EXAM FORMAT: Total 4 questions, EACH WORTH 25 POINTS

- **Two Essay questions: students have to answer both (no choices)**
- **Two Experimental Design questions: students choose one**
- **Two Data Interpretation questions: students choose one**

Time yourself and make sure to submit BEFORE OR BY the deadline of 4pm.

Set a timer and submit whatever you have before or by the 4pm mark.

Essay Questions. YOU HAVE TO ANSWER the 2 ESSAY QUESTIONS.

EACH ESSAY QUESTION IS WORTH 25 POINTS

Clearly indicate the number and the references for each of your essays. The essays must appear in numerical order in your written document.

Essay 1 WORTH 25 POINTS

References:

1. Amoasii L, Hildyard JCW, Li H, Sanchez-Ortiz E, Mireault A, Caballero D, Harron R, Stathopoulou TR, Massey C, Shelton JM, Bassel-Duby R, Piercy RJ, Olson EN. Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Science. 2018 Oct 5;362(6410):86-91. doi: 10.1126/science.aau1549. Epub 2018 Aug 30. PMID: 30166439; PMCID: PMC6205228.

2. Erkut E, Yokota T. CRISPR Therapeutics for Duchenne Muscular Dystrophy. Int J Mol Sci. 2022 Feb 6;23(3):1832. doi: 10.3390/ijms23031832. PMID: 35163754; PMCID: PMC8836469.

Question #1:

Dystrophin is a large scaffolding protein that supports muscle structure and function. Mutations in the gene encoding dystrophin cause Duchenne muscular dystrophy (DMD) which results in a partial transcript of the dystrophin gene. Many of these mutations cluster into "hotspots," in a region that spans exons 45 to 50, resulting in exon 51 to be out of frame with preceding exons. Using the deltaE50 dog model of DMD that harbors a loss of exon 50, Amoasii et al. show that gene editing is an effective approach for restoring dystrophin expression. To explain the success of their approach answer a-c below.

A) Explain **in detail** the gene editing technique that was employed to correct the reading frame in deltaE50 dogs. Include the exons that represent the corrected reading frame.
B) Explain the results of 2 experimental procedures that were employed to demonstrate dystrophin protein correction in vivo.
C) Explain the results of dystrophin expression when different concentrations of the gene editing components were used

Essay 2 WORTH 25 POINTS

References:

1) Alquezar C, Arya S, Kao AW. Tau Post-translational Modifications: Dynamic Transformers of Tau Function, Degradation, and Aggregation. Front Neurol. 2021 Jan 7;11:595532. doi: 10.3389/fneur.2020.595532. PMID: 33488497; PMCID: PMC7817643.

2) Yan Y, Wang X, Chaput D, Shin MK, Koh Y, Gan L, Pieper AA, Woo JA, Kang DE. X-linked ubiquitin-specific peptidase 11 increases tauopathy vulnerability in women. Cell. 2022 Oct 13;185(21):3913-3930.e19. doi: 10.1016/j.cell.2022.09.002. Epub 2022 Oct 4. PMID: 36198316; PMCID: PMC9588697.

Question #2:

Although it is well-known that women are afflicted by Alzheimer's disease (AD) 1.7 times more frequently than men, the mechanistic basis for this increased vulnerability has not been established. It is possible that this could be related to the fact that women endure greater tau burden than men.

Explain how inhibiting USP11-mediated tau deubiquitination may provide an effective therapeutic opportunity to protect women from increased vulnerability to AD and other tauopathies.

You have to cover the following:

  A) Tau post-translational modifications and their functional impacts
  B) X-linked USP11 deubiquitinates tau, enhancing its acetylation and aggregation
  C) USP11 escapes X-inactivation leading to elevated expression in females compared to males
  D) USP11 levels correlate strongly with tau brain pathology in females but not males
  E) Elimination of *usp11* protects females from tau pathology and cognitive impairment

Experimental Design

PICK ONE Experimental Design QUESTION, WORTH 25 POINTS

You must answer 1 of the 2 experimental design choices. Clearly indicate the number and the references for your answers. The answers must appear in numerical order in your written document.

Choice 1. Experimental Design

References:

1) Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu Rev Virol. 2016 Sep 29;3(1):237-261. doi: 10.1146/annurev-virology-110615-042301. Epub 2016 Aug 25. PMID: 27578435; PMCID: PMC5457962.

2) JW, Lagniton PNP, Liu Y, Xu RH. mRNA vaccines for COVID-19: what, why and how. Int J Biol Sci. 2021 Apr 10;17(6):1446-1460. doi: 10.7150/ijbs.59233. PMID: 33907508; PMCID: PMC8071766.

Question #3:

A new coronavirus has emerged that is infecting humans all over the world.

A) Design an experiment (in vitro) to identify the protein(s) the virus uses to enter the host cell.

B) Design an analysis (in silico) to identify the protein(s) the virus uses to enter the host cell.

C) Design an experiment to identify/confirm the host protein that binds to the viral protein.

D) Design an mRNA-based vaccine to the viral protein.

Choice 2. Experimental Design

References:

1) Bensidoun P, Reiter T, Montpetit B, Zenklusen D, Oeffinger M. Nuclear mRNA metabolism drives selective basket assembly on a subset of nuclear pore complexes in budding yeast. Mol Cell. 2022 Oct 20;82(20):3856-3871.e6. doi: 10.1016/j.molcel.2022.09.019. Epub 2022 Oct 10. PMID: 36220102.

2) Bensidoun P, Zenklusen D, Oeffinger M. Choosing the right exit: How functional plasticity of the nuclear pore drives selective and efficient mRNA export. Wiley Interdiscip Rev RNA. 2021 Nov;12(6):e1660. doi: 10.1002/wrna.1660. Epub 2021 May 2. PMID: 33938148.

Question #4:

In the paper "Nuclear mRNA metabolism drives selective basket assembly on a subset of nuclear pore complexes in budding yeast" by Bensidoun et al, Mol Cell. 2022, authors used Mlp1 as the marker for the basket of the nuclear pore complexes (NPCs) in their study. Please answer the following questions concisely but remember to include controls in your experiments. You are not limited to using the methods described in the above references as long as your experiments can address the questions.

A) Please design an experiment to examine whether the NPC basket, such as Mlp1, is dynamic.

B) Mammalian Tpr is the ortholog of yeast Mlp1 and Mlp2 and is considered a basket nucleoporin. Please design an experiment to determine whether the similar NPC heterogeneity also exists in mammalian cells: some nuclear pores contain a basket, and some don't have a basket.

C) Design experiments to determine that the assembly of nuclear pore baskets depends on mRNA, not rRNA, metabolism.

D) To study the functions of the NPC baskets, you want to know which components are preferred to be associated with the basket-containing NPCs. Please design experiments, using budding yeast as a model system, to identify the proteins that are specifically associated with the basket-containing NPCs.

PICK ONE Data Interpretation QUESTION, WORTH 25 POINTS

You must answer 1 of the 2 data interpretation choices. Clearly indicate the number and the references for your answers. The answers must appear in numerical order in your written document.

Choice 1. Data Interpretation

References:

1) Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA. A chromatin landmark and transcription initiation at most promoters in human cells. Cell. 2007 Jul 13;130(1):77-88. doi: 10.1016/j.cell.2007.05.042. PMID: 17632057; PMCID: PMC3200295.

2) Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA. c-Myc regulates transcriptional pause release. Cell. 2010 Apr 30;141(3):432-45. doi: 10.1016/j.cell.2010.03.030. PMID: 20434984; PMCID: PMC2864022.

Question #5:

You are studying the regulation of two genes (gene "X" and gene "Y"). Both of these genes seem to be inactive in un-stimulated cells. However, both of these genes produce large amounts of transcript after cell stimulation with factor "Z". You are interested in understanding how factor Z causes this strong activation of genes X and Y transcript production.

Chromatin immuno-precipitation is employed to study the state of the two gene loci (X and Y) in the presence and absence of factor Z. The following data is obtained:

Cells BEFORE factor Z treatment

Gene	H3K4me3	H3K36me3
X	negative	negative
Y	positive	negative

Cells AFTER factor Z treatment

Gene	H3K4me3	H3K36me3
X	positive	positive
Y	positive	positive

Negative means not detected at the gene locus. *Positive* means readily detected at the gene locus

Please answer the following:

A) Please briefly explain what "H3K4me3" and "H3K36me3" refer to and what their presence at a gene locus is thought to signify.
B) What is your interpretation of the data with respect to the mechanism by which factor Z activates transcript production from gene X?
C) Please describe what other molecular evidence you would expect to find at the gene X locus that would support your initial interpretation (described in part B) from the above data.
D) What is your interpretation of the data with respect to the mechanism by which factor Z activates transcript production from gene Y?
E) Please describe what other molecular evidence you would expect to find at the gene Y locus that would support your initial interpretation (described in part D) from the above data.

References:

1) Chiba K, Johnson JZ, Vogan JM, Wagner T, Boyle JM, Hockemeyer D. Cancer-associated TERT promoter mutations abrogate telomerase silencing. Elife. 2015 Jul 21;4:e07918. doi: 10.7554/eLife.07918. PMID: 26194807; PMCID: PMC4507476.

2) Naxerova K, Elledge SJ. Taking the brakes off telomerase. Elife. 2015 Jul 21;4:e09519. doi: 10.7554/eLife.09519. PMID: 26194808; PMCID: PMC4507575.

Question #6

The article "Cancer-associated TERT promoter mutations abrogate telomerase silencing", by Chiba et al., describe the generation of 4 lines of human pluripotent stem cells with the TERT promoter mutations diagramed below:
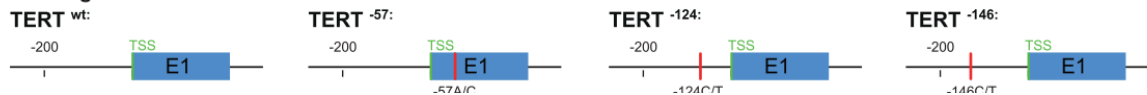

-Referring to panel D below, briefly explain how telomere length is measured, and describe the effect of these mutations on telomere length. Comment on each mutation separately and compare to wild-type.

- Referring to panel F below, describe the effect of each mutation on telomerase activity.

- Referring to panel E below, describe the effect of each mutation on the transcription of TERT.
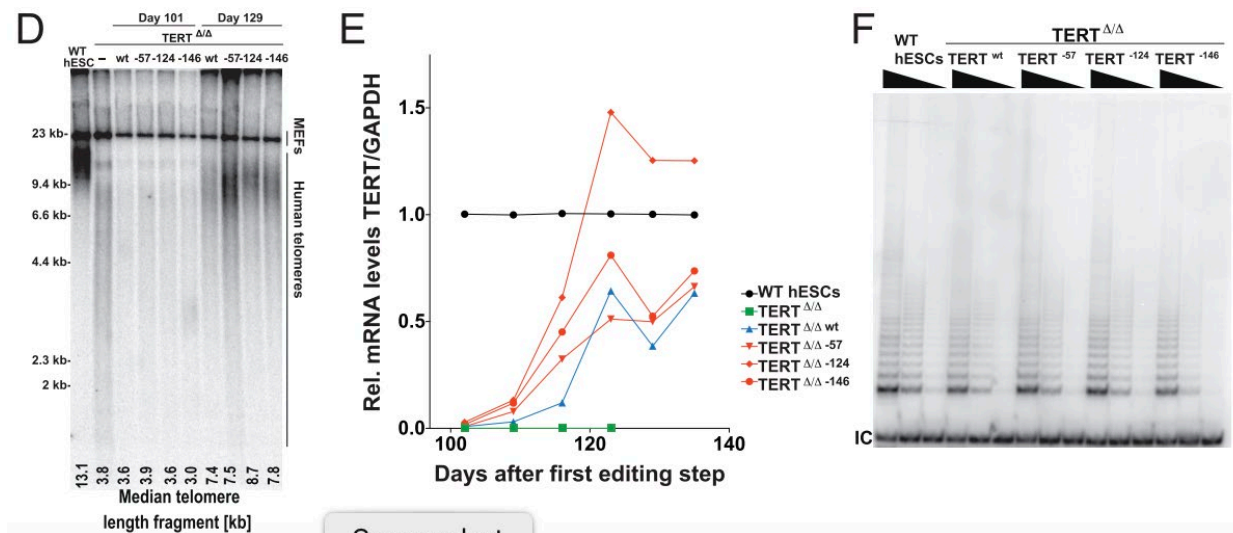
The authors then generate cell lines expressing GFP (G, control), hTERT (T), hTR (R), and hTERT+hTR (T&R). In panel D below, what effect can be seen on each of these conditions on telomerase activity, in the three cell lines examined (hESCs, NPCs and fibroblasts).

D
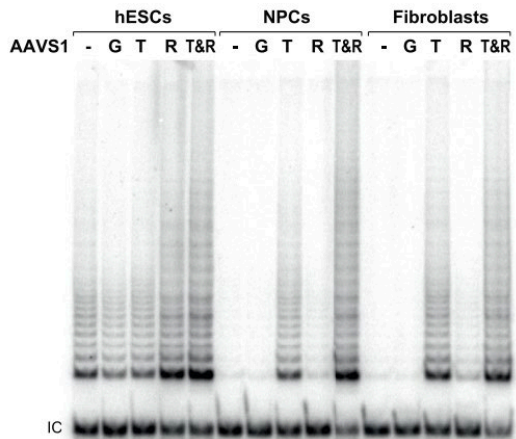
The authors then grow the hESC under differentiating conditions. What are the effects of each mutation in the TERT promoter (-57, -124 and -146) on transcription? Describe first panel A, and then comment on panels B and C in your answer.
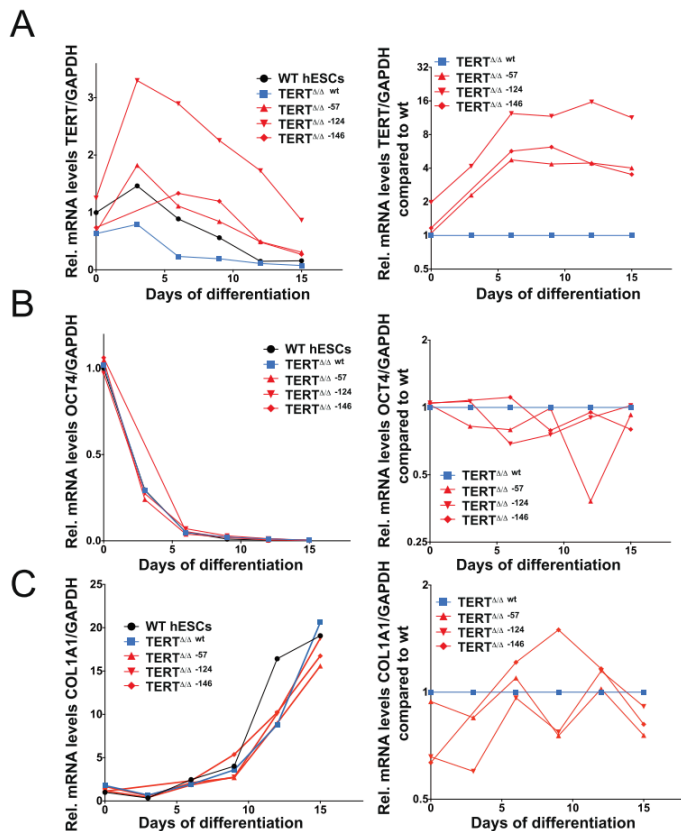
As discussed in the commentary provided (Naxerova and Elledge), the TERT promoter mutations

analyzed are associated with high incidence of tumors, notably melanoma.

What is the model for the mechanism of cellular transformation in cells that contain these mutations in the TERT promoter? How do these mutations affect the expression of telomerase in order to promote tumor formation?