Bio 324 Molecular Biology Final December 14, 2006

This exam is worth a total of 140 points

1. a. Nucleic acids are polymers of nucleotides. What is the name of the bond connecting the nucleotides? **Draw** the chemical structure of this bond. (4 pts.)

Phosphodiester bond...sugar - o - p - o sugar

b. What is **one** advantage of using a cloning strategy in which the foreign DNA is inserted into the vector using two different restriction enzymes? (4 pts.)

-to efficiently amplify the sequence cheaply and accurately
-inefficient to go from DNA in a test tube and have it taken up by a living cell cell have barriers

c. What is the difference between a genomic and a cDNA library? If I wished to determine the location of introns in a given gene, what is the most reliable method to do so? (4 pts.)

genomic library - contains everything, introns, exons, house keeping genes, etc
cDNA - contains the coding region, so it is only exons

Find the specific gene, based off of the cDNA, from there compare that region with the genomic library to find the introns

d. Microarray technology is a method used to determine the expression patterns of thousands of genes at the same time. Descibe how the method works, including what is spotted on the "chip" and how the probe is generated. (4 pts.)

chip is made with single stranded oligonucleotides that is 22 nucleotides long that corresponds to sequence of known genes in a given organism. All the potential RNAs

needed to be produced as single stranded or cDNA are spotted onto the chip, then isolate RNA from two different sources. Now the RNA has to be converted to DNA. Which can be done either by use of reverse transcriptase or amplification step using a primer to make more of the RNA. Use primers that have two different fluorescent tags. Resulting in cDNa with two different fluorescent dyes and then mix together and hybridize. Then wash off and scan via laser

2. a. Bacterial plasmids are the most common "vector" used to clone fragments of double stranded DNA. Such plasmids often carry an antibiotic resistance gene and a gene encoding b-galactosidase within the multiple cloning site. Explain how each facilitates obtaining the desired clone. (4 pts.)

Antibiotic resisteance gene allows to select only bacteria transformed by plasmid carrying resistence gene when plated in appropriate plates containing an antibiotic
B-galactosidase which coverts x-gal to blue wil be disrupted if foreign DNA inserted into polylinker. Therefore plasmid with insert will be white in X-gal plate.

b. Bacteria that lack the enzyme topoisomerase I have difficulty replicating their DNA. What is the role of topoisomerase in cells and how would that affect replication? (4 pts.)

Topoisomerase relaxes the DNA by breaking bonds in DNa so that replication can occur. If Topoisomerases are not present then replication cannot occur.

c. Describe all the processing steps that a mRNA undergoes from the time it is transcribed until it is translated on ribosomes. Indicate which events occur in the nucleus and which in the cytoplasm. (4 pts.)

methyl G cap added to 5' end, poly A tail added to cleaved mRNA, introns removed. All in nucleus.

5' end of the RNA is modified by the addition of a "cap", the 3' end is modified by addition of a series of adenylic acid nucleotides(poly A tail) immediately after its cleavage.

Removal of introns. Only after the completion of all modification and processing events can the mRNA be exported from the nucleus to the cytoplasm.

5' cap is formed by adding a G to the terminal base of the transcript via a 5'-5' link. One to three methyl groups are added to the base or ribose of the new terminal guanosine. The cap is a 5' to 5' bond

poly(A) sequenes is not coded in the DNA, but rater is added to the RNA in the nucleus after transcription.

d. Mammalian chromosomes are linear. During normal DNA replication, about 50-100 bases are lost from the ends of chromosomes each replication cycle. Draw a diagram to explain why this occurs and describe how mammals overcome the loss of the ends of their chromosomes. Be specific. (4 pts.)

After removal of the RNA primer, DNA polymerase I has extended the previous DNA and filled it in. Its going to have a nick, a nick will result 1 in every 1000 bases. While DNA ligase seals the nicks.
The 3 end is longer than the 5' strand by 200 bases, this causes the two proteins to cat-alyze an invasion.
Once RNA primer is removed from the very end of the linear DNA, the gap cannot be filled because there is no initiator .
DNA polymerase only synthesizes 5'-3' so cannot fill in the gap. Telomerases uses its own RNA template to add heximeric repeats to 3' end and new primer to be laid down.

3. a. Both prokaryotic and eukaryotic ribosomes are composed of a large and a small subuit. The amino acylated tRNAs make contact wth both subunits, however, they play different roles in protein translation. What are the roles of the small and large ribosomal subunits? (4 pts.)