

Applications of recombinant DNA technology

Help! It is a Thursday afternoon, problem sets are due and...

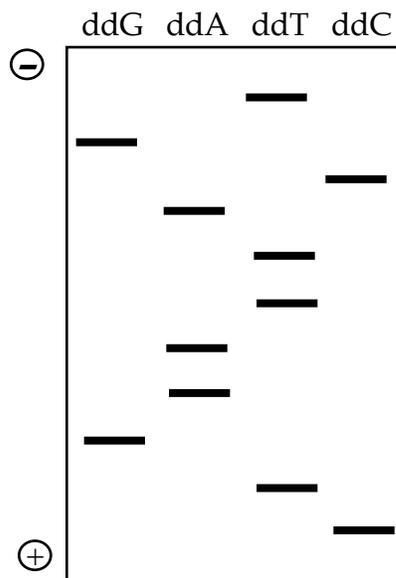
SOMEONE HAS TAKEN THE WOODEN PROBLEM SET BOX! Chaos has ensued. Help us solve the mystery so we can make sure all the problem sets are collected in a peaceful way.

THE FACTS:

- The problem set box was last seen in the Biology building on Wednesday at 5:00 pm. The building was then locked for the night.
- TA1, TA2, TA3, TA4, TA5, and TA6 all have a key to the biology building.
- 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 detectives, John and Ted, 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.



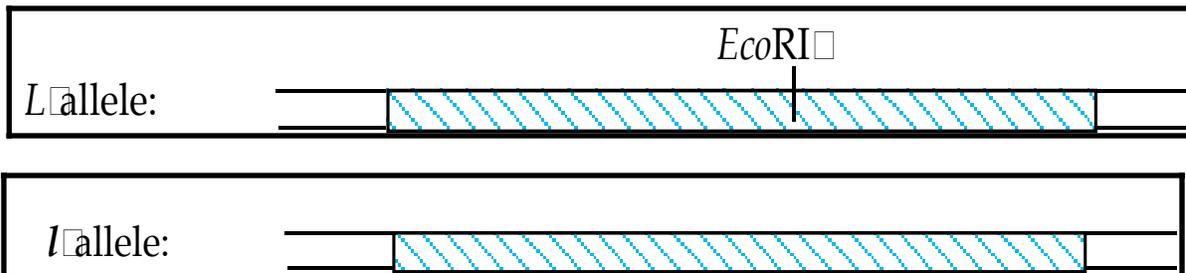
a) Write the DNA sequence for this region of *gene K* as shown in the sequencing gel above. Indicate the 5' and 3' ends of the DNA sequence.

b) One of the suspects, TA6, has the following ds DNA sequence for this same region in both copies of *gene K*:

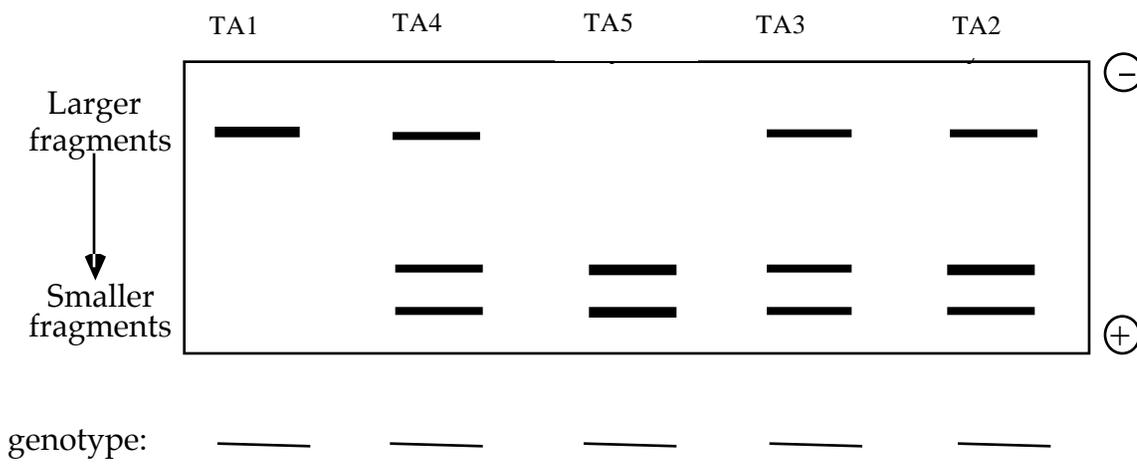
5' CTGAAGTACGT 3'
3' GACTTCATGCA 5'

Is TA6 still considered a suspect in the case? Justify your answer.

John, the detective analyzed a second site, the *gene L* locus (represented by the shaded box below), which he has shown is useful for forensics. The only difference between the two alleles at this locus is that the *l* allele lacks an internal *EcoRI* restriction site present in the *L* allele.



Ted, the detective, 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:



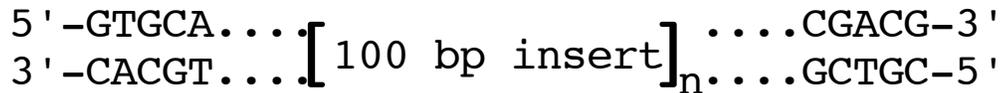
c) In the spaces provided above, indicate the genotypes at the *gene L* locus for these suspects.

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:

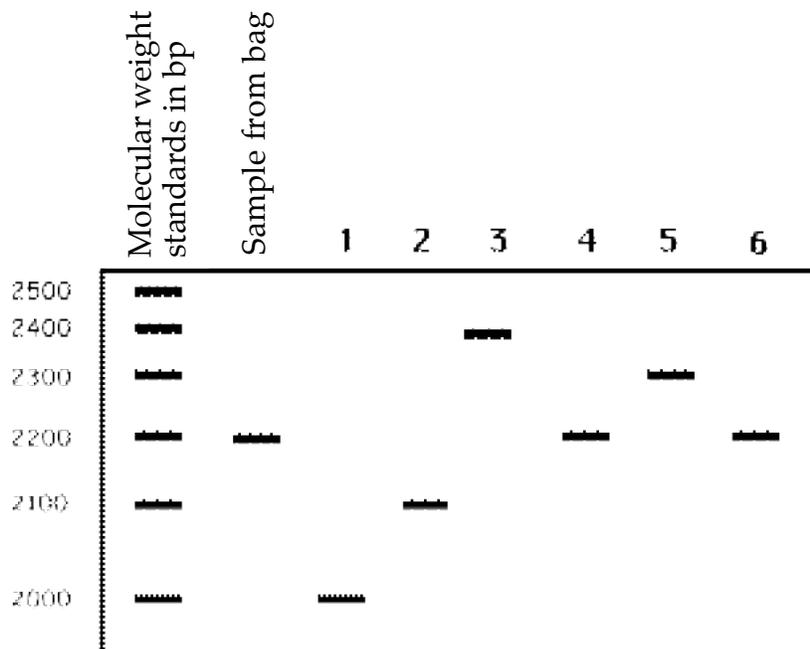
- TA2 has no inserts.
- TA5 has one insert.
- TA3 has two inserts.
- TA4 has three inserts.
- TA1 has four inserts.
- TA6 has two inserts.

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



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 on the gel used to separate the PCR products from the above experiment.



i) Which lane corresponds to which individual?

ii) Ted, the detective says "I know who did it." Who committed this heinous crime?