Laboratory Protocol

INTRODUCTION

Although the DNA from different individuals is more alike than different, there are many regions of human chromosomes that exhibit a great deal of diversity. Such variable sequences are termed "polymorphic" (meaning many forms) and provide the basis for genetic disease diagnosis, forensic identification, and paternity testing. One class of polymorphism results from repeated copies of a DNA sequence that lie next to each other on the chromosome, like the cars of a train. Two common types of repeat polymorphisms are STRs (short tandem repeats) and VNTRs (variable number of tandem repeats). In each type, different numbers of repeats create alleles that differ in size.

British researcher Alec Jeffreys was the first to realize that DNA polymorphisms can be used to establish human identity. He coined the term DNA fingerprinting and was the first to use DNA polymorphisms in paternity, immigration, and murder cases. The discovery, in 1984, of the so-called "Jeffreys' probes" arose from the investigation of the "mini-satellite" fraction of highly repetitive DNA in the human genome.

Many repeat polymorphisms are highly polymorphic, having tens of different alleles. Repeat polymorphisms also typically exhibit a high level of heterozygosity, meaning that most individuals inherit different alleles from each parent. The repeat polymorphisms used in forensic biology are neutral mutations, found primarily in intergenic regions. However, di- and tri-nucleotide repeats are the causative mutations in Huntington disease, Alzheimer disease, and fragile X syndrome.

The Federal Bureau of Investigation (FBI) uses a 13-marker panel of STRs to establish an individual DNA fingerprint. With this number of independently inherited polymorphisms, the probability of even the most common combination is in the tens of billions. Thus, modern DNA testing has the capability to identify each person alive today. As of February 2007, the FBI's Combined DNA Index System (CODIS) contained 4,907,077 profiles and has produced over 45,400 hits assisting in more than 46,300 investigations.

This experiment examines a VNTR on chromosome 1 called D1S80 (or pMCT118), which has a repeat unit of 16 base pairs (bp). Most individuals have between 14 and 40 copies of the repeat on each copy of chromosome 1.

In this experiment, polymerase chain reaction (PCR) is used to amplify a DNA locus from chromosome 1 to determine the number of repeated DNA sequences within a highly variable region of the chromosome. To compare the genotypes from a number of individuals, aliquots of the amplified sample and a DNA molecular ladder are loaded into the wells of a DNA chip. Microfluidic electrophoresis separates and analyzes the PCR products. Following computer analysis, amplification products appear as distinct peaks with assigned base pair sizes on an electropherogram. Computerized microfluidic electrophoresis analyzes the size of PCR products. The base pair length of products can then be used to determine the specific number of repeats within each allele. The amplified samples and a DNA molecular ladder are also loaded into the wells of an agarose gel, to compare sensitivity and specificity of the two methods. One or two bands are visible in each lane, indicating the genotype for an individual.

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PROCEDURE

I. DNA Isolation by Saline Mouthwash

1. Pour 10 ml of the saline solution (0.9% NaCl) into mouth and vigorously swish for 30 seconds.

2. Expel saline solution into a paper cup.

3. Swirl to mix cells in the cup and use a micropipette to transfer 1 ml (1000 µl) of the liquid to 1.5-ml tube.

4. Place your sample tube, together with other student samples, in a balanced configuration in a microcentrifuge, and spin for 1 minute.

5. Carefully pour off supernatant into paper cup or sink. Be careful not to disturb the cell pellet at the bottom of the test tube. A small amount of saline will remain in the tube.

6. Resuspend cells in remaining saline by pipetting in and out. (If needed, 30 µl of saline solution may be added to facilitate resuspension.)

7. Use a micropipette to withdraw 30 µl of cell suspension, and add to tube containing 100 µl of Chelex. Shake well to mix.

8. Boil cell sample for 10 minutes. Use boiling water bath, heat block, or program thermal cycler for 10 minutes at 99°C. Then, cool tube briefly on ice (optional).

9. After boiling, shake tube. Place in a balanced configuration in a microcentrifuge, and spin for 1 minute.

10. Use a micropipette to transfer 30 µl of supernatant (containing the DNA) to clean 1.5 ml tube. Avoid cell debris and Chelex beads. This sample will be used for setting up one or more PCR reactions.

II. DNA Amplification by PCR

1. Use a micropipette with a fresh tip to add 18 µl of the D1S80 primer/loading buffer mix (without cresol red) to a PCR tube.

2. Use a micropipette with a fresh tip to add 2 µl of human DNA (from Part I) to reaction tube, and tap to mix. Pool reagents by pulsing in a microcentrifuge or by sharply tapping tube bottom on lab bench.

3. Label the cap of your tube with a number, as assigned by your teacher.

4. If required by the thermal cycler, add one drop of mineral oil on top of reactants in the PCR tube. Be careful not to touch the dropper tip to the tube or reactants, or subsequent reactions will be contaminated with DNA from your preparation. Note: Thermal cyclers with heated lids do not require use of mineral oil.

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5. Store all samples on ice until ready to amplify. Program thermal cycler for 30 cycles according to the following cycle profiles. Each program may be linked to a 4° C cycle to hold samples after completing the cycle profile, but amplified DNAs also hold well at room temperature.

    1. 94° C, 10 sec
    2. 94° C, 01 sec
    3. 68° C, 30 sec
    4. GOTO step 2, 29X
    5. 72° C, 30 sec
    6. 4° C ∞

IIIa. DNA Analysis by DNA Chip Electrophoresis

1. Use a micropipette with a fresh tip to add 1µl of PCR sample into your assigned well of a DNA chip. Place the tip directly against the bottom of the well and depress the pipet to the first stop only to avoid bubbles.

2. Once all wells are loaded, place DNA chip into the Bioanalyzer. When the Bioanalyzer recognizes the chip is in place, click the "Start" icon. Analyze results once available.

IIIb. DNA Analysis by Gel Electrophoresis

1. Use a micropipette with a fresh tip to add entire PCR sample/loading dye mixture into your assigned well of a 2% agarose gel. Expel any air from the tip before loading, and be careful not to push the tip of the pipet through the bottom of the sample well.

2. Load 5 µl of the 100bp size marker into one lane of each gel.

3. Electrophorese at 140 volts for 30-40 minutes. Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.

4. Gels will contain DNA stain and be ready to view with a UV transilluminator immediately. DNA fragments of identical size in the same lane will appear as a discrete band.

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