F24 Group Activity #2
Group #:_____
The following group members were in attendance and contributed to the assignment:
1.
2.
3.
4.
5.
6.

A patient comes into the clinic with heart failure. An analysis of commonly dysfunctional genes in the heart reveals mutations in the gene for desmoplakin, which encodes a protein essential to maintaining heart cell-to-cell integrity while the heart beats. There are two separate nucleotide mutations, one in each of the two copies of the gene. These are shown in bold and red in the sequences below.

Section of Chromosome 1 ***Coding Strand***:
Assume the first nucleotide shown *is the start site of transcription* into mRNA
Normal: **5'** AGGCATGCACAAATGT **3'**
Patient: **5'** AGGCATGCACA**C**ATGT **3'**

Section of Chromosome 2 ***Template Strand:***
Assume the first nucleotide used for transcription is *already in-frame within the coding sequence*
Normal: **5'** AGGTGACGTGGC **3'**
Patient: **5'** AGGT**T**ACGTGGC **3'**

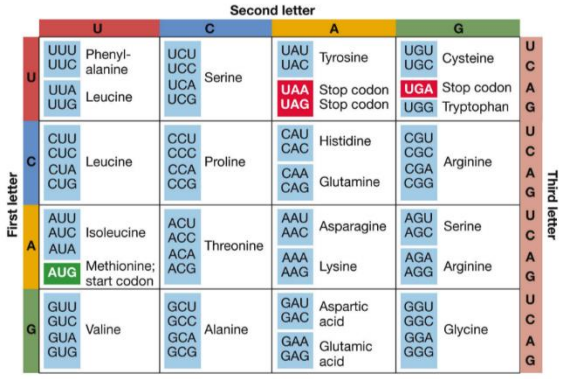
Q1: What would the mRNA transcripts be for these sections of sections of the desmoplakin gene? Please indicate polarity of the mRNA (i.e., 5' and 3' ends; 10 pts)

Chromosome 1:
Normal: **5' AGGCAUGCACAAAUGU 3'** **(or 3' UGUAAACACGUACGGA 5')**
Patient: **5' AGGCAUGCACACAUGU 3'** **(or 3' UGUAAACACGUACGGA 5')**

Chromosome 2:
Normal: **5' GCCACGUCACCU 3'** **(or 3' UCCACUGCACCG 5')**
Patient: **5' GCCACGUAACCU 3'** **(or 3' UCCAAUGCACCG 5')**

Q2: Would you expect these mutations to affect transcriptional efficiency? Why or why not?(5 pts)
No, the transcriptional machinery will not be affected by changes in the nucleotide sequence, and will produce the mRNA as normal.

Q3: Using the codon table shown, what would the translated sequences be for these sections of the mRNA? Recall the directionality of how the ribosome reads the mRNA. (10 pts)



Chromosome 1:
Normal: **Methionine – Histidine – Lysine - Cysteine**
Patient: **Methionine – Histidine – Threonine - Cysteine**

Chromosome 2:
Normal: **Alanine – Threonine – Serine - Proline**
Patient: **Alanine – Threonine - STOP**

Q4: Why would the mutation in Chromosome 1 likely be problematic for this patient? (10 pts)
The amino acid sequence of desmoplakin has changed, with the substitution of lysine (positively charged) for threonine (neutral or negatively charged), which could likely affect the overall protein folding and 3D geometry of the protein, interfering with its function.

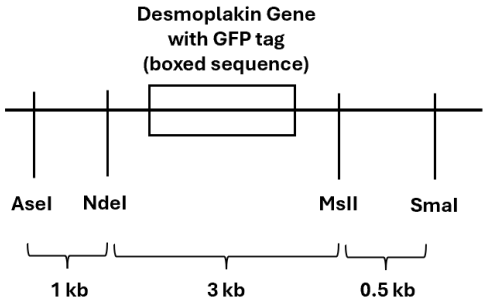
Q5: Why would the mutation in Chromosome 2 likely be problematic for this patient? (10 pts)
The mutation leads to a premature STOP codon, and so would produce a truncated and most likely dysfunctional version of desmoplakin.

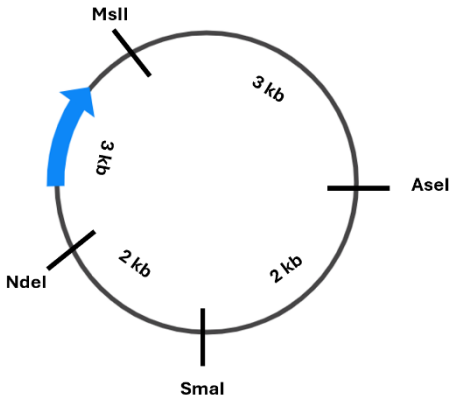
You would like to create a transgenic mouse line to study the cell biology of these specific desmoplakin mutations to try to develop a future therapy for the patient. First, you are curious about whether desmoplakin is expressed in other tissues than the heart, as this could guide your transgene generation.

Q6: What technique would you want to use to study the expression of desmoplakin *at the protein level* in different tissues? (5 pts)
You would want to use a reporter gene construct that can allow you to visualize the expression and activity of desmoplakin in different cell types without affecting its regulation and function.

To accomplish this, you need to clone the desmoplakin gene into a plasmid so you can see whether the gene is expressed in different tissues after transfecting various cell types. For the purposes of this assignment, consider *the blue arrow in the plasmid below to contain all regulatory sequences and the promoter necessary for desmoplakin expression*.

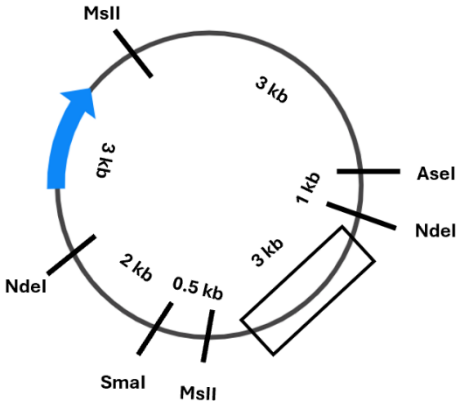
Q7: Given the restriction enzyme sites and the plasmid map below, select the two restriction enzymes that you would use for cloning desmoplakin and state why the others would not work. (10 pts)





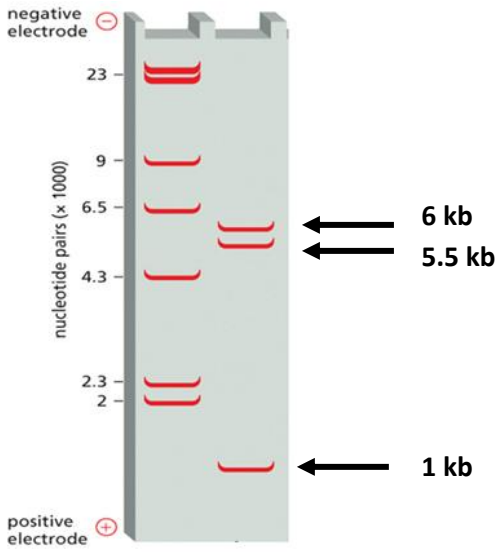
AseI & SmaI would work for cloning desmoplakin, the other combinations would either not include the gene, include the gene in the incorrect direction, or not include the promoter.

Q8: Please draw your recombinant plasmid below, including all restriction enzyme sites (10 pts):



To ensure everything is inserted properly into the plasmid, you perform a second digestion using the restriction enzymes NdeI and AseI, and analyze the result using gel electrophoresis.

Q9: Please draw the expected outcome of this restriction digestion on the gel below, labeling the sizes of your bands (10 pts):



Your transfection experiments demonstrate that desmoplakin is also expressed in skin and lung cells, in addition to the heart. We only want to express the mutant desmoplakin gene in the heart, while leaving non-cardiac desmoplakin as normal, so we can study the heart in isolation within the animal.

Q10: What is an approach we could use to selectively express our mutant desmoplakin in the heart and not in the other tissues? (10 pts)

We can use a tissue specific promoter or transcriptional regulator that will only be active in the heart tissue and not in other tissues. As a result, in non-heart tissues, transcription will not occur and our mutant desmoplakin will not be expressed.

Q11: In overview, what steps would you need to take to go from mouse embryonic stem cells with a normal desmoplakin gene to get to the point where you are ready to inject a pregnant mouse with the mutant desmoplakin genes mimicking our patient and begin breeding for fully transgenic mice? (10 pts)

We would need to perform CRISPR/Cas9 gene editing to create the mutations of interest in the embryonic stem cells prior to injecting into the pregnant mouse. To do this, we need to design a guideRNA that will target Cas9 to the specific location within desmoplakin where we want to introduce the mutations.

For mutation 1, we would need to include a donor DNA template with the nucleotide change so we get precise correction of the nucleotide leading to the altered amino acid.

For mutation 2, we could also use a donor DNA template for this specific change, or simply perform the cutting and see whether non-homologous end joining creates a premature STOP codon on its own, which is a reasonably likely outcome.