7.013 Section Problem:
Applications of recombinant DNA technology

Help! It is a Thursday afternoon, problem sets are due and...
SOMEONE HAS TAKEN THE WOODEN PROBLEM SET BOX! Gone! Missing! Chaos has ensued. Students are piling up pestering the Education Office staff with their problem sets! Let’s try and find the culprit so we can make sure all the problem sets are collected and accounted for.

THE FACTS:

- The problem set box was last seen inside 68-120 on Wednesday at 5:00 pm. The room was then locked for the night.

- Natalie, Brendan, Chris, Ann, James, and Keara, and Alison all have a key to 68-120.

- Someone took the box during the evening and left the following note:

Recombinant DNA techniques were used to solve the crime. Two “CSI-like” detectives, Adriana and Gloria, collected cells left on the ransom note and from these cells extracted DNA. The region encoding the gene K was amplified using polymerase chain reaction (PCR). The DNA sequence of the PCR products was then determined by dideoxy DNA sequencing. Shown in the box below are the results from the sequencing gel.

\[
\begin{array}{cccc}
 & ddG & ddA & ddT & ddC \\
- & & & & \\
+ & & & & \\
\end{array}
\]

a) Write the DNA sequence for this region of gene K as shown in the sequencing gel above. Indicate the 5' and 3' ends of the DNA sequence.

b) One of the suspects, Keara, has the following ds DNA sequence for this same region in both copies of gene K:

\[
\begin{align*}
5' & \text{ CTGAAGTACGT } 3' \\
3' & \text{ GACTTCATGCA } 5'
\end{align*}
\]

Is Keara still considered a suspect in the case? Justify your answer.
Adriana, the detective analyzed a second site, the *gene L* locus (represented by the shaded box below), which she has shown is useful for forensics. The only difference between the two alleles at this locus is that the *l* allele lacks an internal *Eco* RI restriction site present in the *L* allele.

![EcoRI Diagram](image)

Gloria, the detective, performed PCR analysis of the cells taken from the note and determined that the note was handled only by an *Ll* individual. Based on this, the *gene L* locus of some of the suspects, (Ann left early Friday morning for Rome), was amplified by PCR, the PCR products were digested with *Eco* RI, and the resulting fragments were separated by agarose gel electrophoresis as shown below:

![Genotype Diagram](image)

c) In the spaces provided above, indicate the genotypes at the *gene L* locus for these suspects.

d) Based on the PCR analysis of the *gene L* locus, which individual(s) can be excluded as suspects in the crime? Justify your answer.
At chromosomal position Z, it is common to find one or more copies of a 100 bp insert. Fortunately, many of the suspects have different number of inserts at this position:

- Brendan has no inserts.
- James has one insert.
- Chris has two inserts.
- Alison has three inserts.
- Natalie has four inserts.
- Keara has two inserts.
- Ann is unavailable for sample collection (now in Paris).

A partial DNA sequence of position Z is given below. The length of position Z without an insert is 2000 bp.

\[
\begin{align*}
5' &- GTGCA \ldots \text{[100 bp insert]} \ldots CGACG-3' \\
3' &- CACGT \ldots \text{[100 bp insert]} \ldots GCTGC-5'
\end{align*}
\]

e) To determine who stole the problem set box, design a PCR-based strategy to analyze position Z, including the sequences and orientation (5' and 3' ends) of two 5-base primers necessary to PCR amplify this region.

f) The following pattern was seen by Adriana, a DNA technical expert, on the gel used to separate the PCR products from the above experiment:

i) Which lane corresponds to which individual?

ii) Gloria, the detective says “I know who did it.” Who committed this heinous crime?