# Assignment 4: The mutation

## Remember that...

To succeed in this assignment, you should be familiar with the characteristics of genes, mutation types, and how the information in DNA is transcribed and translated into a protein. You should also be familiar with the genome and bioinformatics, polymorphic markers and databases.

## What we wanted to prove...

In the previous assignment, you searched computer databases and found homology between a gene involved in the hearing process in mice and a gene located on chromosome 5 in the human genome. The human gene is now a candidate gene. If this gene is found to be involved in hearing in humans, it will be added to the list of the several dozen known human genes identified as being involved in the hearing process. If we are able to show that all deaf adults in our case family have one mutated allele of the gene, we will prove that the gene that is currently being considered as a candidate is in fact the gene we are after. When both alleles are normal, the individual can hear. When one of the alleles is mutated, the individual carrying it may become deaf in adulthood.

## The assignment: What we'll be doing next

The scientists located a normal allele of the candidate gene in the database. The DNA sequence of the normal allele is known. What do you think the next step should be? What question will the researchers ask? At this stage, the scientists must find the difference between the allele that is considered normal and the one that is considered to be a mutant. In other words, they must locate the mutation in the sequence of the allele that causes deafness in adulthood. They must therefore determine the DNA sequence of the mutated allele that the deaf individuals have (the normal sequence can be found in the database). Now the DNA sequence of a normal allele can be compared with the DNA sequence of a mutated allele. The comparison will involve the BLAST program, which compares two given sequences. For this assignment, we have planted a sequence of the mutant allele in the database. This sequence does not appear in the public database.

## Comparisons using BLAST

Enter [BLAST2seq](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&PROG_DEF=blastn&BLAST_PROG_DEF=megaBlast&BLAST_SPEC=blast2seq)

Enter the accession number NM\_002700 in the "Query Sequence" box. This is the mRNA sequence of the normal allele.

Enter the sequence which appears [here](https://stwww1.weizmann.ac.il/bioinformatics/mrna/) in the "Subject Sequence" box. This is the mRNA sequence of the mutant allele.

Click the "BLAST" button.

Look at the page with the comparison results between the normal and mutant mRNA sequences - and answer the following questions

1. Can you find a difference in the DNA sequence between the normal and mutant allele (mRNA)?
   1. Yes, there appears to be a mutation in the allele characteristic to the deaf individuals.
   2. No. there is no difference.
2. What mutation occurred in the deaf individuals' allele?
   1. Insertion
   2. Point mutation
   3. Other chromosomal aberration
   4. Deletion
3. The length of the normal mRNA sequence is 1017 bases. Based on a comparison between the normal sequence and the mutant sequence, the length of the mutant mRNA is:
   1. 1017 bases
   2. 1025 bases
   3. 1009 bases
   4. 2224 bases
4. Which of the following possibilities is most likely? The mutation occurred:
   1. in the ear cells of all the deaf individuals.
   2. in all of the cells of all the deaf individuals.
   3. in the germ cells of all the deaf individuals.
   4. in the germ cell of one of the ancestors from the pedigree.
5. Of the following possibilities, which is least likely? The mutant allele causes:
   1. a change in the gene's reading-frame.
   2. formation of a protein containing the same amino acids, but in a different order.
   3. the appearance of a translation stop codon in a different place than in the normal gene.
   4. formation of an inactive protein or no protein formed.
6. The mutation effectively causes a reading frame shift, leading to the early appearance of a translation stop codon. The translation ends at codon number 299 instead of codon number 574. Try to explain how such a change in the DNA sequence could cause deafness. Refer to DNA, mRNA, protein synthesis, protein function and phenotype in your answer.

## Answers

1. A
2. D
3. C
4. D
5. B
6. The reading-frame shift causes the early appearance of a stop codon. The mRNA transcribed from the mutant DNA sequence template is shorter than the normal mRNA. The protein translated from the mutant mRNA template is shorter than normal. The gene in question encodes a protein that is a transcription factor. The short protein formed from the mutant allele template is inactive as a transcription factor. For this reason, the genes affected by the transcription factor that we identified are not transcribed, or are over-transcribed.   
   At this time, we do not know what genes are regulated by the transcription factor we identified, what genes it activates or what genes it suppresses. We know that whoever has one such mutated allele will be deaf in adulthood.

## Question 4 – Why the other answers are wrong

* 1. in the ear cells of all the deaf individuals: The mutation is inherited "down", meaning that it is unlikely to have occurred in ear cells.
  2. in all of the cells of all the deaf individuals: The mutation occurred in a progenitor of many cells.
  3. in the germ cells of all the deaf individuals: The chance of an identical mutation occurring in relatives is miniscule.

## What have you learned from this assignment?

You located a mutation that is characteristic of deaf people in this family only, using the BLAST program. You identified the mutation that differentiates a normal sequence carried by hearing people from a mutant sequence found in deaf adults. You showed that there is a significant difference between the normal allele and the mutant allele. This is proof that the candidate gene is the gene involved in the hearing process.

The gene is no longer a "candidate" - you have found what you were looking for!