POPULATION GENETICS OF HUMAN SHORT TANDEM REPEATS

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Background of Study

As forensic DNA lab topics have become popular in high schools, colleges and universities, offering a professional-level forensic DNA lab still faces technical and financial challenges. In this study we attempt to overcome these issues in order to provide concepts in molecular biology, population genetics, and mathematics typically found in a professional forensic DNA procedure. We choose ten of the 13 FBI designated microsatellite markers from its Combined DNA Index System (CODIS, Table 1, Butler, 2005). These markers, also known as short tandem repeats (STRs) in the forensic community, are DNA sequences comprised of tetra-nucleotide repeat units and are located in various sites on human chromosomes. In addition to STR markers, we are also interested in using the amelogenin marker to identify gender of the sample donors (Sasaki & Shimokawa, 1995).

Table 1. Ten STR loci included in the pilot study.

Locus	Chromosome location	Generalized repeat unit	Allele range
TPOX	2p25.3	AATG	4-16
D3S1358	3p21.31	TCTA, TCTG	8-20
FGA	4q29	TTTC, CTTC, CTTT	12.2-51.2
CSF1PO	5q33.1	AGAT	5-16
D7S820	7q21.11	GATA	5-16
D8S1179	8q24.13	TCTA, TCTG	7-20
TH01	11p15.5	AATG	3-14
VWA	12p13.31	TCTA, TCTG	10-25
D13S317	13q31.1	TATC	5-17
D16S539	16q24.1	GATA	4-16

The use of an appropriate DNA extraction kit can potentially overcome the need of DNA quantification and can simplify the extraction procedure. If a fixed amount of magnetic resin beads (for example, from DNA IQ™ System, Promega Corporation) is saturated with DNA molecules and excess DNA is washed away, the released DNA during elution should fall within the range of ideal concentrations for use in the PCR process.

The extraction procedure can be further simplified by the magnetism of the beads when used with a magnetic stand. This allows instant separation of beads and solution without centrifugation (Fig. 1).

Figure 1. A magnetic stand with sample containing magnetic beads.

To lower the costs on multiplexing, we designed forward and reverse primers based on sequences published by Promega Corporation (Butler, 2009) and to be manufactured by Integrated DNA Technologies. As this study involves human subjects and the results are intended for publication, the Institutional Review Board has approved the protocol to safeguard student's privacy.

Methods

The two-week pilot study was conducted in February 2009 in BioG 1104, a freshman course for science majors at Cornell University. Student volunteers came from four lab sections, each taught by a teaching assistant trained for the procedure. Students must sign a consent form before being permitted to conduct DNA extraction. Students were assigned a sample code known only to themselves to be used for DNA sample identification and the code was required before individual students could retrieve their result.

In Week 1 students extracted DNA from their saliva using DNA IQ™ System (Promega Corporation). The use of magnetic stand and magnetic beads replaced centrifugation. Removal of cell debris and excess DNA was accomplished through a series of washes. After beads were dried, elution buffer was added to release DNA while magnetic beads remained as a pellet. PCR procedure was conducted by staff using Qiagen Multiplex PCR Kit. PCR products were submitted to genotyping services available at Cornell University where Applied Biosystems 3100 Genetic Analyzer was used for DNA fragment analysis and Applied Biosystems GeneMapper™ (v3.5) was used for fragment size determination. Student samples were compared with known genotypes from the author (K-CC) to designate alleles of each locus.

In week 2 the genetic profile of each sample was released to the student who presented the ID code. Based on Hardy-Weinberg equilibrium, students estimated the genotypic frequency of each of the ten loci. Using the multiplication rule, they estimated the random match probability of their genetic profile.

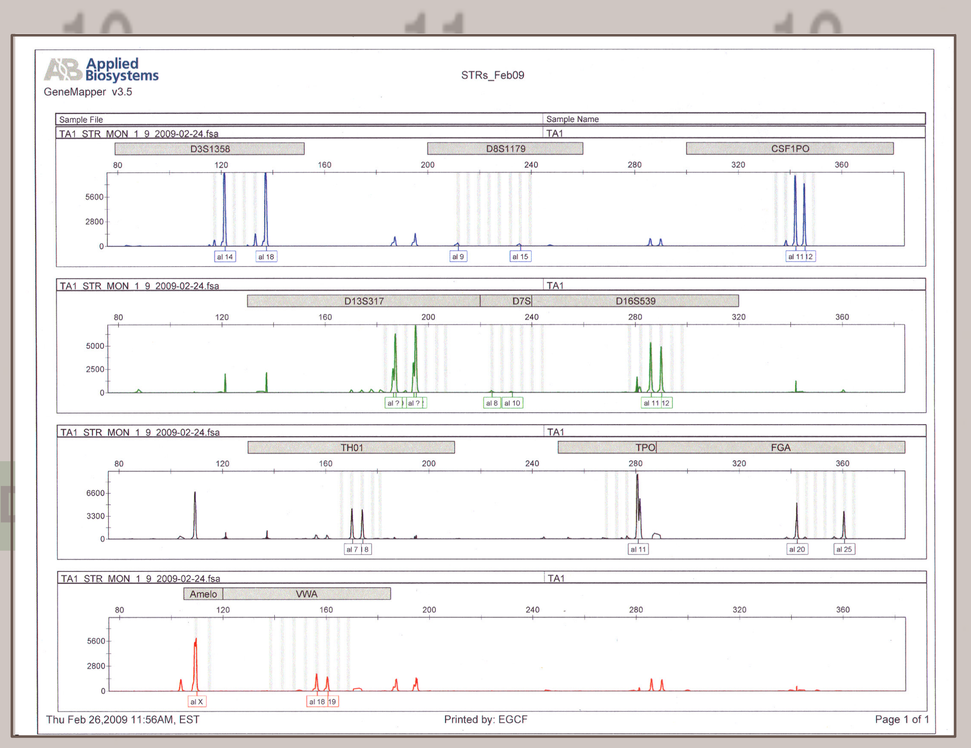

Figure 2. Electropherogram of ten STR loci and the Amelogenin locus. The loci are shown in bars above the peaks and the alleles are indicated as number below each peak.

Results and Discussion

All students have generated at least a partial profile of their genotype and most of the students produced a complete genetic profile of the ten markers. There was no indication of contamination. A representative electropherogram is shown in Figure 2. A typical full profile can reach a random match probability of 10^{-11}.

Barrier pipettor tips appeared to have prevented contamination between samples. High success rate was also contributed by PCR conducted by staff. Based on student suggestions, the future offering of this lab may include student preparation of PCR, DNA mixture analysis and paternity index determination (Evett & Weir, 1998). To expand this lab to a class of 500 students the average of costs for consumables and genotyping service is about $3/student.

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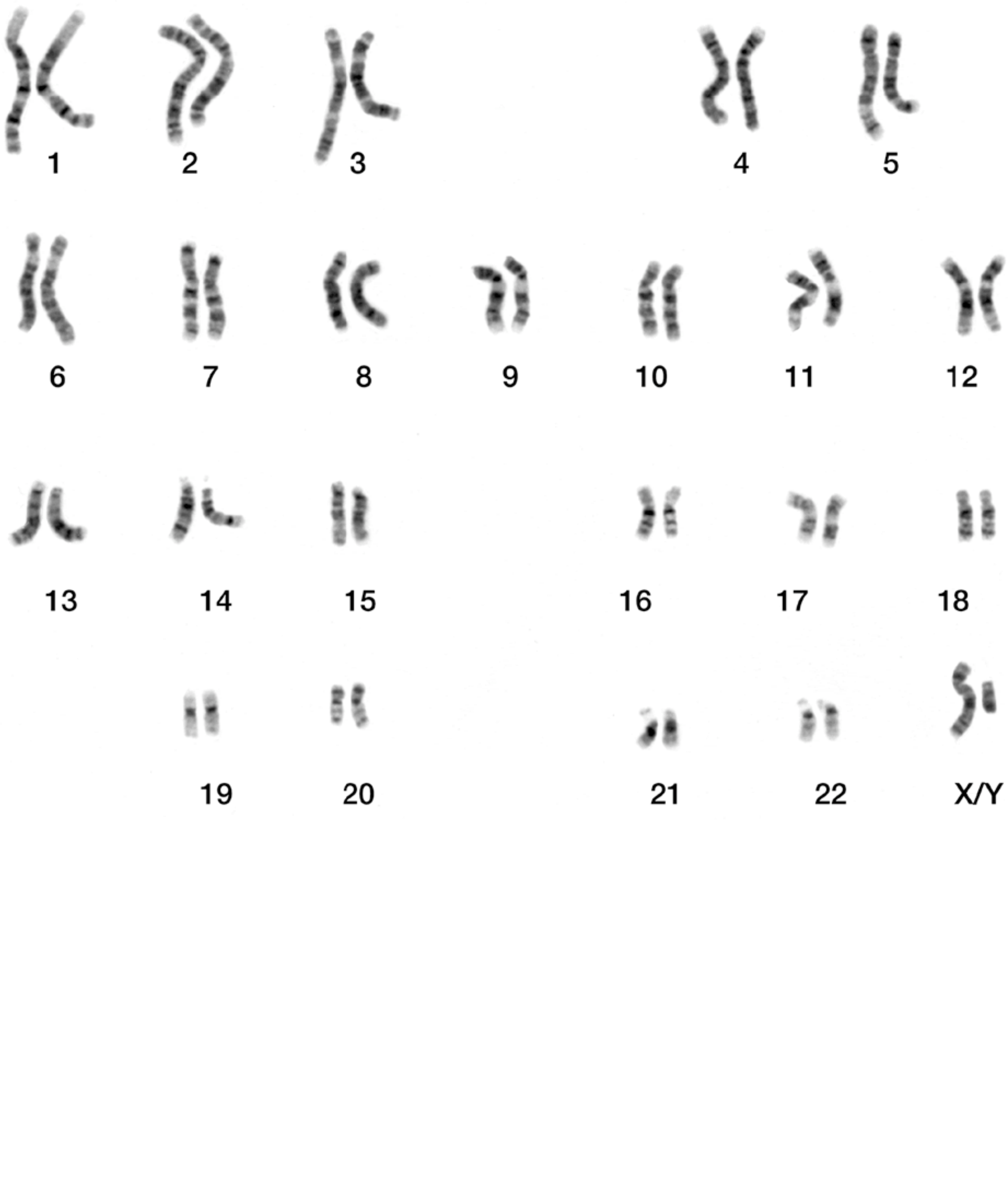
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