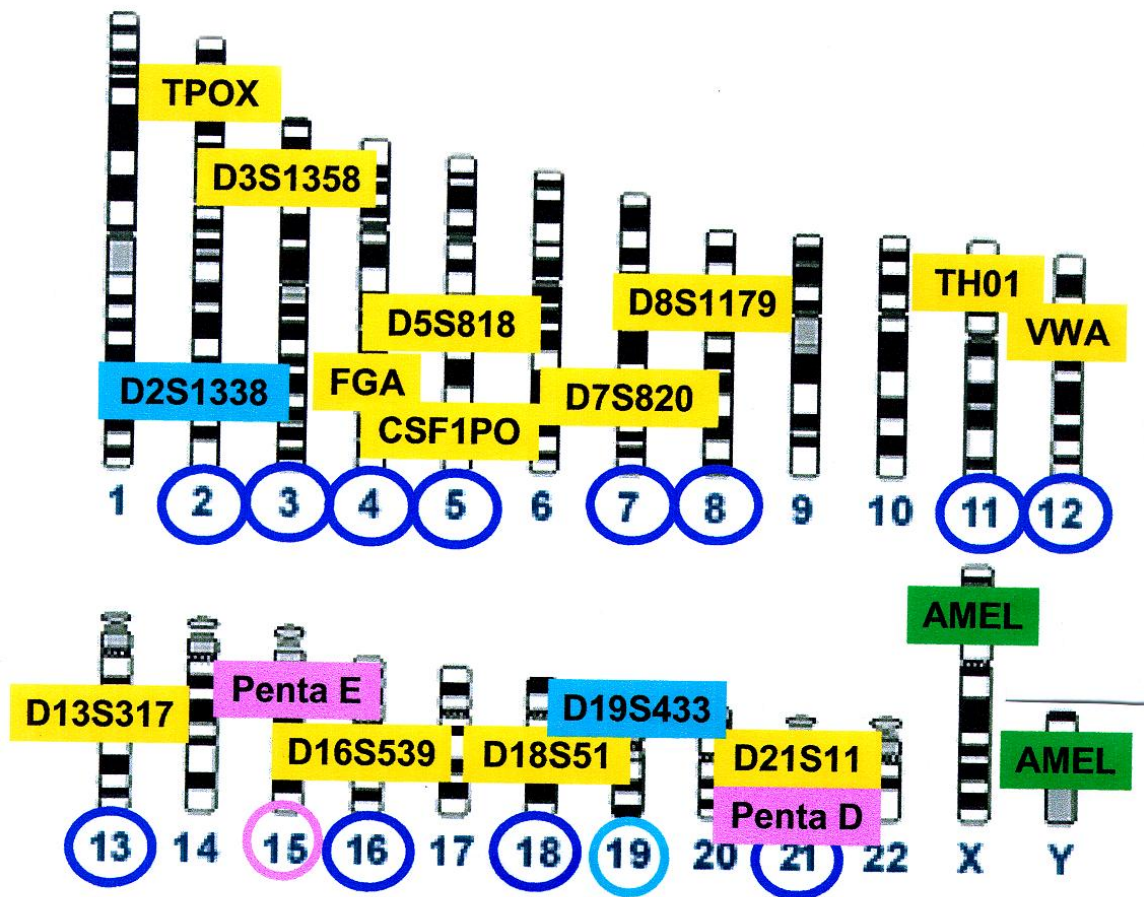


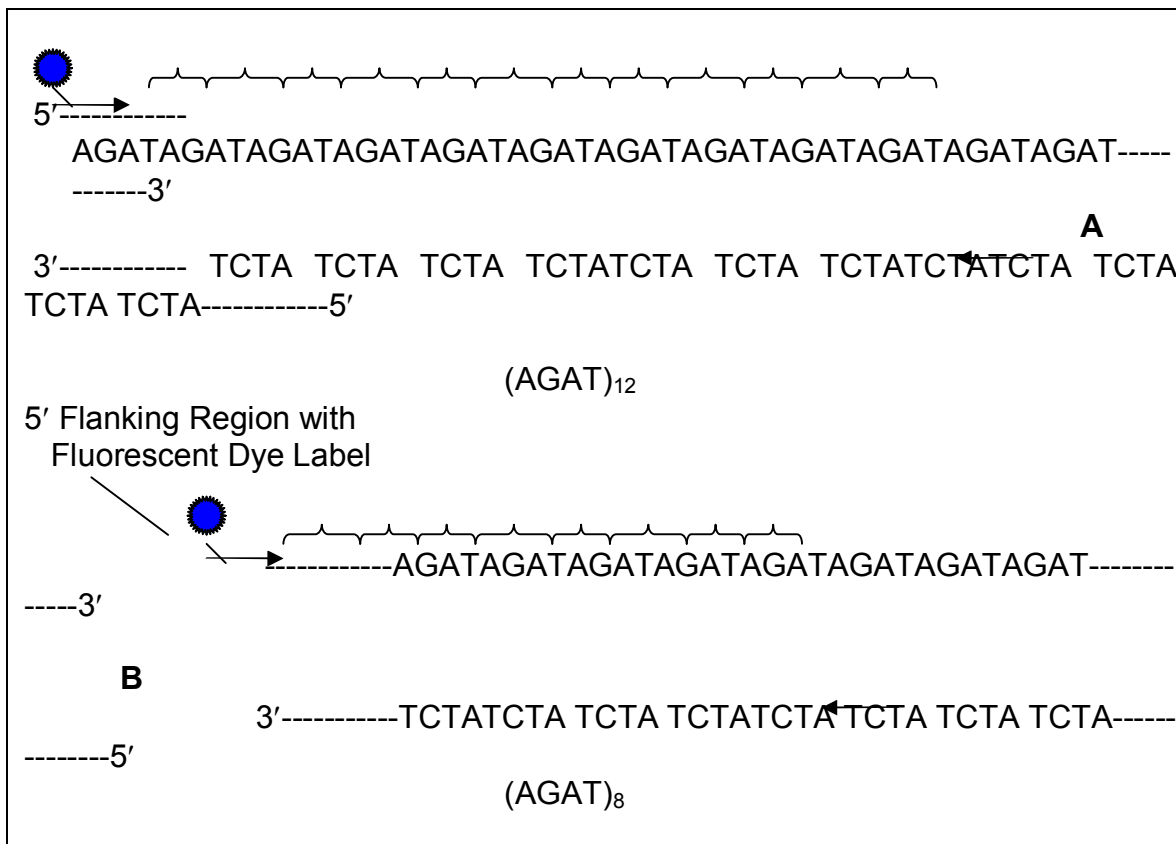
## Genetic Profiling Using STR Analysis

The objective of DNA profiling is to determine the genotype of a person at several highly variable sites in the genome. Thus, its value lies in the fact that it is based on genotype not phenotype. A DNA profile, or genetic fingerprint, can be obtained from saliva left on a stamp, cigarette butt, or even on the mouthpiece of a telephone. Analysts can make a profile of tandem repeats of nucleotides found in small sections of DNA that lay between the genes on chromosomes. Some regions of non-coding DNA sequences are highly **polymorphic**, meaning they vary from person to person in terms of the length of the repeated sequence and the number of times the sequence is repeated. These Variable Number Tandem Repeats (**VNTR**) are scattered throughout the genome. A class of VNTR called Short Tandem Repeats (**STR**) are stretches of DNA containing tandemly repeated nucleotide sequences in which the repeat unit is at least two bases but no more than seven in length. DNA profiling typically targets a four base repeat unit, or **tetranucleotide** sequence. STR occur at various **loci**, or positions on a chromosome as shown in the following diagram.



Position of some Forensic STR Markers on Human Chromosomes, 13 CODIS Core STR Loci.

A specific STR is characterized by the sequence of its repeat unit and the number of times that unit is repeated. Located on chromosome 5, the human c-fms proto-oncogene for CSF-1 receptor gene (CSF1PO), is an example of a tetranucleotide repeat polymorphism which has two alleles as a result of variation in the number of the (AGAT) repeats. The repeat region is variable between homologous chromosomes, while the flanking regions where the primers bind remain constant. One allele contains an STR having 12 repeats (48 base pairs), and the other allele has an STR with 8 repeats (32 base pairs). Thus, the alleles can be resolved from one another based on size. The STR regions for CSF1PO are shown in the following figure.



Repeat regions on homologous chromosomes from a heterozygous individual showing CSF1PO Short Tandem Repeats (STRs). The repeat number is variable between homologous chromosomes, while the flanking regions where the primers bind remain constant. Allele A contains a tetranucleotide STR having 12 repeats (48 base pairs), and allele B has an STR with 8 repeats (32 base pairs).

Inheritance of STRs follows basic Mendelian patterns. The individual shown in the above figure inherited a different allele from each parent and is heterozygous for CSF1PO. Thirty or more different alleles at some STR loci have been identified. This large number of different alleles means that each is relatively rare in a population, with allele frequencies usually only 1% to 5% (0.01-0.05). Thus the probability of any two individuals, except identical twins, having exactly the same alleles at each STR is extremely low. For example, if four STR loci are

screened and each allele at each locus occurred with a frequency of 1% (0.01) in a population, the odds of a chance match between any two random samples showing the same genotype would be one on ten million (.01 x .01 x .01 x .01). Hence, forensic laboratories regularly use STRs for the examination of biological evidence.

Forensic genotyping is highly discriminatory. Assuming no new mutations occur, an individual can be excluded as a suspect with absolute certainty on the basis of one allele mismatch. A positive identification is based on the unlikely probability that agreement in allele constellations is due to chance alone.

In an actual genotyping lab, scientists extract DNA from cells found in teeth, bone, tissue, blood, semen, or commonly from buccal cells that are naturally shed and found in saliva. Once they isolate the DNA, they amplify the target DNA fragments using PCR. The great advantage of PCR is its ability to amplify trace quantities of DNA. Then they separate the fragments from each other using **capillary electrophoresis**. Capillary electrophoresis has replaced gel-electrophoresis with a series of capillaries that separate one sample at a time through an acrylamide polymer that acts as a sieve. DNA fragments move through the capillary according to size, the smallest moving the fastest. Some of the PCR primers carry a fluorescent dye. This results in fluorescently labeled DNA fragments that can be detected and sized on an ABI PRISM® 310 Genetic Analyzer. The fluorescently labeled DNA fragments are excited by a laser as they move past a detector where they are detected and sized to a single base pair. Then, GeneScan® and Genotyper® software is used by the Prism 310 to automatically size and identify the fragments. The result is a plot called an **electropherogram** that indicates the version of each allele. Each peak on the electropherogram represents a fluorescently labeled DNA fragment having a particular size as represented by the number of base pairs, and a particular quantity based on the amount of fluorescent signal. The steps of automated fluorescent detection of DNA fragments are outlined below:

1. Collect material assumed to contain biological sample
2. DNA extracted and purified and quantified
3. PCR amplification and fluorescent labeling of STRs
4. Capillary electrophoresis-detection of alleles with Prism 310
5. Size and identify DNA fragments using software
6. Analysis-interpretation of results, frequency estimation of match, comparison against a database