

Bioc471a/571a Homework 1 - Due at the start of class August 28

Note: These are very thorough answers and intended to teach concepts. Correct answers for the homework assignment need not be so detailed., although I do expect complete sentences (see course objectives).

1. (2 pts) Name two applied molecular genetic techniques that depend on the formation of RNA-DNA hybrids.

Numerous molecular genetic techniques depend on formation of RNA-DNA hybrids including Northern blots (when the probe is cDNA), any method requiring gene transcription or cDNA synthesis, transcript mapping using genomic DNA and cellular RNA (S1 mapping), and hybrid arrest translation (cDNA forms hybrids with poly A RNA in an in vitro translation system).

2. (2 pts.) Why does adding NaCl to a DNA solution increase the T_m of a DNA duplex? How does increasing the NaCl concentration affect the "specificity" of a hybridization reaction?

DNA duplexes are stabilized by hydrogen bonding between base pairs, however, negative charges in the phosphodiester backbone have repulsive properties. Under optimal conditions of ionic strength and temperature, the strength of hydrogen bonding is greater than phosphate repulsion for complementary nucleotides of >10 base pairs. When the temperature is increased in the system, the hydrogen bonds are destabilized and the helix falls apart due in part to repulsion from the negative charges. If NaCl is added to a DNA solution, then the Na^+ ions shield the negative charges on the phosphates and offset interstrand repulsion.

DNA base pair mismatches occur when two strands are not 100% complementary. For example, a G opposite an A or a G, or a C opposite an A or T, are examples of mismatches. If the number of mismatches is relatively high over a given stretch of DNA, then the helix is destabilized due to loss of hydrogen bonding energy. The negative charge in the phosphate backbones have repulsive properties which contribute to the helix destabilization. If NaCl is added to a DNA solution, then DNA duplexes containing some mismatches (non-specific interactions) are tolerated due to neutralization of the repulsive effects.

3. (2 pts.) How could you take advantage of the Cot phenomenon to functionally "inactivate" highly repetitive DNA sequences from a DNA probe that contains both unique and repeated sequences?

The idea is to "block" the repetitive DNA sequence in the probe from hybridizing to complementary sequences in the target (e.g., the target DNA could be a DNA sequence on a Southern blot). This can be done by isolating low Cot DNA from a reassociation experiment and including it at a high molar ratio as unlabeled single strand DNA in the probe mixture. The repetitive DNA sequences (by definition, low Cot material) will form short hybrids with the radioactive probe and thus prevent these regions of the probe from interacting with the repetitive sequences in the target. Importantly, the high Cot material in the probe (unique DNA sequences) is not effected by this pretreatment with low Cot material since there is very little sequence similarity.

4. (2 pts.) Is it possible to ligate a DNA fragment cleaved with BamHI to a heterologous fragment that had been cleaved with the restriction enzyme BglII? (Hint: use Appendix D in the textbook to determine the recognition site sequence of BglII). Can the ligated DNA fragments be cleaved again with both BamHI and BglII? Explain.

Yes, it is possible to ligate a 5' staggered end from a BamHI digested DNA fragment to that of the 5' staggered end of a BglII digested DNA fragment since both enzyme cleavage products share the same complementary GATC core sequence.

The ligated DNA fragments form a hybrid BamHI-BglII site will have the sequence G-GATC-T which is not palindromic in the terminal positions. The hybrid restriction site is not recognized by either a BamHI dimer protein or a BglII dimer protein and therefore the heterologous DNA molecule cannot be cleaved.

5. (2 pts.) Why is formaldehyde used to denature RNA in a Northern blot, isn't RNA single stranded to begin with? Why can't NaOH be used to denature RNA in the Northern blotting procedure as it is in the Southern Blotting procedure?

RNA is a single-stranded molecule in the sense that it is a single polymer of ribonucleotides, however, RNA under physiological conditions contains many regions of intrastrand hydrogen bonding between base pairs. This intramolecular RNA structure greatly influences the "apparent" molecular weight in a standard agarose gel. Therefore, to get a more accurate indication of molecular weight, the RNA molecules must all be denatured so that gel migration is directly proportional to molecular weight (number of nucleotides).

NaOH denatures DNA and RNA by disrupting the hydrogen bonds between base pairs. However, NaOH also catalyzes breakage of the phosphate backbone in RNA because the hydroxyl ion catalyzes cyclization of the ribose sugar between the 2' hydroxyl and 3' phosphodiester bond. DNA does not contain a 2' hydroxyl (deoxyribonucleic acid) and therefore it is safe to use NaOH.