Crime Scene: Highway Motel Rm#13

The motel manager hears loud voices, a woman screams, and a shot rings out. The manager runs to the window in time to see the receding lights of a car leaving in a hurry. The door to room #13 hangs open. The manager runs to the open door, to see a man lying face down in a pool of blood. He calls 911. The police arrive, and begin to examine the crime scene. It looks like an apparent homicide, but with no obvious clues as to who committed the crime.

What kinds of human DNA sequences are used in crime scene investigations?
There are ~3 billion basepairs in the human genome – greater than 99.5% don’t vary between different human beings. However, a small percentage of the human DNA sequence (<0.5%) does differ, and these are the special polymorphic (“many forms”) sequences used in forensic applications. By universal agreement, DNA sequences used for forensic profiling are “anonymous”; that is, they come from regions of our chromosomes (also called loci) that do not control any known traits and have no known functions. Loci are basically genetic addresses or locations. A single locus may have different forms or types; these different forms are called alleles. A locus may be bi-allelic, having only two different forms, or it may be polymorphic, as described above.

The DNA sequences used in forensic labs are non-coding regions that contain segments of Short Tandem Repeats or STRs. STRs are very short DNA sequences that are repeated in direct head-to-tail fashion. The example below shows a locus (known as TH01) found on chromosome 11; its specific DNA sequence contains four repeats of [TCAT].

...CCC TCAT TCAT TCAT TCA...

For the TH01 STR locus, there are many alternate polymorphic alleles that differ from each other by the number of [TCAT] repeats present in the sequence. Although more than 20 different alleles of TH01 have been discovered in people worldwide, each of us still has only two of these, one inherited from our mother and one inherited from our father. For example as shown below, suspect A has one allele with 5 repeats, and one allele with 3 repeats, giving a DNA profile for the TH01 locus of 5-3.

Suspect A’s DNA type for the TH01 locus: CCC ■ ■ ■ ■ AAA
                                CCC ■ ■ ■ AAA

Suspect B’s DNA type for the TH01 locus: CCC ■ ■ ■ ■ ■ ■ ■ ■ AAA
                                CCC ■ ■ ■ ■ ■ ■ ■ ■ AAA
Imagine a scenario in which Suspect A and Suspect B are accused of being involved in a love triangle and committing the murder of a third person in the Highway Motel Room #13; the person who actually pulled the trigger is unknown. In addition to DNA samples from the crime scene, the forensic specialist will isolate DNA from suspects, victims, and others present to genotype as controls. Using PCR-based analysis, the samples will be examined at 13 different genetic loci, using software to interpret the results from the amplification products. In real crime scene analysis DNA profiling is performed at many loci to improve the power of discrimination of the testing. The power of discrimination is the ability of the profiling to tell the genetic difference between different individuals. The larger the number of loci profiled, the more powerful the ability to discriminate.

You are about to conduct real world forensic DNA profiling. As a crime scene investigator, you will use the polymerase chain reaction (PCR) and agarose gel electrophoresis to analyze the DNA samples obtained from a hypothetical crime scene and four suspects. Your job is to identify the perpetrator. A genotype is the particular set of genetic markers, or alleles, in a DNA sample. Every person’s genotype is their own uniquely personal genetic barcode. In this experiment, you’ll be revealing the genetic barcodes of several individuals by looking at a single locus BXP007 and looking for whodunit!

**Polymerase Chain Reaction:**

**Materials:**
- Ice bath containing tubes
- Master Mix + primers (MMP, blue liquid)
- Tubes of DNA (Crime Scene and Suspect A-D DNAs)
- PCR tubes (small tubes)
- Marking pen
- P20 pipette
- Pipette tips

**Protocol:**
1) Keep tubes on ice during the entire procedure. Only remove them to remove or add a solution.
2) Label the PCR tubes CS, A, B, C and D and include your group name as well.
3) Set your pipettes to 20µL and transfer 20µL of MMP into each of your tubes.
4) Next transfer 20µL of template DNA into the appropriately labeled tube. For example, transfer 20 µL from the Crime Scene tube to the tube labeled ‘CS’. **Important: use a fresh pipette tip for each DNA sample!**
5) The solution in your PCR tubes should be blue now. Be sure to cap the tubes securely and keep them on ice.
6) When instructed to do so, place your tubes in the thermal cycler.
7) Write down the cycle that will be used in the thermal cycler.
Questions:

1) What kind of materials obtained from a crime scene might contain DNA?

2) What might you see if you ran a DNA sample extracted from evidence on a gel before PCR? Why do you need to perform PCR on DNA evidence?

3) What is a genotype?

4) What is the difference between an allele and a locus?
5) Why do forensic labs analyze non-coding DNA and not genes?

6) What components do you need to perform PCR and why do you need each component?

7) What steps make up a PCR cycle, and what happens at each step?