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CRIMINAL PATERNITY DNA TESTING OF MICROSCOPICALLY-IDENTIFIED CHORIONIC VILLI IN FORMALIN-FIXED PARAFFIN-EMBEDDED PRODUCTS OF CONCEPTION

presented by

ANN ELIZABETH-CHAMBERLAIN GORDON

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Major Professor's Signature

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CRIMINAL PATERNITY DNA TESTING OF MICROSCOPICALLY-IDENTIFIED CHORIONIC VILLI IN FORMALIN-FIXED PARAFFIN-EMBEDDED PRODUCTS OF CONCEPTION

By

Ann Elizabeth-Chamberlain Gordon

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ABSTRACT

CRIMINAL PATERNITY DNA TESTING OF MICROSCOPICALLY-IDENTIFIED CHORIONIC VILLI IN FORMALIN-FIXED PARAFFIN-EMBEDDED PRODUCTS OF CONCEPTION

By

Ann Elizabeth-Chamberlain Gordon

Embryonic/fetal tissue was not easily discerned from maternal decidua in early abortion materials; therefore, sampling for criminal paternity DNA analysis was problematic. Microscopic identification of chorionic villi (embryonic/fetal placental tissue) for subsequent DNA testing was pursued. The resultant tissues from sixteen early-term elective and spontaneous abortions were examined with no embryonic/fetal anatomy identified. Fifty specimens were formalin-fixed, paraffin-embedded, sectioned, stained, mounted on slides, and microscopically examined. Chorionic villi were identified for STR DNA analysis. Xylene deparaffinisation and tissue lysis buffer digestion were employed prior to the comparison of two DNA extraction methods—Chelex® and organic—in recovery of DNA for amplification.

Significantly higher quantities of DNA and higher quality DNA profile information (full Profiler Plus™/COfiler™ profiles) were obtained after Chelex® extraction. Varying proportions of offspring and maternal alleles—single-source profiles to equal mixtures of contributors—were observed. Discernment of offspring profiles, aided by maternal profile information, for comparison to putative fathers would be possible for determination of association. Since implementation of the Chelex® extraction method for this application at the Michigan State Police Lansing Laboratory in 2004, convictions were handed down in five cases as a direct result of analysis.

To my family

ACKNOWLEDGEMENTS

I would like to thank Dr. Joyce deJong, DO, Medical Director of Forensic Pathology at Sparrow Hospital, Lansing, MI for the continued partnership in pursuing criminal paternity DNA analysis in mid-Michigan and especially in the implementation of the microscopic identification process of chorionic villi. For the purposes of this research, Dr. deJong prepared the samples from abortion materials for subsequent DNA testing. Preparation of the tissue included the initial examination, formalin-fixation, paraffin-embedding, sectioning, and microscopic identification of the chorionic villi.

I would also like to thank thesis advisor, Dr. David Foran, PhD, Director– Forensic Science Program, School of Criminal Justice and Department of Zoology, Michigan State University for approving the proposed research, guiding the methodology and manipulation of data, and painstakingly overseeing the writing of this research paper. Thank you for the encouragement and support especially in the final stages of project completion.

Thank you to Dr. Christina DeJong Schweitzer, PhD, Associate Professor, School of Criminal Justice, Michigan State University for guiding the statistical portions of this research. The patience, effort, and accessibility afforded were greatly appreciated.

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INTRODUCTION

Rape Offenses in Michigan

Michigan law outlines in detail what constitutes rape or criminal sexual conduct (CSC). CSC offenses are assigned one of four degrees. First and third degree CSC offenses (CSC I and CSC III) involve penetration. Second and fourth degree offenses (CSC II and CSCIV) involve contact in a sexual manner. The degree of a CSC offense must be accompanied by at least one statutory circumstance. Some of the statutory circumstances that apply to CSC I and CSC II are as follows: the victim is under 13 years of age, the victim is at least 13 but less than 16 and the perpetrator is a member of the victim's household or related by blood or in a position of authority over the victim, the sexual act involves commission of any other felony, and the perpetrator is armed with a weapon or an article fashioned so as to lead a person to reasonably believe it is a weapon. A different set of statutory circumstances apply to CSC III and CSC IV. Some of these are as follows: the victim is at least 13 years of age but less than 16 (CSC III only), the victim is at least 13 but less than 16 years of age and the perpetrator is five or more years older than the victim (CSC IV), the perpetrator uses force, and perpetrator knows or has reason to know the victim is mentally incapable, mentally incapacitated, or physically helpless. Complete lists of statutory circumstances can be found in Appendix A and Appendix B.

Statutory rape (non-forcible intercourse with or between individuals, which would otherwise be legal, if not for their ages) differs from forcible rape in the matter of consent. Intercourse with a minor is illegal in all 50 states, but the age of consent varies.

In Michigan, as previously described, any sexual intercourse, even non-forcible, with an individual under the age of 16 is illegal and prosecutable.

Occurrence and Reporting of Rape

For a variety of reasons, females do not always report sexual crimes in a timely manner, or at all. Some of these include the desire to avoid embarrassment, fear of further harm (Kilpatrick et al., 1992; Kilpatrick, 2000), a statutory rape situation, or forcible rape of a minor in which discovery was prevented by the perpetrator and/or the circumstances. All of the aforementioned can make evidence recovery difficult or impossible.

Transfer of Biological Evidence in Rape Cases

Physical evidence of interest in the prosecution of rape or criminal sexual conduct cases can vary with the circumstances. The best evidence is the direct transfer of bodily fluids (vaginal secretions, semen, seminal fluid, saliva, or perspiration containing epithelial cells) between the victim and perpetrator during intercourse and the transfer of fluids to the environment or to a condom. In the crime lab, bodily fluids are located, identified, isolated, and individualized using DNA analysis methods. The results are compared and the evidence can be declared a match to the exemplar, or the exemplar can be excluded as a possible donor to the evidence.

A small percentage of the time a victim becomes pregnant as a result of the assault, and in some of these cases, the pregnancy is the only evidence that a forcible or statutory rape occurred. The Rape, Abuse, and Incest National Network (RAINN) (2006) calculated that out of the 247,730 rapes that occurred in 2002, approximately 4,315 pregnancies (~1.7% of reported rapes) resulted. The calculation is based on information from the U.S. Department of Justice, Bureau of Justice Statistics' 2002 National Crime Victimization Survey and medical reports.

Collection of Embryonic, Fetal, and Full-Term Offspring Samples for DNA Analysis

If a pregnancy resulting from rape is not aborted, a sample can be obtained from the embryo, fetus or the full-term baby and compared using paternity DNA analysis with the victim and suspect samples. Samples of chorionic villi—tiny outgrowths from the outer membrane chorion surrounding an embryo that grow into the womb wall and help to form the placenta (see below)—can be obtained during gestation (Lobbiani et al., 1991; Karger et al., 2001; Mingjun et al., 1993; Reshef et al., 1999). After birth, a sample can be obtained by swabbing the infant's mouth or by drawing a whole blood sample for analysis. If the mother decides to terminate the pregnancy, DNA analysis of the embryonic or fetal tissue obtained during an abortion procedure is possible. The present study focused on the viability of the latter method.

Abortion Techniques and Possible Effects on Sample Collection, Preservation, and DNA Testing

Initial Screening and Gestational Age Determination

The first step in the abortion process is the actual confirmation of pregnancy (Smith, 1982). The pregnancy hormone human chorionic gonadotropin (hCG) can be detected approximately two weeks after fertilization or four weeks since the last menstrual period. A positive result from a simple 5-minute urine test is an indication of pregnancy. Ultrasound and pelvic examination will attempt to confirm pregnancy and to identify growth stage (Smith, 1982). Many women have irregular menses or have experienced bleeding that was interpreted as menses, which can throw off the estimation of gestational age (Hern, 1984). Documentation of the gestational age of the pregnancy at the time of abortion is important to law enforcement, as well as pathology and forensic laboratory scientists. Once the gestational age is determined, the appropriate abortion procedure can be performed.

Abortion Techniques

According to the World Health Organization (2003), the vacuum aspiration technique generally performed through 12 weeks utilizes a vacuum, either manual or electric, to empty the uterine contents. An aspirator or syringe is connected to a cannula ranging in size from 4–12 mm in diameter which is connected to the vacuum source. Dilation of the cervix may be necessary for insertion of the cannula and/or syringe. The vacuum process takes 3–10 minutes and is performed under local anesthesia or analgesics. During the vacuum process, the tissues may suffer gross trauma, distorting them beyond recognition (Karger et al., 2001), making it difficult or impossible to identify fetal components for DNA analysis.

Another procedure, dilatation and curettage (D&C) is used through the 12th week of pregnancy. This involves the dilation of the cervix with mechanical dilators or pharmacological agents and the use of sharp metal curettes to remove tissue from the walls of the uterus. The procedure may also distort the tissue due to gross trauma (Karger et al., 2001). The dilatation and evacuation (D&E) procedure is used after the 12th week of pregnancy until the 23rd week. The cervix is dilated and the uterus is evacuated using a vacuum and a 14–16 mm diameter cannula and forceps. The dilation procedure may take 2 hours to one full day. An alternative to the aforementioned procedures is the chemical induction method. It utilizes an anti-progesterone drug to interfere with the continuation of the pregnancy, and a prostaglandin to enhance uterine contractions to expel the products of conception. This method is far less destructive to tissues.

Evidence Handling of Abortion Materials in Mid-Michigan Prior to 2002

Prior to 2002, the aborted embryo or fetus (resultant from rape) and the maternal materials were frozen or fixed in formalin and shipped directly to the Michigan State Police Lansing Laboratory for analysis at any stage of development. Well-developed, whole fetuses were often received even though swabs of the mouth or a blood sample could easily have been collected—causing adverse psychological stress to the analyst, as well as storage and disposal problems. Materials from very early abortions (approximately 2–7 weeks post-conception) exhibiting amorphous tissue were also frequently received. Identification of embryonic or fetal tissue for DNA analysis relied on visual recognition of fetal anatomy within or among the materials. According to Moore and Persaud (1993), the embryo or fetus may not be readily visible to the naked eye before the 8th or 9th week. This is especially true if the anatomy was badly distorted due to the abortion process (Karger et al., 2001). Due to the difficulty in visual identification of the embryonic or early fetal parts in early abortions, random samples from the materials would be used for genetic testing and often results were limited to the maternal profile.

Evidence Handling of Abortion Materials in Mid-Michigan from 2002 to Early 2003

Difficulties with storage, disposal, and analyst stress provoked an agreement between Dr. Joyce deJong, Medical Director of Forensic Pathology at Sparrow Hospital in Lansing, Michigan and the Michigan State Police Lansing Laboratory Biology Unit, with regards to processing of abortion materials. Forensic Pathology received all aborted material cases and screened them for embryonic/fetal anatomy. If such anatomy was located, samples were collected and forwarded to the Michigan State Police Lansing Laboratory. If anatomy was not located, it was agreed that the case would not be pursued for DNA analysis.

Evidence Handling of Abortion Materials in Mid-Michigan in Early 2003

In early 2003, a study conducted by Karger et al. (2001) was discovered, which discussed a procedure to identify very early embryonic tissue structures, chorionic villi, for forensic genetic testing. An agreement was reached between Dr. deJong and the Michigan State Police Lansing Laboratory to conduct further research on the topic and to attempt implementation. This procedure suggested formalin-fixation, paraffin-embedding, sectioning, and hematoxylin-eosin staining of early-term abortion materials. DNA analysis would be conducted on sections demonstrating the presence of chorionic villi.

Chorionic Villi Development

The stages of embryonic and fetal development are important in microscopically identifying chorionic villi—cells from the fetal side of the placenta (Fig. 1). According to Moore and Persaud (1993), the primitive chorionic villi appear by the end of the 2nd week following conception. The embryonic period begins at the beginning of the 3rd week just after the primitive chorionic villi appear. The primary chorionic villi (Fig. 2) begin to branch, and in several days they cover the entire chorionic sac becoming secondary chorionic villi (Fig. 3). Within a few days the venous networks are present, and the structures become tertiary chorionic villi or stem villi (Fig. 4). Blood begins to flow through the villi by the end of the 3rd week. The limb buds start to develop at the end of week four and beginning of week five. At the end of week five, the embryo is often still too small (8 mm) to identify with the naked eye. By the beginning of week six distinct fingers are beginning to form, and if left intact the embryo (13 mm) may be visually identified at the end of this week (Fig. 5). The embryo will grow to be approximately 30 mm by the end of the 8th week, when it can frequently be identified visually. During the 9th week, the fetus is easily identified visually as it is 50 mm in length. To identify the chorionic villi from an early aborted pregnancy, the maternal decidua and/or embryonic/fetal material must be chemically fixed, embedded in paraffin, sectioned, stained, and mounted. Microscopic evaluation identifies slides containing chorionic villi.

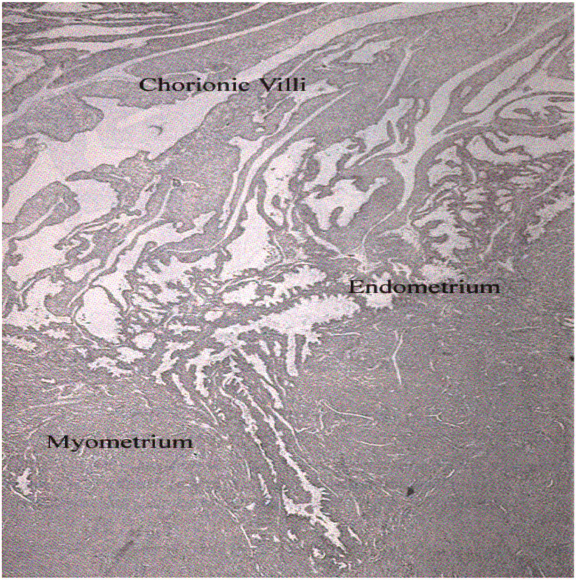


FIG. 1—*Microscopic cross-section of chorionic villi and maternal tissues. Three layers of tissue, chorionic villi which are finger-like in appearance with lacunae (empty spaces that would contain maternal blood), maternal endometrium (spent glands exhibit saw-tooth edges—to the immediate left of the identifier), and myometrium (maternal) are represented from the top down (Duker, 2003).*

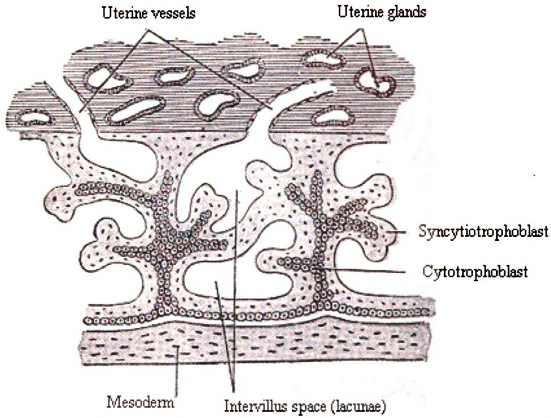


FIG. 2—*Primary chorionic villi surrounded by a thin layer of mesoderm (connective tissue). The mesoderm is covered by cytotrophoblast and superficially with syncytiotrophoblast (cells that contact maternal blood) (Gray, 2000 (1918)).*

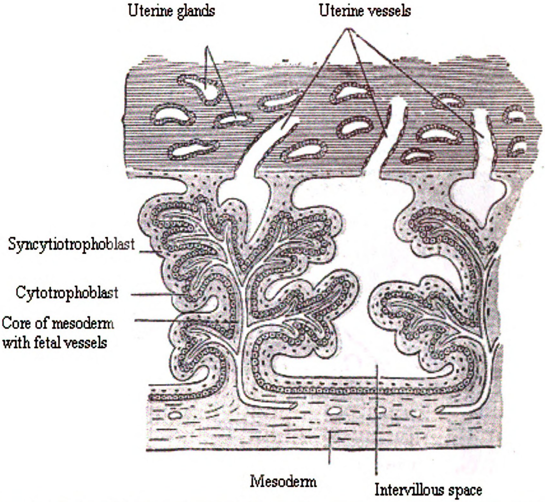


FIG. 3—*Secondary chorionic villi surrounded by a thin layer of mesoderm (connective tissue). The mesoderm is covered by cytotrophoblast and superficially with syncytiotrophoblast (cells that contact maternal blood). The venous networks are present within the villi (Gray, 2000 (1918)).*

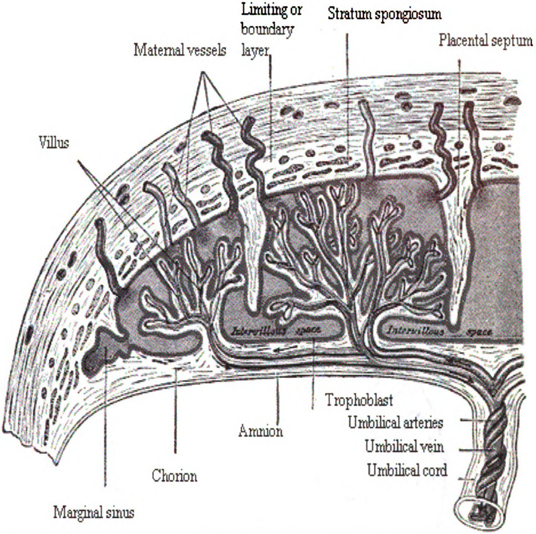


FIG. 4—*Tertiary villi and intervillous spaces facilitate placental circulation. Fetal and maternal blood does not intermingle—the delicate walls of the villi facilitate the exchange of waste products, and oxygen and nutritive materials. After the exchange, blood is carried back to the fetus by the umbilical vein (Gray, 2000 (1918)).*

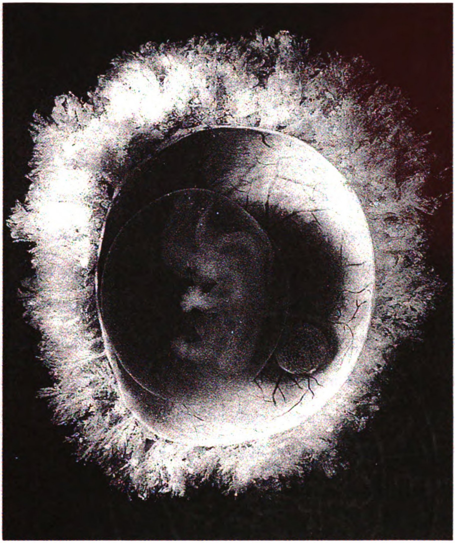


FIG. 5—*Photograph of an embryo at 7 weeks contained within the chorionic sac. The chorionic villi are evenly distributed—covering the chorionic sac (O'Rahilly and Muller, 2001).*

Forensic STR Analysis

Today, most forensic DNA typing focuses on DNA regions with repeating units referred to as short tandem repeats, or STRs (Butler, 2001). STRs can be highly variable due to the repeat unit composition and length of the alleles. The 13 CODIS (Combined DNA Index System) STR loci (CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11) were selected because of their high discriminating power (in combination), separate chromosomal locations (to avoid linkage), result reproducibility and robustness in multiplexing (simultaneous amplification of alleles), low mutation and stutter rates, and allele lengths between 90–500 base pairs (smaller sizes are best for degraded samples encountered in forensic testing) (Butler, 2005).

Commercial kits are available that allow multiplex amplification of the CODIS loci. Profiler Plus™ and COfiler™ (manufactured by Applied Biosystems) are examples of these, with Profiler Plus™ amplifying nine of the loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820) and COfiler™ six (D3S1358, D16S539, TH01, TPOX, CSF1PO, and D7S820). An allelic ladder, an artificial mixture of all of the common alleles present at each locus, is used as a "measuring stick" to determine the alleles present within a sample. The combination of allelic information from the loci comprises a genetic profile. Profiles from evidence samples can be compared to known samples from victims and suspects, and conclusions regarding the source of the profiles can be drawn.

Unfortunately, challenges in interpretation and comparison of forensic STR results often occur. Microvariants, mutations, DNA degradation, inhibition of amplification, and mixtures of alleles from multiple DNA contributors are commonly encountered (Butler, 2001). Microvariants are rare alleles differing from a common allele by one or more nucleotides. These often generate "off-ladder" alleles (OL alleles) as they are not present in the allelic ladder. Amplification must be repeated to confirm the "OL allele" status of a suspected microvariant. If re-analysis verifies that the allele is a true microvariant, further comparison with the allelic ladder is necessary. The allele designation can be interpolated if the microvariant falls within two ladder alleles, or extrapolated if it falls outside of the allele range. Mutations in STRs usually result in single base changes or changes in repeat unit. A mutational event can result in a mismatch between a parent's DNA profile and a child's (see the Limitations in Interpretation of Genetic Testing Results section for further explanation). Degradation (random breakdown of DNA molecules due to environmental exposure), which is common in forensic samples, can result in incomplete or failed amplification. Larger-sized loci are more likely to disappear due to degradation, rendering amplification of intact sequences impossible. Partial profiles (comprised mainly of results from smaller-sized loci) are sometimes obtained from degraded samples but often there are no results observed (false negative). Inhibition (interference of amplification), caused by substances contaminating the DNA, can result in partial or no profile results as well. A summary by Wilson (1997) identified three mechanisms by which inhibitors act—inactivation of the DNA polymerase, degradation or capture of nucleic acids, and interference with the lysis of cells during DNA extraction. Numerous substances (e.g. textile dyes (Shutler et al., 1999), hemoglobin (Akane et. al., 1994; Mercier et al., 1990), melanin in tissue and hair (Eckhart et al., 2000), polysaccharides and bile salts in feces (Lantz et al., 1997; Monteiro et al., 1997), humic compounds in soil (Tsai and Olson, 1992), heparin (Beutler et al., 1990), phenol (Katcher and Schwartz, 1994), plant polysaccharides (Demeke and Adams, 1992), polyamines (spermine and spermadine) (Ahokas and Erkkela, 1993), urea in urine (Khan et al., 1991; Mahoney et al., 1998), detergents (i.e. SDS) (Gelfand, 1989), and calcium alginate swab fibers and aluminum swab shafts (Wadowsky et al., 1994)) have been identified as PCR inhibitors. Careful consideration and selection of sample preparation methods which adequately reduce inhibitory effects is crucial for optimal amplification results (Radstrom et al., 2004). Finally, mixtures of alleles from multiple contributors can render source attribution difficult or impossible (see the Limitations in Interpretation of Genetic Testing Results section for further explanation).

Factors Affecting DNA Recovery from Tissue Samples

Romero et al. (1997) stated that the extraction of DNA from formalin-fixed tissue embedded in paraffin has historically shown little success. Opinions as to the effects of formalin on tissue have varied over time. Formalin was originally thought to damage DNA molecules causing strand breakage. It is now understood that the formation of methyl bridges between amino groups of purine and pyrimidine bases as well as between the bases and histones, can be facilitated by formalin (Brutlag et al., 1969; Feldman, 1973; Moerkerk et al., 1990; Romero et al., 1997). The formation of these cross-links can inhibit extraction of DNA from fixed samples. Karger et al. (2001) were successful at obtaining genetic profile information in a limited study of six samples of fetal/maternal decidua from an abortion. These were obtained from microscope slides prepared from formalin-fixed paraffin-embedded abortion materials. Two of these generated fetal results.

Organic and Chelex® 100 DNA Extraction of DNA

A popular method for DNA isolation employed in forensic laboratories uses Chelex® 100. Chelex® 100 is composed of paired iminodiacetate ions attached to styrene divinylbenzene copolymers (plastic beads), which chelate (bind) polyvalent metal ions such as magnesium and iron; these ions can help degrade DNA, or inhibit its subsequent analysis. The Chelex® procedure may be more successful at isolating higher quantities of DNA for STR-PCR testing (Walsh et al., 1991) than traditional organic extractions involving phenol/chloroform/isoamyl alcohol (see below). Further, Chelex® 100 resin is added directly to the sample tube and no DNA transfers are required; this reduces the chance of sample loss and contamination (Walsh et al., 1991). The sample is boiled in the presence of the Chelex® beads, which according to Singer-Sam et al. (1989) protect the DNA from degradation. Exposure to boiling (100°C) destroys the cell membranes—releasing DNA into the solution and denaturing the DNA.

Organic extraction includes the use of proteinase K, a detergent, (e.g., SDS or Tween 20) and exposure to hot temperatures (e.g., 56 °C) to break down cell membranes—releasing DNA into solution. The addition of phenol/chloroform/isoamyl alcohol facilitates a physical separation of the hydrophilic DNA from the hydrophobic protein materials. (The DNA is more soluble in the aqueous phase of the solution, whereas, the proteins remain in the organic phase.) Centricon-100™ concentrators or similar devices can be used to purify the DNA, removing small molecules such as hemoglobin that may inhibit DNA analyses. A risk with this method is the need to transfer the aqueous DNA containing solution from one tube to another, resulting in potential sample loss.

Limitations in Interpretation of Genetic Testing Results

According to Karger et al. (2001), the interpretation of DNA analysis results from aborted tissues can be challenging; often the results are a mixture of embryo or fetus and mother. Genetic inheritance is based on the combination of allelic information from both parents, thus when results are a mixture of embryo/fetus and mother, determination of the embryo/fetal profile can be difficult (Butler, 2001). A known sample from a parent is crucial to the process of identifying the embryonic or fetal profile. Once the profile is identified, suspected father profiles can be compared to determine the likelihood of paternity.

Comparison of potential suspect profiles to the profile of the embryo/fetus can also pose challenges, and association of the suspect to the embryo/fetus is never conclusive. The strongest association possible is that "the suspect cannot be excluded as a parent of the offspring". Generation of a paternity likelihood ratio supports the association—estimating the likelihood that the suspect is a parent of the offspring versus another random individual. However, results may or may not be easy to interpret. Sometimes one or more mismatch occurs between the suspect and the offspring, and the suspect sample is readily excluded as the father. On the other hand, a mismatch between the offspring and suspect may result from a mutational event and the suspect's sample still cannot be excluded. Paternity testing generally allows for one mismatch between the potential father and offspring due to mutational events (Butler, 2001). Most often this mutation will result in a difference ± one STR repeat unit.

According to Brinkmann et al. (1998), paternal mutations are more common than maternal mutations, with a ratio of 17:3, due to the different numbers and types of cell division. The oogonia divide approximately 22 times before meiosis begins and the oocyte is formed. The spermatogonia are constantly renewed by mitosis and some continue to divide through meiosis before becoming sperm cells. The rate of mutation in older men is even higher than in younger men due to more cell divisions (Brinkmann et al., 1998).

Goal of this Research

The research presented here was designed to evaluate a combination of techniques for obtaining useful DNA profile information from the analysis of aborted embryonic or fetal tissue. It was proposed that microscopic identification of embryonic structures (chorionic villi) would reduce the chances of sampling maternal tissue during DNA testing. The exposure of the tissue to formalin fixative during the paraffin embedding process, although necessary for the preparation of quality microscopic specimens, is generally not favorable for genetic profiling. Chelex® and organic DNA extraction methods are both designed to generate analyzable DNA, therefore comparison of these was conducted to determine their relative effectiveness in obtaining DNA, and in minimizing the negative effects caused by formalin exposure. The success of either extraction method was determined based on the quantity of DNA recovered and ability to retrieve full genetic profiles from the abortion material. The generation of full profiles would enable comparison to maternal and paternal profiles to reveal paternity status. Ultimately, the goal was to apply the most effective combination of techniques to forensic casework for the purpose of enabling the most discriminating comparison of evidence to putative father profiles, thus assisting in accurate prosecution of applicable rape crimes.

MATERIALS AND METHODS

Institutional Review Board (IRB) Approval

Per UCRIHS (University Committee on Research Involving Human Subjects), this research project involved only the in vitro use of de-identified human tissues; therefore, it did not require IRB approval. A certification form was submitted to Dr. Peter Vasilenko, IRB Chair, Office of Research Ethics and Standards, Michigan State University and approved under certification #CT06-002.

Sample Collection and Preparation

Prior to this experiment, early-term abortions were conducted at undisclosed facilities in Michigan and the aborted tissue was sent to Dr. Joyce deJong, Medical Director of Forensic Pathology at Sparrow Hospital, Lansing, MI. The resultant decidua and embryonic/fetal material from each abortion were examined for identifiable fetal parts. None were located. The tissue from each abortion was sliced into segments less than one centimeter in any dimension and placed into separate standard tissue embedding cassettes. Three sets of cassettes (Test Set 1 (TS1-1–10), Test Set 2 (TS2-1–20), and Test Set 3 (TS3-A–F, I–K, M–O, Q–W)) were prepared and labeled accordingly. Each set of cassettes was transferred to the Sparrow Hospital Department of Histology for processing.

Fixation

The histologist placed the cassettes containing tissue into a basket and then into a chamber containing 10% formalin at neutral pH. The tissue was fixed in formalin for a period of approximately 10–12 hours (recommended by Greer et al. (1991) and Rogers et al. (1990)).

Tissue Processing

A tissue processor was used to gradually dehydrate the formalin-fixed tissue. The tissue was washed with 10% formalin and then passed through increasing strengths of ethyl alcohol (70%, 80%, 95%, and 100%). Following dehydration, xylene was used to clear the tissue of the ethyl alcohol. The cassettes were placed into metal cassette holders and passed through several changes of melted paraffin until completely embedded (in blocks of paraffin within the cassettes).

Sectioning

The paraffin embedded tissue was sectioned into 4.0 μm slices using a microtome. The sections were floated on a warm water bath to remove wrinkles and folds. They were mounted on slides by placing the slide underneath the section and lifting it out of the water. A fixative on the slides enabled the tissue to adhere.

Staining

Paraffin was removed from the sectioned tissue on the slides with xylene, followed by 100% ethyl alcohol and water. The tissue was exposed to the stain hematoxylin, followed by 80% ethyl alcohol, 100% ethyl alcohol, the stain eosin, 100% ethyl alcohol again and finally xylene. The slides were placed into a processor for permanent placement of cover slips. Slides were scanned for quality control and transferred back to the pathologist.

Microscopic Examination

Microscopic evaluation of the slides identified cassettes containing chorionic villi. These cassettes were noted. All of the cassettes (and slides) were transferred to the Michigan State Police Biology Unit for DNA analysis.

Preparation of Paraffin-Embedded Embryonic or Fetal Tissue and Maternal Decidua for DNA Extraction

Sample Preparation

Fifty microscope slides corresponding to specific paraffin blocks (Fig. 6) (sets identified as TS1, TS2, and TS3) were visually analyzed and locations containing chorionic villi were identified (see Appendix C for complete protocol). Two 2–4 mm^3 tissue segments were cut from each paraffin block (areas corresponding to chorionic villi identified on the slide) using a sterile razor blade, and were placed into separate labeled microcentrifuge tubes for paraffin removal followed by digestion and Chelex® or organic extraction. A blank tube (no tissue segment) was prepared for each extraction method, and was carried through the entire extraction process with the purpose of identifying contamination, if present, in the reagents used. The tubes containing tissue segments and the reagent blank tubes were collectively referred to as 'samples' from this point forward in the experiment.

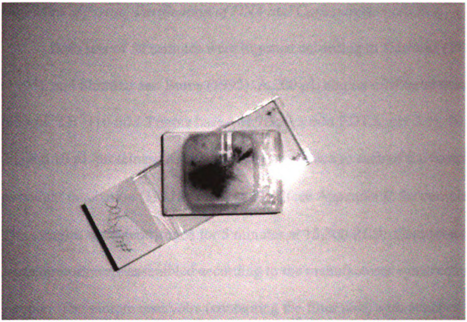


FIG. 6—*Photograph of the paraffin block containing sample TS1-4A. The slide was orientated over the paraffin block consistent with the corresponding tissue on the slide and in the block.*

Deparaffinisation

A xylene/ethanol deparaffinisation method (Coombs et al., 1999; Goelz et al., 1985) was utilized on both sets of fifty samples. A 1 mL aliquot of xylene was added to each of the samples to remove the paraffin wax (see Appendix C for complete protocol); the samples were then incubated for 30 minutes at room temperature, and then centrifuged for 2–5 minutes at 15,300 relative centrifugal force (RCF). The liquid portion was discarded and the process repeated. A 1 mL aliquot of ethanol was added to each of the samples to remove the remaining xylene from the tissue. The samples were centrifuged at 15,300 RCF for 2–5 minutes. The liquid portion was discarded and the process repeated. The samples were dried in a Hetovac vacuum apparatus at 15–20 in. Hg for 10–20 minutes.

Digestion of Tissue, Purification of DNA and Concentration of DNA

Both sets of 50 samples were digested according to Kawaski (1990), Sepp et al. (1994), and Shimizu and Burns (1995). A 200 µL aliquot of filtered tissue lysis buffer (189 µL TE^{-4} (10 mM Trizma base, pH 7.5; 0.5 mM EDTA, pH 7.5), 10 µL 0.5% Tween 20, and 10 µL proteinase K (20 mg/mL)) was added to each of the samples prior to overnight incubation (12–18 hours) at 37°C (see Appendix C for complete protocol). The samples were centrifuged for 5 minutes at 15,300 RCF. Centricon-100™ concentrators were assembled according to the manufacturer's instructions for each sample. The sample reservoirs (containing the filter unit) with attached rententate vials were fitted to filtrate vials. The liquid portion of each digested sample (approximately 200 µL) was placed into a separate Centricon-100™ concentrator sample reservoir (the tissue was discarded), and centrifuged for 30-60 minutes at 2000 RCF. The filtrate from each was discarded. A 2 mL aliquot of TE^{-4} was added to each sample, and the samples were centrifuged for 30-60 minutes at 2000 RCF. The filtrate was discarded and the process repeated. Following the second wash, the filtrate vials were removed from the concentrators and discarded. The sample reservoirs with attached retentate vials were inverted and centrifuged at 1000 RCF for 3 minutes. The concentrated DNA (rententate) was captured in the retentate vials and transferred to clean, labeled, microcentrifuge tubes was used for Chelex® 100 extraction and the other for organic extraction.

<u>DNA Extraction of Embryonic or Fetal Tissue and Maternal Decidua</u>

One sample from each of the 50 cassettes was extracted with Chelex® (Walsh et al., 1991) and one was extracted organically (phenol/chloroform/isoamyl alcohol) (Sambrook et al., 1989).

Chelex® Extraction

A solution of 5% Chelex® 100 was prepared by adding 2.5 g of Chelex® resin beads to 50 mL of sterile water and mixing until evenly distributed (see Appendix D for complete protocol). The pH was verified at 9.0 using a Corning 220 pH Meter and buffer solutions of pH 7.0 and pH 10.0. A 20 µL aliquot of the Chelex® 100 solution was added to each tube. The samples were vortexed briefly and incubated at 56°C for 30 minutes. The samples were then vortexed at high-speed for 5–10 seconds and placed into a boiling water bath for 8 minutes. These were vortexed again at high-speed for 5–10 seconds and centrifuged for 3 minutes at 15,300 RCF.

Organic (phenol/chloroform/isoamyl alcohol) Extraction

A 200 µL aliquot of phenol/chloroform/isoamyl alcohol 25:24:1 was added to each sample (see Appendix E for complete protocol). The samples were vortexed until a milky emulsion was achieved (5–10 seconds) and then centrifuged at 15,300 RCF for 5 minutes. After centrifugation the components were separated into a lower organic solution, an interface layer of protein and cellular material, and an upper aqueous portion. The aqueous portions were transferred to clean Centricon-100™ concentrators and centrifuged for 30-60 minutes at 2000 RCF (the filtrate was discarded). A 2 mL aliquot of TE^{-4} was added to each sample, and the samples were centrifuged for 30-60 minutes at 2000 RCF. The filtrate from each was discarded and the process repeated. Following the second wash, the filtrate vials were removed from the concentrators and were discarded. The sample reservoirs with attached retentate vials were inverted and centrifuged at 1000 RCF for 3 minutes. The concentrated DNA (rententate) was captured in the retentate vials and was transferred to clean, labeled, microcentrifuge tubes.

Quantification of DNA

The Chelex® and organically extracted samples and kit standards (known DNA quantities of 10 ng, 5 ng, 2.5 ng, 1.25 ng, 0.625 ng, 0.3125 ng, and 0.15625 ng) were quantified using a Quantiblot™ kit (manufactured by Applied Biosystems) according to the manufacturer's instructions. The chemiluminescent method of detection was used. Membranes were placed on Kodak® X-Omat LS film and exposed overnight (24 hours). The films were processed using a medical film processor and compatible chemistry. An estimate of DNA quantity was made in relation to the standards. If no DNA was detected, those samples were concentrated to 10 µL using Microcon-100™ Micro-concentrators according to manufacturer's instructions.

DNA Amplification

A PCR master mix was prepared with primers, reaction mix, and AmpliTaq Gold™ DNA polymerase from AmpFlSTR Profiler Plus™ and AmpFlSTR CoFiler™ kits with one minor modification to the manufacturer's instructions (total reaction volume per sample was lowered to 25 µL). Samples with no detectable DNA based on Quantiblot™ results were amplified with the Profiler Plus™ kit only using any/all available DNA. Dilutions or concentrations of the samples (whichever was appropriate given the estimated quantity of DNA detected) and the positive control samples were prepared; targeting 1.0 ng of DNA per 10 µL of sample based on the Quantiblot™ estimates. Fifteen microliters of master mix (10.5 µL of PCR Reaction mix, 5.5 µL of the Profiler Plus™ or COfiler™ kit primers, and 0.5 µL of AmpliTaq Gold™) was combined with 10 µL of each sample and control. The negative controls were prepared with 10 µL of sterile water replacing the DNA. All were amplified using ABI 9700 thermocyclers with an initial incubation at 95°C for 11 minutes to activate the AmpliTaq Gold™ enzyme. Denaturing was conducted at 94°C for 1 minute, primer annealing at 59°C for 1 minute, and extension at 72°C for 1 minute. This sequence was repeated for a total of 28 cycles. The final extension was conducted at 60°C for 45 minutes, and the plates were held at 25°C until removal from the thermocycler.

Sample Preparation for Electrophoresis and GeneScan Analysis

One microliter of each amplified sample, control product, or allelic ladder standard (Profiler Plus™ and COfiler™) was added to 24 µL of deionized formamide (Bio-Rad Laboratories) and 1 µL of GS-500™ ROX internal size standard. The samples, controls and ladders were denatured at 95°C for 3–5 minutes and snap-cooled on ice for a minimum of 3 minutes. An ABI Prism™ 310 Genetic Analyzer and ABI Prism™ 310 Collection Software were used according to manufacturer's instructions to obtain raw data of genetic profiles. These were analyzed with GeneScan® Analysis Software, while Genotyper® software was used to obtain final allele designations at each locus.

Data Interpretation

Determination of Full vs. Partial Single-Source Profiles

The guidelines for interpretation of acceptable single-source genetic profile results were:

1. Each allele peak must fall within a minimum threshold of 150 relative fluorescent units (RFUs) and a maximum threshold of 4500 RFUs (relative fluorescent units), with the exception of the amelogenin locus which has a maximum threshold of 7500 RFUs to qualify for interpretation.

2. Allelic balance for heterozygosity must equal or exceed 70 percent. Single-source profiles were considered full (complete) if alleles at each of the loci (Profiler Plus—D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820; COfiler—D3S1358, THO1, TPOX, CSF1PO, D7S820, and D16S539) fell within the 150–4500 RFUs range. The sample was also considered to have generated a full profile if enhancement utilizing a 3 μL input of amplified DNA or a decreased injection time of 1–4 seconds was expected to place all alleles within the range (defined above). Compilation of results from multiple electropherograms from the same sample was acceptable as well. Samples which generated interpretable results at the amelogenin locus and at least one allele were considered partial profiles. A sample was also considered a partial profile if enhancement utilizing a 3 μL preparation of amplified DNA was expected to place the allele(s) at the amelogenin locus and at least one additional allele above the minimum threshold of 150 RFUs.

Determination of Full vs. Partial Mixture Profiles

A mixture profile (DNA types detected from more than one donor) was considered full if alleles from at least one of the two contributors fell within the established range of RFUs. Samples which did not meet these criteria were considered full profiles if enhancement utilizing a 3 μL preparation of amplified DNA or a decreased injection time of 1–4 seconds was expected to place all alleles from at least one contributor within the interpretable range of RFUs. Successful interpretation of loci over multiple electropherograms from the same sample was also acceptable for mixture samples. Partial profiles included interpretable amelogenin, in addition to, a minimum of one allele at one locus. A mixture sample was also considered a partial profile if enhancement utilizing a 3 μL preparation of amplified DNA was expected to place the allele(s) at the amelogenin locus and at least one additional allele above the minimum threshold of 150 RFUs.

Mixture Allele Relationships Representative of the Mother and a Full Offspring

Profiles which exhibited a mixture of maternal and fetal alleles were recognized by the presence of several different allele configurations. If three alleles were present at one locus, one of them should be shared by the mother and fetus. This allele would be consistent with the proportion of the contribution from the mother and from the fetus (the other two alleles) combined. In a two–allele result the mother and fetus must be heterozygous and share the same two alleles, or one must be homozygous and the other must be heterozygous. In the former circumstance, the alleles would be equal in contribution; in the latter circumstance, the shared allele of the homozygous contributor and the heterozygous contributor should be three times as large as the remaining allele contribution (assuming a 50/50 mixture of contributors). A single allele result would indicate that the embryo/fetus and mother were both homozygous sharing the same allele. If the maternal profile is known, which it was not in this study, it is generally simple to discern the fetal profile. Additionally, if both the maternal and putative father's profiles are known (also not known in this research), this task becomes even easier with fewer assumptions.

Sample Concordance

Samples were compared between amplification sets at overlapping loci D3S1358, D7S820 and amelogenin, as well as between extraction sets at all loci to determine if the results were concordant. Allele designations and ratios were expected to be identical for

the overlapping loci of Profiler Plus™- and COfiler™- amplified samples with the exception of the presence of additional minor contributor alleles in COfiler™ due to greater observed sensitivity at the Lansing Laboratory. It was anticipated that the Chelex®-extracted and organically-extracted samples could vary in the ratio of allelic contribution between mother and offspring as a result of adjacent sampling. Both sets of samples needed to exhibit at least one identical allele designation at each locus to indicate concordance.

While comparing samples for concordance, relationships among samples were identified. Allele designations within each extraction set and between extraction sets were compared and samples that shared one or more alleles at each locus were considered to be associated. Some samples were compared using only one amplification system or one extraction method due to limited profile information.

Statistical Analyses

DNA yields resulting from the organic and Chelex® extraction methods were compared. A mean of the DNA yield results was calculated for each of the methods. This value was used to conduct a two-tailed t test using the separate variance estimate (Bachman and Paternoster, 1997), indicating whether a significant difference in recovery of DNA existed between extraction methods. The following formula was used:

$$t_{obt} = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{(n_1 - 1)} + \frac{s_2^2}{(n_2 - 1)}}}$$

x_1 = sample mean from first sample set
x_2 = sample mean from second sample set
s_1 = sample standard deviation of first set
s_2 = sample standard deviation of second set
n_1 = sample size first set
n_2 = sample size second set

The following formula was used to calculate the degrees of freedom (df):

$$df \cong \left[\frac{\left(\frac{s_1^2}{n_1 - 1} + \frac{s_2^2}{n_2 - 1} \right)}{\left(\frac{s_1^2}{n_1 - 1} \right)^2 \left(\frac{1}{n_1 + 1} \right) + \left(\frac{s_2^2}{n_2 - 1} \right)^2 \left(\frac{1}{n_2 + 1} \right)} \right] - 2$$

s_1 = sample standard deviation of first set
s_2 = sample standard deviation of second set
n_1 = sample size first set
n_2 = sample size second set

The result was rounded to the nearest integer to obtain the approximate degrees of freedom. The null hypothesis to be tested was that no significant difference in DNA recovery existed between the two extraction processes.

A z test (Bachman and Paternoster, 1997) based on a proportion calculation of extraction attempts and the actual recovery of a full genetic profile was used to determine whether or not a significant difference existed between methods in obtaining full genetic profiles. The following formula was used:

$$z_{obt} = \left(\frac{(\hat{p}_1 - \hat{p}_2) - (p_1 - p_2)}{\sigma_{p1-p2}} \right)$$

$\hat{p}_1$ = the sample proportion for the first sample
$\hat{p}_2$ = the sample proportion for the second sample
p_1 = the first population proportion
p_2 = the second population proportion
σ_{p1-p2} = the standard error of the difference between proportions

The pooled standard error was calculated with the following formula:

$$\sigma_{p1-p2} = \sigma \sqrt{\frac{n_1 + n_2}{n_1 n_2}}$$

The result was then used to calculate the z_{obt} value. A 95% confidence interval was selected with a critical region z score of ± 1.96. The null hypothesis to be tested was that no significant difference existed between extraction processes in obtaining full genetic profiles.

RESULTS

Paraffin Extraction

Upon sampling, the embryonic/fetal tissue was firm in texture and solid in appearance due to the support of the paraffin wax. Xylene exposure effectively solubilized the paraffin wax—releasing it from the tissue. At this point, the tissue was pliable and soft with a sponge-like appearance. The addition of alcohol cleared the xylene from the tissue. After removal of the final alcohol solution, the xylene odor was not detectable. Traces of alcohol were removed from the tissue through evaporation.

DNA Quantification

Human DNA was detected in all of the 50 Chelex®-extracted samples utilizing the Quantiblot™ kit (Tables 1–3). The quantity of DNA recovered ranged from 25 ng to 1500 ng. DNA was detected in 36 of the 50 organically-extracted samples (72%) (Tables 4–6). Samples exhibited a range of DNA recovery from 0 ng to approximately 400 ng. Figures 7–9 illustrate the comparison of DNA quantity recovered from each Chelex® and organically extracted sample. Calculation of the percent difference in recovery revealed that 73.1% more DNA was acquired using the Chelex® method than with the organic method.

TABLE 1—*Quantiblot™ results of human DNA recovered from Chelex® extraction of Test Set 1 samples*

Sample Id#	µL*	ng/µL[†]	ng total[‡]
TS1 1	100	2.0	200
TS1 2	100	2.0	200
TS1 3	100	10.0	1000
TS1 4A	100	2.5	250
TS1 4B	100	10.0	1000
TS1 5	100	10.0	1000
TS1 6	100	5.0	500
TS1 7	100	2.5	250
TS1 8	125	0.625	78
TS1 9	100	10.0	1000
TS1 10	100	15.0	1500

*Volume of supernatant recovered from the Chelex® extraction method
[†] Quantity of DNA contained within one microliter of DNA sample
[‡] Total quantity of DNA contained within the DNA sample

TABLE 2—*Quantiblot™ results of human DNA recovered from Chelex® extraction of Test Set 2 samples*

Sample Id#	µL*	ng/µL†	ng total‡
TS2 1	100	3.0	300
TS2 2	100	2.5	250
TS2 3	100	12.5	1250
TS2 4	100	0.625	62.5
TS2 5	100	1.5	150
TS2 6	100	12.5	1250
TS2 7	100	0.625	62.5
TS2 8	100	1.5	150
TS2 9	100	2.5	250
TS2 10	100	0.5	50
TS2 11	100	2.5	250
TS2 12	100	2.5	250
TS2 13	100	10.0	1000
TS2 14	150	0.625	93.8
TS2 15	100	1.0	100
TS2 16	100	15.0	1500
TS2 17	150	1.0	150
TS2 18	100	2.5	250
TS2 19	100	2.0	200
TS2 20	350	2.0	700

*Volume of supernatant recovered from the Chelex® extraction method

†Quantity of DNA contained within one microliter of DNA sample

‡Total quantity of DNA contained within the DNA sample

TABLE 3—*Quantiblot™ results of human DNA recovered from Chelex® extraction of Test Set 3 samples*

Sample Id#	µL*	ng/µL†	ng total‡
TS3 A	100	2.0	200
TS3 B	175	2.0	350
TS3 C	100	3.5	350
TS3 D	150	2.5	375
TS3 E	100	5.0	500
TS3 F	100	2.5	250
TS3 I	175	3.5	612.5
TS3 J	100	2.0	200
TS3 K	100	3.5	350
TS3 M	150	2.5	375
TS3 N	100	7.5	750
TS3 O	100	3.5	350
TS3 Q	100	0.5	50
TS3 R	100	2.5	250
TS3 S	100	2.5	250
TS3 T	100	3.5	350
TS3 U	100	2.0	200
TS3 V	150	3.5	525
TS3 W	200	2.0	400

*Volume of supernatant recovered from the Chelex® extraction method
†Quantity of DNA contained within one microliter of DNA sample
‡Total quantity of DNA contained within the DNA sample

TABLE 4—*Quantiblot™ results of human DNA recovered from organic extraction of Test Set 1 samples*

Sample Id#	µL*	ng/µL†	ng total‡
TS1 1	100	0.3125	31.25
TS1 2	150	0.3125	46.88
TS1 3	125	0.3125	39.06
TS1 4A	100	1.75	175
TS1 4B	75	0	0
TS1 5	100	0.3125	31.25
TS1 6	100	0	0
TS1 7	125	0.3125	16.56
TS1 8	100	0	0
TS1 9	125	0.5	62.5
TS1 10	125	0.15625	19.531

*Volume of supernatant recovered from the organic extraction method
†Quantity of DNA contained within one microliter of DNA sample
‡Total quantity of DNA contained within the DNA sample

TABLE 5—*Quantiblot™ results of human DNA recovered from organic extraction of Test Set 2 samples*

Sample Id#	µL*	ng/µL[†]	ng total[‡]
TS2 1	175	0	0
TS2 2	100	0	0
TS2 3	100	1.75	175
TS2 4	100	0	0
TS2 5	100	0.3125	31.25
TS2 6	100	0.15625	15.625
TS2 7	100	0	0
TS2 8	100	0	0
TS2 9	100	0.3125	31.25
TS2 10	100	0	0
TS2 11	100	0	0
TS2 12	100	0	0
TS2 13	100	0	0
TS2 14	150	1.25	125
TS2 15	100	0	0
TS2 16	150	0.3125	46.88
TS2 17	75	1.25	93.8
TS2 18	75	1.75	131.3
TS2 19	50	1.25	62.5
TS2 20	100	0	0

*Volume of supernatant recovered from the organic extraction method
[†] Quantity of DNA contained within one microliter of DNA sample
[‡] Total quantity of DNA contained within the DNA sample

TABLE 6—*Quantiblot™ results of human DNA recovered from organic extraction of Test Set 3 samples*

Sample Id#	µL*	ng/µL[†]	ng total[‡]
TS3 A	175	0.3125	54.69
TS3 B	300	0.3125	93.75
TS3 C	200	0.5	100
TS3 D	150	0.5	75
TS3 E	300	0.3125	93.75
TS3 F	125	0.5	62.5
TS3 I	150	0.625	93.8
TS3 J	75	1.75	131
TS3 K	175	0.625	109
TS3 M	75	5	375
TS3 N	75	1.75	131
TS3 O	75	2.5	187.5
TS3 Q	75	1.25	93.8
TS3 R	75	2	150
TS3 S	75	2	150
TS3 T	75	1.25	93.8
TS3 U	75	1.5	112.5
TS3 V	75	2.5	187.5
TS3 W	75	1.5	112.5

*Volume of supernatant recovered from the organic extraction method

[†] Quantity of DNA contained within one microliter of DNA sample

[‡] Total quantity of DNA contained within the DNA sample

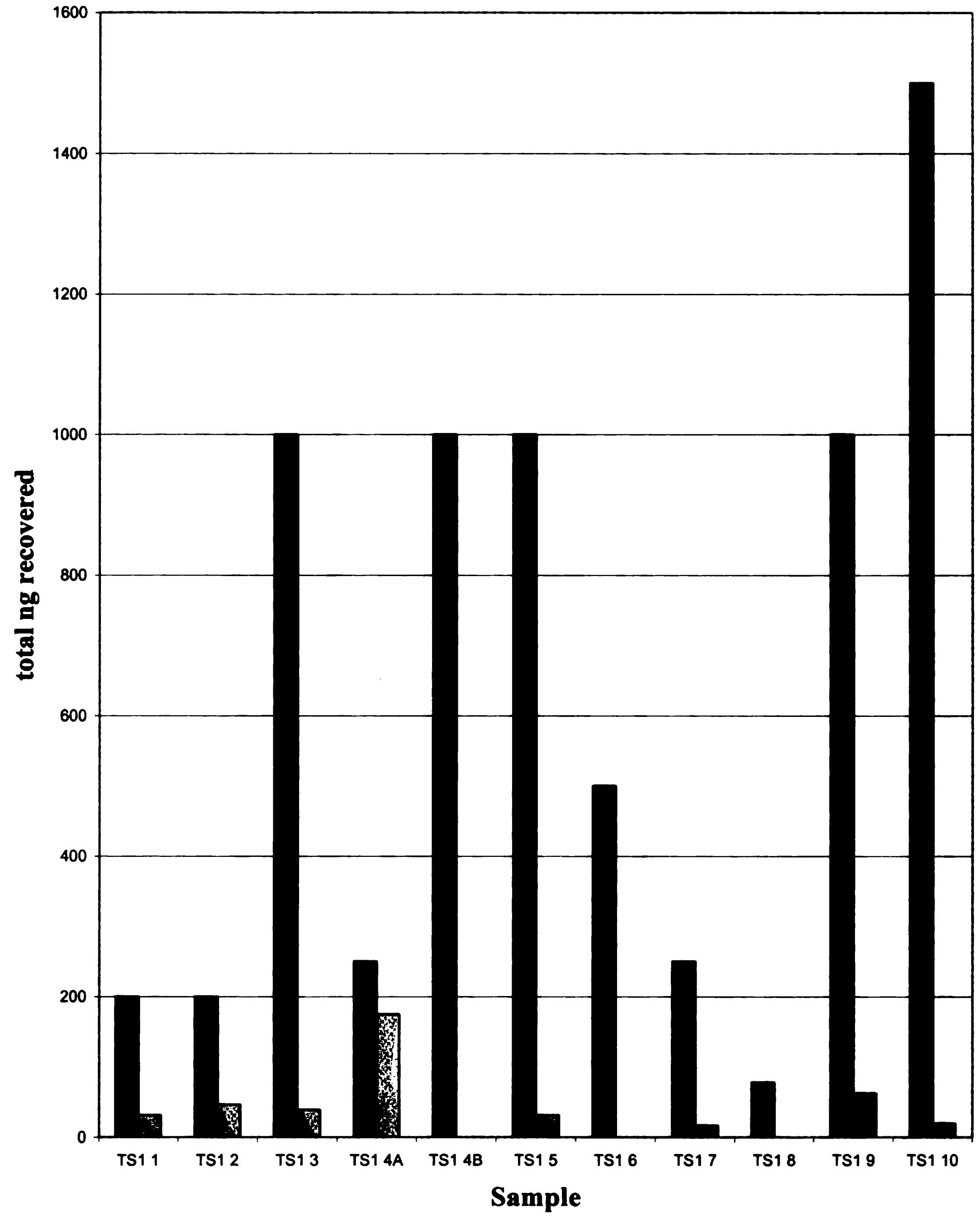


FIG. 7—*Comparison of DNA recovery from TS-1 Chelex®- and organically-extracted samples. Odd columns (black) represent Chelex®-extracted samples. Even columns (gray) represent organically-extracted samples. Sample quantities were obtained using the Quantiblot™ kit procedure and were measured in ng.*

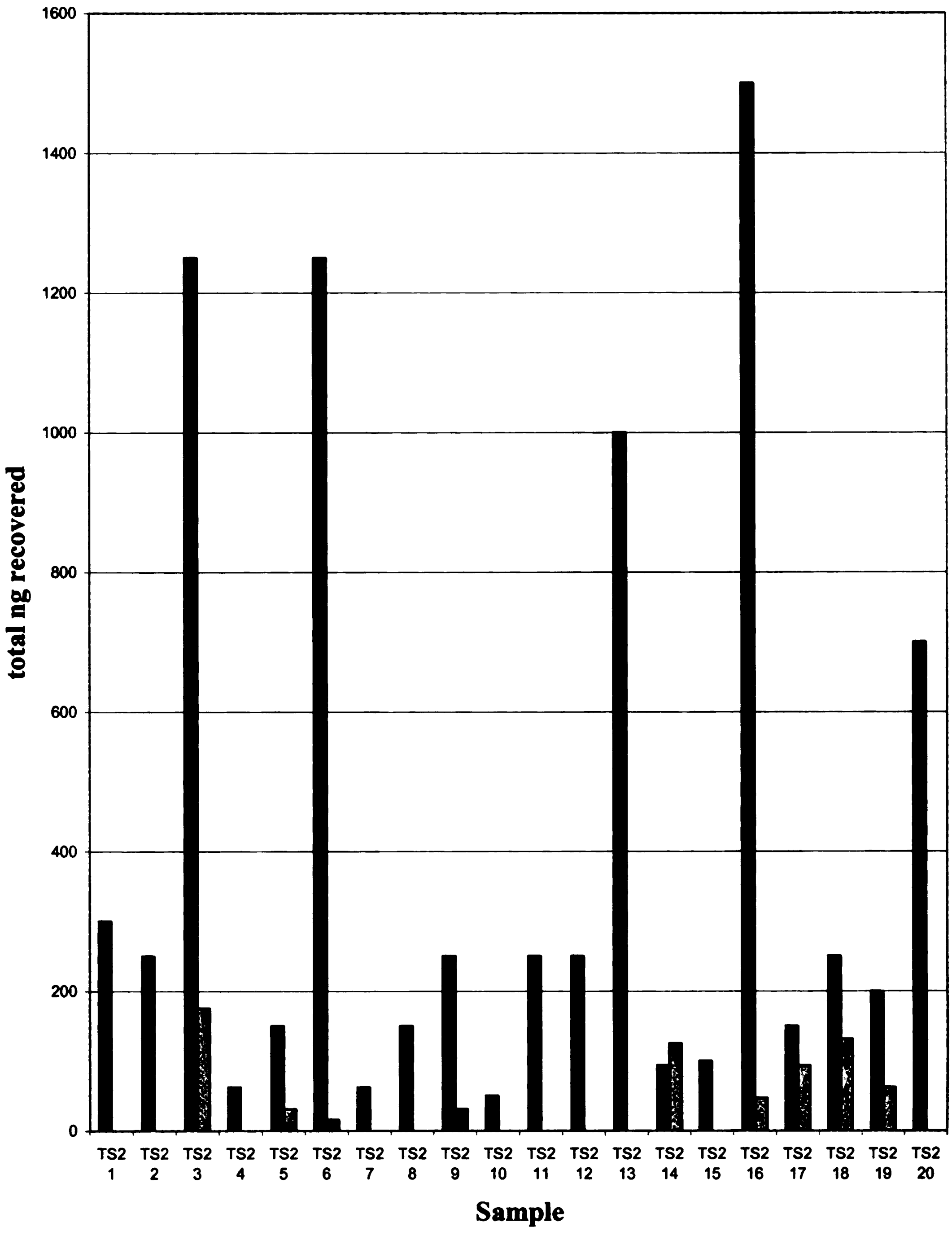


FIG. 8—*Comparison of DNA recovery from TS-2 Chelex®- and organically-extracted samples. Odd columns (black) represent Chelex®-extracted samples. Even columns (gray) represent organically-extracted samples. Sample quantities were obtained using the Quantiblot™ kit procedure and were measured in ng.*

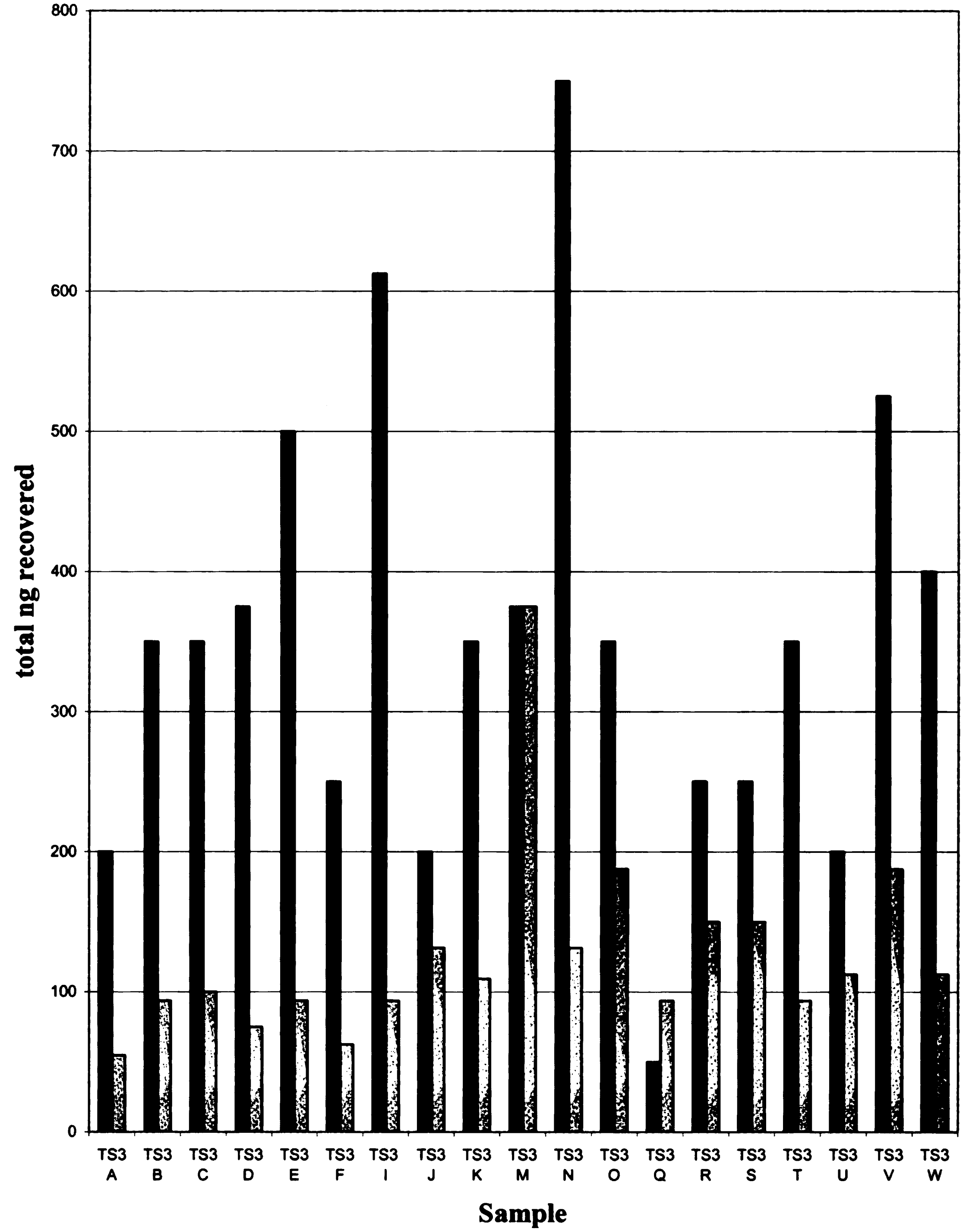


FIG. 9—*Comparison of DNA recovery from TS-3 Chelex®- and organically-extracted samples. Odd columns (black) represent Chelex®-extracted samples. Even columns (gray) represent organically-extracted samples. Sample quantities were obtained using the Quantiblot™ kit procedure and were measured in ng.*

Amplification and Electrophoresis of Purified DNA

Results for all of the thirteen CODIS core loci and amelogenin were obtained from 48 of the Chelex®-extracted samples (96%) amplified with the Profiler Plus™ Amplification kit, while 37 of the organically-extracted samples (74%) generated full profiles (Fig. 10; Tables 7–9). Results using COfiler™ included 48 of the samples extracted with Chelex® (96%) and 15 of the organically-extracted samples (30%). All control samples performed as expected.

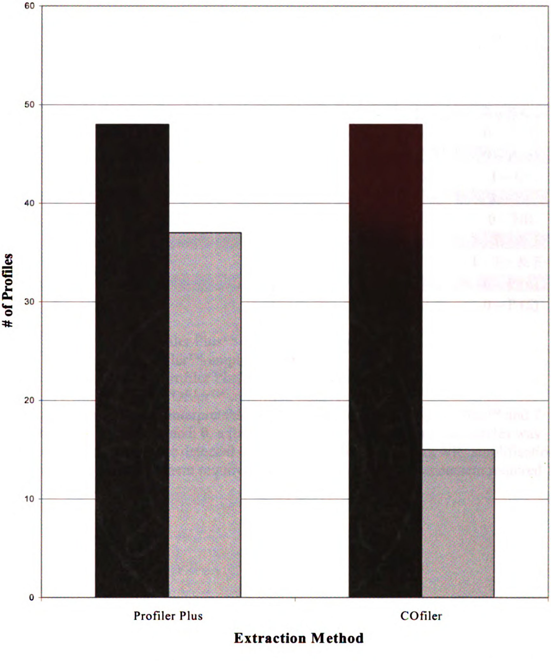


FIG. 10—*Number of full profiles generated for Chelex® extraction (black–1st and 3rd columns) and organic extraction (gray–2nd and 4th* columns). *Full profiles were generated for 48 samples (96%) extracted with Chelex® and amplified with the Profiler Plus™ amplification kit (black–1st column). Full profiles were generated for 37 samples (74%) extracted organically and amplified with the Profiler Plus™ amplification kit (white–2nd column). Full profiles were generated for 48 samples (96%) extracted with Chelex® and amplified with the COfiler™ amplification kit (black–3rd column). Full profiles were generated for 15 samples (30%) extracted organically and amplified with the COfiler™ amplification kit (white–4th column).*

TABLE 7—*Full genetic profiles generated for Test Set 1 samples*

Sample Id#	Chelex® Profiler Plus™*	Chelex® COfiler™†	Organic Profiler Plus™‡	Organic COfiler™§
TS1 1	**1** – E>	**1** – E>	**1** – E>	**1** – E>
TS1 2	**1** – E>	**1** – E>	**0** – P (9)	**0** – P (3)
TS1 3	**1**	**1**	**0** – P (10)	**0** – P (7)
TS1 4A	**1**	**1** – E>	**0** – P (8)	**0** – P (5)
TS1 4B	**1**	**1**	**1** – E> & E<	**1** – E<
TS1 5	**1**	**1**	**1** – E>	**1** – E>
TS1 6	**1** – E>	**1**	**0** – P (5)	**0** – NR
TS1 7	**1**	**1** – E<	**1**	**1** – E> & E<
TS1 8	**1** – E>	**1** – E<	**1** – E>	**1** – E> & E<
TS1 9	**1**	**1**	**1** – E>	**0** – P (6)
TS1 10	**1**	**1**	**1** – E>	**0** – P (5)

*Chelex®-extracted and Profiler Plus™-amplified samples
†Chelex®-extracted and COfiler™-amplified samples
‡Organically-extracted and Profiler Plus™-amplified samples
§Organically-extracted and COfiler™-amplified samples
1, a full genetic profile with interpretable alleles at all 10 loci for Profiler Plus™ and 7 loci for COfiler™ was obtained; **0**, a full genetic profile with interpretable alleles was not obtained; P, partial profile detected & ( ), complete loci generated; NR, amplification not conducted; E>, enhancement required 3 µL preparation; E<, enhancement required 1–4 second injection

TABLE 8—*Full genetic profiles generated for Test Set 2 samples*

Sample Id#	Chelex® Profiler Plus™*	Chelex® COfiler™†	Organic Profiler Plus™‡	Organic COfiler™§
TS2 1	**1**	**1**	**1** – E>	**0** – NR
TS2 2	**1** – E<	**1** – E<	**0** – P (8)	**0** – NR
TS2 3	**1**	**1** – E<	**1**	**1** – E<
TS2 4	**1** – E>	**1** – E>	**1** – E>	**0** – NR
TS2 5	**1** – E>	**0** – P (5)	**1** – E<	**1** – E<
TS2 6	**1** – E<	**1** – E<	**1**	**1**
TS2 7	**0** – P (2), E>	**1** – E>	**1** – E>	**0** – NR
TS2 8	**1**	**1**	**1**	**0** – NR
TS2 9	**1**	**1**	**0** – ND	**0** – ND
TS2 10	**1**	**1**	**1** – E<	**0** – NR
TS2 11	**1**	**1**	**1**	**0** – NR
TS2 12	**1**	**1**	**1** – E<	**0** – NR
TS2 13	**1** – E<	**1** – E<	**1** – E>	**0** – NR
TS2 14	**1** – E>	**1**	**0** – ND	**0** – NR
TS2 15	**1**	**1**	**1**	**0** – NR
TS2 16	**1** – E<	**1**	**0** – P (2)	**0** – P (6)
TS2 17	**0** – P (8)	**1**	**0** – P (9)	**0** – NR
TS2 18	**1**	**1**	**0** – P (3), E>	**0** – NR
TS2 19	**1**	**1**	**1** – E>	**1**
TS2 20	**1** – E<	**1**	**0** – ND	**0** – NR

*Chelex®-extracted and Profiler Plus™-amplified samples
†Chelex®-extracted and COfiler™-amplified samples
‡Organically-extracted and Profiler Plus™-amplified samples
§Organically-extracted and COfiler™-amplified samples
1, a full genetic profile with interpretable alleles at all 10 loci for Profiler Plus™ and 7 loci for COfiler™ was obtained; **0**, a full genetic profile with interpretable alleles was not obtained; P, partial profile detected & ( ), complete loci generated; NR, amplification not conducted; E>, enhancement required 3 μL preparation; E<, enhancement required 1–4 second injection

TABLE 9—*Full genetic profiles generated for Test Set 3 samples*

Sample Id#	Chelex® Profiler Plus™*	Chelex® COfiler™†	Organic Profiler Plus™‡	Organic COfiler™§
TS3 A	1 – E<	1 – E<	0 – P (5)	0 – NR
TS3 B	1 – E<	1	1 – E>	0 – NR
TS3 C	1	1	0 – P (9)	0 – NR
TS3 D	1 – E>	1	1 – E<	0 – NR
TS3 E	1 – E<	1	1 – E>	0 – NR
TS3 F	1	1 – E<	1 – E>	0 – NR
TS3 I	1 – E<	1	1 – E<	0 – NR
TS3 J	1	1 – E<	1 – E<	1 – E<
TS3 K	1 – E>	1	1 – E>	0 – NR
TS3 M	1 – E<	1 – E<	1 – E<	1 – E<
TS3 N	1 – E<	1	1 – E<	1 – E<
TS3 O	1 – E<	1 – E<	1 – E<	0 – NR
TS3 Q	1 – E> & E<	1 – E<	1 – E<	0 – NR
TS3 R	1	1 – E<	1 – E<	0 – NR
TS3 S	1	1 – E<	1 – E<	0 – P (5)
TS3 T	1 – E<	1 – E<	1 – E<	0 – NR
TS3 U	1	0 – P (5)	1 – E>	1 – E<
TS3 V	1 – E<	1	1	1
TS3 W	1	1	1	1 – E<

*Chelex®-extracted and Profiler Plus™-amplified samples
†Chelex®-extracted and COfiler™-amplified samples
‡Organically-extracted and Profiler Plus™-amplified samples
§Organically-extracted and COfiler™-amplified samples
1, a full genetic profile with interpretable alleles at all 10 loci for Profiler Plus™ and 7 loci for COfiler™ was obtained; **0**, a full genetic profile with interpretable alleles was not obtained; P, partial profile detected & ( ), complete loci generated; NR, amplification not conducted; E>, enhancement required 3 µL preparation; E<, enhancement required 1–4 second injection

TS1—Profile Results

All of the Chelex®-extracted TS1 samples amplified using the Profiler Plus™ and COfiler™ kits and each exhibited full profile results. Likewise, all organically-extracted TS1 samples amplified using the Profiler Plus™ kit, however only seven generated full profiles while four were partial. Ten of the organically-extracted TS1 samples amplified using the COfiler™ kit; five resulted in full profiles and five in partial profiles.

TS2—Profile Results

The 20 TS2 samples extracted with Chelex® amplified using the Profiler Plus™ and COfiler™ kits. Eighteen samples amplified using Profiler Plus™ and nineteen samples amplified using COfiler™ produced full profiles, while two and one produced partial profiles respectively. The partial profiles originated from different samples (Table 8).

Of the 20 organically-extracted samples amplified using the Profiler Plus™ kit—13 produced full profiles, four were partial, and three showed no results (reasons undetermined). Only six organically-extracted samples were amplified using the COfiler™ kit—four generated full profiles, one was partial and no results were detected for one. Amplification of the other 14 samples was not conducted due to insufficient quantities of DNA. These samples were concentrated and consumed for Profiler Plus™ amplification due to the limited quantity of DNA present.

TS3—Profile Results

All 19 of the Chelex®-extracted TS3 samples amplified using the Profiler Plus™ and COfiler™ kits. Nineteen samples amplified with Profiler Plus™ produced full profiles. Eighteen samples amplified using COfiler™ generated full profiles and one produced a partial profile.

Nineteen of the organically-extracted TS3 samples amplified using the Profiler Plus™ kit and full profiles were generated for seventeen of them; two produced partial profiles. Of seven samples amplified using the COfiler™ kit, six resulted in full profiles and one a partial profile. Due to the limited quantity of DNA present, the other 12 samples were not amplified, and instead were concentrated and consumed for Profiler Plus™ amplification.

Genetic Profile Composition

Electropherograms of Chelex®- and organically-extracted samples were evaluated and compared. Based on the results, six categories were appropriate for explanation of the genetic information obtained—single-source female (Fig. 11), single-source female with additional activity (Fig. 12), single-source male (Fig. 13), single-source male with additional activity (Fig. 14), mixture of females (Fig. 15), and mixture of a female and a male (Fig. 16). Samples were categorized by allele presence and balance at the amelogenin locus, by the number of alleles present at the other loci and the balance between those alleles. Additionally, the presence of any allele at any locus which did not meet the minimum threshold for reporting (150 RFUs) and was not an artifact (pull-up, fluorescent spike, or noisy baseline) was termed 'activity' and suggested an additional DNA contributor. Results which could be attributed to an embryo/fetus were observed in the following sample categories: single-source male, single-source male or female with additional activity, mixture of females, and mixture of a female and a male. Source attribution (embryo/fetus or mother) of the single-source female profiles and the single-source female with additional activity profiles was not discernable without comparison to known samples from the mothers.

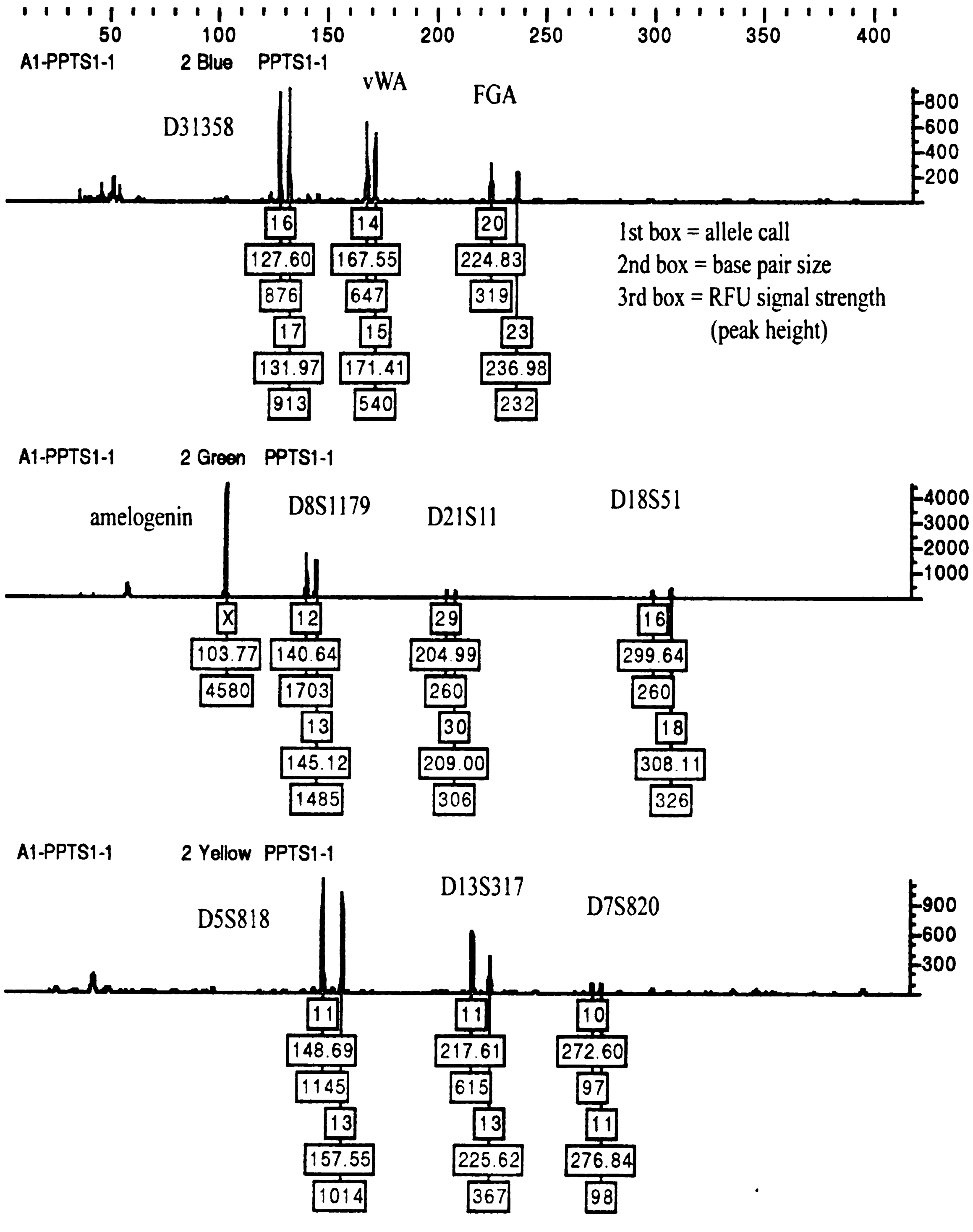


FIG. 11—*Sample TS1-1 (extracted with Chelex® and amplified with the Profiler Plus™ amplification kit) exhibited a full single-source female profile. Alleles at the D13S317 locus did not fit the 70% rule for interpretation. With 3 μL enhancement, this profile was expected to be interpretable at all loci shown and the imbalance at D13S317 may be resolved.*

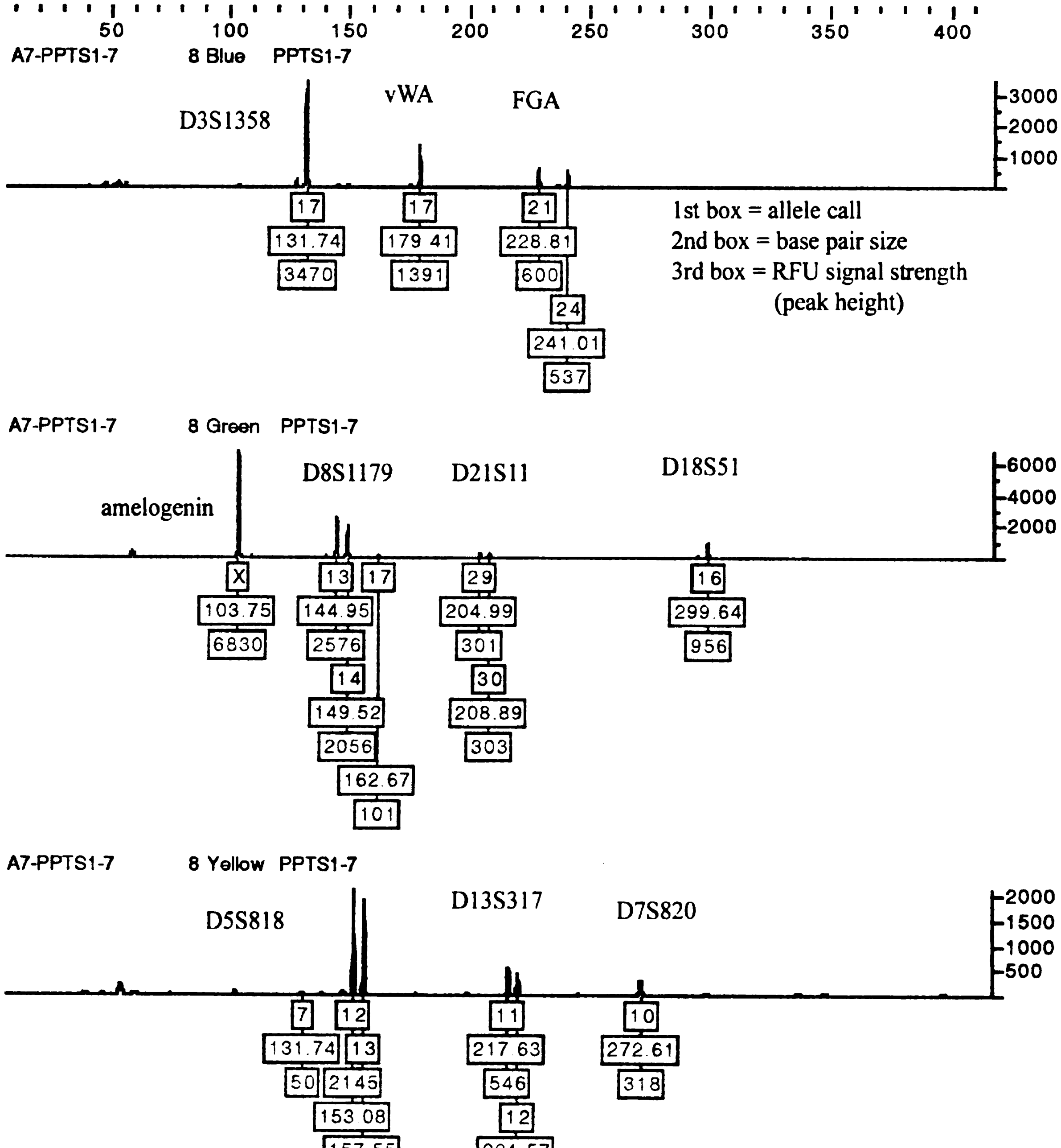


FIG. 12—*Sample TS1-7 (extracted with Chelex® and amplified with the Profiler Plus™ amplification kit) exhibited a full single-source female profile with additional activity. Three allele calls, 13, 14, and 17, were observed at the D8S1179 locus. The 17 allele fell below the minimum threshold of 150 RFUs for interpretation; therefore, this allele was not considered. An additional allele (7) was present at the D5S818 locus. Since the base pair size matches the 17 allele at the D3S1358 locus, this call may have resulted from pull-up. It was not considered to be an allele or activity for this reason.*

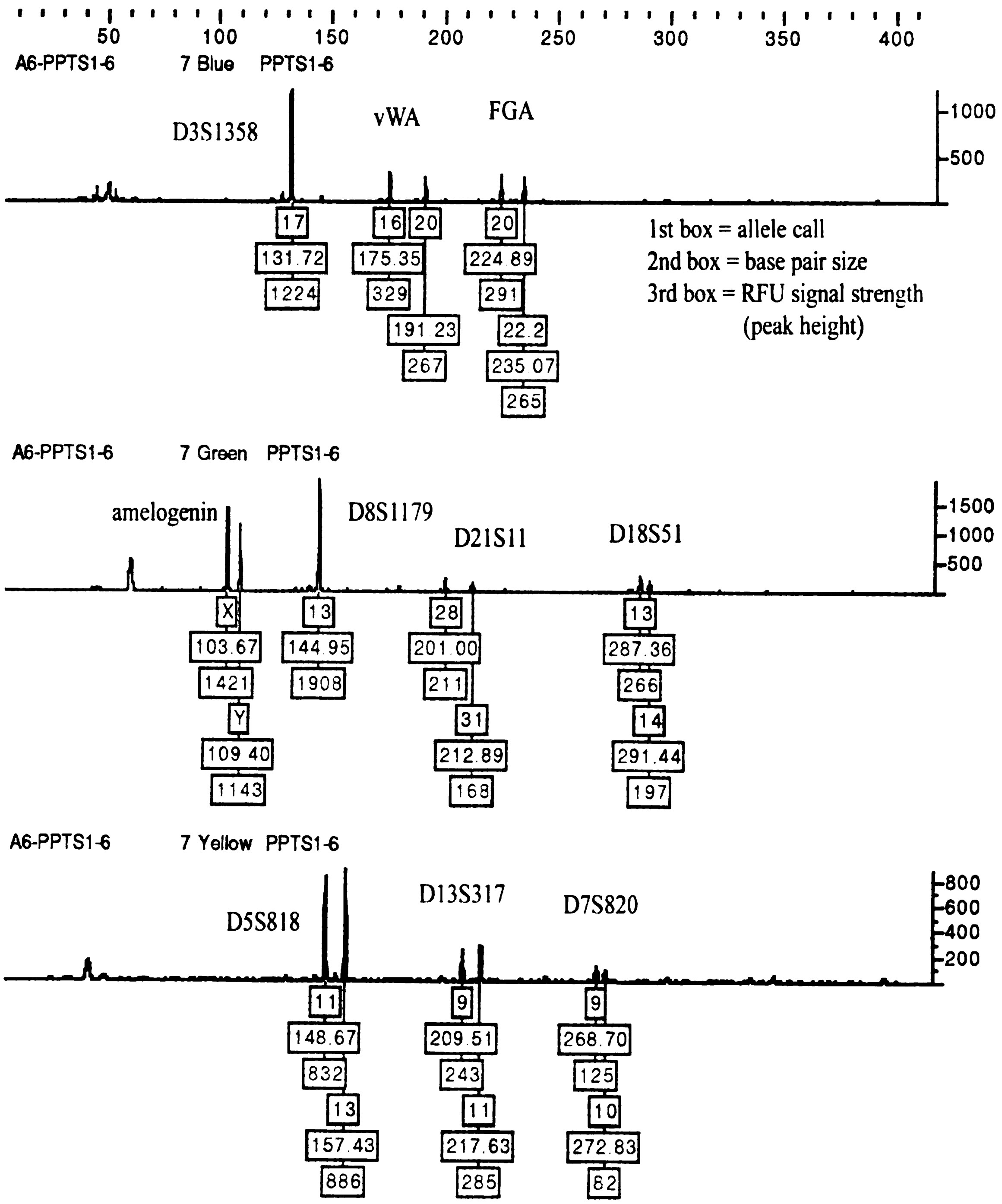


FIG. 13—*Sample TS1-6 (extracted with Chelex® and amplified with the Profiler Plus™ amplification kit) exhibited a single-source male profile. With 3 μL enhancement, this profile was expected to be interpretable at all loci shown.*

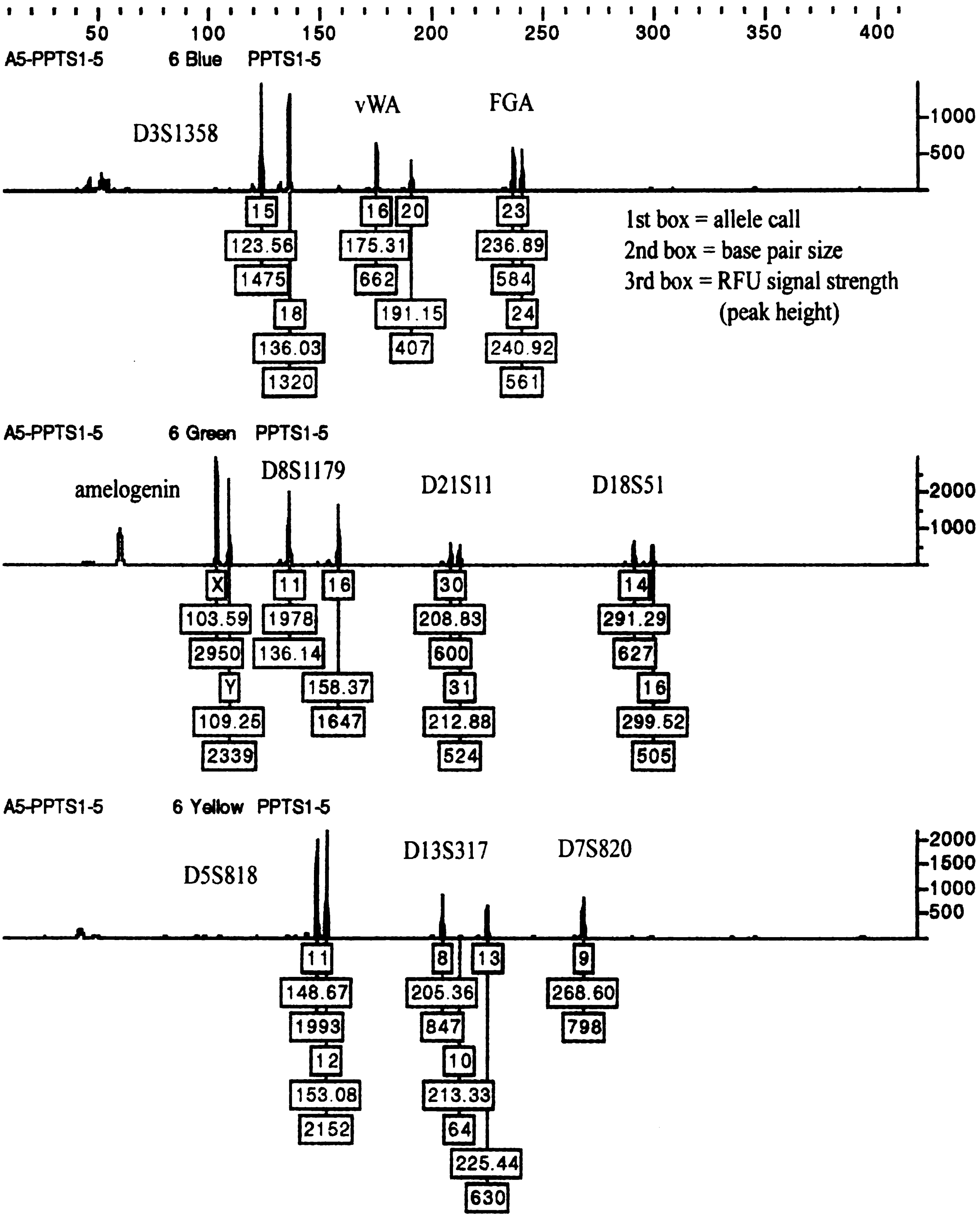


FIG. 14—*Sample TS1-5 (extracted with Chelex® and amplified with the Profiler Plus™ amplification kit) exhibited a single-source male profile with additional activity. Three allele calls, 8, 10, and 13, were observed at the D13S317 locus. The 10 allele fell below the minimum threshold of 150 RFUs for interpretation; therefore, it was classified as activity and was not considered.*

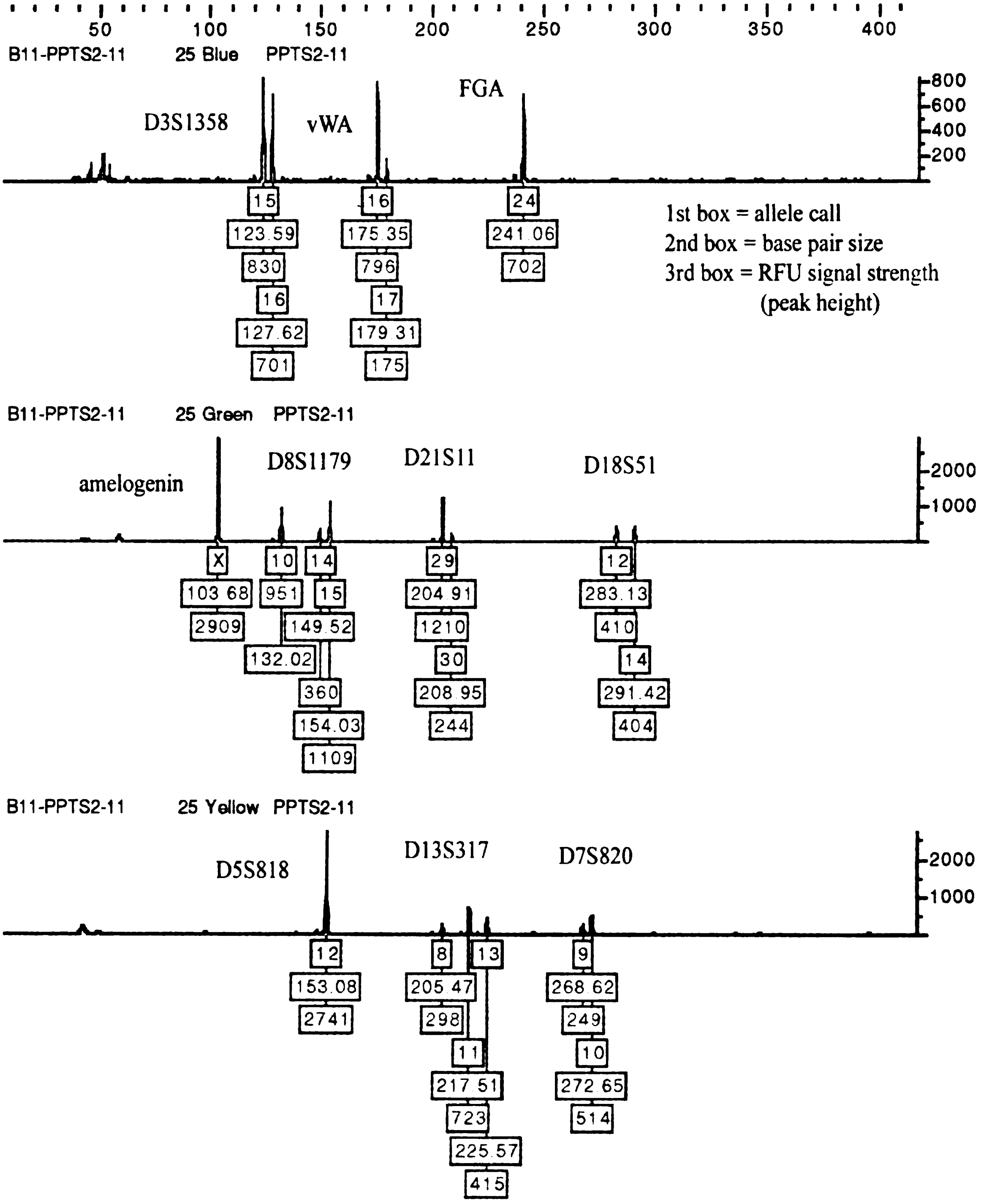


FIG. 15—*Sample TS2-11 (extracted with Chelex® and amplified with the Profiler Plus™ amplification kit) exhibited a mixture of female DNA. The presence of three alleles at two of the loci (D8S1179 and D13S317) and the imbalance of alleles at the vWA, D21S11, and D7S820 loci indicate two donors with shared alleles consistent with heredity by a full offspring. The ratio of contribution (female to female) is approximately 2:1.*

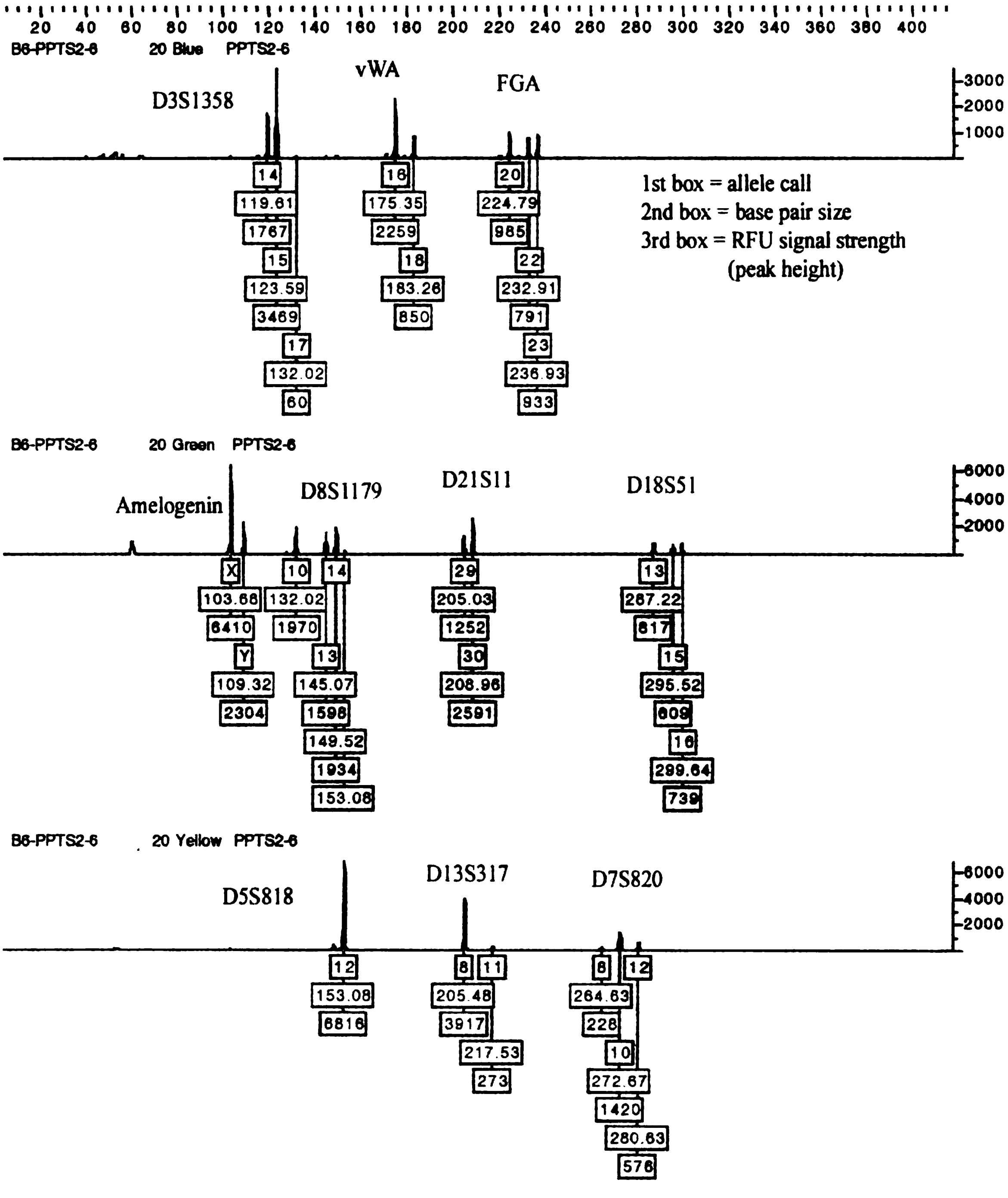


FIG. 16—*Sample TS2-6 (extracted with Chelex® and amplified with the Profiler Plus™ amplification kit) exhibited a mixture of female and male DNA (D3S1358, vWA, FGA, amelogenin, D8S1179, D21S11, and D18S51 are depicted above). The presence of the Y allele at amelogenin, the imbalance of the X and Y allele at amelogenin, the presence of three alleles at three of the loci (FGA, D8S1179, and D18S51) and the imbalance of the remaining alleles indicate two donors with shared alleles consistent with heredity by a full offspring. The ratio of contribution (male to female) is approximately 2:1.*

While the isolation of embryonic/fetal DNA was indicated in results from both extraction methods, the profiles generated from organically-extracted samples were frequently deemed insufficient for comparison purposes or suitable only for limited comparison. Significantly less DNA was recovered from the organically-extracted samples (see Statistical Estimates below) and there was a possible relationship between unsuccessful organic results and the amount of DNA observed in the Quantiblot™ procedure; partial profile or no profile results were more prevalent for samples that contained less than the target 1.0 ng of DNA. With fewer interpretable alleles identified, assuming many were attributable to the embryo/fetus; comparative power of the sample was diminished.

Chelex®-Extracted and Profiler Plus™-Amplified Samples

Data (electropherograms) from samples extracted with Chelex® and amplified with Profiler Plus™ were examined for embryonic/fetal profiles (Tables 10–12). Two samples, TS1-6 (Fig. 13) and TS1-9, generated single-source male profiles. One sample, TS1-5, produced a single-source male profile with additional allelic activity detected that fell below reporting threshold. Eight samples, TS1-7 (Fig. 12), TS2-10, TS2-14, TS3-A, TS3-B, TS3-E, TS3-J, and TS3-V, generated single-source female profiles with additional allelic activity that fell below reporting threshold. The following 25 samples were mixtures of two females: TS1-2, TS1-3, TS2-8, TS2-9, TS2-11 (Fig. 15), TS2-12, TS2-13, TS2-15, TS2-16, TS2-17, TS2-18, TS2-19, TS3-C, TS3-D, TS3-F, TS3-I, TS3-K, TS3-M, TS3-N, TS3-O, TS3-Q, TS3-S, TS3-T, TS3-U, and TS3-W. Nine samples were mixtures of a male and female (Fig. 16), TS1-4A, TS1-4B, TS2-1, TS2-2, TS2-3, TS2-4, TS2-5, TS2-6 (Fig. 17), and TS2-20. Five samples, TS1-1 (Fig. 11), TS1-8, TS1-10, TS2-7, and TS3-R, produced single source female profiles.

TABLE 10—*Genetic profile composition of Test Set 1 samples*

Sample Id#	Chelex® Profiler Plus™*	Chelex® COfiler™†	Organic Profiler Plus™‡	Organic COfiler™§
TS1 1	SS-F	SS-F	SS-F	SS-F
TS1 2	MX-F	MX-F	SS-F (A)	SS-F (A)
TS1 3	MX-F	MX-F	SS-F (A)	SS-F (A)
TS1 4A	MX-M	MX-M	MX-M	MX-M
TS1 4B	MX-M	MX-M	SS-M (A)	SS-M (A)
TS1 5	SS-M (A)	SS-M	SS-M (A)	SS-M (A)
TS1 6	SS-M	SS-M	SS-F	
TS1 7	SS-F (A)	SS-F	MX-M	MX-M
TS1 8	SS-F	SS-F (A)	SS-F (A)	SS-F
TS1 9	SS-M	SS-M	SS-F	SS-F
TS1 10	SS-F	SS-F	SS-F	SS-F

*Chelex®-extracted and Profiler Plus™-amplified samples
†Chelex®-extracted and COfiler™-amplified samples
‡Organically-extracted and Profiler Plus™-amplified samples
§Organically-extracted and COfiler™-amplified samples
SS, single-source; MX, mixture; F, female; M, male; (A), allelic activity below reporting threshold; empty field, amplification not conducted—no sample results

TABLE 11—Genetic profile composition of Test Set 2 samples

Sample Id#	Chelex® Profiler Plus™*	Chelex® COfiler™†	Organic Profiler Plus™‡	Organic COfiler™§
TS2 1	MX-M	MX-M	SS-M	
TS2 2	MX-M	MX-M	MX-M	
TS2 3	MX-M	MX-M	MX-M	MX-M
TS2 4	MX-M	MX-M	MX-M	
TS2 5	MX-M	MX-M	MX-M	MX-M
TS2 6	MX-M	MX-M	MX-M	MX-M
TS2 7	SS-F	SS-F (A)	SS-F (A)	
TS2 8	MX-F	SS-F (A)	MX-F	
TS2 9	MX-F	MX-F		
TS2 10	SS-F (A)	SS-F	MX-F	
TS2 11	MX-F	SS-F (A)	MX-F	
TS2 12	MX-F	SS-F	MX-F	
TS2 13	MX-F	MX-F	MX-F	
TS2 14	SS-F (A)	SS-F (A)		
TS2 15	MX-F	SS-F (A)	MX-F	
TS2 16	MX-F	SS-F (A)	SS-F (A)	SS-F
TS2 17	MX-F	SS-F (A)	SS-F (A)	
TS2 18	MX-F	MX-F	SS-F	
TS2 19	MX-F	MX-F	MX-F	MX-F
TS2 20	MX-M	MX-M		

*Chelex®-extracted and Profiler Plus™-amplified samples
†Chelex®-extracted and COfiler™-amplified samples
‡Organically-extracted and Profiler Plus™-amplified samples
§Organically-extracted and COfiler™-amplified samples
SS, single-source; MX, mixture; F, female; M, male; (A), allelic activity below reporting threshold; empty field, amplification not conducted—no sample results

TABLE 12—Genetic profile composition of Test Set 3 samples

Sample Id#	Chelex® Profiler Plus™*	Chelex® COfiler™†	Organic Profiler Plus™‡	Organic COfiler™§
TS3 A	SS-F (A)	SS-F (A)	SS-F	
TS3 B	SS-F (A)	SS-F (A)	SS-F	
TS3 C	MX-F	MX-F	SS-F (A)	
TS3 D	MX-F	MX-F	MX-F	
TS3 E	SS-F (A)	SS-F (A)	MX-F	
TS3 F	MX-F	MX-F	MX-F	
TS3 I	MX-F	MX-F	MX-F	
TS3 J	SS-F (A)	SS-F (A)	SS-F (A)	SS-F (A)
TS3 K	MX-F	MX-F	SS-F (A)	
TS3 M	MX-F	MX-F	MX-F	MX-F
TS3 N	MX-F	MX-F	MX-F	MX-F
TS3 O	MX-F	MX-F	MX-F	
TS3 Q	MX-F	MX-F	SS-F (A)	
TS3 R	SS-F	SS-F	SS-F (A)	
TS3 S	MX-F	MX-F	SS-F (A)	SS-F
TS3 T	MX-F	MX-F	MX-F	
TS3 U	MX-F	MX-F	MX-F	MX-F
TS3 V	SS-F (A)	SS-F (A)	MX-F	MX-F
TS3 W	MX-F	MX-F	SS-F (A)	SS-F (A)

*Chelex®-extracted and Profiler Plus™-amplified samples
†Chelex®-extracted and COfiler™-amplified samples
‡Organically-extracted and Profiler Plus™-amplified samples
§Organically-extracted and COfiler™-amplified samples
SS, single-source; MX, mixture; F, female; M, male; (A), allelic activity below reporting threshold; empty field, amplification not conducted—no sample results

Chelex®-Extracted and COfiler™-Amplified Samples

Electropherograms from samples extracted with Chelex® and amplified with COfiler™ were examined for embryonic/fetal profiles (Tables 10–12). Three samples—TS1-5, TS1-6, and TS1-9 are single-source male profiles. Results from 13 samples were single-source female profiles with additional allelic activity that fell below reporting threshold (TS1-8, TS2-7, TS2-8, TS2-11, TS2-14, TS2-15, TS2-16, TS2-17, TS3-A, TS3-B, TS3-E, TS3-J, and TS3-V). Nineteen were mixtures of two females (TS1-2, TS1-3, TS2-9, TS2-13, TS2-18, TS2-19, TS3-C, TS3-D, TS3-F, TS3-I, TS3-K, TS3-M, TS3-N, TS3-O, TS3-Q, TS3-S, TS3-T, TS3-U, and TS3-W). Nine were mixtures of a male and a female (TS1-4A, TS1-4B, TS2-1, TS2-2, TS2-3, TS2-4, TS2-5, TS2-6, and TS2-20). Six samples, TS1-1, TS1-7, TS1-10, TS2-10, TS2-12, and TS3-R, were single-source female profiles.

Organically-Extracted and Profiler Plus™-Amplified Samples

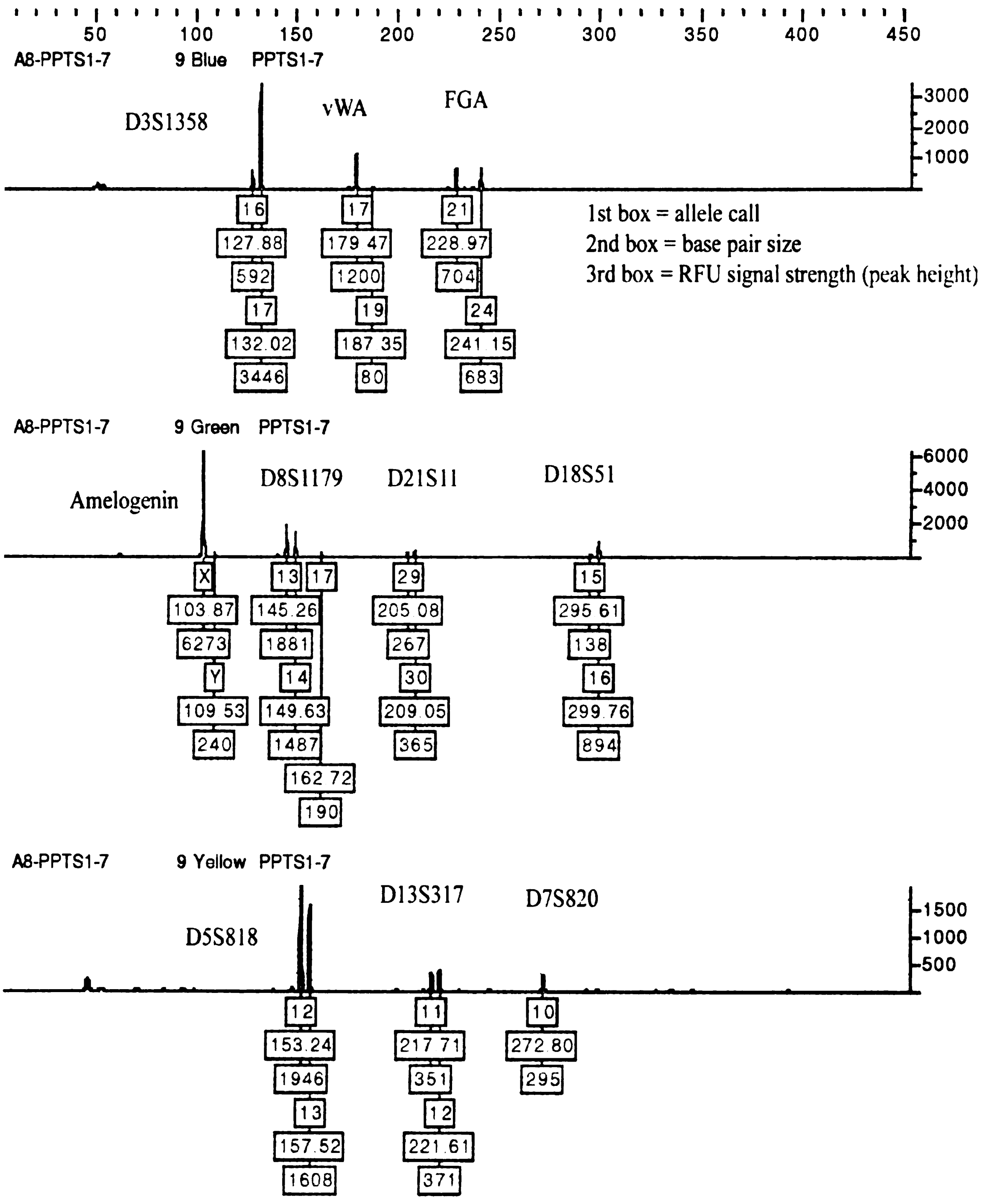
Results for samples organically-extracted and amplified with Profiler Plus™ were examined for embryonic/fetal profiles (Tables 10–12). Sample TS2-1 was a single-source male profile. Two, TS1-4B and TS1-5, were single-source male profiles with additional activity detected which did not meet reporting threshold. Thirteen samples, TS1-2, TS1-3, TS1-8, TS2-7, TS2-16, TS2-17, TS3-C, TS3-J, TS3-K, TS3-Q, TS3-R, TS3-S, and TS3-W, were single-source female profiles with additional activity detected which fell below reporting threshold. Seventeen were mixtures of two females (TS2-8, TS2-10, TS2-11, TS2-12, TS2-13, TS2-15, TS2-19, TS3-D, TS3-E, TS3-F, TS3-I, TS3-M, TS3-N, TS3-O, TS3-T, TS3-U, and TS3-V). Seven were mixtures of a male and a female (TS1-4A, TS1-7, TS2-2, TS2-3, TS2-4, TS2-5, and TS2-6). Seven samples, TS1-1, TS1-6, TS1-9, TS1-10, TS2-18, TS3-A, and TS3-B, were single-source female profiles. No results were obtained for the following samples: TS2-9, TS2-14 and TS2-20.

Organically-Extracted and COfiler™-Amplified Samples

Organically-extracted sample sets were amplified with COfiler™ and examined for embryonic/fetal profiles (Tables 10–12). Two samples, TS1-4B and TS1-5, were single-source male profiles with additional allelic activity which did not meet reporting threshold. Samples TS1-2, TS1-3, TS3-J, and TS3-W were single-source female profiles with additional activity detected which fell below reporting threshold. Five samples, TS2-19, TS3-M, TS3-N, TS3-U and TS3-V, were mixtures of two female profiles. Five were mixtures of a male and a female profile (TS1-4A, TS1-7, TS2-3, TS2-5, and TS2-6). Six samples were single-source female profiles (TS1-1, TS1-8, TS1-9, TS1-10, TS2-16, and TS3-S). There were no results observed for the following samples: TS1-6, TS2-1, TS2-2, TS2-4, TS2-7, TS2-8, TS2-9, TS2-10, TS2-11, TS2-12, TS2-13, TS2-14, TS2-15, TS2-17, TS2-18, TS2-20, TS3-A, TS3-B, TS3-C, TS3-D, TS3-E, TS3-F, TS3-I, TS3-K, TS3-O, TS3-Q, TS3-R, and T3S-T.

Concordance of Profiles Between Amplification Systems and Extraction Methods

Each Chelex®-extracted Profiler Plus™-amplified sample was compared to the same Chelex®-extracted COfiler™-amplified sample at overlapping loci D3S1358 and D7S820. Results were consistent for all samples that exhibited this information. The same comparison was made between the organically-extracted Profiler Plus™ and COfiler™ samples. This comparison was not conducted for the following samples based on the lack of results for one or both amplifications: TS1-6, TS2-1, TS2-3, TS2-7, TS2-8, TS2-9, TS2-10, TS2-11, TS2-12, TS2-13, TS2-14, TS2-15, TS2-17, TS2-18, TS2-20, TS3-A, TS3-B, TS3-C, TS3-D, TS3-E, TS3-F, TS3-I, TS3-K, TS3-O, TS3-Q, TS3-R, and TS3-T. There were no inconsistencies observed between the remaining samples that exhibited results at the D3S1358 and D7S820 loci. All samples were examined for the presence of additional alleles. None of the samples exhibited more than the expected maximum of three alleles at any locus. Further comparisons of the collective profile information from the Chelex®-extracted Profiler Plus™- and COfiler™-amplified samples and the organically-extracted samples were conducted. The alleles generated from analysis of the Chelex®- and the organically-extracted samples were concordant, however, not identical. The Chelex®- (Fig. 12) and organically- (Fig. 17) extracted Profiler-Plus™ amplified TS1-7 samples illustrate this point. The Chelex® TS1-7 result is a single-source female profile with additional activity and the organic is a mixture of female and male DNA. The Y allele at amelogenin, the 15 allele (activity) at D18S51, and the 16 allele at D3S1358 dropped out of the Chelex® sample.



1st box = allele call
2nd box = base pair size
3rd box = RFU signal strength (peak height)

FIG. 17—*Sample TS1-7 (extracted organically and amplified with the Profiler Plus™ amplification kit) exhibited a mixture of female and male DNA (D3S1358, amelogenin, D8S1179). The presence of the Y allele at amelogenin, the imbalance of the X and Y allele at amelogenin, the presence of three alleles at the D8S1179 locus and the imbalance of the D3S1358 and D18S51 alleles indicate two donors with shared alleles.*

Identification of Associated Samples

Based on the comparison of the sample profile results (all loci—Profiler Plus™ and COfiler™) for both extraction methods, 16 different abortions were identified (Table 13). Some determinations were made utilizing results from only one extraction procedure due to the lack of results for the other. Some were made based on results from one amplification system (Profiler Plus™ or COfiler™) due to the lack of results from the other. Figures 15 and 18 illustrate the association of two profiles from abortion number 12.

TABLE 13—*Associated samples from Test Sets 1, 2, and 3*

Abortion #	Test Set Samples Associated
1	TS1-1, TS1-2
2	TS1-3
3	TS1-4A, TS1-4B
4	TS1-5
5	TS1-6
6	TS1-7
7	TS1-8
8	TS1-9
9	TS1-10
10	TS2-1, TS2-2, TS2-3, TS2-4
11	TS2-5, TS2-6, TS2-7
12	TS2-8, TS2-9, TS2-10, TS2-11, TS2-12, TS2-13, TS2-14
13	TS2-17, TS2-18
14	TS2-19
15	TS2-20
16	All samples from TS3

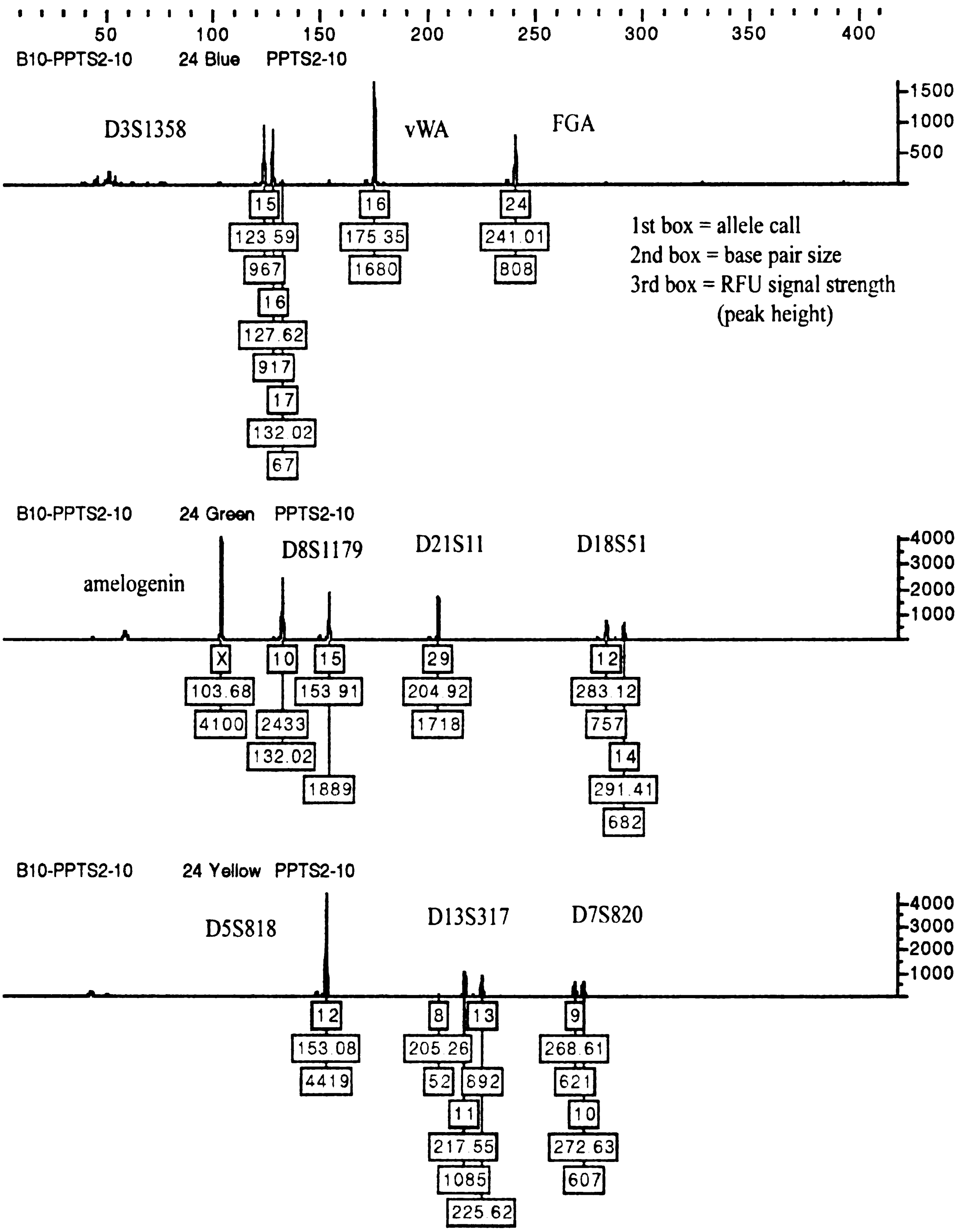


FIG. 18—*Sample TS2-10 (extracted with Chelex® and amplified with the Profiler Plus™ amplification kit) exhibited a single-source female profile with additional activity. The presence of activity at one locus (D13S317) indicates two donors. The additional activity allele at D3S1358 was due to pull-up and was not considered.*

Comparison of Single-Source Female Profiles to Associated Samples

Results from four of the Chelex®-extracted Profiler Plus™-amplified samples were single-source female profiles (TS1-1, TS1-8, TS1-10, and TS3-R). TS1-1 originated from the same abortion as TS1-2. Results for sample TS1-2, however, revealed a mixture of females. Because of this, it was not possible to discern if TS1-1 could be attributed to the mother or the female fetus. TS1-8 and TS1-10 were the only samples from two different abortions; therefore, no further comparison to aid in the determination of the profile source was possible. All of the samples from TS3 were from one abortion. Other samples from this abortion revealed mixtures of the same two female profiles (TS3-C, D, E, F, I, K, M, N, O, Q, S, T, U, V and W), therefore, no further source determination was possible. Six of the Chelex®-extracted COfiler™-amplified samples, TS1-1, TS1-7, TS1-10, TS2-10, TS2-12, and TS3-R, were single-source female profiles. The sources of TS1-1 and TS3-R were not identifiable (see above). Samples TS1-7 and TS1-10 were the sole samples from two separate abortions. No further information regarding source was obtained. Single source female samples TS2-10 and TS2-12 were from the same abortion; however, other samples indicated a mixture of female DNA. The comparison did not further aid in source determination.

Results from six samples extracted with the organic method and amplified with Profiler Plus™ were single-source female profiles. These included TS1-1, TS1-6, TS1-9, TS1-10, TS3-A, and TS3-B. Comparisons of TS1-1, TS3-A and TS3-B to associated samples were not helpful (previous paragraph). TS1-6, TS1-9, and TS1-10 were the only samples from three different abortions, so, there were no samples to compare.

Six of the organically-extracted COfiler™-amplified samples were single-source female profiles (TS1-1, TS1-8, TS1-9, TS1-10, TS2-16, and TS3-S). Other samples from the abortion which resulted in TS2-16 were from a mixture of female profiles.

Peak Height Imbalance Between Loci

Samples that were extracted organically displayed more severe imbalances in peak height between loci than those extracted with Chelex®. In the most extreme example of this—organically-extracted Profiler Plus™-amplified TS1-4B—the smaller-sized loci D3S1358, D8S1179, D5S818, and amelogenin (base pair size ranges less than 171) had large peak heights near or exceeding the upper threshold for interpretation (4500 RFUs), while the FGA, D18S51, and D7S820 loci (base pair size ranges greater than 215) exhibited RFU values near or below the minimum interpretation guideline (150 RFUs). Comparison of Chelex®-extracted (Fig. 19) and the organically-extracted (Fig. 20) sample results for TS1-4B illustrate imbalances of peak height between loci. The highest peak height allele for the D5S818 locus and the lowest peak height allele for the D7S820 locus were considered for both TS1-4B samples. The lowest at D7S820 for the Chelex® extracted sample was 291 RFUs and the highest at D5S818 was eight times larger at 2477 RFUs. The lowest for the organically-extracted sample was 72 RFUs. The highest was approximately 83 times larger at 5952 RFUs. The difference between the allelic ratios of the two samples is approximately 10 fold. Further evidence for this difference was observed upon comparison of single-source result Profiler Plus™-amplified samples where the alleles at the D5S818 and D7S820 loci were designated as either heterozygous at each (peak height relationship must be at least 70%) or homozygous at each. Chelex®-extracted samples fit these criteria: TS1-1, TS1-4B, TS1-

6, TS1-9, TS1-10, TS2-14, TS3-A, TS3-B, TS3-E, TS3-J, TS3-R and TS3-V. The organically-extracted samples that fit the criteria were as follows: TS1-1, TS1-4B, TS3-B, TS3-C, TS3-E, TS3-F, TS3-J, TS3-K, TS3-Q, TS3-R, TS3-S, and TS3-U. Ratios were calculated for the highest allele peak height observed at D5S818 and the lowest at D7S820 for each. The average ratio of the Chelex®-extracted samples was 1 to 6 while the average for the organically-extracted samples was 1 to 14.

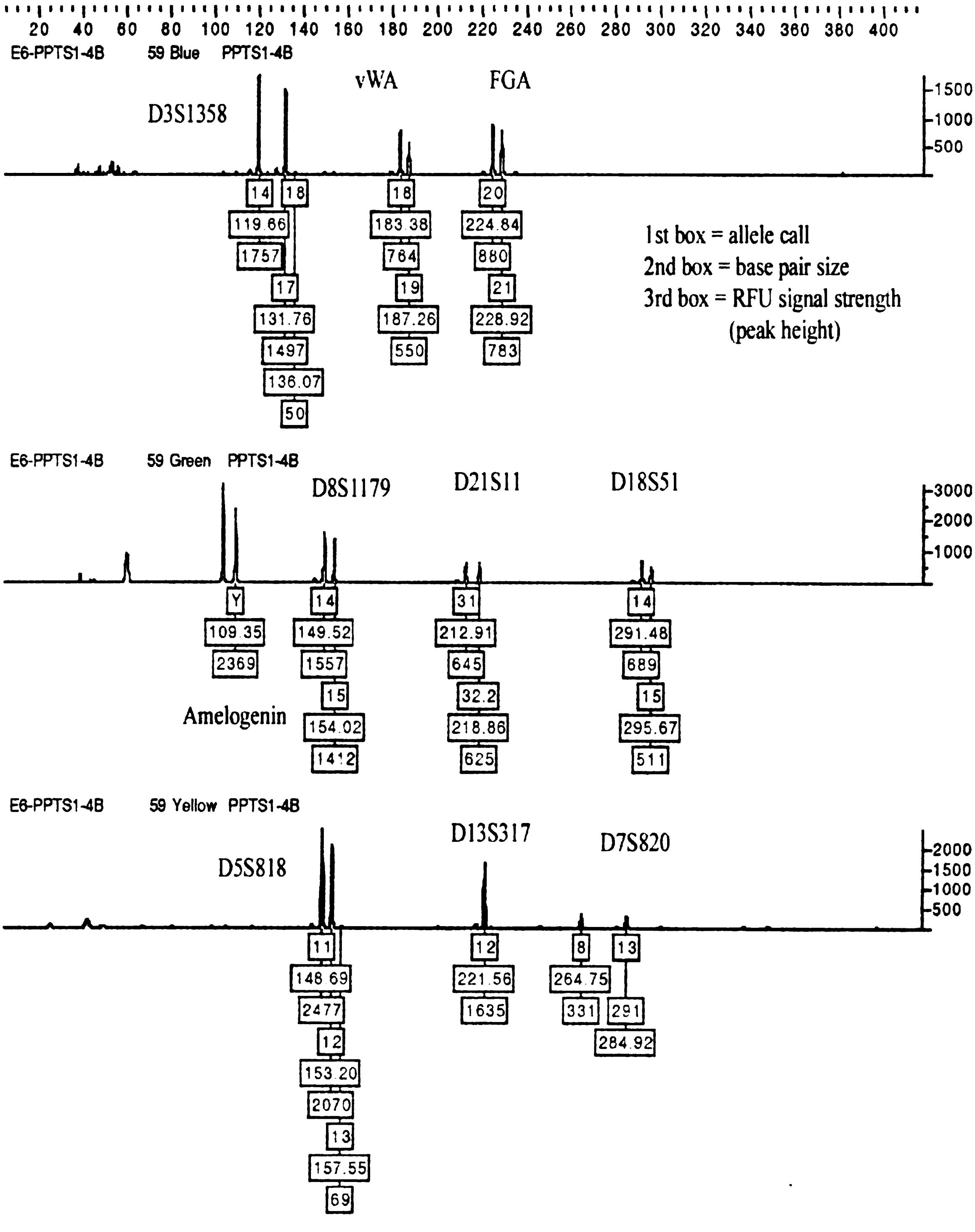


FIG. 19—*Sample TS1-4B extracted with the Chelex® method and amplified with the Profiler Plus™ amplification kit. The 11 allele at the D5S818 locus had a peak height of 2477 RFUs which was 8.5 times larger than the 13 allele at the D7S820 locus which had a peak height of 291 RFUs. All alleles from the major DNA contributor fell within interpretational guidelines.*

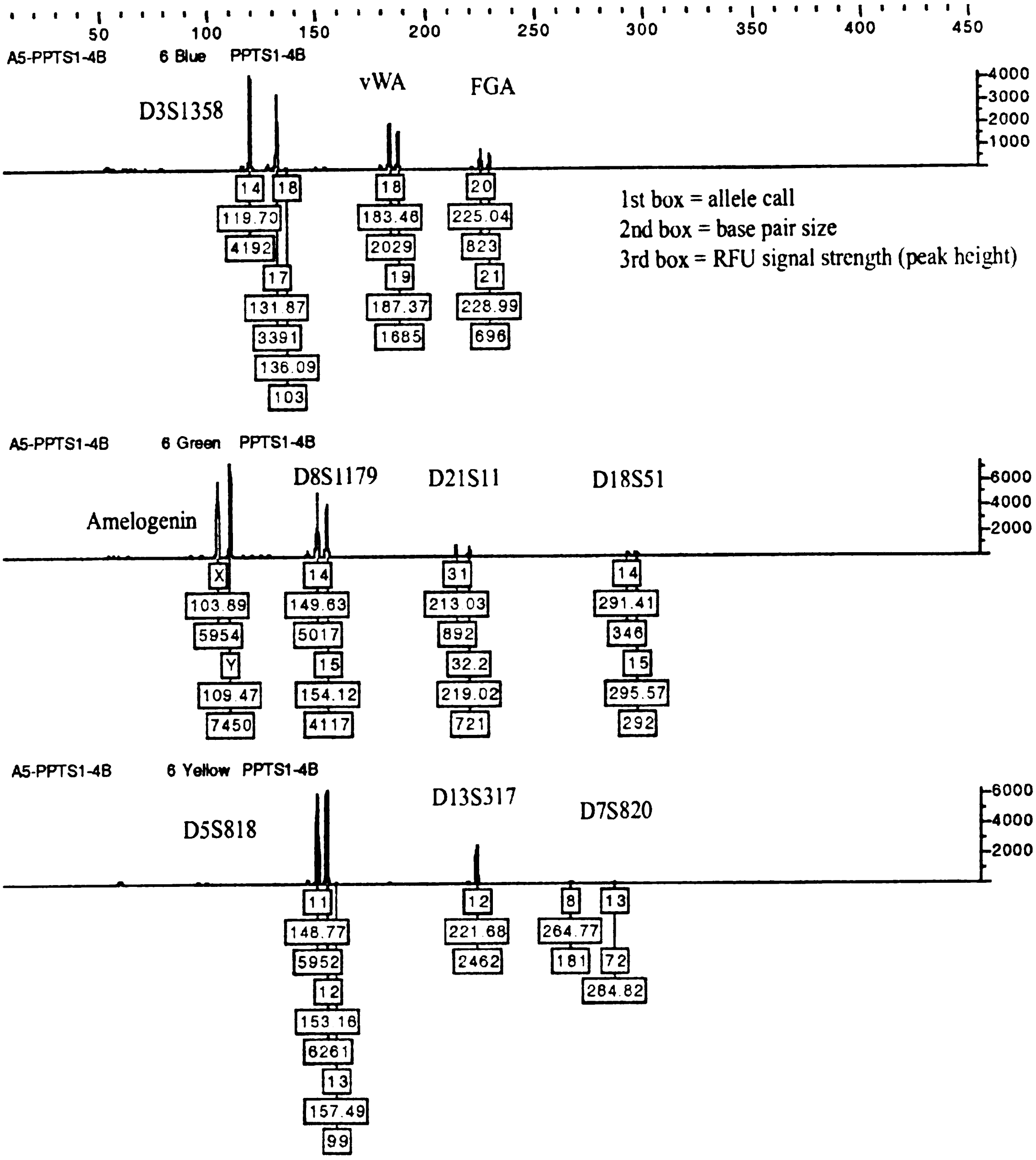
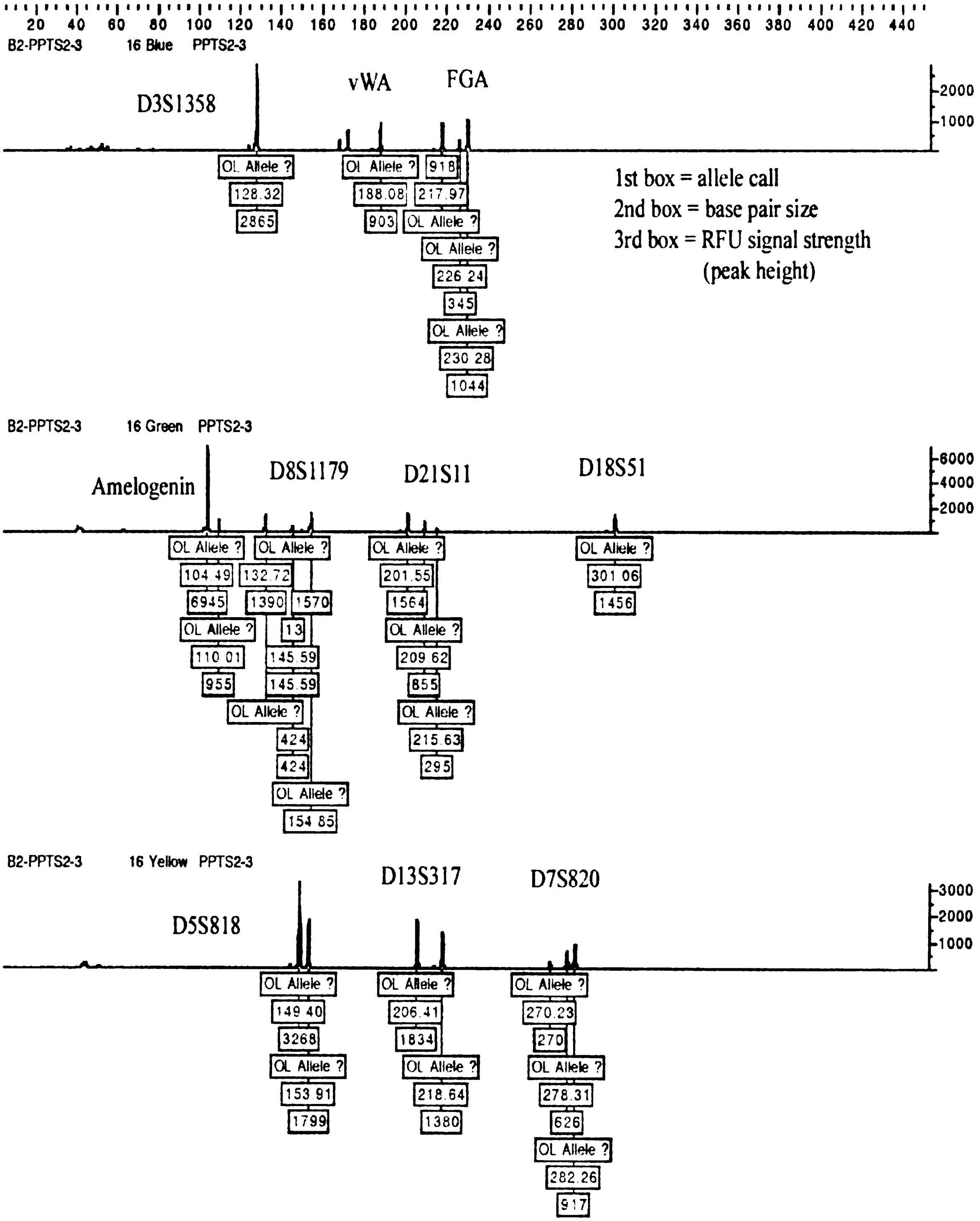


FIG. 20—*Sample TS1-4B extracted with the organic method and amplified with the Profiler Plus™ amplification kit. The 11 allele at the D5S818 locus had a peak height of 5952 RFUs which was 83 times larger than the 13 allele at D7S820 which had a peak height of 72 RFUs. The 11 allele at D5S818 exceeded the maximum threshold for reporting (4500 RFUs). The 13 allele did not meet the minimum threshold for reporting (150 RFUs). Enhancement of this sample to reduce the D5S818 allele RFUs (1–4 seconds) and to increase the D7S820 allele RFUs (3 µL preparation of amplified DNA) was necessary but would require interpretation to be conducted over two electropherograms.*

Off Ladder Alleles

The Genotyper® software assigned off-ladder allele designations (OL alleles) to all of the peaks generated for organically-extracted, Profiler Plus™-amplified samples TS2-1, TS2-2, TS2-3 (Fig. 21) and TS2-4. No definitive conclusions could be made for three of these, as TS2-1, TS2-2, and TS2-4 were consumed for amplification on the initial attempt. The samples should have been re-injected immediately, or the amplified product should have been re-prepared and injected. Neither option was available at the time of analysis. Since sample TS2-3 was not consumed, it was re-amplified with the Profiler Plus™ kit. The peak pattern remained the same; however, the base pair sizes of the alleles differed by approximately 0.5–1.5 base pairs between amplifications. Allele designations were assigned for each of the peaks (Fig. 22).



1st box = allele call
2nd box = base pair size
3rd box = RFU signal strength
(peak height)

FIG. 21—*Sample TS2-3 (organically-extracted) with OL allele designations. The Genotyper® software was not able to designate alleles for the D3S1358, FGA, vWA, amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 loci due to the .5–1.5 base pair shift of the alleles in comparison to the Profiler Plus™ ladder.*

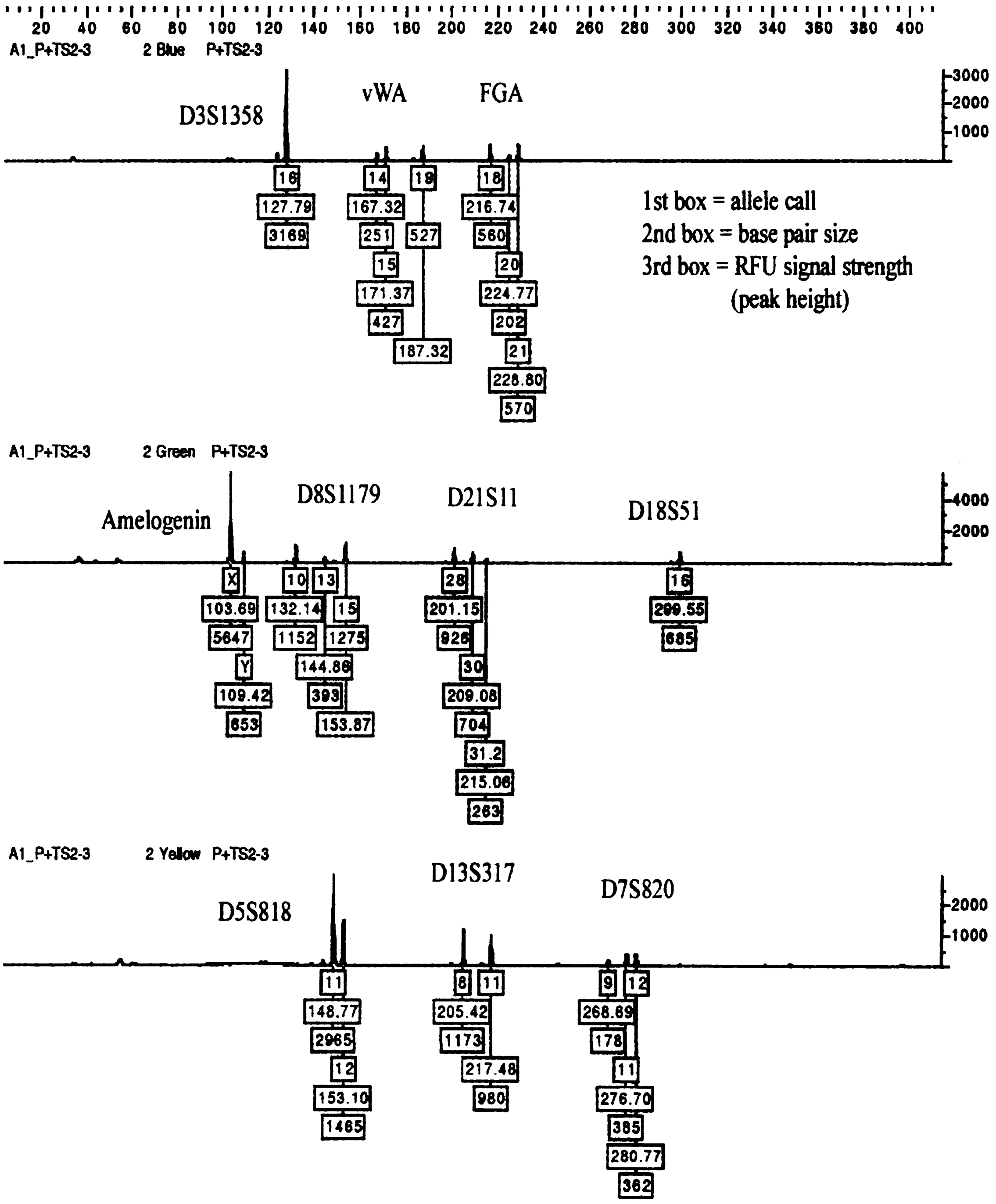


FIG. 22—*Sample TS2-3 (organically-extracted) with allele designations. The sample was re-amplified with the Profiler Plus™ kit. Upon comparison to the ladder, the D3S1358, FGA, vWA, amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 alleles were designated correctly.*

Statistical Estimates

A two-tailed t test using the separate variance estimate (Bachman and Paternoster, 1997) was used to determine whether a significant difference in recovery of DNA existed between extraction methods. The following were used to calculate the t_{obt} value and degrees of freedom:

$x_1 = 438.69\ \mu L$
$x_2 = 70.85\ \mu L$
$s_1 = 386.50$
$s_2 = 73.48$
$n_1 = 50$ samples extracted with Chelex®
$n_2 = 50$ samples extracted organically

The average amount of DNA recovered from the Chelex®-extracted samples was 438.69 µL compared to 70.85 µL from organically-extracted. DNA recovery with Chelex® was approximately six times higher than organic extraction. The calculated t_{obt} value was 6.54, with 55 degrees of freedom. The critical value of t with 55 degrees of freedom and alpha value of .05 is 2.00. The calculated value of t_{obt} falls outside the critical region of ±2.00. Thus, the null hypothesis was rejected and a significant difference in recovery of DNA existed between extraction methods.

A z test (Bachman and Paternoster, 1997) for the difference between proportions was applied to the attempts made at extraction versus the actual recovery of a full genetic profile. The z test was used to determine whether a significant difference existed between extraction methods in obtaining full genetic profiles (Tables 7–9). The following were used to calculate the z_{obt} and σ_{p1-p2} values:

$\hat{p}_1 = .96$
$\hat{p}_2 = .52$
$\sigma_{p1-p2} = .062$

Ninety-six Chelex-, but only fifty-two organically-extracted samples resulted in full profiles. The associated sample proportions where .96 and .52, respectively. The pooled standard error was calculated as .062. The calculated z_{obt} value was 7.09. When using a 95% confidence interval, the z scores of the critical region are ± 1.96. The calculated value of z_{obt} falls outside the critical region of ±1.96. Therefore, the Chelex® and organic extraction methods differ significantly in recovery of full genetic profiles.

DISCUSSION

Historical Issues and Procedures Developed

Sampling from early abortion materials collected within two to twelve weeks of conception for criminal DNA paternity testing poses multiple problems. The ability to visually discern embryonic or fetal tissue from maternal tissue is generally not possible before eight weeks. The Michigan State Police Lansing Laboratory frequently utilized random tissue sampling techniques during these time periods, which often failed to detect embryonic/fetal profiles due to the presence of overwhelming amounts of uterine tissue. Karger et al. (2001) developed a procedure to identify chorionic villi among recovered tissues for DNA testing in an effort to ensure embryonic/fetal profile results while saving time and money. The tissue was formalin-fixed and suspended in paraffin blocks for microsectioning and microscopic identification of the chorionic villi, followed by DNA testing. Six samples from one abortion—one with identified chorionic villi—were amplified targeting ten STR loci, nine of which were CODIS core loci. Profiles were obtained from the six samples—one was a mixture and five were single source. Four of the samples only produced partial profiles (4 loci) and were consistent with the mother. One sample was a mixture of alleles consistent with the mother and an offspring. Analysis of the sample in which chorionic villi were identified resulted in a full profile consistent with an offspring of the mother and putative father. Karger et al. (2001) referenced extraction methods from two papers (Iwasa et al. (1997) and Klintschar et al. (1999)) each of which utilized a different extraction method—Chelex® and modified alkaline lysis respectively, although it is not clear which of these Karger et al. (2001)

used. Likewise, their sampling source—tissue from prepared slides or paraffin blocks—was not outlined. There was no indication of quantity and quality of recovered DNA. Finally, since few samples were tested, it was not clear if the level of success could be reproduced.

DNA Recovery and Isolation of Embryonic/Fetal DNA

The goals of the research presented here were to test the microscopic identification of chorionic villi across a broad scope of samples, to compare the Chelex® and organic extraction methods in their ability to generate the highest quantity of DNA from the embedded tissue, and to attempt amplification of the recovered DNA with Profiler Plus™ and COfiler™ kits in an effort to generate full CODIS profiles representative of the embryo/fetus. The method which resulted in the most complete embryonic/fetal DNA would provide greater discriminatory power upon comparison with suspect samples. Upon comparison of the quantification data from samples comprised of formalin-fixed embryonic/fetal tissue and maternal decidua it was found that significantly greater quantities of DNA were recovered using the Chelex® method (Tables 1–3) than the organic method (Tables 4–6). Further comparison of profile data following electrophoresis revealed significantly more full profiles were produced with Chelex® extraction than with the organic extraction method (Tables 7–9). A relationship appeared to exist between the quantity of DNA recovered and the generation of a full profile. Many of the organically-extracted samples did not yield enough DNA for amplification with both kits; therefore, the maximum number of loci was nine instead of thirteen.

Data were compared from each extraction method. The organically-extracted samples exhibited more severe imbalances of peak height between loci. The most illustrative example of this was sample TS1-4B, in which the ratio of the lowest peak height from the D7S820 locus to the highest peak height from the D5S818 locus was 1:83 (Figure 20). The same ratio for the Chelex-extracted sample TS1-4B was 1:8 (Figure 19). The average ratio of the lowest peak height at D7S820 to the highest peak height at D5S818 was 1:6 for the Chelex®-extracted samples and 1:14 for the organically-extracted samples. Reasons for imbalance of peak height between loci include DNA degradation and/or the presence of a PCR inhibitor (see Explanation of Chelex® Success below). The difference in average peak height ratio obtained for the Chelex®-extracted samples was less drastic than that of the organically-extracted samples, indicating DNA of higher quality.

The electropherograms from Chelex®- and organically-extracted samples were examined for the presence of embryonic/fetal profile information. Six classifications of profile results were observed—single-source female, single-source female with additional activity, single-source male, single-source male with additional activity, mixture of females, and mixture of a male and a female. All classifications confirmed the presence of embryonic/fetal alleles except single-source female profiles, in which case the alleles may be attributed to the embryo/fetus or to the mother. It was impossible to determine the donor (embryo/fetus or mother) of the single-source female profiles, unless other associated abortion samples (Table 13) contained male DNA. In those instances it was clear that the male DNA was attributed to the embryo/fetus and the female DNA to the mother. All of the single-source female profiles were compared to associated abortion samples if they existed (Table 13), but these yielded no further information for discernment.

The presence of embryonic/fetal profile information was indicated in the majority of samples; however, the usefulness of the profile information could not be determined in many circumstances. Results classified as single-source female profiles with activity could contain interpretable profile information from either the embryo/fetus or the mother. If the activity portion of the profile was attributed to the embryo/fetus, the information would be useless for associative purposes in a criminal proceeding. Incomplete mixtures of two females could contain the embryonic/fetal profile as the major donor (complete) and the maternal profile as the minor component (incomplete) or the reverse. Associations would be less discriminating if the minor contributor was the embryo/fetus.

Single-source female profiles may contain exclusively embryonic/fetal DNA or maternal DNA; no confirmations were possible. If these profiles were confirmed as embryonic/fetal, ay be very useful for association if was not determined. The profiles may be attributed to either the embryo/fetus or the mother. the profile would be useful only if it was attributed to the embryo/fetus.

Concordance of Samples Between Amplification Systems and Extraction Methods

Allelic information obtained from Chelex®-extracted samples was concordant with that of the organically-extracted samples in all cases. However, in many instances the allele calls at each locus were not identical, but were consistent with heredity relationships between an individual and its full offspring in varying proportions. This result is likely due to the tissue sampling technique. Tissue sections were taken from adjacent areas of the same paraffin block in these experiments, and obtaining an identical ratio of embryonic/fetal tissue to maternal decidua was improbable due to the variation present in each block. Allelic information at shared loci (D3S1358, D7S820, and amelogenin) between Profiler Plus™ and COfiler™ amplified samples was also concordant. Less variation was observed with DNAs between amplification sets than between extraction sets, as these were amplified from the same extracted sample.

Identification of Associated Samples

All electropherograms were compared and profile relationships discerned in order to identify related samples among the 50 cassettes analyzed. It was determined that the three test sets of formalin-fixed, paraffin-embedded embryonic/fetal tissue and maternal decidua were comprised of materials from sixteen abortions (Table 13). Since this discernment was possible, it is further evidence of the utility of the research methods in obtaining useful allelic information for interpretation. The combination of sample data from each abortion most often generated more profile information—increasing the comparative power of the samples for paternity determination. Electropherograms from TS2-11 (Fig. 15) and TS2-10 (Fig. 18), which are associated abortion samples, illustrate this point.

Explanation of Superior Chelex® Results

Explanations for the greater success observed with Chelex® extraction compared to organic extractions include more effective removal of inhibitors such as iron in heme molecules present in blood, decrease in nuclease activity catalyzed by magnesium, and reduction in formalin-protein and formalin-DNA interaction. According to Wilson (1997), inhibitors interfere with PCR amplification (inactivation of the DNA polymerase), degrade or capture nucleic acids, and interfere with cell lysis in extraction. It is possible that the Chelex® process is more successful in removing common PCR inhibitors such as porphyrin compounds in blood (Walsh et al., 1997) than is the organic preparation. Hemoglobin, a protein containing heme, is found in blood and is released by proteinase K (used in both extraction procedures of this experiment) during the extraction process. Chelex® beads can bind iron (carried by heme) separating it from the DNA (Walsh et al., 1997), whereas the organic procedure may not effectively reduce or remove enough iron to facilitate complete PCR amplification.

Romero et al. (1997) stated that historically, DNA from formalin-fixed tissues does not amplify well due to degradation. During the fixation process, DNA degrades rapidly, the effects of which can cause poor amplification of larger sized loci. Chung et al. (2004) observed poor amplification for loci within the 300 to 500 base pair (bp) range in samples exposed to formalin. Shibata et al. (1994) found that PCR amplification of formalin-fixed tissue was optimal between target lengths of 80 and 170 bp. In the research presented here, nine of the Profiler Plus™ and COfiler™ amplicons exceed 170 bp in length; however, the effects of degradation were exhibited in loci greater than 200 bp (considered larger sized loci). It is possible that the use of mini-STR sets, with smaller amplicon sizes of approximately 100 bp (Butler et al., 2003), could be utilized to reduce or eliminate the effects of degradation (Wiegand and Kleiber, 2001). The use of multiplex systems such as Profiler Plus™ and COfiler™ (used in this research) require fewer amplifications of the DNA sample and are therefore less labor intensive than mini-STR sets. However, the loss of data for larger loci at the minimum threshold (150 RFUs) and smaller loci at the maximum threshold (4500 RFUs) is a risk when using the multiplex systems. In this research, the differences in peak height of alleles at small versus large loci of the Chelex®-extracted samples were less pronounced than in samples extracted organically. Samples extracted with Chelex® contained more interpretable allelic information at more loci than did samples extracted organically. Both extraction processes include digestion with tissue lysis buffer that contains EDTA, which binds magnesium, blocking it from activating nucleases that destroy DNA. The iminodiacete ions, attached to plastic Chelex® beads, chelate (bind) more of the remaining divalent metal ions. The beads are collected at the bottom of the extraction tube by centrifugation, separating them from DNA in the solution, further reducing degradation in these samples.

During the fixation process, exposure of tissues to formalin results in the formation of methyl bridges (cross-links) between the amino groups of purine and pyrimidine bases (DNA) and proteins, and also between proteins (Brutlag et al., 1969; Feldman, 1973; Kiernan 2000; Moerkerk et al., 1990; Romero et al., 1997). Cross-linking may occur in many different configurations, comprised of primarily linked proteins or of a combination of DNA and proteins, and both may reduce input DNA for PCR. This reduction may be purely mechanical, chemical, or a combination of both. The organic extraction method relies on phase separation of organic solvents and aqueous components and is effective due to the hydrophobic affinity of proteins and hydrophilic affinity of DNA. The formation of long cross-linked chains of proteins could cause mechanical interference during phase separation, trapping DNA molecules in the organic and interface layers. Likewise, cross-linked molecules containing DNA and proteins may have a hydrophobic affinity, chemically interfering with phase separation and carrying DNA molecules into the organic and interface layers. In both circumstances, the upper aqueous layer would be collected, and the interface and organic solvent layers, containing some DNA, discarded. In this research, samples extracted organically recovered significantly less DNA than those extracted with Chelex®. The Chelex® extraction method does not require separation; therefore more DNA may remain in the extraction solution.

The digestion of tissue prior to either extraction method was accomplished with tissue lysis buffer containing Tween 20 (detergent) and proteinase K to break open cells and denature proteins. Cross-linked proteins caused by formalin exposure may not be as easily denatured with proteinase K, possibly reducing the quantity of available DNA for amplification. The Chelex® extraction method utilizes a boiling step (100°C) to denature proteins and break open cells. This additional denaturing of proteins and subsequent release of DNA from cells could have increased DNA recovery. According to Overton and McCoy (1996), temperatures of 75°C are known to disrupt cross-links between formalin and DNA, which may also have increased input DNA for PCR.

Data Anomaly Observed

In this study, four samples—TS2-1, TS2-2, TS2-3 and TS2-4—extracted organically and amplified with Profiler Plus™ exhibited off-ladder allele (OL allele) designations for many or most of the alleles (Fig. 21). According to Applied Biosystems (1997), variation commonly occurs between samples injected with the same capillary. However, variation causing measurement errors greater than ± .5 base pairs results in the designation of OL allele assignments. Six sample injections of the Profiler Plus™ ladder were attempted. The first two (PPLADDER and PPLADDER-2) failed. These were injected nearest samples TS2-1, TS2-2, TS2-3 and TS2-4. Allele peak heights for both injections fell below the minimum threshold (150 RFU) for interpretation, therefore, could not be used. PPLADDER also exhibited OL allele designations for many of the alleles due to failure of the ROX internal size standard. The ROX peaks came off at higher scan numbers consistently and the resolution of the peaks became progressively broader throughout the electrophoresis. According to Applied Biosystems (1997) this problem was most likely due to syringe malfunction—polymer did not fill the capillary before injection (Applied Biosystems, 2006). This could occur if air bubbles were present in the syringe, if the pump arm was not aligned correctly, or if the capillary fitting was malfunctioning (Applied Biosystems, 2006).

Samples TS2-1, TS2-2, TS2-3, and TS2-4 exhibited ROX peaks which came off at lower scan numbers consistently throughout the electrophoresis. According to Applied Biosystems (1997) this problem occurs when water is present in the syringe. This can be avoided if the syringe is primed correctly with polymer to help remove water before the final polymer filling. The aforementioned problems worked themselves out by the sixth injection. Under normal circumstances those six samples would have been re-injected immediately; however, the 310 instrument was not available. Alleles present in samples TS2-1, TS2-2, TS2-3, and TS2-4 were not designated upon comparison to PPLADDER-3, PPLADDER-4, PPLADDER-5 or PPLADDER-6 due to inaccurate sizing of the ROX peaks. The samples all exhibited allele peak shifts of +0.5–1.5 base pairs. Applied Biosystems (1997), recommends re-injection of samples that contain OL alleles above minimum reporting threshold to verify reproducibility. If the OL alleles are reproduced after re-injection, re-amplification is recommended and reproducibility would confirm the presence of a true OL allele. As was mentioned previously, re-injection of the aforementioned samples was not possible due to equipment constraints. At a later date, sample TS2-3 (the only sample with the appropriate quantity of DNA remaining for amplification) was re-amplified and analyzed. Results revealed acceptable internal size standard peaks and allele call designations (Fig. 22), none were reproduced as OL alleles.

Implementation of Chelex® Extraction Method for Embryonic/Fetal Tissue in the Forensic Laboratory

The research presented utilized the following combination of methods: formalin-fixation, paraffin-embedding, microscopic identification of chorionic villi, sampling of the chorionic villi, xylene deparaffinisation, digestion (tissue), Chelex® or organic extraction, quantification, amplification with Profiler Plus™ and COfiler™ amplification kits, electrophoresis, and analysis of the data. Since Chelex® extraction proved superior to organic extraction, it in combination with the aforementioned methods was adopted as standard operating procedure (SOP) and implemented at the Michigan State Police Lansing Laboratory. Validation was completed in February 2004 and technical review accepted it in May of 2004. This was a simple process as it involved only the comparison of extraction methods (all other procedures were previously validated) and minimal expense for reagents. Since May of 2004, numerous cases have been successfully adjudicated in Michigan Circuit Courts.

Six of these cases were analyzed from the two year period since implementation. Of the six, five had associated known samples from the mother and putative father. Associations to the putative father were made on all of these. One of the cases was analyzed in June of 2006 and known samples have not been received by the submission date of this research. However, the case had defined results which indicated the maternal profile and the embryo/fetal profile. Data from these cases were included to illustrate the types of profiles that were generated—all identifying information has been removed.

Case 1

Sample 1 (cassette containing formalin-fixed, paraffin-embedded embryonic/fetal tissue and maternal decidua from an abortion) generated a mixture profile with the most embryonic/fetal alleles (Table 14A). Sample 3 was a single-source female profile consistent with the mother. Samples 2 and 4 produced mixture profiles which exhibited some embryonic/fetal alleles. Upon comparison with the mother and putative father, sample 1 was consistent with a full offspring of the mother and putative father (Table 14B). The allelic mismatch at D18S818 was most likely due to a mutation (either paternal or maternal). Both parents have a 17 allele as does the offspring and the offspring's 15 allele is one repeat greater than the additional paternal allele and one repeat less than the additional maternal allele.

TABLE 14A—*Genetic data at 13 CODIS core loci for Case 1*

Loci	Sample 1	Sample 2	Sample 3	Sample 4
D3S1358	16,17	16,(17)	16	16,(17)
vWA	14,17,(19)	17,19	17,19	(14),17,19
FGA	(19),20,28	19,20	19,20	19,20
Amelogenin	X	X	X	X
D8S1179	(10),13	10,13	10,13	10,13
D21S11	27,30.2,(31.2)	30.2,31.2	30.2,31.2	a,30.2,31.2
D18S51	15,17	16,17	A	16,17
D5S818	12	12	12	12
D13S317	8,11	11	11	11,a
D7S820	8,a	8	8	8,a
TH01	7,(9),9.3	9,9.3	9,9.3	9,9.3,a
TPOX	8,10,a	8,11	8,11	8,11,a
CSF1PO	11	A	A	11
D16S539	9,12	9,12	9,12	9,12

( ), allelic activity of a minor contributor; a, additional activity detected that failed to meet reporting standards; comma, separates multiple alleles present; period, indicates an allele with deletion of one or more bases

TABLE 14B—*Genetic data at 13 CODIS core loci for Case 1*

Loci	Mother	Putative Father	Discerned Offspring Profile
D3S1358	16	15,17	16,**17**
vWA	17,19	14,15	**14**,17
FGA	19,20	20,28	20,**28**
amelogenin	X	X,Y	X
D8S1179	10,13	12,13	13
D21S11	30.2,31.2	27,28	**27**,30.2
D18S51	16,17	14,17	15*,17
D5S818	12	8,12	12
D13S317	11	8,13	**8**,11
D7S820	8	8,10	8
TH01	9,9.3	7,8	**7**,9.3
TPOX	8,11	8,10	8,**10**
CSF1PO	11	7,11	11
D16S539	9,12	9,12	9,12

underlined, allele contributed by mother; bold, allele contributed by father; *, mutational event suspected

Case 2

Sample 1 generated a mixture profile of female DNA with the most embryonic/fetal alleles (Table 15A). Sample 3 was a single-source female profile consistent with the mother. Sample 2 did not generate adequate allelic information for a useful comparison. Sample 3 produced a single-source female profile. Upon comparison with the mother and putative father, samples 1 and 3 were consistent with a full offspring of the mother and putative father (Table 15B).

TABLE 15A—*Genetic data at 13 CODIS core loci for Case 2*

Loci	Sample 1	Sample 2	Sample 3	Sample 4
D3S1358	16,18	18	18	16,18
vWA	14,(16)	A	14,16	14
FGA	20,21	A	20,21	20,21
Amelogenin	X	X	X	X
D8S1179	13,15	13	13	13,15
D21S11	27,29,(31)	A	29,31	27,29
D18S51	12,14,a	ND	A	a
D5S818	11,(12)	A	11,12	11
D13S317	(8),11,13	ND	8,a	a
D7S820	8,11,a	ND	A	a
TH01	6,(8),9	ND	8,9	6,9
TPOX	8,11	ND	A	8,11
CSF1PO	10,11	ND	ND	ND
D16S539	11	ND	11	11

( ), allelic activity of a minor contributor; a, additional activity detected that failed to meet reporting standards; comma, separates multiple alleles present; period, indicates an allele with deletion of one or more bases; ND, no alleles detected for this locus

TABLE 15B—*Genetic data at 13 CODIS core loci for Case 2*

Loci	Mother	Putative Father	Discerned Offspring Profile
D3S1358	18	14,16	**16**,18
vWA	14,16	14,18	14
FGA	20,21	20,21	20,21
amelogenin	X	X,Y	X
D8S1179	13	13,15	13,**15**
D21S11	29,31	27,29	**27**,29
D18S51	12,15	12,14	12,**14**
D5S818	11,12	11,12	11
D13S317	8,13	11	**11**,13
D7S820	8,10	10,11	8,**11**
TH01	8,9	6,7	**6**,9
TPOX	8,11	8	**8**,11
CSF1PO	11,12	10	**10**,11
D16S539	11	11,12	11

underlined, allele contributed by mother; bold, allele contributed by father

Case 3

Samples 1, 2, and 3 generated mixture results of female and male DNA (Table 16A). Sample 4 produced a single-source male profile. Upon comparison with the mother and putative father, sample 1 generated the most complete embryonic/fetal profile and was consistent with a full offspring of the mother and putative father (Table 16B). The allelic mismatch at CSF1PO was most likely due to a mutation (maternal or paternal). Both parents and the offspring have a 13 allele, and the offspring's 11 allele is one repeat greater than the additional maternal allele and one repeat less than the additional paternal allele.

TABLE 16A—*Genetic data at 13 CODIS core loci for Case 3*

Loci	Sample 1	Sample 2	Sample 3	Sample 4
D3S1358	16,18	16,(17),18	16,18	16,18
vWA	16,17	16,17,a	16,17	16,17
FGA	20,23,a	20,23	ND	20,23
Amelogenin	X,Y	X,Y	X,Y	X,Y
D8S1179	10,(13),16	10,(13),16	10,(13),16	10,16
D21S11	29,31.2,a	29,31.2,a	A	29,31.2
D18S51	12,15	12,15	ND	12,15
D5S818	10,12,(13)	10,12,(13)	10,12	10,12,a
D13S317	9,13,a	9,13,a	ND	9,13
D7S820	10,11	10,a	ND	11,a
TH01	6,7	6,7	A	6,7
TPOX	9,11	9,11	ND	9,11
CSF1PO	13	13	ND	A
D16S539	11,13	11,13	ND	11,a

( ), allelic activity of a minor contributor; a, additional activity detected that failed to meet reporting standards; comma, separates multiple alleles present; period, indicates an allele with deletion of one or more bases; ND, no alleles detected for this locus

TABLE 16B—*Genetic data at 13 CODIS core loci for Case 3*

Loci	Mother	Putative Father	Discerned Offspring Profile
D3S1358	16	16,18	16,**18**
vWA	17,19	16,19	**16**,17
FGA	20,23	22,23	20,**23**
amelogenin	X	X,Y	X,Y
D8S1179	10,13	12,16	10,**16**
D21S11	29,32.2	30,31.2	29,**31.2**
D18S51	15,19	12,20	**12**,15
D5S818	12,13	10,13	**10**,12
D13S317	9,12	12,13	9,**13**
D7S820	11	10	**10**,11
TH01	6,7	6,7	6,7
TPOX	11	9,11	**9**,11
CSF1PO	10,13	12,13	**11***,13
D16S539	11	11,12	11

underlined, allele contributed by mother; bold, allele contributed by father; *, mutational event suspected

Case 4

Sample 1 generated a partial single-source female profile (COfiler™ only) (Table 17A). Sample 2 generated a mixture of male and female DNA (Profiler Plus™ only). Sample 3 produced a mixture of female DNA (full profile Profiler Plus™ and COfiler™). Sample 2 contained the most complete embryonic/fetal allelic information even though it was a partial profile including only Profiler Plus™ data. Upon comparison to the mother and putative father samples, sample 2 was consistent with at least one full offspring of the mother and putative father (Table 17B). The presence of the Y allele and additional allelic presence and balance in sample 2 indicates that this may have been a multiple pregnancy. Sample 1 was consistent with a full offspring of the mother and putative father. Since the overlapping D3S1358 allele matches the Discerned Offspring Profile 1, it most likely reflects the same source. Sample 3 was consistent with the mother and a full offspring of the mother and putative father.

TABLE 17A—*Genetic data at 13 CODIS core loci for Case 4*

Loci	Sample 1	Sample 2	Sample 3
D3S1358	17	17	17
vWA	ND	15,16	14,15,16
FGA	ND	22.2,a	19,22.2,23.2
amelogenin	X	X,(Y)	X
D8S1179	ND	8,12,16	8,12,16
D21S11	ND	29,30	29,30
D18S51	ND	ND	13,15,a
D5S818	ND	11,13	11,12,13
D13S317	ND	9,13	9,13
D7S820	9	ND	9,10
TH01	5,6	ND	5,6,9.3
TPOX	8,12	ND	8,12
CSF1PO	12	ND	12,14
D16S539	11,12	ND	11,12

( ), allelic activity of a minor contributor; a, additional activity detected that failed to meet reporting standards; comma, separates multiple alleles present; period, indicates an allele with deletion of one or more bases; ND, no alleles detected for this locus; alleles detected in Sample 1 are from the COfiler™ amplification only; alleles detected in Sample 2 are from the Profiler Plus™ amplification only

TABLE 17B—*Genetic data at 13 CODIS core loci for Case 4*

Loci	Mother	Putative Father	Discerned Offspring Profile 1	Discerned Offspring Profile 2
D3S1358	17	15,17	**17**	**15,**17
vWA	14,15	14,16	15,**16**	**15**
FGA	19,23.2	21,22.2	**22.2,**23.2	**UK**
Amelogenin	X	X,Y	**X**	**X**
D8S1179	12,16	8,15	**8,**16	**8,**12
D21S11	30	29,31	**29,**30	**29,**30
D18S51	13,15	12,20	**UK**	**UK**
D5S818	12,13	11,13	**11,**13	**UK**
D13S317	9,13	11,13	9,**13**	**UK**
D7S820	9,10	9,10	9	**UK**
TH01	6,9.3	5,9.3	5,6	**UK**
TPOX	8	8,12	8,**12**	**UK**
CSF1PO	12,14	11,12	12	**UK**
D16S539	11,12	11	**11,**12	**UK**

underlined, allele contributed by mother; bold, allele contributed by father

Case 5

Sample 1 was a single-source female profile and was consistent with a full offspring of the mother and putative father (Table 18).

TABLE 18—*Genetic data at 13 CODIS core loci for Case 5*

Loci	Sample 1	Mother	Putative Father	Discerned Offspring Profile
D3S1358	15,16	15,16	16	15,**16**
vWA	14,17	17,18	14,17	**14**,17
FGA	23	19,23	20,23	23
Amelogenin	X	X	X,Y	X
D8S1179	13	13	13,14	13
D21S11	29,30	29,30	28,29	**29**,30
D18S51	14	14,15	14,15	14
D5S818	11,12	11,12	7,11	11,**12**
D13S317	12,13	13	11,12	**12**,13
D7S820	10,11	10	8,11	10,**11**
TH01	9,9.3	6,9	9.3	9,**9.3**
TPOX	8,11	8	8,11	8,**11**
CSF1PO	10	10	10,12	10
D16S539	11,12	11,12	9,12	11,**12**

( ), allelic activity of a minor contributor; a, additional activity detected, but it failed to meet reporting standards; ND, no alleles detected at this locus; underlined, allele contributed by mother; bold, allele contributed by father

Case 6

Sample 1 generated a single-source male profile with additional activity (Table 19). Samples 2 and 3 generated mixtures of female and male DNA and were comprised of higher ratios of maternal to embryo/fetal DNA. Sample 3 was predominantly maternal and was used to compare to sample 1. Profiles of the mother and offspring were discernable. Known samples would of course be necessary for confirmation. Alleles contributed by the father were able to be determined also.

TABLE 19—*Genetic data at 13 CODIS core loci for Case 6*

Loci	Sample 1	Sample 2	Sample 3	Discerned Offspring Profile
D3S1358	16,17,a	17	(16),17	**16**,17
vWA	14,15	14,18,a	14,(15),18	14,**15**
FGA	20,23	20,22,a	20,22,(23)	20,**23**
Amelogenin	X,Y	X,Y	X,Y	X,Y
D8S1179	12,13,a	12,(13)	12,(13)	12,**13**
D21S11	30,31.2	30,(31.2)	30,(31.2)	30,**31.2**
D18S51	13,13.2,a	12,13,a	12,13,(13.2)	13,**13.2**
D5S818	11	11	11	11
D13S317	8,12	(8),11,12	(8),11,12	**8**,12
D7S820	10,11	10,a	10,(11)	10,**11**
TH01	6,7	6,(7),9.3	6,(7),9.3	6,7
TPOX	8	8	8	8
CSF1PO	12	11,12	11,12	12
D16S539	12	12,13	12,13	12

( ), allelic activity of a minor contributor; a, additional activity detected that failed to meet reporting standards; comma, separates multiple alleles present; period, indicates an allele with deletion of one or more bases; underlined, allele contributed by mother; bold, allele contributed by father

Recommendations for Handling Aborted Materials in Criminal Cases

A detailed protocol for handling rape complaints resulting in pregnancy is necessary. The law enforcement officer, doctor performing the abortion, forensic pathologist, DNA testing laboratory scientist, and prosecutor have crucial roles in evidence collection, preservation, and viability. Establishment of a clear method—agreed upon by all potential parties involved—would logically aid in proper handling of rape resulting in pregnancy cases. It would seem that the education of law enforcement personnel would be of the utmost importance as these individuals are usually responsible for evidence transfer facilitation. A list of recommendations for law enforcement personnel has been developed (Fig. 23).

1. Obtain written consent or a search warrant to allow confiscation of the aborted embryonic or fetal materials.
2. Contact the medical facility performing the abortion to request the following:
   A. Written results of the estimated term of pregnancy from an ultrasound procedure
   B. The abortion procedure used
   C. That the tissue be placed into a clean, sterile container and secured with evidence tape, and initialed and dated by the collector
   D. That the tissue be frozen fresh and <u>not</u> placed into fixative.
   E. Obtain written chain of custody information at the time of receipt
3. Transport the materials frozen or maintain at the coldest temperature possible—use a cooler with ice if the facility does not provide something comparable.
4. If the term of pregnancy is 12 weeks or less or unknown, contact the facility that performs autopsies for your agency and request an examination of the materials.
5. If the term of pregnancy is greater than 12 weeks take the materials directly to the forensic laboratory that services your jurisdiction (skip #7).
6. Arrange to transport the processed fetal/embryonic tissue from the pathologist to the forensic laboratory.
7. Notify the forensic laboratory of the contents so that they are stored appropriately(frozen) prior to and following analysis.
8. Obtain victim known buccal swabs or known whole blood in an EDTA (purple-topped) blood collection tube and transport it to the laboratory with a request for comparison to the aborted embryonic or fetal materials.
9. Obtain a warrant or written consent for the suspect's known buccal swabs.
10. Collect buccal swabs from the putative father and transport them to the laboratory for analysis with a request for comparison to the aborted embryonic or fetal materials.

FIG. 23—*List of recommendations for law enforcement personnel. If followed, these ensure proper handling, documentation, and analysis of the embryonic/fetal tissues.*

Recommendation 1 ensures that the embryonic/fetal materials have been legally seized and will be admissible in court proceedings. The medical facility will demand this for release of the materials as well. Recommendation 2A is necessary to determine which facility will receive the materials for further processing (see 5 and 6). 2B provides information as to the possible state of the materials after the abortion procedure, which may assist the pathologist in screening. 2C will aid in tracking of the materials for court admissibility purposes. 2D will reduce the risk of additional DNA degradation (Butler, 2005). Recommendation 3 is necessary because this may not be recorded at the medical facility, and the officer may be creating the sole record of the transfer (necessary for admissibility in court). The fourth recommendation for frozen transport will reduce the risk of additional DNA degradation (Butler, 2005). Recommendations 5, 6, and 7 ensure that the appropriate professional is receiving the materials for preparation for DNA analysis. Recommendation 8, if followed by the receiving agency, ensures that the materials are identified and stored correctly to avoid additional DNA degradation or inhibition. Recommendations 9 and 11 provide known samples from the mother and putative father for DNA analysis and comparison to the embryonic/fetal tissues. The known samples are necessary for determination of paternity and calculation of the supporting likelihood ratio. Recommendation 10 ensures court admissibility of the suspect's DNA profile.

Conclusions

The samples tested contained varying proportions of formalin-exposed, microscopically identified chorionic villi and maternal tissues from abortion procedures. All samples were subjected to xylene deparaffinisation, extracted using either the Chelex® method or the organic method, amplified with Profiler Plus™/COfiler™ kits and analyzed using capillary electrophoresis. Samples extracted with the Chelex® method resulted in significantly higher quantities of DNA than samples extracted organically. Likewise, the Chelex®-extracted samples exhibited significantly higher quality DNA with less severe peak height imbalances between loci (presumably caused by degradation or inhibition) and more complete profile information. The source (embryo/fetus or mother) of the profile was not discernible in some circumstances (i.e. single-source female profiles and single-source female profiles with additional activity). Mixture results exhibited embryonic/fetal profile information, however, the usefulness of the information is dependant on the ratio of the allelic contribution from the donors determined by the presence and balance of alleles. Comparisons were conducted on all test set sample data and resulted in the identification of sixteen different abortions. This further illustrates the utility of the microscopic examination and identification of chorionic villi. Experiments including known maternal and paternal samples are necessary for identification of the contributors to each sample and, in turn, the likelihood of obtaining full embryonic/fetal genetic information from analysis. Results of data to date from implementation of the procedures with Chelex® extraction have been favorable. In five of six cases, association of embryonic/fetal results with putative father samples was possible. Based on the results of this research, both extraction methods isolated STR DNA profile information from abortion materials; however, the Chelex® extraction method was superior in DNA quantity and quality recovered for criminal paternity comparison.

APPENDICES

APPENDIX A

STATUTORY CIRCUMSTANCES FOR CSC I and CSC II

- Victim under 13 years of age.

- Victim at least 13 but less than 16 years of age, AND any of the following:

    - Perpetrator is a member of the same household as victim;
    - Perpetrator is related to victim by blood or affinity to the fourth degree; OR,
    - Perpetrator is in a position of authority over the victim, AND used this authority to coerce the victim to submit.

- Sexual act involves the commission of any other felony.

- Perpetrator aided or abetted by one or more other persons, AND either of the following:

    - Perpetrator knows or has reason to know that the victim is mentally incapable, mentally incapacitated, or physically helpless, OR
    - Perpetrator uses force OR coercion.

- Perpetrator armed with a weapon OR an article fashioned so as to lead a person to reasonably believe it is a weapon.

- Perpetrator uses force or coercion AND causes personal injury.

- Perpetrator causes personal injury and knows or has reason to know the victim is mentally incapable, mentally incapacitated or physically helpless.

- Victim is mentally incapable, mentally disabled, mentally incapacitated or physically helpless and one of the following:

    - Perpetrator is related to the victim by blood or affinity to the fourth degree; OR,
    - Perpetrator is in a position of authority over the victim and used this authority to coerce the victim to submit.

- Perpetrator is an employee, contractual employee, OR volunteer with the Department of Corrections AND knows that the victim is under its jurisdiction (CSC II only).

- Perpetrator is an employee, contractual employee, OR volunteer with a private vendor that operates a youth correctional facility AND knows that the victim is under the jurisdiction of the Department of Corrections (CSC II only).

- Perpetrator is an employee, contractual employee, OR volunteer with a county or the Department of Corrections AND knows that the victim is prisoner or probationer under the jurisdiction of the county (CSC II only).

- Perpetrator is an employee, contractual employee, OR volunteer with the facility in which the victim is detained awaiting trial OR hearing OR in which the victim is committed as a result of having been found responsible for committing an act that would be a crime if committed by an adult (CSC II only).

APPENDIX B

STATUTORY CIRCUMSTANCES FOR CSC III and CSC IV

- Victim is at least 13 but less than 16 years of age (CSC III only).

- Victim is at least 13 but less than 16 years of age AND the perpetrator is five OR more years older than the victim (CSC IV only).

- Perpetrator uses force OR coercion.

- Perpetrator knows OR has reason to know the victim is mentally incapable, mentally incapacitated, OR physically helpless.

- Perpetrator is related to the victim by blood OR affinity to the third degree AND sexual penetration OR contact occurs under circumstances not otherwise prohibited by the CSC Act.

- Perpetrator is a mental health professional AND sexual contact occurs during OR within two hears after victim was patient OR client of perpetrator AND victim was not the perpetrator's spouse (CSC IV only).

APPENDIX C

PROTOCOL FOR THE PREPARATION OF FORMALIN-FIXED PARAFFIN-EMBEDDED EMBRYONIC/FETAL TISSUE AND MATERNAL DECIDUA FOR DNA EXTRACTION

Sample Preparation

1. Observe microscope slides which correspond to paraffin blocks identified as containing chorionic villi and locate the areas with villi for sampling.

2. Cut away the outer layer of the paraffin block with a sterile blade and obtain a small segment of tissue (approximately 2–4 mm^3) from the block and place in a labeled microcentrifuge tube.

Deparaffinisation

1. Add 1.0 mL of xylene to each tube (perform in chemical hood) to remove paraffin wax.

2. Incubate for 30 minutes at room temperature and centrifuge for 2–5 minutes at 15,300 RCF. Remove and discard the supernatant into the appropriate waste container.

3. **Repeat steps 1 and 2** for a total of 2 xylene washes.

4. Add 1.0 mL of ethanol to each tube to remove xylene.

5. Centrifuge for 2-5 minutes at 15,300 RCF. Remove and discard the ethanol into the appropriate waste container.

6. **Repeat steps 4 and 5** for a total of 2 ethanol washes.

7. Dry tissue for 10–20 minutes at 15–20 in. Hg in a Hetovac vacuum apparatus.

Digestion

1. Prepare Tissue Lysis Buffer (fresh daily). Add 200 µL of Tissue Lysis Buffer to each tube.

   Tissue Lysis Buffer

1.89 mL	TE^{-4} Buffer (10 mM, pH 7.5)
10 µL	Tween 20 (0.5%)
100 µL	Proteinase K (20 mg/mL)
2.0 mL	Total

2. Incubate at 37°C overnight. Centrifuge for 5 minutes at 15,300 RCF.

Collection and Purification

1. Remove the solution (sample) from the digestion tube and transfer to a Centricon-100™ concentrator assembled according to the manufacturer's instructions (sample reservoir with attached retentate vial was fitted to a filtrate vial). Discard any remaining tissue.

2. Centrifuge for 30–60 minutes or longer if necessary (until most of the liquid passes through filter into the filtrate vial leaving the filter membrane moist) at 2000 RCF. Discard filtrate.

3. Add 2.0 mL of TE buffer to the sample reservoir. Centrifuge 30–60 minutes or longer if necessary at 2000 RCF. Discard filtrate. **Repeat once.**

4. Remove and discard the filtrate vial, invert the sample reservoir with retentate vial attached and centrifuge for 3 minutes at 1000 RCF.

5. Transfer the retentate (concentrated sample) directly from the Centricon-100™ concentrator to a labeled microcentrifuge tube. The samples are ready for extraction.

APPENDIX D

PROTOCOL FOR CHELEX EXTRACTION OF DNA

Chelex® Extraction Protocol

1. Add 20 μL of 5% Chelex® solution to the sample tube and vortex briefly.
2. Incubate at 56° C for 30 minutes then vortex at high speed for 5–10 seconds.
3. Incubate in a boiling water bath for 8 minutes then vortex at high speed for 5–10 seconds.
4. Centrifuge for 3 minutes at 15,300 RCF. Samples are ready for quantification.
5. For short-term storage place at 2–8°C on the Chelex® beads. Repeat steps 2, 3 and 4 before use. For long term storage, transfer the supernatant from the Chelex® beads to a new tube and freeze at -20°C.

APPENDIX E

PROTOCOL FOR PHENOL/CHLOROFORM/ISOAMYL ALCOHOL (ORGANIC) EXTRACTION OF DNA

Organic Extraction Protocol

1. Add 200 µL of phenol/chloroform/isoamyl alcohol to the sample tube.
2. Vortex until a milky emulsion is produced.
3. Centrifuge in microcentrifuge for 5 minutes at 15,300 RCF.
4. Assemble a Centricon-100™ concentrator according to manufacturer's guidelines (sample reservoir with attached retentate vial must be fitted to a filtrate vial).
5. Draw off the top aqueous layer of solution and transfer to the concentrator.
6. Centrifuge for 30–60 minutes or longer if necessary (until most of the liquid passes through filter into the filtrate vial leaving the filter membrane moist) at 2000 RCF. Discard filtrate.
7. Add 2.0 mL of TE buffer to the sample reservoir. Centrifuge 30–60 minutes or longer if necessary at 2000 RCF. Discard filtrate. **Repeat once.**
8. Remove and discard the filtrate vial, invert the sample reservoir with retentate vial attached and centrifuge for 3 minutes at 1000 RCF.
9. Transfer the retentate directly from the Centricon-100™ concentrator to a labeled microcentrifuge tube. The samples are ready for quantification.

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