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Human Microsatellite DNA: Population Genetics and Forensic Application

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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 teach students concepts and techniques in molecular biology, population genetics, and mathematics typically encountered in a professional forensic DNA laboratory. Consented students extract their DNA and amplify ten of the 13 FBI designated microsatellite markers from its Combined DNA Index System (CODIS) and the amelogenin locus for sex determination. The CODIS microsatellite, commonly referred to as short tandem repeats (STRs) in the forensic community, are DNA sequences comprised of tetra-nucleotide repeat units and are located at various sites on human chromosomes. These loci are amplified via a multiplex PCR system with all 11 loci amplified simultaneously in one reaction. The fragment sizes of PCR products are determined by DNA Analyzer 3730 (Applied Biosystems, Inc.) and the results are analyzed with Peak Scanner v.1.0 (Applied Biosystems, Inc., free download).

A common requirement in PCR is the knowledge of the template DNA concentration so that the template DNA to primer ratio in the reaction is optimum. We bypass the step of quantifying template DNA by using magnetic beads together with a magnetic stand. The fixed amount of resin beads provides a near constant total surface area. Sufficient DNA from student cheek cells saturates the surfaces of the magnetic beads to ensure a near constant total DNA adsorbed to the magnetic beads. Using magnetic stand to pool magnetic beads to the bottom of the sample tube, the excess amount of DNA not adsorbed to the beads may be readily pipetted out and further eliminated by wash buffer. When the DNA is eluted from the beads, its yield should fall within the range of ideal concentrations for use in the PCR process.

Because the allele is determined by the size of the amplified DNA fragment, alleles from different loci with overlapping allelic sizes need to be distinguished by primers labeled with different florescent molecules. Standard touchdown PCR protocol produce fragments that are readily distinguished using DNA fragment analyzing instrument. To produce the genotyping chromatogram the free downloadable software Peak Scanner is used, and all alleles are represented as peaks. An example of a chromatogram is shown in Figure 1.

To make students understand how to calculate the random match probability, students will need to learn the principle of Hardy-Weinberg equilibrium of the frequency of the genotype at each locus. Students then using the product rule to multiply the genotypic frequency of the ten loci to produce a random match probability. This lab can be further expanded to deducing full genotypic profile of DNA samples contributed by two donors. Depending on student interest, paternity index can also be determined by a hypothetical mother-child-alleged father trio.

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Table 1. Primer sequences of ten STR markers. Notice one of each primer sequence pair is labeled with a fluorescent color dye as indicated in brackets.

Locus	Primers
TPOX	5’-[Yellow]-ACTGGCACAGAACAGGCACTTAGG-3’ 5’-GGAGGAACTGGGAACCACACAGGTTA-3’
D3S1358	5’-[Blue]-ATGAAATCAACAGAGGCTTGC-3’ 5’-ACTGCAGTCCAATCTGGGT-3’
FGA	5’-[Yellow]-GGCTGCAGGGCATAACATTA-3’ 5’-ATTCTATGACTTTGCGCTTCAGGA-3’
CSF1PO	5’-CCGGAGGTAAAGGTGTCTTAAAGT-3’ 5’-[Blue]-ATTTCCTGTGTCAGACCCTGTT-3’
D7S820	5’-[Green]-ATGTTGGTCAGGCTGACTATG-3’ 5’-GATTCCACATTTATCCTCATTGAC-3’
D8S1179	5’-ATGTTGGTCAGGCTGACTATG-3’ 5’-[Blue]-GATTCCACATTTATCCTCATTGAC-3
TH01	5’-[Yellow]-ATTCAAAGGGTATCTGGGCTCTGG-3’ 5’-GTGGGCTGAAAAGCTCCCGATTAT-3’
VWA	5’-[Red]-GCCCTAGTGGATGATAAGAATAATCAGTATGTG-3’ 5’-GGACAGATGATAAATACATAGGATGGATGG-3’
D13S317	5’-[Green]-GATTACAGAAGTCTGGGATGTGGAGGA-3’ 5’-GGCAGCCCAAAAAGACAGA-3’
D16S539	5’-[Green]-GGGGGTCTAAGAGCTTGTAAAAAG-3’ 5’-GTTTGTGTGTGCATCTGTAAGCATGTATC-3’

Figure 1. A typical chromatogram from ten microsatellite loci. The y-axis in each of the four panels represents the fluorescent unit as indication of quantity of DNA fragments carrying that specific color label. The x-axis is the size of fragments in base pairs and the color bar at top of each panel shows the range of the allele sizes in the population. The allele of each locus is shown as a peak with base pair size indicated in the box below the peak. All but TH01 locus are heterozygous. This sample is from a male as indicated by two peaks for the amelogenin (Amel) locus (bottom panel in red).

Selected References

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