Part 1- General Essay: This section of the exam is worth 60% of the entire grade. Answer any **four** of the next six questions**-** do not answer all six- only answer **FOUR.** Make sure to develop your thoughts with fully developed and complete sentences. This section requires a general essay that must answer the questions below. Each question is worth 15 points.

1. Question about CRISPRs.
A. What are CRISPRs? What are the different types of CRISPRs and how does each type work at the molecular level? (7 points)

B. Describe at least two mechanisms by which phages evade CRISPR? (3 points)

C. How does the recently uncovered type III CRISPR molecular function help discriminate lytic versus lysogenic phage infection? (2 points)

D. What implications do CRISPRs have for bacterial evolution? (3 points)

Reading List:
Goldberg, G.W. et al., 2014. Conditional tolerance of temperate phages via transcription-dependent CRISPR-Cas targeting. Nature 514: p.633.

Marraffini, L.A., 2015. CRISPR-Cas immunity in prokaryotes. Nature 526: p.55.

2. The topic is ribosome structure and the mechanism of translation.
A. Describe in a general way how messenger RNAs are translated into proteins by the ribosome. (5 points)

B. Describe the 50S ribosomal subunit and the mechanism of peptidyl transfer. (5 points)

C. Describe the 30S ribosomal subunit and the mechanism of decoding. (5 points)

For parts (B) and (C), the research use of antibiotics should be included in your answer.

Reading List:
Steitz, T. 2009. From the structure and function of the ribosome to new antibiotics.

Ramakrishnan, V. 2010. Unraveling the Structure of the Ribosome. Angew. Chem. Int. Ed. 2010, 49, p.4355.

3**.** The *p53* gene produces a wild-type p53 protein that controls many cellular functions. A high proportion of human tumors have mutations in the *p53* gene and express mutant p53 protein. It has become clear that different mutant p53 proteins have different functions. Answer the following questions based on your understanding of the p53 pathway and based on the assigned readings.

A. Describe the different types of mutations that are found in the *p53* gene and why they result in cancers that have variable checkpoints and targets that are downregulated and up-regulated. Make sure to discuss the central biochemical function of wild-type p53 and contrast this to the different types of mutant p53 proteins that arise (5 points)

B. Explain how cancer cells with an R273H or an R172H mtp53 are genetically reprogrammed in their nuclei as compared to cells with wild-type p53. What experimental method was used to elucidate the co-opted pathway. (5 points)

C. What precision medicine approach has been proposed to target this co-opted molecular pathway? What regulators must be targeted? How was this achieved pharmacologically? (5 points)

Reading Lists:
Zhu et al., 2015. Gain of function p53 mutants co-opt chromatin pathways to drive cancer growth. Nature: 525, p. 206.

Prives and Lowe, 2015. Mutant p53 and Chromatin Regulation. Nature 525: p199.

4. In *C. elegans*, the conserved bone morphogenetic protein (BMP) signaling pathway, the Sma/Mab pathway, controls several developmental processes in development, including body size, male-tail morphogenesis, immunity, and reproductive aging. Transport of the receptors from the plasma membrane to endosomes has been proposed to promote signaling and result in BMP-signaling gradients.

A. How does BMP signaling occur? What are the players involved? (5 points)

B. How does post endocytic trafficking of BMP receptors contribute to the regulation of the signal? Which trafficking pathways are involved? (8 points)

C. Does the receptor internalization depend on ligand binding? (2points)

Reading list:
Gleason et al., 2014. BMP signaling requires retromer dependent recycling of the type I receptor. PNAS 111, no. 7, p. 2578.

Patterson and Padgett, 2000. TGF beta-related pathways. Roles in *Caenorhabditis elegans* development. Trends in Genetics. Jan;16 (1):27-33.

5. The DNA damage response (DDR) is constituted by a number of pathways responding to specific types of damage. Of those, ATM and ATR are central kinases essential for genome stability.

Based on your assigned readings, explain:

A. The molecular structure of ATM and ATR, including the general features of the protein family they belong to, important interaction domains, and relevant interacting factors. (3 points)

B. For ATM describe the following three features: (4.5 points)

- the type(s) of DNA damage it responds to
- the modalities of its activation
- known downstream factors important for function

C. For ATR describe the following three features: (4.5 points)

- the type(s) of DNA damage it responds to
- the modalities of its activation
- known downstream factors important for function

D. Are the ATM and ATR pathways independent, or is there crosstalk between the two? Explain. (3 points)

Reading lists:

Maréchal and L. Zou, 2013. DNA Damage Sensing by the ATM and ATR Kinases. Cold Spring Harbor Perspect Biol 5:a012716

Lovejoy, C.A. and D. Cortez, 2009. Common mechanisms of PIKK regulation. DNA Repair 8: 1004–1008.

6. Steinberg et al. reported their studies on the motility of the nuclear pore complexes (NPCs) in three different fungi, *Ustilago Maydis, Aspergillus nidulans* and *Saccharomyces cerevisiae*. Based on their work, answer the following questions.

A. Why are the NPCs in *U. Maydis, A. nidulans* and *S. cerevisiae* mobile, while the NPCs in the interphase nuclear envelope of animal cells do not have the motility? What are the functional significances of the NPC's motility in fungi? What are the consequences when the motility of NPCs in fungi is inhibited? (6 points)

B. Different molecular machineries have been shown to control the motility of the NPCs in fungi. What proteins are important for the motility of the NPCs in *U. Maydis* and *S. cerevisiae, respectively?* What are the characteristic features of the motility of NPCs in fungal cells? (5 points)

C. How do different motor proteins in *U. Maydis* control the motility of the NPCs? How does deletion or inactivation of one motor protein affect the motility and distribution of the NPCs? (4 points)

Reading list:

Steinberg G et al. 2012. Motor-driven motility of fungal nuclear pores organizes chromosomes and fosters nucleocytoplasmic transport. J Cell Biol. Aug 6;198(3):343-55. doi: 10.1083/jcb.201201087. Epub 2012 Jul 30.

Casey AK[1], Wente SR. 2012. Nuclear transport: shifting gears in fungal nuclear and cytoplasmic organization. Curr Biol. 2012 Oct 9;22 (19):R846-8. doi: 10.1016/j.cub.2012.08.043.

Part 2A - Experimental Design: This section of the exam is worth 20% of the total grade of the exam. Answer either question 7 or question 8- **DO NOT ANSWER BOTH.**

7. Rice Tech, a biotechnology company specializing in genetically modified rice crops, has identified a mutation (S145A) of a gene (*drout*) that makes one variety of rice (*Oryza sativa*) more drought resistant than another (*Oryza glaberrima*). Cells from *O. sativa* can grow for two days in the absence of water, unlike cells from *O. glaberrima*. To confirm their findings, Rice Tech scientists want to knock-in the mutant *drout* allele into cells of *O. glaberrima* using CRISPR-Cas9 (Cong et al, *Science* 2013). (20 points)

A. Describe how the scientists can edit the *drout* gene of *O. glaberrima* to contain the S145A mutation using CRISPR-Cas9. Specify how the scientists will select and screen for the mutation and how they will determine if they have heterozygous or homozygous mutant cells. (12 points)

B. Describe how the scientists can identify genes that complement the S145A mutation of *drout* in *O. sativa* using the CRISPR-Cas9 technology in a genetic screen. (8 points)

Reading List:

Le Cong, F.A.R. et al., 2013. Multiplex Genome Engineering Using CRISPR/Cas systems. Science 339; p. 819.

Malina, A. et al., 2014. Adapting CRISPR/Cas9 for Functional Genomics Screens. Methods in Enzymology, 546: p. 193.

8. You are studying a large genomic region X that is normally inactive in a particular mouse cell type. You suspect a role for small RNA in keeping this region in a generally silent state. Please answer the following:

A. Design an experiment to seek evidence that region X could conceivably be targeted for silencing by small RNA.

B. Assuming a positive result in part A of this question, design experiments that could provide at least two pieces of evidence that a small RNA directed pathway is involved in the silencing of region X.

C. Assuming a positive result in part B of this question, design experiments to provide at least three different pieces of evidence that a small RNA-mediated region X silencing process is happening at the level of the region X locus in the genome.

For the experiments you design, please make clear the rationale for your selection of procedures and reagents, the expected results and their interpretation. Also please make clear what controls are being included in each experiment you design.

Reading List:

Giles, K.E. et al., 2010. Maintenance of a constitutive heterochromatin domain in vertebrates by a Dicer-dependent mechanism. *Nature Cell Biol*. 12: 94.

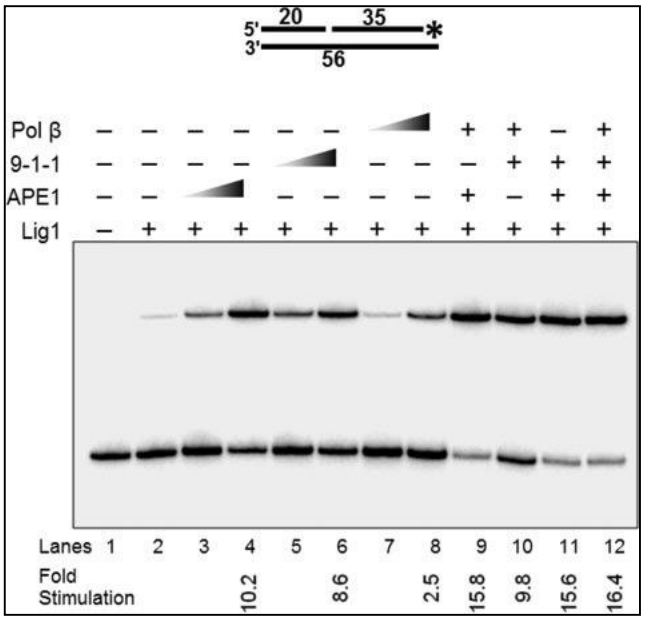
Castel, S.E. and Martienssen, R.A. RNA interference (RNAi) in the nucleus: roles for small RNA transcription, epigenetics and beyond. *Nat Rev Genet* 12(2): p. 100.

Part 2B - Data Interpretation: This section of the exam is worth 20% of the total grade of the exam. Answer either question 9 or question 10- **DO NOT ANSWER BOTH QUESTIONS.**

9. The data presented below is from Balakrishnan et al., 2009. This manuscript examined a role for Long-patch base excision repair in mammalian cells by demonstrating that the polymerase used in short-patch BER is also involved in a pathway that requires flap creation and cleavage in LP-BER.
Analysis of the activity of various Long patch base excision repair sub-pathway (LP-BER) (abbreviated names listed below) proteins and DNA Ligase I (LigI). A nicked DNA substrate (D5:T2:U1) was used to examine the activity of various LP-BER components on LigI. When present, LigI was added at a concentration of 2 fmol per reaction. APE1 was added at 5 and 10 fmol. 9-1-1 was added at a concentration of 250 or 500 fmol, and pol□β was added at a concentration of 10 and 20 fmol. + in the reaction lane signifies the addition of 10 fmol of APE1, 500 fmol of 9-1-1, and 20 fmol of pol β.
Oligonucleotides:
D5, 5'**-** ACTTGCCCGTGCCACCATCCCGACGCCACCTCCTG -3'
T2,
3'GCTGGCACGGTCGGATTTTGTGAACGGGCACGGTGGTAGGGCTGCGGTGGAGG ACG- 5'
U1: 5'- CGACCGTGCCAGCCTAAAAC - 3'

Abbreviations: LP-BER, Long patch base excision repair sub-pathway; Lig1, DNA Ligase I; APE1, apurinic/apyrimidinic endonuclease 1; Pol β, DNA polymerase □; 9-1-1, Rad9-Rad1-Hus1 complex.

Summarize the results from the figure below and address the following questions in your answers.



A. Define the LP-BER pathway. In your answer, include a brief discussion of the relevance of the proteins examined in this pathway and in the assay shown in the figure. State the questions this study is designed to address. (8 points)

B. Explain the particular nucleotide substrate used in this assay. Please briefly describe the assay. (3 points)

C. Which proteins stimulate LigI in this assay and to what extent do they do so? Be specific about what the products are and how they are detected. (4 points)

D. Which of the reactions showed limited or no effect on the activity of LigI? Why do you think this was the case? (3 points)

E. Describe one additional protein that has an important role in this pathway, but was apparently not required for this particular assay and explain why it was not needed. Briefly describe two cellular functions for this protein. (2 points)

Reading List:

Balakrishnan L, Brandt PD, Lindsey-Boltz LA, Sancar A, and Bambara RA, 2009. Long patch base excision repair proceeds via coordinated stimulation of the multienzyme DNA repair complex. *J. Biol. Chem*. 284:15158–15172.

Wang W, Lindsey-Boltz LA, Sancar A, and Bambara RA, 2006. Mechanism of stimulation of human DNA Ligase I by the Rad9-Rad1-Hus1 checkpoint complex. *J. Biol. Chem.* 281: 20865–20872.

10. Mycobacterial toxin–antitoxin (TA) systems are implicated in the down-regulation of bacterial cell growth associated with stress survival and latent tuberculosis infection. Cruz et al. recently published a study investigating the activity and intracellular target of the VapC-mt4 toxin / VapB-mt4 antitoxin system of *Mycobacterium tuberculosis* (Nat. Commun. 2015; 6:7480). The results shown in the figures below are from such a publication. Guided by the examination of the data presented in these figures and the background and methodological knowledge you acquired from the analysis of the publication of Cruz et al., please answer the following questions.

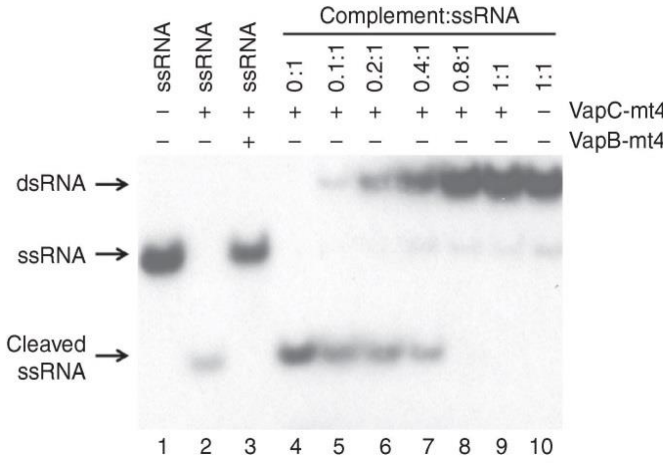


Fig. 5. Gel image showing cleavage assay results. A 20-nucleotide 5' end-labelled RNA containing the VapC-mt4 cleavage consensus sequence ACGC alone (lane 1), after incubation with VapC-mt4 (lane 2), or after incubation with VapB-mt4 and VapC-mt4 (lane 3). This 20- nucleotide RNA was also preincubated with increasing amounts of an RNA complement lacking an ACGC consensus (lanes 4–10) followed by incubation with VapC-mt4. The positions of the double stranded RNA (dsRNA), single stranded RNA (ssRNA) and cleaved ssRNA are shown on the left. Reactions were incubated at 37°C for 3 h.

A. Describe the results presented in Figure 5 from Cruz *et al.* Based on the results shown in Figure 5, what can you infer about the cleavage activity of VapC-mt4? What is the preferred substrate of VapC-mt4? What is the effect of VapB-mt4? Explain your reasoning. (8 points)

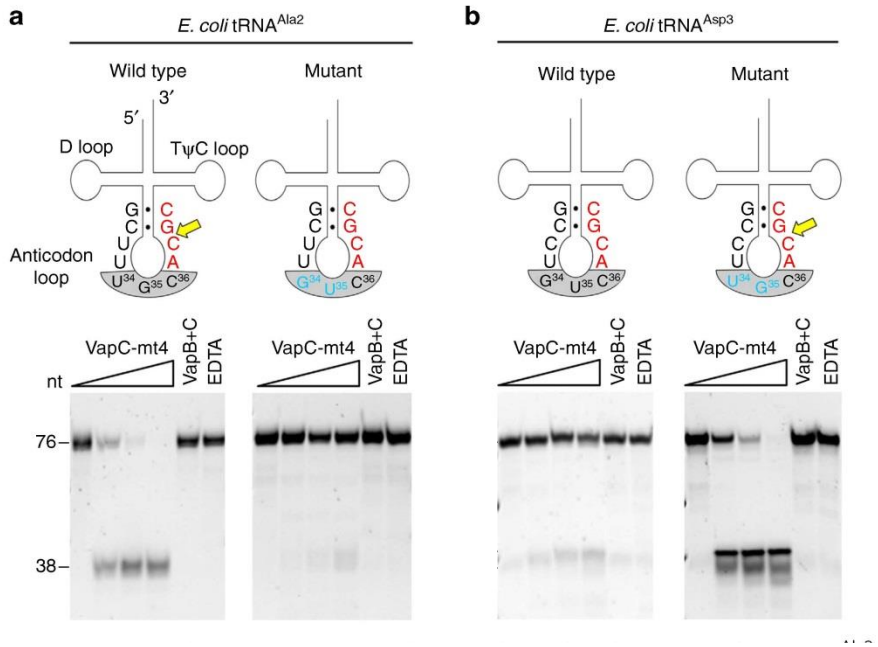


Fig. 6. Gel images showing cleavage assay results. **(a)** Left panel, wild-type $tRNA^{Ala2}$; right panel, $tRNA^{Ala2}$ with mutation of two bases (blue) in the anticodon to match those in $tRNA^{Asp3}$. **(b)** Left panel, wild-type $tRNA^{Asp3}$; right panel, with mutation of two bases (blue) in the anticodon to match those in $tRNA^{Ala2}$. Cleavage site (yellow arrow), consensus cleavage site sequence (red), mutated bases (blue), anticodon (grey shaded), base pairing represented by black dots (•). The vitro-synthesized tRNAs were incubated with increasing amounts of VapC-mt4 (ratios of toxin to RNA were 0:1, 1.25:1, 2.5:1 and 5:1). Control reactions on the right contained the highest concentration of VapC-mt4 preincubated with VapB antitoxin or EDTA before addition of the respective tRNAs. Reactions were incubated at 37°C for 3 h. Sizes of full-length and cleaved tRNA products on the left. Note that in some cases extra bands are visible for the cleavage products because the T7 RNA polymerase used to synthesize the tRNAs frequently leads to 3' end heterogeneity (usually ±1 nucleotide).

B. Describe the results presented in Figure 6 from Cruz *et al.* Based on the results shown in Figure 6, what additional features are important for VapC-mt4 cleavage activity? What is the effect of EDTA in the reaction? Please speculate as to possible explanations for the effect of EDTA. Explain your reasoning. (12 points)

Reading List:

Cruz et al., 2015. Growth-regulating Mycobacterium tuberculosis VapC-mt4 toxin is an isoacceptor-specific tRNase. Nat Communications; 6:7480.

Yamaguchi et al., 2011. Toxin-Antitoxin systems in Bacteria and Archae. Annu Rev Genet.; 45:61