# Assignment 3 – Human gene *PAX2*

The paired box genes are encoding proteins that are important in the development and proliferation of multiple cell lines, organs and central nervous system. PAX2 is a transcription factor that is controlled by other signalling molecules.

PAX2 misexpression is observed in kidney disorders. High expression seems to fuel cell cycling, inhibit cell death and resistance to chemotherapy. This last feature makes it an attractive therapeutic candidate.

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 *PAX2* gene. If there are multiple isoforms available, take isoform 1 (or isoform a).

**Question 6**: What is the NM-number of the sequence (including the version number)?

Example: NM\_123456789.0

Load the mRNA sequence of the *PAX2* 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 *ACTB*

ACTB is one of several β-actin isoforms and are highly conserved proteins. The proteins are involved in cell motility, structure and integrity. The protein is often used in Western blotting as control as well as in qPCR where the transcript is used as a housekeeping gene standard. Mutations in the gene have been associated with diffuse large B-cell lymphoma.

In the zip-file you will find the sequence of the *ACTB* 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. Import the primers (‘*Oligo’* → ‘*Import DNA/RNA sequences from spreadsheet*’ → choose folder and click ‘*Next’* → choose correct file and click ‘*Next’* → ‘*Next’*.

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.

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

Create a protein alignment of the wildtype and mutated ACTB 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)