Answer key

BB2950 Molecular Biology, Exam 2, 11-30-17 100 points total
Please write your name on the back of **EACH** page

1. *(6 points)* In the following sequence, the end of a newly replicated linear chromosome is on the right. The top strand is the parental strand (lagging strand template) and the bottom strand is the lagging strand.

5'…GTGAGTATCGAACCGCGGGTTGGGGTTGGGGTTGGGGTTGGGGTTG 3'
3'…CACTCATAGCTTGGCG 5'

A. Which strand will be directly extended by telomerase?

2pts Top strand | parental strand | lagging strand template

B. How will the other strand get extended?

2pts The replication machinery will extend it using the lengthened top strand as a template. (Primase + DNA polymerase)

C. How does telomerase determine what sequence to add to the end of the chromosome?

2pts The RNA portion of telomerase serves as a template.

2 pts 2pts 2 pts

2. *(6 points)* What is an abasic site, what causes them, and what happens if the DNA replication fork encounters one before it is repaired?

An abasic site is a position in a DNA strand where there is no base, just the phosphodiester backbone. They result from spontaneous hydrolysis (reactions with water). They cause replication forks to stall.

3. *(6 points)* Describe the role of translesion synthesis (TLS) polymerases *(1 sentence or bullet point each)*:

A. When would a cell use a TLS polymerase?

2pts When the normal replicative polymerase cannot insert a nt because of damage in the template (when the replication fork stalls due to DNA damage)

B. Why are TLS polymerases useful to cells?

2pts They allow cells to avoid death by completing DNA replication even in the presence of DNA damage.

C. What negative consequences result from TLS polymerase usage?

2pts They often introduce mutations.

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4. *(5 points)* Suppose the cytosine nucleotide in the top strand of the following sequence gets spontaneously deaminated.

```
5'...GGACATT...3'
3'...CCTGTAA...5'
```

A. Write out the sequence of the **damaged DNA** that will result.

3 pts

5' GGAUATT 3'
3' CCTGTAA 5'

Bottom strand missing: -1
Bottom strand has "A": -1

B. If the damaged DNA is replicated **before** being repaired, what mutation will be present in the descendants of one of the daughter cells?

2 pts

i. G->T
ii. C->T
iii. C->A
iv. C->G

5. *(6 points)*

A. What is a ddNTP, and how does it differ from a dNTP?

Dideoxynucleoside triphosphate; has 3' H instead of 3' OH.

0.5 pts 2.5 pts

B. In what common molecular biology technique are ddNTPs used, and what role do they serve?

Used as chain terminators in Sanger sequencing; produces mix of products of different lengths that can be resolved on a capillary gel to determine the order of nts

1.5 pts 1.5 pts

Ok to explain this idea in different words

6. *(5 points)* Give an example of a hypothetical scenario in which you would want to measure gene expression. (Note there are many possible correct answers!)

7. *(2 points)* Which activity is responsible for degrading RNA primers during DNA replication?

A. DNA Pol I 5'-> 3' exonuclease
B. DNA Pol I 3'-> 5' exonuclease
C. DNA Pol III 3'-> 5' exonuclease
D. DNA Pol I endonuclease

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8. *(4 points)* You suspect that a particular mutation in your favorite gene causes a phenotype of interest. You have obtained ten new cell lines from collaborators, and you want to determine which cell lines have this mutation. Would you choose to use Sanger sequencing or Illumina sequencing, and why? 2pts 2pts

Sanger sequencing, because it is cheaper and more cost effective for sequencing a small number of genes (here we just care about a particular gene and don't need the whole genome sequence)

9. *(5 points)* Why does quantitative PCR measure product abundance after each cycle rather than at the end after all of the cycles are finished?

measuring product abundance after each cycle allows you to collect data from the early cycles of PCR where efficiency is high and the product abundance is directly proportional to the abundance of cDNA in your original sample. It also allows for comparison between multiple samples by determining when each sample crosses a product abundance threshhold

10. *(6 points)* During each cycle of an Illumina sequencing reaction, a picture is taken of the flow cell revealing a field of colored dots.

2pts A. What does each dot represent?

i. A gene
ii. A cluster of identical DNA molecules
iii. A cluster of random DNA molecules
iv. The expression level of a gene

2pts B. What does the **color** of each dot represent?

The identity of the most recently incorporated nt.

2pts C. In consecutive cycles, do you expect the color of each dot to stay the same or to change, and why?

If the next nt in the sequence is different, the color will change. If the next nt is the same, the color will be the same. (Most often, the color will change)

11. *(2 points)* Which eukaryotic RNA polymerase is responsible for transcribing protein-coding genes?

A. RNA Pol I
B. RNA Pol II
C. RNA Pol III

Answer key

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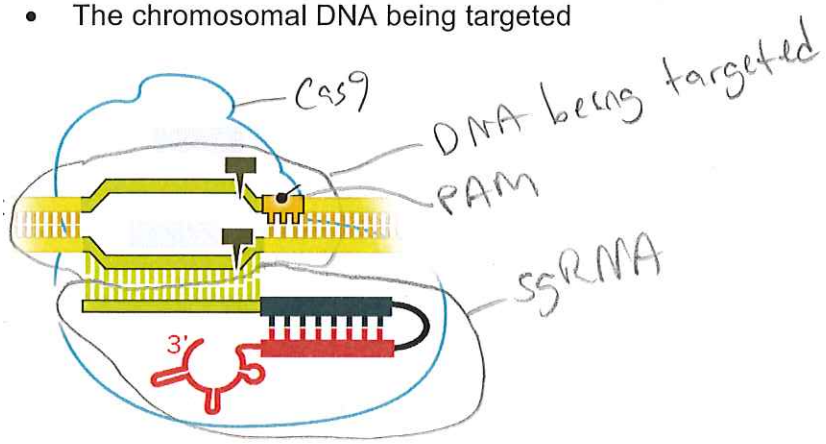
12. *(8 points)* On the picture below, label the following:

- Cas9
- sgRNA
- PAM
- The chromosomal DNA being targeted



13. *(6 points)* Eukaryotic cells can repair double-strand breaks by homologous recombination (HR) or by non-homologous end joining (NHEJ). When performing CRISPR-Cas9 genome editing, how can you bias cells toward using one pathway or the other? How can you use this to disrupt a gene of interest or insert a specific genetic change into a cell line?

If you transfect cells with a repair template (linear DNA with homology to the sequence on either side of the cut site), they will likely use it as a template for repair of the ds break by HR. When you want to disrupt a gene's function, omit the repair template to encourage the cells to repair with NHEJ which will often cause a frameshift. To introduce a specific genetic changes use a repair template that includes the desired sequence.

14. *(4 points)* Below is part of a bacterial gene sequence. The transcription start site is bolded and indicated with a bent arrow.

promoter

```
5'-GTACAATCACCAGGTCCGAGGAGGCGGACATGCTTCGACTCC-3'
3'-CATGTTAGTGGTCCAGGCTCCTCCGCCTGTACGAAGCTGAGG-5'
```

2pts A. Indicate the approximate region where you expect the promoter is located.

2pts B. Write the first five nt of the mRNA that will be made, from 5' to 3'.

GGUCC

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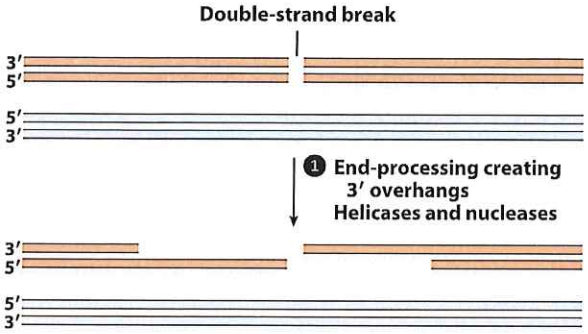
15. *(6 points)* The figure below shows the first step of homologous recombination to repair a double-strand break.



A. What is the next step, and what enzyme or type of enzyme is required to carry it out?

Strand invasion (single-stranded overhangs base pair with strands in the blue chromosome). Requires a recombinase (eg, RecA or RAD51)

B. Why is it important that 3' overhangs were made in step 1 in the figure, rather than 5' overhangs?

After strand invasion, the 3' ends serve as primers for DNA polymerases to extend the strand.

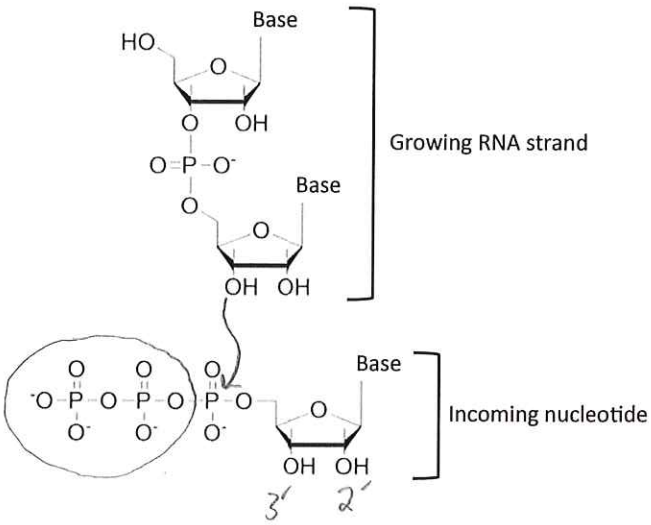
16. *(8 points)* In RNA polymerization, a nucleophilic atom in the growing RNA strand attacks an atom in the incoming nucleotide to form a new phosphodiester bond.

A. Draw an arrow from the nucleophile to the atom being attacked.

B. Label the 2' and 3' hydroxyl groups on the incoming nucleotide.

C. Circle the portion of the incoming nt that will be released after the new phosphodiester bond is formed.

D. What would be different if this were DNA polymerization rather than RNA polymerization?



The chemistry would be the same (only difference is absence of 2'OH)

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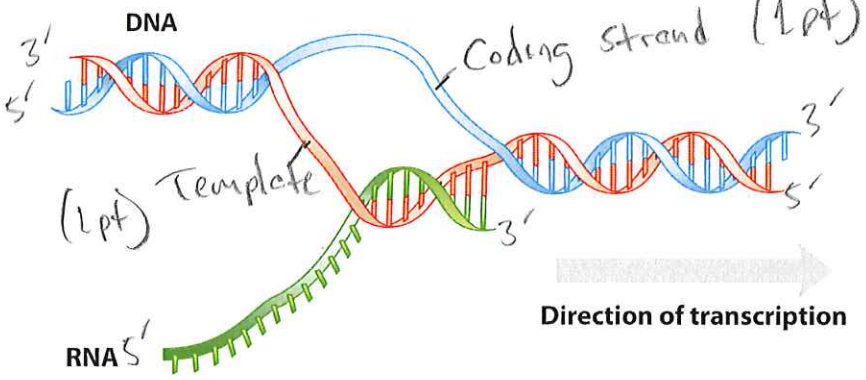
17. (5 points) The figure below shows an RNA being transcribed from a DNA.

A. Label the 3' and 5' ends of each of the three strands shown (2 DNA strands and 1 RNA strand).

0.5 pts per end

B. Label the template strand of the DNA and the coding strand of the DNA (also called the non-template strand).



18. *(6 points)* Bacterial transcription requires sigma factors.

A. What proteins and/or nucleic acids do sigma factors physically interact with?

1 pt – RNA polymerase and promoter sequences (core promoter sequences) 1 pt (0.5 pt for "DNA")

B. Circle the stage(s) of transcription for which a sigma factor required:

(Initiation) **Elongation** **Termination**

2 pts D. What group of eukaryotic proteins performs functions analogous to sigma factors?

General transcription factors (IF TFs only. 1 pt)

19. *(4 points)* What is the role of the mediator complex, and in what group of organisms is it present?

2 pts Mediator serves as a bridge between general TFs and transcriptional activators (specific TFs) in order to substrate transcription.

2 pts In eukaryotes