# Assignment 3 – Human gene *FANCA*

Fanconi anaemia complementation, group A (FANCA) belongs to the FANC family of genes. These proteins are involved in post-replication repair and the maintenance of chromosome stability that regulates the differentiation of haematopoietic stem cells into mature blood cells. FANCA binds to single and double stranded DNA, with higher preference for double stranded DNA. Mutations in the gene are the cause of Fanconi’s anaemia. The main features of this disease are aplastic anaemia, congenital abnormalities and susceptibility to several kinds of cancer.

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 *FANCA* 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 *FANCA* 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 *PEX5*

The peroxisomal targeting signal 1 receptor is encoded by the *PEX5* gene. The protein is involved in the specific transport of molecules for oxidation in the peroxisome. The peroxisome targeting signal SKL binds to PTS1R in the cytosol followed by binding to the Pex14p receptor allowing importation of the peroxisomal protein through the pexsubunit transporter. Diseases associated with dysfunctional receptors include X-linked adrenoleukodystrophy and Zellweger syndrome.

In the zip-file you will find the sequence of the *PEX5* 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 *PEX5* 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)