7.012 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! Chaos has ensued. Students are piling up pestering Sue with their problem sets! Help us solve the mystery so we can make sure all the problem sets are collected in a peaceful way.

THE FACTS:
• The problem set box was last seen in 68-120 on Wednesday at 5:00 pm. The room was then locked for the night.
• Susan, Winston, Chia, Divya, Michel, and Yasemin all have a key to 68-120.
• Someone took the box and left the following note:

HA! HA! HA! I HAVE TAKEN THE WOODEN BOX FOR MY OWN NEFARIOUS PURPOSE.
IF YOU WANT IT BACK, AS I’M SURE YOU WILL THEN YOU MUST MEET MY DEMANDS.
I WILL CONTACT YOU SOON.

Recombinant DNA techniques were used to solve the crime. Two “CSI-like” detectives, Irena and Cydney, collected cells left on the ransom note and from these cells extracted DNA. The region encoding gene K was amplified by 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.

\[
\begin{align*}
\text{ddG} & \quad \text{ddA} & \quad \text{ddT} & \quad \text{ddC} \\
\hline
\text{ ddG} & \quad & \quad & \\
\text{ ddA} & \quad & \quad & \\
\text{ ddT} & \quad & \quad & \\
\text{ ddC} & \quad & \quad & \\
\hline
\end{align*}
\]

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, Yasemin, has the following ds DNA sequence for this same region in both copies of gene K:

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

Is Yasemin still considered a suspect in the case? Justify your answer.
Sarah, 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 I allele lacks an internal EcoRI restriction site present in the L allele.

![Gene L locus diagram]

Cydney, the codetective, performed PCR analysis of the cells taken from the note and determined that the note was handled only by a LI individual. Based on this, the gene L locus of the suspects was amplified by PCR, the PCR products were digested with EcoRI, and the resulting fragments were separated by agarose gel electrophoresis as shown below:

![PCR analysis diagram]

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:

- Winston has no inserts.
- Michel has one insert.
- Chia has two inserts.
- Divya has three inserts.
- Susan has four inserts.
- Yasemin has two inserts.

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

$$5'\text{-GTGCA} \ldots \left[100 \text{ bp insert}\right] \ldots \text{CGACG-3'}$$

$$3'\text{-CACGT} \ldots \text{GCTGC-5'}$$

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 Jim, the DNA technical expert, on the gel used to separate the PCR products from the above experiment.

![Gel Image]

i) Which lane corresponds to which individual?

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