1. 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, with the codon for Phe507 highlighted in grey, is shown below. The DNA sequencing primer is highlighted in cyan. The translation of this sequence gives the following protein sequence for this region of the protein (these are one letter codes for the amino acids, e.g. F=Phe)

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tgg att atg cct ggc acc att aaa gaa aat atc atc ttt ggt gtt tcc tat gat gaa tat
 W   I   M   P   G   T   I   K   E   N   I   I   F   G   V   S   Y   D   E   Y
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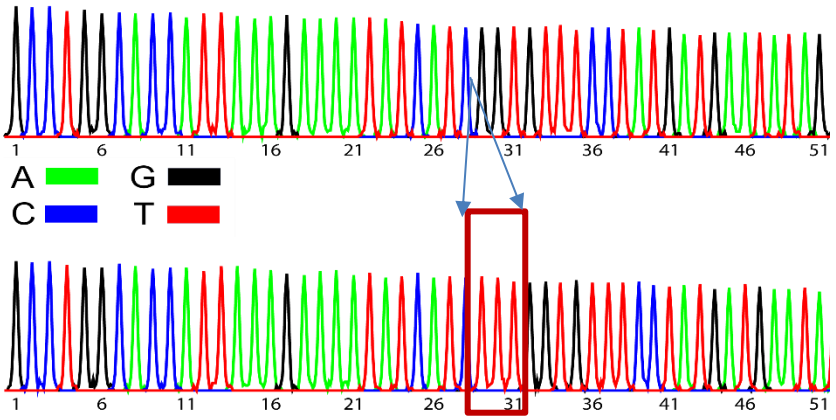
The disease is recessive, meaning that an affected individual must have two copies of the mutation to have the disease. Heterozygous individuals do not have the disease but are carriers. There is a one-in-four chance that a child produced from two carriers will have the disease. Therefore, genetic testing for heterozygotes is very important if there is a family history of the disease.

You sequence DNA samples from a heterozygous individual, each sequence corresponds to the gene on one of their chromosomes. One of these sequences corresponds to the normal gene sequence and the other corresponds to the mutation that is responsible for cystic fibrosis. The DNA sequencing data is given to the right.



i) Briefly describe how the 2nd blue peak was generated, give its DNA sequence.

ii) What is the change in DNA sequence (mutation) associated with this disease?

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

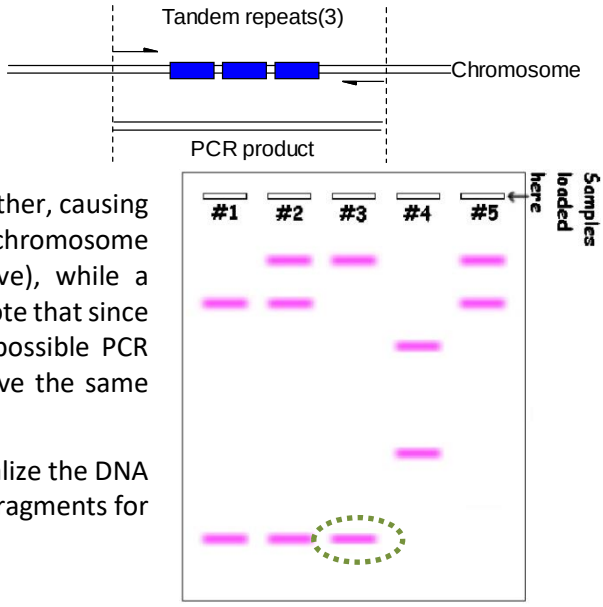
Note, when converting the above DNA sequences to codons, skip the first base, i.e. the first codon is CCT, coding for proline (P). Your translation of the DNA sequence should produce the correct protein sequence.

i) This DNA fragment was generated by the addition of a normal dGTP to the primer, followed by the addition of ddCTP. The ddCTP caused the chain to terminate and it also colored the fragment based on its fluorescence.

ii) The lower trace is the normal DNA sequence, the three T bases that code for Phe507 are present (indicated with the red square.)

iii) The upper trace shows that those three T bases are missing (see arrows), causing a deletion of the Phenylalanine residue. This missing residue affects the folding of the ion channel, making it less functional by preventing it movement of the protein to the cell membrane.

2. 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) definitely guilty?

Each person will give rise to two PCR products, one from the chromosome they inherited from their mother, and one they inherited from their father.

The size of the PCR products will be different if there are a different number of repeats. They will be the same if the number of repeats is the same. If the number of repeats is the same on both chromosomes, only one band will be seen after electrophoresis.

Lane #2: Contains PCR products from two people, the victim and the culprit. Only three bands are seen because both the victim and the culprit have the same number of repeats on one of their chromosomes. So it is not possible to determine unambiguously which of the lower bands come from the culprit. The top band must come from the culprit.

i) Lane 4, or suspect 2, cannot be a suspect since the DNA from this person has different repeats that the culprit.

ii) Lanes 3 and 5 remain as suspects since the number of repeats they have could be the same as the culprit. They both have the upper product, and their other band matches the other two bands in lane 2.

iii) No, they are not necessarily guilty because many other people in the population will have the same repeats as lane 3 or lane 5.

3. The smallest PCR product (circled in the above image) was sequenced, giving the following (top strand only):
GCTGTAGCCATTGGGCCCTTTAAA**GGGCCCTTTAAA**GGGCCCTTTAAACCGCGTGAGTCA

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.

i) GGGCCCTTTAAA (bold and underlined)

ii) three

iii) The left primer is the highlighted sequence on the left, the right primer is the complement of the highlighted region on the right (since it would be the sequence of the lower strand)

Left primer 5′ GCTGTAGC Right primer 5′ TGACTCAC

4. 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).

Mutations in the virus have changed the sequence where one or both of the PCR primers bind. This change could include deletion of the site(s) where the primers bind.

5. The human immunodeficiency virus (HIV) infects and kills T_H cells. Why would this lead to an acquired immunodeficiency? *Please do **not** use the web for this question.*

The HIV virus infects TH cells and kills them. T-helper cells are required to activate both B-cells and T_C cells. No antibodies can be produced, nor can T_C cells be activated. There is no antibody based or cellular immunity and the individual has developed AIDS, or acquired immune-deficiency.

6. What disease is the drug Trastuzumab used to treat? Briefly describe how it works to cure the patient (*please use the web and provide the appropriate citation*).

Trastuzumab is an antibody that is used to treat breast and stomach cancer. It binds to the HER2 receptor which is a growth factor receptor. This receptor is over-expressed in these cancers, leading to increased growth of the cancer cell in response to normal levels of the growth hormone. The antibody prevents the growth hormone from binding to the receptor, therefore preventing growth of the cancer cell.
Source: Wikipedia

7. There are two antigens found on red blood cells, type-A or type-B. A person can be type-A (only have the A antigen), type-B (only have the B-antigen), type AB (have both antigens), or type O (neither A or B). An incompatible blood transfusion occurs if a patient is given blood that has a different antigen than theirs due to the presence of pre-existing antibodies against the antigen. For example, type A blood cannot be given to a type B person, because the type-B person will have pre-existing antibodies against the type A-blood. Hint: You want to consider the process of antibody generation in the bone marrow.

i) Explain why a person with type B blood cannot make antibodies that recognize B-antigen.

ii) Explain why a person with type B blood can accept blood from a type O person.

iii) Explain why a person who is AB can accept blood from anyone.

i) They would be making antibodies against their own cells, any B-cells that recognize the B-antigen should be destroyed.

ii) The type O blood has no antigens on the surface of the red blood cells, so there is nothing for the anti-A antibodies to bind to.

iii) Since they have both blood types they would not make any antibodies against the blood group antigens.