Name: _____

7.013 EXAM 3

TA:_____ **Recitation: _____**

Write your name on this page and your initials on all the other pages in the space provided. This exam has **7 pages** including the coversheet. Check that you have all the pages **1-7**.

NOTE: You can detach Page 7.

Only writing on the **FRONT** of every page will be graded. You may use the backs of the pages, only as scratch paper.

Questions	Points	Score
1	27	
2	14	
3	20	
4	25	
5	14	
TOTAL	100	

Initials: _____

Question 1 (27 points)
During the cell cycle, the anaphase-promoting complex (APC) is necessary for progression from metaphase → anaphase as diagrammed and outlined on **Page 7**.

a) You create the following **homozygous** mutants in **diploid cells (ploidy: 2n).**

- **Mutant 1:** Tubulin loss-of-function mutation (where the spindle fiber does not form).
- **Mutant 2:** Cells lack the transcription factor (TF) required for the transcription of Separase.
- **Mutant 3:** M-Cdk lacks the M-Cyclin binding amino acid sequence.

Based on the information above, complete the table for each of the mutants during cell cycle.

Mutant	APC active (Yes/ No)?	Separase active (Yes/ No)?	Cell enters anaphase (Yes/ No)?	Ploidy of mutant cell (n/ 2n/ 4n)?
1				
2				
3				

b) M-Cdk protein is inhibited by p27Kip1. You grow the M-Cdk temperature sensitive mutant at the permissive temperature (37^{O}C, Plate A) or non-permissive temperature (42^{O}C, Plate B) both in the presence of p27Kip1. Would the cells in Plate A grow **more rapidly/ less rapidly/ at the same rate** as the cells in Plate B? **Explain** why you selected this option.

c) Name the modification on the target protein that the proteasome recognizes: _____

d) The proteasome-mediated hydrolysis of the target protein is an example of a reaction that hydrolyzes **peptide bond/ hydrogen bond/ phosphodiester bond**? Circle as appropriate.

e) You make a cell line that expresses M-Cdk as an in-frame GFP-Cdk fusion protein and M-cyclin as an in-frame RFP-M Cyclin fusion protein. ***Note:*** *GFP fluoresces green and RFP fluoresces red.*

i. Which part in a dividing cell would fluoresce **red**: **Cytoplasm/ Nucleus/ Cell membrane/ Golgi body/ Lysosomes**?

ii. **Circle** the phase(s) of the cell cycle when the cells would fluoresce **green: G1/ S/ G2/ M/ all**

f) If you had a mutant cell that shows a homozygous loss-of-function mutation of M-Cdk gene and a constitutively active APC protein, would you characterize it as oncogenic? **Explain.**

g) Classify the **M-Cyclin/ M-Cdk/ APC/ Securin/ Separase** genes as …

i. **Proto-oncogene(s):** _____

ii. **Tumor suppressor gene(s):** _____

Initials: _____

Question 2 (14 points)

a) Coal tar is a known carcinogen. Exposure to coal tar can form multiple tumors in mice. Would these tumor cells, when grown in culture plates, form a monolayer or multiple foci (piles of cells)? **Explain** why you selected this option.

Histidinal dehydrogenase (HD) is needed for histidine synthesis in bacteria**.** The following bacterial mutants have mutations in the DNA sequence for the amino acids 1-4 of HD. ***Note:*** *The start codon is underlined in wild type (WT), mutants 1 and 2. Each alternative codon is shaded. A codon chart is on* ***Page 7****.*

WT:	5′-A**ATG**ATAGATATG--3′ 3′-TTACTATCTATAC--5′
#1:	5′-A**ATG**TAGATATGG--3′ 3′-TTACATCTATACC--5′
#2:	5′-A**ATG**ATTGATATG--3′ 3′-TTACTAACTATAC--5′

b) Which DNA strand is the **template for transcription** in the WT DNA sequence: **Top or bottom?**

c) On the WT DNA, **show the direction of transcription** by an arrow.

d) Which bacterial mutant is His- such that it could be used in the Ames test: **1 OR 2**? **Explain** why you selected this mutant and not the other.

e) You find that coal tar is **NOT** mutagenic per the standard Ames test although it is mutagenic and forms tumor in mice. Provide an **explanation** for this discrepancy.

Question 3 (20 points)

a) Fill in the table below for a non-small cell lung carcinoma (NSLC) patient who has been treated with the following chemotherapeutic drugs.

Drug	Drug activity	Process **targeted first**: **replication/ transcription/ translation/ mitosis**?
Taxol	Inhibits microtubules assembly	
Cycloheximide	Inhibits eukaryotic ribosomal function	

b) The NSLC patient in part (a) relapses with secondary tumors in lymph nodes and brain after 2 years.

i. Are the cancer cells of the primary tumor in **epithelial (E) OR mesenchymal (M)** state?

ii. Which **"cell state transition"** did the cancer cells of the primary tumor undergo to cause the formation of secondary tumors: **EMT OR MET**?

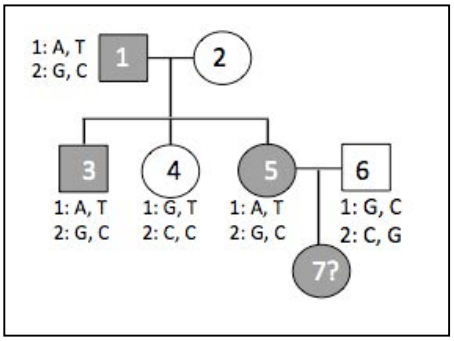
iii. Give **two** differences between the "cell state" in **part (i)** and the "cell state transition" in **part (ii).**

iv. Would the above patient following relapse have a good prognosis? **Why or why not?**

Initials: _____

Question 3 continued

The following pedigree shows the inheritance of **predisposition** to NSLC that shows an **autosomal dominant mode** of inheritance**.** This disease is caused by a gain-of-function mutation in the *ALK* proto-oncogene**.** ***Note:*** *The profile of SNPs 1 and 2 that are in the ALK gene, for some individuals is shown.*



c) Give the genotype of the NSLC cells for **Individual 3** for the **ALK gene** using ALK+ for the wild-type allele and ALK^M for the mutant allele: _____

d) The microarray shows the following SNP profile of Individual 7 **at birth**. **Explain** why the SNP profile of Individual 7 is different from his parents.

1: A, C
2: G, C

e) Would this SNP profile predispose Individual 7 to NSLC? **Why or why not?**

Question 4 (25 points)

The lung develops from embryonic cells called 'endoderm' in the following stages described in mice.

- At day **E5:** The endoderm is present**.**
- At day **E8:** The endoderm forms a tube and expresses the transcription factor Osr1.
- From **E10** on: The dorsal (back) side of the tube makes pharynx and expresses Sox2, an essential transcription factor for pharynx development.
- From **E12** on: The ventral (belly) side of the tube makes lung buds and expresses Nkx2.1, an essential transcription factor for lung bud development.

a) You ask when endodermal cells are committed to form pharynx and lung buds. You isolate a small piece of tissue (explant) from **E5**, **E8** and **E9.5** endoderm of a mouse embryo and grow in a culture dish until each explant is 12 days old. Complete the table below.

Stage of isolated explants	Genes expressed at day E12.0	Committed tissue: **Pharynx/ lung/ both/ neither?**
E5.0	Osr1	
E8.0	Sox2	
E9.5	Nkx2.1	

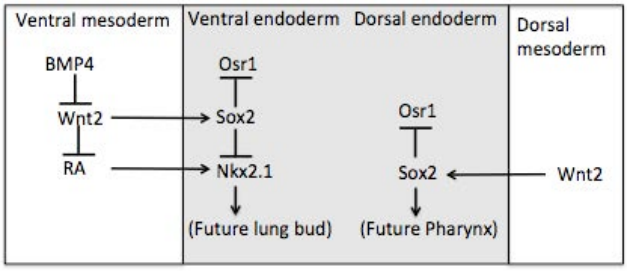
b) Give the potency of the endodermal cells that give rise to pharynx and lung bud. **Explain** your answer.

c) The endodermal tube is surrounded by cells called mesoderm. Following removal of the mesoderm at day E8, neither lung buds nor pharynx form. What role does mesoderm play in lung bud and pharynx formation?

Initials: _____

Question 4 continued

The signaling pathways involved in lung bud and pharynx formation are shown below. Ventral mesoderm lies next to future lung bud, dorsal mesoderm next to future pharynx.



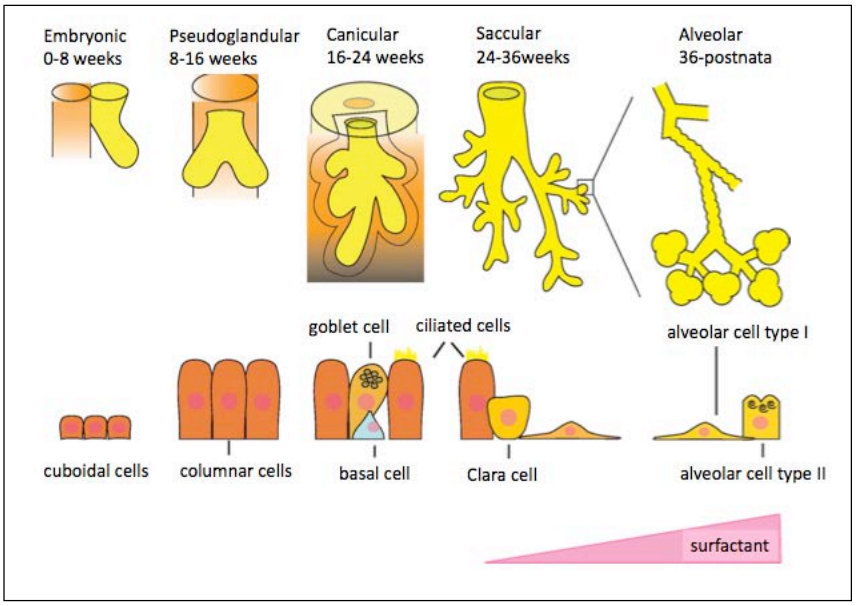
d) Where do you expect the BMP receptor to be expressed: **Ventral mesoderm OR Ventral endoderm**? **Explain** why you selected this option.

e) You create the following **homozygous null (loss of function) mutants**. Predict what would happen to lung bud and pharynx formation in this mutant by choosing from **normal/ none/ excess.**

Mutant	**Lung bud?**	**Pharynx?**
BMP4		
Wnt2		
BMP4 and Wnt 2 double mutant		

f) Is the formation of lung bud versus pharynx an example of **sequential induction/ co-induction/ migration? Why?**

The sequence of human lung development is shown below (*Sunday et al, 2016*). As the lung develops, the lung bud forms the trachea that splits into the bronchi and the lobules of the lung that contain alveoli. The alveolar cells secrete a surfactant protein into the fluid that prevents cells from sticking and allows inflation with air, and gas exchange.



Leibel S and Post M (2016) Endogenous and Exogenous Stem/Progenitor Cells in the Lung and Their Role in the Pathogenesis and Treatment of Pediatric Lung Disease. *Front. Pediatr.* 4:36. doi: 10.3389/fped.2016.00036. License CC-BY

g) For the physical transition of cuboidal to columnar cells, what part of the cell is most important: **Nucleus/ mitochondria/ cytoskeleton/ lysosome?**

h) Between the pseudoglandular and cannicular stages there is huge expansion of the lung tubes. For each mutant below, indicate what cellular process(s) is impaired during lung tube expansion: **Apoptosis/ Cell division/ cell migration/ cell shape change**. Include as appropriate.

i. Null mutation in DNA polymerase: _____

ii. Partial loss of function of actin, a microfilament protein: _____

Initials: _____

Question 4 continued

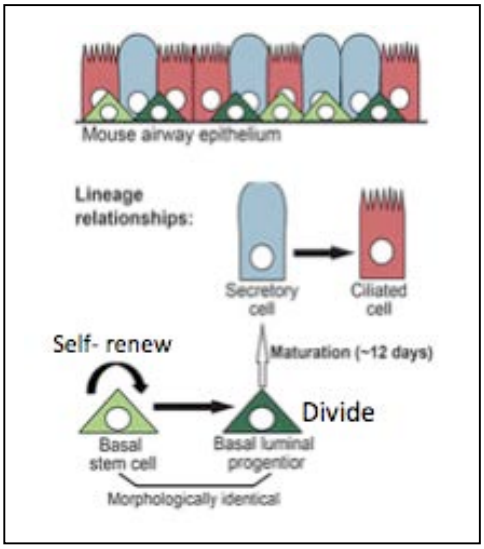
i) Explain why each of the following treatments, applied at the Saccular stage (24 weeks) will prevent or reduce **function of alveoli**, which are responsible for the gas exchange.

#1: Hydrourea, an S phase inhibitor

#2: An inhibitor of tight cell-cell junctions

Question 5 (14 points)

The schematic below shows the lung lining and respiratory stem cell lineage.



a) The Basal stem cells (BSC) express **Trp63** and **K15** cell surface markers. How would you use this information to purify the BSCs from a mixed cell population?

b) The BSCs lie next to 'stromal cells' and are usually quiescent (in G0). They enter the cell cycle in response to stress or inflammation and this activates Notch (ligand)-delta (receptor) signaling. Which cells would make the…

i. **Notch ligand: BSCs OR stromal?**

ii. **Delta: BSCs OR stromal cells?**

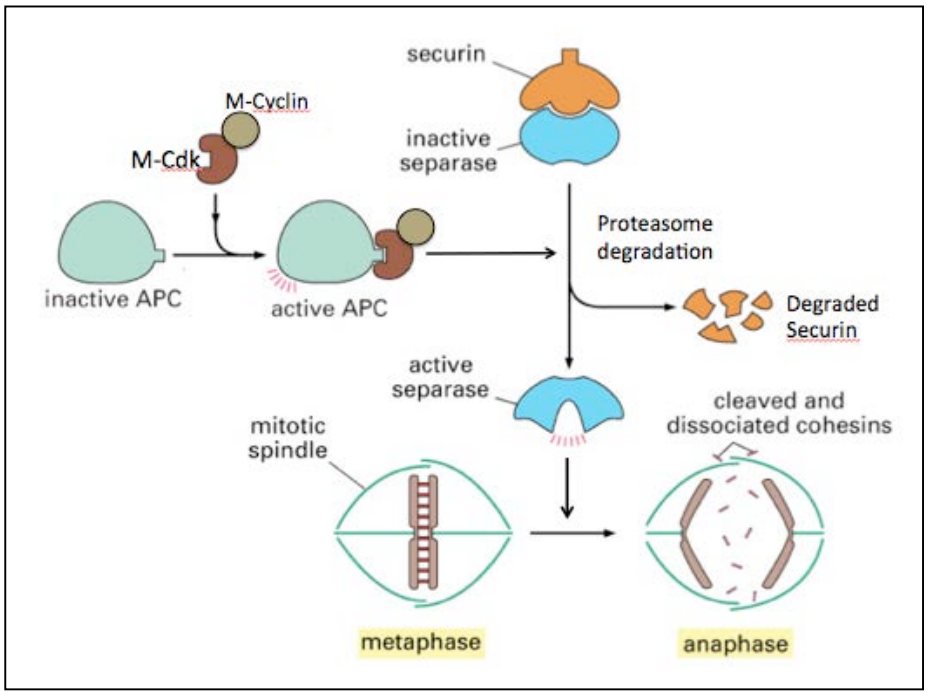
c) Which are potential transit amplifying cells? **Explain your choice.**

d) Cystic fibrosis (CF) is a genetic disorder that is caused by a mutation that inactivates the cystic fibrosis transmembrane receptor (cftr) gene. You want to alleviate the symptoms of CF in adult mice by replacing mutant cells with normal cells that have a wild-type copy of the cftr gene either by using iPS (derived from this mouse) or ES cells. Which of these two cells (iPS or ES) is a better option? **Why?**

e) CF model mice are difficult to breed so you decide to propagate the CF mouse strain through somatic cell nuclear transfer (SCNT). Which nucleus would you preferentially use: **ciliated cell/ basal stem cell/ secretory cell/ stromal cell**? **Explain** your choice.

f) The trachea is the tube that directs air into the lungs, built of cartilage that is secreted by chondrocytes. The cartilage organizes into rings that keep the tube open. In order to get chondrocytes to form a tube, you can seed them into a strong, rigid plastic tube of the right diameter. You can also culture them on extracellular matrix from a decellularized trachea. Which option would you choose and **why**?

Diagram for Question 1 (You can detach this page)



1: The **M cyclin**, expressed at G2→ M checkpoint, phosphorylates and activates **M-Cdk**.

2. The M-cyclin-M-Cdk complex binds to and activates **APC**.

3: Activated APC causes the proteasomal-mediated degradation of **Securin**. This frees Separase from securin and activates **Separase**.

4: Active Separase degrades **Cohesin** proteins that hold the duplicated chromosomes together at the metaphase plate. So the duplicated chromosomes separate in anaphase and the dividing cell progresses *from metaphase → anaphase.*

Codon chart for Question 2d)

	U	C	A	G	
U	UUU **phe**	UCU **ser**	UAU **tyr**	UGU **cys**	U
	UUC **phe**	UCC **ser**	UAC **tyr**	UGC **cys**	C
	UUA **leu**	UCA **ser**	**UAA STOP**	**UGA STOP**	A
	UUG **leu**	UCG **ser**	**UAG STOP**	UGG **trp**	G
C	CUU **leu**	CCU **pro**	CAU **his**	CGU **arg**	U
	CUC **leu**	CCC **pro**	CAC **his**	CGC **arg**	C
	CUA **leu**	CCA **pro**	CAA **gln**	CGA **arg**	A
	CUG **leu**	CCG **pro**	CAG **gln**	CGG **arg**	G
A	AUU **ile**	ACU **thr**	AAU **asn**	AGU **ser**	U
	AUC **ile**	ACC **thr**	AAC **asn**	AGC **ser**	C
	AUA **ile**	ACA **thr**	AAA **lys**	AGA **arg**	A
	AUG met	ACG **thr**	AAG **lys**	AGG **arg**	G
G	GUU **val**	GCU **ala**	GAU **asp**	GGU **gly**	U
	GUC **val**	GCC **ala**	GAC **asp**	GGC **gly**	C
	GUA **val**	GCA **ala**	GAA **glu**	GGA **gly**	A
	GUG **val**	GCG **ala**	GAG **glu**	GGG **gly**	G

7.013 Introductory Biology

Spring 2018