# Assignment 3 – Human gene *EVC*

The EVC gene is one of two genes associated with the rare genetic disorder Ellis-van Creveld syndrome. There are several symptoms present in patients, including teeth present at birth, congenital heart defects, short-limbed dwarfism and short ribs. EVC acts as a positive mediator for three hedgehog signalling molecules. These hedgehog molecules are involved in the early embryonic cell proliferation.

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 *EVC* 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 *EVC* 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 *TINF2*

TERF1-interacting nuclear factor 2 is a protein that in humans is encoded by the *TINF2* gene. TINF2 is a component of the shelterin protein complex found at the end of telomeres. This complex protects the telomeres by allowing the cell to distinguish between telomeres and regions of DNA damage. Mutations in this gene cause dyskeratosis congenita (DKC), an inherited bone marrow failure syndrome

In the zip-file you will find the sequence of the *TINF2* 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 TINF2 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)