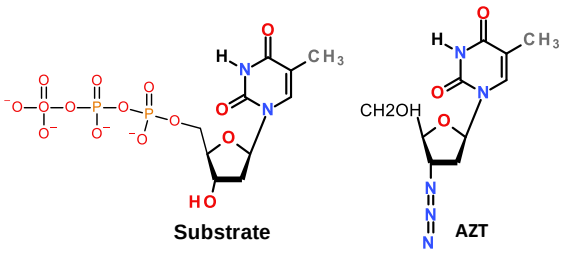
1. A person shows symptoms of tyrosinemia 1, with a high level of 4-fumarylacetoacetate in the blood. Please answer the following questions:

i) What enzyme is likely missing or defective in people with tyrosinemia 1?

ii) What medical conditions arise due to this disease (please use the web)?

2. Shown to the right is one of the substrates for HIV reverse transcriptase, a polymerase that is used by HIV during viral replication. To the right of the substrate is the drug AZT, one of the first antiviral drugs used to treat HIV.



i) Briefly describe the role of reverse transcriptase in the HIV lifecycle (*please use the internet*)

ii) What modification of the drug must occur in order for it to be used as a substrate?

iii) Once the modified AZT is used by the polymerase, replication stops. Explain why.

3. Cystic fibrosis is a genetic disease that is caused by a single mutation in a chloride ion transporter (CFTR). The transporter is necessary to move Cl ions across mucus membranes. The chloride then causes water to move across the membrane to reduce the viscosity of mucus. When the transporter is defective the mucus is very thick, causing difficulty in breathing.

The gene for the CFTR protein is very long (over 180,000 bases). A segment of the DNA that codes for the middle section of the protein is shown below, beginning at the codon for residue 505 in the protein

```
atc atc ttt ggt gtt
```

The disease is recessive, meaning that an affected individual must have two copies of the mutation to have the disease. Heterozygous individuals do not have the disease but are carriers. There is a one-in-four chance that a child produced from two carriers will have the disease. Therefore, genetic testing for heterozygotes is very important if there is a family history of the disease.

You sequence DNA samples from an affected individual and find the following sequence for the same region of the DNA and find:

```
atc atc ggt gtt
```

i) What is the amino acid sequence of the normal and mutant proteins.

ii) How does this mutation affect the protein sequence?

iii) How might this change affect the structure of the protein?

4. Fragile X-syndrome is due to an excessive number of CGG repeats in a gene called FMR1. Normal individuals have between 5 and 40 repeats while affected individuals have more than 200. The beginning of the gene is shown below with the CGG repeats highlighted in yellow and the start codon for the protein in green.

```
   1 ctcagtcagg cgctcagctc cgtttcggtt tcacttccgg tggagggccg cctctgagcg
  61 ggcggcgggc cgacggcgag cgcgggcggc ggcggtgacg gaggcgccgc tgccaggggg
 121 cgtgcggcag cgcggcggcg gcggcggcgg cggcggcggc ggaggcggcg gcggcggcgg
 181 cggcggcggc ggctgggcct cgagcgcccg cagcccacct ctcgggggcg ggctcccggc
 241 gctagcaggg ctgaagagaa gatggaggag ctggtggtgg aagtgcgggg
```

During the normal replication of DNA, you would find the following replication structure (the CGG repeats are highlighted and alternate bold/not bold).

```
5'----TGCCAGGGGGCGTGCGGCAGCGCGGCGGCGGCGGCGG
3'----ACGGTCCCCCGCACGCCGTCGCGCCGCCGCCGCCGCCGCCGCCGCCG---5'
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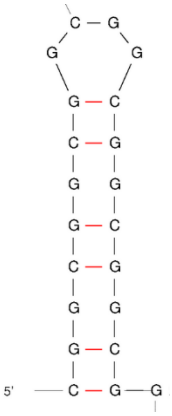
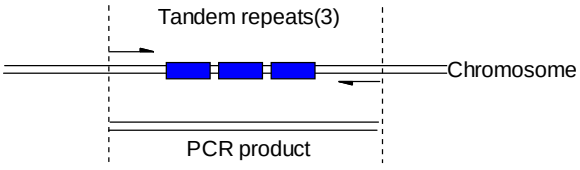


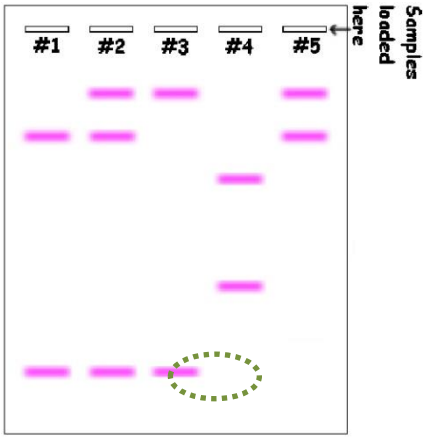
Please answer the following questions:

i) What are the disease symptoms due to fragile x-syndrome (please use the web).

ii) Postulate how DNA polymerase activity could increase the number of repeats. As a hint, the CGG repeats can form stabile hairpin structures, as shown on the right. Please ***do not*** use the web to answer this question.

5. Solve the following forensic case. DNA was isolated from the victim, collected from the crime scene, and from three suspects. The DNA was subjected to PCR using primers that amplify a region of the chromosome that is known to contain tandem repeats, which are sequences that are repeated multiple times on the same chromosome. The number of tandem repeats differs from one individual to another, causing the length of the PCR product to differ. For example, one chromosome could look like this, with three tandem repeat (see above), while a chromosome might have four, giving a larger PCR product. Note that since we have two copies of each chromosome there are two possible PCR products, one from each chromosome. It is possible to have the same number of tandem repeats on each chromosome.





After PCR the DNA was separated by size and stained to visualize the DNA fragments. In the image on the right shows the sizes of DNA fragments for each of the following samples:

- Lane 1 – Victim's blood
- Lane 2 – Blood at crime scene (victim+criminal)
- Lane 3 - Suspect #1
- Lane 4 - Suspect #2
- Lane 5 - Suspect #3

Bands towards the bottom of the diagram are smaller. Bands at the same position have the same size. The circled band will be used for the next problem.

i) Who is no longer a suspect? Please justify your answer.

ii) On the basis of the above data, who is still a suspect for the crime? Please justify your answer.

iii) Is the individual identified in part ii) guilty?

6. The smallest PCR product (circled in the above image) was sequenced, giving the following (top strand only):

GCTGTAGCCATTGGGCCCTTTAAAGGGCCCTTTAAAGGGCCCTTTAAACCGCGTGAGTCA

i) What is the sequence of the tandem repeat?

ii) How many tandem repeats are present in this DNA?

iii) Give the sequence of the left and right PCR primers that would have been used to produce this PCR product. Assume that the PCR primers are 8 bases long.

7. Someone was exposed to Covid19 and shows all of the symptoms of a Covid19 infection. However, when you run a PCR reaction to verify the infection you find that you obtain no PCR product. Explain this result (you should assume that the person is indeed infected with Covid19).