# Assignment 3 – Human gene *TMPRSS6*

The TMPRSS6 gene encodes a protein which is a type II transmembrane serine protease and is attached to the cell surface. The protein is part of a signalling pathway that controls the levels of hepcidin of iron, which is a key regulator of iron balance in the body. Mutations in the gene results in iron shortage and therefore availability for iron in hemoglobine. This in turn results in less oxygen available for cells and causes symptoms of anaemia.

Use NCBI (<https://www.ncbi.nlm.nih.gov/>) and Benchling (<https://www.benchling.com>) to answer the following questions:

Search for the mRNA sequence of the *TMPRSS6* gene. If there are multiple isoforms available, take isoform 1 (or isoform a).

**Question 6**: What is the NM-number of the sequence?

Example: NM\_123456789.0

Load the mRNA sequence of the *TMPRSS6* gene in Benchling and annotate the longest ORF. The minimal ORF length should be 200 codons in your settings.

You want to amplify the gene using PCR and clone the PCR-product in a vector using restriction enzymes. Use Benchling to design primers that will amplify the gene from start to stop codon.

The forward primer should contain the PacI restriction site.

The reverse primer should contain the XbaI restriction site.

**Question 7**: What is the sequence (from 5’ to 3’) of the forward primer? Give only the nucleotides in your answer.

**Question 8**: What is the sequence (from 5’ to 3’) of the reverse primer? Give only the nucleotides in your answer.

# Assignment 4 – Human gene *AIP*

The aryl hydrocarbon receptor-interacting factor protein (AIP) is a protein that interacts with many other proteins. Through these interactions AIP helps regulate several cellular processes such as cell growth and division and is thought to act as a tumor suppressor. Mutations in the gene may cause familial isolated pituitary adenoma.

In the zip-file you will find the sequence of the *AIP* gene containing a mutation. Load the sequence in Benchling. In addition, you will find primer sequences to amplify and clone the gene in the pET32a plasmid (also provided in the zip-file).

Link the primers to the template, create a PCR-product and clone the PCR-product in the plasmid. The primers contain the NotI and BglII restriction sites to clone the PCR-product in the plasmid. Import the primers (‘*Oligo’* → ‘*Import DNA/RNA sequences from spreadsheet*’ → choose folder and click ‘*Next’* → choose correct file and click ‘*Next’* → ‘*Next’*.

**Question 9**: What is the size (in bp) of the construct (insert + plasmid)?

Create a protein alignment of the wildtype and mutated AIP sequences to find the mutation. Use either Benchling or BLAST to find the mutation.

**Question 10**: What is the mutation on protein level? Give the official notation for the mutation.

Example: p.F462Y (a Phenylalanine residue at position 462 in the wildtype is replaced by a Tyrosine in the mutant)

# Assignment 5 – FIJI overlay/movie

In the zip-file you will find files to either create a small movie or an overlay of several microscopic pictures.

Movie: with FIJI create a movie of 7 frames per second from the sequence of pictures. Save the movie as an **avi**-file.

Overlay: with FIJI create an overlay using the correct channels for the colors. Save the overlay as a **png**-file.

**Question 11**: Save your movie/overlay and upload the file with the form. The filename should be your student number and last name.

Example: 123456\_vanDijk.avi (or 123456\_vanDijk.png)