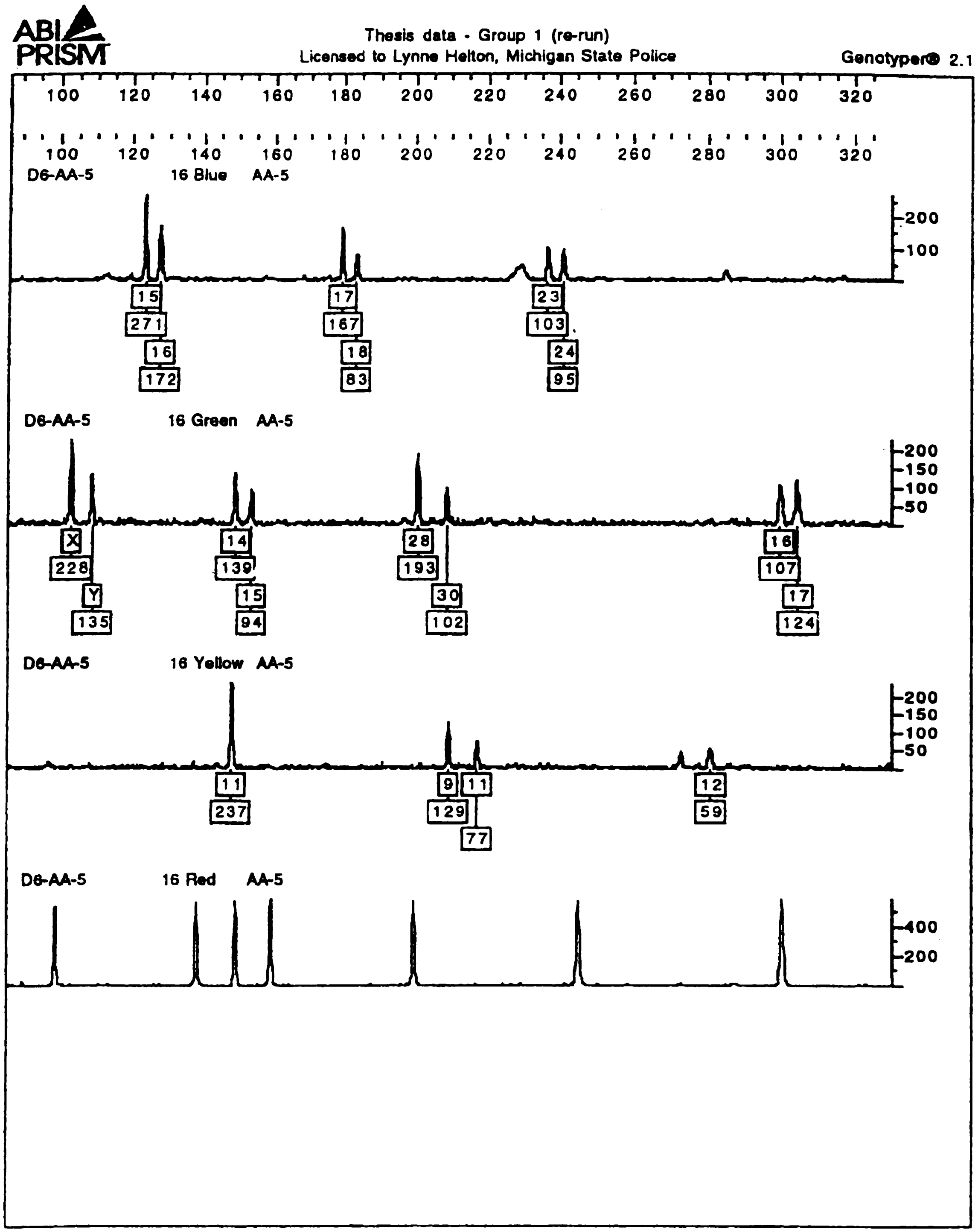
A7-X-15(3UL) 5 Yellow X-15(3UL)

A7-X-15(3UL) 5 Red X-15(3UL)

Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1

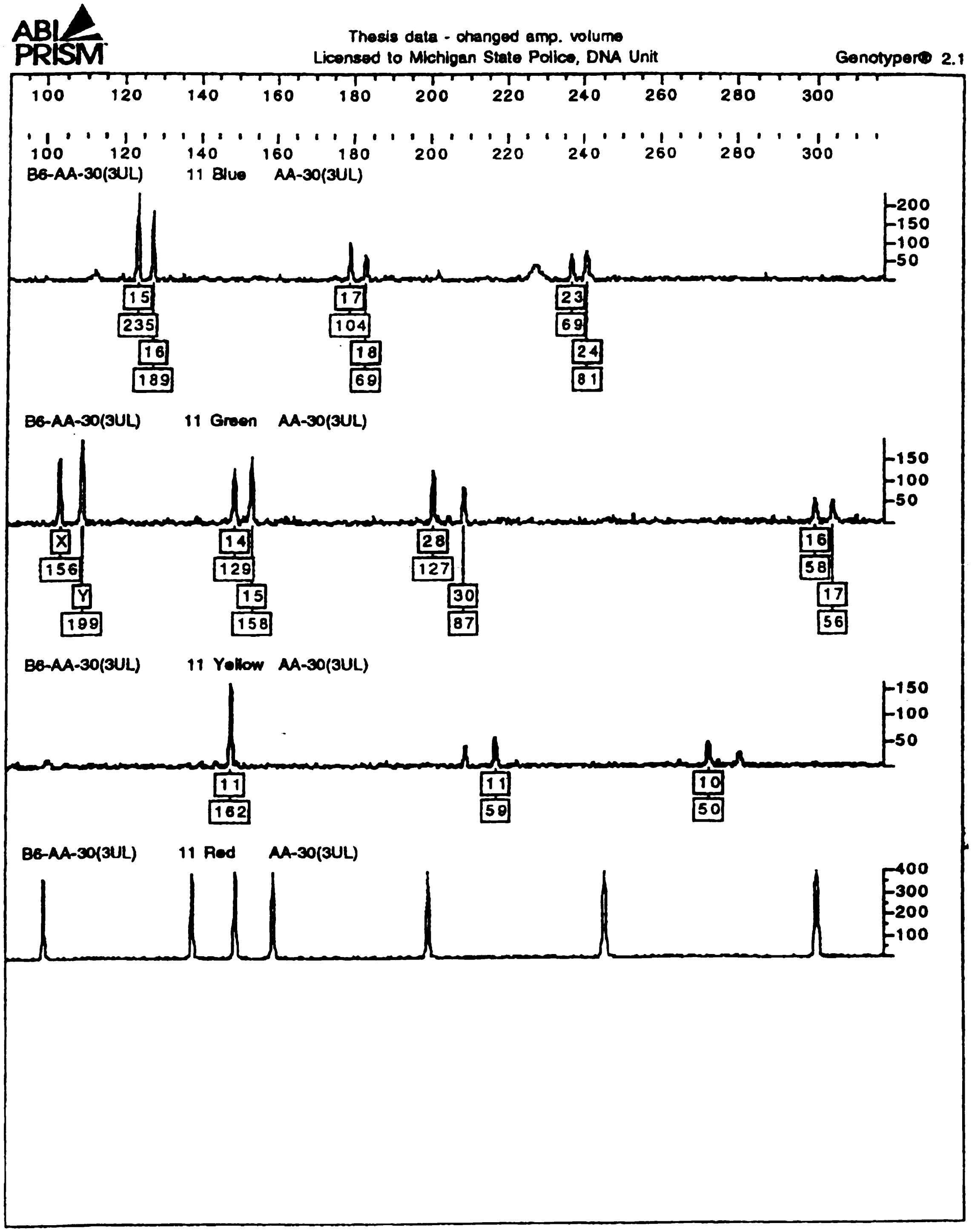


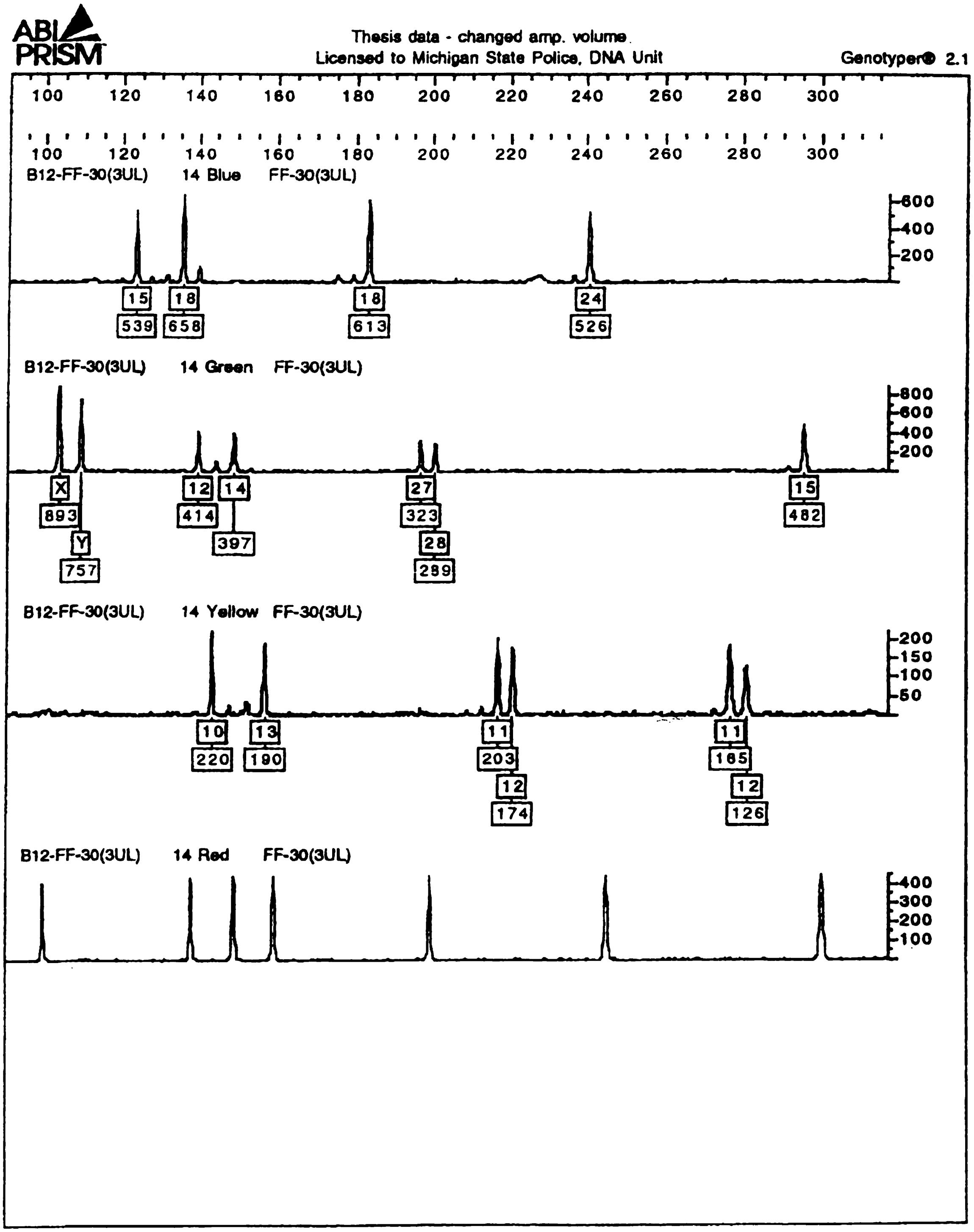


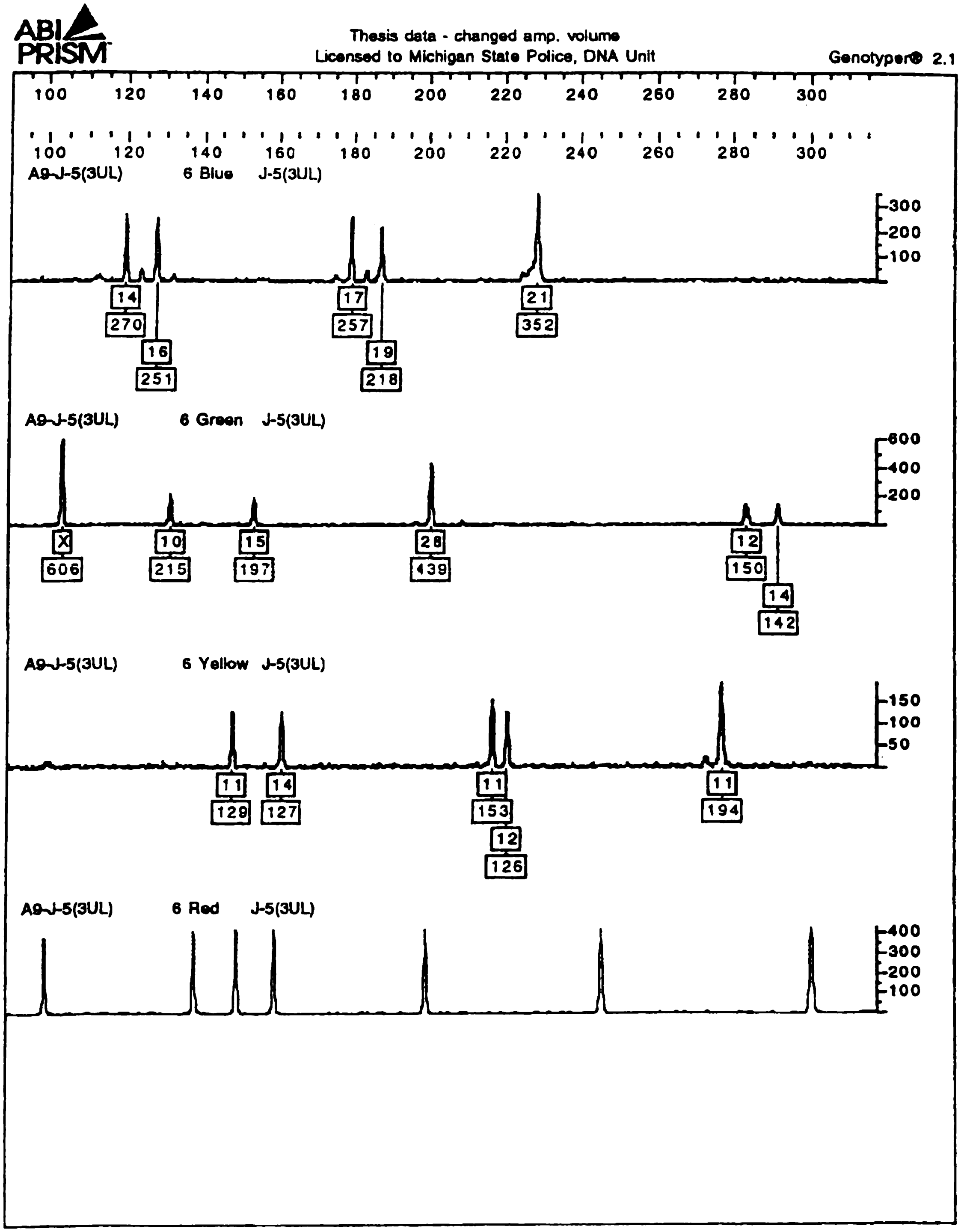
Thesis data - Group 1 (re-run)

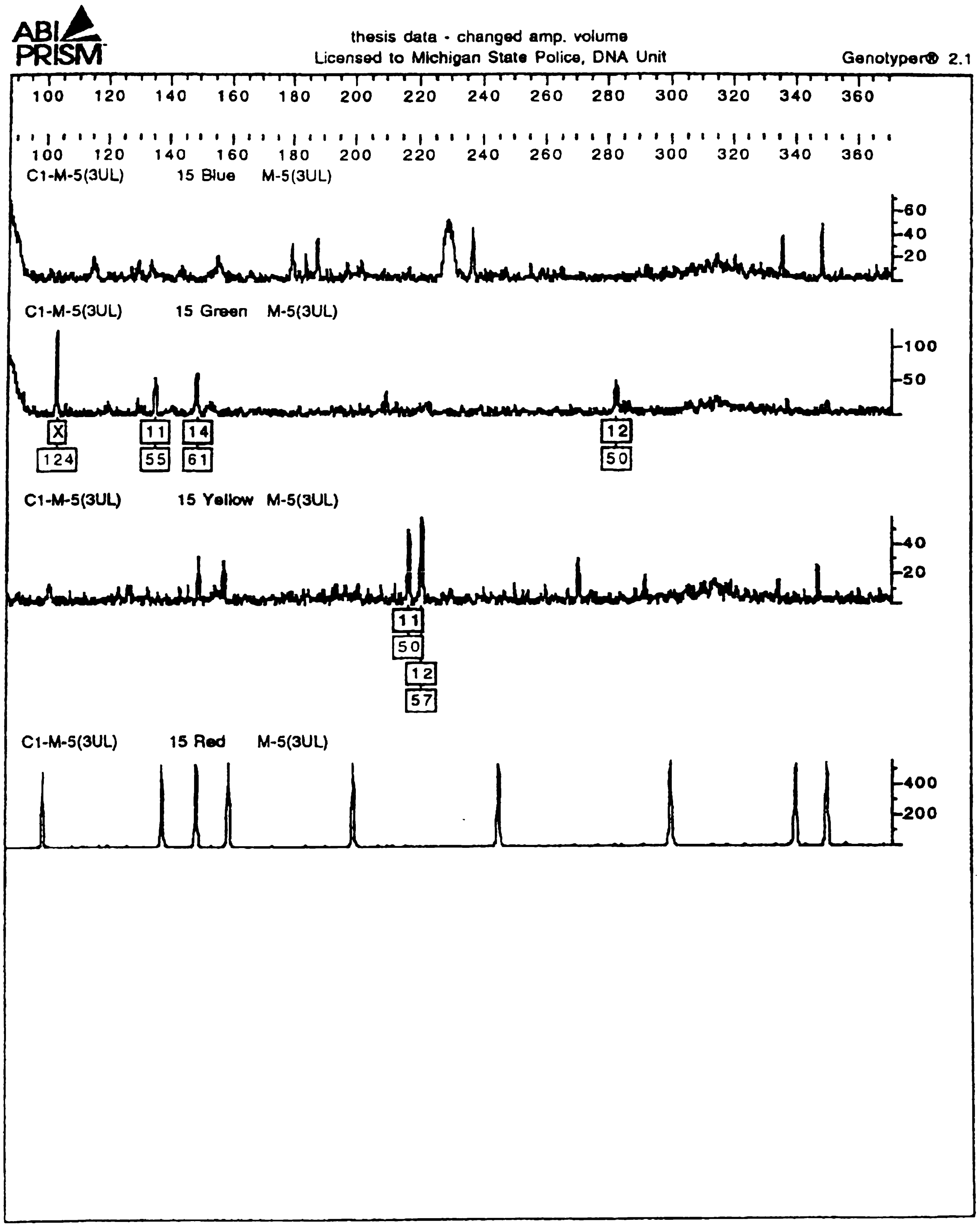
Licensed to Lynne Helton, Michigan State Police

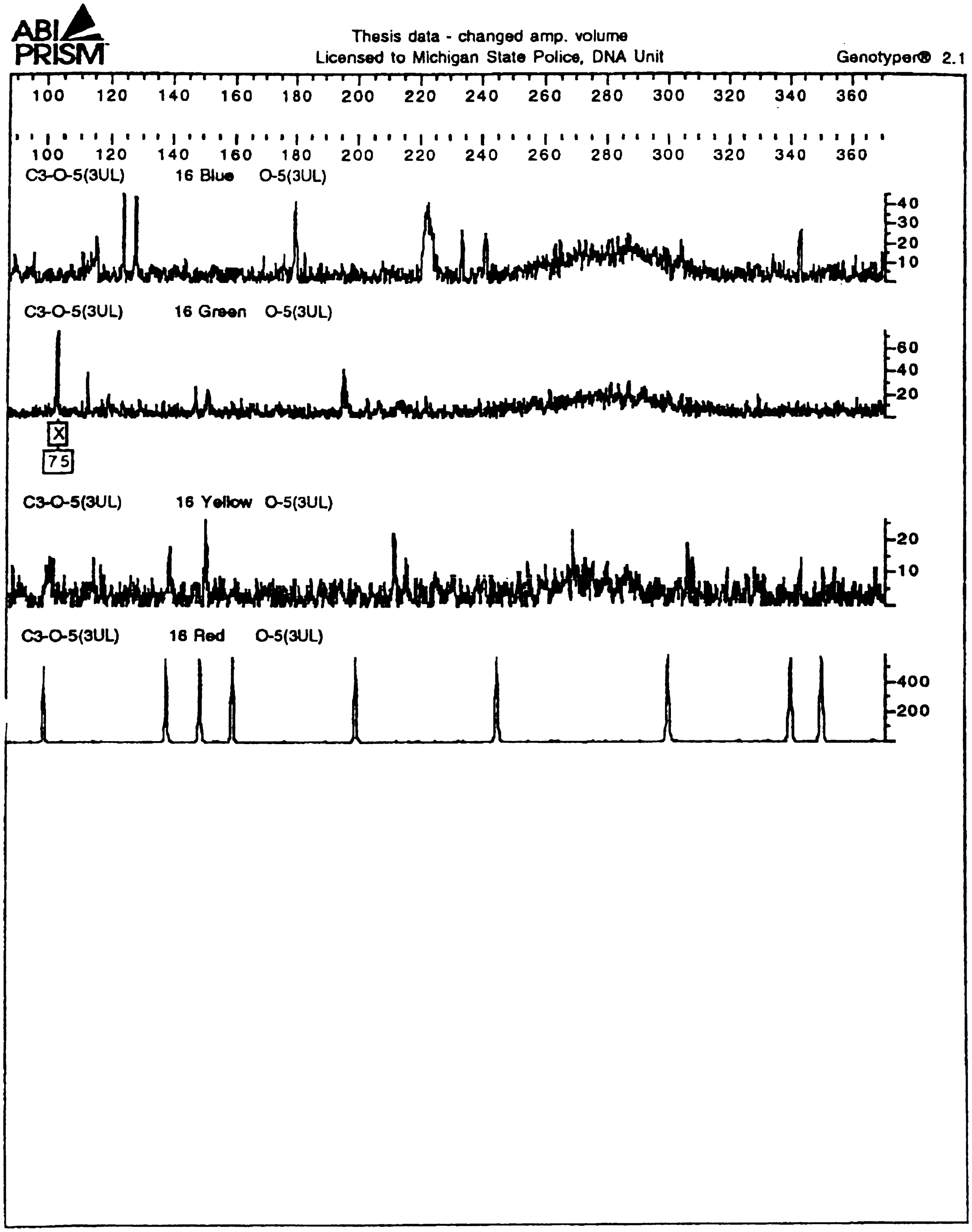
Genotyper® 2.1


Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

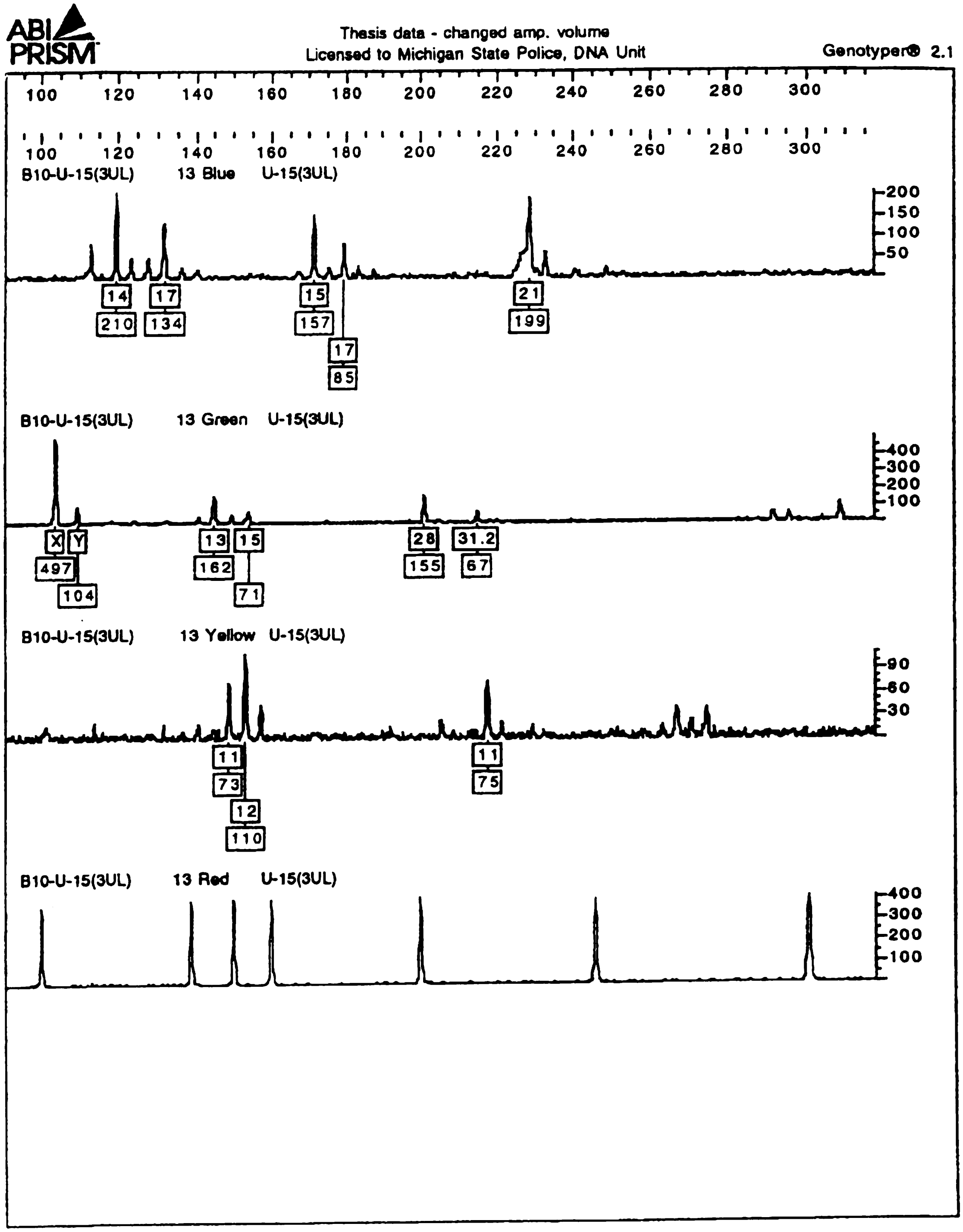

Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1


ABI PRISM
Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300
A9-J-5(3UL) 6 Blue J-5(3UL)
300
200
100
14
270
16
251
17
257
19
218
21
352
A9-J-5(3UL) 6 Green J-5(3UL)
600
400
200
X
606
10
215
15
197
28
439
12
150
14
142
A9-J-5(3UL) 6 Yellow J-5(3UL)
150
100
50
11
129
14
127
11
153
12
126
11
194
A9-J-5(3UL) 6 Red J-5(3UL)
400
300
200
100


ABI PRISM
thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360
100 120 140 160 180 200 220 240 260 280 300 320 340 360
C1-M-5(3UL) 15 Blue M-5(3UL)
60
40
20
C1-M-5(3UL) 15 Green M-5(3UL)
100
50
X
124
11
55
14
61
12
50
C1-M-5(3UL) 15 Yellow M-5(3UL)
40
20
11
50
12
57
C1-M-5(3UL) 15 Red M-5(3UL)
400
200



Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

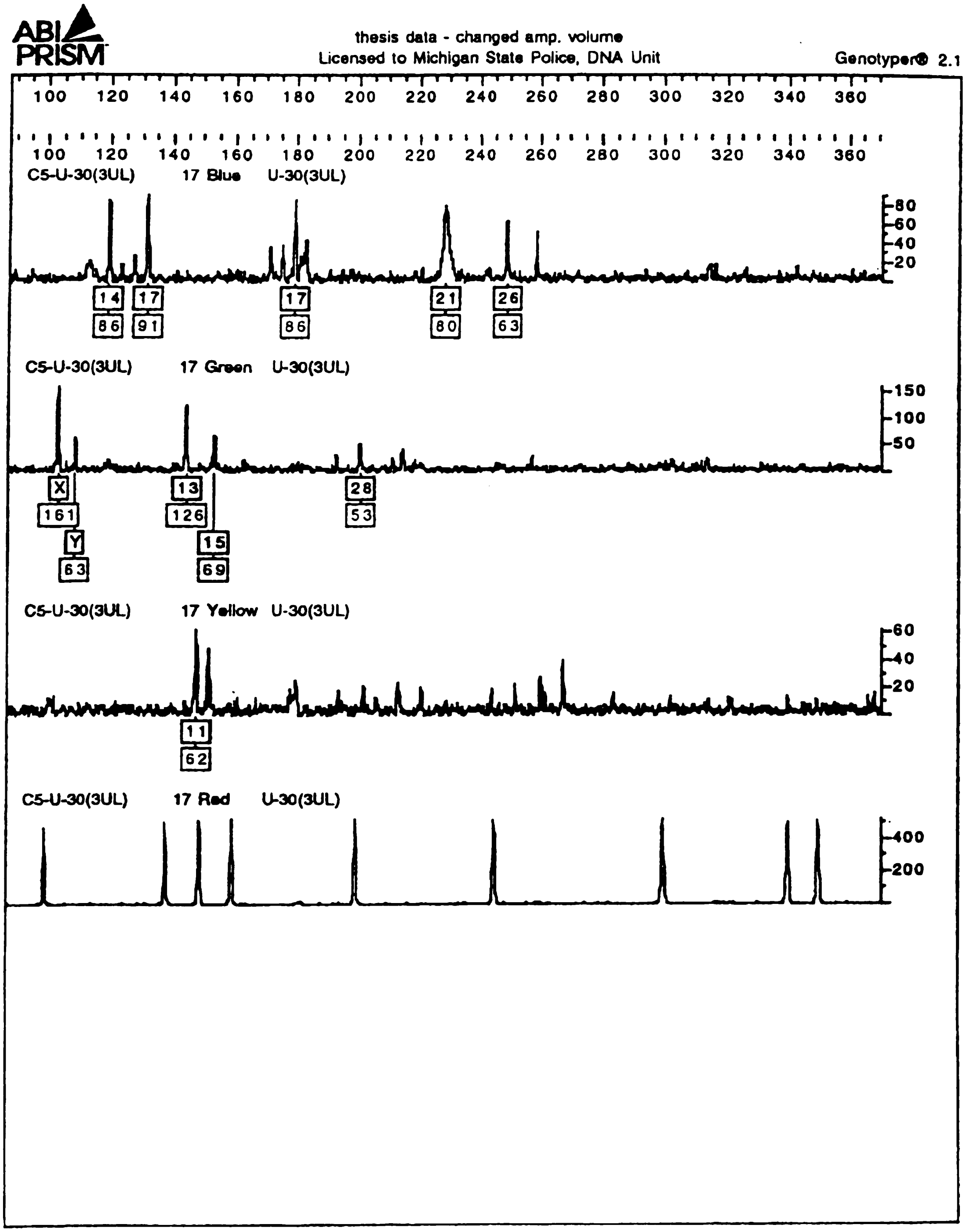

Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

B10-U-15(3UL) 13 Blue U-15(3UL)

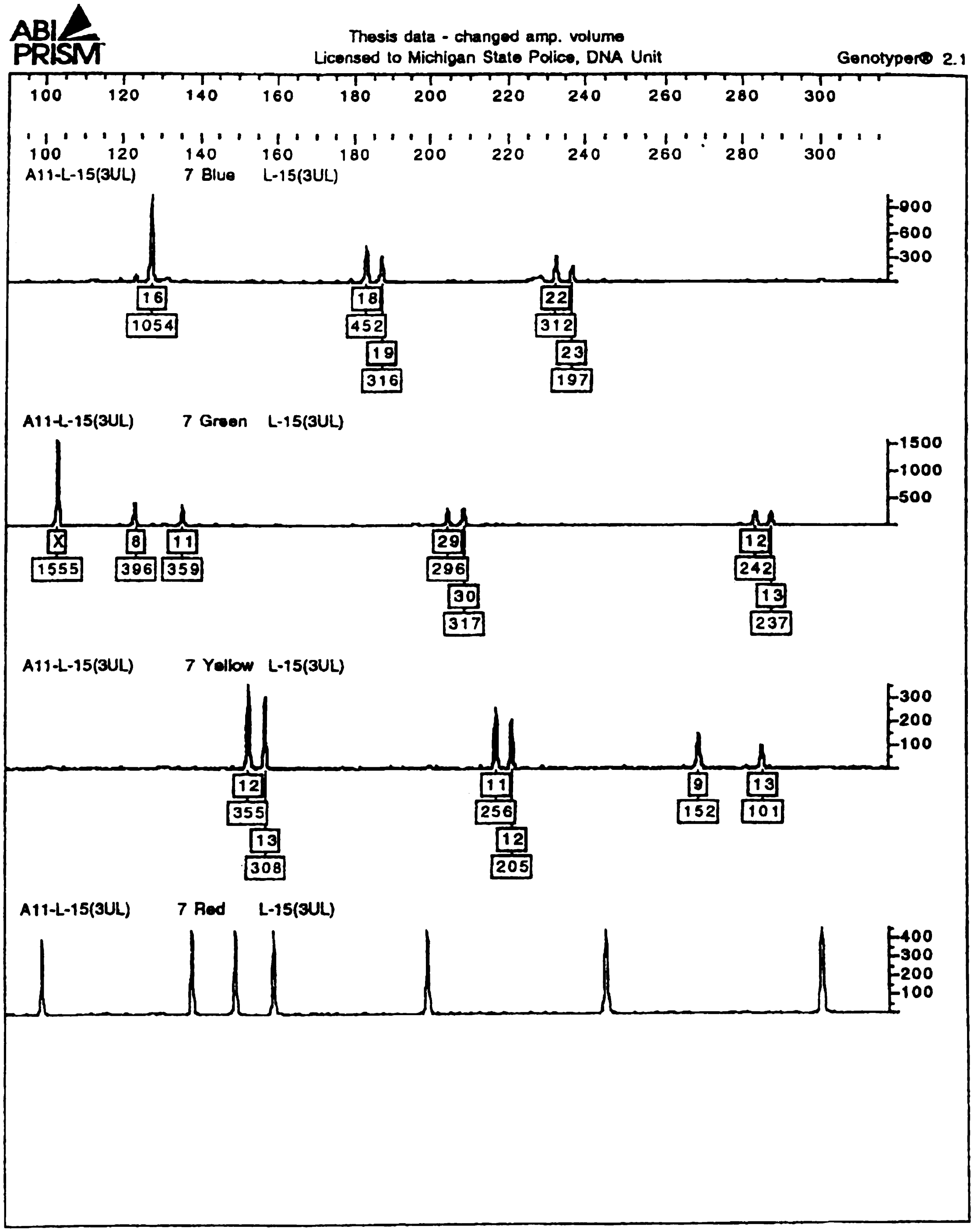
B10-U-15(3UL) 13 Green U-15(3UL)

B10-U-15(3UL) 13 Yellow U-15(3UL)

B10-U-15(3UL) 13 Red U-15(3UL)



thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

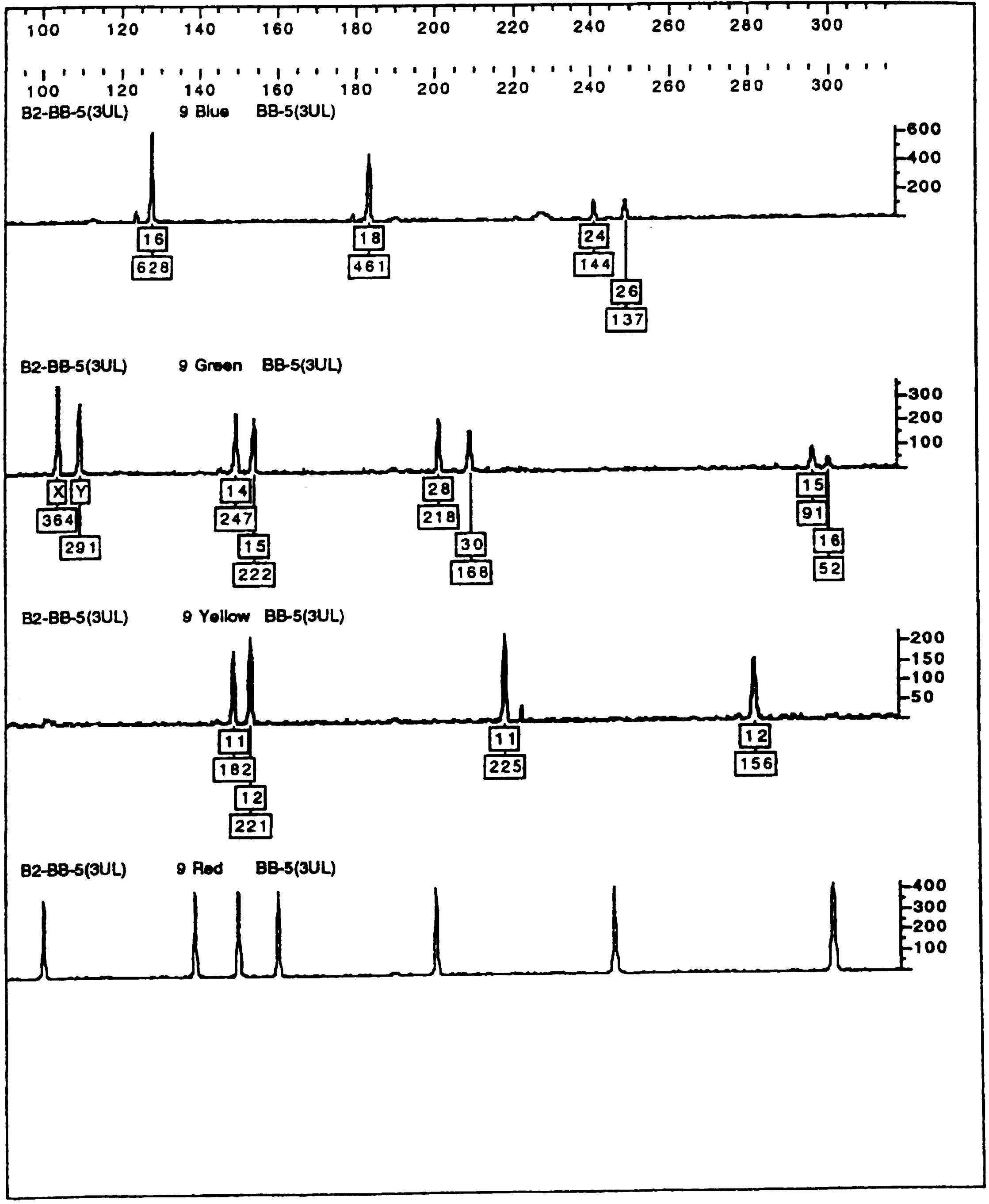


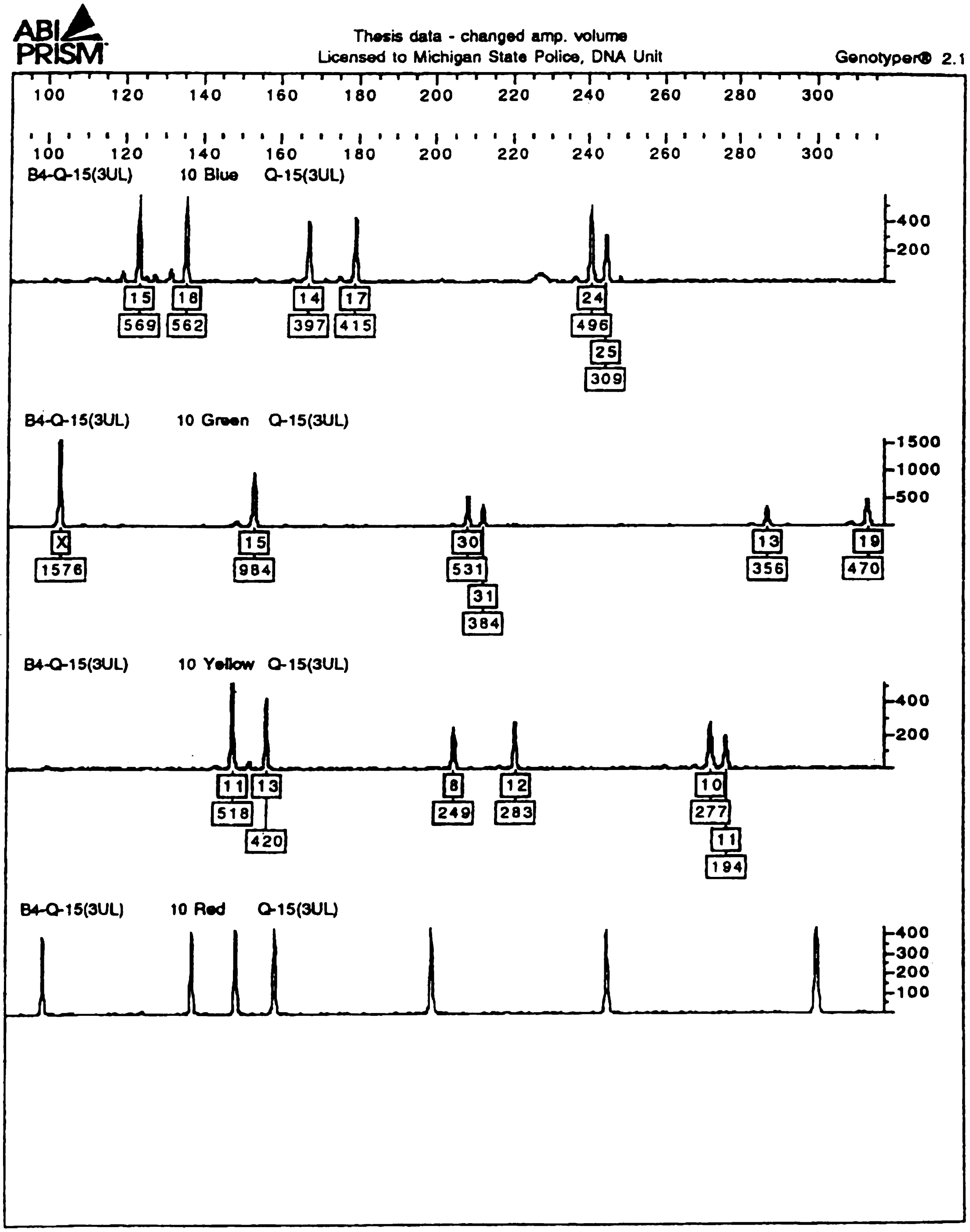
Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

ABI PRISM

Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit

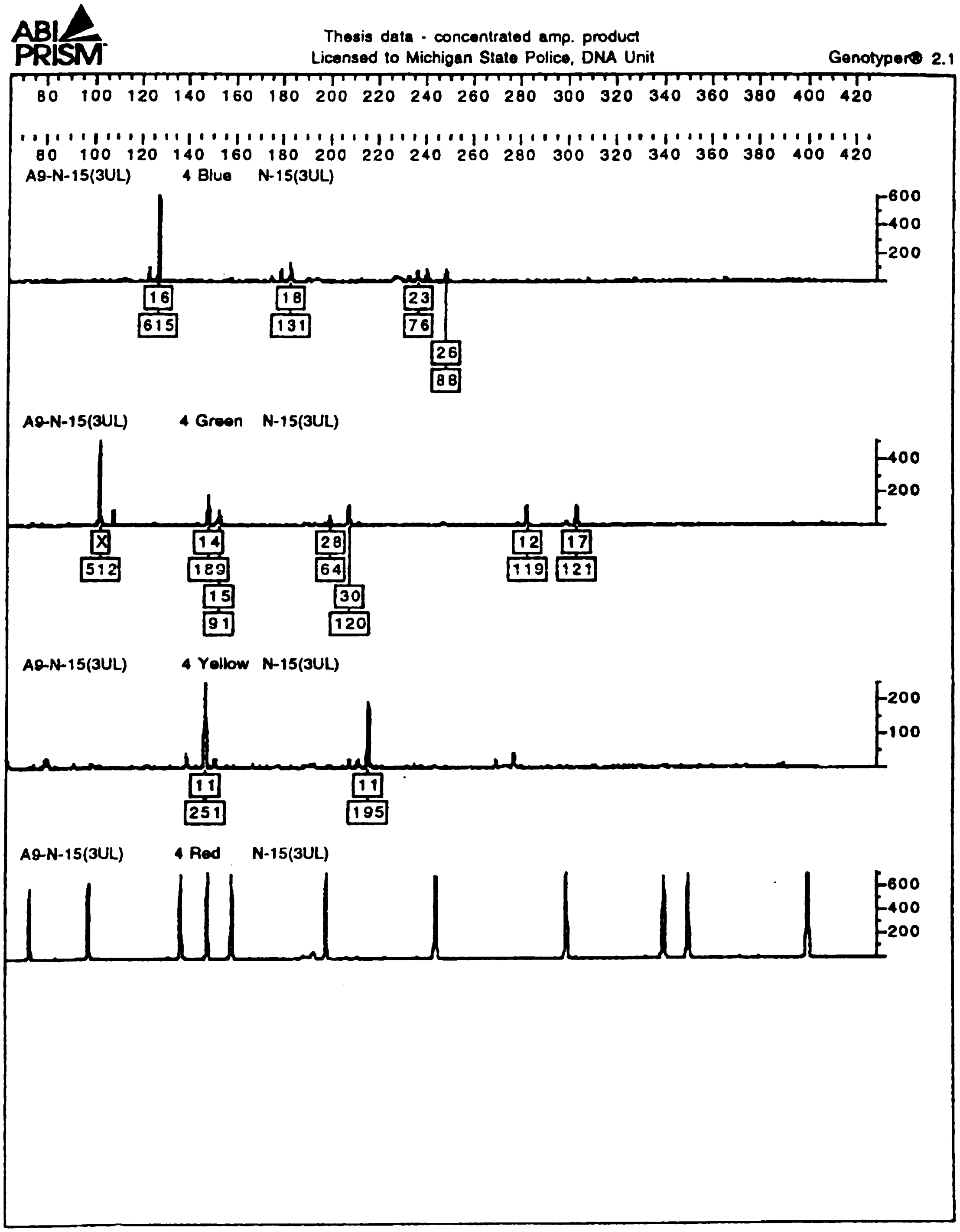
Genotyper® 2.1

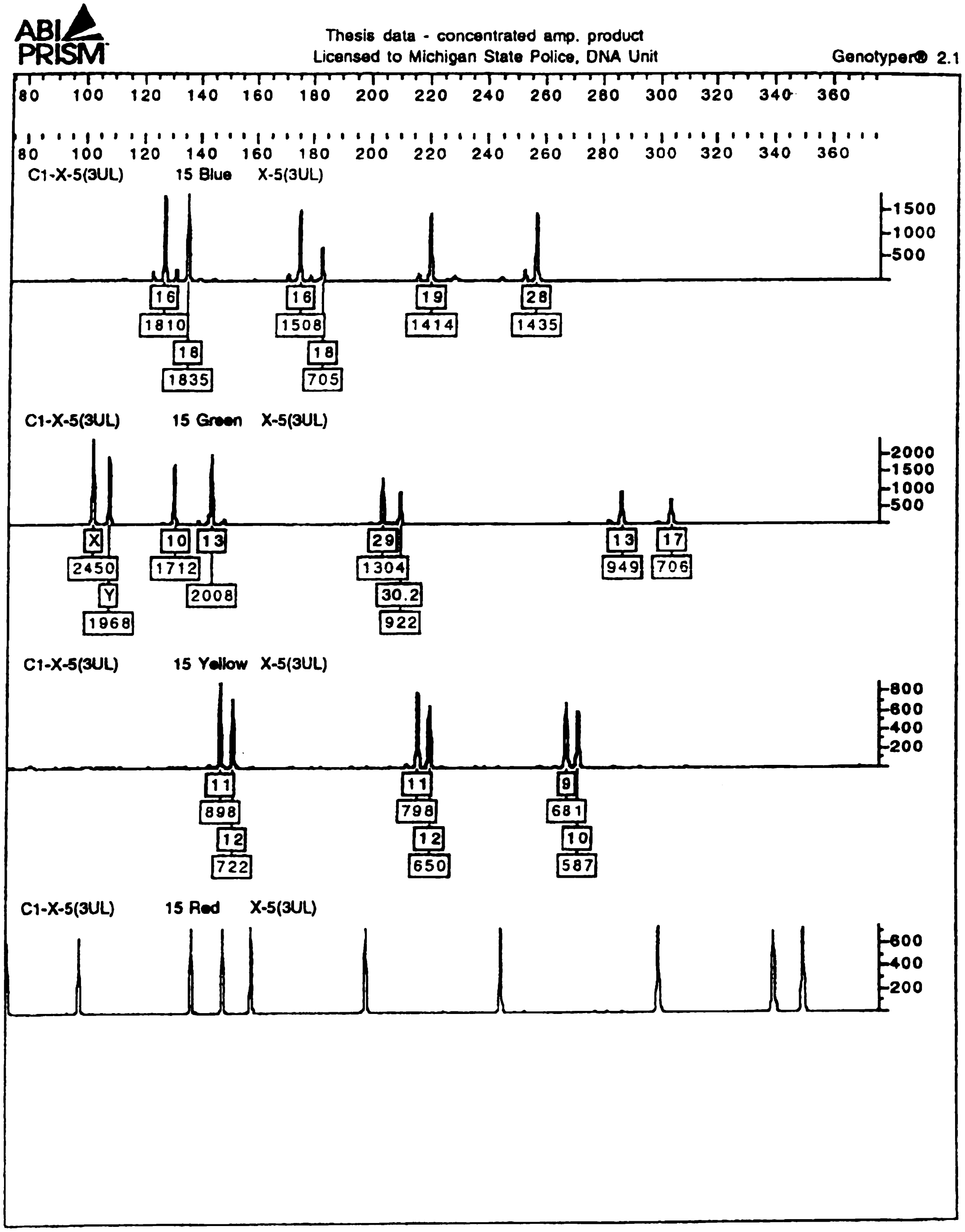



Thesis data - changed amp. volume
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

APPENDIX E

Electropherograms generated from samples containing 3ul of concentrated PCR product.

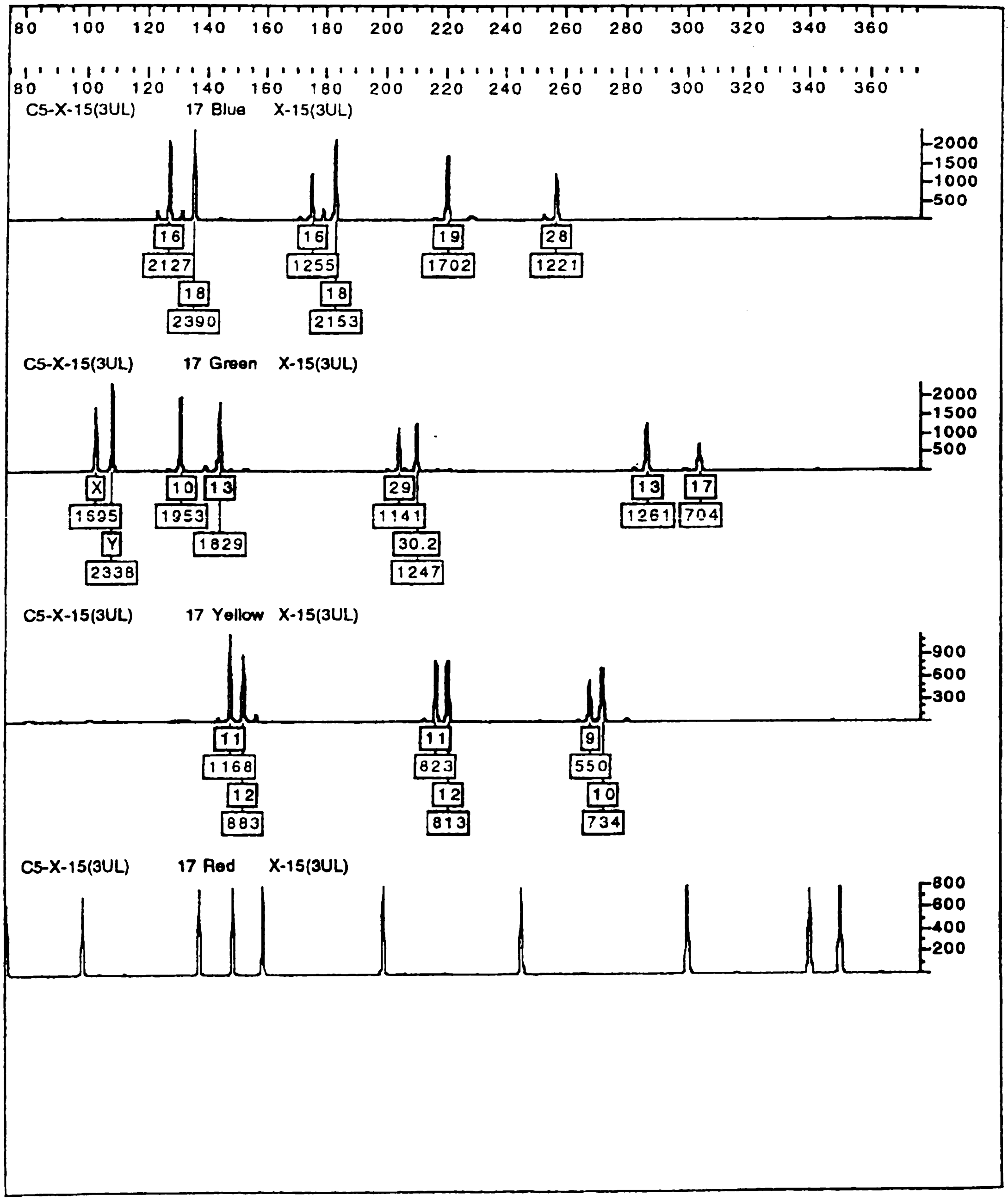

ABI PRISM
Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420
A9-N-15(3UL) 4 Blue N-15(3UL)
600
400
200
16
615
18
131
23
76
26
88
A9-N-15(3UL) 4 Green N-15(3UL)
400
200
X
512
14
189
15
91
28
64
30
120
12
119
17
121
A9-N-15(3UL) 4 Yellow N-15(3UL)
200
100
11
251
11
195
A9-N-15(3UL) 4 Red N-15(3UL)
600
400
200

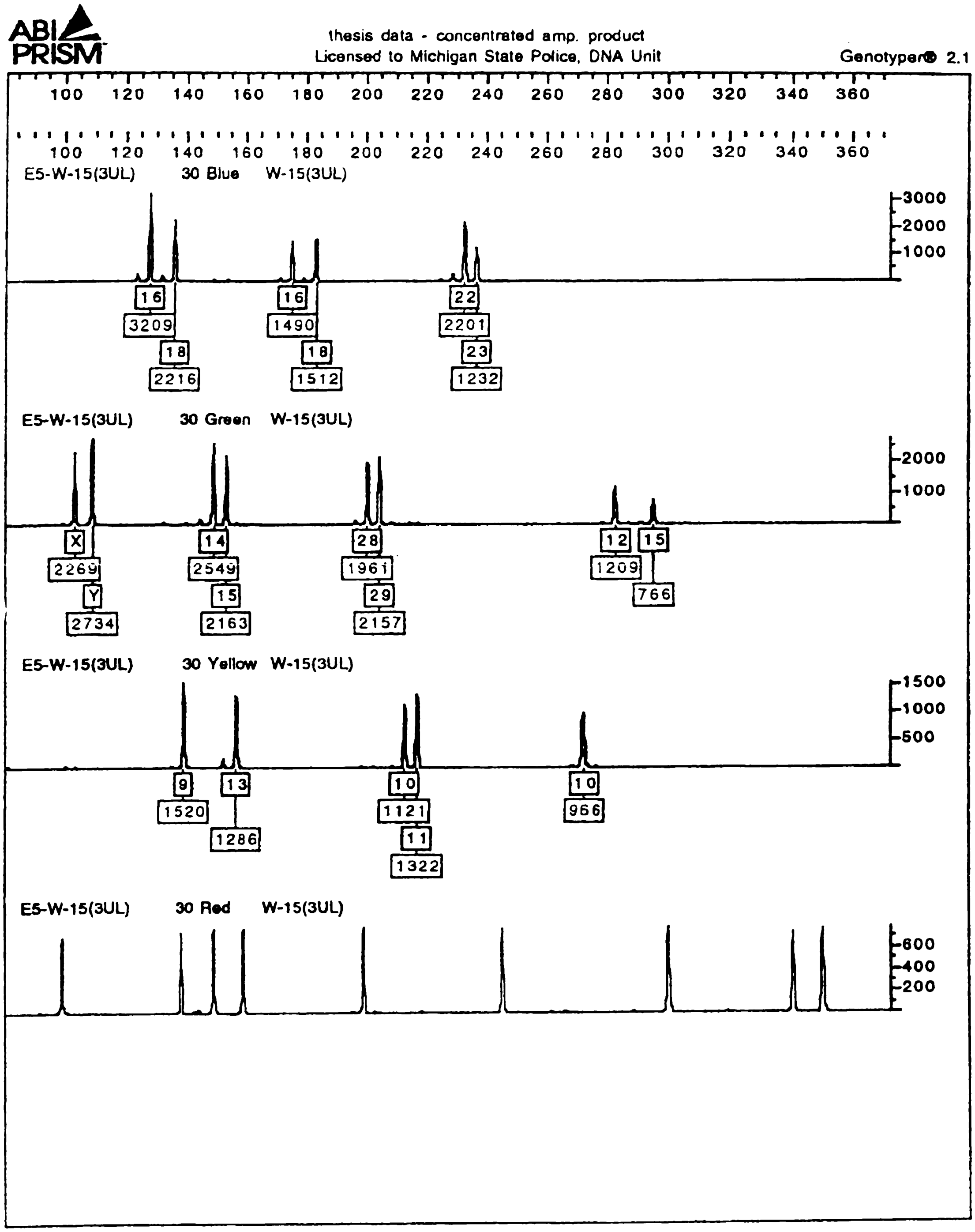

ABI PRISM
Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
C1-X-5(3UL) 15 Blue X-5(3UL)
1500
1000
500
16
1810
18
1835
16
1508
18
705
19
1414
28
1435
C1-X-5(3UL) 15 Green X-5(3UL)
2000
1500
1000
500
X
2450
Y
1968
10
1712
13
2008
29
1304
30.2
922
13
949
17
706
C1-X-5(3UL) 15 Yellow X-5(3UL)
800
600
400
200
11
898
12
722
11
798
12
650
9
681
10
587
C1-X-5(3UL) 15 Red X-5(3UL)
600
400
200

ABI PRISM

Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1

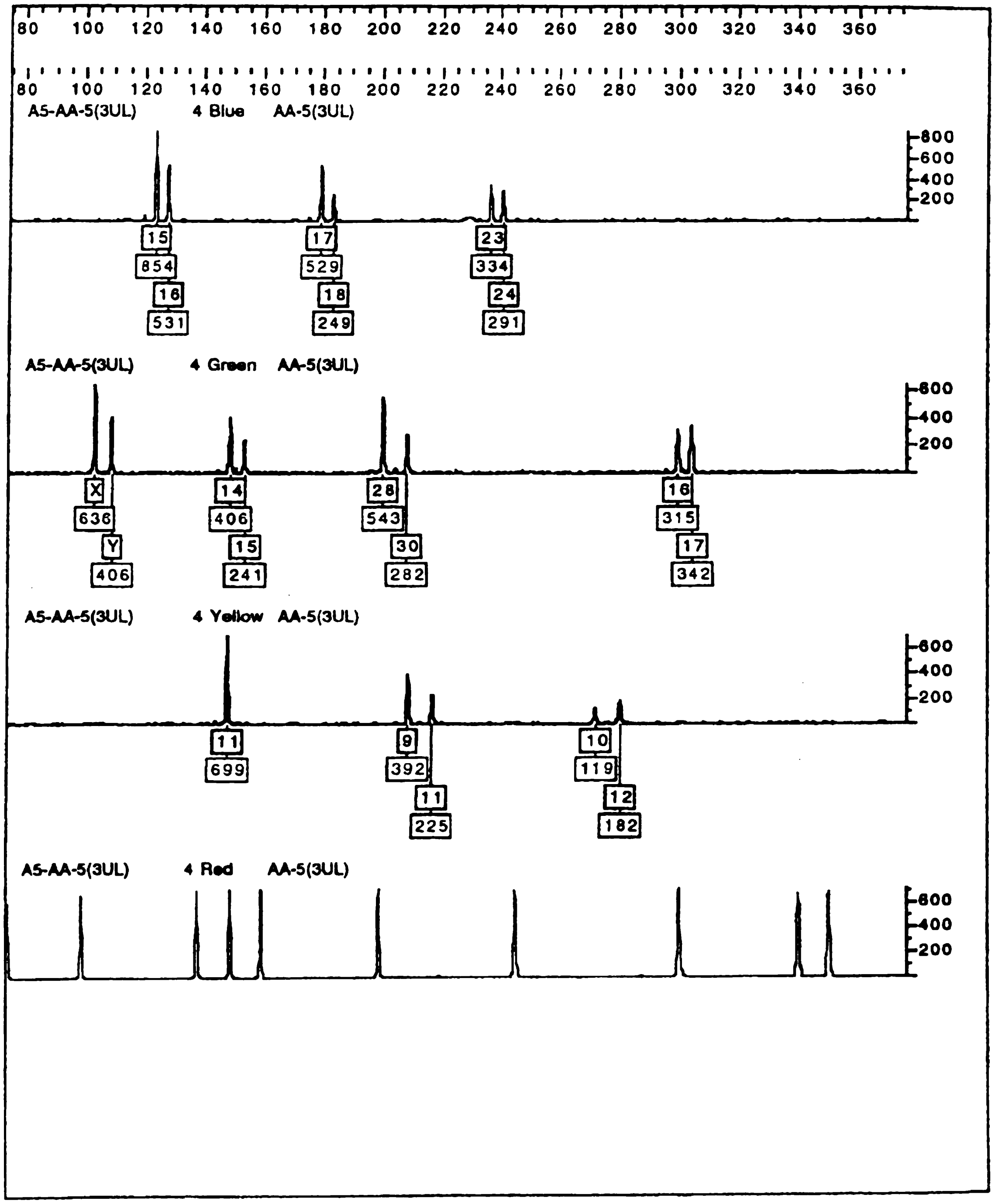


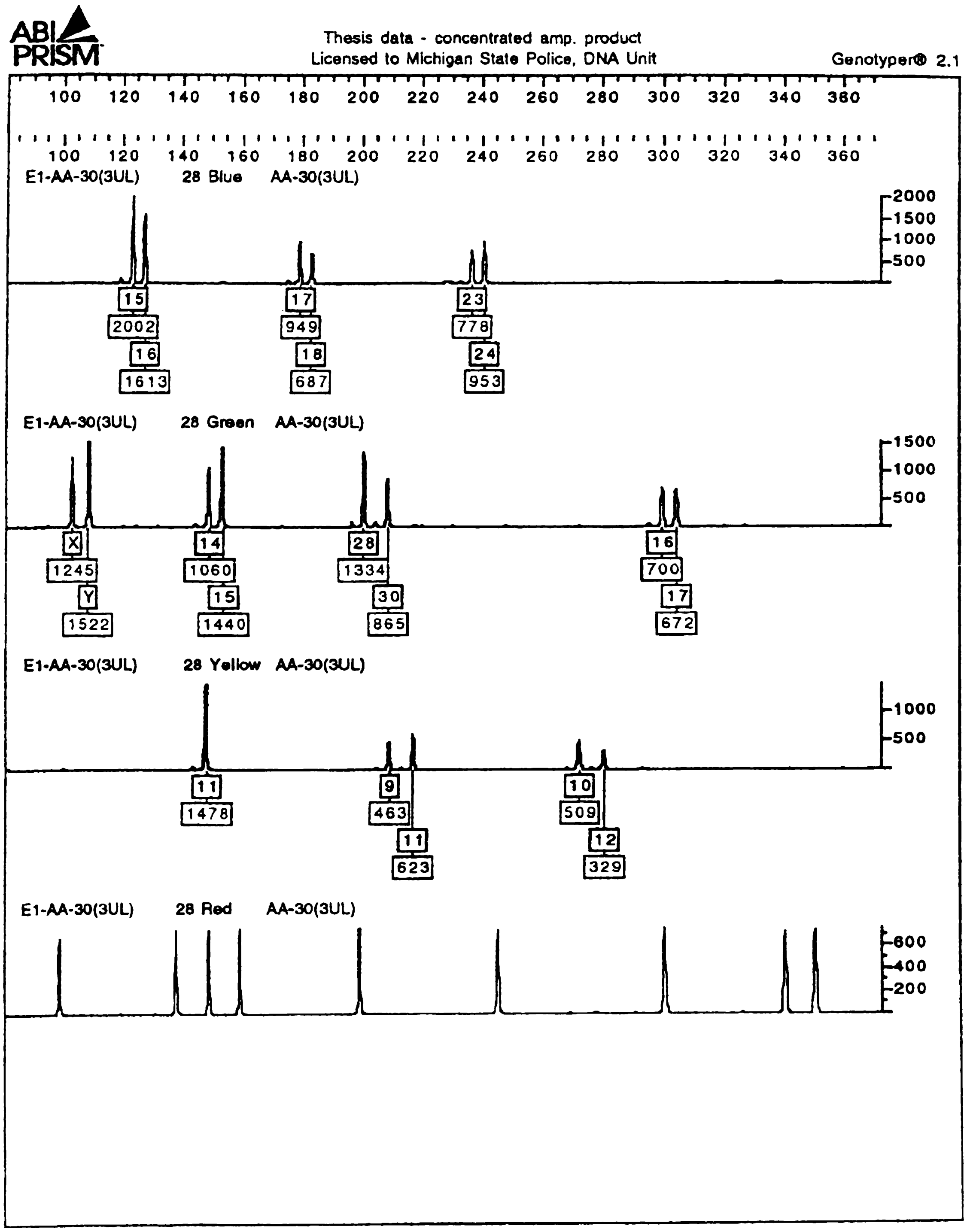


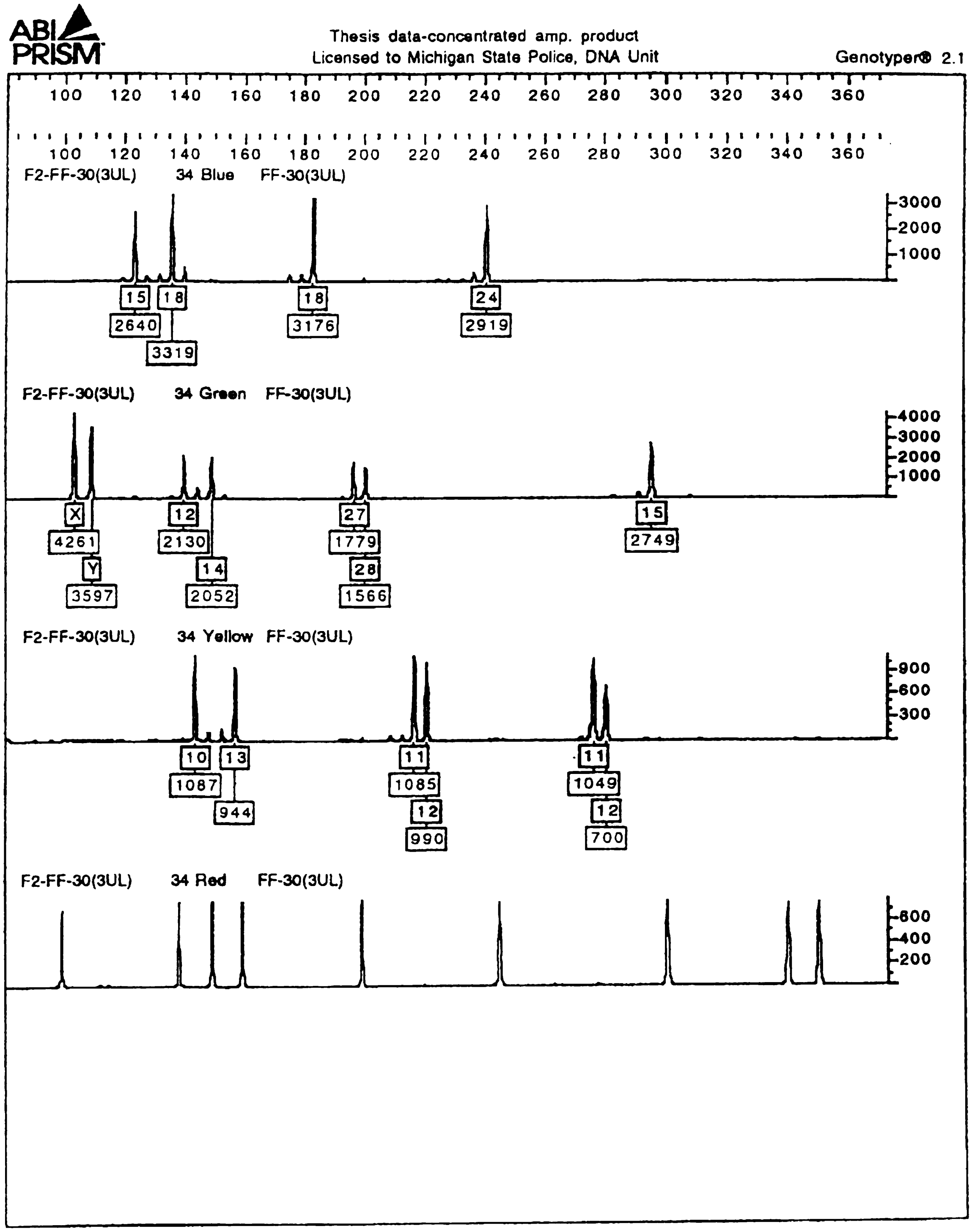
thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

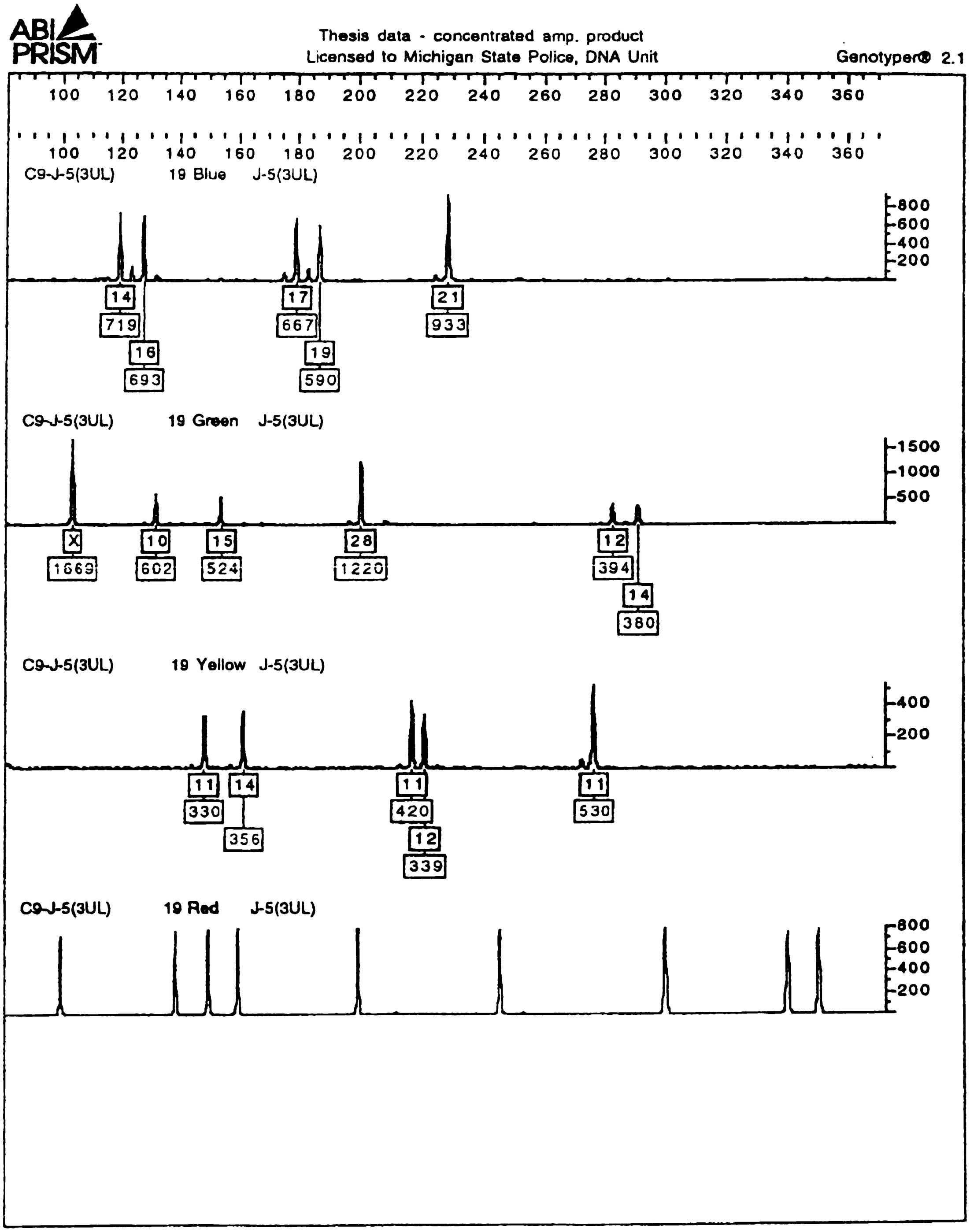
ABI PRISM

Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

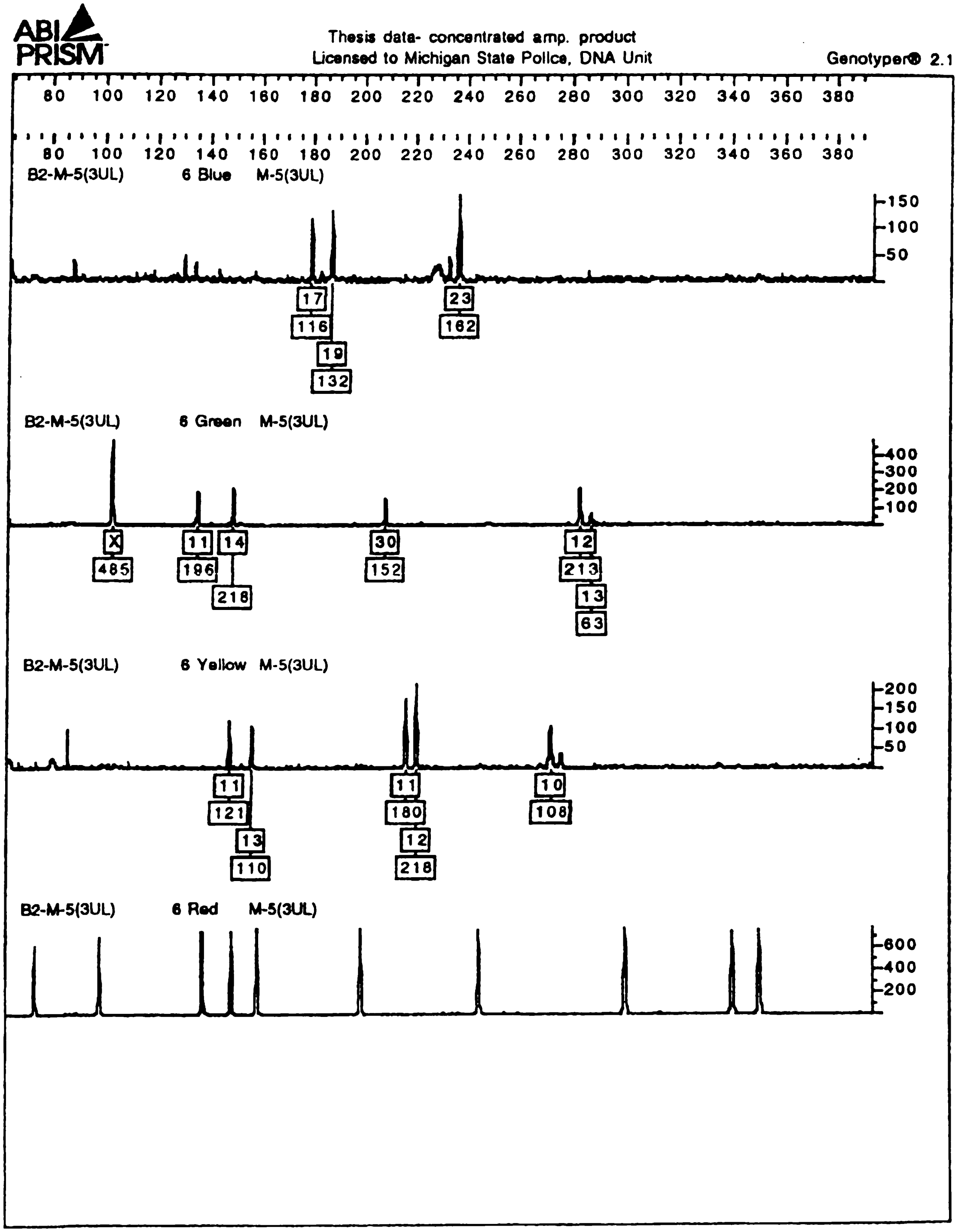


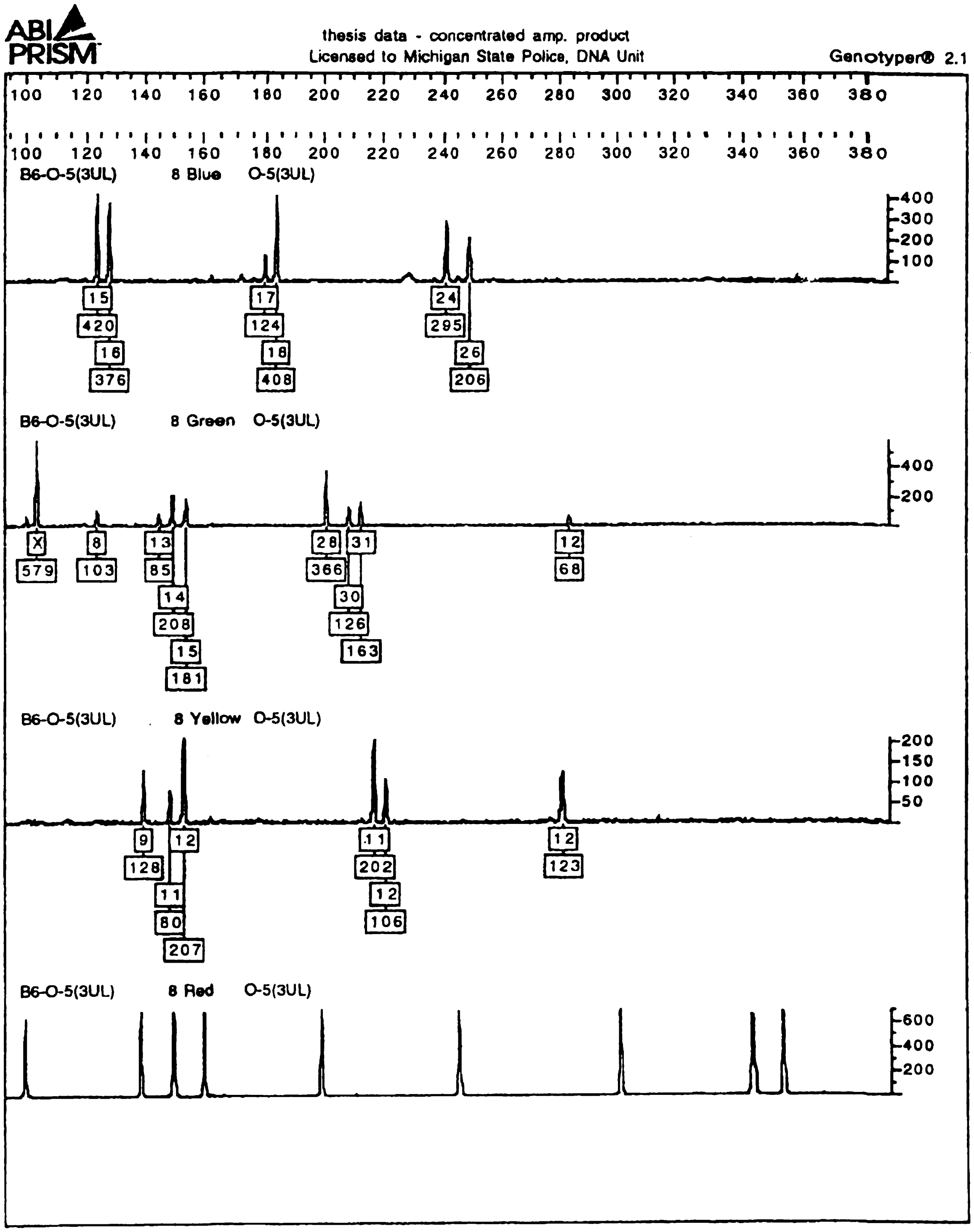

ABI PRISM
Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360
100 120 140 160 180 200 220 240 260 280 300 320 340 360
E1-AA-30(3UL) 28 Blue AA-30(3UL)
2000
1500
1000
500
15
2002
16
1613
17
949
18
687
23
778
24
953
E1-AA-30(3UL) 28 Green AA-30(3UL)
1500
1000
500
X
1245
Y
1522
14
1060
15
1440
28
1334
30
865
16
700
17
672
E1-AA-30(3UL) 28 Yellow AA-30(3UL)
1000
500
11
1478
9
463
11
623
10
509
12
329
E1-AA-30(3UL) 28 Red AA-30(3UL)
600
400
200


ABI PRISM
Thesis data-concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360
F2-FF-30(3UL) 34 Blue FF-30(3UL)
3000
2000
1000
15
2640
18
3319
18
3176
24
2919
F2-FF-30(3UL) 34 Green FF-30(3UL)
4000
3000
2000
1000
X
4261
Y
3597
12
2130
14
2052
27
1779
28
1566
15
2749
F2-FF-30(3UL) 34 Yellow FF-30(3UL)
900
600
300
10
1087
13
944
11
1085
12
990
11
1049
12
700
F2-FF-30(3UL) 34 Red FF-30(3UL)
600
400
200



Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

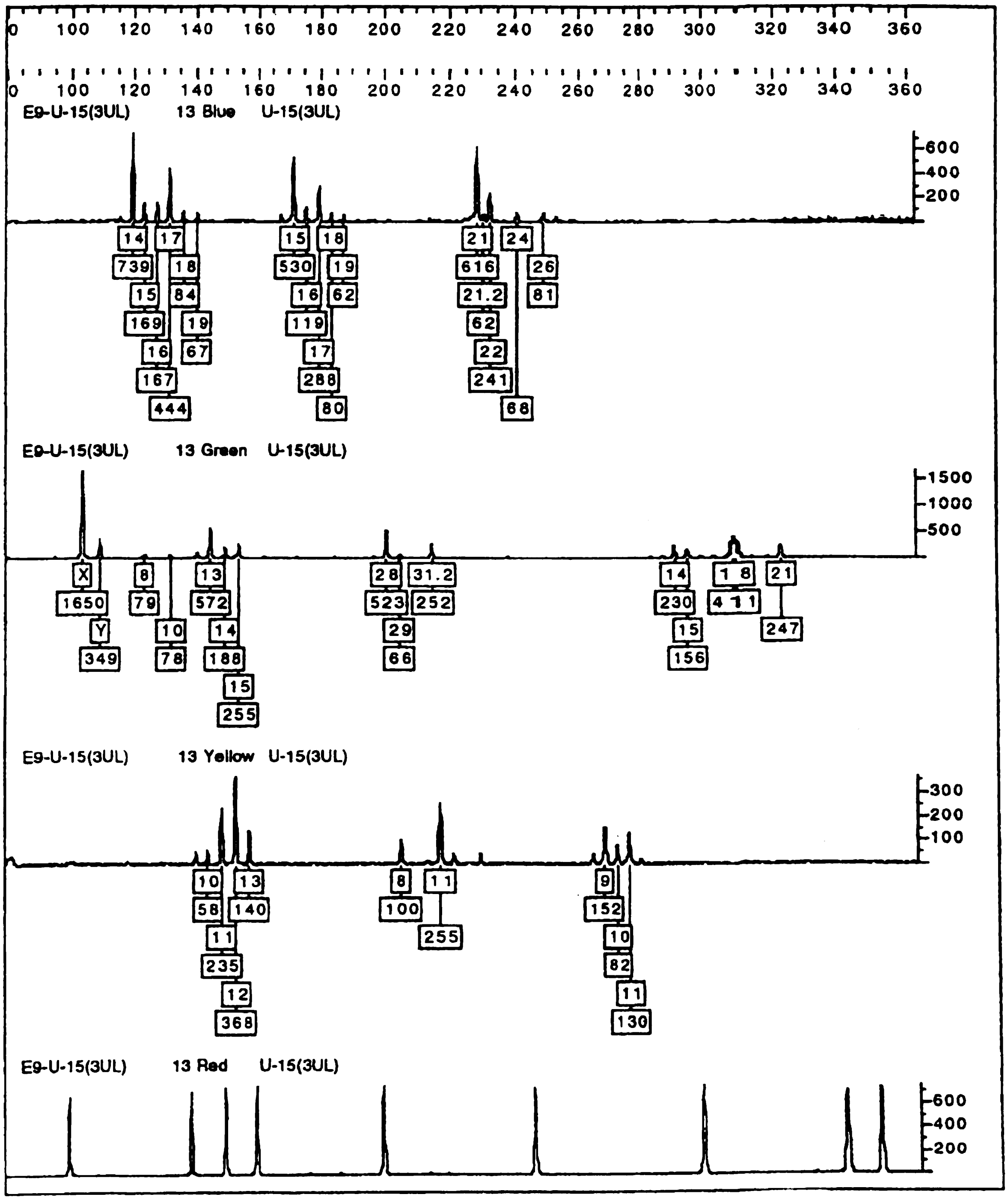

ABI PRISM
Thesis data- concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
B2-M-5(3UL) 6 Blue M-5(3UL)
150
100
50
17
116
19
132
23
162
B2-M-5(3UL) 6 Green M-5(3UL)
400
300
200
100
X
485
11
196
14
218
30
152
12
213
13
63
B2-M-5(3UL) 6 Yellow M-5(3UL)
200
150
100
50
11
121
13
110
11
180
12
218
10
108
B2-M-5(3UL) 6 Red M-5(3UL)
600
400
200

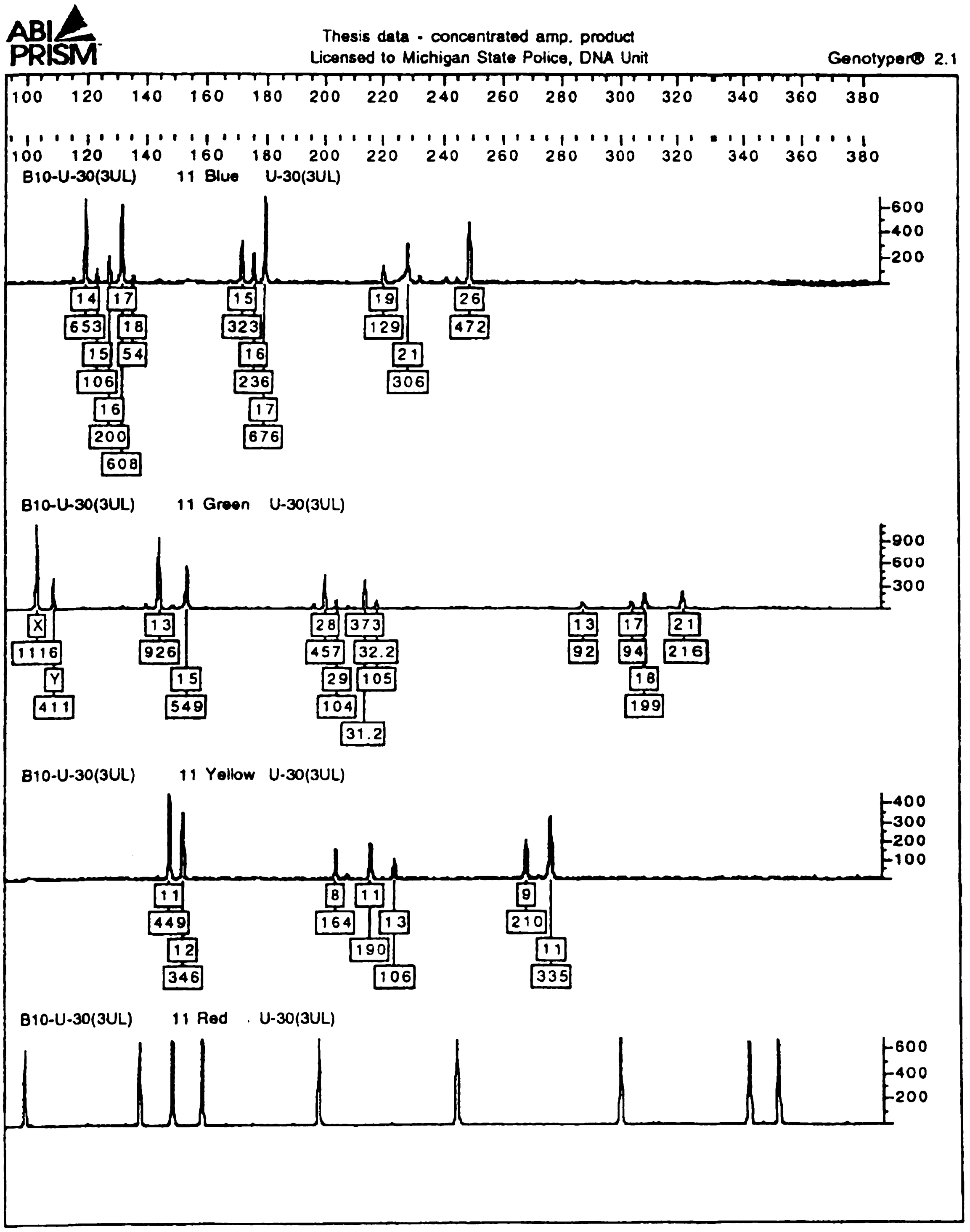

ABI PRISM
thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
B6-O-5(3UL) 8 Blue O-5(3UL)
400
300
200
100
15
420
16
376
17
124
18
408
24
295
26
206
B6-O-5(3UL) 8 Green O-5(3UL)
400
200
X
579
8
103
13
85
14
208
15
181
28
366
31
30
126
163
12
68
B6-O-5(3UL) 8 Yellow O-5(3UL)
200
150
100
50
9
128
12
11
80
207
11
202
12
106
12
123
B6-O-5(3UL) 8 Red O-5(3UL)
600
400
200

ABI PRISM

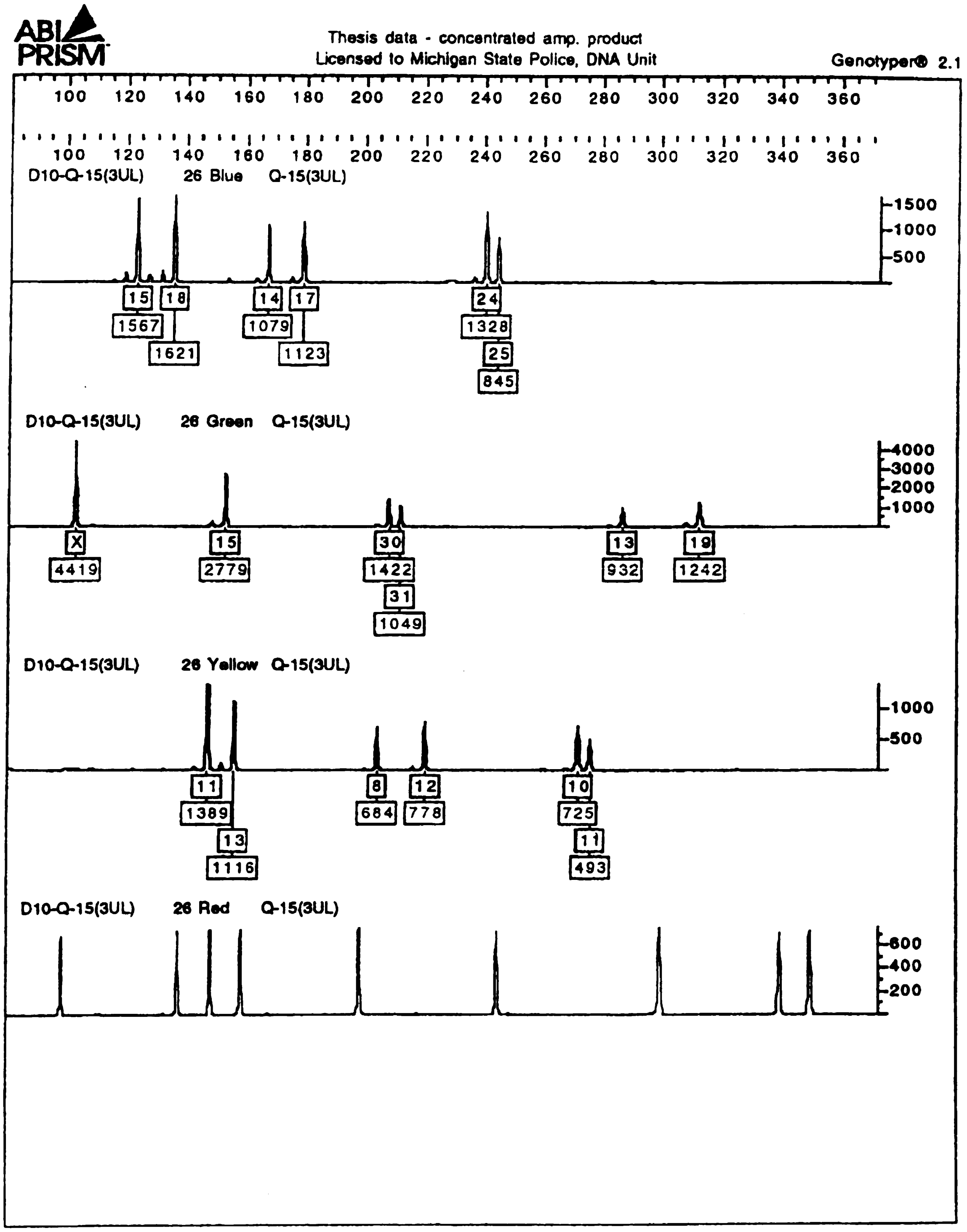
Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit

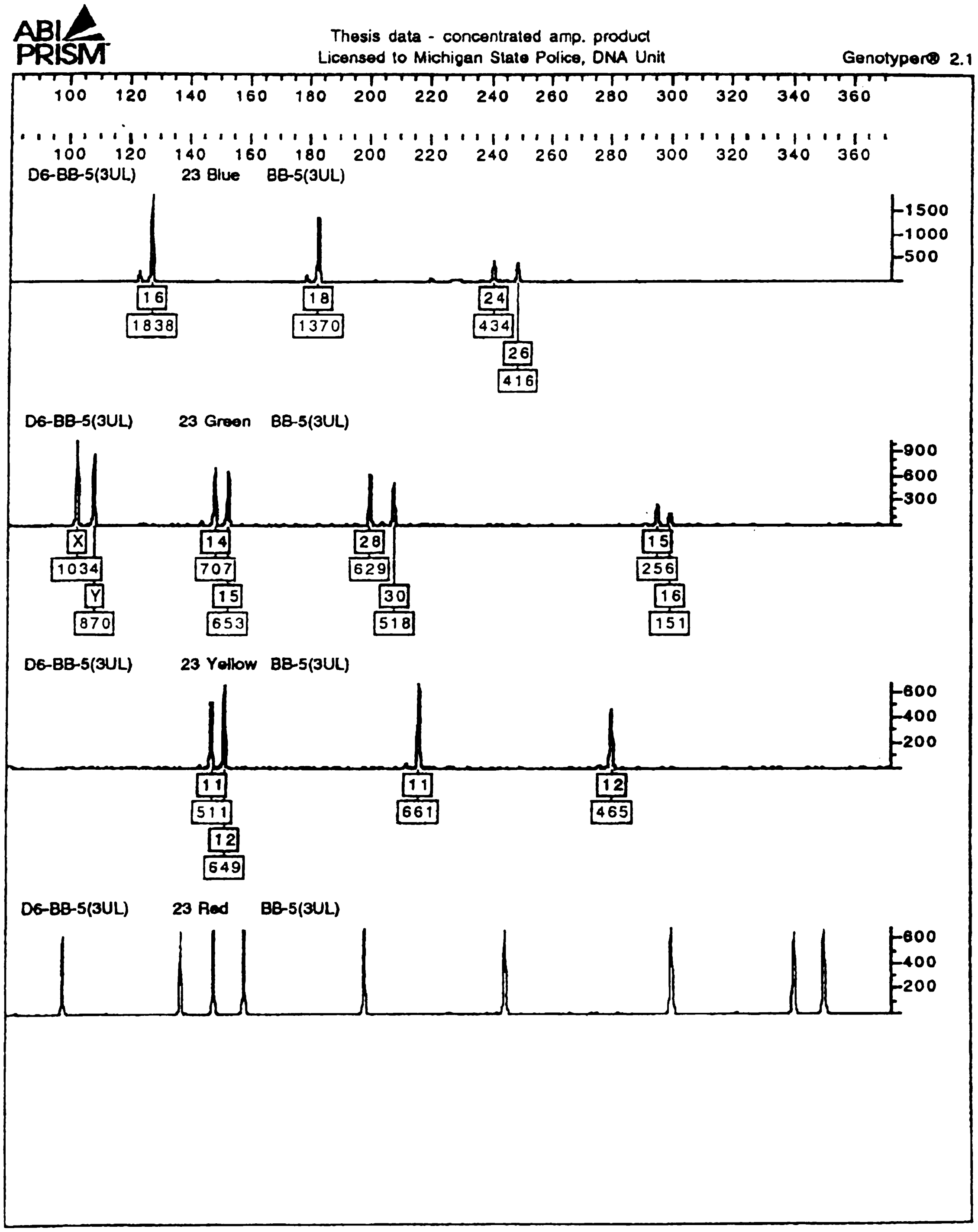
Genotyper® 2.1

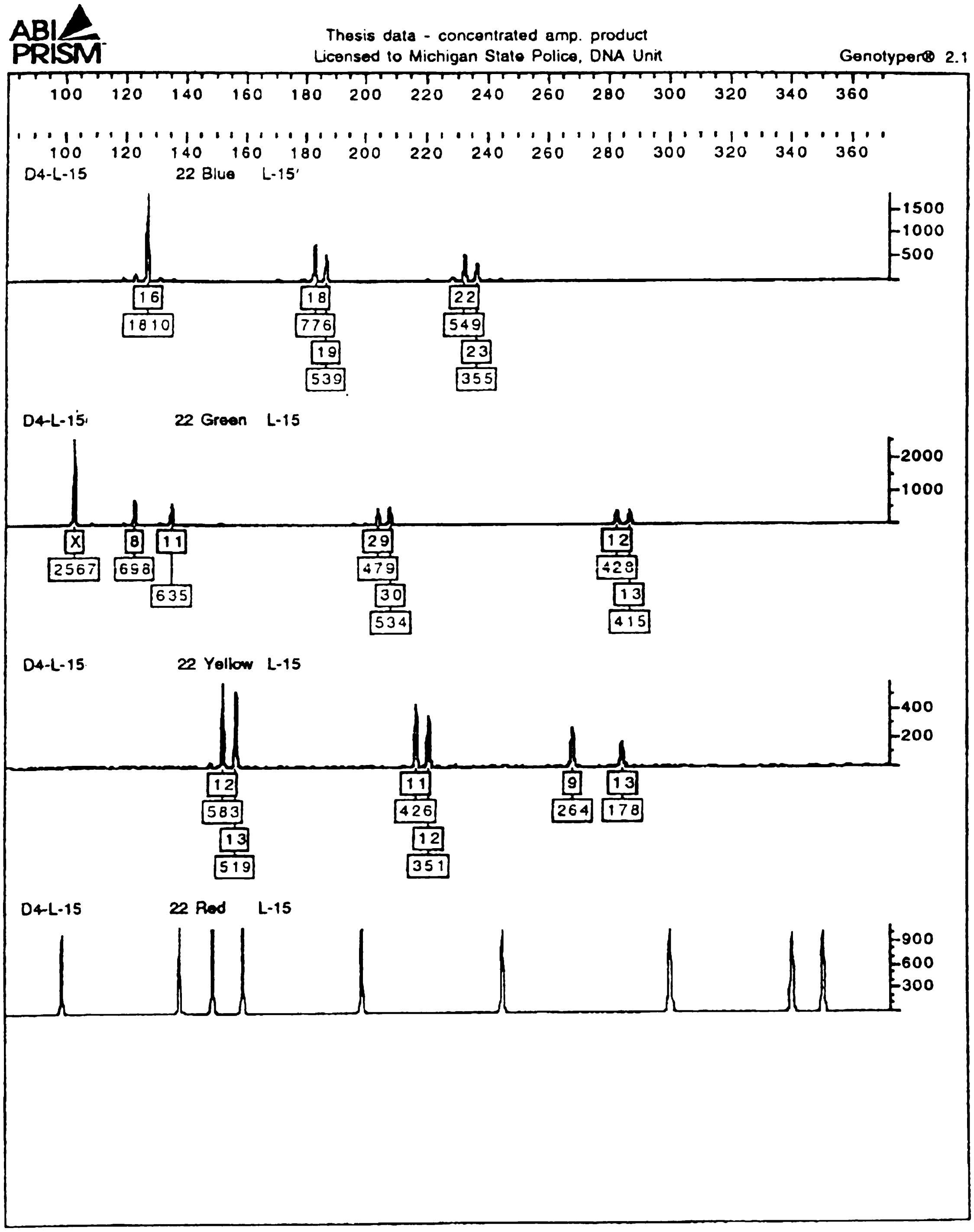




Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

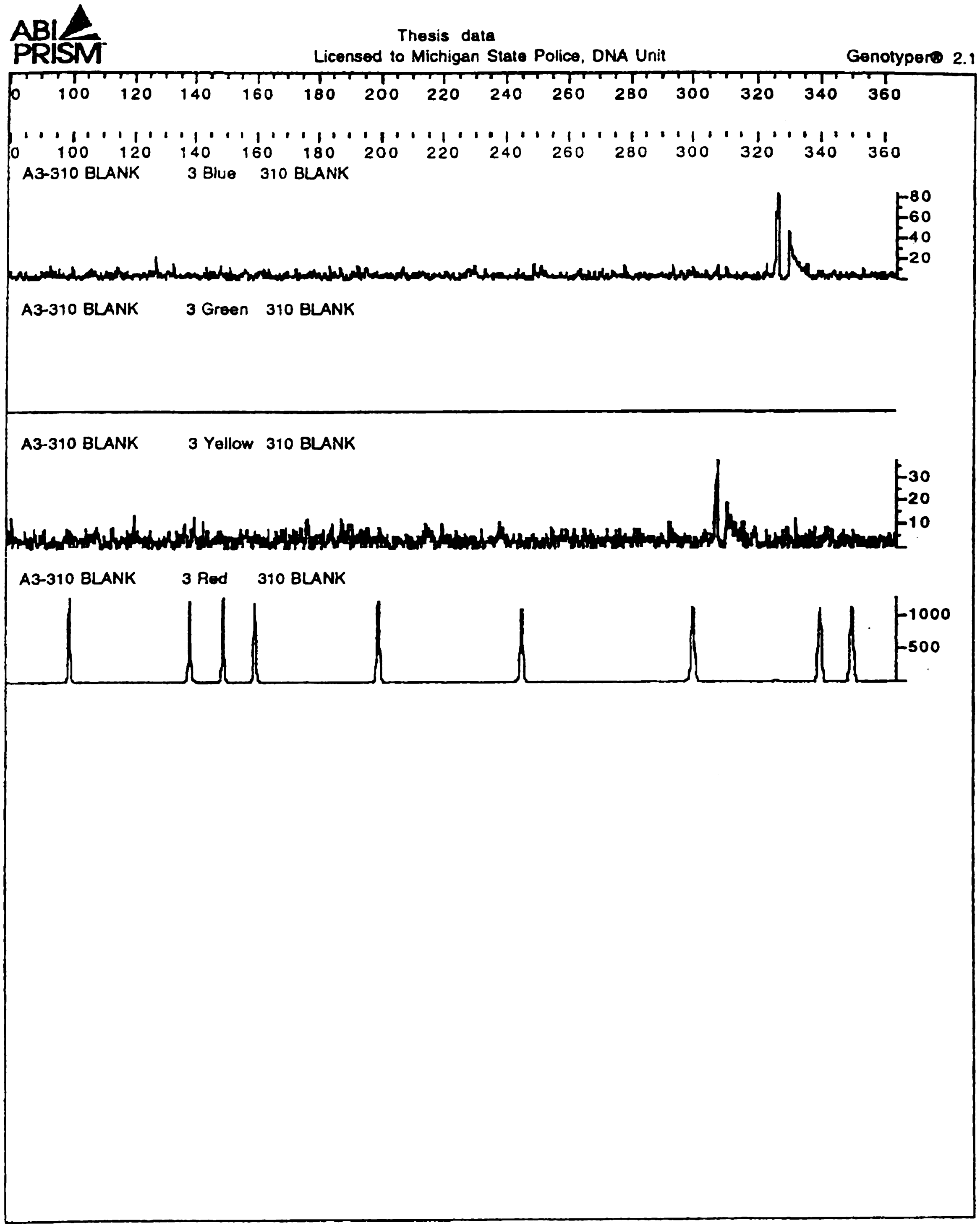

ABI PRISM
Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360
100 120 140 160 180 200 220 240 260 280 300 320 340 360
D10-Q-15(3UL) 26 Blue Q-15(3UL)
1500
1000
500
15
1567
18
1621
14
1079
17
1123
24
1328
25
845
D10-Q-15(3UL) 26 Green Q-15(3UL)
4000
3000
2000
1000
X
4419
15
2779
30
1422
31
1049
13
932
19
1242
D10-Q-15(3UL) 26 Yellow Q-15(3UL)
1000
500
11
1389
13
1116
8
684
12
778
10
725
11
493
D10-Q-15(3UL) 26 Red Q-15(3UL)
600
400
200

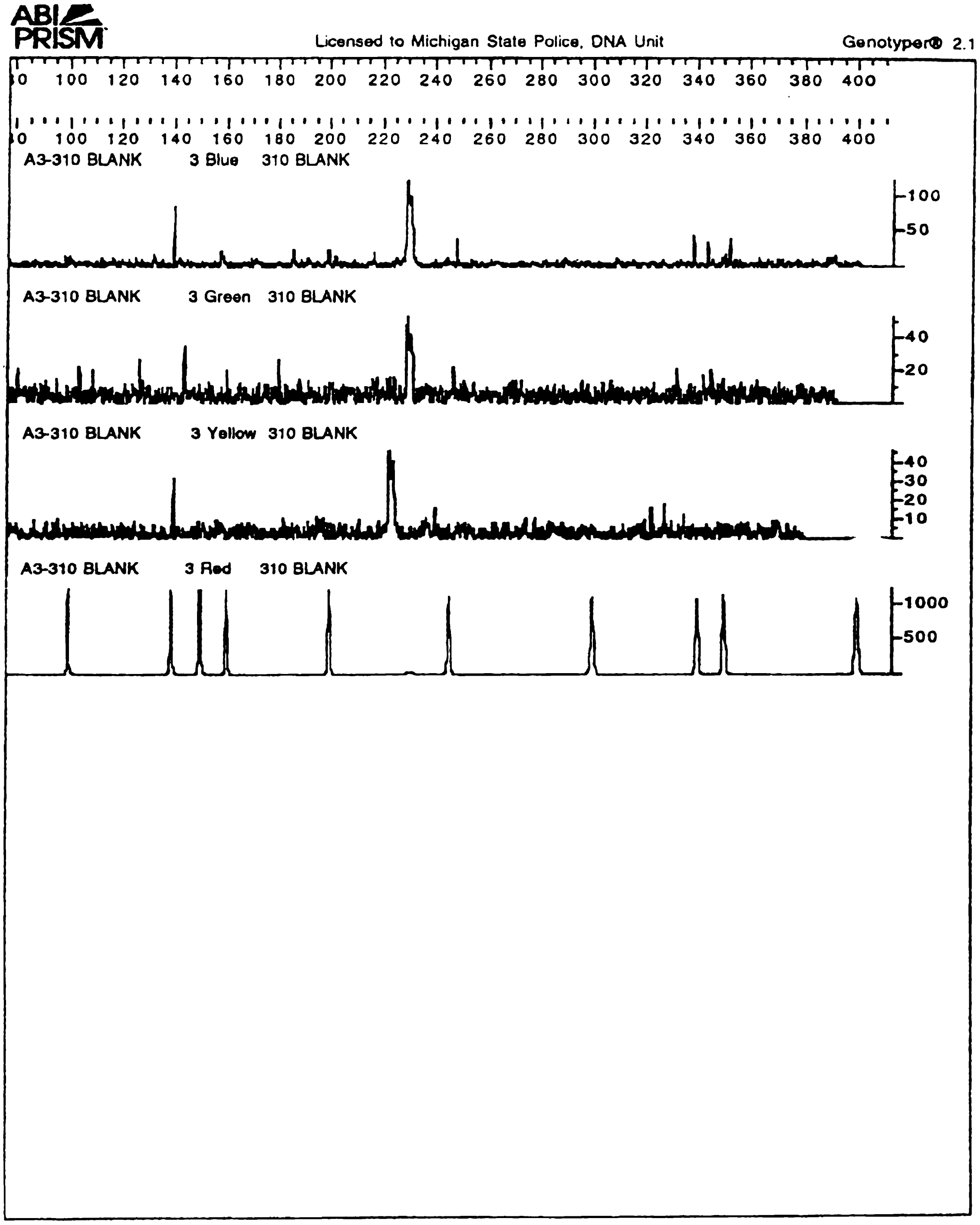

ABI PRISM
Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360
D6-BB-5(3UL) 23 Blue BB-5(3UL)
1500
1000
500
16
1838
18
1370
24
434
26
416
D6-BB-5(3UL) 23 Green BB-5(3UL)
900
600
300
X
1034
Y
870
14
707
15
653
28
629
30
518
15
256
16
151
D6-BB-5(3UL) 23 Yellow BB-5(3UL)
600
400
200
11
511
12
649
11
661
12
465
D6-BB-5(3UL) 23 Red BB-5(3UL)
600
400
200


ABI PRISM
Thesis data - concentrated amp. product
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360
100 120 140 160 180 200 220 240 260 280 300 320 340 360
D4-L-15 22 Blue L-15'
1500
1000
500
16
1810
18
776
19
539
22
549
23
355
D4-L-15 22 Green L-15
2000
1000
X
2567
8
698
11
635
29
479
30
534
12
428
13
415
D4-L-15 22 Yellow L-15
400
200
12
583
13
519
11
426
12
351
9
264
13
178
D4-L-15 22 Red L-15
900
600
300

APPENDIX F

Electropherograms generated from ladders and quality control samples.


ABI PRISM
Thesis data
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340 360
0 100 120 140 160 180 200 220 240 260 280 300 320 340 360
A3-310 BLANK 3 Blue 310 BLANK
80
60
40
20
A3-310 BLANK 3 Green 310 BLANK
A3-310 BLANK 3 Yellow 310 BLANK
30
20
10
A3-310 BLANK 3 Red 310 BLANK
1000
500



Licensed to Michigan State Police, DNA Unit

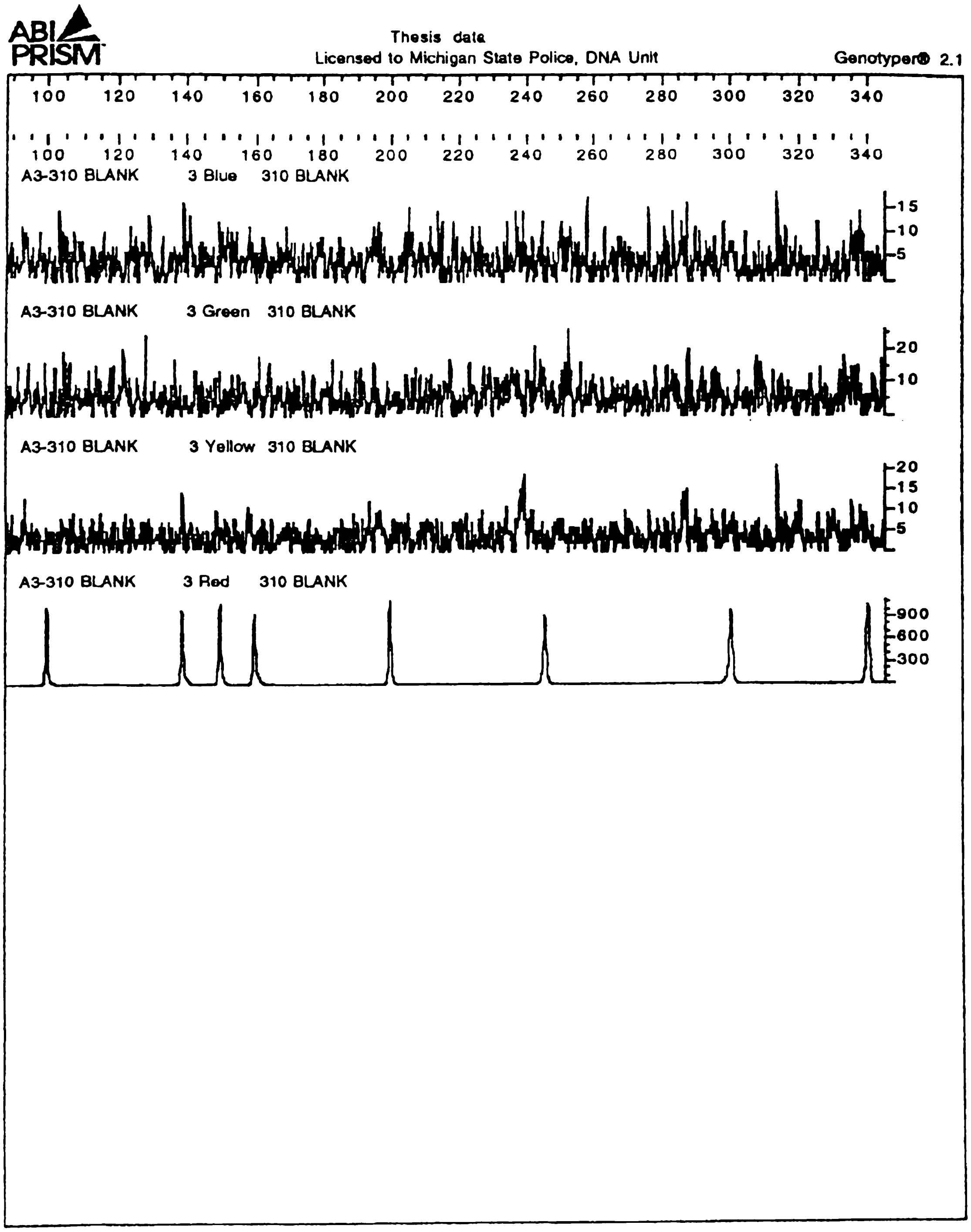
Genotyper® 2.1

A3-310 BLANK 3 Blue 310 BLANK

A3-310 BLANK 3 Green 310 BLANK

A3-310 BLANK 3 Yellow 310 BLANK

A3-310 BLANK 3 Red 310 BLANK



Thesis data

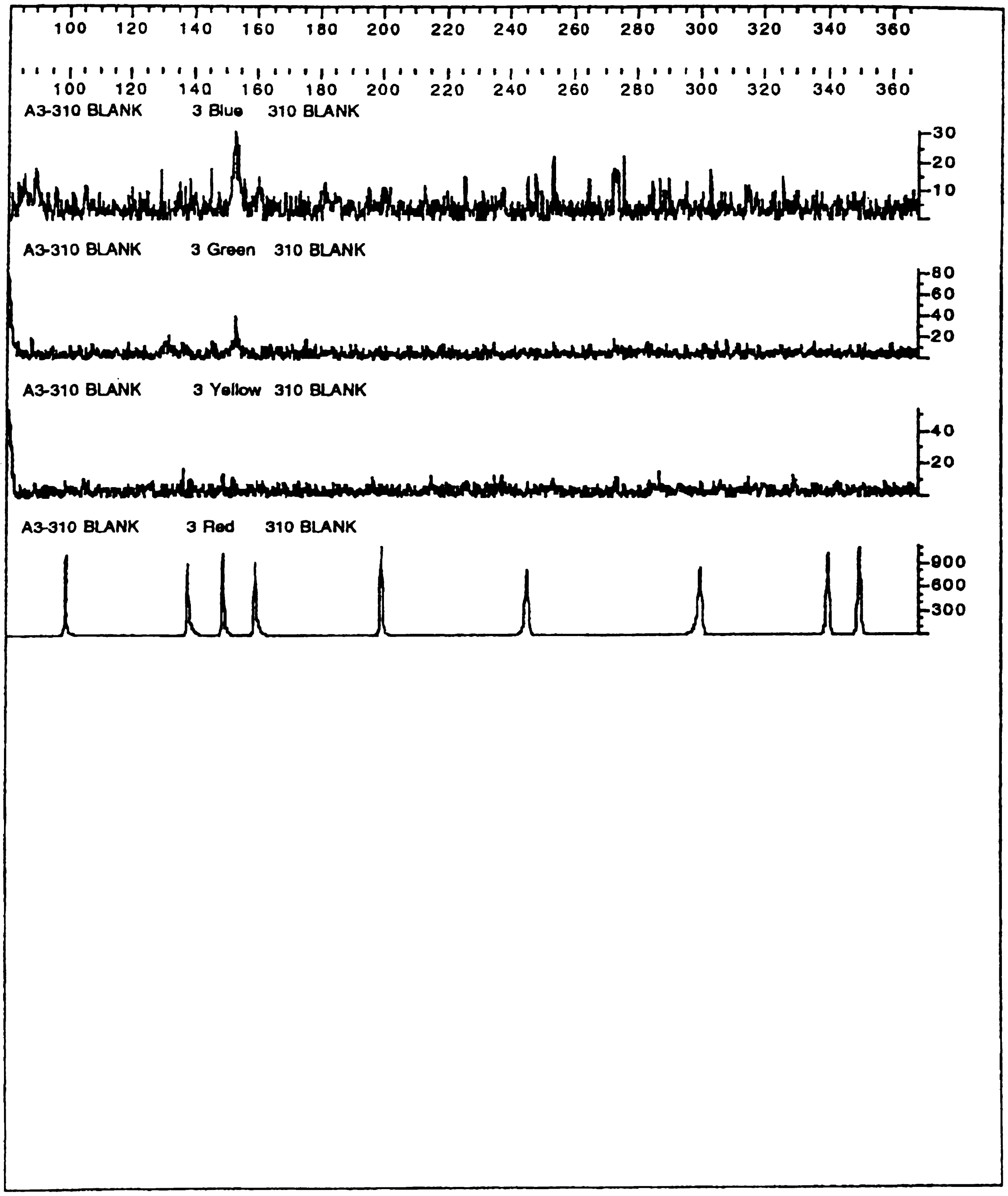
Licensed to Michigan State Police, DNA Unit

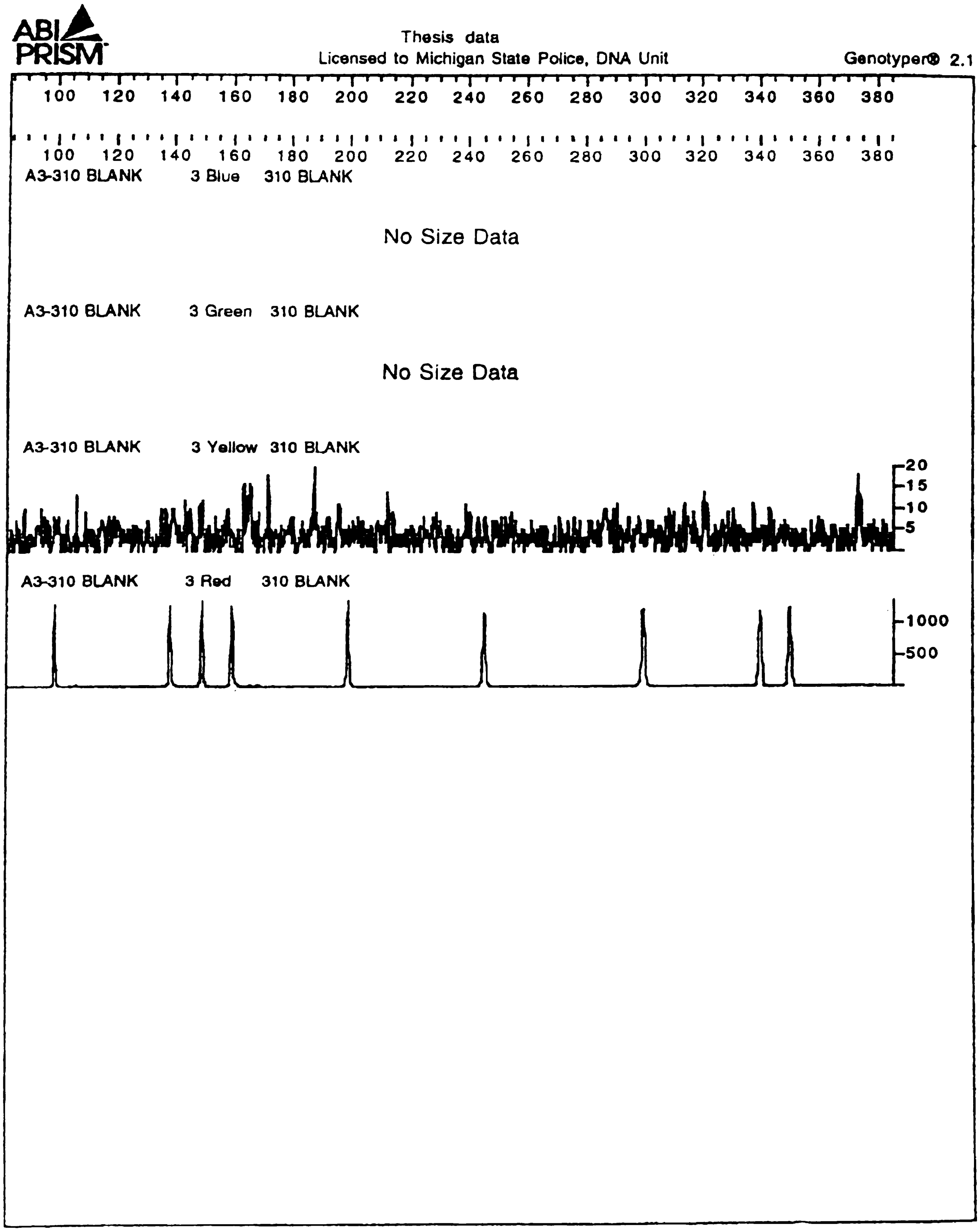
Genotyper® 2.1

Thesis data

Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1





Thesis data
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1

A3-310 BLANK 3 Blue 310 BLANK

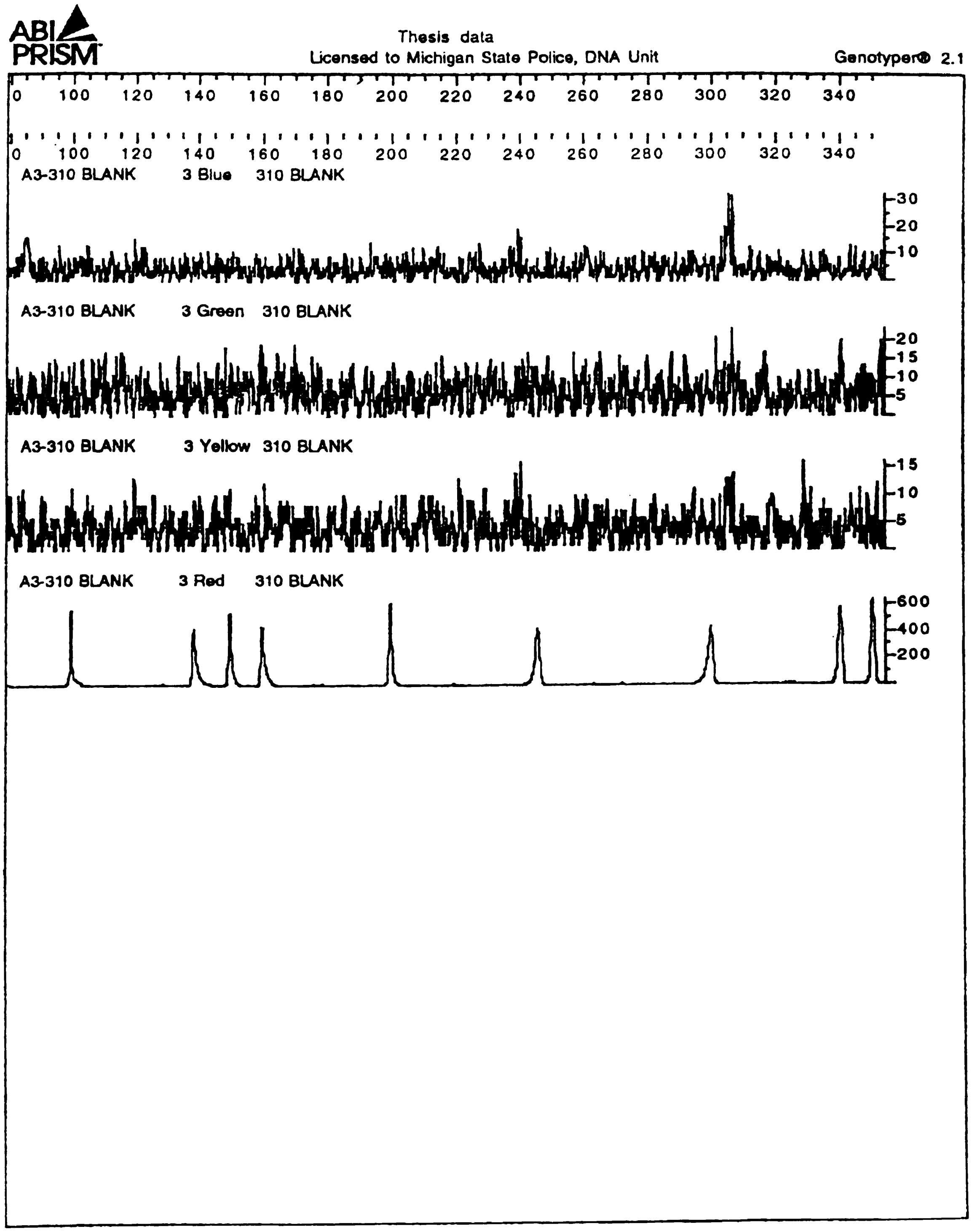
No Size Data

A3-310 BLANK 3 Green 310 BLANK

No Size Data

A3-310 BLANK 3 Yellow 310 BLANK

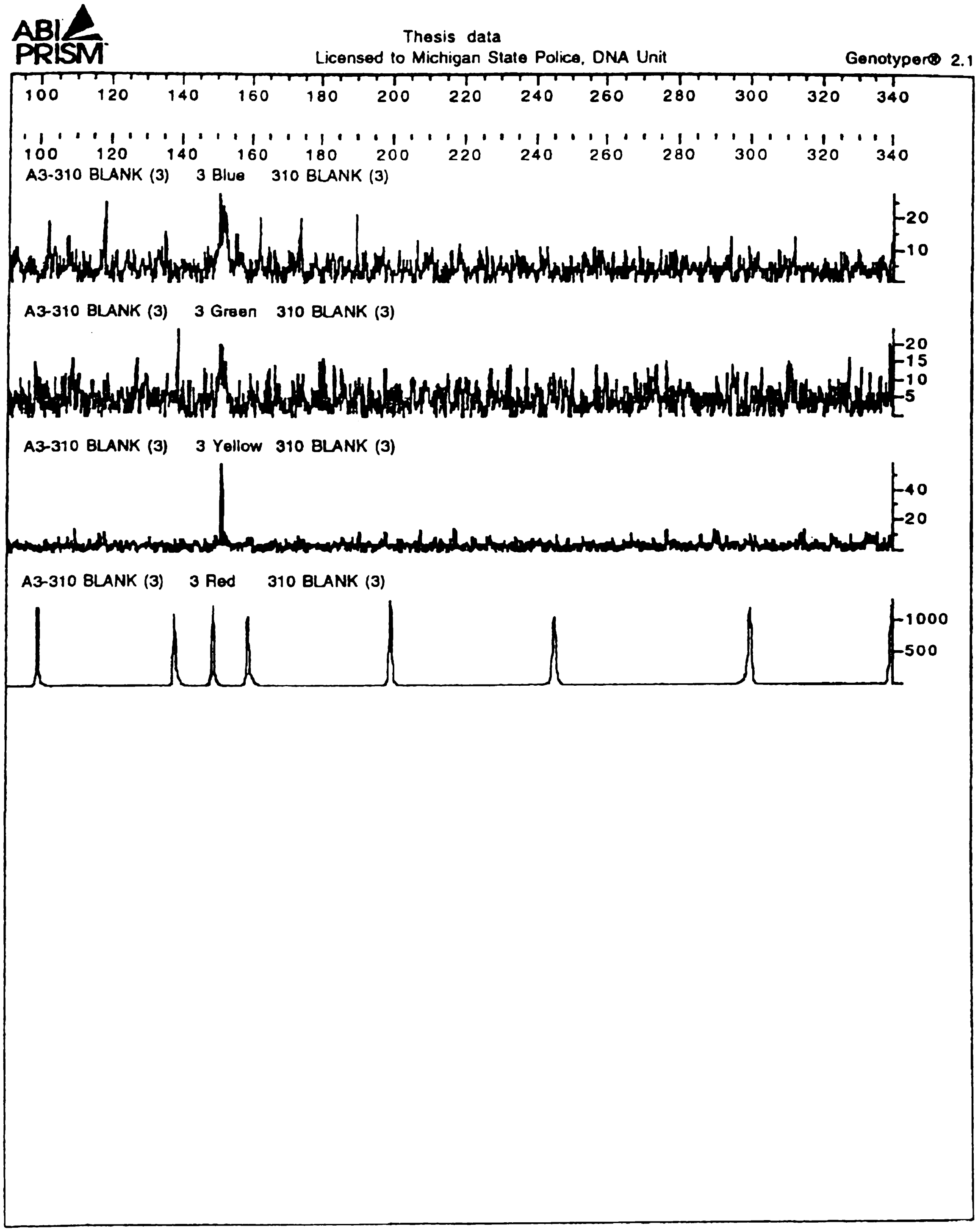
A3-310 BLANK 3 Red 310 BLANK



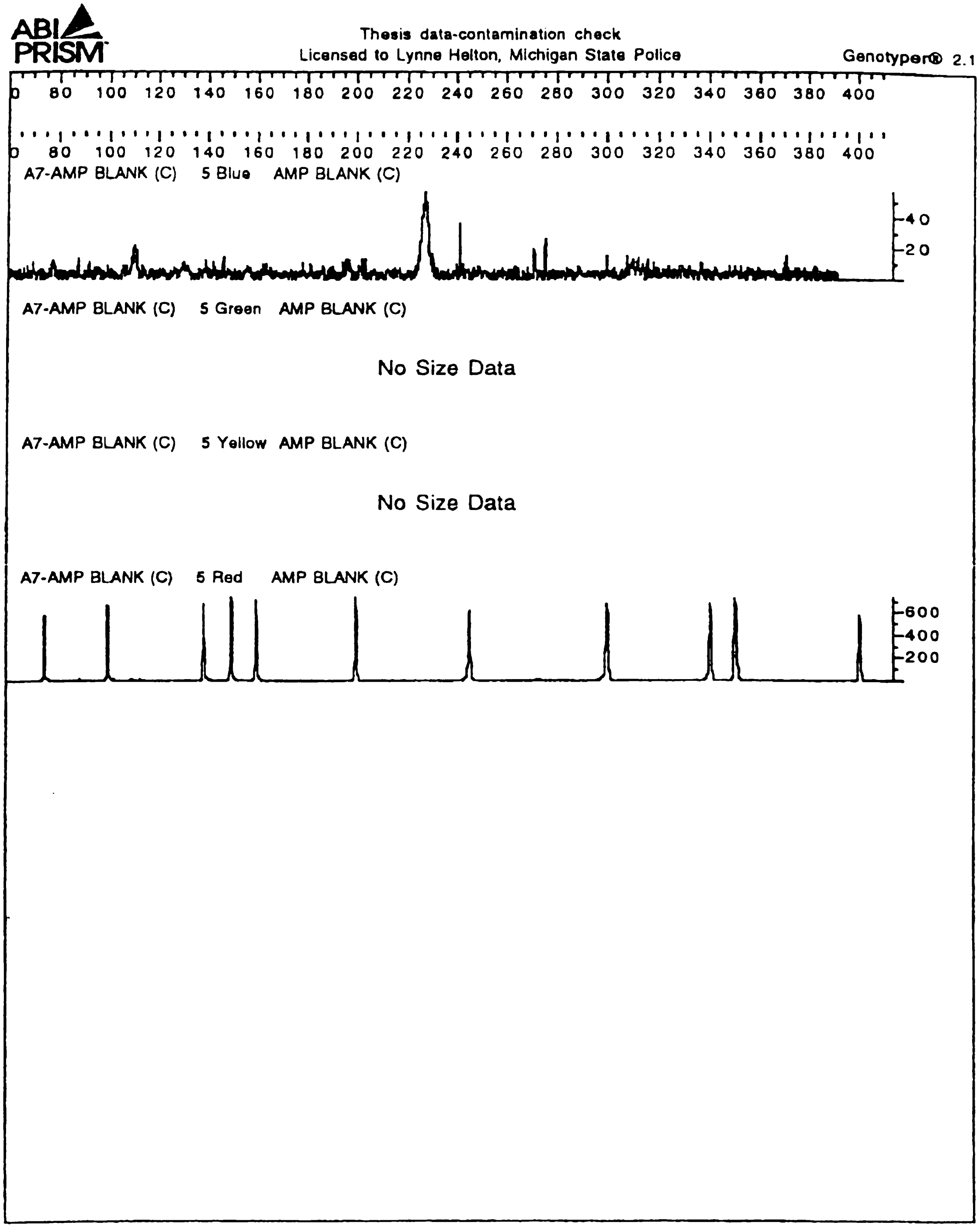
Thesis data

Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1



Thesis data
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1



Thesis data-contamination check

Licensed to Lynne Helton, Michigan State Police

Genotyper® 2.1

A7-AMP BLANK (C) 5 Blue AMP BLANK (C)

A7-AMP BLANK (C) 5 Green AMP BLANK (C)

No Size Data

A7-AMP BLANK (C) 5 Yellow AMP BLANK (C)

No Size Data

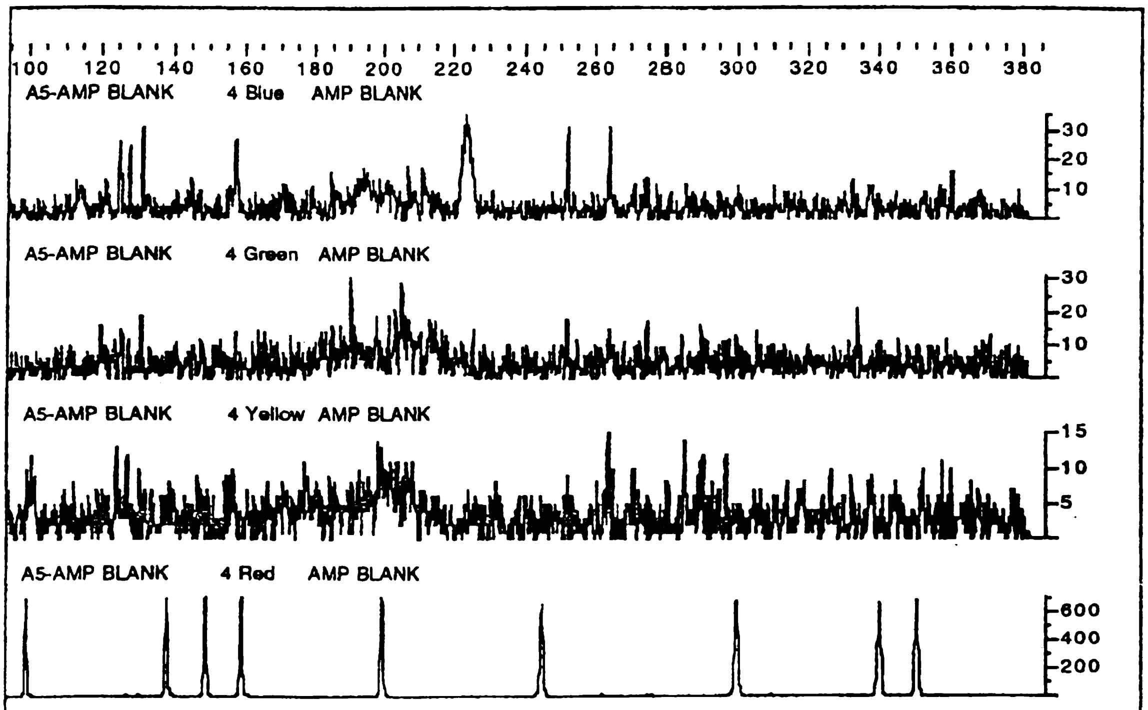
A7-AMP BLANK (C) 5 Red AMP BLANK (C)

ABI PRISM

Thesis data - Group 1

Licensed to Michigan State Police, DNA Unit

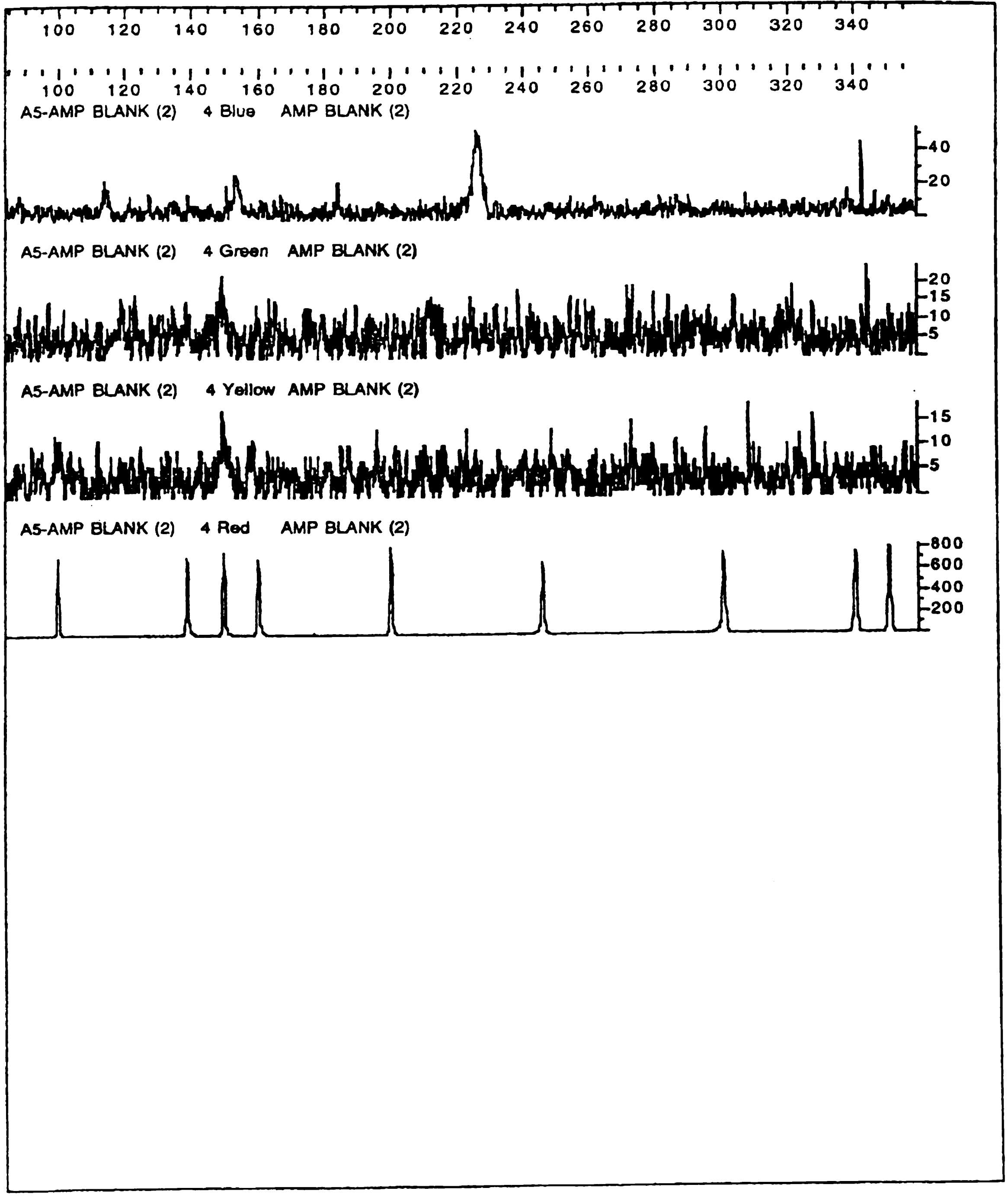
Genotyper® 2.1

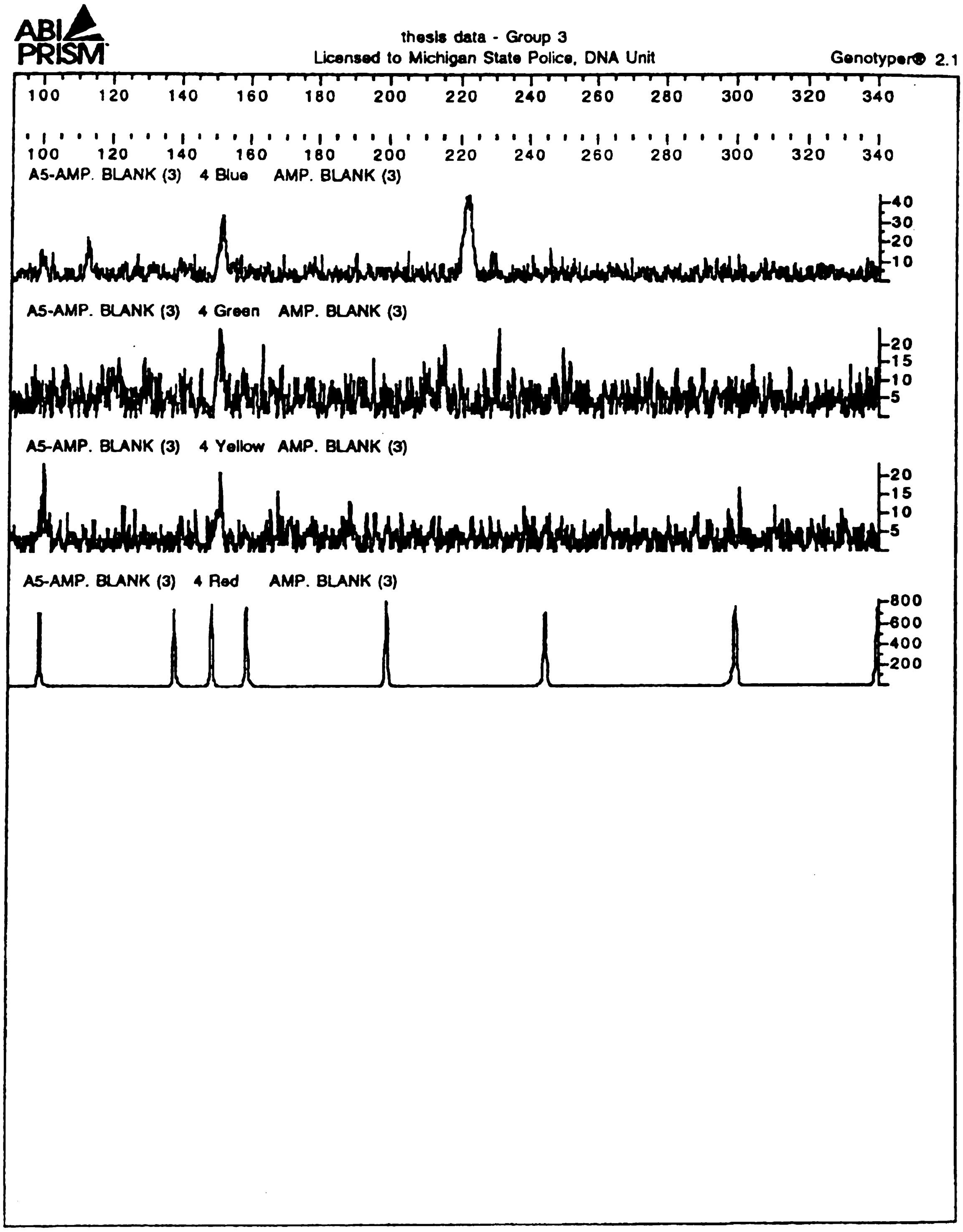


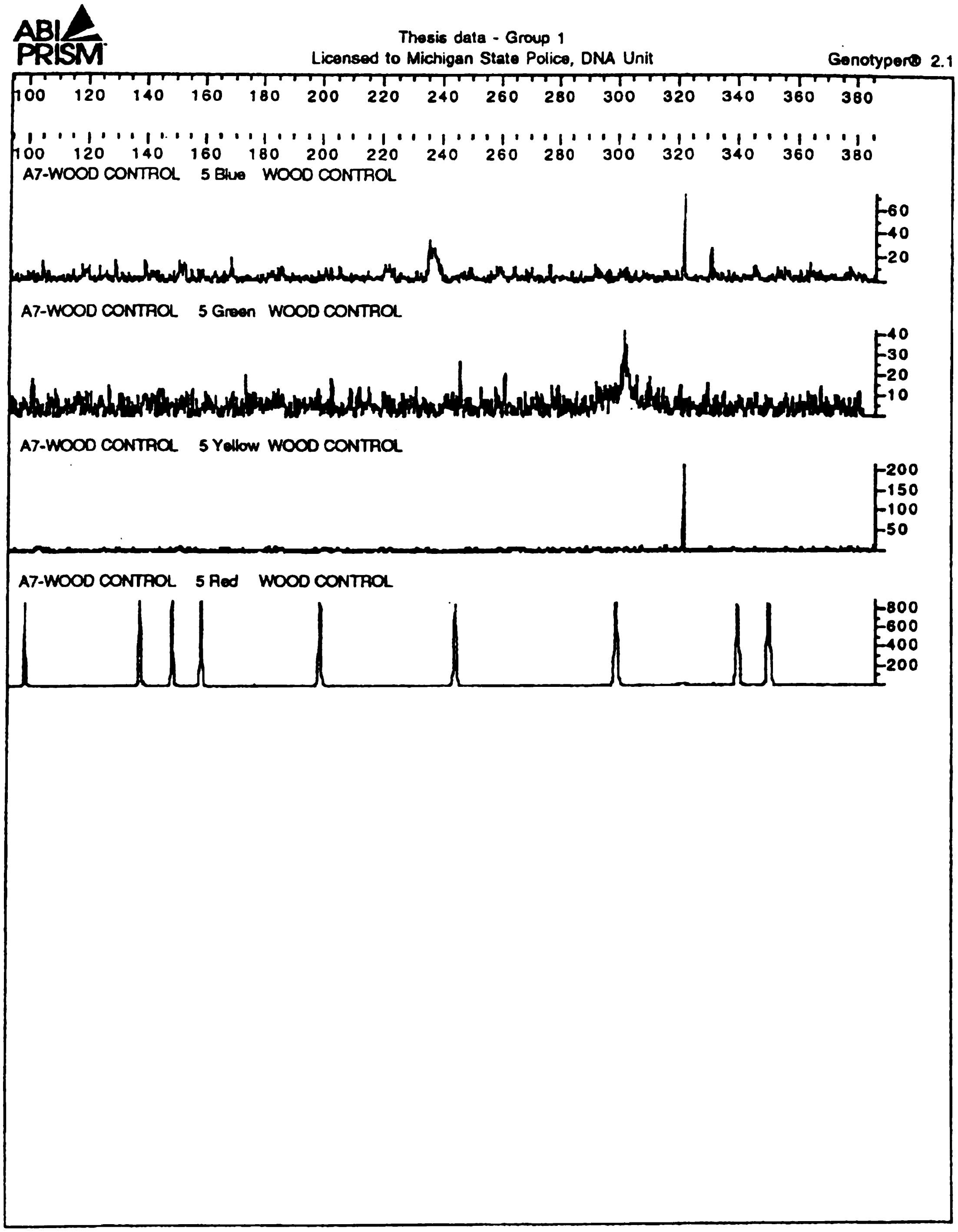
ABI PRISM

Thesis data - Group 2
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1




ABI PRISM
thesis data - Group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
100 120 140 160 180 200 220 240 260 280 300 320 340
A5-AMP. BLANK (3) 4 Blue AMP. BLANK (3)
40
30
20
10
A5-AMP. BLANK (3) 4 Green AMP. BLANK (3)
20
15
10
5
A5-AMP. BLANK (3) 4 Yellow AMP. BLANK (3)
20
15
10
5
A5-AMP. BLANK (3) 4 Red AMP. BLANK (3)
800
600
400
200

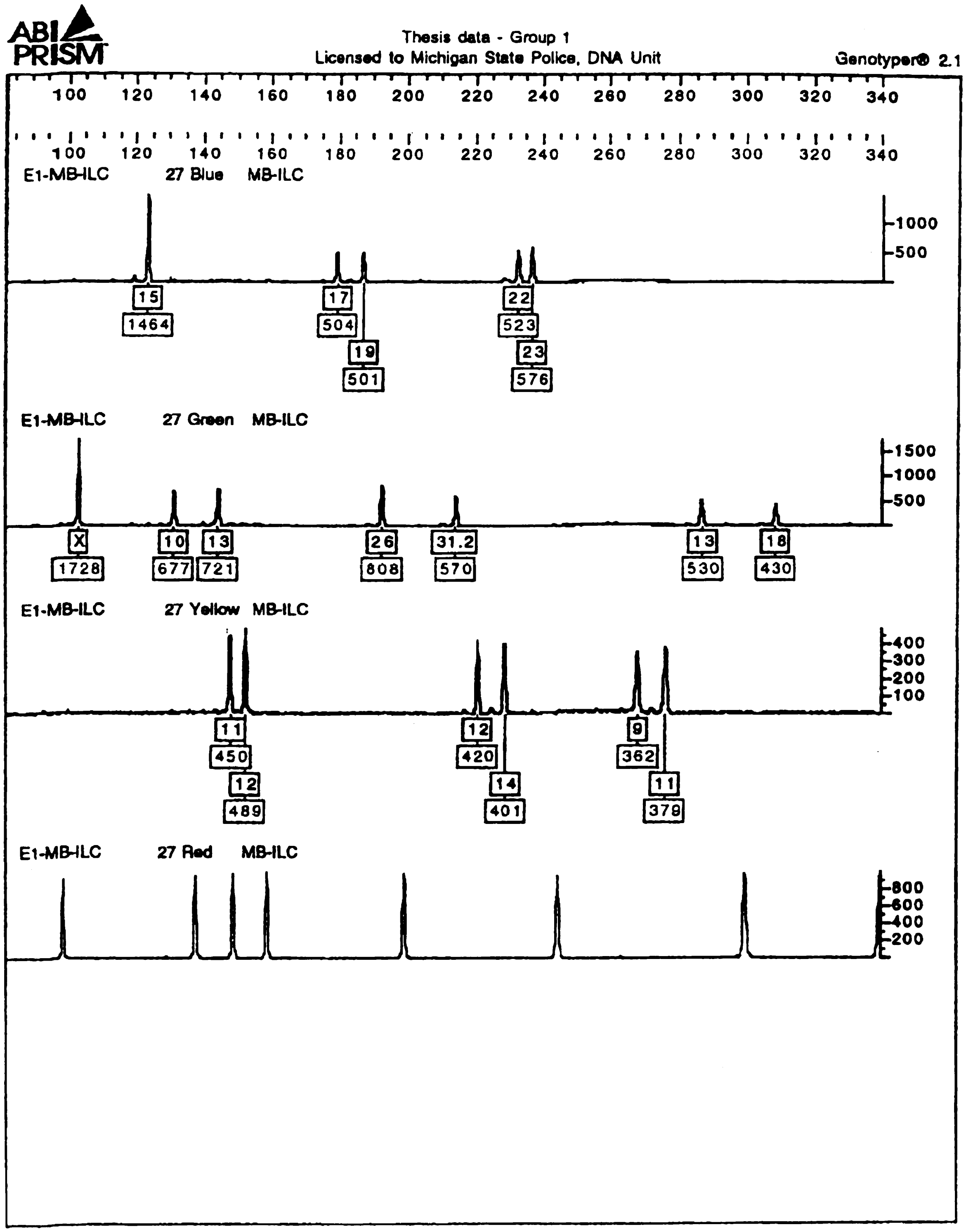


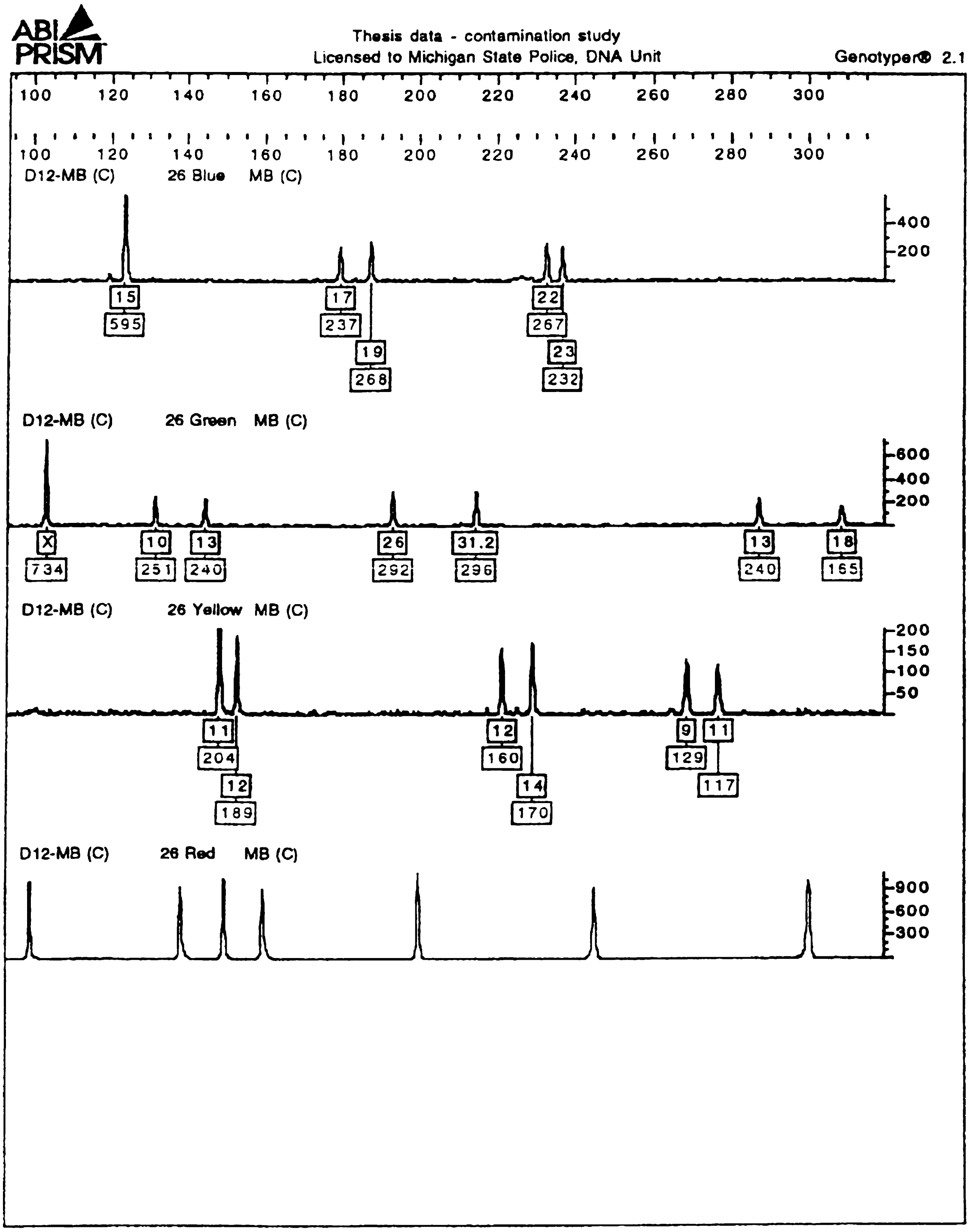
ABI PRISM

Thesis data - Group 1

Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1

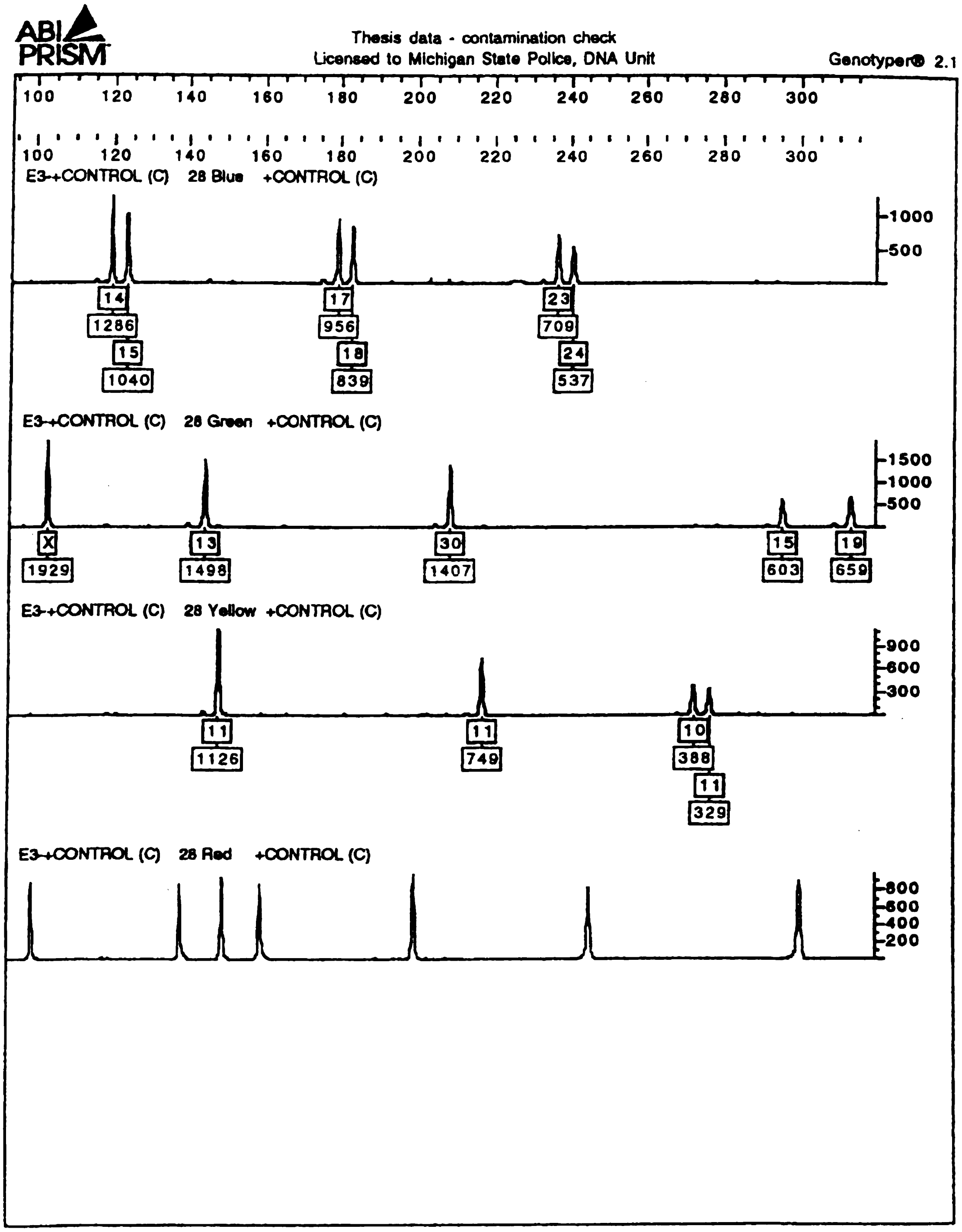

ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340
E1-MB-ILC 27 Blue MB-ILC
1000
500
15
1464
17
504
19
501
22
523
23
576
E1-MB-ILC 27 Green MB-ILC
1500
1000
500
X
1728
10
677
13
721
26
808
31.2
570
13
530
18
430
E1-MB-ILC 27 Yellow MB-ILC
400
300
200
100
11
450
12
489
12
420
14
401
9
362
11
379
E1-MB-ILC 27 Red MB-ILC
800
600
400
200



Thesis data - contamination study

Licensed to Michigan State Police, DNA Unit

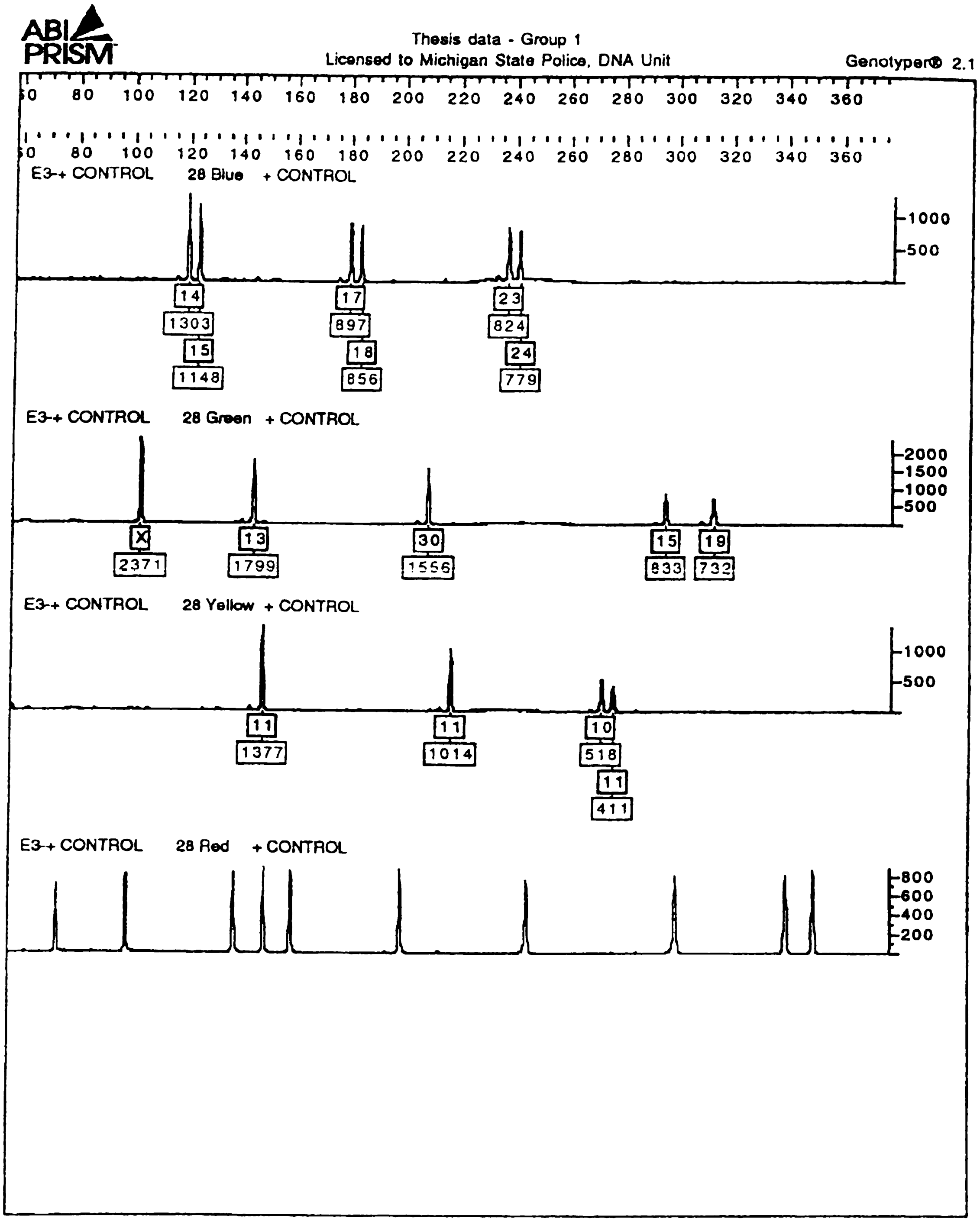
Genotyper® 2.1

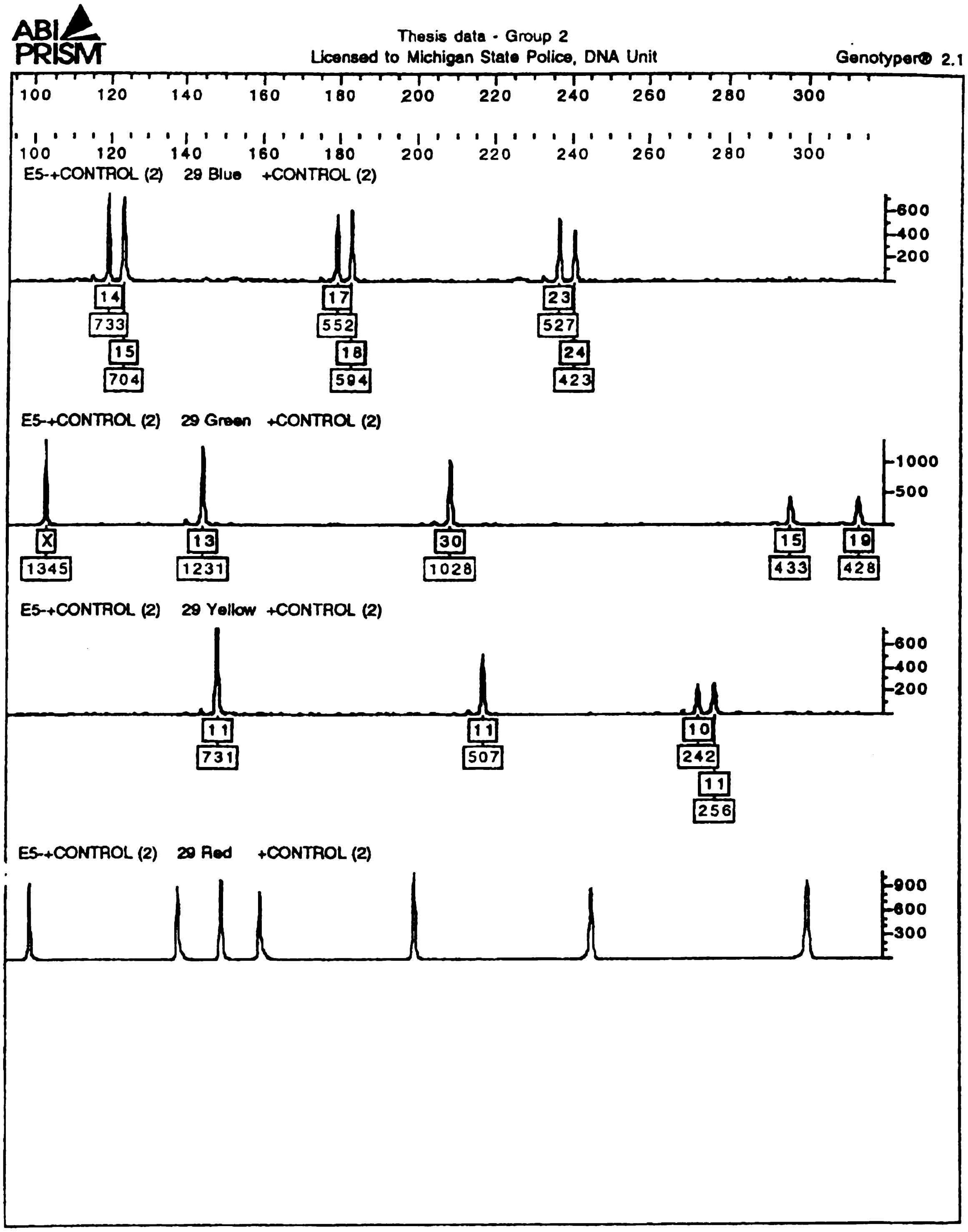


ABI PRISM

Thesis data - contamination check
Licensed to Michigan State Police, DNA Unit

Genotyper® 2.1


ABI PRISM
Thesis data - Group 1
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
E3-+ CONTROL 28 Blue + CONTROL
1000
500
14
1303
15
1148
17
897
18
856
23
824
24
779
E3-+ CONTROL 28 Green + CONTROL
2000
1500
1000
500
X
2371
13
1799
30
1556
15
833
19
732
E3-+ CONTROL 28 Yellow + CONTROL
1000
500
11
1377
11
1014
10
518
11
411
E3-+ CONTROL 28 Red + CONTROL
800
600
400
200


ABI PRISM

Thesis data - Group 2
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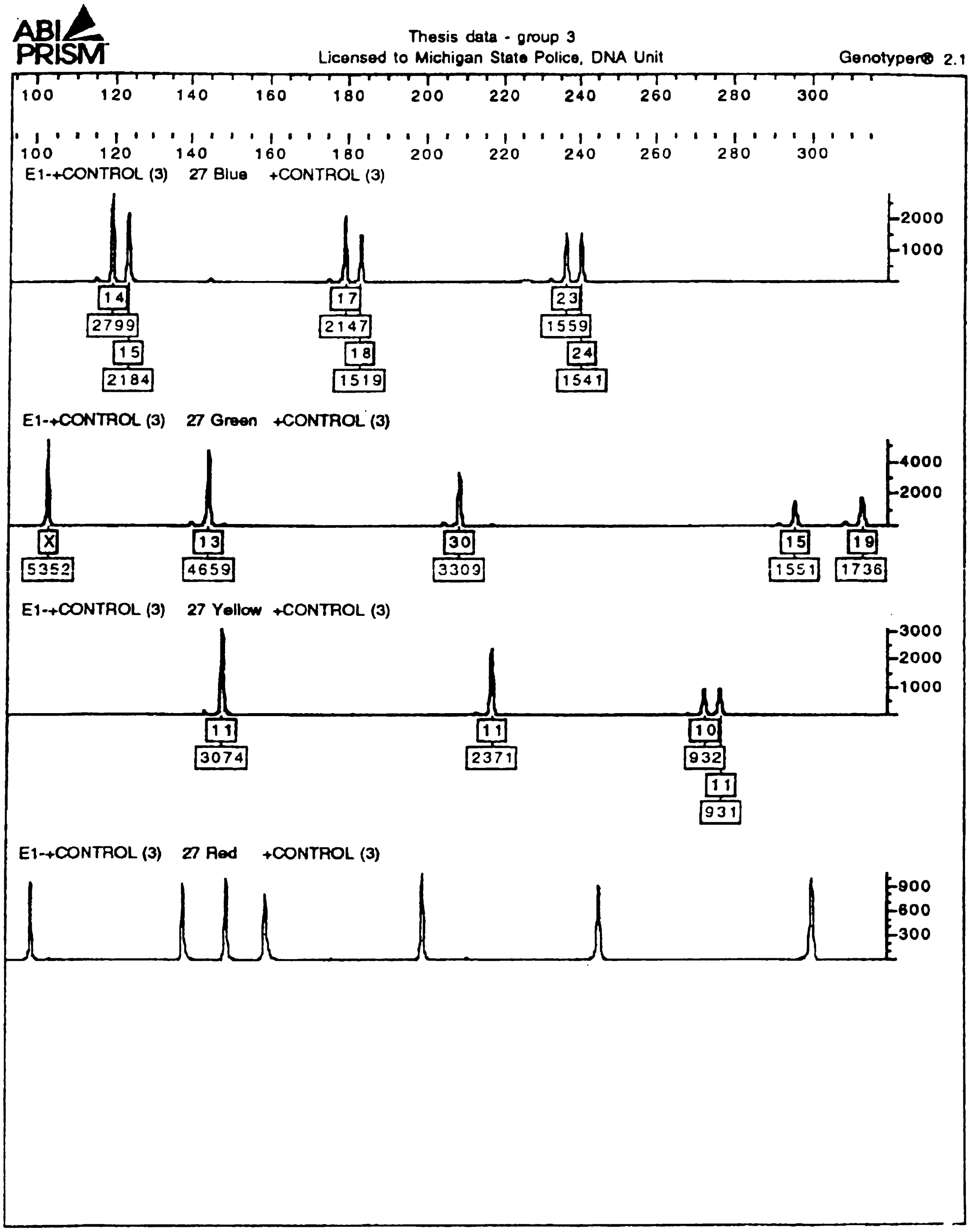
Genotyper® 2.1

E5-+CONTROL (2) 29 Blue +CONTROL (2)

E5-+CONTROL (2) 29 Green +CONTROL (2)

E5-+CONTROL (2) 29 Yellow +CONTROL (2)

E5-+CONTROL (2) 29 Red +CONTROL (2)


ABI PRISM
Thesis data - group 3
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300
E1-+CONTROL (3) 27 Blue +CONTROL (3)
2000
1000
14
2799
15
2184
17
2147
18
1519
23
1559
24
1541
E1-+CONTROL (3) 27 Green +CONTROL (3)
4000
2000
X
5352
13
4659
30
3309
15
1551
19
1736
E1-+CONTROL (3) 27 Yellow +CONTROL (3)
3000
2000
1000
11
3074
11
2371
10
932
11
931
E1-+CONTROL (3) 27 Red +CONTROL (3)
900
600
300

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Thesis data

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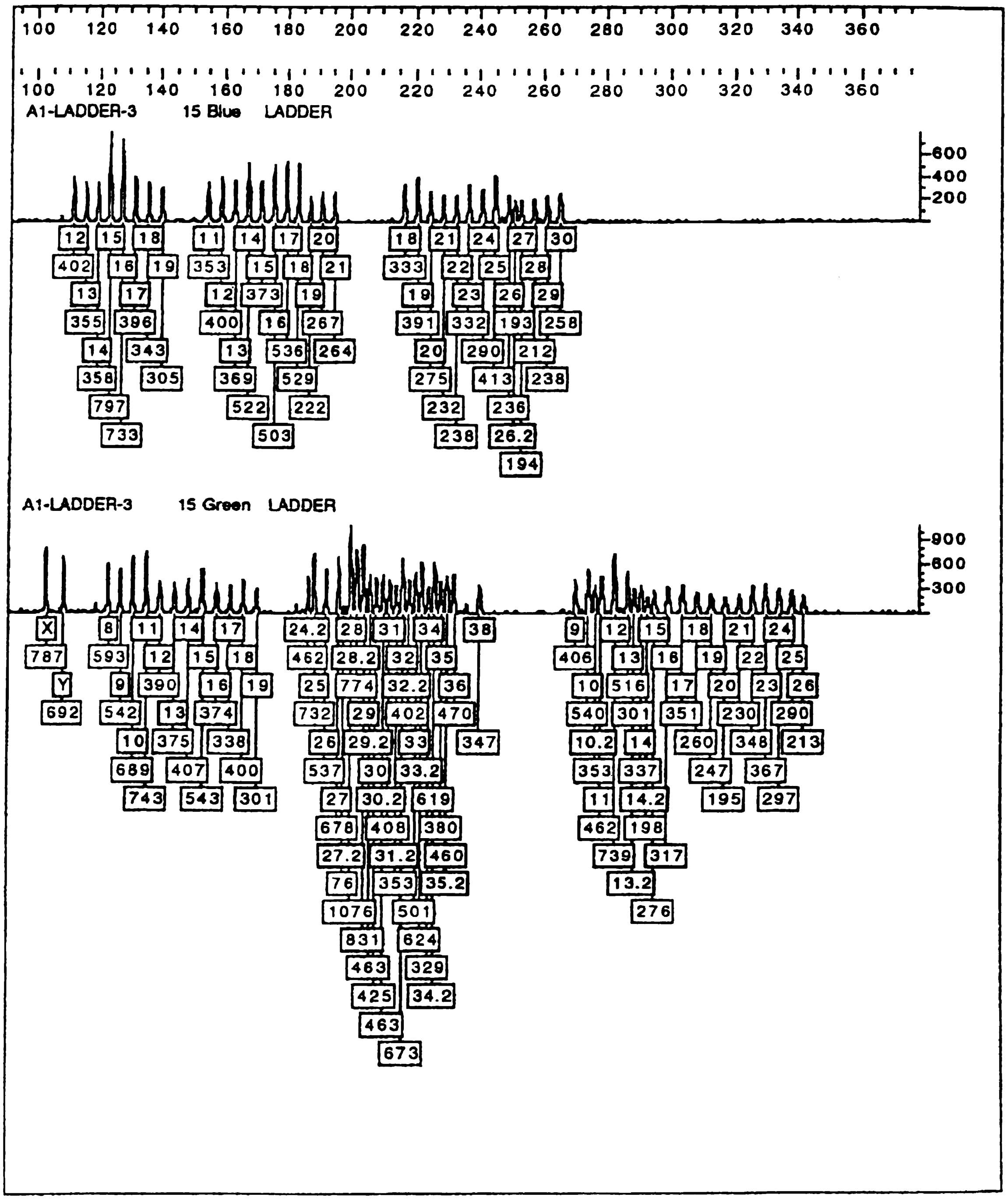
Genotyper® 2.1



ABI PRISM

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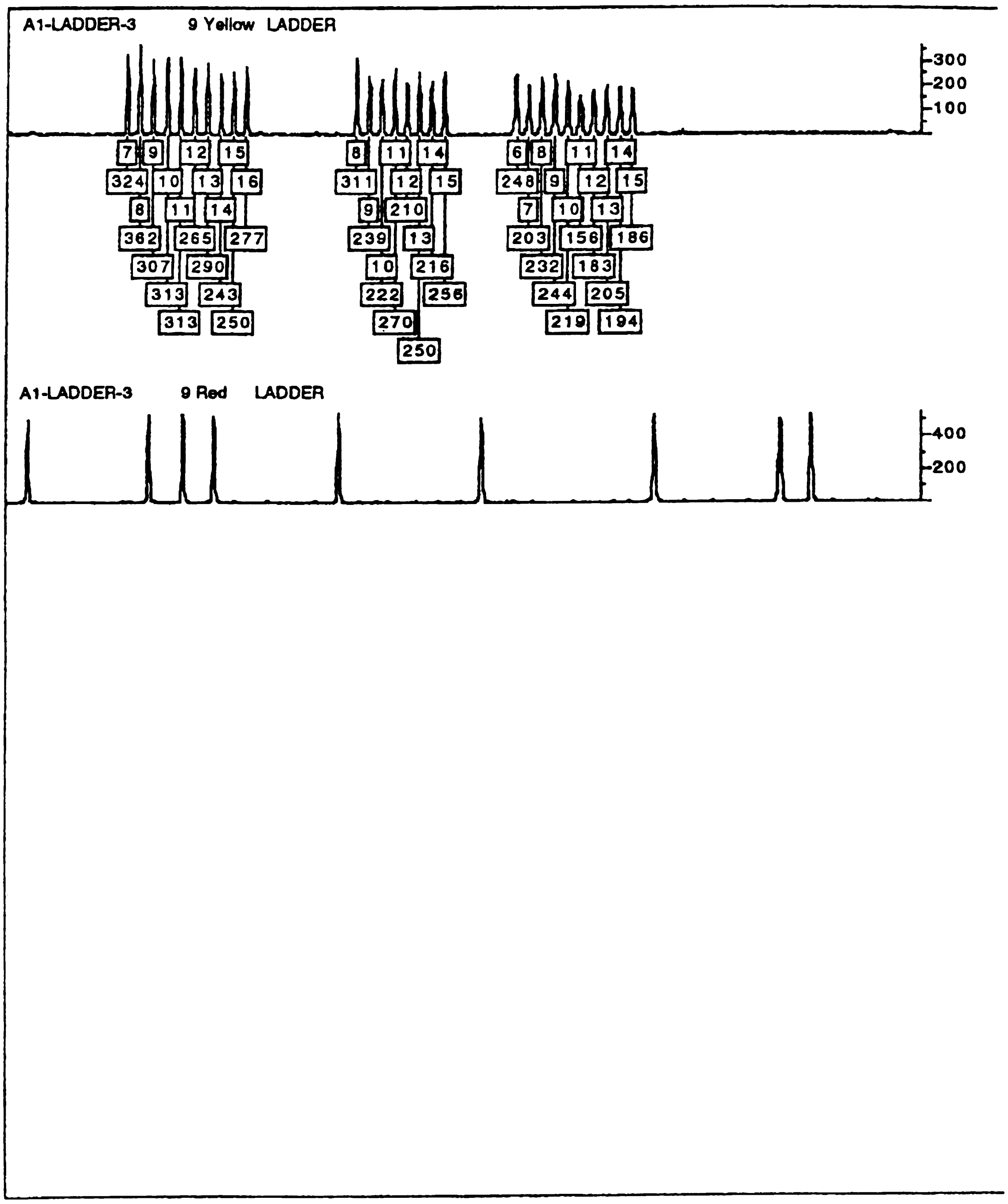
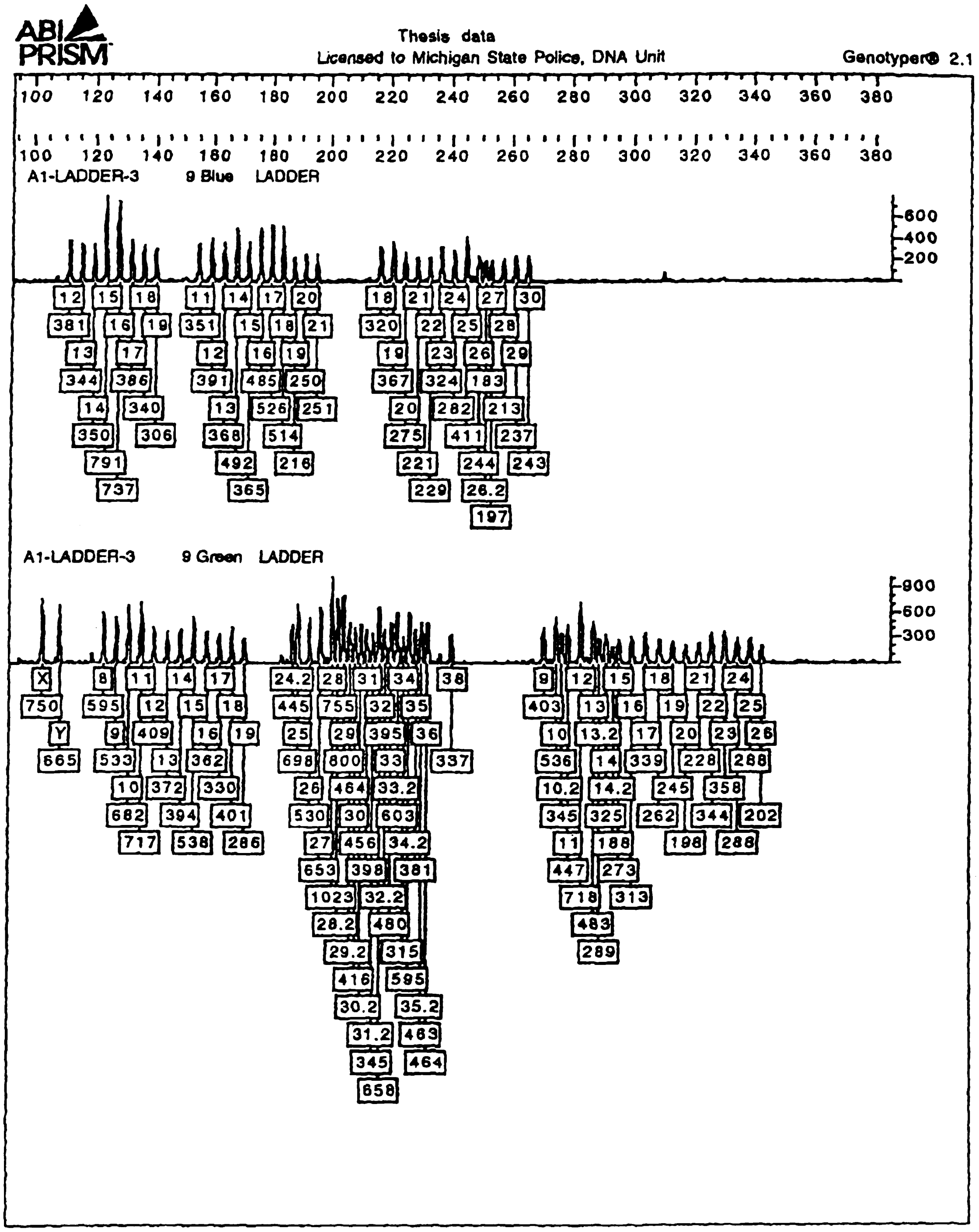
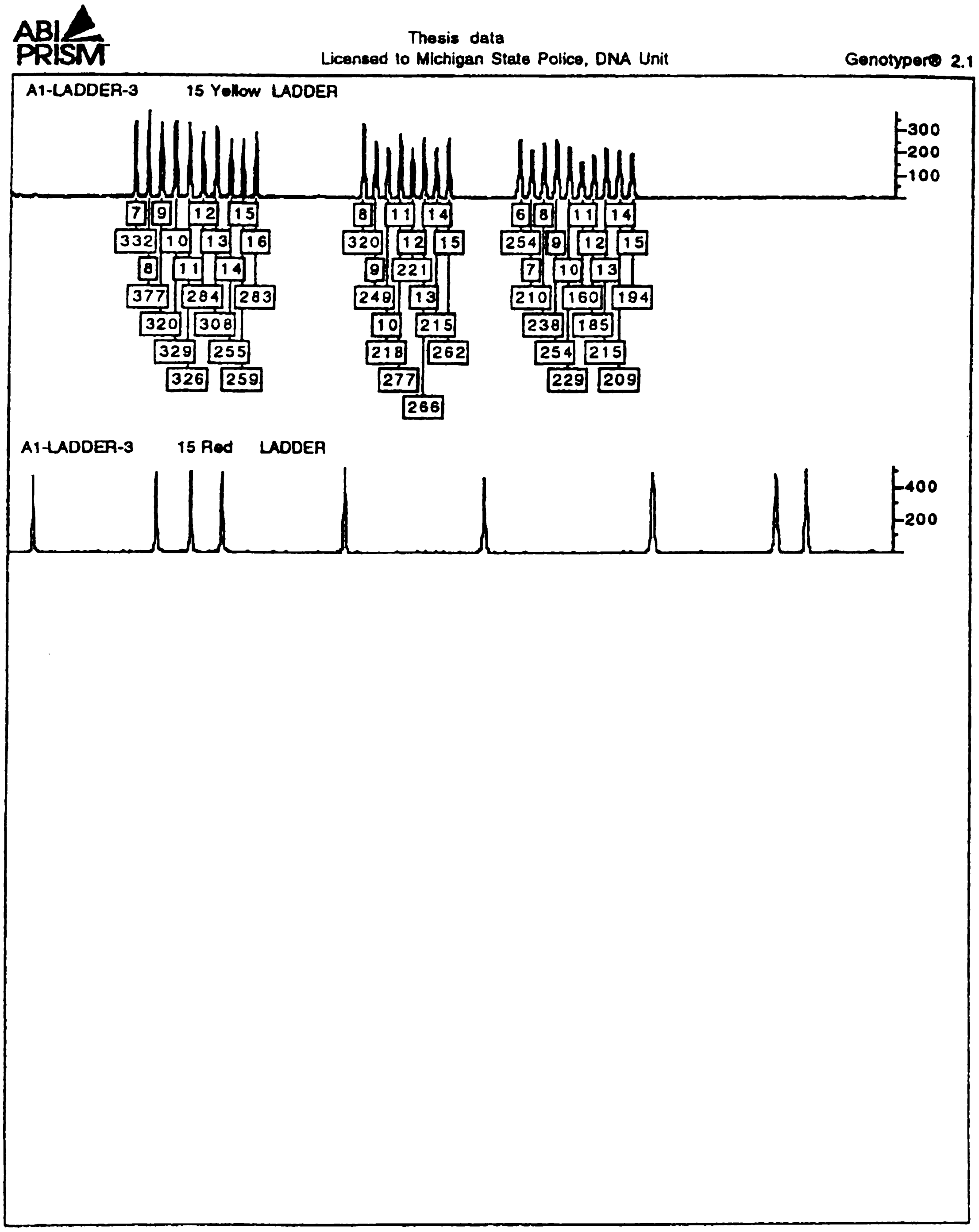
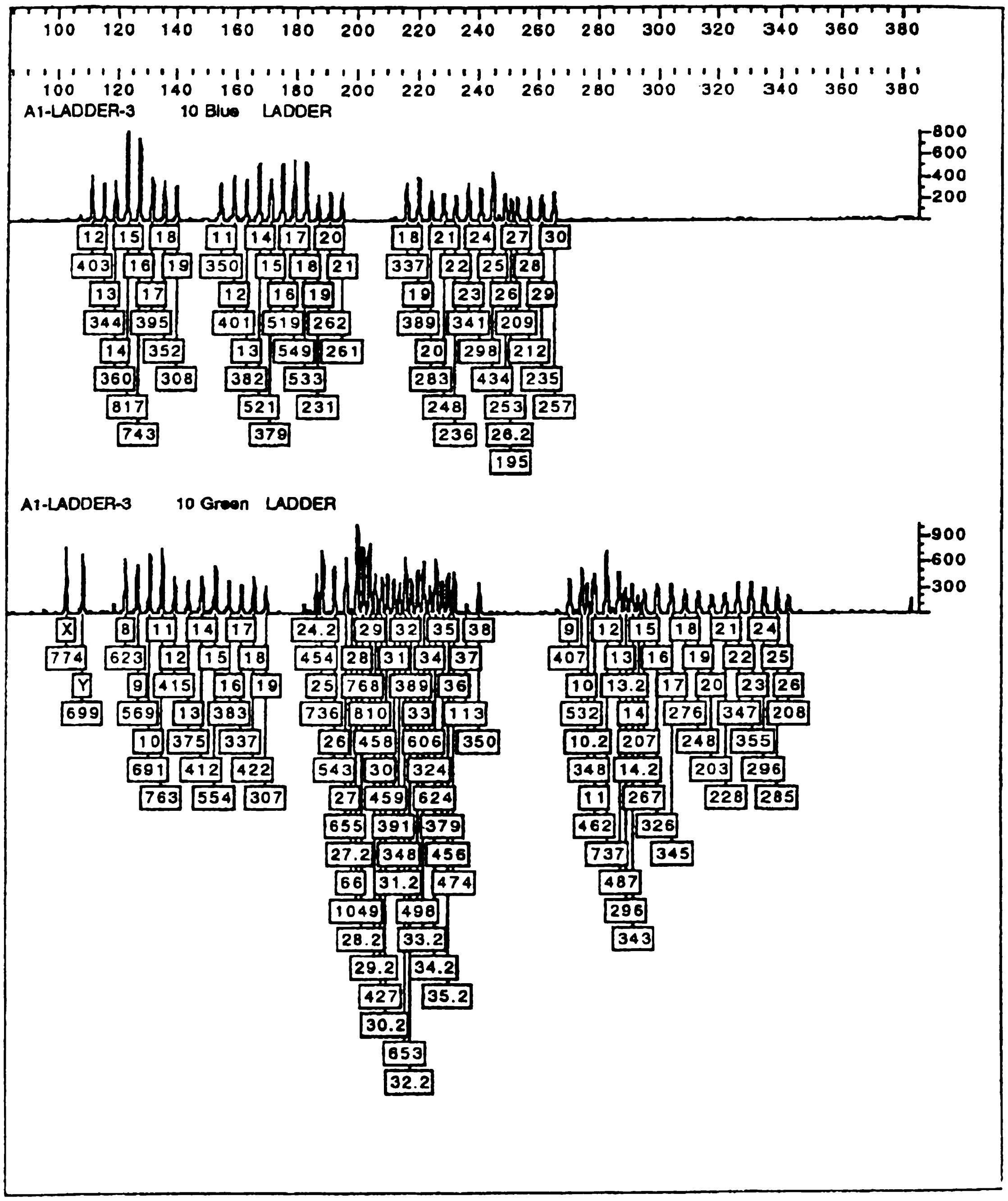
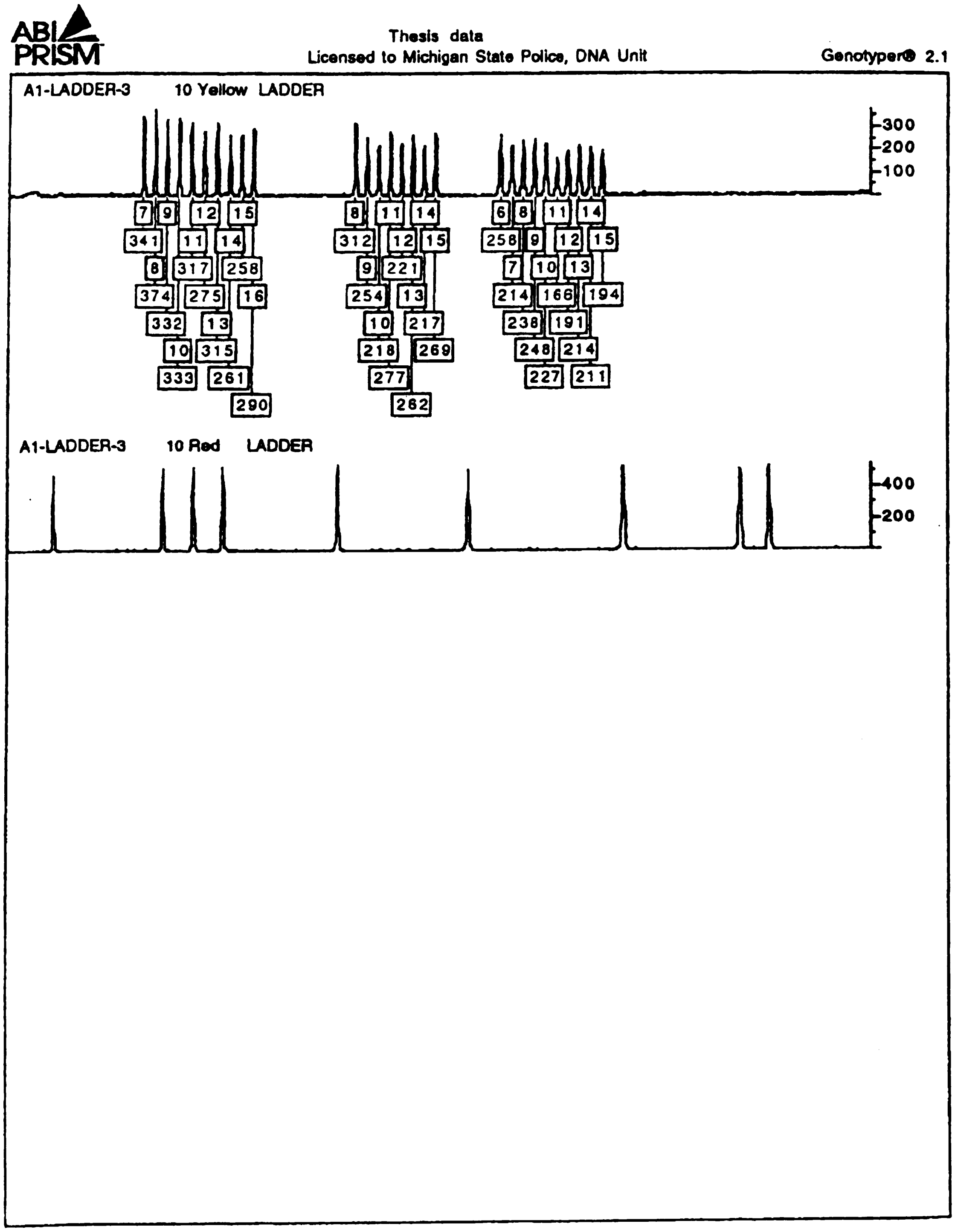



ABI PRISM
Thesis data
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
A1-LADDER-3 9 Blue LADDER
600
400
200
A1-LADDER-3 9 Green LADDER
900
600
300
X
750
Y
665


ABI PRISM
Thesis data
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
A1-LADDER-3 15 Yellow LADDER
300
200
100
7 9 12 15
332 10 13 16
8 11 14
377 284 283
320 308
329 255
326 259
8 11 14
320 12 15
9 221
249 13
10 215
218 262
277
266
6 8 11 14
254 9 12 15
7 10 13
210 160 194
238 185
254 215
229 209
A1-LADDER-3 15 Red LADDER
400
200

Thesis data
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1




Thesis data

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Genotyper® 2.1

ABI PRISM

Thesis data

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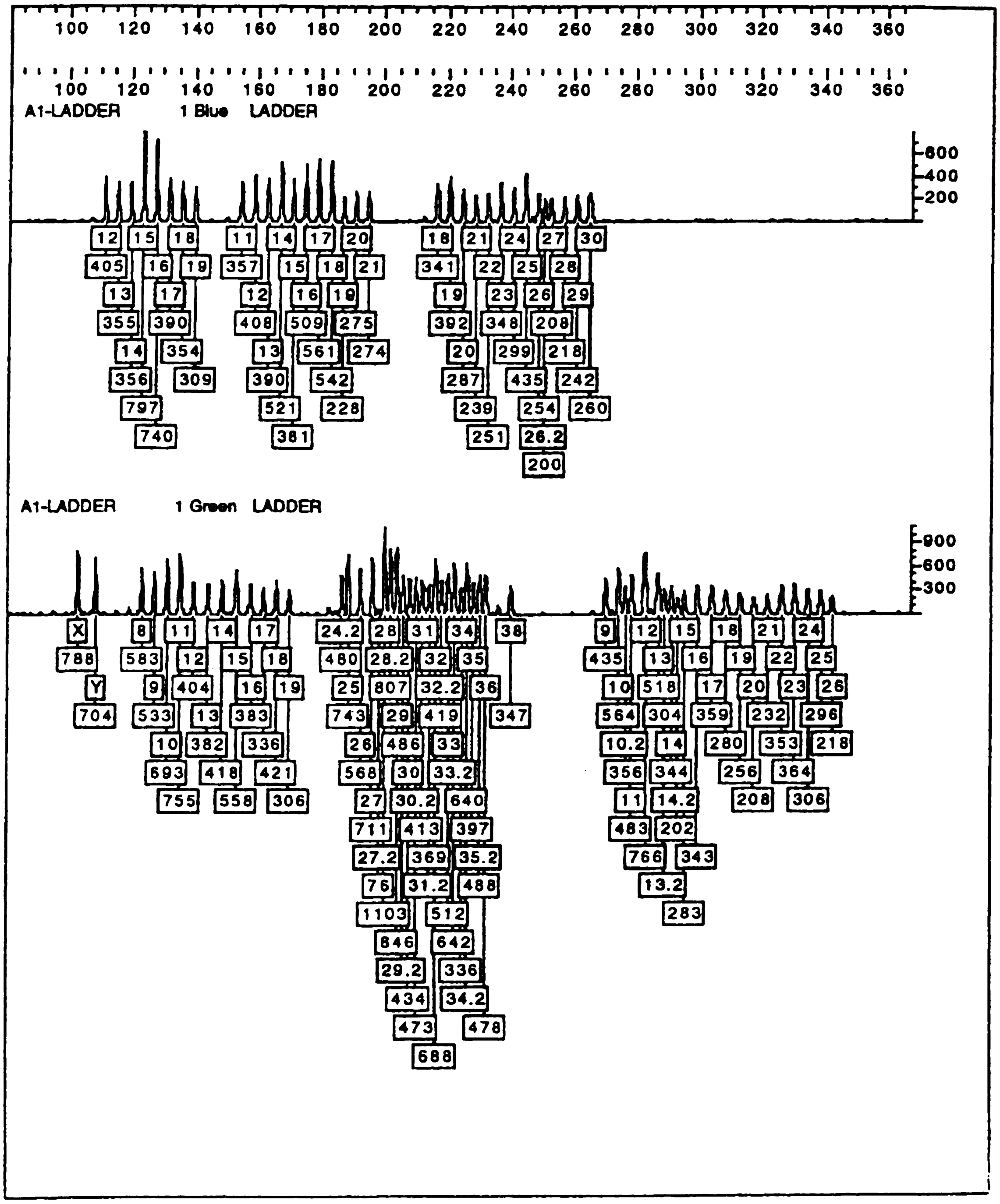
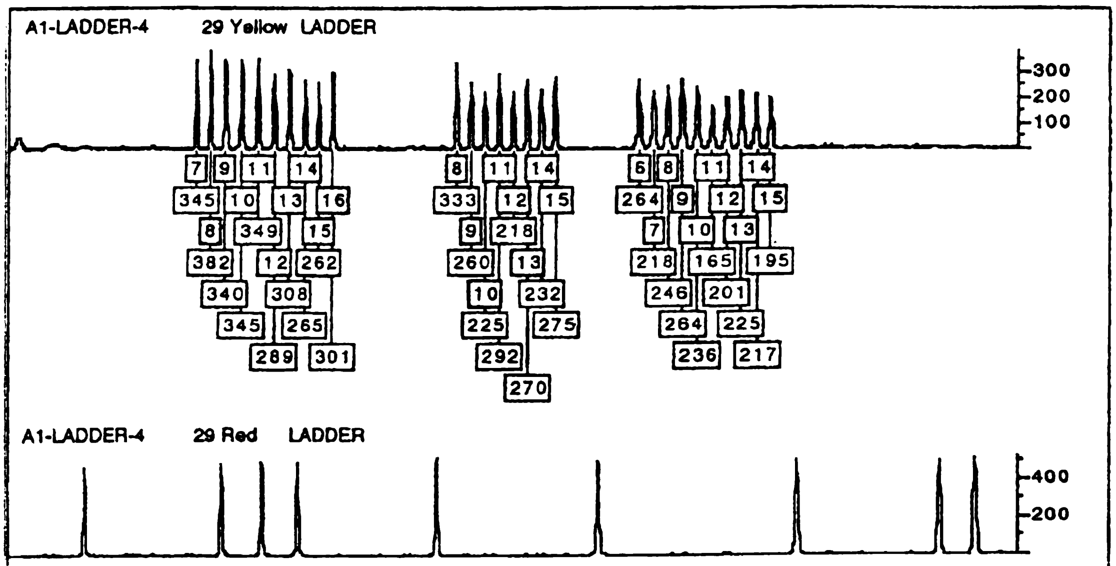
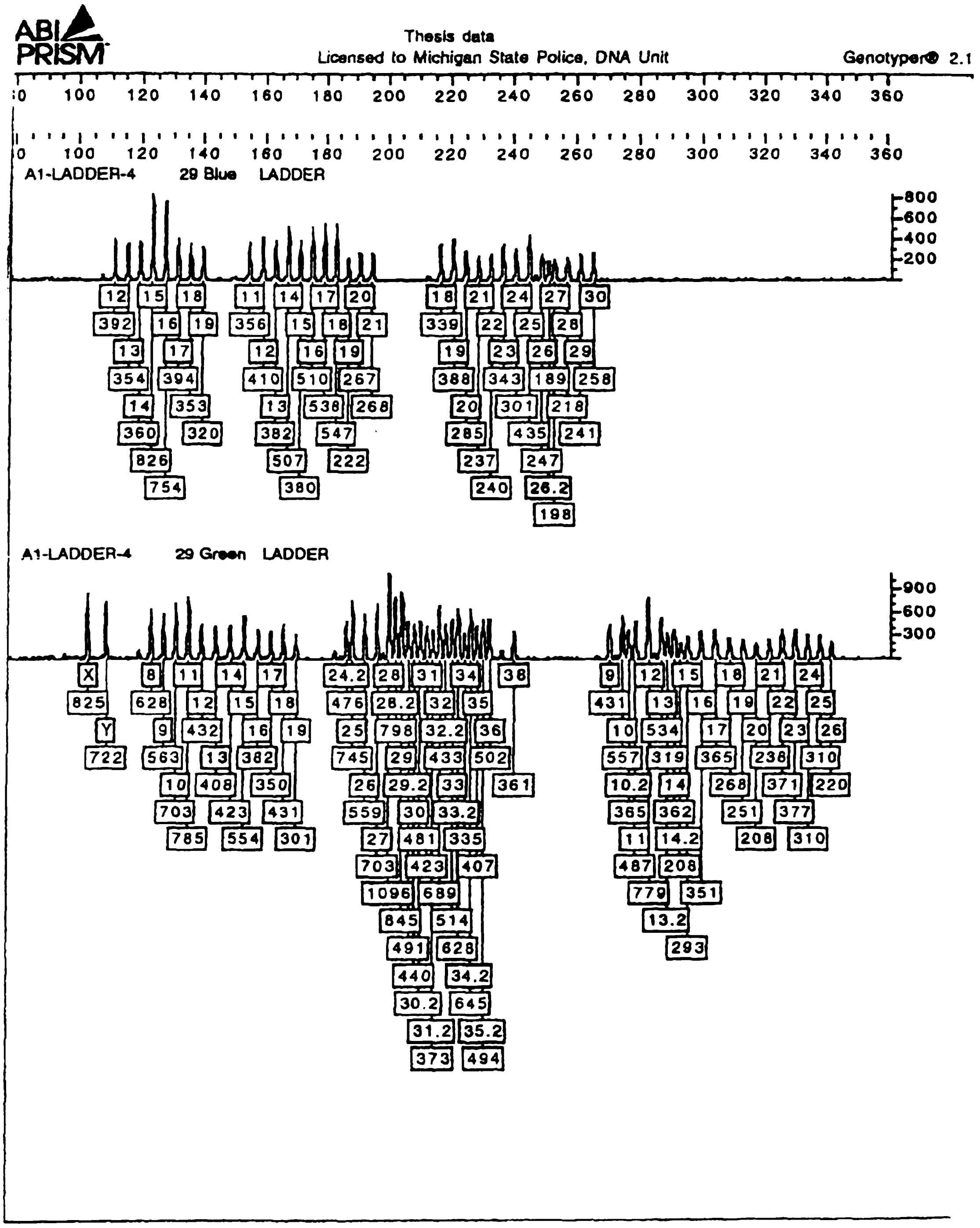
Genotyper® 2.1




A1-LADDER-4
29 Yellow LADDER
-300
-200
-100
7
9
11
14
345
10
13
16
8
349
15
382
12
262
340
308
345
265
289
301
8
11
14
333
12
15
9
218
260
13
10
232
225
275
292
270
6
8
11
14
264
9
12
15
7
10
13
218
165
195
246
201
264
225
236
217
A1-LADDER-4
29 Red LADDER
-400
-200


ABI PRISM
Thesis data
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360
A1-LADDER-4 29 Blue LADDER
800 600 400 200
12 15 18 11 14 17 20 18 21 24 27 30
392 16 19 356 15 18 21 339 22 25 28
13 17 12 16 19 19 23 26 29
354 394 410 510 267 388 343 189 258
14 353 13 538 268 20 301 218
360 320 382 547 285 435 241
826 507 222 237 247
754 380 240 26.2
198
A1-LADDER-4 29 Green LADDER
900 600 300
X 8 11 14 17 24.2 28 31 34 38 9 12 15 18 21 24
825 628 12 15 18 476 28.2 32 35 431 13 16 19 22 25
Y 9 432 16 19 25 798 32.2 36 10 534 17 20 23 26
722 563 13 382 745 29 433 502 557 319 365 238 310
10 408 350 26 29.2 33 361 10.2 14 268 371 220
703 423 431 559 30 33.2 365 362 251 377
785 554 301 27 481 335 11 14.2 208 310
703 423 407 487 208
1096 689 779 351
845 514 13.2
491 628 293
440 34.2
30.2 645
31.2 35.2
373 494

Thesis data
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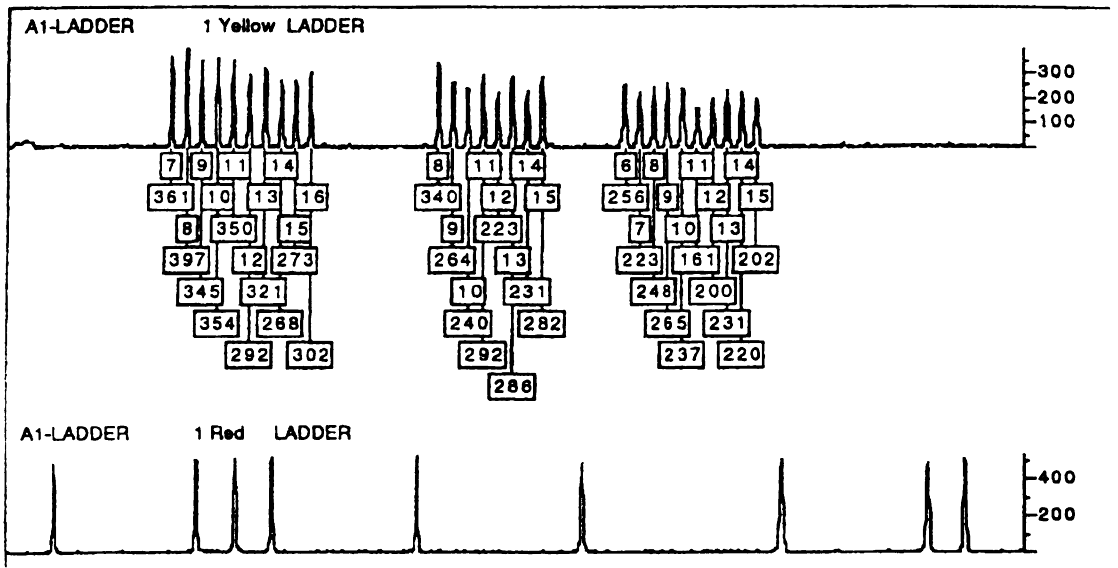

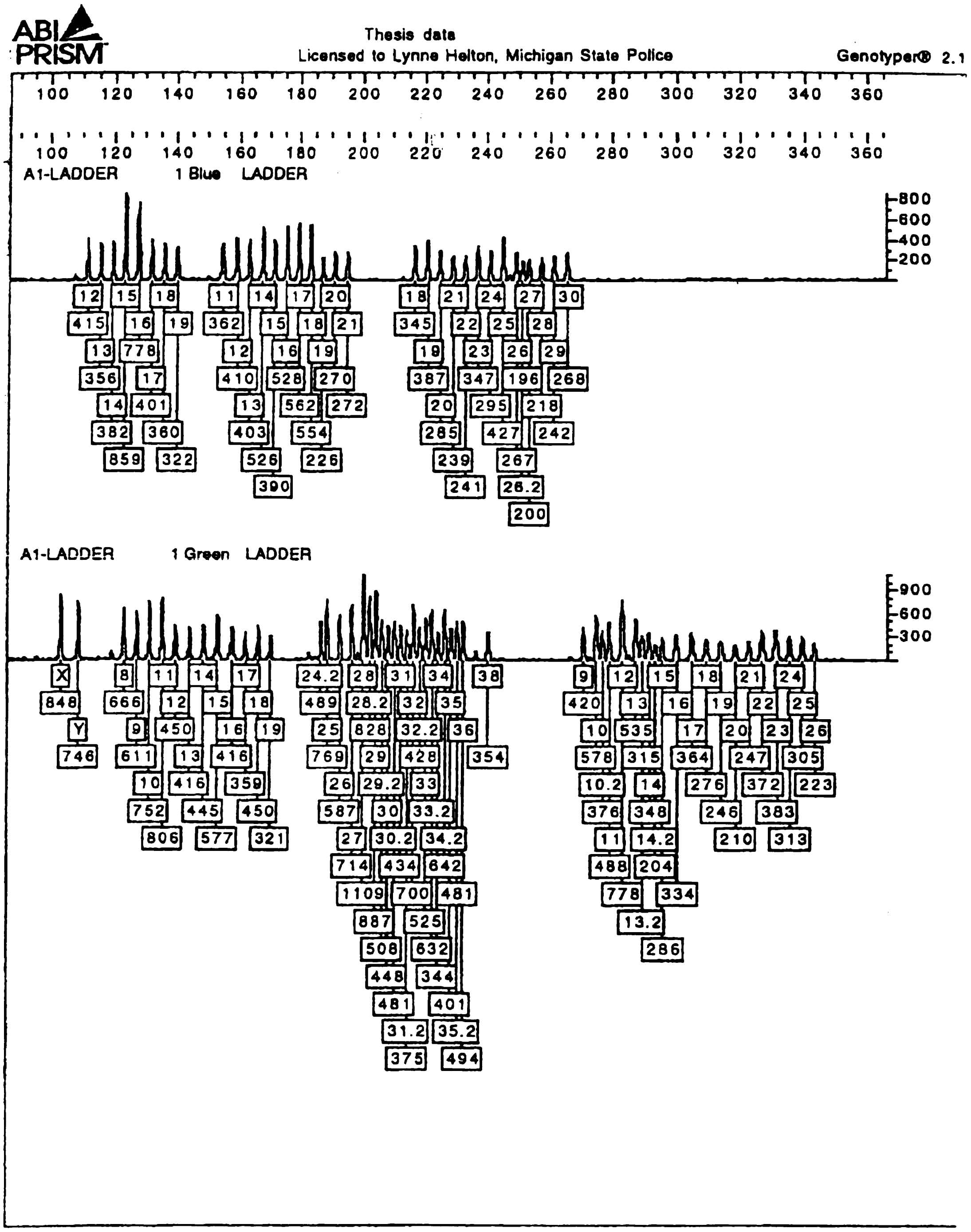
ABI PRISM
Thesis data
Licensed to Lynne Helton, Michigan State Police
Genotyper® 2.1
100 120 140 160 180 200 220 240 260 280 300 320 340 360
100 120 140 160 180 200 220 240 260 280 300 320 340 360
A1-LADDER 1 Blue LADDER
800
600
400
200
12 15 18 11 14 17 20 18 21 24 27 30
415 16 19 362 15 18 21 345 22 25 28
13 778 12 16 19 19 23 26 29
356 17 410 528 270 387 347 196 268
14 401 13 562 272 20 295 218
382 360 403 554 285 427 242
859 322 526 226 239 267
390 241 26.2
200
A1-LADDER 1 Green LADDER
900
600
300
X 8 11 14 17 24.2 28 31 34 38 9 12 15 18 21 24
848 666 12 15 18 489 28.2 32 35 420 13 16 19 22 25
Y 9 450 16 19 25 828 32.2 36 10 535 17 20 23 26
746 611 13 416 769 29 428 354 578 315 364 247 305
10 416 359 26 29.2 33 10.2 14 276 372 223
752 445 450 587 30 33.2 376 348 246 383
806 577 321 27 30.2 34.2 11 14.2 210 313
714 434 642 488 204
1109 700 481 778 334
887 525 13.2
508 632 286
448 344
481 401
31.2 35.2
375 494

Thesis data
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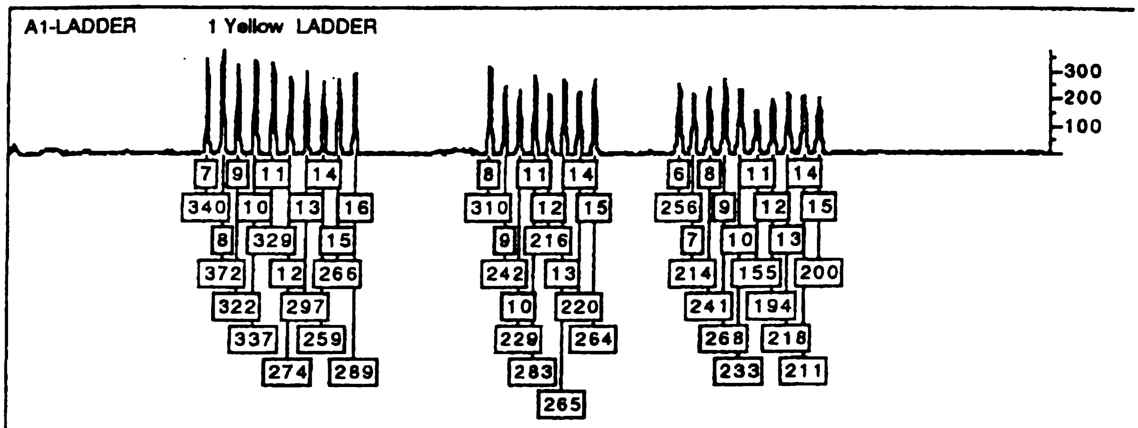
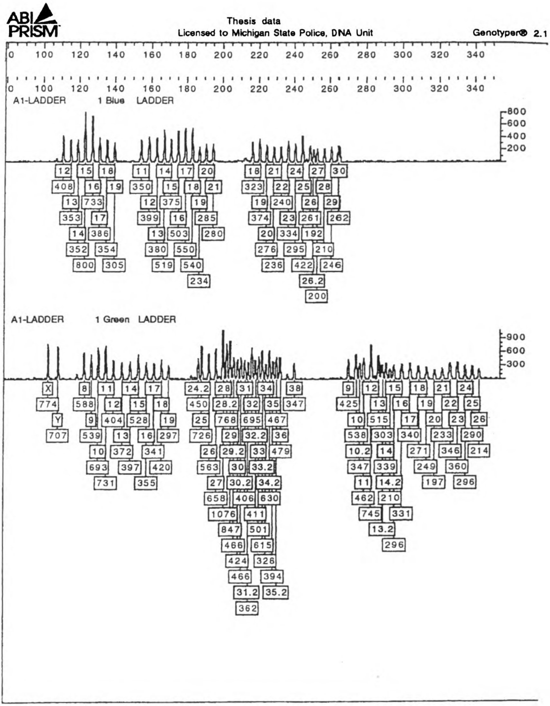
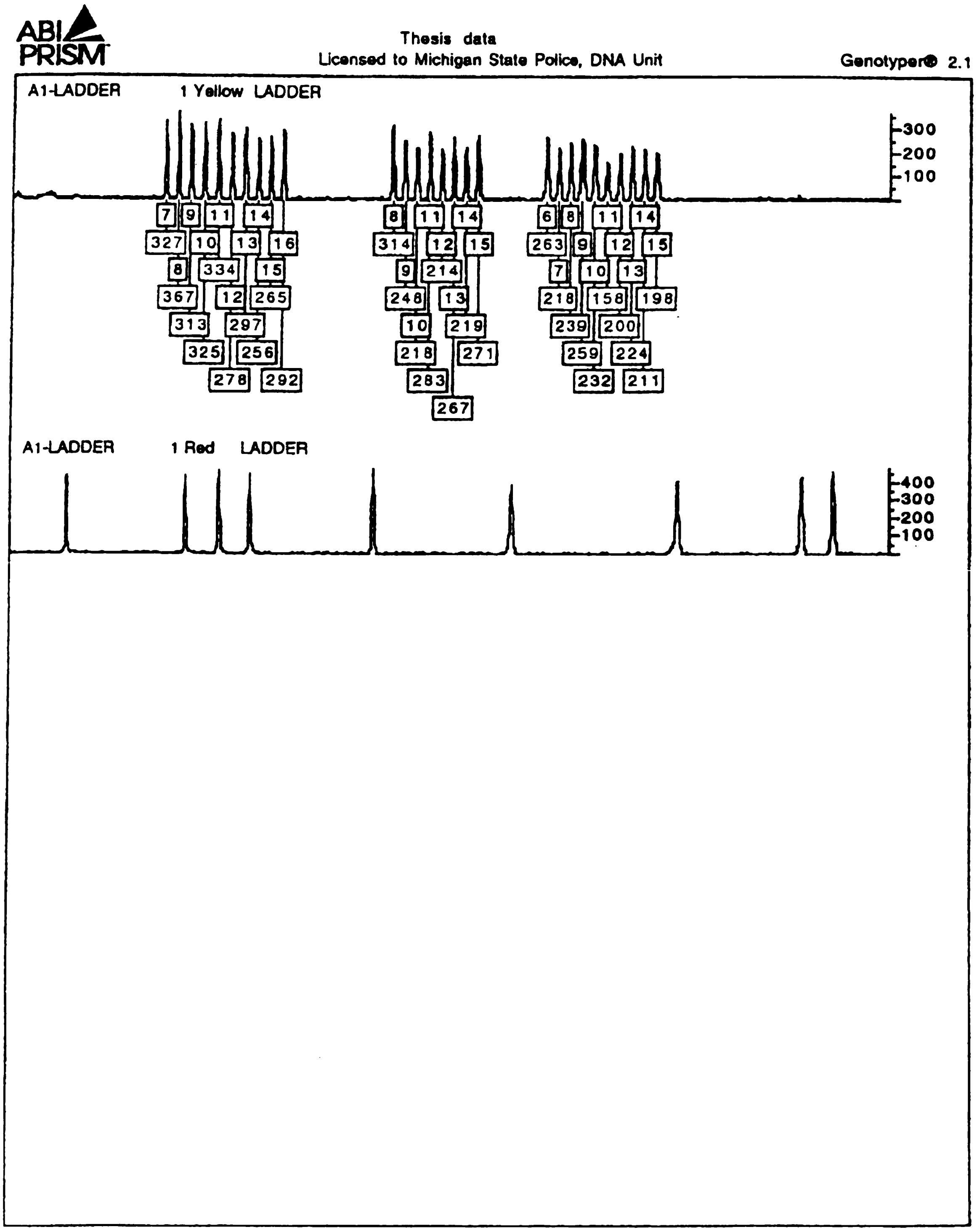
A1-LADDER 1 Yellow LADDER



A1-LADDER 1 Red LADDER


ABI PRISM
Thesis data
Licensed to Michigan State Police, DNA Unit
Genotyper® 2.1
0 100 120 140 160 180 200 220 240 260 280 300 320 340
0 100 120 140 160 180 200 220 240 260 280 300 320 340
A1-LADDER 1 Blue LADDER
800
600
400
200
12 15 18 11 14 17 20 18 21 24 27 30
408 16 19 350 15 18 21 323 22 25 28
13 733 12 375 19 19 240 26 29
353 17 399 16 285 374 23 261 262
14 386 13 503 280 20 334 192
352 354 380 550 276 295 210
800 305 519 540 236 422 246
234 26.2
200
A1-LADDER 1 Green LADDER
900
600
300
X 8 11 14 17 24.2 28 31 34 38 9 12 15 18 21 24
774 588 12 15 18 450 28.2 32 35 347 425 13 16 19 22 25
Y 9 404 528 19 25 768 695 467 10 515 17 20 23 26
707 539 13 16 297 726 29 32.2 36 538 303 340 233 290
10 372 341 26 29.2 33 479 10.2 14 271 346 214
693 397 420 563 30 33.2 347 339 249 360
731 355 27 30.2 34.2 11 14.2 197 296
658 406 630 462 210
1076 411 745 331
847 501 13.2
466 615 296
424 328
466 394
31.2 35.2
362



Thesis data

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Genotyper® 2.1