Appendix 1:

STRs:

STRs: Short Tandem Repeats

The human genome contains about 3000 million base pairs, which are distributed among 23 pairs of chromosomes. Only 90 million base pairs of the human genome are genes (including introns); the rest of the genome consists of non-coding sequences. Comparing human genomes, the similarities are striking; less than 0.1 % (about 3 million bases) differs from one person to the other. These variable differences tend to be concentrated in "variable regions". Forensic scientists use these variable regions as genetic markers to generate a DNA profile of an individual, using samples from blood, bones, saliva, skin, or any other body tissues or products.

Short Tandem Repeats (STR) DNA profiling uses the variability in the number of tandemly repeated copies in microsatellite DNA. The DNA forensic community has moved toward the use of STRs for its greater fidelity and reproducibility. STRs are amplified using polymerase chain reaction (PCR) using a set of primers that flank the STR loci. The patterns of amplified bands seen in the results gels reflect the number of repeats in the alleles on the two chromosomes for each STR type. As both chromosomes originate from different parents, two different lengths of product may result from one set of primers (Figure 1).

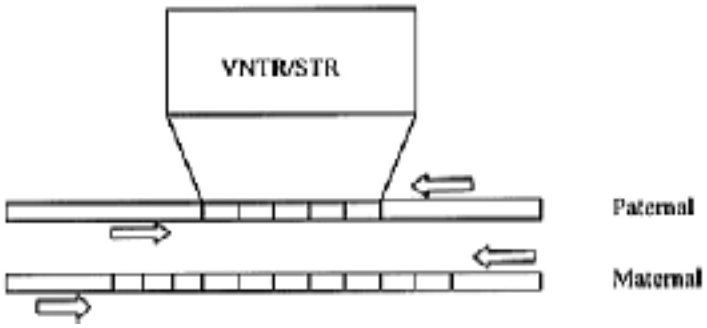


Figure 1

To really distinguish between as little as a 2-5 nucleotide differences in length, you can use polyacrylamide gels for separation of the PCR products. These types of gels more finely separate pieces of DNA, but are not only more difficult to work with but also are a neurotoxin before the liquid form polymerizes into the solid gel form. For our lab, we use agarose gels for our Alu insert analysis. As you know from your text, the best way to detect differences between individuals is to use multiple STRs, just as we used multiple probes for the RFLP analysis in Labs 3-4. The Federal Bureau of Investigations (FBI) adopted the use of 13 loci to constitute the core of the United States CODIS (Combined DNA Index System) database (Figure 2). Usually genomic DNA is used in this analysis. See the figure on the next page for the 13 loci [STR types] used for CODIS, although usually only 4 different types are looked at in a single PCR. For our Alu insert analysis, we looked at only 1 type of difference that is NOT an STR.

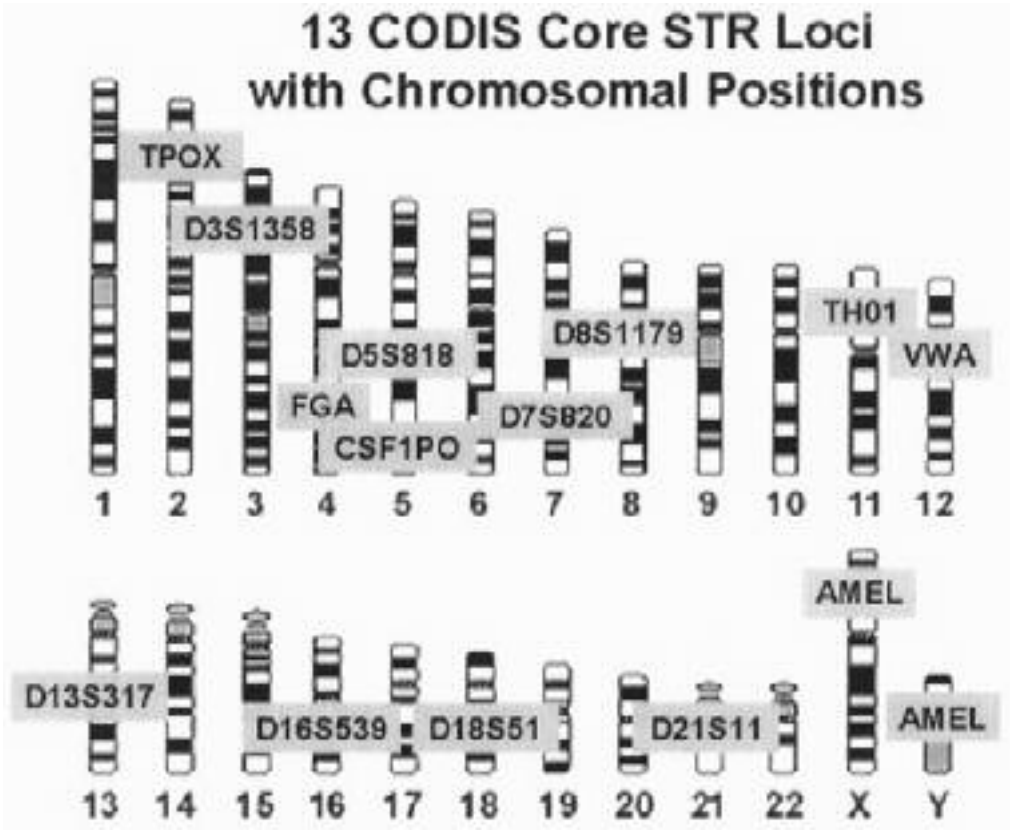


Figure 2

Refer to http://dna-view.com/profile.htm for an example on DNA profile probability calculations.

Often a PCR reaction will be performed on the DNA using multiple primers ("multiplex PCR") to amplify four separate STRs on different chromosomes simultaneously. The lengths of the PCR products are then analyzed on a gel, with a DNA size marker included as a size reference. You should also use at least 1 water only "negative control" in the PCR – adding water only to a reaction instead of DNA helps determine if the reactions are contaminated because a negative control should have NO resulting bands. A set of 4 primer systems that can be used are listed below in Table 1.

Table 1 provides information on the loci that will be amplified in this experiment.

Locus	Chromosome	Repeat Structure	Repeat #	Genbank accession number	Human genome database
D7S820	7	GATA	5-15	G08616	
CSF1PO	5	AGAT	6-16	X1420	
Y-GATA-H4	Y	(TAGAATGGATAGATT A (GATG)pAA(TAGA)q	*	G42676	AC011751
HUMTH01	11	TCAT	3-14	D00269	

* Repeat numbers are not clearly defined

More background information from the lecture modified from your Criminalistics text book:

PCR Advantages:

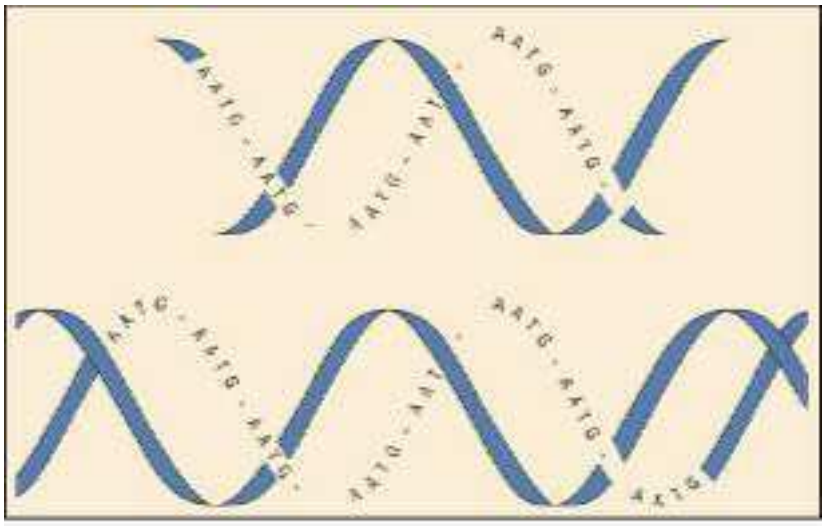
- One advantage in moving to shorter DNA strands is that they would be expected to be more stable and less subject to degradation brought about by adverse environmental conditions.
- The long RFLP strands in restriction enzyme analysis tend to readily break apart under the adverse conditions not uncommon at crime scenes.
- PCR also offers the advantage in that it can amplify minute quantities of DNA, thus overcoming the limited sample size problem often associated with crime scene evidence.

STRs:

- The latest method of DNA typing, short tandem repeat (STR) analysis, has emerged as the most successful and widely used DNA profiling procedure.
- STRs are locations on the chromosome that contain short sequences that repeat themselves within the DNA molecule.
- They serve as useful markers for identification because they are found in great abundance throughout the human genome.

STR advantages:

- STRs normally consist of repeating sequences of 3 to 7 bases in length, and the entire strand of an STR is also very short, less than 450 bases in length.
- This means that STRs are much less susceptible to degradation and may often be recovered from bodies or stains that have been subjected to extreme decomposition.
- Also, because of their shortness, STRs are ideal candidates for multiplication by PCR, thus overcoming the previously mentioned limited-sample-size problem often associated with crime-scene evidence.



Chromosome from Parent 1

+

Chromosome from Parent 2

≈ Combination in 3.5 % of population

Figure 13–12 Variants of the short tandem repeat TH01. The upper DNA strand contains six repeats of the sequence A–A–T–G; the lower DNA strand contains eight repeats of the sequence A–A–T–G. Seven TH01 variants in human genome: 5 – 11 repeats of this sequence. Each person has two possible variants: one from each parent.

Power of STRs:

- What makes STRs so attractive to forensic scientists is that hundreds of different types of STRs are found in human genes.
- The more STRs one can characterize, the smaller will be the percentage of the population from which a particular combination of STRs can emanate.
- This gives rise to the concept of multiplexing.
- Using the technology of PCR, one can simultaneously extract and amplify a combination of different STRs.

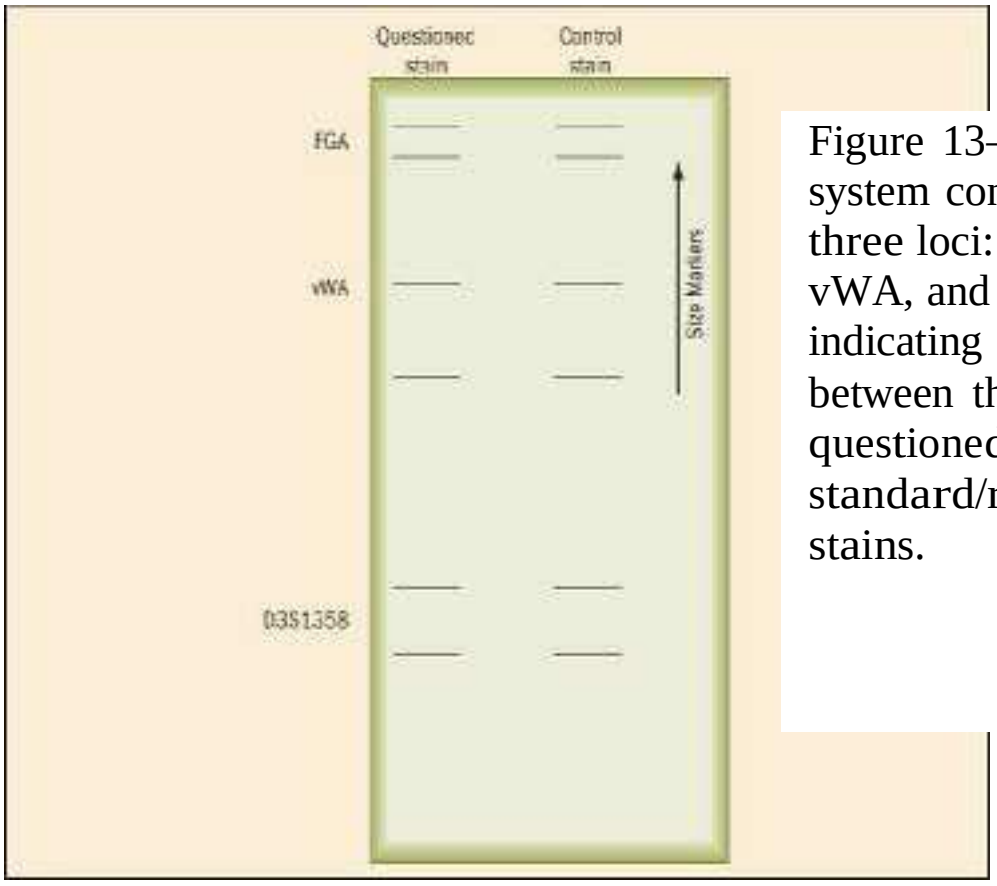


Figure 13–13 Triplex system containing three loci: FGA, vWA, and D3S1358, indicating a match between the questioned and the standard/reference stains.