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**Evaluation of High-Throughput STR Technologies
for Potential Implementation in the National DNA Databank**

by

Susan Borys, B.Sc. (Hons)

**A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements of the degree of
Master of Science**

Ottawa-Carleton Institute of Biology

Carleton University

Ottawa, Ontario

May, 1999

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**The undersigned hereby recommend to the Faculty of Graduate Studies and Research
acceptance of the thesis**

**“Evaluation of High-Throughput STR Technologies for Potential Implementation in the
National DNA Databank”**

**submitted by Susan Borys, B.Sc.
in partial fulfilment of the requirements
for the degree of Master of Science.**

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Thesis Supervisor

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Chairman, Department of Biology

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.....
May 4, 1999
Date

Abstract

With the enactment of Bill C-3 (The DNA Identification Act) in December of 1998, Canadian law enforcement agencies will begin banking an annual estimate of 28,000 to 98,000 profiles (established using STR technology) obtained from individuals convicted of primary and secondary designated offences. A triad of technologies was evaluated for potential implementation in the National DNA Databank. The combination of FTA™ collection cards, multiplex PCR amplification and capillary electrophoresis proved to be a fast, efficient and reliable system for high-throughput databasing. The evaluation was based on the comparison of frequencies and distributions of alleles for the nine STR loci of the AmpFISTR Profiler Plus™ amplification kit. Genotype data from a total of 473 individuals from five populations including African-American, Chinese, Bangladesh, Vietnamese and Japanese was obtained. Each of the five populations were in Hardy-Weinberg and linkage equilibrium. Population differentiation tests revealed no significant genic differentiation between Chinese and Vietnamese populations and these two populations could be amalgamated into one population group. In addition, amalgamation of the Vietnamese and Bangladesh populations was possible.

Two novel off-ladder variants were discovered: allele 9.1 at D7S820 in the Bangladesh population and allele 9.3 at D7S820 in the African-American populations. In addition, a male individual in the African-American population was typed as OY or YY for the gender discrimination locus, amelogenin. Such a finding has not been previously reported.

Collection of biological specimens on FTA collection cards are ideal for establishing

DNA repositories and databanks. Multiplex PCR amplification at a reduced volume (5 μ L compared to 25 μ L) provides high quality DNA typing results and increases the yield of amplification by up to nine-fold. Finally, although capillary electrophoresis provides excellent resolution, precision and sensitivity for the separation of PCR amplified products, its use is hindered by its inability to compete with current slab gel electrophoresis for high-throughput analysis.

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List of Abbreviations

AMP-FLPs	amplifiable fragment length polymorphisms
bp	base pairs
°C	degrees Celsius
CCD	charge coupled device
CE	capillary electrophoresis
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DNA	deoxyribonucleic acid
EDTA	N,N-ethylene diamine tetraacetic acid
EtOH	ethanol
5-FAM	5-carboxyfluorescein
FAD	filtered, autoclaved, deionized
FGA	alpha fibrinogen gene
GS-500	GeneScan-500
HW	Hardy-Weinberg
JOE	6-carboxy-2',7'-dimethoxy-4',5'-dichlorofluorescein
kb	kilobases
kV	kilovolts
L1	LINE1
LD	length-to-detector
LIF	laser-induced fluorescence
NRC	National Research Council
³² P	radioactive isotope of phosphorous
P ₁	Probability of Identity
<i>p</i> -value	probability value
PCR	polymerase chain reaction
POP-4	performance optimized polymer-4
RCMP	Royal Canadian Mounted Police
RFLP	restriction fragment length polymorphism
ROX	6-carboxy-X-rhodamine
SSC	150 mM NaCl, 15 mM sodium citrate (pH 7.0)
S.E.	Standard Error
STR	short tandem repeat
TE	10 mM Tris-HCl; 1 mM EDTA pH 8.0
TWGDAM	Technical Working Group on DNA Analysis Methods
UV	ultraviolet
VNTR	variable number of tandem repeats
vWF	von Willebrand Factor gene

I. INTRODUCTION

A. Genetic polymorphism and its application to forensic science

The polymorphic nature of the human genome has become an important tool for the individualization of biological materials in forensic laboratories. Not since the widespread use of fingerprinting to identify an individual has a technique emerged that provides the potential for near absolute human identification. Over the years, many different human enzymes and other proteins have been screened and found to exhibit variation between individuals and among population groups. Genetic polymorphism was first discovered in blood groups (Landsteiner, 1901). Markers of the red blood cell antigens, particularly those of the ABO system, were exploited as genetic markers because of their ease of classification into different phenotypes, their simple mode of inheritance and their variability in frequency among different populations (Thompson *et al.*, 1991). In the early 1970's, other protein and enzyme markers, including phosphoglucosmutase (PGM), erythrocyte acid phosphatase (EAP), haptoglobin and adenylate kinase (AK) were found useful for forensic evidence (Inman and Rudin, 1997). By examining more than one genetic locus, the percentage of the population who were included as possible donors of an evidentiary stain became smaller and smaller.

Genetic diversity in portions of the genome encoding proteins is estimated to be about 1 in 2500 base pairs (Thompson *et al.*, 1991); these protein-coding regions comprise an estimated five percent of the total genome. The remaining 95% of the total genomic DNA is estimated to contain polymorphic bases for about 1 in 270 base pairs, about ten times higher than the proportions of polymorphic base positions estimated for protein-coding

regions (Cooper *et al.*, 1985). This remarkable variation at the nucleotide sequence level was exploited in the 1980's by Jeffreys *et al.* (1985) with the discovery of DNA fingerprinting. This technique detected DNA polymorphism between individuals due to variation in the distribution of restriction enzyme sites (Restriction Fragment Length Polymorphisms, RFLP) (Botstein *et al.*, 1980). In the interval between two restriction sites a series of variable number of tandemly repeated DNA sequences or VNTRs was first described (Wyman and White, 1980). For the RFLP markers, between 20 and 80 VNTRs could be identified at any one locus, with each type occurring at a fairly low frequency (Nakamura *et al.*, 1987). Immigration and paternity disputes were among the first applications of DNA typing in the legal arena. One of the first forensic applications of the "DNA fingerprinting" method took place in England where an innocent suspect was the first accused murderer to be exonerated based on DNA evidence (Wambaugh, 1989).

In 1986, the biotechnology and the forensic community were revolutionized with the invention of the polymerase chain reaction (PCR) by Kary Mullis (Mullis *et al.*, 1986). Small stretches of DNA, even in a degraded state, could be copied with high fidelity. PCR markers usually have less variation per locus than RFLP loci and therefore offer a lower power of discrimination. However, when several PCR markers are examined in combination, a measure of individuality can be approached.

The progression of the application of genetic polymorphisms for forensic purposes to its present state is shown in Table 1. The following section examines the remarkable architecture of the human genome and how genetic variation and polymorphism in the human genome plays a role in forensic identification.

Table 1. Forensic DNA timeline; a brief history.

- 1901 The discovery of the ABO blood groups by Karl Landsteiner marked the first demonstration that individuals could be systematically differentiated at the biochemical level.
- 1944 Oswald Avery defines the role of DNA (deoxyribonucleic acid) in the generational transference of heritable traits.
- 1953 James Watson and Francis Crick elucidate the structure of DNA as a double helix.
- 1980 Wyman and White describe the use of DNA polymorphisms in disease analysis.
- 1980 Botstein and coworkers exploit the small DNA variations between individuals to construct a human gene map.
- 1984 Alec Jeffreys discovers a unique application of RFLP technology to forensic science. His method is termed a "DNA fingerprint."
- 1985 Immigration disputes were among the first application of DNA typing for human identification.
- 1986 The polymerase chain reaction (PCR) was invented by Kary Mullis.
- 1987 One of the first forensic uses of DNA typing using the "DNA fingerprint" method. Colin Pitchfork was identified as being responsible for the murders of two young girls in England. An innocent suspect was the first accused murderer to be exonerated based on DNA evidence in this case.
- 1991 Caskey and Edwards describe the use of short tandem repeats (STRs) for potential use in forensic DNA identification.
- 1995 Development of a large-scale criminal offender DNA database (Forensic Science Service, Birmingham, UK).
- 1998 High throughput DNA identification of all victims of the SwissAir Flight 111 mass disaster off the coast of Nova Scotia, Canada performed by the RCMP. This was the first mass disaster to utilize DNA typing as the principal method to reassociate all human remains.
- 1998 Bill C-3 (The DNA Identification Act) is passed in December. Canadian law enforcement agencies will begin banking profiles (using established STR technology) obtained from individuals convicted of primary and secondary designated offences.

B. The architecture of the human genome and its significant role in forensics

As outlined in the previous section, a considerable number of polymorphic sequences are known in the human genome (Pascali *et al.*, 1994), representing single-copy sequence differences and repetitive DNA from noncoding regions. The total linear composition of the human genome is approximately three quarters single-copy DNA and approximately one quarter repetitive DNA (Table 2). Although the single-copy sequences represent the major portion of the genome, much of their function is not completely understood since sequences encoding proteins comprise less than 10% of the total genetic makeup. Most single-copy DNA is found in short stretches (several kilobases or less), interspersed with repetitive DNA sequences; long stretches of single-copy DNA (>25 kb) are rarely found in the genome, with the average size of a human gene about 10 kb in length (Thompson *et al.*, 1991).

An example of unique DNA sequence applied to forensic profiling was the single-copy gene HLA DQA1, also known as HLA DQ α (Saiki *et al.*, 1986; Gyllensten and Erlich, 1988; Blake *et al.*, 1992). The DQ α locus encodes a class II human leukocyte cell-surface antigen involved in the regulation of immune responses (Blann and Crichton, 1988). Four major alleles (DQA1, A2, A3 and A4) and six subtypes (DQA1.1, 1.2, 1.3 and DQA4.1, 4.2 and 4.3) have been identified by sequencing the second exon of the DQ α gene (Gyllensten and Erlich, 1988; Horn *et al.*, 1988).

For purposes of human identification, most forensic markers are derived from several classes of repetitive DNA (Frégeau and Fourney, 1993; Moller *et al.*, 1994; Urquhart *et al.*, 1994; Barber *et al.*, 1996). The different families of repetitive DNA can be classified by

Table 2. DNA architecture in the human genome and its application to forensic science (revised from Thompson *et al.*, 1991).

Type of DNA	Proportion of Genome	Features
Single-copy DNA	~75%	<10% encodes genes Located throughout genome e.g. HLA DQ α
Repetitive DNA		
Dispersed DNA	~15%	Interspersed with unique or single-copy DNA Two major families (Alu and L1)
Satellite DNA	~10%	Highly repetitive sequences Minisatellites: 15 to 65 bp core span up to 2 kb e.g. VNTRs, AMP-FLPs Microsatellites: 2 to 7 bp core <350 bp in length e.g. D3S1358

whether the repeated sequences or repeats are clustered in one or a few locations or whether they are dispersed throughout the genome, interspersed with genes and other unique sequences (Thompson *et al.*, 1991). The dispersed category of repetitive DNA is found to constitute approximately 15% of the human genome, with two families comprising approximately six percent of the entire genome: the *Alu* family and the L1 family (Table 2).

The *Alu* family is so named since most members of the family are cleavable by the bacterial restriction enzyme *AluI*. About 500,000 members make up this family, none of which are identical in sequence. They are approximately 300 base pairs in length, have no apparent function and constitute three percent of the entire genome. The second major family of dispersed repetitive DNA is the L1 family. L1 sequences are repetitive elements of six to seven base pairs, spanning up to six kilobases in length and are present in 10,000 copies per genome (Bickmore and Sumner, 1989). *Alu* sequences are 56% guanine plus cytosine and L1 sequences are 58% adenine plus thymine. This distribution of L1 and *Alu* sequences may account for a large part of the alternating dark and light chromosome banding patterns observed when human metaphase chromosomes are examined with staining techniques (Korenberg and Rykowski, 1988; Chen and Manuelidis, 1989).

The category of repetitive DNA consisting of clustered repeats, comprising approximately 10% of the genome, contains highly repetitive DNA sequences which are ideal forensic markers (Table 2). Originally the various tandem repeats were isolated from the rest of the genome by density centrifugation and termed "satellite" fractions of DNA. Satellite DNA families are grouped with regard to their location within the genome, the length of the core repeat units and the total length of the array. Minisatellites or variable

number of tandem repeats (VNTRs) are composed of tandem repetitions of a 15 to 65 base pair core unit that can range up to 20 kilobases in length and are distributed along each chromosome unlike the highly localized satellite arrays (Nakamura *et al.*, 1987). VNTR polymorphisms are isolated through the cleavage of genomic DNA by restriction endonucleases and subsequent Southern hybridization with a probe specific to the VNTR core sequence. The length polymorphisms result from the distance between restriction sites due to the variable number of tandem repeats within, but may infrequently result from restriction site polymorphisms (Waye and Fourney, 1990). VNTR arrays that are short enough to be amplified by the polymerase chain reaction (PCR) are termed amplifiable fragment length polymorphisms or AMP-FLPs (Jeffreys *et al.*, 1990). Although the VNTR approach provided excellent discrimination power, there are several important limitations to its use: 1) large quantities of high molecular weight DNA (50 to 500 ng) are required, 2) due to the limitations of the detection procedures used to identify the VNTR alleles (e.g. ^{32}P -labeled probes and autoradiography), “null” alleles are observed (individuals who were heterozygous for a VNTR locus falsely produced a homozygous profile at that locus due to restriction site polymorphisms or produced fragment sizes that were too small) and 3) the procedure is tedious and not amenable to automation (Frégeau and Fourney, 1993).

Another class of highly repetitive DNA includes microsatellite sequences. Microsatellite polymorphisms were characterized in the early nineties and are widespread throughout the genome, occurring with a frequency of one locus every 6-10 kilobases (Weber and May, 1989; Edwards *et al.*, 1991; Beckman and Weber, 1992). The advent of the polymerase chain reaction (PCR) coupled with the discovery of microsatellite sequences

or short tandem repeats (STRs) allowed a genetic profile to be generated from minuscule amounts of template DNA, even in a degraded state (Saiki *et al.*, 1985). Higuchi *et al.* (1988) showed that amplifying DNA from a single hair root was possible.

C. Short tandem repeats (STRs)

Analysis of short tandem repeat sequences by PCR has become the method of choice for the forensic identification of biological evidence (Frégeau and Fournay, 1993; Lygo *et al.*, 1994; Micka *et al.*, 1996). By definition, microsatellites, or STRs, are comprised of tandem repeats of two to seven base pair core sequences, with total fragment sizes less than 350 base pairs (Brinkmann, 1995). STR alleles are named according to the number of consecutive repeat units each allele contains; e.g., an allele that contains 15 iterations of the core sequence would be designated as allele 15 at that locus. However, the designation of two alleles by the same allele number cannot necessarily be interpreted to mean that they contain the identical nucleotide sequences. STRs can be characterized into three main classes: 1) 'Simple' repeats contain core sequences of identical length and sequence, 2) 'Compound' repeats are comprised of two or more adjacent simple repeats and 3) 'Complex' repeats contain several repeat blocks of variable core sequence length, as well as variable intervening non-core sequences (Urquhart *et al.*, 1994).

Loci of short tandem repeats generally have fewer alleles and genotypes than VNTR loci. Therefore, to obtain an equivalent power of discrimination, more loci are required. The probability of identical alleles at all loci in two individuals is inversely proportional to the number of polymorphic loci examined (Edwards and Gibbs, 1994). Multiplex PCR

amplification, in which several STR loci are simultaneously amplified, is a useful technique for DNA profiling. Co-amplification of several STR loci in one PCR reaction tube also provides the additional benefits of conserving sample, minimizing contamination and expediting manual processing time (Wallin *et al.*, 1998). These advantages are most important for high-throughput laboratories which produce criminal intelligence databases or evaluate the frequencies and distributions of STRs for population studies.

D. Developing an STR Multiplex System for Forensic Applications

The human genome is estimated to contain approximately 500 000 STR loci (Edwards *et al.*, 1991). Although they are abundant, not all STR loci are suitable for implementation in identity testing. To evaluate the reliability of an STR system for forensic applications, the validation process should consider ten parameters as outlined by the Technical Working Group on DNA Analysis Methods (TWGDAM) committee (Technical Working Group on DNA Analysis Methods, 1995):

- 1) *Species Specificity*- DNA samples from non-human sources should be amplified using the multiplex system to determine the species specificity of a DNA assay designed for human use.
- 2) *Reliable performance*- Single source DNA samples should be amplified to ensure balanced peak heights within and across loci. In addition, artifact peaks should be minimal or non-existent. One artifact of particular concern is “stuttering.” Possible slippage of the DNA polymerase during amplification of the short tandem repeats results in stutter or shadow bands which are usually less than the size of the true peak by an integer core repeat length

Figure 1. Slipped strand mispairing model for stutter formation one repeat unit shorter than the main allele band. The template strand (main allele) in this figure is represented by black boxes and contains seven repeat units. In Step 1, the DNA polymerase has extended through four repeat units, represented by the blue boxes. If/when the polymerase falls off the extending strand, the template and extending strand can separate, as shown in Step 2. When the two strands re-anneal in Step 3 the template strand has looped out and the extending strand misaligns by one repeat unit. Thus, the polymerase can only add an additional two repeat units, instead of the correct three repeat units. Step 4 demonstrates that at the completion of synthesis the newly extended strand contains only six repeat units, while the template strand has seven repeat units (reproduced from Walsh *et al.*, 1996).

Step 1

Step 2

Step 3

Step 4

(-4, -8 nucleotides for tetranucleotide repeats) and are typically less than 10% of the height of the true peak (Figure 1). A stutter of one repeat unit larger than the true peak has been observed as well (Walsh *et al.*, 1996). The presence of stutter bands increases as the core repeat size decreases (i.e. pentanucleotide < tetranucleotide < trinucleotide < dinucleotide) and the proportion of stutter bands is highest for alleles containing long stretches of uninterrupted core repeats (Walsh *et al.*, 1996).

3)*Somatic Stability*- DNA from a variety of body tissues and fluids obtained and stored in a controlled manner should be examined to confirm that the same genotype is produced from different tissues of the same donor.

4)*Consistency*- genotyping results should be reproducible both within a particular laboratory and among different laboratories.

5)*Population Studies*- the distributions and frequencies of population data in different racial and/or ethnic groups should be examined to confirm Hardy-Weinberg equilibrium and linkage equilibrium. A population in Hardy-Weinberg equilibrium is sufficiently large and characterized by random mating, with little if any stratification or inbreeding. In addition, the locus under consideration exhibits a constant mutation rate and there is no selection for or against a particular phenotype (Thompson *et al.*, 1991). Linkage equilibrium is characterized by the lack of association between loci whether or not the loci are linked (Weir, 1990).

6)*Inheritance*- the mode of inheritance of alleles (examined by family studies) should be carried out to ensure independent assortment of alleles (Mendelian inheritance).

7)*Mixed Specimen Studies*- studies investigating the ability of a multiplex system to detect

components of mixed DNA specimens should be carried out and definitions of the limitations of interpretation of such samples should be established.

8)*Environmental Studies*- forensic casework specimens may be exposed to a variety of compromising environmental conditions which may result in DNA degradation or PCR inhibition or both. Studies should be carried out which closely resemble such conditions to evaluate and define any limitations of the DNA assay.

9)*Sensitivity Studies*- an imbalance in the yield of alleles in a heterozygous individual may be observed when the total number of template copies added to the PCR reaction is extremely low (Wallin *et al.*, 1998). Since such an imbalance can affect the interpretation of a genotyping result, the quantity of DNA needed to obtain a reliable result must be established.

10)*Evidentiary Specimens*- the multiplex system should be evaluated with actual forensic casework specimens.

The tetrameric and pentameric STRs appear to be the most promising for forensic purposes due to minimal or negligible slippage and the ease of resolving consecutive alleles (Frégeau and Fourney, 1993; Brinkmann, 1995; Wilson-Wilde *et al.*, 1997). Typing of simple STRs has been demonstrated to be highly successful, even with several labs employing a wide range of electrophoretic and detection systems (Gill *et al.*, 1994). Of the three classes of STRs, compound and complex groups are generally the more discriminating since they exhibit a high degree of length polymorphism and would therefore be most suitable for use in a highly discriminating multiplex system (Kimpton *et al.*, 1995; Oldroyd *et al.*, 1995). In addition, the presence of microvariants resulting from insertions and/or

deletions of one, two or three nucleotides within or between repeat blocks provide an increased level of diversity in populations. Another advantage of these groups is reduced stutter occurrence (Frégeau *et al.*, 1998). The ability to resolve alleles differing by one or two bases, however, has been demonstrated to be problematic for many laboratories with different electrophoretic systems (Gill *et al.*, 1994). In addition, alleles of the same size, but having different nucleotide sequences, have been shown to migrate anomalously when analyzed with certain electrophoretic instruments, producing further problems in interpretation (Gill *et al.*, 1994). With newer automated fluorescence technologies, it is now relatively simple to resolve one-base variants and multiplex amplification, coupled with laser-induced fluorescent detection and denaturing gels, has been shown to be a robust system capable of providing reproducible results between different laboratories (Lygo *et al.*, 1994; Kimpton *et al.*, 1995).

E. Population Databases

Numerous STR systems have been documented and used to study the extent of genetic polymorphisms within and between populations by constructing population databases (Edwards *et al.*, 1992; Budowle and Monson, 1994; Möller *et al.*, 1994; Waye *et al.*, 1994; Frégeau *et al.*, 1998). The relative frequencies of alleles in a population database are used to obtain an estimate of the random match probability using the so-called 'product rule' which determines the strength of a match between two DNA profiles in a forensic identification (Balding and Nichols, 1994). If discrepancies between the profile of a suspect and a profile obtained at the scene of a crime exist, the suspect will usually be exonerated.

However, if the two DNA profiles are judged to match, the strength of this evidence must be weighed in view of other evidence and much controversy has centered on methods for calculating the significance of the match, particularly with VNTR data (Balding and Donnelly, 1994). In 1992, the National Research Council (NRC) of the US National Academy of Sciences issued a report (National Research Council, 1992) to identify some of the problems with the product rule and to provide recommendations. The report caused extensive criticism over the "ceiling principle" which was intended to place a lower limit on the size of the profile frequency (Weir, 1993). The ceiling principle involved sampling 100 individuals from each of 15-20 "genetically homogeneous ethnic populations," determining the highest frequency among all groups for each allele, or 5%, whichever is larger and applying these values to estimate the random match probability (National Research Council, 1992). However, there was concern with population substructuring within large databases, that is, when the reference population is composed of groups that are genetically different from each other (e.g. if the samples comprising the Caucasian database were North American Caucasians and European Caucasians and these populations were genetically different from each other). The question did not center on whether substructuring existed, but on the extent of it and how it should be taken into account when estimating the rarity of a particular allele or genetic profile (Lempert, 1993). If population substructure is significant, this can invalidate the assumptions of Hardy-Weinberg equilibrium and linkage equilibrium since the populations utilized are not homogeneous and randomly mating. Also, estimations of allele frequencies based on a reference database may provide an underestimate of the allele frequencies in the ethnic group to which a suspect belongs. Therefore, multiplying together

the frequencies with which each of the individual VNTR genotypes occurs in the reference database may be unjustified (Lewontin and Hartl, 1991). In addition, estimation of population frequencies from VNTR profiles was questioned since resolution of alleles on agarose gels was poor in the upper size ranges and the discrete number of repeat units for some variants could not be accurately assessed. With the PCR approach, the allelic data obtained is more discrete than VNTR alleles typed by RFLP analysis. In addition, PCR-based loci can be resolved to at least one repeat unit, and by some methods to a single base by separation on denatured polyacrylamide gels (Edwards *et al.*, 1991). Numerous authors refuted these initial claims on the effects of population substructuring and demonstrated that major population group databases can be used instead of subpopulation databases to provide meaningful estimates of DNA profile frequencies (Devlin *et al.*, 1990, Chakraborty and Kidd, 1991; Budowle and Monson, 1994). More recent studies have demonstrated that the variability among subpopulations is less than the variability among ethnic groups (Roeder, 1994) and the product rule can be utilized for establishing random match probabilities using the appropriate reference population.

F. The National DNA Databank

Databases consisting of tetranucleotide STR profiles are also useful for linking the DNA profiles of convicted offenders with profiles established from evidentiary samples from crime scene investigations (Dovaston, 1996). With the enactment of Bill C-3 (The DNA Identification Act) in December of 1998, Canadian law enforcement agencies will begin banking an annual estimate of 28,000 to 98,000 profiles (established using STR technology)

obtained from individuals convicted of primary and secondary designated offences. The undertaking of such a project requires consideration of many factors involving the scientific technology, security and privacy, quality assurance, as well as legislative realities. From a practical perspective, cost, processing time and long-term storage of the biological materials become important issues. In addition, the technology which provides high throughput potential for implementation in a large-scale DNA database must be reviewed.

G. Technology considerations for DNA database applications

(i) FTA™ collection cards

In the forensic community, for a DNA database initiative, there is the need for an efficient, valid, reliable and safe method to quickly process and analyse a DNA sample. The use of FTA™ collection cards (Fitzco, Minneapolis, MN) proves to be an excellent approach (Figure 2). FTA™ paper contains a dried chemical reagent mix embedded within the paper matrix (Elliott *et al.*, in press). The reagent mix consists of a strong buffer, with protein denaturants, a chelating agent and a free radical trap. It is non-toxic to humans, protects DNA from bacteria and mold, ultraviolet radiation and oxidation and provides protection against acidic atmospheric gases, such as traces of HCl, HNO₂/HNO₃ and SO₂/SO₃ (Williams *et al.*, 1995; Rogers and Burgoyne, 1997). In addition, the FTA™ collection card inactivates any human blood-borne pathogens instantly. Whole blood or buccal scraping can be applied directly to the FTA™ collection card, which is then sealed in a mylar envelope with dessicant and stored at room temperature for an indefinite number of years (studies are ongoing). In addition to being an excellent archival storage media, FTA™ cards can be transported easily

Figure 2. Processing of biological specimens on FTATM collection cards. A 1.2mm disk is manually punched out of the sample on the collection card and subjected to a series of washes to immobilize the DNA onto the paper matrix. The processed FTA punch is added directly to the PCR reaction tube for amplification. The amplified fragments is then visualized through electrophoresis and analysed with the appropriate software programs. The profile obtained is then entered into a database for further analysis.

FTA processing

3-5 min. washes with FTA
purification reagent

2-5 min. washes with 1X TEBuffer

PCR amplification

Electrophoresis

Data Analysis

Data Entry into Database

15,15; 19,19; 19,23; X,Y; 13,16; 29,33.2; 13,14; 9,11; 8,8; 10,11

and tracked conveniently through the use of an electronic barcode system. A 1.2 mm disk of the dried blood or buccal specimen is taken manually from the FTA™ card and is treated with a series of five simple five minute washes. Since the DNA is immobilized onto the paper matrix while contaminants are washed away, there is no handling of the DNA and subsequently no variable losses due to pipetting. The processed FTA™ disk provides ample DNA template for PCR amplification and is added directly to the amplification reaction. Due to the ease of the FTA™ procedure, the protocol can be incorporated into a robotic process, combined with the use of instruments for automated detection and analysis of fluorescent PCR amplification products (Frégeau and Fourney, 1993; Kimpton *et al.*, 1993; Urquhart *et al.*, 1994; Belgrader *et al.*, 1995; Williams *et al.*, 1995).

(ii) STR analysis

An agreement had been reached by North American law enforcement agencies on a common group of STR loci suitable for casework and database applications (13 core STR loci). These loci have been assembled as PCR megaplex kits by several commercial suppliers. The AmpF/STR Profiler Plus™ PCR amplification kit is a commercial product available from Perkin-Elmer that co-amplifies nine of these STR loci: D3S1358 (Li *et al.*, 1993), vWA (Kimpton *et al.*, 1992), FGA (Mills *et al.*, 1992), D8S1179 (Oldroyd *et al.*, 1995), D21S11 (Sharma and Litt, 1992), D18S51 (Urquhart *et al.*, 1995), D5S818 (Hudson *et al.*, 1995), D13S317 (Hudson *et al.*, 1995) and D7S820 (Green *et al.*, 1991), plus the gender discrimination locus contained on a segment of the X-Y homologous gene amelogenin (Sullivan *et al.*, 1993) (Table 3). The Profiler Plus™ megaplex was validated

Table 3. AmpF/STR Profiler Plus™ loci.

Note: Locus designations are designated by their GenBank locus name except for D21S11 which remains uncharacterized. Abbreviations: vWA, von Willebrand factor A; FGA, α -fibrinogen. 5-FAM, 5-carboxyfluorescein (absorbance at 493nm, emission at 522nm); JOE, 6-carboxy-2',7'-dimethoxy-4',5'-dichlorofluorescein (528nm, 585nm); NED (553nm, 575nm) (proprietary). Letters in parentheses correspond to the color of the product: B, blue; G; green; Y; yellow.

^aBased on actual base pair size of sequenced alleles contained in the AmpF/STR Allelic Ladders, including the 3'-A nucleotide addition.

^bR can represent either an A or G nucleotide.

* not reported whether the six bases are conserved between male individuals

Locus designation	Chromosome location	Core repeat (5'-3')	Amplicon size range(bp) ^a	Fluorescent tag
D3S1358	3p	TCTA(TCTG) ₁₋₃ (TCTA) _n	114-142	5-FAM (B)
vWA	12p12-pter	TCTA(TCTG) ₃₋₄ (TCTA) _n	157-197	5-FAM (B)
FGA	4q28	(TTTC) ₃ TTTT TTCT(CTTT) _n CTCC(TTCC) ₂	219-267	5-FAM (B)
Amelogenin	X: p22.1-22.3	-	107	JOE (G)
	Y: p11.2	-	113	JOE (G)
D8S1179	8	(TCTR) _n ^b	128-168	JOE (G)
D21S11	21	(TCTA) _n (TCTG) _n [(TCTA) ₃ TA (TCTA) ₃ TCA(TCTA) ₂ TCCATA] (TCTA) _n	189-243	JOE (G)
D18S51	18q21.3	(AGAA) _n	273-341	JOE (G)
D5S818	5q21-31	(AGAT) _n	135-171	NED (Y)
D13S317	13q22031	(GATA) _n	206-234	NED (Y)
D7S820	7q	(GATA) _n	258-294	NED (Y)

Figure 3. Genotyper™ plot of an AmpF/STR Profiler Plus™ amplification reaction. The names of the STR loci are labeled beside each corresponding peak. Blue, Green and Yellow colours represent the 5-FAM, JOE and NED fluorescent tags, respectively. The horizontal scale represents the size in bases and the vertical scale depicts the peak height in relative fluorescence units (RFUs). The boxed number below each peak represents the allele designation or number of iterations of the core sequence. The bottom electrophoretogram represents the DNA fragments of the GS-500 [ROX] (red) internal lane standard. Numbers below the red ladder indicate the size in bases of the DNA fragments.

according to the guidelines recommended by TWGDAM (AmpFISTR Profiler Plus™ PCR Amplification Kit User's Manual, 1997) and offers an excellent system for forensic DNA profiling. The availability of four distinguishable fluorescent dyes permits the differentiation of loci in an STR multiplex PCR system with overlapping allele size ranges. The Profiler Plus™ kit is designed such that one primer of each primer set is fluorescently tagged with either a 5-FAM, JOE or NED dye (blue, green and yellow, respectively) (Figure 3). A fourth fluorescent tag, ROX, (6-carboxy-X-rhodamine 585nm, 605nm) displayed as red, is reserved for DNA fragments of an internal lane size standard (Figure 3). The internal lane standard is co-analyzed by electrophoresis with each PCR sample and controls for inter- and intra- lane variation.

The 'blue' system is composed of D3S1358, vWA and FGA (Wallin *et al.*, 1998). Both D3S1358 and vWA are compound STRs and FGA is classified in the complex group of STRs (Wallin *et al.*, 1998). The polymorphic region examined in vWA is found in intron 40 of the human von Willebrand Factor (vWF) gene (Kimpton *et al.*, 1992) and has been shown to be highly reliable and robust in multiplex PCR amplification (Kimpton *et al.*, 1996). The FGA locus resides in the third intron of the human alpha fibrinogen locus and the repetitive sequence exhibits a high degree of length polymorphism which proves highly informative (Mills *et al.*, 1992; Frégeau *et al.*, 1998). Several two-base variants have been identified at the FGA locus which could be related to the high AT composition of the core repeat (Table 3).

The 'green' system is made up of the gender discrimination marker, amelogenin, as well as D8S1179, D21S11 and D18S51. The amelogenin gene codes for an extracellular

matrix protein in the developing tooth bud (Taylor *et al.*, 1997). The amplified target is six base pairs shorter on the X chromosome (106 bp) than the homologous region on the Y chromosome (112 bp); this sequence length variation aids in sex determination (Nakahori *et al.*, 1991). A female profile (X,X) will contain only the 106 bp amplicon, whereas a male profile (XY) will contain both the 106 bp and 112 bp products. The D8S1179 locus is included in the compound STR class, the D21S11 locus is classified as a complex STR and D18S51 represents a simple STR (Barber and Parkin, 1996; Brinkmann *et al.*, 1996; Zhou *et al.*, 1997). The D21S11 locus is invaluable for forensic profiling, combining increased discriminating power due to its sequence complexity with reduced potential for the formation of stutter bands (Gill *et al.*, 1995).

The 'yellow' system consists of D5S818, D13S317 and D7S820, all of which are grouped into the simple class of STRs (Lins *et al.*, 1998). These three loci demonstrate a relative lack of stutter product, with a calculated average of seven percent stutter (Leclair, in preparation).

The STR loci examined by the Profiler Plus™ kit are highly informative and collectively offer a high value of Probability of Identity (P_I). The P_I value is the probability that two individuals, randomly selected, have an identical Profiler Plus™ genotype (Sensabaugh, 1982). The P_I value calculated for all nine Profiler Plus loci combined is approximately $1/6.8 \times 10^{10}$ in the African-American population and $1/9.6 \times 10^{10}$ in the U.S. Caucasian population (AmpF/STR Profiler Plus™ PCR Amplification Kit User's Manual, 1997).

Figure 4. Schematic of a typical capillary electrophoresis (CE) instrument.

(iii) Capillary electrophoresis

Capillary electrophoresis (CE) has generated considerable interest as an analytical technique for the automated analysis of fluorescently labelled alleles (Thormann *et al.*, 1994; Isenberg *et al.*, 1996; Zhang and Yeung, 1997; Mansfield *et al.*, 1998; Wenz *et al.*, 1998). While conventional agarose or polyacrylamide slab gel electrophoresis have been reliable techniques for many years, they are labor-intensive and difficult to fully automate (Butler *et al.*, 1995). A schematic of a typical CE instrument is shown in Figure 4. Fused-silica capillaries of 25-75 μm inner diameter (ID) and 15-100 cm in length are used as the separation channel (von Heeren and Thormann, 1997). Compared to slab gel electrophoresis, the high surface area-to-volume ratio of a capillary permits higher voltages (up to 10X higher than slab gel electrophoresis) to be used, resulting in rapid (less than 30 min.), high resolution separations (Paulus and Hüsken, 1993; Mansfield *et al.*, 1996). The buffers and electrodes are located at the terminal ends of the capillary and a high-voltage power supply delivering up to 30 kV provides the force for separation along the column (Krull and Mazzeo, 1992). A small amount of sample (a few nL) is introduced into the capillary typically through electrokinetic injection. An electrokinetic injection involves the brief application of high voltage which causes the negatively-charged DNA molecules to enter the capillary and migrate towards the positive electrode at the other end of the capillary. The basis of separation is usually by size through a sieving gel matrix.

Early separations of amplified DNA products using capillary electrophoresis employed chemical gels, including polyacrylamide (Paulus *et al.*, 1990; Guttman *et al.*, 1990) and agarose (Bocek and Chrambach, 1991), which were chemically linked to the

capillary wall. Although the chemical gel matrices were highly effective in DNA separations, several problems associated with their routine use became apparent. Since the matrix was permanently "fixed" to the capillary wall, when the gel was no longer functional, neither was the capillary. In addition, when filling the capillaries with the chemical gel, great care was required to avoid the introduction of air bubbles which would cause a subsequent loss of conductance (Baba *et al.*, 1992).

The current alternative to chemical gels is the use of physical gels or polymer networks (Grossman and Soane, 1991; Rosenblum *et al.*, 1997; Tietz *et al.*, 1992; Vainer *et al.*, 1997). The viscous solution contains entangled polymers which are not cross-linked, but interact forming a mesh-like structure. This solution can be introduced into the capillary and replaced with fresh media for each run, providing the opportunity for automation. In addition, high temperatures (up to 70°C) and high voltages (1000 V/cm) can be applied without damage to the matrix (Sanchez and Smith, 1997). Examples of entangled polymers include linear polyacrylamide (Chiari *et al.*, 1992; Pariat *et al.*, 1993), polyethylene oxide (Kim and Yeung, 1997) and cellulosic polymers such as methyl cellulose (Strege and Lagu, 1991; Figeys *et al.*, 1994) and hydroxyethyl cellulose (Nathakarikool *et al.*, 1992; Butler *et al.*, 1994).

Detection of the DNA fragments is performed on-column by a sensor placed toward the opposite end of the capillary inlet, with data transmitted digitally to a computerized station (Figure 4). The sensitivity and enhanced performance of laser-induced fluorescence detection (LIF) compared to ultraviolet absorbance detection make it the preferred method for DNA analysis (Arakawa *et al.*, 1994; Oda *et al.*, 1996). A laser (typically argon-ion)

focuses the excitation light onto the detection window of a capillary (Schwartz *et al.*, 1994). The fluorescence emitted by the fluorescently tagged DNA molecules as they are passing through the detection window is then collected by a series of optical components and focused onto a spectrograph. A diffraction grating in the spectrograph separates the emitted light by wavelength and focuses it onto a charge coupled device (CCD) detector. The CCD detector integrates the light and converts it into a voltage signal that the instrument acquires and analyses (ABI PRISM™ 310 Genetic Analyzer User's Manual, 1995).

H. Thesis Objectives

The attractive characteristics that FTA™ paper, multiplex PCR amplification and capillary electrophoresis provide may constitute an interesting integration of tools for forensic DNA analysis. Particularly, this combination could provide a fast, efficient and automatable approach for database purposes, both in the construction of population databases to examine allelic variation among individuals and for the generation of DNA profiles for the National DNA Databank (from convicted offenders and evidentiary crime scene samples). The objective of the study was to evaluate the combination of these technologies for large-scale database potential, principally for its application to the National DNA Databank. The objective was carried out by using these tools to examine and compare the frequencies and distributions of nine short tandem repeat (STR) loci in African-American, Chinese, Vietnamese, Bangladesh and Japanese populations. By processing a large number of samples in this fashion, the efficiency of these methods for the collection and storage of biological materials and the amplification and electrophoresis of PCR generated DNA fragments can be ascertained, and at the same time, useful information regarding the frequencies and distributions of STR loci among these five populations can be obtained.

II. MATERIALS AND METHODS

A. Sample populations

Bloodstained FTA™ collection cards (US Patent 5,496,562 Fitzco, Minneapolis, Minnesota) from 151 unrelated individuals of African-American descent were obtained from Dr. Arthur Eisenberg (University of North Texas Health Science Center, Fort Worth, Texas). The stains were derived from non-frozen whole blood specimens containing chelating anticoagulants.

Bloodstained FTA™ collection cards consisting of 50 unrelated individuals for each of Chinese, Bangladesh and Vietnamese ethnicity were provided by Jennifer Clay (Helix Biotech, Richmond, British Columbia). The FTA™ collection cards were spotted with previously frozen whole blood samples containing chelating anticoagulants.

Bloodstained absorbent cotton swatches from 113 unrelated Japanese individuals were obtained from Dr. Jungi Miyakoshi, (Kyoto University, Kyoto, Japan). DNA from 67 of those cotton swatches had been previously extracted by Paul Roussy (RCMP, Ottawa, Ontario) using established RCMP protocols (Biology Section Methods Guide, 1998). An additional 59 Japanese samples were provided by Ryoji Iwamoto (Scientific Investigation Laboratory, Aomori, Japan). A one cm² piece of bloodstained gauze was obtained for 58 of those individuals and a bloodstained FTA™ collection card from a fingertip puncture was provided for one individual. A total of 172 unrelated individuals were sampled from the Japanese population.

B. Isolation of DNA

A 1.2 mm disk (Harris MicroPunch, Fitzco, Minneapolis, Minnesota) was taken manually from each of the FTA™ collection cards and was processed according to established RCMP protocols (Appendix 1).

A triangular area of approximately 10 mm² was cut from each of the bloodstained cotton swatches and gauze samples and processed in the same fashion as the FTA™ collection cards, except the volume of purification and wash solutions was doubled.

C. Quantitation of DNA extracts

The processing of FTA™ samples permits a sufficient and relatively constant amount of target DNA to be immobilized on the paper matrix such that a reliable PCR typing result can be obtained without the need of DNA quantitation. The DNA extracts from the 67 Japanese individuals were diluted ten-fold with FAD (filtered, autoclaved, deionized) water and quantified using a chemiluminescent slot blot procedure according to the established RCMP protocol (Appendix 2). Quantitation of DNA was determined by hybridization with a primate specific α -satellite DNA probe and direct assessment of the unknown DNA samples with a standard reference set of quantified DNA (Waye *et al.*, 1991). Kodak XRP5 film (no screen) was used, with an exposure of 2.5 hours at room temperature. The appropriate dilutions with FAD water were prepared to yield a DNA concentration of 1ng/ μ L.

D. PCR amplification

All PCR reactions were carried out using the AmpF/STR Profiler Plus™ PCR Amplification Kit (Perkin-Elmer, Foster City, California) and the GeneAmp PCR System 9600 Thermocycler (Perkin-Elmer, Foster City, California). The processed 1.2 mm FTA punches were transferred into the PCR microamp tubes (Perkin-Elmer, Foster City, California) and amplified in a 5 μ L PCR reaction volume consisting of 2 μ L of FAD water and 3 μ L of PCR master mix (1.92 μ L AmpF/STR PCR Reaction Mix (containing $MgCl_2$, dATP, dGTP, dCTP and dTTP, bovine serum albumin and 0.05% sodium azide in buffer and salt) + 0.1 μ L AmpliTaq Gold DNA Polymerase (5 U/ μ L) + 1 μ L AmpF/STR Profiler Plus™ Primer Set). The FTA collection cards which were stained with non-frozen bloodstains were amplified using the following conditions: 95°C, 11 min. initial incubation; followed by 23 cycles of a three temperature PCR consisting of denaturation at 94°C, 1 min.; annealing at 59°C, 1 min.; and extension at 72°C, 1.5 min. A final extension at 60°C, 45 min., followed by an overnight hold at 22°C, concluded the amplification. The FTA collection cards spotted with frozen whole blood underwent the same amplification conditions, except 25 cycles were applied.

The processed cotton swatches and gauze were amplified in a 25 μ L PCR reaction volume containing 10 μ L of FAD water and 15 μ L of PCR master mix (9.6 μ L AmpF/STR PCR Reaction Mix (containing $MgCl_2$, dATP, dGTP, dCTP and dTTP, bovine serum albumin and 0.05% sodium azide in buffer and salt) + 0.5 μ L AmpliTaq Gold DNA Polymerase (5 U/ μ L) + 5 μ L AmpF/STR Profiler Plus Primer Set), for 28 and 25 cycles, respectively. A total of 2.5 ng of quantified DNA was amplified in a 25 μ L PCR reaction

volume containing 7.5 μL of FAD water and 15 μL of PCR master mix, for 28 cycles.

Each amplification round included a positive control (2.0 ng female cell line 9947A; Perkin-Elmer, Foster City, California; Frégeau *et al.*, 1995) and a negative control (2 μL or 10 μL FAD water) in either a 5 μL or 25 μL PCR reaction volume, respectively. All amplified products were stored at -20°C .

E. Capillary electrophoresis

Separation of the amplified DNA fragments was carried out through capillary electrophoresis using the ABI PRISM™ 310 Genetic Analyzer (Perkin-Elmer, Foster City, California). A silica-fused 47 cm, 36 cm length-to-detector (LD), 50 μm inner diameter capillary was filled automatically with performance optimized polymer, POP-4 (Perkin-Elmer, Foster City, California) sieving matrix (Rosenblum *et al.*, 1997) before each sample injection. The running buffer, 10X Buffer with EDTA, (Rosenblum *et al.*, 1997) was purchased from Perkin-Elmer (Foster City, California) and diluted ten-fold with FAD water. An aliquot of PCR product between 0.25 μL and 2 μL was added to each sample tube with 1 μL GeneScan-500 [ROX] (GS-500) internal lane standard (Perkin-Elmer, Foster City, California) and up to a total volume of 26 μL with deionized formamide (Appendix 3).

For every group of samples analyzed on the 310 instrument, the AmpF/STR Profiler Plus™ Allelic Ladder (Perkin-Elmer, Foster City, California) was injected in the first, second, last and every 13th position in-between (Appendix 4). The allelic ladder was prepared by mixing equal volumes of the AmpF/STR Blue, Green II and Yellow allelic ladders and aliquoting 2 μL of this mixture into a sample tube containing 23 μL deionized

formamide and 1 μ L GS-500 [ROX] internal lane standard.

After denaturation of samples at 95°C for three minutes and snap cooling on ice for three minutes, samples were electrokinetically injected in sequence for five seconds at 15 kV and electrophoresed at 15 kV through the capillary for 24 min., at a constant temperature of 60°C (Run Module GS STR POP-4 A).

F. Sizing and designation of alleles

Data was analysed using the ABI Prism™ GeneScan™ Analysis 2.1 software (Perkin-Elmer, Foster City, California). Sizes (in bases) were assigned to the electrophoresed PCR products using the Local Southern method of size calling. Based on the number of iterations of the core sequence, expressed as allele size in bases and migration time of the DNA fragments from the GS-500 [ROX] internal lane size standard, a linear relationships from 75 to 400 bases is graphed and used to determine size estimates of unknown DNA fragment sizes. The 250 base and 340 base peaks in the GS-500 [ROX] internal lane size standard were not used. Due to incomplete denaturation of these double stranded molecules, their migration is altered on the CE instrument and consequently their sizes are unreliable as standards. Each allele was carefully reviewed to ensure that a valid peak size was determined based on size, low background, lack of stutter or other PCR attributable artifacts. The allele size data, for both the allelic ladders and population samples, were converted to allele designations for each locus by importing the analyzed data into the DNA fragment analysis software Genotyper™ 2.0 (Perkin-Elmer, Foster City, California). Genotyper™ 2.0 utilizes the allelic ladder as a reference for

assigning alleles to the questioned DNA fragments. A table of allele designations for each locus was generated for each individual, and this tabular data was then used for further statistical analysis.

G. Population genetics

The software program GenepopTM version 3.1b (Raymond and Rousset, 1995a) was used to perform statistical analyses of the population data. Three main tests were performed: Hardy-Weinberg exact tests, linkage disequilibrium tests and tests for population differentiation. The Hardy-Weinberg (HW) test for each locus in each population was performed using an unbiased estimation of the exact HW probability by an algorithm defined by the Genepop program (Guo and Thompson, 1992). For each test, the p -value of the test and the standard error were calculated by GenepopTM (Robertson and Hill, 1984; Weir and Cockerham, 1984). Other parameters calculated include: expected genotypic proportions, allele frequencies, as well as observed and expected number of heterozygotes and homozygotes. In addition, to use the product rule for estimating random match probabilities, tests for genotypic disequilibria were carried out for each pair of loci in each population by the GenepopTM program. Tables were constructed for all pairs of loci in each population by the GenepopTM program, then a probability test was performed for each table using an algorithm. An unbiased estimate of the p -value was indicated as well as its standard error. Tests for population differentiation were concerned with the allelic distribution of alleles in the various populations. Allelic differentiation for all pairs of populations for all loci was tested. An unbiased estimate of the p -value of the probability test and its standard error was

performed as described in Raymond and Rousset (1995b).

The software program DNAtype™ (Chakraborty, Zhong and Stivers, 1998 version, Human Genetics Center, School of Public Health, University of Texas Health Science Center, Houston, Texas) was utilized to provide a global test of independence for all nine Profiler Plus™ loci based on the distribution of the number of heterozygous loci across individuals (Brown *et al.*, 1980; Chakraborty, 1984).

III. RESULTS

A. DNA detection and quantitation by chemiluminescence

Quantitation of the 46 DNA extracts from Japanese individuals (previously extracted by Paul Roussy, RCMP, Ottawa, Ontario) using chemiluminescence detection yielded ample amounts of DNA, ranging from 20 ng to 750. Figure 5 shows the results from a slot blot of 12 Japanese individuals. Dilutions of DNA from the female standard present in the first two columns of the membrane were used to visually estimate the amount of DNA present in each of the Japanese samples. If greater than 100 ng of DNA was present on the slot blot lumigraph for any of the DNA extracts, the DNA for that sample was diluted and re-quantified to obtain a more accurate estimation of yield.

B. Efficiency of DNA recovery, PCR amplification and capillary electrophoresis

The DNA from all 473 individuals in the five populations was successfully amplified with complete profiles obtained across the 10 loci (Appendix 5). The peaks were sharp, distinct and well-balanced across all loci. Examination of the DNA profiles revealed no duplicate profiles. Samples that were amplified in a 5 μ L PCR reaction volume produced excellent results. In fact, amplification yields increased by up to nine-fold when a 5 μ L PCR reaction volume was used compared to the established 25 μ L PCR reaction volume stated in the RCMP protocol (Borys *et al.*, in preparation).

Of the 151 bloodstained FTA™ cards from the African-American population sample, eight samples had to be reprocessed by re-sampling the original stain and one sample had to be repeated twice, due to insufficient amplification yield. In the Vietnamese group of 50

Figure 5. Slot blot quantitation of DNA by chemiluminescence detection. Column A (Rows 1 to 8) and Column B (Rows 1 to 4) represents two-fold serial dilutions of the standard female cell line (9947A) beginning at 100 ng. Column B (Rows 5 to 8) and Column C, Row 8 represent negative controls. Column C (Rows 1 to 7) represent previously extracted DNA samples (Paul Roussy, RCMP, Ottawa, ON) from 7 Japanese individuals. Quantitation was estimated by visual comparison of the questioned samples to the known amounts of the standard female cell line.

individuals, FTA processing was repeated twice with one sample, finally requiring the addition of two disks to the amplification cocktail. With the 50 individuals sampling the Chinese population, seven FTA samples were reprocessed, two of which were repeated twice. The Bangladesh group of 50 individuals required the repetition of eight FTA samples, with three repeats each containing two disks in the amplification mix. Two of the bloodstains on cotton swatches from the 46 Japanese individuals were reprocessed twice. There were no initial amplification failures for the gauze specimens or for the previously extracted DNA samples from the Japanese population.

Injection failures on the capillary electrophoresis instrument were very rare, occurring at most five times. In such instances, the sample was simply added onto the end of the injection list and re-injected.

C. Statistical analyses

Exact tests for Hardy-Weinberg equilibrium, for population differentiation and for genotypic disequilibrium among pairs of loci were computed by the GenepopTM program. No significant deviation from Hardy-Weinberg equilibrium was detected for each of the nine STR loci in each of the five populations examined; that is, p values were greater than 0.05 (Table 4). Genotypic linkage disequilibrium tests between pairs of loci in each population showed no evidence supporting a correlation between any pairs of loci (Table 5). Out of the 180 tests done examining pairwise comparisons between loci, a total of nine were below the five percent significance level, when nine were expected by chance alone. A global test of independence across all nine loci carried out by the DNATYPETM program showed no

Table 4. Results for the Hardy-Weinberg probability test performed by GenepopTM. The p -value column indicates the value for the X^2 test for HW equilibrium with its accompanying standard error in the following column. For each population, the results of a combined X^2 test using Fisher's method for the overall significance of the p -values is shown.

¹ standard error

² degrees of freedom

African-American

Loci	P-value	S.E. ¹
-----	-----	-----
D3S1358	.3457	.0075
vWA	.6697	.0067
FGA	.2557	.0086
D8S1179	.1564	.0052
D21S11	.5805	.0109
D18S51	.6957	.0086
D5S818	.8045	.0046
D13S317	.9858	.0007
D7S820	.0731	.0041

Chi2: 16.9
Df²: 18
Prob: .532

Chinese

Loci	P-val	S.E. ¹
-----	-----	-----
D3S1358	.7746	.0041
vWA	.5377	.0043
FGA	.2804	.0075
D8S1179	.9132	.0019
D21S11	.7710	.0064
D18S51	.2664	.0077
D5S818	.9510	.0013
D13S317	.1343	.0029
D7S820	.6369	.0044

Chi2: 12.7
Df²: 18
Prob: .811

Vietnamese

Loci	P-val	S.E. ¹
-----	-----	-----
D3S1358	.6859	.0036
vWA	.0809	.0025
FGA	.4875	.0102
D8S1179	.1473	.0031
D21S11	.3466	.0093
D18S51	.2775	.0078
D5S818	.7933	.0025
D13S317	.1252	.0032
D7S820	.3839	.0059

Chi2: 22.3
Df²: 18
Prob: .220

Bangladesh

Loci	P-val	S.E. ¹
-----	-----	-----
D3S1358	.6802	.0027
vWA	.7349	.0035
FGA	.3950	.0070
D8S1179	.0770	.0028
D21S11	.7349	.0045
D18S51	.1533	.0069
D5S818	.5967	.0038
D13S317	.2069	.0040
D7S820	.1410	.0035

Chi2: 20.8
Df²: 18
Prob: .288

Japanese

Loci	P-val	S.E. ¹
-----	-----	-----
D3S1358	.5774	.0064
vWA	.2373	.0057
FGA	.3225	.0090
D8S1179	.2108	.0061
D21S11	.0639	.0046
D18S51	.6687	.0101
D5S818	.5346	.0067
D13S317	.8984	.0029
D7S820	.7617	.0051

Chi2: 17.7
Df²: 18
Prob: .478

Table 5. Results for linkage equilibrium tests performed by GenepopTM. Numbers represent the p -values obtained for each population for pairwise combinations of loci.

African-American

	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317
vWA	0.856							
FGA	0.937	0.416						
D8S1179	0.376	0.804	0.851					
D21S11	0.291	0.824	0.906	0.335				
D18S51	0.988	0.896	0.296	0.826	0.994			
D5S818	0.579	0.583	0.864	0.104	0.896	0.565		
D13S317	0.579	0.561	0.620	0.154	0.325	0.379	0.425	
D7S820	0.449	0.036	0.726	0.655	0.505	0.988	0.931	0.136

Chinese

	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317
vWA	0.582							
FGA	0.964	0.524						
D8S1179	0.456	0.040	1.000					
D21S11	0.756	0.317	0.479	0.752				
D18S51	0.975	0.235	0.316	0.906	0.822			
D5S818	0.991	0.906	0.827	0.557	0.865	0.943		
D13S317	0.941	0.693	0.761	0.760	0.104	0.483	0.795	
D7S820	0.456	0.596	0.056	0.471	0.130	0.115	0.628	0.603

Vietnamese

	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317
vWA	0.042							
FGA	0.383	0.306						
D8S1179	0.524	0.250	1.000					
D21S11	0.003	0.209	0.660	1.000				
D18S51	0.973	0.466	0.821	0.519	0.820			
D5S818	0.246	0.336	0.901	0.942	0.697	0.810		
D13S317	0.377	0.365	0.151	0.924	0.979	0.607	0.429	
D7S820	0.845	0.480	1.000	0.457	0.908	0.530	0.600	0.041

Bangladesh

	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317
vWA	0.644							
FGA	0.647	0.739						
D8S1179	0.565	0.988	0.806					
D21S11	0.162	0.112	0.045	0.274				
D18S51	0.684	0.040	0.767	1.000	1.000			
D5S818	0.075	0.331	0.214	0.484	0.391	0.724		
D13S317	0.554	0.708	0.095	0.275	0.729	0.938	0.341	
D7S820	0.262	0.666	0.928	0.695	0.968	1.000	0.185	0.510

Japanese

	D3S1358	vWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317
vWA	0.946							
FGA	0.453	0.692						
D8S1179	0.009	0.794	0.203					
D21S11	0.366	0.317	0.613	0.822				
D18S51	0.701	0.668	0.458	0.060	0.207			
D5S818	0.715	0.689	0.163	0.025	0.480	0.069		
D13S317	0.610	0.038	0.093	0.104	0.145	0.330	0.543	
D7S820	0.134	0.292	0.457	0.801	0.294	0.220	0.827	0.701

evidence of linkage disequilibrium (Tables 6,7,8,9,10). Population differentiation tests (Tables 11,12,13) revealed that each of the five populations are very different in allele composition and cannot be amalgamated into one population group. The Chinese and Vietnamese populations revealed no significant genic differentiation and could be amalgamated into one population group. The Vietnamese and Bangladesh populations revealed no significant genic differentiation except for the locus D21S11 ($p= 0.004$, S.E. 0.00037). Since 1/20 (5%) is the expectation of getting a significant value by chance alone, this deviation can be overlooked and the Vietnamese and Bangladesh populations may be combined into one group. Amalgamation of the Vietnamese, Bangladesh and Chinese populations revealed significant deviation from Hardy-Weinberg equilibrium for two out of the nine loci: D18S51 ($p= 0.041$, S.E. 0.0034) and D7S820 ($p= 0.023$, S.E. 0.002).

The Japanese population database was comprised of 172 individuals from two separate regions, Kyoto (113 individuals) and Aomori (59 individuals). Statistical tests for Hardy-Weinberg equilibrium, linkage disequilibrium and population differentiation were also carried out with the separating of the Japanese population database into two distinct population databases (the Kyoto group and the Aomori group). Therefore, six population databases were created instead of five. No evidence for Hardy-Weinberg disequilibrium or for linkage disequilibrium between pairs of loci was observed (data not shown). Tests for population differentiation revealed that amalgamation between the following groups was also possible: 1) Chinese database and Aomori group, 2) Vietnamese database and Aomori group and 3) Kyoto group and Aomori group (data not shown).

Table 6. Distribution of the number of heterozygous loci for the African-American population to determine independence across all nine loci performed by DNAtype™.

African-American population

Heterozygosity at Loci		Observed		Expected Freq. Based on	
x		Number	Freq.	Obs. Het.	Expected Het.
0		0	0.00000	0.00000	0.00000
1		0	0.00000	0.00001	0.00001
2		0	0.00000	0.00015	0.00024
3		1	0.00662	0.00168	0.00244
4		3	0.01987	0.01174	0.01551
5		7	0.04636	0.05345	0.06458
6		18	0.11921	0.15916	0.17650
7		50	0.33113	0.29919	0.30534
8		51	0.33775	0.32254	0.30342
9		21	0.13907	0.15208	0.13195
Mean			7.31788	7.31788	7.20404
Variance			1.39161	1.33941	1.40971
95% C.I. of Variance:					
Lower Limit				1.03423	1.09067
Upper Limit				1.64460	1.72876

Table 7. Distribution of the number of heterozygous loci for the Chinese population to determine independence across all nine loci performed by DNAtype™.

Chinese Population

Heterozygosity at Loci		Observed		Expected Freq. Based on	
x		Number	Freq.	Obs. Het.	Expected Het.
0		0	0.00000	0.00000	0.00000
1		0	0.00000	0.00001	0.00001
2		0	0.00000	0.00019	0.00020
3		1	0.02000	0.00203	0.00205
4		1	0.02000	0.01388	0.01338
5		2	0.04000	0.06133	0.05783
6		10	0.20000	0.17497	0.16514
7		16	0.32000	0.30962	0.30032
8		8	0.16000	0.30754	0.31541
9		12	0.24000	0.13043	0.14567
Mean			7.22000	7.22000	7.27657
Variance			1.97102	1.36360	1.37660
95% C.I. of Variance:					
Lower Limit				0.82810	0.83136
Upper Limit				1.89910	1.92185

Table 8. Distribution of the number of heterozygous loci for the Bangladesh population to determine independence across all nine loci performed by DNAtype™.

Bangladesh population

Heterozygosity at Loci x	Observed		Expected Freq. Based on	
	Number	Freq.	Obs. Het.	Expected Het.
0	0	0.00000	0.00000	0.00000
1	0	0.00000	0.00001	0.00001
2	0	0.00000	0.00017	0.00014
3	0	0.00000	0.00192	0.00158
4	0	0.00000	0.01340	0.01111
5	5	0.10000	0.06043	0.05133
6	9	0.18000	0.17509	0.15552
7	13	0.26000	0.31226	0.29775
8	16	0.32000	0.30868	0.32651
9	7	0.14000	0.12804	0.15605
Mean		7.22000	7.22000	7.34000
Variance		1.44041	1.34520	1.32507
95% C.I. of Variance:				
Lower Limit			0.81656	0.79862
Upper Limit			1.87384	1.85153

Table 9. Distribution of the number of heterozygous loci for the Vietnamese population to determine independence across all nine loci performed by DNAtype™.

Vietnamese population

Heterozygosity at Loci		Observed		Expected Freq. Based on	
x		Number	Freq.	Obs. Het.	Expected Het.
0		0	0.00000	0.00000	0.00000
1		0	0.00000	0.00001	0.00001
2		0	0.00000	0.00025	0.00016
3		0	0.00000	0.00256	0.00171
4		1	0.02000	0.01622	0.01172
5		4	0.08000	0.06696	0.05290
6		6	0.12000	0.18042	0.15738
7		18	0.36000	0.30672	0.29743
8		16	0.32000	0.29914	0.32390
9		5	0.10000	0.12772	0.15480
Mean			7.18000	7.18000	7.32748
Variance			1.33429	1.41960	1.34192
95% C.I. of Variance:					
Lower Limit				0.86343	0.80878
Upper Limit				1.97578	1.87505

Table 10. Distribution of the number of heterozygous loci for the Japanese population to determine independence across all nine loci performed by DNAtype™.

Japanese population

Heterozygosity at Loci		Observed		Expected Freq. Based on	
x		Number	Freq.	Obs. Het.	Expected Het.
0		0	0.00000	0.00000	0.00000
1		0	0.00000	0.00002	0.00002
2		0	0.00000	0.00039	0.00028
3		2	0.01163	0.00349	0.00270
4		1	0.00581	0.02003	0.01652
5		13	0.07558	0.07612	0.06680
6		33	0.19186	0.19134	0.17863
7		54	0.31395	0.30684	0.30450
8		51	0.29651	0.28496	0.30019
9		18	0.10465	0.11680	0.13036
Mean			7.09884	7.09884	7.18683
Variance			1.44631	1.48489	1.43209
95% C.I. of Variance:					
Lower Limit				1.17163	1.12819
Upper Limit				1.79816	1.73599

Table 11. Results from population differentiation tests performed by GenepopTM for the AmpF/STR ProfilerTM Plus 'blue' system.

Locus	Populations	Probability	S.E.
D3S1358	Afr-American & Chinese	0.042	0.002
D3S1358	Afr-American & Vietnamese	0.504	0.004
D3S1358	Afr-American & Bangladesh	0.086	0.002
D3S1358	Afr-American & Japanese	0.002	0.000
D3S1358	Chinese & Vietnamese	0.599	0.004
D3S1358	Chinese & Bangladesh	0.051	0.002
D3S1358	Chinese & Japanese	0.150	0.003
D3S1358	Vietnamese & Bangladesh	0.202	0.003
D3S1358	Vietnamese & Japanese	0.692	0.003
D3S1358	Bangladesh & Japanese	0.592	0.004

Locus	Populations	Probability	S.E.
vWA	Afr-American & Chinese	0.000	0.000
vWA	Afr-American & Vietnamese	0.000	0.000
vWA	Afr-American & Bangladesh	0.000	0.000
vWA	Afr-American & Japanese	0.000	0.000
vWA	Chinese & Vietnamese	0.258	0.003
vWA	Chinese & Bangladesh	0.165	0.003
vWA	Chinese & Japanese	0.164	0.003
vWA	Vietnamese & Bangladesh	0.523	0.003
vWA	Vietnamese & Japanese	0.712	0.003
vWA	Bangladesh & Japanese	0.987	0.000

Locus	Populations	Probability	S.E.
FGA	Afr-American & Chinese	0.075	0.002
FGA	Afr-American & Vietnamese	0.087	0.003
FGA	Afr-American & Bangladesh	0.234	0.004
FGA	Afr-American & Japanese	0.000	0.000
FGA	Chinese & Vietnamese	0.192	0.003
FGA	Chinese & Bangladesh	0.150	0.003
FGA	Chinese & Japanese	0.027	0.001
FGA	Vietnamese & Bangladesh	0.228	0.004
FGA	Vietnamese & Japanese	0.006	0.001
FGA	Bangladesh & Japanese	0.068	0.002

Table 12. Results from population differentiation tests performed by GenepopTM for the AmpF/STR ProfilerTM Plus 'green' system.

Locus	Populations	Probability	S.E.
-----	-----	-----	-----
D8S1179	Afr-American & Chinese	0.000	0.000
D8S1179	Afr-American & Vietnamese	0.000	0.000
D8S1179	Afr-American & Bangladesh	0.000	0.000
D8S1179	Afr-American & Japanese	0.000	0.000
D8S1179	Chinese & Vietnamese	0.279	0.003
D8S1179	Chinese & Bangladesh	0.527	0.003
D8S1179	Chinese & Japanese	0.046	0.002
D8S1179	Vietnamese & Bangladesh	0.523	0.004
D8S1179	Vietnamese & Japanese	0.189	0.003
D8S1179	Bangladesh & Japanese	0.084	0.002

Locus	Populations	Probability	S.E.
-----	-----	-----	-----
D21S11	Afr-American & Chinese	0.000	0.000
D21S11	Afr-American & Vietnamese	0.000	0.000
D21S11	Afr-American & Bangladesh	0.001	0.000
D21S11	Afr-American & Japanese	0.000	0.000
D21S11	Chinese & Vietnamese	0.594	0.004
D21S11	Chinese & Bangladesh	0.039	0.001
D21S11	Chinese & Japanese	0.068	0.002
D21S11	Vietnamese & Bangladesh	0.004	0.000
D21S11	Vietnamese & Japanese	0.033	0.001
D21S11	Bangladesh & Japanese	0.000	0.000

Locus	Populations	Probability	S.E.
-----	-----	-----	-----
D18S51	Afr-American & Chinese	0.000	0.000
D18S51	Afr-American & Vietnamese	0.000	0.000
D18S51	Afr-American & Bangladesh	0.000	0.000
D18S51	Afr-American & Japanese	0.000	0.000
D18S51	Chinese & Vietnamese	0.289	0.004
D18S51	Chinese & Bangladesh	0.395	0.004
D18S51	Chinese & Japanese	0.699	0.004
D18S51	Vietnamese & Bangladesh	0.495	0.004
D18S51	Vietnamese & Japanese	0.892	0.002
D18S51	Bangladesh & Japanese	0.170	0.004

Table 13. Results from population differentiation tests performed by Genepop™ for the AmpF/STR Profiler™ Plus 'yellow' system.

Locus	Populations	Probability	S.E.
-----	-----	-----	-----
D5S818	Afr-American & Chinese	0.000	0.000
D5S818	Afr-American & Vietnamese	0.000	0.000
D5S818	Afr-American & Bangladesh	0.001	0.000
D5S818	Afr-American & Japanese	0.000	0.000
D5S818	Chinese & Vietnamese	0.967	0.001
D5S818	Chinese & Bangladesh	0.258	0.004
D5S818	Chinese & Japanese	0.035	0.002
D5S818	Vietnamese & Bangladesh	0.150	0.003
D5S818	Vietnamese & Japanese	0.150	0.003
D5S818	Bangladesh & Japanese	0.523	0.004

Locus	Populations	Probability	S.E.
-----	-----	-----	-----
D13S317	Afr-American & Chinese	0.000	0.000
D13S317	Afr-American & Vietnamese	0.000	0.000
D13S317	Afr-American & Bangladesh	0.000	0.000
D13S317	Afr-American & Japanese	0.000	0.000
D13S317	Chinese & Vietnamese	0.327	0.003
D13S317	Chinese & Bangladesh	0.663	0.003
D13S317	Chinese & Japanese	0.493	0.004
D13S317	Vietnamese & Bangladesh	0.257	0.003
D13S317	Vietnamese & Japanese	0.459	0.004
D13S317	Bangladesh & Japanese	0.558	0.004

Locus	Populations	Probability	S.E.
-----	-----	-----	-----
D7S820	Afr-American & Chinese	0.000	0.000
D7S820	Afr-American & Vietnamese	0.000	0.000
D7S820	Afr-American & Bangladesh	0.005	0.000
D7S820	Afr-American & Japanese	0.000	0.000
D7S820	Chinese & Vietnamese	0.528	0.004
D7S820	Chinese & Bangladesh	0.062	0.002
D7S820	Chinese & Japanese	0.917	0.002
D7S820	Vietnamese & Bangladesh	0.319	0.004
D7S820	Vietnamese & Japanese	0.050	0.002
D7S820	Bangladesh & Japanese	0.002	0.000

D. Comparison of alleles across all populations

(i) Overview

The frequencies and distributions of alleles in the five populations for the nine loci are shown in Figures 6 through 14. Across all populations, the greatest number of allelic variants occurred in FGA and D21S11, each with 20 alleles. Ten D3S1358 alleles, ten vWA alleles, 11 D8S1179 alleles, 18 D18S51 alleles, ten D5S818 alleles and 11 D7S820 alleles were detected across all populations. The least number of alleles observed, across all populations was within the D13S317 locus, with eight alleles. The total number of alleles in common among all populations for each locus are as follows: five alleles for D3S1358, seven alleles for vWA, nine alleles for FGA, eight alleles for D8S1179, seven alleles for D21S11, ten alleles for D18S51, five alleles for D5S818, seven alleles for D13S317 and seven alleles for D7S820.

Two-base variants were represented in D3S1358, FGA, D21S11, D18S51. A three-base variant (30.3) and a one-base variant (33.1) were observed in D21S11. In addition, a three-base variant (9.3) and two one-base variants (8.1, 9.1) were represented in D7S820. The remaining systems contained alleles which differ consistently by four base increments. The minimum number of iterations of the tetranucleotide core repeat was seven, for D5S818, D13S317 and D7S820 and the maximum number of iterations was 36 for D21S11. Between populations, the greatest number of alleles, across all loci combined, occurred in the African-American population (110 alleles) and the Japanese population (92 alleles).

Figure 6. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ D3S1358 locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

D3S1358

- AA (N=151 individuals)
- CH (N=50 individuals)
- VT (N=50 individuals)
- BG (N=50 individuals)
- JP (N=172 individuals)

Figure 7. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ vWA locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

vWA

- AA (N=151 individuals)
- CH (N=50 individuals)
- VT (N=50 individuals)
- BG (N=50 individuals)
- JP (N=172 individuals)

Figure 8. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ FGA locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

FGA

- AA (N=151 individuals)
- CH (N=50 individuals)
- VT (N=50 individuals)
- BG (N=50 individuals)
- JP (N=172 individuals)

Figure 9. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ D8S1179 locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

D8S1179

- AA (N=151 individuals)
- CH (N=50 individuals)
- VT (N=50 individuals)
- BG (N=50 individuals)
- JP (N=172 individuals)

Figure 10. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ D21S11 locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

D21S11

Figure 11. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ D18S51 locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

D18S51

Figure 12. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ D5S818 locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

D5S818

Figure 13. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ D13S317 locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

D13S317

Figure 14. Frequencies and distributions of alleles of the AmpF/STR Profiler Plus™ D7S820 locus in five population groups. Alleles are numbered according to the number of iterations of the tetranucleotide core repeat (allele designation). AA= African-American, CH= Chinese, VT= Vietnamese, BG= Bangladesh and JP= Japanese. The total number of individuals in each population group is indicated in the legend.

D7S820

- AA (N=151 individuals)
- CH (N=50 individuals)
- VT (N=50 individuals)
- BG (N=50 individuals)
- JP (N=172 individuals)

(ii) D3S1358

The number of different alleles detected at D3S1358 for the African-American, Chinese, Vietnamese, Bangladesh and Japanese populations were nine, seven, six, five and seven, respectively. At this locus, three alleles, 15, 16 and 17, represented greater than 80% of the total allelic variation for each of the five populations. Alleles with a low number of core repeats, such as 11, 12, 13 and 14, were observed very infrequently, in most cases occurring in less than three percent of the populations. A two-base variant (15.2) was detected in the African-American population at a frequency of less than one percent and was not observed in the other four populations. Allele 11 was also observed solely in the African-American population occurring with a frequency of less than one percent. The largest allele seen at D3S1358 was 19, which was detected only in the Chinese and Japanese populations, with a frequency of less than one percent for each group.

(iii). vWA

At the vWA locus, the following number of alleles were detected for the African-American, Chinese, Vietnamese, Bangladesh and Japanese populations, respectively: ten, seven, seven, seven and eight. The number of iterations of the core repeat ranged from 11 to 21 sequentially, except for allele 12 which was not detected in any of the five populations. Alleles with one, two or three base additions to the tetranucleotide core repeat were not identified. The most common alleles within the vWA locus for all five populations were 14, 16, 17 and 18, except for the African-American population in which allele 14 was underrepresented with only a seven percent frequency, compared to greater than 18% in the

other four populations. Allele 15 occurred quite frequently in the African-American population, with a frequency greater than 20%. In contrast, the other four populations each exhibited frequencies of less than six percent for allele 15. Alleles 11 and 13 were detected only in the African-American population with a frequency of less than one percent for each allele. Allele 21 was identified in the African-American and Japanese populations only, occurring at a frequency of less than one percent for each population.

(iv). FGA

A total number of 15, 12, 15, 11 and 14 alleles were detected in the African-American, Chinese, Vietnamese, Bangladesh and Japanese populations, respectively. The number of iterations of the core repeat sequence ranged from 17 to 29, with several two-base variants observed in the lower to mid-range of the series. In FGA, three alleles (22, 23 and 24) represented greater than 50% of the frequency of alleles for all five populations, whereas the frequencies of alleles at the low and high end of the range for FGA were quite low.

Across all five populations, seven two-base variants were found. Four of these variant alleles (21.2, 23.2, 24.2 and 25.2) were present in the Vietnamese population, occurring with a frequency of less than two percent for each allele. The other four populations had only one or two of these two-base variants represented in the distribution, with a frequency less than two percent for each allele. Alleles 18.2 and 19.2 were observed only in the African-American population, allele 22.2 was detected in only the Bangladesh population and allele 24.2 was present only in the Vietnamese population.

(v). D8S1179

Alleles at the D8S1179 locus ranged sequentially from eight to eighteen iterations of the core sequence. The number of alleles detected in the African-American, Chinese, Vietnamese, Bangladesh and Japanese populations totaled ten, eight, eight, nine and ten, respectively. The frequency of alleles in D8S1179 appeared to be quite evenly distributed across the mid-range of allele sizes, with a low representation of alleles at the opposite ends of the series. At the low end of the series, allele 8 was present only in the African-American and Bangladesh population groups, each with a frequency of about one percent. Allele 9 was detected at a frequency of less than one percent for the African-American and Japanese populations and was not detected in the other three population groups. At the high end of the series, allele 18 was detected only in the Japanese population with a frequency of less than one percent. In the mid-range of alleles at D8S1179, alleles 10 and 11 were each represented with frequencies between 10% and 20% for the Chinese, Vietnamese, Bangladesh and Japanese populations. For the African-American population, allele 10 was observed with a frequency of approximately two percent and allele 11 had a frequency of less than six percent. Allele 14 occurred at a frequency of greater than 32% in the African-American population with the remaining four populations exhibiting frequencies of less than 22% for this allele.

(vi). D21S11

The number of iterations of the core sequence at D21S11 ranged from 26 to 36, with several variants observed in the mid-range of allele sizes. The total number of alleles

detected in the African-American, Chinese, Vietnamese, Bangladesh and Japanese populations were 17, 11, 13, 9 and 13, respectively. Alleles in the mid-range of the series were observed most frequently, primarily alleles 28, 29, 30 and 32.2. Allele 28 was frequent among the African-American (31%) and Bangladesh (17%) populations but occurred at a frequency of less than eight percent in the Chinese, Vietnamese and Japanese populations. Allele 29 was observed frequently in each of the five populations with frequencies between 18% (African-American population) and 27% (Chinese population). Allele 30 was quite frequent in the Japanese population with a frequency of greater than 36%. However, in the African-American population, allele 30 was observed in only nine percent of the population. The remaining three populations had a frequency of between 16% and 26% for allele 30.

Several alleles that deviated from the tetranucleotide core sequence were seen. A one-base variant (33.1) was detected only in the African-American population, with a frequency of less than one percent. Seven two-base variants were found. They included 1) allele 28.2, which was present only in the Japanese population with a frequency of less than one percent, 2) allele 29.2, which was present in both the Vietnamese (two percent) and Japanese populations (less than one percent), 3) allele 30.2 which was seen in all populations except the Japanese population, with a frequency of less than four percent for each group, 4) allele 31.2 which was observed in all five populations, with a frequency of less than 11%, 5) allele 32.2 which was detected in all five populations, with a frequency ranging between seven percent and 18%, 6) allele 33.2 which was seen in all five population groups with a frequency less than eight percent and 7) allele 34.2 which was found only in the African-American, Vietnamese and Japanese populations, with a frequency of less than one percent

for each group. Finally, a three-base variant (30.3) was observed at a frequency of one percent in only the Chinese population.

Alleles at the low and high ends of the D21S11 series were found to occur very infrequently in all five populations groups. For example, at the low end of the allele size range, allele 26 was only observed in the African-American populations with a frequency of less than one percent and allele 27 was observed in the African-American, Vietnamese and Bangladesh populations with frequencies of less than four percent. At the high end of the series, alleles 32, 33, 34, 35 and 36 were not detected in the Bangladesh population at all. Alleles 34, 35 and 36 were not found in the Chinese and Vietnamese groups. In the Japanese population, no STR profiles generated showed alleles 35 and 36. The only population group that had a representation of all the alleles at the high end of the series (alleles 32, 33, 34, 35 and 36) was the African-American population; however, the frequency was less than four percent for each one.

(vii) D18S51

The total number of alleles observed at the D18S51 locus for the African-American, Chinese, Vietnamese, Bangladesh and Japanese populations were 15,12,12,14 and 16, respectively. The number of iterations of the core sequence ranged between 10 and 25 with two two-base variants detected. The most frequently observed alleles were found in the mid-range of the allele size series (alleles 13, 14, 15 and 16). In the Chinese, Bangladesh, Vietnamese and Japanese populations, alleles 13, 14, 15 and 16 represented a combined frequency of approximately 70% or greater. Allele 14 had a frequency of three percent in the

African-American population, whereas the frequency of allele 14 in the other four populations was greater than 11%. Allele 15 in the Chinese population was represented by a frequency of greater than 30%, with the remaining populations having frequencies of 20%. In the African-American population, the most frequently occurring alleles were 15, 16, 17 and 18, representing greater than 60% of the total number of alleles for D18S51.

Alleles at the lower and upper ends of the series at D18S51 were represented with a lower frequency than those in the mid-range. At the smaller size range, alleles 10 and 11 were not detected in the Chinese and Vietnamese populations and were present in the other three populations, but at a frequency of less than four percent for each allele in each population. At the upper end of the series, for those populations which contained alleles 22, 23, 24 and 25, the frequency of occurrence was less than two percent in all instances. In the African-American population, alleles 22, 24 and 25 were not observed. The Chinese population was void of alleles 22 and 25, the Vietnamese population did not have any representation of alleles 23 and 24 and the Bangladesh population did not contain alleles 24 and 25. Finally, the Japanese population did contain alleles 22, 23, 24 and 25.

Two two-base variants at D18S51 were isolated in the mid-range of the series (13.2 and 14.2). Both of these variants were observed in the African-American population, with a frequency of approximately one percent for each allele. These variants were not detected in any of the four remaining populations.

(viii). D5S818

The number of iterations of the core sequence at D5S818 ranged sequentially from

seven to 17, with the exception of allele 16, which was not detected in any of the four populations. The total number of alleles observed were eight, seven, six, six and eight for the African-American, Chinese, Vietnamese, Bangladesh and Japanese populations, respectively. At D5S818, four alleles (10, 11, 12 and 13) from the mid-range of the series, represented greater than 88% of the combined frequency for each of the five populations. For allele 10, the frequency was approximately seven percent in the African-American population, whereas the frequency in the rest of the populations was greater than 14%.

The frequencies of alleles at the lower and upper ends of the series for D5S818 were low. At the lower end, allele 7 was not detected in the Bangladesh and Japanese populations and was present in the three remaining populations at a frequency of less than four percent. Allele 8 in the African-American population was present at a frequency of approximately eight percent and was not detected in any other populations, except for the Japanese group where it was observed at a frequency of less than one percent. At the upper end of the series, alleles 14, 15 and 17 were observed in some of the populations with a frequency of less than two percent in all instances. The African-American and Bangladesh populations did not contain alleles 15 or 17, the Chinese population did not have the alleles 14 and 15 present, the Vietnamese population was void of alleles 14, 15 and 17 and finally the Japanese population did not have any representation of allele 17.

(ix). D13S317

The total number of alleles present at the D13S317 locus for the African-American, Chinese, Vietnamese, Bangladesh and Japanese populations included seven, seven, seven

eight and eight, respectively. The number of iterations of the core sequence ranged from seven to 14, consecutively. The D13S317 locus was represented primarily by alleles 8,9,10,11 and 12 for the Chinese, Vietnamese, Bangladesh and Japanese populations. In the African-American population, alleles 11 and 12 represented greater than 75% of the allelic composition at D13S317. Alleles 8, 9 and 10 occurred very infrequently in the African-American population, with frequencies of less than four percent for each of these alleles. Allele 7 was observed only in the Bangladesh and Japanese populations, with a frequency of less than two percent for each population.

(x). D7S820

The number of iterations of the core sequence at D7S820 ranged from seven to 14, consecutively, with the presence of three variants at the lower end of the range. The total number of alleles in each population were nine (African-American), seven (Chinese), nine (Vietnamese), eight (Bangladesh) and eight (Japanese). The D7S820 locus had four alleles (8, 10, 11 and 12) that together provided a frequency of greater than 80% for each of the populations. In the African-American population, allele 12 had a frequency of approximately eight percent, whereas the rest of the populations had frequencies of greater than 18%. The frequencies of alleles at the lower and upper end of the range for D7S820 were low. Allele 7 in the lower range was present in all five populations, but at a frequency of less than three percent for each population. At the upper end of the spectrum, allele 14 was present only in the African-American, Vietnamese and Japanese populations, all with frequencies less than

two percent.

Two one-base variants were identified at the D7S820 locus. An 8.1 allele was detected in only the Bangladesh population, with a frequency of one percent. The 9.1 allele was seen in the Vietnamese population only, with a frequency of one percent. One three-base variant, 9.3, was observed in the African-American populations, with a frequency of less than one percent.

E. Examination of heterozygote excess

Genotypic matrices of allelic combinations for each locus within each population are represented in Tables 14 through 22, along with the observed and expected number of heterozygotes. Across all five populations, 21 out of 45 matrices showed an excess of heterozygotes. Within the African-American population, 5 of the 9 loci (D3S1358, D21S11, D18S51, D13S317 and D7S820) showed an excess of heterozygotes. In the Chinese population, 6 of the 9 loci (FGA, D8S1179, D21S11, D18S51, D5S818 and D13S317) had an excess of heterozygotes. For the Japanese population, 3 of the 9 loci showed an excess of heterozygotes (D3S1358, FGA and D13S317). An excess of heterozygotes in the Vietnamese population was observed for 4 of the 9 loci (D3S1358, FGA, D18S51 and D13S317). The Bangladesh population contained an excess of heterozygotes in 3 of the 9 loci (vWA, FGA and D7S820). Examining each locus across all populations, FGA and D13S317 had the greatest excess of heterozygotes, occurring in 4 out of 5 populations. The least excess of heterozygotes across loci, each occurring in 1 of the 5 populations were vWA, D8S1179 and D5S818.

Table 14. Genotypic matrices: D3S1358. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

D3S1358

African-American										total number
	11	12	13	14	15	15.2	16	17	18	of alleles
11	0									2
12	0	0								2
13	0	0	0							2
14	1	0	0	0						28
15	1	2	1	11	7					90
15.2	0	0	0	0	1	0				2
16	0	0	0	10	28	1	16			90
17	0	0	1	5	27	0	17	4		65
18	0	0	0	1	7	0	4	7	1	21
										302

N= 151 individuals

Heterozygotes observed: 123

Heterozygotes expected: 115.51

Chinese										total number
	13	14	15	16	17	18	19			of alleles
13	0									2
14	0	0								1
15	1	0	5							30
16	1	1	10	8						38
17	0	0	6	7	5					23
18	0	0	3	2	0	0				5
19	0	0	0	1	0	0	0			1
										100

N= 50 individuals

Heterozygotes observed: 32

Heterozygotes expected: 35.84

Japanese								total number
	13	14	15	16	17	18	19	of alleles
13	0							1
14	0	0						10
15	1	4	25					133
16	0	3	36	18				103
17	0	2	33	24	3			73
18	0	1	8	4	8	1		23
19	0	0	1	0	0	0	0	1
								344

N= 172 individuals

Heterozygotes observed: 125

Heterozygotes expected: 122.56

Vietnamese							total number
	12	14	15	16	17	18	of alleles
12	0						1
14	0	0					3
15	0	1	7				35
16	1	1	9	3			31
17	0	1	8	12	2		25
18	0	0	3	2	0	0	5
							100

N= 50 individuals

Heterozygotes observed: 38

Heterozygotes expected: 36.13

Bangladesh						total number
	14	15	16	17	18	of alleles
14	0					3
15	1	11				44
16	1	11	6			29
17	1	5	3	2		14
18	0	5	2	1	1	10
						100

N= 50 individuals

Heterozygotes observed: 30

Heterozygotes expected: 34.94

Table 15. Genotypic matrices: vWA. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

vWA

African-American											total number of alleles
11	13	14	15	16	17	18	19	20	21		
11	0										2
13	0	0									7
14	0	0	2								21
15	1	3	4	5							63
16	1	3	2	21	11						83
17	0	1	6	12	12	8					61
18	0	0	1	6	14	6	3				36
19	0	0	4	6	5	6	3	0			24
20	0	0	0	0	3	1	0	0	0		4
21	0	0	0	0	0	1	0	0	0	0	1
											302

N= 151 individuals
Heterozygotes observed: 122
Heterozygotes expected: 123.33

Chinese								total number of alleles
14	15	16	17	18	19	20		
14	6							30
15	1	0						3
16	5	0	4					21
17	6	1	2	1				18
18	5	1	3	5	2			19
19	0	0	3	2	1	1		8
20	1	0	0	0	0	0	0	1
								100

N= 50 individuals
Heterozygotes observed: 36
Heterozygotes expected: 39.90

Japanese								total number of alleles
14	15	16	17	18	19	20	21	
14	9							64
15	0	0						12
16	7	3	8					58
17	23	5	14	14				102
18	12	4	12	20	6			71
19	2	0	5	12	9	1		31
20	2	0	1	0	2	0	0	5
21	0	0	0	0	0	1	0	1
								344

N= 172 individuals
Heterozygotes observed: 134
Heterozygotes expected: 137.46

Vietnamese								total number of alleles
14	15	16	17	18	19	20		
14	3							22
15	0	0						1
16	1	0	2					14
17	3	0	5	7				29
18	11	1	2	4	1			24
19	1	0	2	2	2	0		7
20	0	0	0	1	2	0	0	3
								100

N= 50 individuals
Heterozygotes observed: 37
Heterozygotes expected: 39.62

Bangladesh							total number of alleles
14	15	16	17	18	19	20	
14	0						16
15	1	0					5
16	4	0	1				18
17	3	3	8	4			31
18	6	1	3	7	1		21
19	1	0	1	2	2	1	8
20	1	0	0	0	0	0	1
							100

N= 50 individuals
Heterozygotes observed: 43
Heterozygotes expected: 41.09

Table 16. Genotypic matrices: FGA. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

FGA

African-American

	17	18	18.2	19	19.2	20	21	22	23	24	25	26	27	28	29	total number of alleles
17	0															1
18	0	0														2
18.2	0	0	0													5
19	0	0	0	0												19
19.2	0	0	0	0	1	0										1
20	0	0	0	0	1	0	1									15
21	1	0	0	0	2	0	0	2								36
22	0	1	2	7	0	3	8	7								65
23	0	1	1	0	0	3	6	10	8							50
24	0	0	1	2	0	4	9	8	9	1						40
25	0	0	0	2	0	1	1	5	2	4	2					22
26	0	0	1	2	0	1	3	5	0	6	1	2				24
27	0	0	0	1	0	0	0	0	2	4	1	1	0			9
28	0	0	0	1	0	0	0	2	0	0	1	0	0	0		4
29	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
																302

N= 151 individuals

Heterozygotes observed: 128

Heterozygotes expected: 131.36

Chinese

	17	18	19	20	21	21.2	22	23	24	25	25.2	26	total number of alleles
17	0												1
18	0	0											4
19	1	0	0										9
20	0	0	0	0									1
21	0	0	1	0	0								11
21.2	0	0	0	0	0	0							1
22	0	0	0	0	4	0	1						20
23	0	3	1	1	1	0	5	3					21
24	0	0	3	0	3	1	3	3	3				19
25	0	0	1	0	1	0	4	1	0	0			7
25.2	0	0	1	0	0	0	0	0	0	0	0		1
26	0	1	1	0	1	0	2	0	0	0	0	0	5
													100

N= 50 individuals

Heterozygotes observed: 43

Heterozygotes expected: 42.94

Japanese

	17	18	19	20	21	21.2	22	23	23.2	24	25	26	27	28	total number of alleles
17	0														1
18	0	0													12
19	0	1	1												20
20	0	1	1	3											33
21	0	4	1	8	2										51
21.2	0	0	0	0	0	0									1
22	0	2	6	4	6	1	4								61
23	0	1	5	8	17	0	17	10							98
23.2	0	0	0	0	0	0	1	0	0						1
24	0	1	0	2	8	0	9	12	0	2					40
25	0	1	3	2	2	0	2	8	0	3	2				25
26	1	1	1	1	1	0	3	0	0	1	0	0			9
27	0	0	0	0	0	0	1	0	0	0	0	0	0		1
28	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
															344

N= 172 individuals

Heterozygotes observed: 148

Heterozygotes expected: 146.25

FGA

Vietnamese																total number
	17	18	19	20	21	21.2	22	23	23.2	24	24.2	25	25.2	26	27	of alleles
17	0															1
18	1	0														2
19	0	0	0													3
20	0	0	1	0												4
21	0	0	2	1	0											5
21.2	0	0	0	0	0	0										1
22	0	0	1	3	2	0	4									27
23	0	0	4	0	1	0	5	0								15
23.2	0	1	0	0	0	1	0	0	0							2
24	0	0	1	2	3	0	0	2	0	0						10
24.2	0	0	0	0	0	0	1	0	0	0	0					1
25	0	0	0	1	0	0	2	1	0	1	0	0				6
25.2	0	0	0	0	0	0	1	0	0	0	0	0	0			1
26	0	0	0	0	0	0	2	1	0	1	0	1	0	0		5
27	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	3
																100

N= 50 individuals
Heterozygotes observed: 46
Heterozygotes expected: 43.63

Bangladesh												total number of alleles	
	17	18	20	21	22	22.2	23	24	25	26	28		
17	0												2
18	0	1											3
20	2	0	0										10
21	0	1	0	0									11
22	0	1	0	0	2								15
22.2	0	0	0	1	0	0							1
23	0	2	3	2	5	0	1						19
24	0	2	2	4	3	0	2	1					18
25	0	0	3	2	2	0	2	0	0				9
26	0	1	0	1	0	0	1	2	0	0			5
28	0	0	0	0	0	0	0	1	0	0	0		1
													100

N= 50 individuals
Heterozygotes observed: 45
Heterozygotes expected: 43.82

Table 17. Genotypic matrices: D8S1179. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

D8S1179

African-American											total number of alleles
8	9	10	11	12	13	14	15	16	17		
8	0										2
9	0	0									1
10	0	0	0								7
11	0	0	2	0							17
12	0	0	1	3	2						29
13	0	0	0	1	2	9					54
14	0	1	3	6	9	18	18				97
15	1	0	1	3	7	9	21	9			69
16	1	0	0	1	2	6	2	8	1		22
17	0	0	0	1	1	0	1	1	0	0	4
											302

N= 151 individuals

Heterozygotes observed: 112

Heterozygotes expected: 120.32

Chinese											total number of alleles
10	11	12	13	14	15	16	17				
10	2										21
11	3	2									16
12	3	1	0								7
13	6	2	0	1							17
14	1	2	1	2	0						13
15	2	3	1	4	4	2					18
16	1	1	1	1	2	0	0				6
17	1	0	0	0	1	0	0	0			2
											100

N= 50 individuals

Heterozygotes observed: 43

Heterozygotes expected: 42.63

Japanese											total number of alleles
9	10	11	12	13	14	15	16	17	18		
9	0										3
10	0	3									36
11	0	6	6								46
12	0	0	6	3							37
13	1	10	7	14	11						84
14	2	5	10	5	14	12					75
15	0	8	3	5	12	9	1				41
16	0	3	2	1	4	3	2	0			16
17	0	0	0	0	0	2	0	1	0		3
18	0	0	0	0	0	1	0	0	0	0	1
											344

N= 172 individuals

Heterozygotes observed: 136

Heterozygotes expected: 143.98

Vietnamese											total number of alleles
10	11	12	13	14	15	16	17				
10	0										11
11	4	1									17
12	3	3	1								16
13	1	2	2	3							17
14	1	2	1	1	3						14
15	0	4	3	4	0	0					14
16	2	0	2	0	3	3	0				10
17	0	0	0	1	0	0	0	0			1
											100

N= 50 individuals

Heterozygotes observed: 42

Heterozygotes expected: 43.19

Bangladesh											total number of alleles
8	10	11	12	13	14	15	16	17			
8	0										1
10	0	0									19
11	0	4	0								8
12	0	1	1	1							13
13	0	2	2	1	3						16
14	0	6	1	1	3	1					16
15	1	4	0	2	1	3	2				15
16	0	1	0	5	3	0	0	0			9
17	0	1	0	0	0	0	0	0	0	0	1
											100

N= 50 individuals

Heterozygotes observed: 43

Heterozygotes expected 43.02

Table 18. Genotypic matrices: D21S11. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

D21S11

African-American																total number of alleles	
26	27	28	29	30	30.2	31	31.2	32	32.2	33	33.1	33.2	34	34.2	35	36	
26	0																2
27	1	0															13
28	1	4	11														92
29	0	3	17	3													53
30	0	2	5	7	1												28
30.2	0	1	4	3	2	0											11
31	0	1	7	10	4	0	1										29
31.2	0	0	8	1	3	0	0	1									18
32	0	0	3	0	0	0	2	1	0								7
32.2	0	0	11	3	1	0	2	1	0	0							21
33	0	0	1	0	0	0	0	0	0	0	0						2
33.1	0	1	0	0	0	0	0	0	0	0	0	0					1
33.2	0	0	3	1	0	0	1	1	0	1	0	0	0				7
34	0	0	0	0	0	0	0	0	1	0	0	0	0	0			1
34.2	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0		3
35	0	0	4	2	1	1	0	1	1	0	0	0	0	0	0		11
36	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	3
																	302

N= 151 individuals

Heterozygotes observed: 134

Heterozygotes expected: 127.91

Chinese												total number of alleles
28	29	30	30.2	30.3	31	31.2	32	32.2	33	33.2		
28	0											8
29	1	3										27
30	2	10	2									23
30.2	0	0	0	0								1
30.3	0	0	1	0	0							1
31	0	4	1	0	0	0						7
31.2	3	2	1	0	0	1	0					10
32	0	1	1	0	0	0	0	0				2
32.2	2	2	3	1	0	1	3	0	2			18
33	0	0	0	0	0	0	0	0	1	0		1
33.2	0	1	0	0	0	0	0	0	1	0	0	2
												100

N= 50 individuals

Heterozygotes observed: 43

Heterozygotes expected: 41.38

D21S11

Japanese														total number
	28	28.2	29	29.2	30	31	31.2	32	32.2	33	33.2	34	34.2	of alleles
28	0													16
28.2	0	0												2
29	0	1	10											74
29.2	0	0	0	0										1
30	8	1	19	1	28									125
31	3	0	10	0	9	1								33
31.2	0	0	8	0	7	1	0							22
32	1	0	1	0	2	0	1	0						8
32.2	2	0	14	0	16	3	2	2	1					43
33	0	0	0	0	1	0	0	0	0	0				1
33.2	2	0	1	0	5	5	3	0	1	0	0			17
34	0	0	0	0	0	0	0	0	1	0	0	0		1
34.2	0	0	0	0	0	0	0	1	0	0	0	0	0	1
														344

N= 172 individuals

Heterozygotes observed: 132

Heterozygotes expected: 135.85

Vietnamese														total number
	27	28	29	29.2	30	30.2	31	31.2	32	32.2	33	33.2	34.2	of alleles
27	0													2
28	0	0												4
29	0	2	5											26
29.2	0	0	0	0										2
30	1	2	3	2	4									26
30.2	0	0	0	0	1	0								1
31	0	0	3	0	3	0	0							6
31.2	0	0	2	0	0	0	0	0						6
32	1	0	1	0	2	0	0	1	0					5
32.2	0	0	3	0	3	0	0	1	0	3				14
33	0	0	1	0	0	0	0	0	0	1	0			2
33.2	0	0	0	0	1	0	0	2	0	0	0	1		5
34.2	0	0	1	0	0	0	0	0	0	0	0	0	0	1
														100

N= 50 individuals

Heterozygotes observed: 37

Heterozygotes expected: 41.92

Bangladesh										total number of alleles
	27	28	29	30	30.2	31	31.2	32.2	33.2	
27	0									2
28	0	2								17
29	0	6	5							28
30	2	2	3	0						16
30.2	0	0	1	2	0					5
31	0	0	0	1	0	0				3
31.2	0	3	2	2	1	1	0			11
32.2	0	1	2	3	0	0	2	1		12
33.2	0	1	2	1	1	1	0	2	0	8
										100

N= 50 individuals

Heterozygotes observed: 42

Heterozygotes expected: 42.48

Table 19. Genotypic matrices: D18S51. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

D18S51

African-American														total number of alleles	
10	11	12	13	13.2	14	14.2	15	16	17	18	19	20	21	23	
10	0														1
11	0	0													4
12	1	0	0												12
13	0	0	0	0											10
13.2	0	0	0	0	0										3
14	0	1	1	1	0	0									24
14.2	0	0	0	0	0	0	0								2
15	0	1	2	1	0	5	1	5							56
16	0	0	2	1	1	2	0	13	5						52
17	0	2	4	2	0	5	0	9	4	3					48
18	0	0	2	1	1	6	0	5	9	4	1				35
19	0	0	0	3	0	1	0	3	5	6	2	0			25
20	0	0	0	0	1	1	0	3	5	5	3	3	0		21
21	0	0	0	1	0	1	1	3	0	1	0	1	0	0	8
23	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
															302

N= 151 individuals

Heterozygotes observed: 137

Heterozygotes expected: 132.65

Chinese												total number of alleles	
12	13	14	15	16	17	18	19	20	21	23	24		
12	0												2
13	0	0											14
14	0	7	1										16
15	1	6	4	5									31
16	0	1	3	2	0								11
17	0	0	1	1	3	0							7
18	0	0	0	2	0	1	0						5
19	0	0	0	2	1	0	1	0					4
20	1	0	0	1	0	0	0	0	0				2
21	0	0	1	1	0	0	1	0	0	0			3
23	0	0	0	0	1	1	0	0	0	0	0		2
24	0	0	0	1	0	0	0	0	0	0	0	0	1
													100

N= 50 individuals

Heterozygotes observed: 44

Heterozygotes expected: 41.85

D16S51

Japanese																	total number	
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	of alleles	
10	0																1	
11	0	0															2	
12	0	0	0														17	
13	0	0	4	9													68	
14	0	0	5	15	9												74	
15	0	2	3	12	14	7											71	
16	1	0	1	9	8	7	3										42	
17	0	0	2	0	2	8	2	0									18	
18	0	0	2	6	3	2	2	0	0								16	
19	0	0	0	1	1	5	2	0	1	0							11	
20	0	0	0	2	4	1	1	1	0	0	0						9	
21	0	0	0	0	1	2	1	1	0	1	0	0					6	
22	0	0	0	0	0	0	1	0	0	0	0	0	0				1	
23	0	0	0	0	2	0	1	2	0	0	0	0	0	0			5	
24	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		1	
25	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	
																	344	

N= 172 individuals

Heterozygotes observed: 144

Heterozygotes expected: 146.19

Vietnamese											total number				
	12	13	14	15	16	17	18	19	20	21	22	25	of alleles		
12	0												7		
13	0	3											19		
14	3	2	0										21		
15	1	4	6	2									19		
16	0	3	5	3	2								16		
17	1	1	2	0	0	0							4		
18	2	0	1	0	1	0	0						5		
19	0	1	0	0	0	0	0	0					2		
20	0	1	0	0	0	0	0	0	0				1		
21	0	1	1	1	0	0	0	0	0	0			3		
22	0	0	0	0	0	0	0	1	0	0	0		1		
25	0	0	1	0	0	0	1	0	0	0	0	0	2		
															100

N= 50 individuals

Heterozygotes observed: 43

Heterozygotes expected: 42.79

D18S51

Bangladesh														total number of alleles	
	10	11	12	13	14	15	16	17	18	19	20	21	22	23	
10	0														1
11	0	0													4
12	0	1	0												4
13	0	0	1	0											11
14	0	0	0	5	2										21
15	0	1	2	2	3	3									19
16	0	0	0	1	4	5	2								18
17	0	0	0	1	1	0	3	0							7
18	0	0	0	1	1	0	0	1	0						4
19	1	1	0	0	0	0	1	1	0	0					5
20	0	1	0	0	0	0	0	0	0	0	0				1
21	0	0	0	0	2	0	0	0	0	0	0	0			2
22	0	0	0	0	0	0	0	0	1	1	0	0	0		2
23	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
															100

N= 50 individuals

Heterozygotes observed: 43

Heterozygotes expected: 43.54

Table 20. Genotypic matrices: D5S818. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

D5S818

African-American										total number
7	8	9	10	11	12	13	14			of alleles
7	0									1
8	0	2								24
9	0	0	0							7
10	0	1	1	0						20
11	0	5	2	6	10					75
12	1	9	2	3	28	14				92
13	0	5	2	9	16	22	11			79
14	0	0	0	0	0	1	3	0		4
										302

N= 151 individuals

Heterozygotes observed: 114

Heterozygotes expected: 116

Chinese										total number
7	8	9	10	11	12	13	17			of alleles
7	0									4
9	0	0								7
10	1	1	1							18
11	1	1	6	4						29
12	1	1	5	8	1					22
13	1	4	3	5	4	1				19
17	0	0	0	0	1	0	0			1
										100

N= 50 individuals

Heterozygotes observed: 43

Heterozygotes expected: 40.02

Japanese										total number
8	9	10	11	12	13	14	15			of alleles
8	0									2
9	0	0								23
10	0	2	9							75
11	2	6	23	18						109
12	0	11	15	21	9					74
13	0	4	14	17	9	5				54
14	0	0	3	3	0	0	0			6
15	0	0	0	1	0	0	0	0		1
										344

N= 172 individuals

Heterozygotes observed: 131

Heterozygotes expected: 133.92

Vietnamese							total number
7	9	10	11	12	13		of alleles
7	0						3
9	0	0					7
10	0	1	4				23
11	0	3	5	5			27
12	2	1	3	6	2		20
13	1	2	6	3	4	2	20
							100

N= 50 individuals

Heterozygotes observed: 37

Heterozygotes expected: 39.82

Bangladesh							total number
9	10	11	12	13	14		of alleles
9	0						4
10	1	2					14
11	2	3	8				39
12	1	2	9	4			22
13	0	4	8	2	3		20
14	0	0	1	0	0	0	1
							100

N= 50 individuals

Heterozygotes observed: 33

Heterozygotes expected: 37.28

Table 21. Genotypic matrices: D13S317. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

D13S317

African-American								total number
8	9	10	11	12	13	14		of alleles
8	0							6
9	0	0						8
10	0	0	0					10
11	3	2	3	15				96
12	3	5	6	42	23			131
13	0	1	1	10	19	0		33
14	0	0	0	6	10	2	0	18
								302

N= 151 individuals

Heterozygotes observed: 113

Heterozygotes expected: 105.01

Chinese								total number
8	9	10	11	12	13	14		of alleles
8	4							26
9	6	0						14
10	1	4	1					18
11	6	2	4	2				19
12	3	1	7	2	0			15
13	1	1	0	0	2	1		6
14	1	0	0	1	0	0	0	2
								100

N= 50 individuals

Heterozygotes observed: 42

Heterozygotes expected: 41.30

Japanese								total number
7	8	9	10	11	12	13	14	of alleles
7	0							1
8	1	13						96
9	0	8	1					43
10	0	9	5	2				36
11	0	24	13	8	8			79
12	0	20	11	6	15	7		68
13	0	6	4	2	2	1	0	15
14	0	2	0	2	1	1	0	6
								344

N= 172 individuals

Heterozygotes observed: 141

Heterozygotes expected: 138.26

Vietnamese								total number
8	9	10	11	12	13	14		of alleles
8	8							38
9	4	0						9
10	7	0	0					13
11	6	3	3	2				23
12	5	0	2	7	0			14
13	0	2	0	0	0	0		2
14	0	0	1	0	0	0	0	1
								100

N= 50 individuals

Heterozygotes observed: 40

Heterozygotes expected: 38.26

Bangladesh								total number
7	8	9	10	11	12	13	14	of alleles
7	0							2
8	0	4						25
9	0	1	1					11
10	0	5	1	0				13
11	2	4	2	0	2			19
12	0	6	4	6	3	2		23
13	0	1	0	0	3	0	0	4
14	0	0	1	1	1	0	0	3
								100

N= 50 individuals

Heterozygotes observed: 41

Heterozygotes expected: 41.24

Table 22. Genotypic matrices: D7S820. The number of individuals displaying a particular genotype is identified, as well as the number of observed and expected heterozygotes in the African-American, Chinese, Japanese, Vietnamese and Bangladesh populations.

African-American											total number	
	7	8	9	9.3	10	11	12	13	14		of alleles	
7	0										1	
8	0	8									63	
9	0	3	0								34	
9.3	0	0	0	0							1	
10	1	28	12	1	16						108	
11	0	11	9	0	28	4					64	
12	0	3	8	0	5	7	1				25	
13	0	1	2	0	1	1	0	0			5	
14	0	1	0	0	0	0	0	0	0		1	
											302	

N= 151 individuals

Heterozygotes observed: 122

Heterozygotes expected: 115.72

Chinese											total number	
	7	8	9	10	11	12	13				of alleles	
7	0										1	
8	1	2									14	
9	0	1	0								3	
10	0	2	1	2							18	
11	0	5	0	7	8						38	
12	0	1	1	4	8	3					22	
13	0	0	0	0	2	2	0	4			100	

N= 50 individuals

Heterozygotes observed: 35

Heterozygotes expected: 38.01

Japanese											total number	
	7	8	9	10	11	12	13	14			of alleles	
7	0										1	
8	0	4									41	
9	0	1	0								14	
10	1	9	5	7							68	
11	0	14	6	19	19						118	
12	0	7	1	15	36	12					86	
13	0	2	1	4	4	3	0				14	
14	0	0	0	1	1	0	0	0			2	
											344	

N= 172 individuals

Heterozygotes observed: 130

Heterozygotes expected: 131.65

Vietnamese											total number	
	7	8	9	9.1	10	11	12	13	14		of alleles	
7	0										2	
8	0	1									16	
9	0	1	0								8	
9.1	1	0	0	0							1	
10	0	7	2	0	1						17	
11	0	3	3	0	3	7					30	
12	1	2	1	0	2	5	2				18	
13	0	1	0	0	1	2	2	0			6	
14	0	0	1	0	0	0	1	0	0		2	
											100	

N= 50 individuals

Heterozygotes observed: 39

Heterozygotes expected: 41.02

Bangladesh											total number	
	7	8	8.1	9	10	11	12	13			of alleles	
7	1										3	
8	1	2									21	
8.1	0	0	0								1	
9	0	2	0	0							6	
10	0	9	0	3	1						27	
11	0	1	1	1	4	3					19	
12	0	4	0	0	7	4	2				19	
13	0	0	0	0	2	2	0	0			4	
											100	

N=50 individuals

Heterozygotes observed: 41

Heterozygotes expected: 40.64

F. Discovery of novel STR variants

Several novel off-ladder variants were recognized and reamplified for confirmation. One individual in the Bangladesh population sample was heterozygous for an 8.1 repeat at the D7S820 locus. In the African-American population sample, a 9.3 repeat was discovered in a heterozygous state at the D7S820 locus in one individual. A male in the African-American population sample was typed as YY or OY, rather than the typical XY alleles for the gender discrimination locus amelogenin (Figure 15). The variant in the amelogenin locus on the X chromosome may be a result of mispriming at the 3'-end due to a point mutation or alternatively, the individual may contain a deletion within the amelogenin locus on the X chromosome. None of the novel variants were confirmed by sequencing.

Figure 15. Genotyper™ plots of the AmpF/STR Profiler Plus™ ‘green’ loci. Top panel shows a male profile (amelogenin: X,Y), middle panel shows a profile of a male containing a null X allele (amelogenin: OY or YY) and bottom panel is a profile of a female donor (amelogenin: XX). The horizontal scale represents the size of the peaks, in bases and the vertical scale represents the peak height, in relative fluorescence units (RFUs). Boxed numbers below each peak represent the allele designations. The loci represented are labeled below the horizontal scale at the top.

AA4044

AA4048

AA4049

IV. DISCUSSION

A. Comparisons of allele frequencies and distributions among populations

The main objective of this study was to evaluate the use of FTATM collection cards, Profiler PlusTM multiplex STR amplification and capillary electrophoresis on five population groups for potential implementation of this combination in the creation of the National DNA Databank. In the process, the frequencies and distributions of various short tandem repeat loci in African-American, Chinese, Vietnamese, Bangladesh and Japanese populations were compared and examined. Statistical analyses of the five population groups showed that all groups were in Hardy-Weinberg equilibrium. Tests for linkage disequilibrium revealed that alleles at separate loci showed no associations, allowing the product rule to be applied to generate the frequency of a genetic profile. Population differentiation tests on the five population groups showed that amalgamation of two sets of populations was possible: 1) Vietnamese and Chinese populations and 2) Vietnamese and Bangladesh populations. Since the Japanese database was comprised of individuals from both the Aomori and Kyoto regions, statistical analyses including Hardy-Weinberg equilibrium, linkage disequilibrium and population differentiation were also performed with the splitting of the Japanese database into these two groups. No evidence for Hardy-Weinberg disequilibrium or for linkage disequilibrium between pairs of loci was observed. Tests for population differentiation showed that amalgamation between three groups was possible: 1) Chinese database and Aomori group, 2) Vietnamese database and Aomori group and 3) Kyoto group and Aomori group.

The Profiler PlusTM system proved to be an efficient, fast and powerful tool for the

investigation of alleles from randomly selected African-American, Vietnamese, Chinese, Bangladesh and Japanese individuals, as well as for the individualisation of biological specimens. The loci of the Profiler Plus™ system have a large number of alleles, as reflected in the greater variability within populations than between populations. Allele frequency differences were found between populations but overall were quite similar for common alleles. The two most frequent alleles for each locus of the Profiler Plus™ system in each population are shown in Table 23. At the D3S1358 locus, the two most frequent alleles across all populations were 15 and 16, with a combined frequency ranging between 60% and 73%. Alleles 17 and 18 of the vWA locus were most commonly observed within the Vietnamese, Bangladesh and Chinese populations, each with a combined frequency around 50%. The two most frequent alleles at vWA for the African-American population included alleles 15 and 16 and the two most frequent alleles in the Chinese group included alleles 14 and 16. Alleles 22 and 23 were most frequently observed for FGA across almost all of the five population groups, each with a combined frequency of about 40%. The locus D8S1179 exhibited the most variation for the two most frequent alleles across the five populations and included alleles 10, 11, 13, 14 and 15. Alleles 28, 29 and 30 were most frequent at the D21S11 locus. In addition, the two-base variant allele 32.2 at D21S11 was observed in the Vietnamese population at a frequency of about 15%. At D18S51, alleles 14 and 15 were quite frequent across all five populations. At the D5S818 locus, allele 11 was most frequently observed in the Chinese, Vietnamese, Bangladesh and Japanese populations whereas allele 13 was observed most often in the African-American population. The D13S317 locus was most represented by alleles 8, 11 and 12 for all five populations. Finally,

Table 23. The two most frequent alleles identified for each of the AmpF/STR Profiler Plus™ loci in each of the five populations.

	African-American	Chinese	Vietnamese	Bangladesh	Japanese
D3S1358	15 (30%) 16 (30%) 17 (22%)	15 (30%) 16 (38%)	15 (35%) 16 (30%)	15 (44%) 16 (29%)	15 (39%) 16 (30%)
vWA	15 (21%) 16 (28%)	14 (30%) 16 (21%)	17 (29%) 18 (24%)	17 (31%) 18 (21%)	17 (30%) 18 (21%)
FGA	22 (22%) 23 (17%)	22 (20%) 23 (22%)	22 (27%) 23 (15%)	23 (19%) 24 (18%)	22 (18%) 23 (26%)
D8S1179	14 (32%) 15 (23%)	10 (21%) 15 (18%)	11 (17%) 13 (17%) 14 (14%) 15 (14%)	10 (19%) 13 (18%)	13 (24%) 14 (22%)
D21S11	28 (31%) 29 (18%)	29 (27%) 30 (23%)	29 (26%) 30 (26%) 32.2 (14%)	28 (17%) 29 (26%)	29 (22%) 30 (36%)
D18S51	15 (19%) 16 (17%)	14 (18%) 15 (31%)	13 (19%) 14 (21%) 15 (19%)	14 (21%) 15 (19%)	13 (20%) 14 (22%) 15 (20%)
D5S818	12 (31%) 13 (26%)	11 (29%) 12 (22%)	10 (23%) 11 (27%)	11 (39%) 12 (22%)	10 (22%) 11 (32%) 12 (22%)
D13S317	11 (32%) 12 (43%)	8 (26%) 11 (19%)	8 (38%) 11 (19%)	8 (25%) 12 (23%)	8 (28%) 11 (23%)
D7S820	8 (21%) 10 (36%) 11 (21%)	11 (38%) 12 (22%)	11 (30%) 12 (18%)	8 (21%) 10 (27%)	11 (34%) 12 (25%)

at the D7S820 locus, allele 10 was observed most often in the African-American and Bangladesh populations, whereas allele 11 was most common in the Chinese, Vietnamese and Japanese populations.

Tables 24 through 32 display the distribution of alleles for each of the nine STR loci studied in this report for the five populations, as well as a comparison of the data to published African-American, Chinese, Japanese and Caucasian population databases. Allele frequency distributions between the two African-American populations, between the two Chinese populations and between the two Japanese populations, are very similar across all nine loci with almost identical allele frequencies.

Population specific alleles were also noted in this study. At the D3S1358 locus, alleles 11, and 15.2 were detected only in the African-American populations. At the vWA locus, allele 15 occurred at a frequency of approximately 20% in the African-American population but at a frequency of between one percent and less than 12% in the remaining populations. Allele 19.2 at the FGA locus was detected only in the African-American populations. At D8S1179, allele 18 was observed only in the Japanese population. At D18S51, allele 13.2 was detected only in the African-American population.

B. Rare allelic variants detected

Interesting variant alleles were observed which occur at a rare frequency but which have been previously discovered. These included alleles 30.3 (Chinese population) and 33.1 (African-American population) at D21S11 and at D7S820, allele 9.1 (Vietnamese population). Novel variants which have not been identified in the scientific community were

Table 24. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: D3S1358.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

D3S1358						
	African-American (This study) (N=151)	African-American (N=195) ¹	Chinese (This study) (N=50)	Chinese (N=500) ²	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
9	0.000	0.003	0.000	0.000	0.000	0.000
10	0.000	0.000	0.000	0.000	0.000	0.000
11	0.007	0.003	0.000	0.000	0.000	0.000
12	0.007	0.005	0.000	0.002	0.010	0.000
13	0.007	0.000	0.020	0.000	0.000	0.000
14	0.093	0.118	0.010	0.040	0.030	0.030
15	0.298	0.280	0.300	0.359	0.350	0.440
15.2	0.007	0.003	0.000	0.000	0.000	0.000
16	0.298	0.323	0.380	0.316	0.310	0.290
17	0.215	0.218	0.230	0.235	0.250	0.140
18	0.070	0.046	0.050	0.047	0.050	0.100
19	0.000	0.003	0.010	0.001	0.000	0.000

D3S1358		Japanese (This study) (N=172)	Japanese (N=206)²	Canadian Caucasian (N=328)⁴
9		0.000	0.000	0.000
10		0.000	0.000	0.000
11		0.000	0.000	0.000
12		0.000	0.000	0.003
13		0.003	0.000	0.006
14		0.029	0.034	0.134
15		0.387	0.415	0.290
15.2		0.000	0.000	0.000
16		0.299	0.291	0.229
17		0.212	0.199	0.162
18		0.067	0.056	0.162
19		0.003	0.005	0.015

Table 25. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: vWA.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

VWA	African-American (This study) (N=151)	African-American (N=195) ¹	Chinese (This study) (N=50)	Chinese (N=500) ²	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
11	0.007	0.003	0.000	0.001	0.000	0.000
12	0.000	0.000	0.000	0.002	0.000	0.000
13	0.023	0.015	0.000	0.001	0.000	0.000
14	0.070	0.077	0.300	0.232	0.220	0.120
15	0.209	0.221	0.030	0.044	0.010	0.060
16	0.275	0.269	0.210	0.160	0.140	0.150
17	0.202	0.169	0.180	0.254	0.290	0.230
18	0.119	0.139	0.190	0.198	0.240	0.280
19	0.079	0.085	0.080	0.092	0.070	0.110
20	0.013	0.021	0.010	0.012	0.030	0.050
21	0.003	0.000	0.000	0.004	0.000	0.000
22	0.000	0.003	0.000	0.000	0.000	0.000

VWA	Japanese		
	(This study) (N=172)	Japanese (N=206) ²	Canadian Caucasians (N=328) ⁴
11	0.000	0.000	0.000
12	0.000	0.000	0.003
13	0.000	0.000	0.000
14	0.186	0.189	0.131
15	0.035	0.019	0.119
16	0.169	0.175	0.186
17	0.297	0.262	0.277
18	0.206	0.240	0.189
19	0.090	0.102	0.088
20	0.015	0.012	0.006
21	0.003	0.000	0.000
22	0.000	0.000	0.000

Table 26. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: FGA.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

FGA	African-American (This study) (N=151)	African-American (N=195) ¹	Chinese (This study) (N=50)	Chinese (N=500) ²	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
16.2	0.000	0.003	0.000	0.000	0.000	0.000
17	0.003	0.003	0.010	0.001	0.010	0.020
18	0.007	0.005	0.040	0.024	0.020	0.000
18.2	0.017	0.000	0.000	0.000	0.000	0.000
19	0.063	0.046	0.090	0.059	0.090	0.090
19.2	0.003	0.003	0.000	0.000	0.000	0.000
20	0.050	0.044	0.010	0.050	0.080	0.100
20.2	0.000	0.000	0.000	0.000	0.000	0.110
21	0.116	0.133	0.110	0.126	0.090	0.000
21.2	0.000	0.003	0.010	0.006	0.010	0.000
22	0.215	0.190	0.200	0.163	0.270	0.150
22.2	0.000	0.003	0.000	0.002	0.000	0.010
23	0.166	0.180	0.210	0.214	0.150	0.190
23.2	0.000	0.000	0.000	0.004	0.020	0.000
24	0.162	0.164	0.190	0.167	0.100	0.180
24.2	0.000	0.000	0.000	0.008	0.010	0.000
25	0.073	0.123	0.070	0.102	0.060	0.090
25.2	0.000	0.000	0.010	0.000	0.010	0.000
26	0.079	0.041	0.050	0.041	0.050	0.050
27	0.030	0.041	0.000	0.011	0.030	0.000
28	0.013	0.008	0.000	0.002	0.000	0.010
29	0.003	0.003	0.000	0.000	0.000	0.000

FGA	Japanese (This study) (N=172)	Japanese (N=208) ²	Canadian Caucasian (N=328) ⁴
16.2	0.000	0.000	0.000
17	0.003	0.005	0.000
18	0.035	0.022	0.009
18.2	0.000	0.000	0.000
19	0.058	0.073	0.061
19.2	0.000	0.000	0.000
20	0.096	0.121	0.125
20.2	0.000	0.000	0.000
21	0.148	0.138	0.180
21.2	0.003	0.002	0.000
22	0.177	0.216	0.229
22.2	0.000	0.002	0.000
23	0.256	0.163	0.131
23.2	0.003	0.000	0.000
24	0.116	0.143	0.146
24.2	0.000	0.002	0.000
25	0.073	0.068	0.095
25.2	0.000	0.005	0.000
26	0.026	0.029	0.018
27	0.003	0.010	0.006
28	0.000	0.000	0.000
29	0.003	0.000	0.000

Table 27. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: D8S1179.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

D8S1179					
	African-American (This study) (N=151)	African-American (N=195) ¹	Chinese (This study) (N=50)	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
8	0.007	0.000	0.000	0.000	0.010
9	0.003	0.005	0.000	0.000	0.000
10	0.023	0.031	0.210	0.110	0.190
11	0.056	0.044	0.160	0.170	0.080
12	0.096	0.105	0.070	0.160	0.130
13	0.179	0.197	0.170	0.170	0.180
14	0.321	0.333	0.140	0.140	0.160
15	0.228	0.210	0.170	0.140	0.150
16	0.073	0.064	0.060	0.100	0.090
17	0.013	0.010	0.020	0.010	0.010
18	0.000	0.000	0.000	0.000	0.000

D8S1179	Japanese (This study) (N=172)		Canadian Caucasian (N=328)*	
8		0.000		0.018
9		0.009		0.006
10		0.110		0.095
11		0.134		0.070
12		0.108		0.162
13		0.244		0.335
14		0.218		0.189
15		0.119		0.095
16		0.047		0.027
17		0.009		0.003
18		0.003		0.000

Table 28. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: D21S11.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

D21S11						
	African-American (This study) (N=151)	African-American (N=195) ¹	Chinese (This study) (N=50)	Chinese (N=500) ²	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
26	0.007	0.003	0.000	0.000	0.000	0.000
27	0.043	0.059	0.000	0.000	0.020	0.020
28	0.305	0.218	0.080	0.033	0.040	0.170
28.2	0.000	0.000	0.000	0.004	0.000	0.000
29	0.175	0.200	0.270	0.271	0.260	0.260
29.2	0.000	0.000	0.000	0.000	0.020	0.000
29.3	0.000	0.003	0.000	0.000	0.000	0.000
30	0.093	0.162	0.230	0.275	0.260	0.160
30.2	0.036	0.026	0.010	0.004	0.010	0.050
30.3	0.000	0.000	0.010	0.000	0.000	0.000
31	0.096	0.090	0.070	0.108	0.060	0.030
31.2	0.060	0.059	0.100	0.063	0.060	0.110
32	0.023	0.008	0.020	0.050	0.050	0.000
32.2	0.070	0.069	0.180	0.133	0.140	0.120
33	0.007	0.005	0.010	0.004	0.020	0.000
33.1	0.003	0.003	0.000	0.000	0.000	0.000
33.2	0.023	0.041	0.020	0.038	0.050	0.080
34	0.003	0.003	0.000	0.004	0.000	0.000
34.2	0.010	0.000	0.000	0.008	0.010	0.000
35	0.036	0.036	0.000	0.000	0.000	0.000
35.2	0.000	0.000	0.000	0.004	0.000	0.000
36	0.010	0.015	0.000	0.000	0.000	0.000
38	0.000	0.003	0.000	0.000	0.000	0.000

D21S11	Japanese (This study) (N=172)	Japanese (N=214)⁴	Canadian Caucasian (N=328)⁴
26	0.000	0.000	0.003
27	0.000	0.000	0.024
28	0.047	0.056	0.155
28.2	0.006	0.005	0.000
29	0.215	0.231	0.213
29.2	0.003	0.035	0.003
29.3	0.000	0.000	0.003
30	0.363	0.318	0.207
30.2	0.000	0.000	0.076
30.3	0.000	0.000	0.000
31	0.096	0.103	0.095
31.2	0.064	0.051	0.095
32	0.023	0.040	0.018
32.2	0.125	0.122	0.082
33	0.003	0.005	0.000
33.1	0.000	0.000	0.000
33.2	0.049	0.028	0.021
34	0.003	0.002	0.000
34.2	0.003	0.000	0.003
35	0.000	0.000	0.000
35.2	0.000	0.005	0.000
36	0.000	0.000	0.000
38	0.000	0.000	0.000

Table 29. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: D18S51.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

D18S51					
	African-American (This study) (N=151)	African-American (N=195) ¹	Chinese (This study) (N=50)	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
10	0.003	0.005	0.000	0.000	0.010
10.2	0.000	0.005	0.000	0.000	0.000
11	0.013	0.015	0.000	0.000	0.040
12	0.040	0.056	0.020	0.070	0.040
13	0.033	0.041	0.140	0.190	0.110
13.2	0.010	0.005	0.000	0.000	0.000
14	0.079	0.067	0.180	0.210	0.210
14.2	0.007	0.005	0.000	0.000	0.000
15	0.185	0.177	0.310	0.190	0.190
16	0.172	0.174	0.110	0.160	0.180
17	0.159	0.164	0.070	0.040	0.070
18	0.116	0.110	0.050	0.050	0.040
19	0.083	0.103	0.040	0.020	0.050
20	0.070	0.049	0.020	0.010	0.010
21	0.026	0.010	0.030	0.030	0.020
22	0.000	0.010	0.000	0.010	0.020
23	0.003	0.003	0.020	0.000	0.010
24	0.000	0.000	0.010	0.000	0.000
25	0.000	0.000	0.000	0.020	0.000

D18S51	Japanese (This study) (N=172)	Canadian Caucasian (N=328)^c
10	0.003	0.012
10.2	0.000	0.000
11	0.006	0.009
12	0.049	0.095
13	0.198	0.110
13.2	0.000	0.000
14	0.215	0.146
14.2	0.000	0.000
15	0.206	0.180
16	0.122	0.183
17	0.052	0.113
18	0.047	0.067
19	0.032	0.034
20	0.026	0.037
21	0.017	0.012
22	0.003	0.003
23	0.015	0.000
24	0.003	0.000
25	0.006	0.000

Table 30. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: D5S818.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

D5S818						
	African-American (This study) (N=151)	African-American (N=195) ¹	Chinese (This study) (N=50)	Chinese (N=500) ²	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
7	0.003	0.003	0.040	0.029	0.030	0.000
8	0.079	0.051	0.000	0.005	0.000	0.000
9	0.023	0.021	0.070	0.071	0.070	0.040
10	0.066	0.074	0.180	0.204	0.230	0.140
11	0.248	0.254	0.290	0.300	0.270	0.390
12	0.305	0.326	0.220	0.242	0.200	0.220
13	0.262	0.249	0.190	0.143	0.200	0.200
14	0.013	0.021	0.000	0.006	0.000	0.010
15	0.000	0.003	0.000	0.000	0.000	0.000
17	0.000	0.000	0.010	0.000	0.000	0.000

D5S818	Japanese (This study) (N=172)		
	Japanese (N=206) ²	Japanese (N=328) ⁴	Canadian Caucasian
7	0.000	0.005	0.000
8	0.006	0.007	0.000
9	0.067	0.083	0.034
10	0.218	0.214	0.073
11	0.317	0.252	0.348
12	0.215	0.272	0.363
13	0.157	0.146	0.168
14	0.017	0.019	0.012
15	0.003	0.002	0.003
17	0.000	0.000	0.000

Table 31. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: D13S317.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

D13S317						
	African-American (This study) (N=151)	African-American (N=195) ¹	Chinese (This study) (N=50)	Chinese (N=500)²	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
7	0.000	0.000	0.000	0.002	0.000	0.020
8	0.020	0.036	0.260	0.282	0.380	0.250
9	0.026	0.023	0.140	0.137	0.090	0.110
10	0.033	0.023	0.180	0.167	0.130	0.130
11	0.318	0.272	0.190	0.221	0.230	0.190
12	0.434	0.444	0.150	0.153	0.140	0.230
13	0.109	0.141	0.060	0.026	0.020	0.040
14	0.060	0.062	0.020	0.011	0.010	0.030
15	0.000	0.000	0.000	0.001	0.000	0.000

D13S317	Japanese (This study) (N=172)		Japanese (N=206) ^a	Canadian Caucasian (N=328) ^a					
	7	8	9	10	11	12	13	14	15
	0.003	0.279	0.125	0.105	0.230	0.198	0.044	0.017	0.000

Table 32. Comparison of AmpF/STR Profiler Plus™ allele frequencies generated for the five populations of this study to published data: D7S820.

¹ AmpF/STR Profiler Plus™ Amplification Kit User's Manual, 1997.

² Pu *et al.*, 1998

³ Yamamoto *et al.*, 1998

⁴ RCMP Caucasian database (provided by Benoît Leclair, RCMP Central Forensic Lab)

D7S820						
	African-American (This study) (N=151)	African-American (N=198) ¹	Chinese (This study) (N=50)	Chinese (N=500) ²	Vietnamese (This study) (N=50)	Bangladesh (This study) (N=50)
6	0.000	0.005	0.000	0.000	0.000	0.000
7	0.003	0.000	0.010	0.004	0.020	0.030
8	0.209	0.180	0.140	0.131	0.160	0.210
8.1	0.000	0.000	0.000	0.000	0.000	0.010
9	0.113	0.118	0.030	0.067	0.080	0.060
9.1	0.000	0.000	0.000	0.000	0.010	0.000
9.3	0.003	0.000	0.000	0.000	0.000	0.000
10	0.358	0.336	0.180	0.146	0.170	0.270
11	0.212	0.228	0.380	0.358	0.300	0.190
12	0.083	0.095	0.220	0.246	0.180	0.190
13	0.017	0.036	0.040	0.043	0.060	0.040
14	0.003	0.003	0.000	0.005	0.020	0.000

D7S820			
	Japanese (This study) (N=172)	Japanese (N=206)²	Canadian Caucasian (N=328)⁴
6	0.000	0.000	0.000
7	0.003	0.002	0.018
8	0.119	0.090	0.162
8.1	0.000	0.000	0.000
9	0.041	0.051	0.131
9.1	0.000	0.000	0.000
9.3	0.000	0.000	0.000
10	0.198	0.216	0.262
11	0.343	0.350	0.250
12	0.250	0.243	0.128
13	0.041	0.049	0.046
14	0.006	0.000	0.003

discovered in two populations in this study: an 8.1 repeat for the D7S820 locus in the Bangladesh population and a 9.3 repeat for D7S820 in the African-American population. Both variants were represented in the heterozygous state for that specific STR locus. These variants were reamplified and reanalysed using polyacrylamide slab gel electrophoresis (ABI Prism™ 377 DNA Sequencer) according to RCMP protocols and confirmed. Sequencing of these rare variants will provide conclusive information about their size.

A fascinating discovery was the STR profile of an individual, known to be male, in the African-American population genotyped as YY or OY for the amelogenin locus. Normally, the presence of the X-derived fragment and the Y-derived fragment in a sample denotes a male individual and the presence of solely the X-derived fragment signifies a female individual. Studies have shown that the amelogenin assay can incorrectly type some males as females because they lack the Y copy of the amelogenin gene as a result of a deletion polymorphism; this mistyping occurs at a very low frequency (Sanlos *et al.*, 1998). However, there have not been any published reports of a male individual in which amplification of the X-derived amelogenin fragment was not observed, as shown in this study. Null amplification could be the result of a point mutation or deletion of one or both of the primer binding sites. In addition, null amplification could result from a partial or complete deletion or duplication of the amelogenin locus. Sequencing of the X-homologue at the amelogenin locus will determine whether the null allele was due to mismatch of the primer as a result of a point mutation at the 3'-binding site, or whether a deletion was present within the amplification region.

C. Evaluation of tools for potential use in high-throughput databasing

(i). FTA™ collection cards, cotton swatches and gauze

The main objective of this study was to evaluate the use of FTA™ collection cards, Profiler Plus™ amplification and capillary electrophoresis as potential tools in the construction of the National DNA Databank. The use of FTA™ bloodstained collection cards proved to be a safe, fast and efficient procedure for the isolation of DNA. No carryover products were detected when the same cleaned Micropunch was used for all samples. Storage of the FTA™ collection cards was facilitated by their compact nature and the capability of archival storage at room temperature. Although most of the 1.2 mm disks remained intact during the five processing stages, some disks frayed, produced minimal PCR yield and needed to be reprocessed. In addition, FTA™ disks containing previously frozen whole blood required reprocessing and reamplification more often (15% of samples were repeated) than disks prepared from non-frozen whole blood (six percent of samples were repeated). This was mostly likely due to the persistence of heme which is a recognized PCR inhibitor. Four of the 150 samples from previously frozen whole blood were amplified using two processed FTA™ disks in the microamp tubes. An ample amount of DNA was available from the processed FTA™ disks for PCR amplification, but a greater number of cycles was required for the bloodstains prepared from previously frozen blood (25 cycles) than those prepared from non-frozen blood (23 cycles) to generate comparable amplicon yields. Ross *et al.* (1990) demonstrated that the yield of DNA is reduced by more than 25% in blood samples frozen a single time due to the lysing or rupture of cells during the freezing/thawing cycle.

An increasing number of law enforcement agencies are using the FTA™ collection cards to establish DNA repositories and databanks for convicted offenders. The use of FTA™ collection cards also proved invaluable as a repository of DNA from next-of-kin for the recent identification of victims of the SwissAir Flight 111 disaster by DNA typing performed by the RCMP. To further reduce the number of hours and manual manipulation required for such high throughput systems for DNA purification, a robotic system can be easily incorporated into the FTA™ purification procedure and coupled with subsequent PCR amplification (Belgrader and Marino, 1996). Belgrader and Marino (1996) demonstrated that up to 2400 FTA™ samples can be automatically purified and provide results suitable for DNA amplification and typing per day, with no trace of contamination due to carry-over using a robotic workstation.

Bloodstains from the cotton swatches were the most difficult to work with since the material was thick and hard to cut. Processing volumes were doubled and the heme was very persistent and difficult to remove from the triangular wedges. Therefore, more material was inserted into the microamp tubes than the tubes containing the FTA™ disks and as a result, the amplification volume was increased to 25 μ L and the cycle number was increased to 28 cycles. The bloodstained cotton batons were bulky to archive and required freezer storage.

The bloodstains collected on gauze amplified very successfully, with no amplification failures. Triangular wedges were processed with double the amount of reagents in an amplification volume of 25 μ L. The gauze material does not contain any chemical ingredients to prevent DNA degradation so long-term archival storage especially at room temperature using the gauze medium is questionable.

(ii). PCR volume reduction: 25 μ L vs 5 μ L reaction volumes

Amplification with the AmpF/STR Profiler Plus™ kit was successful in producing complete profiles across the nine STR systems and the gender discrimination locus, amelogenin. The RCMP forensic laboratories are presently performing amplification using the Profiler Plus™ kit in a 25 μ L volume for routine casework analysis. To manage costs involved in databasing, the validity of reducing the amplification final reaction volume to 5 μ L was evaluated and demonstrated excellent results. Essentially, the same concentration of PCR amplification reagents is present in the reaction tube whether a 25 μ L or a 5 μ L PCR volume is utilized. The concentration of DNA template, however, is increased by five-fold. For routine casework analysis, approximately 2 ng of DNA template is added to the PCR reaction mix in a total volume of 25 μ L. With the volume reduction protocol, 2 ng of DNA template is contained in a total PCR reaction volume of 5 μ L. Borys *et al.* (1998) showed that the quality of the amplification was comparable when using a 25 μ L or a 5 μ L PCR volume. Quality of the amplification was assessed by the percentage of stutter detected for the 25 μ L and 5 μ L volumes, as well as by the relative balance of peaks at heterozygous loci. Ideally, two peaks representative of a heterozygous profile at a single locus should have exactly the same height. In most cases, this ideal is not reached and preferential amplification to some extent is observed. Heterozygote ratios are calculated by dividing the lowest value (in relative fluorescence units, RFUs) of the two peaks by the highest value of the two peaks. Examination of the heterozygote ratios when a 25 μ L or a 5 μ L PCR reaction volume is used revealed that overall, lower heterozygote ratios were obtained when using a 5 μ L PCR reaction than a 25 μ L volume, but this difference was not substantial. The yield

of PCR product, however, was dramatically enhanced, by up to nine-fold, when a 5 μ L PCR reaction volume rather than a 25 μ L PCR reaction volume was amplified for 25 cycles. As a result, the number of PCR amplification cycles was reduced by two to compensate for the high yield of PCR product produced from the 5 μ L PCR reaction volume. The reduced amplification volume combined with the use of FTATM collection cards offer tremendous potential for the construction of the National DNA Databank.

(iii) Capillary electrophoresis

The separation of amplified STR products using capillary electrophoresis (CE) was demonstrated to be an excellent alternative to slab gel electrophoresis. With the auto-sampling capabilities of the instrument, the amount of manual preparation is minimized. CE provides better sensitivity and resolution than conventional slab gel electrophoresis due to the minimal amount of sample required and the high separation voltages employed. However, capillary electrophoresis instruments currently available and validated for forensic applications do not provide the high-throughput required to process the volume of samples expected in the construction of the National DNA Databank. Each sample is sequentially electrophoresed through a single capillary. This current level of throughput cannot compete with slab gel analysis where 34 samples can be electrophoresed simultaneously in less than three hours. However, the capillary electrophoresis instrument can handle high voltages which results in a single sample being electrophoresed in less than 30 minutes. In addition, the CE instrument can be run 24 hours a day without any human intervention and is amenable to robotic automation. Current advancements in technology to address the

requirement for high-throughput capabilities are offered for both slab gel and capillary electrophoresis. For example a 96-lane system upgrade has been developed for the ABI Prism™ 377 DNA Sequencer and multichannel capillary electrophoresis instruments, with up to 100 capillaries per instrument, are currently being tested with potential implications for large-scale databasing. The future of forensic DNA analysis by capillary electrophoresis looks promising.

V. CONCLUSIONS

The genotype data for a total of 473 individuals in five population groups (African-American, Chinese, Vietnamese, Bangladesh and Japanese) have been used to calculate the allele frequencies and distributions for the nine STR loci of the AmpF/STR Profiler Plus™ amplification kit. Each of the five populations demonstrated Hardy-Weinberg equilibrium and independence of alleles between pairs of loci, as well as a global test across all nine loci. Population differentiation tests revealed no significant genic differentiation between Chinese and Vietnamese populations illustrating that these two populations could be amalgamated into one population group. In addition, amalgamation between the Vietnamese and Bangladesh populations was valid. Since the Japanese population consisted of individuals from the Kyoto and Aomori regions, the same statistical analyses were performed with the separating of the Japanese population into two groups. No evidence for Hardy-Weinberg disequilibrium or linkage disequilibrium was found. Tests for population differentiation showed that amalgamation between the following groups was possible: 1) Chinese and Aomori groups, 2) Vietnamese and Aomori groups and 3) Kyoto and Aomori groups.

Two novel off-ladder variants were discovered: allele 8.1 at D7S820 in the Bangladesh population and allele 9.3 at D7S829 in the African-American population. In addition, a male individual in the African-American population was typed as OY or YY for the gender discrimination locus, amelogenin. Such a finding has not been identified in the scientific community.

Evaluation of bloodstained FTA™ collection cards, multiplex PCR amplification and

capillary electrophoresis revealed that these tools would be suitable for use in high-throughput laboratories performing large-scale databasing. Collection of biological specimens, such as blood, buccal or hair roots on FTA™ collection cards is an ideal method for establishing DNA repositories and databanks. Multiplex PCR amplification at a reduced volume (5 μ L versus 25 μ L) provides high quality DNA typing results and increases the yield of amplification by up to nine-fold. Finally, the use of capillary electrophoresis for the separation of PCR amplified products was ascertained to provide excellent resolution, precise results and high sensitivity. However, the use of capillary electrophoresis is hindered by its inability to compete with current slab gel electrophoresis for high-throughput analysis.

Appendix 1

Procedure for the Amplification of DNA from Blood Spotted on FTA™ Paper

As a sample of blood is spotted onto the FTA™ paper, cells are lysed, and the DNA from the white cell nuclei are trapped and immobilized within the matrix of the stain card. After the blood has dried onto the paper, a 1.2 mm disk is placed in a 0.6 mL eppendorf tube. The immobilized DNA is then purified by simply washing out the heme and other PCR inhibitors. After processing the FTA™ paper, the immobilized DNA is amplified by adding the disk directly to the amplification mix.

Blood collected by venipuncture in EDTA vacutainer tubes should be thoroughly mixed prior to spotting on FTA™ Paper.

1. Spot a minimum of 2 μ L of whole blood onto the FTA™ paper. For all PCR amplification mixes tested, a sufficient amount of DNA is obtained from this amount of whole blood.
2. Allow each blood sample to air dry for at least one hour at RT in a BioSafety cabinet.
3. Punch a 1.2 mm disk of the bloodstained FTA™ paper (Fitzco, Minneapolis, Minnesota) into a 0.6 mL microcentrifuge tube using a clean 1.2 mm Harris Micro-Punch (Canadian Life Technologies, Burlington, Ontario).

NOTE: It is important to ensure that no residual paper is carried from one punch to the next.

Clean the punch and cutting mat between samples with 70% EtOH and a Kimwipe.

4. Add 200 μ L of Fitzco Sample DNA Purification Reagent (Fitzco, Minneapolis, Minnesota) to each tube. Briefly vortex for one to two seconds at low speed.
5. Allow the tubes to sit for five minutes at RT, with a second brief vortexing half-way through this step.
6. Vortex briefly and using a pipette tip, carefully remove as much of the reagent as possible.
7. Repeat the wash procedure 2X (steps 4 to 6).
8. Add 200 μ l TE buffer (10mM Tris-HCl; 1mM EDTA pH 8.0) to each tube and briefly vortex at low speed.
9. Allow the tubes to sit for five minutes at RT, with a second brief vortexing half-way through this step.
10. Remove the TE buffer, as described in Step 6 and add 200 μ l of fresh TE buffer. Briefly vortex at low speed.
11. Allow the tubes to sit for five minutes at RT, with a second brief vortexing half-way through this step.
12. Remove the TE buffer, as described in Step 6 and allow the paper disk to dry completely (at least one hour at 56°C).
13. Transfer the processed FTA disk into the appropriate microamp tube for PCR amplification.

Appendix 2

Chemiluminescent Slot Blot Quantification of Human Genomic DNA (*ACES*[™] 2.0+ Human DNA Quantification System (Gibco BRL, Gaithersburg, Maryland)).

1. To quantify DNA, use 1/50 (1 μ L) of the sample in question.
2. Denature the samples for 20 min. by adding 100 μ L of 0.5 M NaOH, 0.5 M NaCl solution containing 0.05% bromophenol blue.
3. Prepare the DNA standard:
 - a. Make serial 2-fold dilutions of human genomic DNA (female cell line 9947A-Perkin Elmer, Foster City, California) of known concentration.
 - b. The dilution series should begin at the 100 ng level or less and can extend down to concentrations as low as 10 pg.
 - c. Take the required amount of the known standard and bring to a final volume of 200 μ L with 0.5 M NaOH, 0.5 M NaCl, containing 0.05% bromophenol blue.
 - d. After 20 min., remove 100 μ L of this solution and add it to a 100 μ L aliquot of 0.5 M NaOH, 0.5 M NaCl containing 0.05% bromophenol blue dye to prepare a 2-fold standard dilution.
 - e. Repeat the dilution process to complete the series.
 - f. The serial dilutions can be conveniently prepared in microtiter plates.
4. Prepare a negative control of 100 μ L 0.5M NaOH, 0.5 M NaCl, 0.05% bromophenol blue solution.
5. Prepare a slot blot membrane using Pall Biodyne A nylon membrane (Gibco BRL,

Gaithersburg, Maryland). Presoak the membrane in 2X SSC for a minimum 2 min. Wet two Whatman 3 MM chromatography papers (Bio-Rad, Hercules, California) in 2X SSC and place onto the slot blot vacuum apparatus.

6. Apply the DNA solutions (100 μ L) to the membrane using a Bio-Dot SF Microfiltration Apparatus (Bio-Rad, Hercules, California) in accordance with the manufacturer's instructions. By visual inspection of the dye coloured DNA solution, ensure that the DNA sample has filtered through the manifold prior to disassembly of the manifold (typically five to eight minutes under vacuum). Successful application of the DNA to the membrane will be apparent by the presence of uniform dye-coloured sample well marks.
7. Neutralize the membrane by rinsing in 2X SSC, 0.2 M Tris, pH 7.5 for five min. on a rotator at RT.
8. Blot the membrane on Whatman 3 MM chromatography paper and fix the DNA to the membrane by exposing the membrane to UV light (0.15 Joules/cm² , 254 nm) in a Stratalinker UV Crosslinker (Stratagene, La Jolla, California) set for 90 sec.
9. Have a shaking water bath warmed to 50°C. Equilibrate the ACES 2.0 hybridization buffer (0.5 M Sodium Phosphate pH 7.3, 0.5% (v/v) Tween 20TM, 1% (w/v) Hammerstein Casein, 0.02% (w/v) Sodium Azide) to 50°C. Prepare 250 mL of Wash Buffer 1 (1:5 dilution of ACES 2.0 Wash Buffer 1 Concentrate- 0.1M Sodium Phosphate pH 7.2, 1% (v/v) Tween 20) and heat to 50°C.
10. Prepare 250 mL of 1X Final Wash buffer (1:10 dilution of 10X Final Wash Buffer- 0.1 M Tris/HCl, pH 8.6, 1.5 M NaCl) and keep at RT.
11. Place the membrane, DNA side up, in 10 mL ACES 2.0 hybridization solution in a

Hybriboat™ to prehybridize for 20 min. at 50°C in a shaking water bath.

12. Centrifuge the Human DNA Probe, D17Z1, alkaline phosphatase labeled oligonucleotide, for three to five minutes at 13,000 rpm just prior to use.
13. Prepare the hybridization solution and probe mixture by adding 20 ng of probe to 10 mL hybridization solution. Keep this preparation at 50°C until ready to hybridize.
14. Decant pre-hybridization solution and add hybridization mixture. Incubate at 50°C for 20 min.
15. Wash the membrane for 10 min. in 125 mL Wash Buffer 1 at 50°C. Decant solution and repeat with fresh solution.
16. Wash the membrane for 5 min. in 125 mL 1X Final Wash Buffer at RT on a rotator. Decant and repeat with fresh solution.
17. Blot the membrane on Whatman 3MM paper. Do not let the membrane dry completely.
18. Dim the lights, place the membrane in a Hybriboat™ and add 4 mL Lumi-Phos Plus (Gibco BRL, Gaithersburg, Maryland) per membrane. Rotate for 5 min. at RT.
19. Remove the membrane from the container, place in a clear plastic bag and squeeze out excess Lumi-Phos Plus and any air bubbles. Heat seal and clean the outside of the bag with a water-dampened laboratory wipe.
20. Expose the membrane to Kodak™ XRP 5 film (Kodak Canada, Ottawa, Ontario) at RT (no screens). Develop the film after one to two hours. Lumi-Phos Plus will ramp to its peak output in approximately three hours. Film exposure after ramping is 1-40 min. Ensure that the DNA side is facing the X-ray film. If necessary repeat the exposure, adjusting the time to obtain an optimal lumigraph. The standard DNA dilutions should be detectable down to

the 20 pg level. Use the relative intensities of the lumigraphic image in the standard DNA dilutions to assess the approximate concentration of human DNA present in the evidentiary samples.

Appendix3

Formamide Deionization

1. Weigh out 0.15g mixed bed resin beads (Amberlite MB150 Resin, Perkin-Elmer, Foster City, California) and deposit beads into a 1.5mL polypropylene tube for each mL of formamide to be deionized.
2. In a fume hood, add 1.0ml formamide (Amresco, Solon, Ohio) to the beads. Securely cap the tubes and vortex vigorously for two to three minutes.
3. Centrifuge tubes at high speed for one or two minutes.
4. Transfer formamide to larger polypropylene tube leaving all beads behind. Combine all formamide deionized in a given batch.
5. Check pH of deionized formamide. If pH is 6.0 or higher, aliquot formamide to 0.2mL amplification tubes. If pH is below 6.0, repeat the deionization process using fresh resin beads.
6. Store aliquots of deionized formamide in freezer (-20°C). Unused portions of thawed aliquots should be discarded.

AmpFISTR Profiler Plus profiles for individuals in each of the five population groups

Individual	D3S1358	D3S1358	vWA	vWA	FGA	FGA	AMEL	AMEL	D8S1179	D8S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
AA4000	15	18	11	16	21	25	X	Y	12	13	28	31.2	17	19	12	13	11	12	10	11
AA4001	15	17	15	15	24	26	X	X	14	14	28	33.2	16	16	11	13	12	12	10	11
AA4002	14	16	17	17	23	27	X	Y	13	13	29	31	13	15	11	13	11	11	8	11
AA4003	15	16	15	17	21	24	X	X	13	14	29	35	15	16	11	13	10	13	8	11
AA4004	15	15	14	19	20	24	X	Y	15	15	30	31	17	20	12	12	12	13	8	10
AA4005	15	16	15	18	22	23	X	X	13	14	29	31	15	19	11	13	11	13	10	10
AA4006	15	17	14	19	22	23	X	Y	14	14	28	28	16	18	10	11	11	11	9	11
AA4007	16	18	15	17	21	24	X	X	11	14	28	32.2	16	19	12	13	12	14	10	10
AA4008	14	17	14	17	19	22	X	Y	13	16	28	29	14	15	12	12	12	12	9	13
AA4009	15	16	15	16	22	26	X	X	14	15	29	31	15	20	8	11	12	14	10	11
AA4010	15	17	13	16	26	27	X	Y	13	15	28	28	15	17	11	12	12	13	11	11
AA4011	15	17	17	17	18.2	22	X	X	12	16	28	29	12	17	10	13	11	13	9	11
AA4012	15	15	16	18	22	24	X	Y	15	15	28	32.2	16	17	8	12	10	12	8	10
AA4013	16	16	17	21	23	27	X	X	14	15	28	29	14	18	12	13	11	12	8	8
AA4014	16	16	16	16	21	26	X	Y	14	15	31	32.2	17	17	11	12	11	12	8	12
AA4015	14	15	16	18	24	24	X	X	13	14	30	30.2	15	16	10	13	11	12	9	11
AA4016	14	15	15	16	21	26	X	Y	14	15	28	31	17	21	11	12	11	13	10	11
AA4017	17	17	15	18	22	22	X	X	12	15	27	30	15	15	13	13	8	11	9	11
AA4018	16	17	15	16	23	23	X	Y	14	15	29	33.2	15	16	12	12	11	12	10	11
AA4019	15	16	14	17	23	24	X	X	14	15	26	28	11	14	10	11	11	11	10	11
AA4020	16	16	16	17	22	22	X	Y	14	15	29	31.2	15	17	12	13	11	11	10	10
AA4021	14	15	17	19	20	22	X	X	13	15	28	29	16	18	11	12	11	12	9	10
AA4022	15	15	15	15	21	24	X	Y	14	14	32.2	33.2	15	15	12	12	11	12	10	12
AA4023	15	17	15	17	23	24	X	X	13	14	26	27	17	17	13	13	11	11	10	11
AA4024	16	16	18	18	24	26	X	Y	11	15	28	31	15	18	8	10	12	13	10	11
AA4025	17	17	16	19	24	26	X	X	14	15	28	29	15	16	11	12	12	12	10	11
AA4026	16	16	14	19	23	25	X	Y	15	16	27	29	18	20	11	13	11	11	8	9
AA4027	14	18	15	17	22	25	X	X	14	15	28	32.2	18	20	11	13	11	11	9	11
AA4028	16	17	16	17	20	20	X	Y	13	15	28	28	15	16	13	13	11	12	10	10
AA4029	15	17	13	17	22	23	X	X	12	13	28	32.2	19	23	10	11	11	12	8	10
AA4030	14	16	15	17	22	22	X	Y	15	15	28	31	12	17	11	13	11	12	8	10
AA4031	15	17	15	17	24	26	X	X	10	15	28	35	18	19	8	13	12	13	9	10
AA4032	14	16	15	19	22	22	X	Y	13	15	28	28	16	20	13	14	12	13	8	9
AA4033	16	17	15	16	19	22	X	X	14	14	30	31.2	10	12	13	13	12	12	8	10
AA4034	17	18	14	18	22	25	X	Y	13	14	28	31	12	16	9	13	12	14	8	10
AA4035	15	17	15	19	21	22	X	X	13	13	29	30.2	13	16	12	13	12	12	8	10
AA4036	17	17	16	16	22	23	X	Y	13	13	31	33.2	17	20	10	12	12	12	10	11
AA4037	16	17	16	18	23	24	X	X	14	17	27	29	15	15	12	13	10	12	8	11
AA4038	14	15	15	16	21	24	X	Y	11	13	30	31	15	18	11	13	11	12	8	10
AA4039	16	16	17	18	22	24	X	X	11	17	28	34.2	16	16	8	13	12	12	8	10
AA4040	16	17	17	17	24	25	X	Y	15	15	28	29	14.2	15	11	13	11	12	11	11
AA4041	15	16	15	16	21	24	X	X	10	11	30	31	15	17	13	14	11	11	10	11
AA4042	15	18	16	18	21	24	X	Y	14	15	29	30.2	14	16	13	13	12	12	10	10
AA4043	14	15	16	17	24	27	X	X	12	12	28	31.2	15	19	11	11	11	12	8	8
AA4044	15	15	15	16	22	24	X	Y	13	14	28	33.2	14	15	10	13	11	12	8	10
AA4045	15	16	15	18	19	22	X	X	15	16	28	28	15	21	10	13	11	12	9	11
AA4046	15	16	18	18	21	23	X	Y	13	13	29	35	14	17	11	13	12	13	9	12
AA4047	16	17	14	14	20	22	X	X	11	12	27	28	11	17	11	13	8	12	9.3	10
AA4048	15	16	16	16	22	22	Y	Y	14	14	28	32.2	12	14	9	11	11	11	11	11
AA4049	17	18	15	17	23	23	X	X	14	16	28	31	15	16	12	13	12	12	9	12
AA4050	15	16	17	19	23	24	X	Y	13	14	32.2	35	16	16	8	12	12	13	8	8

Individual	D3S1358	D3S1358	vWA	vWA	FGA	FGA	AMEL	AMEL	D6S1179	D6S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
AA4051	15	16	16	16	23	23	X	X	14	14	29	29	13.2	20	12	12	12	12	10	11
AA4052	16	17	16	19	25	25	X	Y	12	14	32	35	16	17	12	13	11	12	10	12
AA4053	15	15.2	16	18	22	24	X	X	14	14	30	31.2	16	18	8	12	12	13	11	12
AA4054	14	15	16	17	23	23	X	Y	15	16	28	30	12	15	11	11	12	12	8	10
AA4055	16	17	14	15	22	22	X	X	12	17	28	31.2	15	16	12	13	12	13	10	11
AA4056	15	17	17	20	22	22	X	Y	12	14	28	35	14	15	11	12	11	14	11	12
AA4057	16	17	16	19	20	22	X	X	13	16	29	30	14.2	21	12	12	11	11	10	11
AA4058	16	16	16	16	22	25	X	Y	13	13	28	28	13	14	11	12	11	12	10	10
AA4059	15	17	16	20	24	25	X	X	13	13	28	28	12	18	9	12	8	11	8	11
AA4060	14	16	13	16	22	23	X	Y	13	16	28	29	16	20	12	12	11	11	8	10
AA4061	15	17	16	19	23	23	X	X	11	15	29	29	15	16	9	13	12	12	8	8
AA4062	14	15	15	17	21	24	X	Y	14	15	30	31.2	16	16	10	12	12	14	9	10
AA4063	13	15	15	18	23	24	X	X	13	14	29	30	16	18	13	13	12	12	10	12
AA4064	16	16	13	16	18.2	22	X	Y	14	15	27	31	12	18	8	8	13	14	8	11
AA4065	14	15	15	17	19	20	X	X	10	14	28	32	17	20	11	12	9	12	10	10
AA4066	14	17	15	16	23	23	X	Y	15	16	28	30.2	15	16	12	13	12	13	10	11
AA4067	15	17	14	14	21	22	X	X	14	15	28	35	14	18	8	12	12	14	9	10
AA4068	15	17	14	15	24	27	X	Y	13	16	28	29	16	19	10	11	13	14	9	10
AA4069	16	17	17	17	21	23	X	X	8	15	28	28	14	19	12	12	11	12	8	10
AA4070	15	17	14	17	26	26	X	Y	14	15	27	28	12	16	12	13	11	11	8	10
AA4071	17	18	16	16	23	25	X	X	12	14	29	30.2	17	18	12	13	8	12	10	10
AA4072	15	15	14	16	24	26	X	Y	11	14	31	32	15	19	11	12	12	14	8	8
AA4073	16	17	16	17	21	26	X	X	10	14	31.2	36	15	16	7	12	11	11	9	10
AA4074	16	16	14	17	18.2	26	X	Y	14	15	32.2	34	15	21	11	12	12	13	8	10
AA4075	17	18	13	15	23	24	X	X	14	16	35	36	12	17	10	13	11	13	10	11
AA4076	16	17	16	17	20	24	X	Y	14	14	29	31	16	20	8	11	9	12	8	10
AA4077	12	15	15	18	21	22	X	X	12	15	28	32.2	13	18	12	12	10	12	8	12
AA4078	15	17	16	18	23	23	X	Y	10	11	28	33	12	15	13	13	12	14	11	12
AA4079	16	17	15	18	19	22	X	X	14	14	28	29	17	19	8	12	11	12	9	12
AA4080	16	16	15	16	26	26	X	Y	13	14	28	31.2	19	21	8	11	12	13	8	10
AA4081	16	17	15	15	19	25	X	X	14	15	30	35	14	18	11	12	9	13	9	12
AA4082	14	15	16	17	22	25	X	Y	8	16	29	31	14	17	13	13	12	13	9	10
AA4083	14	16	17	19	22	23	X	Y	12	15	29	32.2	16	16	8	11	12	13	8	12
AA4084	16	16	16	18	19	22	X	X	13	14	30	32.2	15	20	11	13	9	12	8	8
AA4085	15	18	15	15	23	23	X	Y	15	16	27	28	17	17	10	13	11	14	8	10
AA4086	12	15	16	19	18.2	24	X	X	15	15	28	31.2	15	16	11	12	9	11	8	11
AA4087	15	17	15	19	22	24	X	Y	16	16	28	30	14	18	11	12	12	12	8	10
AA4088	16	17	15	17	22	23	X	X	14	14	30	31	18	18	11	12	11	13	10	11
AA4089	14	16	18	19	22	24	X	Y	15	16	29	31	14	18	11	13	10	11	8	11
AA4090	15	18	17	18	19	25	X	X	15	16	29	29	16	18	12	13	10	11	12	12
AA4091	16	18	17	19	21	22	X	Y	14	14	31	32	16	19	12	12	11	12	9	10
AA4092	15	15	17	18	22	24	X	X	11	12	29	30	17	19	11	11	11	13	10	11
AA4093	15	17	16	18	19	28	X	Y	15	15	29	30	15	17	11	12	12	12	11	12
AA4094	15	18	15	16	22	23	X	X	15	15	28	32	15	17	9	12	11	13	9	10
AA4095	15.2	16	18	19	21	22	X	Y	14	14	31	31	12	17	12	12	12	12	10	11
AA4096	16	16	16	18	18	23	X	X	12	16	29	30	13.2	16	8	12	12	12	10	11
AA4097	15	17	16	20	22	28	X	Y	10	14	28	28	14	15	8	8	11	12	8	11
AA4098	15	16	15	17	22	23	X	X	14	14	31.2	32.2	16	17	13	14	12	14	10	10
AA4099	15	17	14	17	19	26	X	Y	13	16	28	29	13	17	10	11	8	11	9	12
AA4100	15	16	16	17	22	26	X	X	12	14	29	31	17	20	11	11	11	14	9	10
AA4101	15	17	14	17	22	28	X	Y	11	12	28	32.2	13.2	18	11	11	11	13	10	11

Individual	D3S1358	D3S1358	VWA	VWA	FGA	FGA	AMEL	AMEL	D8S1179	D8S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
AA4102	15	17	16	16	19	27	X	X	13	14	28	29	17	19	10	13	11	12	9	11
AA4103	14	17	15	16	21	22	X	Y	13	13	28	31.2	16	17	11	12	12	12	10	11
AA4104	14	16	15	17	24	27	X	X	13	15	31.2	33.2	15	20	9	10	10	12	9	12
AA4105	14	16	15	16	22	26	X	Y	14	14	27	30	14	16	11	12	12	13	8	10
AA4106	13	17	17	19	22	26	X	X	13	15	30	34.2	18	20	10	13	11	12	9	12
AA4107	15	16	11	15	21	22	X	Y	14	14	28	30.2	15	18	12	12	12	13	11	11
AA4108	15	16	14	19	25	26	X	X	13	14	27	30.2	11	15	11	11	8	12	8	11
AA4109	15	18	15	16	21	24	X	Y	13	13	29	32.2	17	18	13	13	12	12	8	10
AA4110	15	16	16	18	24	27	X	X	12	14	28	33.2	13	19	12	13	11	13	9	11
AA4111	14	17	17	17	21	23	X	Y	15	17	28	32	15	17	12	13	11	12	8	14
AA4112	15	16	15	16	23	24	X	X	11	14	28	34.2	14	20	12	14	11	12	11	12
AA4113	11	15	15	19	23	24	X	Y	12	14	27	29	17	19	12	13	10	12	10	10
AA4114	17	18	15	16	21	29	X	X	14	14	28	30.2	14	18	8	13	11	12	10	12
AA4115	16	17	16	17	20	25	X	Y	12	14	28	32.2	15	15	12	12	11	12	8	8
AA4116	16	18	16	18	24	25	X	X	15	16	31.2	32	14	21	11	13	11	12	8	10
AA4117	15	17	15	16	20	26	X	Y	14	15	28	29	13	21	11	11	11	12	11	12
AA4118	11	14	17	18	21	23	X	X	15	15	28	29	19	20	13	13	12	13	7	10
AA4119	16	16	13	15	23	24	X	Y	12	15	28	29	13	19	8	11	12	12	8	10
AA4120	15	16	17	17	25	27	X	X	13	14	30	30.2	15	15	11	11	11	12	9	12
AA4121	15	16	16	16	21	21	X	Y	14	15	28	35	17	20	9	11	11	12	8	11
AA4122	14	17	17	17	21	24	X	X	12	14	30	30	15	18	8	13	11	12	8	13
AA4123	15	16	16	18	24	26	X	Y	11	14	29	31	15	21	12	13	11	14	10	10
AA4124	14	16	15	19	18	22	X	X	12	12	33	36	16	18	11	12	9	12	10	11
AA4125	15	16	16	17	25	28	X	Y	14	15	28	32.2	15	17	13	13	12	12	8	10
AA4126	15	17	16	16	20	24	X	X	10	12	27	33.1	16	18	12	13	11	12	10	11
AA4127	15	17	15	19	19	22	X	Y	13	15	28	32.2	15	16	11	13	11	12	10	10
AA4128	17	18	15	16	20	23	X	X	11	14	29	31	19	20	8	12	12	12	9	10
AA4129	16	18	15	16	17	21	X	Y	12	14	27	28	16	18	11	11	11	12	10	10
AA4130	17	18	17	18	19	21	X	X	13	15	28	28	14	17	8	12	11	11	10	11
AA4131	15	18	13	15	25	25	X	Y	13	14	31.2	31.2	15	16	11	12	11	12	8	10
AA4132	15	15	18	18	19	26	X	X	13	14	28	31.2	15	17	11	13	12	14	8	10
AA4133	15	16	16	17	22	25	X	Y	14	15	28	28	16	20	11	12	9	11	8	11
AA4134	16	17	15	16	19	22	X	X	12	15	28	29	18	19	8	12	11	14	10	10
AA4135	15	16	14	15	18.2	23	X	Y	14	14	28	30	17	19	12	13	11	12	8	9
AA4136	14	15	16	18	20	23	X	X	11	16	28	31.2	16	19	12	13	10	11	9	11
AA4137	15	17	16	17	19	19.2	X	Y	11	14	28	31	16	19	11	12	11	12	11	13
AA4138	14	15	16	16	24	25	X	X	9	14	28	32.2	15	17	11	12	9	12	10	10
AA4139	17	17	15	16	20	24	X	Y	11	15	28	30	17	18	11	12	10	12	9	10
AA4140	15	16	17	18	19	21	X	X	15	15	29	30	14	17	10	13	11	14	10	10
AA4141	15	16	15	16	22	23	X	Y	13	16	31	32.2	16	20	11	11	12	14	10	11
AA4142	15	17	18	19	21	21	X	X	13	14	28	29	17	18	12	13	11	12	11	12
AA4143	16	16	16	16	21	23	X	Y	12	15	28	31	14	17	11	12	12	13	10	13
AA4144	16	16	14	15	19	24	X	X	13	15	29	31	16	18	10	11	11	13	9	13
AA4145	15	16	16	20	19	24	X	Y	14	14	29	30	15	18	12	12	12	13	8	10
AA4146	16	16	15	15	21	22	X	X	13	14	28	29	11	17	12	13	11	12	8	8
AA4147	15	17	14	16	22	26	X	Y	13	13	29	32.2	13	19	10	12	12	12	8	10
AA4148	18	18	16	18	22	24	X	X	14	15	28	30	19	20	8	13	11	12	10	11
AA4149	15	16	17	19	21	23	X	Y	13	14	28	30.2	14	15	11	12	11	12	10	12
AA4150	14	16	17	17	20	23	X	X	12	15	30.2	35	13	17	11	12	11	11	10	11
JP0	15	16	17	19	21	22	X	Y	10	13	30	32.2	13	14	9	12	10	12	8	12
JP1	16	18	16	18	22	24	X	Y	10	14	28	30	13	15	11	11	8	11	9	10

Individual	D3S1358	D3S1358	VWA	VWA	FGA	FGA	AMEL	AMEL	D8S1179	D8S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
JP2	15	16	18	19	20	25	X	Y	11	14	28	30	13	15	10	12	9	11	10	11
JP3	15	18	18	18	22	26	X	Y	10	11	29	30	11	15	10	12	10	12	8	8
JP4	15	16	14	19	22	23	X	Y	10	11	32	32.2	15	17	11	14	8	11	12	13
JP5	15	17	16	18	21	24	X	Y	14	15	29	31.2	15	16	10	11	8	12	10	13
JP6	17	18	18	19	23	25	X	Y	14	14	29	31.2	10	16	8	11	8	11	12	12
JP7	17	18	14	17	23	24	X	Y	14	14	29	30	15	15	9	12	10	11	10	11
JP8	15	15	17	18	20	23	X	Y	10	16	30	30	13	18	11	12	9	11	12	12
JP9	14	16	14	17	20	20	X	Y	13	13	30	32	13	13	10	11	10	11	10	11
JP10	16	17	17	18	22	27	X	Y	11	14	29	29	13	15	12	13	10	12	9	10
JP11	16	17	14	17	24	26	X	Y	11	14	29	31	14	25	10	10	8	8	10	11
JP12	16	16	17	17	18	19	X	Y	13	15	31	33.2	16	16	11	12	9	12	8	11
JP13	15	15	18	19	19	22	X	Y	10	14	30	30	14	18	11	13	8	12	11	11
JP14	15	17	14	17	19	23	X	Y	13	14	29	32	13	18	11	13	10	14	11	13
JP15	15	16	16	17	21	23	X	Y	12	12	29	31	14	14	10	13	12	12	8	13
JP16	14	15	14	16	21.2	22	X	Y	11	15	30	31.2	15	19	11	13	8	8	11	12
JP17	15	16	14	18	21	23	X	Y	12	14	30	30	13	14	11	11	8	13	11	12
JP18	15	16	16	18	21	26	X	Y	13	14	30	32.2	13	13	12	13	8	11	10	12
JP19	16	17	17	18	20	23	X	Y	11	14	29.2	30	13	16	10	11	10	10	11	12
JP20	15	16	14	20	19	23	X	Y	11	14	30	32.2	15	19	10	13	8	11	9	11
JP21	16	17	18	19	23	23	X	Y	10	15	31.2	33.2	14	18	10	13	11	12	10	11
JP22	15	16	18	18	20	25	X	Y	11	13	30	30	15	17	10	13	8	13	10	12
JP23	15	15	17	18	20	21	X	Y	11	15	29	30	13	13	12	12	10	13	10	10
JP24	15	18	14	17	22	23.2	X	Y	12	13	31.2	33.2	16	17	10	10	8	11	11	12
JP25	15	15	16	17	24	24	X	Y	11	12	29	30	15	17	10	11	8	12	12	13
JP26	15	15	14	17	21	24	X	Y	10	11	30	30	15	18	10	10	11	12	11	11
JP28	16	17	19	21	20	23	X	X	10	13	30	30	14	15	10	11	11	12	8	13
JP30	15	16	17	18	23	24	X	X	12	15	30	31.2	14	14	10	11	8	13	10	10
JP32	16	16	17	18	22	22	X	X	12	15	30	33.2	13	15	9	10	11	11	11	12
JP33	16	17	16	17	18	25	X	Y	12	12	29	30	14	20	10	11	9	10	10	12
JP34	14	15	17	18	22	23	X	Y	12	12	29	31	13	14	11	12	8	10	8	11
JP35	15	17	17	17	24	24	X	Y	14	15	30	30	13	18	10	11	8	12	10	12
JP36	17	17	14	17	18	24	X	Y	11	16	30	30	13	14	10	12	9	13	11	12
JP37	16	17	18	18	22	24	X	Y	13	16	29	31	12	18	10	13	8	8	8	11
JP38	16	16	15	17	19	22	X	Y	11	12	30	32.2	15	18	11	13	10	12	10	12
JP39	14	18	17	19	22	24	X	Y	10	15	28	30	16	16	12	13	8	11	11	11
JP40	16	16	14	18	19	23	X	Y	11	14	30	30	16	19	10	11	12	13	8	10
JP41	16	16	14	19	23	24	X	Y	12	15	30	30	14	16	12	12	12	12	8	11
JP42	15	17	17	17	22	22	X	Y	11	13	30	30	13	14	10	13	8	9	8	11
JP43	16	16	14	14	22	23	X	Y	11	13	31	33.2	15	21	11	12	9	12	9	11
JP44	15	15	16	16	19	19	X	Y	14	14	30	30	13	24	11	12	12	12	12	13
JP45	15	16	16	17	23	25	X	Y	11	12	29	29	13	15	10	12	8	12	11	12
JP47	15	16	16	18	23	24	X	Y	13	14	30	32.2	12	15	10	12	10	10	11	12
JP48	15	16	15	17	21	24	X	Y	16	17	29	31.2	12	14	12	13	8	9	11	12
JP50	15	17	16	17	21	25	X	Y	10	15	30	31	13	18	10	13	8	12	11	13
JP51	15	17	18	19	21	24	X	Y	13	13	29	33.2	13	15	9	11	11	11	8	12
JP52	15	17	14	18	22	22	X	Y	13	15	28	32	15	17	10	12	9	11	11	12
JP53	17	18	16	19	20	21	X	Y	13	14	29	30	13	13	9	12	9	12	11	11
JP54	14	17	17	19	22	25	X	Y	14	15	30	31.2	14	17	12	12	8	12	11	12
JP55	15	17	14	18	22	22	X	Y	13	15	28	32	15	17	10	12	9	11	11	12
JP56	15	18	14	16	23	23	X	Y	14	14	32.2	33.2	13	14	10	11	8	11	11	11
JP57	15	15	15	16	17	26	X	X	13	14	29	32.2	14	15	10	10	8	10	10	12

Individual	D3S1358	D3S1358	vWA	vWA	FGA	FGA	AMEL	AMEL	D8S1179	D8S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
JP58	16	16	15	17	24	25	X	Y	14	15	29	29	13	16	11	12	9	10	10	13
JP59	17	17	16	16	23	25	X	Y	13	13	30	32.2	12	16	11	13	8	12	10	11
JP60	15	16	14	18	21	23	X	Y	12	13	29	32.2	14	15	11	12	11	13	8	10
JP61	15	19	15	16	22	24	X	Y	10	16	30	30	14	21	10	11	8	11	11	12
JP62	16	17	18	19	23	23	X	Y	14	16	29	32.2	13	13	8	11	8	11	8	10
JP63	15	15	14	14	21	24	X	Y	11	14	28	31	13	15	10	12	8	11	12	12
JP64	15	16	14	17	21	22	X	Y	9	13	29	30	14	23	11	13	8	8	11	12
JP65	15	16	17	18	19	22	X	Y	13	14	30	30	14	14	11	12	11	11	12	12
JP66	15	16	16	16	21	23	X	X	12	13	30	30	13	16	11	13	10	11	11	12
JP67	15	17	14	16	20	23	X	Y	13	15	29	32.2	13	20	11	11	8	11	11	12
JP68	15	15	14	16	25	25	X	Y	13	13	29	30	15	15	12	12	8	8	10	11
JP69	17	18	17	18	18	22	X	Y	11	11	28	31	14	16	11	12	8	8	12	12
JP70	15	15	17	17	21	23	X	Y	13	13	28	30	13	16	9	10	9	12	10	10
JP71	15	17	15	18	20	21	X	Y	12	13	31	31.2	15	16	9	12	12	12	10	12
JP72	15	16	17	18	21	23	X	X	12	14	30	31	15	19	13	13	9	11	11	12
JP73	15	16	17	18	22	23	X	Y	11	16	30	31.2	15	15	11	11	10	11	11	12
JP74	14	16	16	16	19	23	X	Y	11	12	30	32.2	13	15	10	11	11	12	8	12
JP75	16	16	17	18	23	23	X	Y	13	15	30	33.2	17	23	9	11	9	11	10	12
JP76	16	17	14	14	21	23	X	Y	14	17	29	32.2	16	17	12	12	8	12	11	11
JP77	16	17	17	19	22	23	X	Y	11	12	28.2	29	14	15	12	12	8	10	8	12
JP78	17	18	14	17	21	25	X	Y	13	13	31	33.2	12	13	11	12	9	12	11	12
JP79	15	16	14	17	22	22	X	X	14	14	32	34.2	14	16	10	13	10	12	11	12
JP80	17	17	16	17	20	23	X	X	9	14	30	31	13	16	10	11	8	11	10	11
JP81	16	17	18	18	18	21	X	Y	12	13	30	32.2	13	13	10	13	9	11	11	12
JP82	16	16	16	16	25	25	X	X	13	15	29	30	14	20	9	12	8	10	8	10
JP83	17	18	14	17	23	25	X	Y	14	18	30	31.2	16	23	11	11	9	10	11	12
JP84	15	17	14	14	20	23	X	Y	14	15	30	30	15	16	10	10	8	11	11	12
JP85	15	15	16	18	21	22	X	Y	10	13	32.2	34	13	20	10	11	11	12	11	12
JP86	15	15	16	20	23	24	X	Y	10	13	29	30	13	15	9	11	8	9	10	12
JP87	18	18	16	18	23	24	X	Y	10	14	30	31	17	20	12	12	8	9	10	11
JP88	15	16	15	18	20	20	X	Y	12	14	28	30	14	14	11	12	8	8	10	11
JP89	15	17	17	17	21	23	X	Y	12	13	30	31	14	15	12	12	8	12	12	12
JP90	15	16	17	18	21	24	X	Y	12	13	28.2	30	14	14	11	14	9	10	11	12
JP92	13	15	14	14	19	23	X	Y	14	17	30	30	14	15	11	13	8	11	8	8
JP93	16	17	14	14	22	24	X	Y	11	11	29	30	16	18	10	11	9	9	8	8
JP94	16	17	16	19	22	23	X	Y	15	16	30	32.2	14	20	11	13	8	8	10	11
JP95	15	17	17	18	23	25	X	Y	11	12	29	29	11	15	10	12	9	12	11	12
JP96	16	17	14	18	22	23	X	Y	11	11	29	30	13	15	10	10	11	11	11	12
JP97	16	16	14	17	22	24	X	X	12	13	30	33.2	14	19	12	13	8	11	10	11
JP98	15	17	16	16	23	25	X	X	14	14	30	33.2	16	21	10	12	11	11	11	11
JP99	16	17	14	17	21	24	X	Y	11	11	31	33.2	15	16	10	11	11	12	8	10
JP100	15	16	17	17	20	21	X	Y	10	15	30	32	15	17	12	13	11	12	11	11
JP101	15	17	19	19	22	23	X	Y	13	15	31	33.2	14	16	11	11	8	10	10	10
JP102	15	16	18	20	23	25	X	Y	9	14	29	31.2	12	13	12	13	9	13	9	11
JP103	16	16	14	16	24	25	X	Y	10	13	29	31	13	14	9	12	11	14	8	11
JP104	15	16	18	20	23	25	X	Y	9	14	29	31.2	12	13	12	13	9	13	9	11
JP105	15	16	18	19	21	23	X	X	12	16	29	30	14	15	10	14	8	11	8	12
JP106	14	15	14	17	23	24	X	Y	13	15	31.2	32.2	16	22	9	13	9	12	9	11
JP107	15	17	16	17	21	21	X	Y	12	15	29	29	14	23	11	11	11	13	10	10
JP108	15	16	18	18	20	21	X	Y	10	13	30	30	13	14	11	11	8	10	9	11
JP109	15	17	16	19	22	23	X	X	10	13	31.2	33.2	16	20	10	12	7	8	11	11

Individual	D3S1358	D3S1359	VWA	VWA	FGA	FGA	AMEL	AMEL	D6S1179	D6S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
JP110	15	17	17	19	21	23	X	X	12	13	30	32.2	14	16	12	13	10	11	8	11
JP111	16	16	15	18	22	23	X	X	13	13	30	32.2	14	15	11	11	8	11	10	12
JP112	15	15	15	17	22	23	X	Y	13	14	29	29	13	14	9	12	8	9	11	12
JP113	15	15	18	19	23	23	X	X	13	16	29	32.2	15	15	12	12	8	11	10	11
JP114	15	16	16	17	22	26	X	X	14	15	32	32.2	13	16	11	12	8	12	11	11
JP115	15	17	15	17	22	23	X	X	13	14	29	29	12	17	11	12	11	12	10	12
JP116	16	17	17	17	23	23	X	Y	10	14	31	32.2	15	15	10	13	8	8	11	11
JP117	17	18	17	18	23	25	X	Y	13	16	29	30	15	15	10	13	8	8	10	14
JP118	16	18	14	17	18	21	X	X	11	15	29	32.2	15	17	10	11	8	11	8	10
JP119	15	17	14	14	20	21	X	Y	10	10	30	32.2	13	14	10	10	10	12	10	12
JP120	15	16	14	14	23	24	X	Y	10	15	30	30	13	14	10	11	8	12	8	11
JP121	15	16	16	18	20	26	X	Y	10	11	28	30	15	15	9	11	8	12	8	11
JP122	15	17	17	17	23	24	X	Y	14	16	30	31	12	14	10	13	8	12	11	12
JP123	16	17	16	19	19	25	X	Y	13	13	30	30	14	16	11	11	9	11	10	10
JP124	15	15	14	18	20	23	X	Y	13	15	29	31	16	16	10	11	9	12	10	11
JP125	16	17	16	17	23	24	X	Y	13	15	30	30	13	14	10	11	10	13	10	12
JP126	16	16	17	19	22	26	X	Y	12	14	29	31	17	23	10	11	9	12	10	13
JP127	15	17	16	16	18	21	X	Y	10	13	31.2	32.2	13	15	11	11	8	9	11	11
JP128	16	17	14	20	20	21	X	Y	12	13	28	30	16	19	11	12	8	11	9	12
JP129	15	18	16	17	19	21	X	X	10	14	29	31	12	17	11	13	10	11	10	10
JP130	15	18	16	18	21	23	X	Y	15	16	30	30	12	18	11	13	8	14	11	12
JP131	16	16	17	17	23	23	X	Y	10	13	29	30	12	15	10	12	9	10	8	8
JP132	15	16	17	18	19	20	X	Y	10	10	29	30	14	16	13	13	12	12	11	12
JP133	15	17	14	17	19	22	X	Y	14	15	29	31.2	13	14	11	11	8	12	8	11
JP134	15	16	15	18	22	23	X	Y	11	11	29	32.2	12	14	9	13	8	8	11	11
JP135	15	18	16	17	22	23	X	Y	12	13	30	33	13	14	11	11	8	13	12	12
JP136	16	16	14	17	20	22	X	Y	11	13	29	32.2	12	14	10	11	11	12	12	12
JP137	15	17	17	18	23	23	X	Y	11	13	29	32.2	14	15	13	13	9	11	11	11
JP138	15	17	14	18	20	21	X	Y	12	13	29	31	14	15	11	11	8	8	8	11
JP139	15	18	14	18	22	23	X	Y	10	16	31	31	13	16	11	12	9	13	10	11
JP140	15	17	16	19	23	24	X	Y	13	15	30	33.2	13	14	9	11	8	11	11	12
JP141	15	16	17	17	22	25	X	Y	14	16	30	31	13	13	11	15	8	13	9	10
JP142	17	18	17	19	20	22	X	Y	10	15	29	29	13	18	11	13	10	11	12	12
JP143	14	16	17	18	21	21	X	Y	13	14	28	32.2	16	18	10	14	8	10	10	13
JP144	15	15	17	19	20	22	X	Y	14	14	31	32.2	12	13	11	12	10	11	11	12
JP145	16	16	14	18	20	24	X	Y	11	14	31.2	32	12	14	9	13	9	11	12	12
JP146	15	15	14	17	21	23	X	Y	13	14	30	30	13	19	11	12	8	12	12	12
JP147	16	17	14	16	24	25	X	Y	11	14	29	32.2	15	16	10	13	8	8	8	11
JP148	15	17	16	17	23	24	X	Y	14	14	28	31	15	19	9	13	12	12	12	12
JP149	16	16	17	17	21	23	X	Y	10	13	30	31	14	15	11	13	8	9	10	11
JP150	15	17	14	17	23	23	X	Y	13	14	30	32.2	15	20	9	12	11	12	11	11
JP151	16	17	17	17	19	25	X	Y	12	14	30	32.2	14	14	10	14	11	12	8	10
JP152	14	17	17	17	18	21	X	Y	14	15	30	30	14	14	9	12	11	12	11	11
JP153	15	17	17	18	20	20	X	Y	12	13	30	30	12	15	11	14	9	13	8	10
JP154	16	18	14	14	20	23	X	Y	11	13	30	32.2	13	18	13	13	8	9	11	13
JP155	15	15	17	19	21	22	X	Y	10	15	29	32.2	13	13	11	13	11	12	11	11
JP156	15	15	14	17	22	29	X	Y	14	14	29	32.2	14	14	9	12	8	13	10	11
JP157	15	17	16	17	22	23	X	Y	13	14	29	31	12	13	13	13	11	11	11	12
JP158	16	16	14	18	22	24	X	Y	10	11	31	32.2	15	17	10	10	9	12	11	14
JP159	16	17	18	18	18	26	X	Y	12	15	28	33.2	15	25	11	11	8	10	10	11
JP160	15	15	17	19	22	24	X	Y	14	14	30	31.2	19	21	10	12	8	14	9	10

Individual	D3S1358	D3S1358	vWA	vWA	FGA	FGA	AMEL	AMEL	D8S1179	D8S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
JP161	15	15	16	18	19	25	X	Y	11	14	29	32.2	15	16	10	12	12	14	10	12
JP162	15	15	14	17	22	23	X	Y	13	13	30	31	14	16	11	12	10	14	9	10
JP163	16	17	17	17	19	22	X	Y	13	16	32.2	32.2	13	15	10	12	8	11	8	9
JP164	15	18	14	17	21	23	X	Y	10	11	29	31.2	15	17	9	11	9	11	10	12
JP165	15	17	14	18	19	22	X	Y	10	15	29	30	13	16	11	12	11	11	11	12
JP166	15	15	16	18	20	24	X	Y	14	14	30	30	14	15	10	10	8	12	9	11
JP167	15	16	16	18	21	22	X	Y	10	10	29	31.2	18	19	11	11	11	12	7	10
JP168	16	18	14	17	21	23	X	Y	13	13	28	30	14	15	11	12	9	11	8	12
JP169	15	16	17	18	18	23	X	Y	13	13	28	32.2	13	16	11	13	9	11	11	11
JP170	15	17	17	19	19	26	X	Y	14	14	29	29	14	14	10	11	11	12	8	11
JP171	14	15	17	19	18	20	X	Y	13	15	29	29	13	13	10	12	9	11	11	12
JP172	15	17	16	16	22	24	X	Y	15	15	28	33.2	14	18	12	13	9	12	11	13
JP173	15	16	16	17	20	22	X	Y	13	14	29	30	14	20	11	13	8	11	8	12
JP174	15	15	18	19	21	22	X	Y	11	13	30	31.2	15	21	10	13	8	10	10	11
JP175	15	16	14	16	21	23	X	X	13	14	29	30	17	21	9	12	12	12	8	11
JP176	16	17	16	18	21	23	X	Y	12	13	29	31.2	15	16	11	11	8	12	11	11
JP177	15	16	18	20	23	23	X	Y	11	11	30	32.2	14	17	11	12	8	12	8	10
JP178	15	17	14	18	21	24	X	X	14	15	30	30	14	15	11	11	11	11	11	12
JP179	15	15	15	16	18	22	X	X	13	15	30	30	15	19	11	13	8	12	9	13
CH5100	16	18	14	14	22	25	X	X	15	15	29	32.2	13	14	7	13	11	11	12	12
CH5101	17	17	14	17	24	24	X	Y	10	13	29	30	15	21	10	13	8	9	11	11
CH5102	16	16	14	14	20	23	X	X	11	14	28	31.2	15	16	10	13	10	11	10	12
CH5103	15	17	16	17	22	23	X	Y	10	12	28	32.2	13	14	11	11	8	12	10	12
CH5104	15	16	16	17	21	25	X	X	10	16	28	32.2	13	16	12	13	10	11	11	13
CH5105	16	16	16	19	23	23	X	Y	13	16	29	30	18	21	9	13	8	12	8	11
CH5106	16	17	15	18	22	22	X	Y	12	14	29	31.2	15	18	7	11	8	9	11	11
CH5107	17	17	17	18	21	26	X	Y	11	15	30	30	14	15	11	13	8	11	7	8
CH5108	16	16	16	16	23	25	X	X	10	13	30	32	14	16	10	11	11	12	11	11
CH5109	15	16	16	18	23	24	X	Y	10	15	29	33.2	13	14	10	13	9	10	11	12
CH5110	17	17	18	19	22	25	X	X	13	13	29	31.2	14	16	9	12	9	13	10	11
CH5111	15	16	14	14	22	24	X	Y	14	15	32.2	32.2	15	18	7	12	12	13	9	12
CH5112	13	16	17	17	21	22	X	X	10	13	29	31	14	16	10	10	8	11	10	11
CH5113	15	16	18	18	21	23	X	Y	14	15	29	30	15	16	11	12	8	8	9	10
CH5114	17	17	14	14	24	24	X	X	10	10	30.2	32.2	15	15	10	12	10	12	8	9
CH5115	16	16	17	18	19	25	X	Y	11	12	31	31.2	15	15	10	12	8	13	8	10
CH5116	14	16	14	14	19	21	X	X	10	12	31.2	32.2	15	15	11	12	9	10	10	11
CH5117	16	17	14	17	21	22	X	Y	13	15	29	29	12	20	10	11	8	10	11	11
CH5118	15	17	16	18	21	24	X	X	14	16	30	32.2	14	15	12	13	9	11	12	13
CH5119	15	17	17	19	21	22	X	X	10	11	29	30	16	23	11	12	10	11	11	11
CH5120	16	19	16	16	23	23	X	Y	13	15	29	31	16	17	12	13	8	9	10	11
CH5121	17	17	14	16	18	26	X	X	11	13	29	30	15	15	11	13	8	8	12	12
CH5122	13	15	14	17	22	24	X	Y	10	13	31.2	32.2	15	17	9	13	8	9	10	11
CH5123	16	16	14	17	22	26	X	X	10	13	32.2	33	14	15	12	17	13	13	8	8
CH5124	16	17	14	18	22	23	X	Y	14	16	29	30	14	21	12	13	8	9	11	12
CH5125	16	16	17	18	18	23	X	X	11	13	32.2	32.2	16	17	11	12	10	10	8	11
CH5126	16	17	15	17	21.2	24	X	Y	14	17	29	31	13	14	9	13	10	12	10	12
CH5127	15	18	16	19	22	26	X	X	13	15	28	29	16	19	10	11	8	11	8	10
CH5128	15	16	14	18	23	23	X	Y	10	11	29	30	15	20	10	12	8	11	8	11
CH5129	16	17	14	16	22	23	X	X	13	15	28	31.2	13	15	10	12	10	11	11	12

Individual	D3S1358	D3S1358	vWA	vWA	FGA	FGA	AMEL	AMEL	D8S1179	D8S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
CH5130	15	17	16	16	18	23	X	Y	10	13	29	29	16	17	10	12	12	13	12	12
CH5131	16	16	14	14	21	24	X	Y	11	15	32.2	33.2	13	14	11	12	10	12	10	11
CH5132	15	18	16	16	19	23	X	X	13	14	30	32.2	13	14	11	13	8	12	11	13
CH5133	15	16	14	18	22	25	X	Y	13	14	30	31.2	12	15	13	13	8	11	10	10
CH5134	15	15	14	17	22	23	X	X	15	15	29	29	14	15	9	13	9	10	8	11
CH5135	15	16	14	16	21	24	X	Y	12	15	29	30	13	14	11	12	8	14	10	12
CH5136	16	16	14	17	24	24	X	X	11	14	30	31	15	19	7	10	11	14	11	11
CH5137	16	17	14	20	19	26	X	Y	10	10	31	32.2	14	14	11	13	8	8	11	12
CH5138	15	16	14	16	17	19	X	X	11	16	29	32	18	19	10	11	10	12	12	13
CH5139	16	17	14	15	22	25	X	Y	14	15	29	30	14	17	11	11	11	11	11	12
CH5140	16	18	17	18	22	24	X	X	10	15	28	31.2	13	15	11	13	10	12	11	12
CH5141	15	16	16	18	22	23	X	Y	11	11	29	31	13	15	10	11	11	12	8	11
CH5142	15	15	17	18	23	24	X	X	11	15	28	30	17	18	11	11	10	12	11	11
CH5143	15	17	18	18	23	24	X	X	14	15	28	30	13	15	9	10	9	10	8	12
CH5144	15	16	14	16	19	25.2	X	X	10	14	30	30	13	15	11	11	8	11	10	10
CH5145	15	17	14	18	19	24	X	Y	10	12	29	32.2	17	23	10	11	9	12	10	11
CH5146	15	15	16	19	18	23	X	X	10	11	30	30.3	13	15	11	12	10	12	11	12
CH5147	15	15	19	19	19	24	X	Y	12	16	31.2	32.2	15	15	12	12	8	8	8	8
CH5148	15	18	14	18	19	24	X	X	11	11	30	32.2	15	24	11	12	9	11	11	12
CH5149	15	15	17	19	21	22	X	Y	10	17	29	30	15	19	9	11	8	9	11	11
VT5200	12	16	16	19	22	27	X	X	15	16	30	31	14	15	10	11	8	8	8	8
VT5201	16	17	14	18	19	23	X	X	14	16	29	32	13	13	12	13	8	11	10	12
VT5202	17	17	18	20	19	20	X	X	10	11	31.2	32.2	14	16	10	11	8	12	11	11
VT5203	16	18	16	18	22	26	X	X	12	12	27	32	13	15	11	12	9	11	10	11
VT5204	15	15	17	17	19	21	X	Y	12	15	30	33.2	14	25	9	11	8	10	8	10
VT5205	15	15	17	17	22	23	X	Y	13	15	30	32	14	17	10	10	8	12	9	14
VT5206	15	16	17	18	19	22	X	Y	11	15	29	31.2	14	15	10	13	8	10	11	11
VT5207	15	16	16	16	19	24	X	Y	12	13	29	33	18	25	10	10	9	11	8	11
VT5208	15	16	16	19	22	25	X	Y	12	16	30	30.2	13	14	9	11	8	12	8	10
VT5209	16	18	18	18	22	26	X	Y	13	17	30	30	12	18	11	12	11	11	11	12
VT5210	16	17	17	19	21.2	23.2	X	X	11	11	29	29	16	16	11	12	9	11	8	11
VT5211	15	17	14	19	23	27	X	X	14	14	29.2	30	13	21	9	12	8	10	11	11
VT5212	15	17	14	18	21	23	X	Y	10	11	31.2	33.2	15	15	10	12	10	14	10	10
VT5213	15	17	16	17	21	22	X	X	13	13	29.2	30	15	15	10	13	8	9	10	12
VT5214	15	16	17	17	22	24.2	X	X	14	14	30	30	13	15	7	13	11	12	12	13
VT5215	15	17	16	17	22	23	X	X	10	13	30	32.2	12	17	10	11	8	10	11	12
VT5216	15	17	14	14	22	22	X	Y	10	12	32.2	32.2	12	14	12	12	8	10	8	10
VT5217	14	15	16	17	23	26	X	X	11	14	29	29	14	16	12	13	11	12	11	12
VT5218	15	18	14	18	22	22	X	Y	12	16	29	29	14	21	10	13	8	10	8	9
VT5219	15	17	14	17	21	22	X	X	10	11	30	32.2	14	16	11	13	8	11	9	10
VT5220	16	16	17	20	19	23	X	Y	11	12	29	31	14	15	11	13	8	8	8	12
VT5221	16	17	14	18	25	26	X	X	11	14	31.2	32	14	15	12	12	8	8	11	13
VT5222	16	17	14	14	23	24	X	Y	12	15	29	30	14	16	10	10	8	8	11	11
VT5223	15	15	16	16	22	22	X	X	11	15	32.2	32.2	13	15	13	13	8	11	11	12
VT5224	17	17	14	18	22	25.2	X	Y	10	11	29	32.2	13	16	10	13	11	12	12	13
VT5225	16	17	14	18	22	22	X	X	12	14	29	32.2	13	19	10	10	8	8	7	9.1

Individual	D3S1358	D3S1358	vWA	vWA	FGA	FGA	AMEL	AMEL	D8S1179	D8S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
VT5226	15	15	14	17	21	24	X	Y	13	15	28	30	13	14	13	13	11	11	12	12
VT5227	16	17	16	17	17	18	X	X	11	15	30	30	15	16	10	11	8	11	11	12
VT5228	15	16	14	14	22	25	X	Y	14	16	30	32.2	12	14	9	13	8	9	9	11
VT5229	16	16	14	18	20	22	X	X	14	16	32.2	33	12	14	11	11	11	12	7	12
VT5230	15	17	17	18	19	23	X	Y	11	13	31.2	33.2	15	16	7	12	9	13	11	11
VT5231	16	17	14	18	20	25	X	X	10	14	30	30	13	16	11	12	8	8	9	11
VT5232	15	18	15	18	22	23	X	Y	13	14	29	31	13	15	9	13	8	9	8	13
VT5233	14	16	14	17	22	27	X	X	12	13	28	29	13	13	9	10	8	8	8	12
VT5234	16	17	14	18	18	23.2	X	X	13	13	29	29	14	15	10	11	10	12	11	13
VT5235	15	15	17	17	24	25	X	Y	13	15	29	31	14	15	9	11	10	11	9	10
VT5236	15	15	17	18	20	22	X	Y	11	13	30	32	12	18	10	13	8	9	8	10
VT5237	15	16	18	19	22	23	X	X	10	16	32.2	32.2	13	16	11	12	8	12	10	11
VT5238	16	17	18	19	24	26	X	X	14	14	29	30	13	13	10	12	10	11	8	11
VT5239	15	16	16	18	22	23	X	Y	12	15	30	31	15	16	10	13	11	12	11	11
VT5240	14	17	14	18	19	21	X	Y	13	15	29	32.2	15	21	7	12	8	11	12	12
VT5241	16	17	17	18	20	22	X	Y	11	12	29	30	14	18	11	13	11	12	9	12
VT5242	15	15	17	17	20	24	X	X	10	12	30	31	13	20	12	13	10	11	12	14
VT5243	16	17	16	17	21	24	X	Y	15	16	29	29	12	15	12	13	11	12	10	11
VT5244	15	16	17	17	20	21	X	X	10	12	29	34.2	16	16	11	11	8	12	10	13
VT5245	16	16	17	17	20	24	X	X	11	12	28	29	13	17	11	11	9	13	11	11
VT5246	15	18	14	16	21	24	X	Y	13	13	27	30	16	18	11	12	10	12	8	10
VT5247	16	17	17	19	23	25	X	X	10	16	33.2	33.2	14	17	10	12	8	10	8	10
VT5248	15	17	14	18	19	23	X	Y	11	15	28	30	19	22	11	11	8	8	9	11
VT5249	15	16	18	20	23	24	X	Y	15	16	29	31.2	14	16	11	11	8	11	8	10
BG5300	15	15	19	19	19	23	X	Y	13	16	29	33.2	13	14	9	11	8	8	10	11
BG5301	16	16	16	18	22	23	X	X	10	11	28	29	14	21	11	12	11	14	8.1	11
BG5302	15	15	17	19	23	26	X	Y	13	14	29	30.2	14	16	11	13	9	14	8	12
BG5303	15	18	14	16	22	23	X	X	11	13	30	32.2	14	16	10	13	9	12	8	11
BG5304	15	16	16	17	22	23	X	Y	11	14	29	29	13	14	11	12	9	11	11	12
BG5305	15	16	17	18	21	24	X	X	13	16	28	31.2	13	15	13	13	8	8	9	10
BG5306	16	16	16	16	21	24	X	X	10	16	28	29	14	14	10	10	9	12	9	11
BG5307	16	16	17	19	23	24	X	X	10	11	28	30	11	20	12	13	10	12	11	12
BG5308	15	16	18	18	21	24	X	X	14	15	28	29	13	18	11	13	8	8	10	12
BG5309	15	17	17	17	22	24	X	X	11	12	29	29	18	22	10	13	10	12	11	13
BG5310	14	15	18	19	20	24	X	Y	13	13	32.2	33.2	14	21	11	13	8	12	10	12
BG5311	16	17	14	16	24	28	X	X	10	14	30.2	31.2	16	17	11	11	11	12	10	12
BG5312	15	16	17	17	19	22	X	Y	12	13	29	30	10	19	13	13	11	13	9	10
BG5313	14	17	15	17	19	21	X	X	12	16	31.2	32.2	15	16	10	11	10	12	8	9
BG5314	15	15	14	15	21	24	X	X	12	15	30	31	13	17	11	11	12	12	11	11
BG5315	15	15	17	18	19	24	X	X	10	15	28	31.2	14	18	13	13	11	13	11	12
BG5316	15	18	18	19	21	26	X	X	10	15	32.2	32.2	17	19	12	12	7	11	11	13
BG5317	15	15	15	18	22	23	X	X	13	13	28	31.2	16	16	11	13	8	10	8	10
BG5318	15	15	16	17	24	26	X	X	10	14	30.2	33.2	17	18	10	11	8	11	8	10
BG5319	15	15	14	18	21	23	X	Y	15	15	29	29	12	13	11	12	8	8	7	8
BG5320	16	16	14	18	17	20	X	X	10	14	27	30	15	16	11	12	9	10	10	12
BG5321	15	16	16	17	17	20	X	Y	10	12	30	31.2	16	17	10	13	8	9	8	8
BG5322	16	17	15	17	23	25	X	Y	10	13	30	30.2	13	15	9	10	8	12	10	11

Individual	D3S1358	D3S1358	VWA	VWA	FGA	FGA	AMEL	AMEL	D8S1179	D8S1179	D21S11	D21S11	D18S51	D18S51	D5S818	D5S818	D13S317	D13S317	D7S820	D7S820
BG5323	15	16	14	16	21	23	X	X	12	16	28	32.2	14	16	11	13	8	11	8	10
BG5324	16	18	16	17	21	25	X	X	13	15	29	33.2	14	16	11	11	11	11	10	12
BG5325	18	18	14	17	21	25	X	Y	8	15	32.2	33.2	14	23	10	11	10	12	8	10
BG5326	15	15	14	18	19	24	X	X	13	13	31	33.2	16	19	11	11	11	11	8	12
BG5327	15	15	17	18	22	24	X	Y	15	15	29	29	14	15	11	12	10	14	9	10
BG5328	15	17	17	17	20	24	X	X	11	13	29	30	12	15	11	11	11	13	10	13
BG5329	15	17	16	17	24	24	X	Y	10	11	28	29	19	22	10	12	8	10	8	10
BG5330	15	18	16	18	23	25	X	X	14	15	29	31.2	11	12	11	13	9	12	10	12
BG5331	16	16	17	17	22	24	X	Y	14	14	28	33.2	15	15	10	13	10	12	8	12
BG5332	15	16	14	19	20	23	X	X	12	15	30	32.2	15	15	11	12	11	12	8	10
BG5333	16	18	14	20	20	25	X	Y	12	16	31.2	32.2	13	16	11	11	8	11	8	12
BG5334	15	18	14	17	20	25	X	Y	12	16	30	30.2	13	14	12	12	8	10	10	12
BG5335	15	15	15	17	23	23	X	X	10	15	31	31.2	13	14	10	12	8	12	8	10
BG5336	16	16	17	18	20	23	X	Y	13	14	29	32.2	15	16	9	12	9	9	12	12
BG5337	15	17	17	18	23	24	X	X	10	13	28	30	16	17	9	11	10	12	8	9
BG5338	15	16	14	16	22	25	X	X	10	11	27	30	14	14	11	14	8	12	11	11
BG5339	15	15	14	18	22	22	X	X	12	16	30	33.2	16	16	11	12	9	11	10	13
BG5340	15	16	14	18	22	23	X	Y	13	16	29	30	11	19	11	12	9	12	8	10
BG5341	15	16	16	17	20	25	X	X	12	12	28	29	15	16	11	13	8	12	8	8
BG5342	17	17	17	18	19	26	X	Y	12	14	30	32.2	14	15	10	10	12	12	10	11
BG5343	15	18	16	17	22	25	X	X	14	15	30	31.2	15	16	12	12	8	13	7	7
BG5344	15	16	14	18	21	22.2	X	Y	10	15	29	29	14	17	12	12	8	12	10	10
BG5345	14	16	16	17	24	26	X	X	10	17	28	28	12	15	11	11	11	12	12	12
BG5346	16	17	17	18	19	23	X	Y	13	14	29	31.2	14	15	11	11	8	10	11	12
BG5347	15	17	16	19	19	19	X	Y	10	14	28	29	11	15	12	13	8	11	8	10
BG5348	17	18	14	17	22	22	X	Y	10	14	28	28	13	14	11	12	8	10	11	11
BG5349	17	17	16	18	20	23	X	Y	10	14	29	32.2	15	15	11	13	7	11	10	11

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