Answer key

BB2950 Molecular Biology, Quiz 4: 11-21-17, 25 points total
PLEASE WRITE YOUR NAME ON THE BACK OF EACH PAGE

1. *(4 points)* In what circumstances would you would choose to use RNAseq rather than quantitative PCR, and why?

Use RNAseq when you want to measure expression of a large number of genes, because it is more cost-effective and faster for large scale projects. Or: when you don't know which genes will be differentially expressed, or to get additional information such as splice variants.

2. *(4.5 points)* What, if anything, differs between Illumina RNAseq and Illumina genomic DNA sequencing with respect to:

A. Library construction

In RNAseq, libraries are made from cDNA that was made by reverse transcription of RNA. Genomic DNA sequencing libraries are made from genomic DNA.

B. Cluster formation and the sequencing reactions

No differences

B. Data analysis

In RNAseq, reads are aligned to a reference genome and the number of reads aligning to each gene are counted and compared; In DNA sequencing, reads are aligned and non-matching positions are identified to identify mutations, or reads are aligned to each other to determine the sequence of an unknown genome.

3. *(4.5 points)* Telomerase is a protein-RNA complex.

A. What role does the RNA play?

The RNA is a template for DNA synthesis

B. What enzymatic activity is carried out by the protein?

Reverse transcriptase (DNA synthesis)

C. What will change about the chromosome after each cycle of DNA replication if telomerase is NOT expressed?

The telomeres (ends of the chromosome) lagging strand will get shorter.

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4. (*4 points*) Briefly state how quantitative PCR and standard PCR differ with respect to template and how/when the products are detected.

	Quantitative PCR	Standard PCR
Template	cDNA (made from RNA)	Double-stranded DNA (dsDNA)
How/when products are detected	Products are detected after every cycle by fluorescent dye	Products are detected by running on a gel after all cycles are completed.

5. *(3 points)* The diagram below shows part of a newly replicated strand of DNA in which a polymerase slippage event has occurred. The dashes between nucleotides indicate the phosphodiester bonds. If the mismatch is **not repaired** prior to the next round of DNA replication, what are the sequences of the **two** new double-stranded DNAs that will be produced?

```
        A-A
        | |
5' G-A-A A-A-A-T 3'   Newly replicated strand
3' C-T-T-T-T-T-A 5'   Template strand
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5' GAAAAAAT 3'
3' CTTTTTTA 5'

5' GAAAAAT 3'
3' CTTTTTA 5'

6. *(2 points)* The mutation caused by the slippage event above is an example of:

A. Trinucleotide repeat expansion
B. A point mutation
C. An indel
D. Cytosine deamination

7. (*3 points*) The nts used in Illumina sequencing have **two** major chemical differences compared to regular dNTPs. Briefly state the ROLES of each of these two modifications.

Fluorescent dyes allow the instrument to identify which nt was incorporated at each cycle

3' reversible blocking groups allow nts to be incorporated one at a time, with an image taken between each addition.