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MIDTERM EXAMINATION (October 7, 2014)
BIOE150. Introduction to Bio-Nanoscience & Bio-Nanotechnology
Fall Semester, 2014

0. Write down your name and the last 4 digits of your SID on all the pages (1)

1. Define the following terms briefly: (each 4, total 16)

a) Holliday DNA junction

A mobile junction between four DNA strands involved in homologous recombination during the meiosis process. Homologous sequences cause the junction to be able to slide up and down the DNA.

b) Staple DNA

Oligonucleotides complementary to a scaffold template in DNA origami approach developed by Dr. Paul Rothemund. Staple DNA are designed specifically to fold scaffold DNA into complex 2D and 3D shapes to enable DNA origami to be assembled.

c) B-Z DNA switch

DNA sequences with high G-C pair content can undergo B- to Z- DNA switch at high ionic strength. Ions mask negatively charged phosphate groups from repelling each other as in the normal B-DNA and this masking allows close packing of the phosphate backbone to stably form Z-DNA.

d) SPM

Scanning Probe Microscopy

A field of microscopy in which images are generated based on physical mapping of interactions between substrate surface and probes. A nanoscale probe tip is utilized to measure the surface forces and its position and interaction is visualized as an image of the substrate surface.

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2) Describe the difference briefly (each 4, total 16)

a) Top-down approach vs. bottom-up approach

Top-down – Fabricating nanostructures starting from bulk materials to detail nanostructure.
Bottom-up – Fabricating nanostructures using individual particles (molecules and atoms) primarily via self-assembling processes.

b) Directed evolution vs. natural evolution

Directed evolution – This is a relatively fast laboratory technique comprising diversification, selection, and amplification to find a biomolecule or an organism with desired properties. The process begins by creating a large library of gene variants and their mutagenesis during amplification, and ends with selection of a product with the best characteristics.

Natural evolution – This is a natural process comprising of variation, inheritance, and natural selection. All modern life forms have evolved from simple biological systems over millions of years of genetic mutations, inheritance of genes and survival of the fittest organisms.

c) Holiday DNA junction vs. synthetic DNA junction

Holliday DNA junction – A mobile junction involved in homologous recombination. The homologous sequences involved allow the branch point to migrate up and down the DNA
Synthetic DNA junction – Designed to avoid symmetry to block mobility of junction branch point. Used to form DNA-based molecular LEGO blocks able to self-assemble into intended shapes.

d) DNA brick vs. DNA origami

DNA brick – Single stranded DNA with 4 domains designed to bind to complementary domains of other bricks. These modular bricks can self-assemble into 3D structures by binding to 4 local neighbors and can be added or removed independently.
DNA origami – Origami inspired technique in which a single stranded genome (any source) is folded into desired shapes by designing complementary DNA staple strands.

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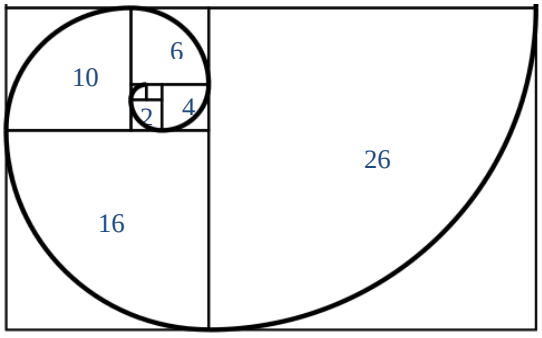
3. Suppose that there are two adult rabbits in closed environment. Suppose that a pair of adult rabbits produce a pair of baby rabbits once every month. Each pair of baby rabbits require one month to grow to be adults and subsequently produce one pair of baby rabbits each month thereafter. It is assumed the rabbits are immortal, and inbreeding is OK! (16)

a. Write down total number of rabbits in the closed environment for six months by filling the table below (4):

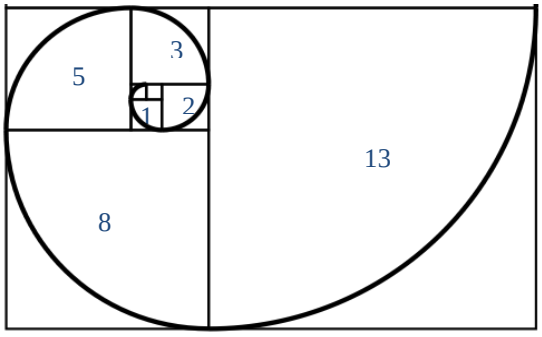
Month	1st	2nd	3rd	4th	5th	6th
Number of rabbits	2	4	6	10	16	26

If you look at the number of pairs (# of rabbits/2), you can see the Fibonacci sequence: 1, 2, 3, 5, 8, 13…

b. Based on the above number, draw a golden rectangle and indicate the golden spiral (4)



Or



Same as if you were using the Fibonacci sequence

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c. Write down an equation related to golden ratio (phi) and solve the equation to obtain the phi value (4)

From the golden rectangle, we know that: $\frac{(a+b)}{a} = \frac{b}{(a-b)}$

$\therefore a^2 - b^2 = ab$

$\therefore \frac{a}{b} - \frac{b}{a} = 1$

Assuming $(a/b) = \varphi$,

$\varphi - \frac{1}{\varphi} = 1$ [You can start the answer with this equation]

$\therefore \varphi^2 - \varphi - 1 = 0$

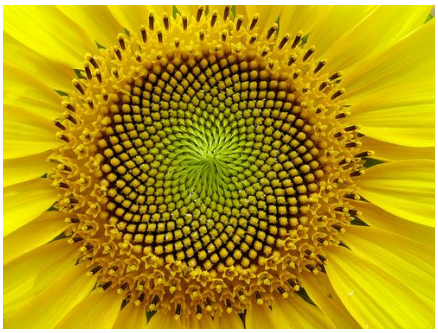
Plug this into the quadratic formula and you get,

$\varphi = \frac{1 \pm \sqrt{5}}{2}$

Since the golden ratio is a positive number,

$\varphi = \frac{1 + \sqrt{5}}{2} = 1.618 \ldots$

d. Explain how the golden ratio can manifest in the growth of sunflower seeds (pictured below) (4).



The seeds grow in a spiral arrangement with each new seed growing at a fraction of a rotation away from the old. This fraction is called the 'golden angle'. The spiral arrangement manifests naturally as new seeds grow at the center and push out the others.

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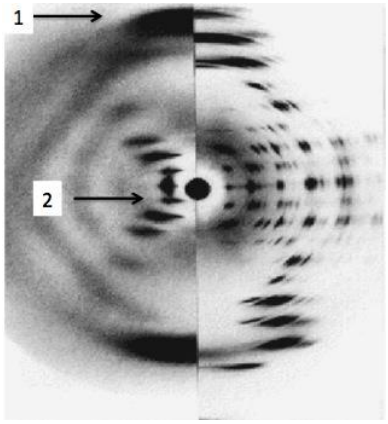
4. DNA structure determination

The following two images are x-ray crystallographic data from A and B DNA structures (total 10).

(Left) (Right)



(**B**)-DNA (**A**)-DNA

a) A-DNA is a right handed and dehydrated form of DNA that has a more packed structure than B-DNA, while B-DNA is a hydrated and most stable form of the DNA structures. Among the two DNA x-ray crystallographic data, determine which DNA structure is which by writing down A or B in ( ) under the image (4)

b) Explain the reason for your decision for A- and B-DNA structures using Bragg's law (4).

Clear X-ray diffraction spots are present in the highly crystalline dehydrated A-DNA. On the other hand, B-form DNA exhibits an x-pattern which indicates DNA double helix. The spacing of outer bands (peak 1) correlates to the spacing of base pairs and is ~10x the size of spacing of inner bands (peak 2) related to the pitch of the DNA helix. According to Bragg's law ($n\lambda = d \sin\theta$), the angle θ will be large for smaller interplane distance, d, and this is in agreement with the X-ray diffraction images shown.

c) In the above x-ray crystallographic data, explain what are the corresponding DNA structural features that are correlated with x-ray peaks 1 and 2 indicated by two arrows (4).

Signal 1 – 3.4 angstroms for the change in height per base pair within a double stranded DNA helix.

Signal 2 – 34 angstrom signal for the height of 1 turn of double stranded DNA helix.

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5. Drugs are not equally effective on all patients. Drugs can also have serious side effects; in the worst case, a drug used to treat a disease can produce a fatal outcome. By examining genetic differences among individuals and administering drugs on the basis of such findings, the impact of side effects can be reduced. Scientists are making advances in personalized medicine with research into how drugs can be tailored to a patient's genetic information through the analysis of single nucleotide polymorphisms (SNPs). Identify the single nucleotide polymorphism (SNP) that plays a key role in drug screening (total 10).

Suppose that "GGA TTA TTG TTA AAT ATT GAT AAG GAT" is a marker gene sequence that exhibits the SNP for an antibiotic called 'tetracycline'. The underlined nucleotide has multiple SNPs including as follows: "GGA TTA TTG TTA AGT ATT GAT AAG GAT". Design a colorimetric assay to detect this SNP using gold nanoparticles and explain how it works.

SNP detection can be achieved by using a gold nanoparticle based spot assay. First, we can design two sequences complementary to the target gene GGA TTA TTG TTA AAT ATT GAT AAG GAT. These can be modified at 5' or 3' end to contain a reactive thiol group. The modified DNA fragments can then be attached to 10 um gold nanoparticles through Au-thiol coupling.

DNA fragment A, 5'-ATC CTT ATC AAT ATT-3' with 5' end thiol modification, is attached to particle A.

DNA fragment B, 5'-TAA CAA TAA TCC-3' with 3' end thiol modification, is attached to particle B.

The two particles can be spotted on a silica substrate. In the absence of target, the spot will show a blue color. In the presence of the target, particles A and B will aggregate and the color will change to red.

In order to detect an SNP, we can scan the substrate through a range of temperatures encompassing the melting temperature of the perfectly matched target. Any mismatch will lower the melting temperature. In this case, G-T mismatch will have a lower melting temperature causing the spot color to change from red to blue as we get close to the melting temperature of the target gene. If other mismatches are present (T-T or C-T), they will likely have different melting temperatures and can be detected.

Additionally, we can also make another test with DNA fragment A' having the sequence 5'-ATC CTT ATC AAT ACT-3'. We can compare the colors of A-B spots and A'-B spots with changing temperature and determine which target has original versus SNP sequences.

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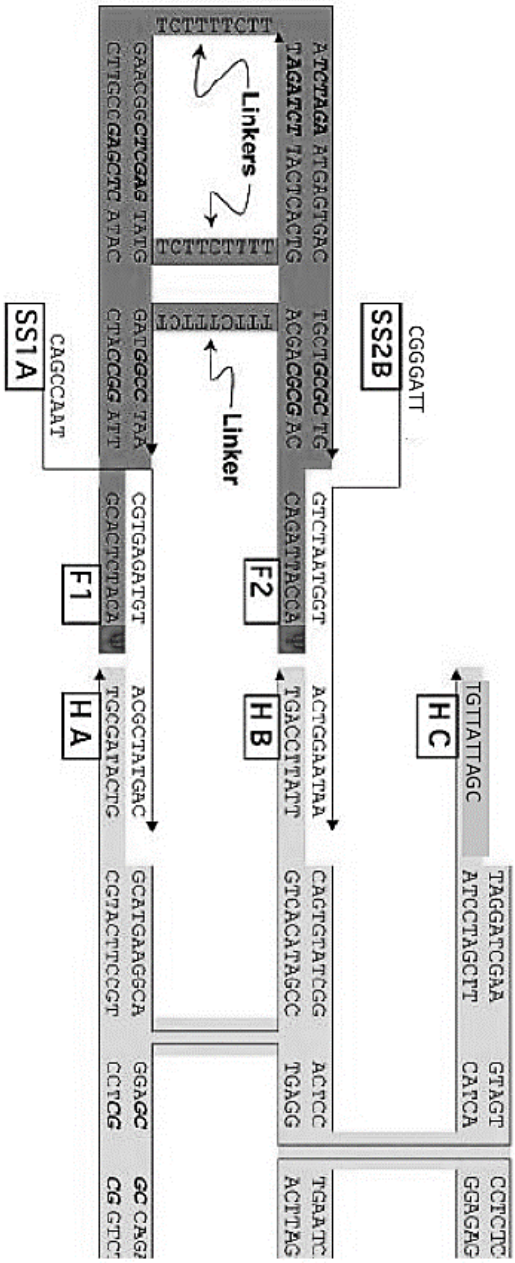
6. Dr. Nadrian Seeman developed various DNA-based nanostructures and their functional machines. Among them, the below image represents DNA-based biped-walking machine that can move its DNA structures depending on input or output of specific DNA sequences that can complement their foot (F) or hold (HC) DNA structures (total 15).



a) Describe a possible scheme to move Foot (F2) to the next hold (HC) (4).

In order to move F2 to HC, we first need to unset the set strand. The unset strand (US2B) should be complementary to SS2B. US2B will first bind to the overhanging region of SS2B and hybridize with the remaining DNA setting F2 free from HB. Next, a new set strand SS2C can be inserted. SS2C can have a 7-8 nucleotide overhanging region along with complements to F2 and HC. The free F2 will localize to HC when SS2C hybridizes with the two sticky ssDNA.

b) Design necessary DNA sequences to complete the biped motion of the DNA (6).

To complete 1 full motion, we need 2 unset strands and 2 set strands which will have to be added in the following order:

1. Release F2 from HB using US2B: 5'-TTATTCCAGT-ACCATTAGAC-AATCCCG-3'
2. Bind F2 to HC using SS2C: 5'-CGGGATT-GTCTAATGGT-ACAATAATCG-3'
3. Release F1 from HA using US1A: 5'-GTCATAGCGT-ACATCTCACG-ATTGGCTG-3'
4. Bind F1 to HB using SS1B: 5'-CAGCCAAT-CGTGAGATGT-ACTGGAATAA-3'

These sequences can be used to complete one biped cycle by the DNA.

(c) Design an assay to monitor the above biped motion induced by the sequential treatment of the complementary assay using a fluorescent dye (5).

This can be done in several ways. One of assays is as follows:

Hold strands can be modified with fluorescent dye #1 at the 3'end and Foot strands can be modified with fluorescent dye #2 at the 5' end such that the two fluorophores can undergo FRET. Movement of the biped structure can then be tracked using FRET imaging. Normally, the fluorophores on foot and hold strands will only absorb and emit their respective wavelengths. However, when a foot is bound to a hold using a set strand, the decrease in proximity of the two fluorophores will cause energy transfer. Thus, molecule #1 will absorb its wavelength but the pair will emit at the wavelength of #2. Locating this phenomenon can help localize the biped and track it over repeated stepping cycles.

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7. Phage display is a method to identify functional peptide motifs by exploiting phage biology and bacterial metabolism. Using the phage display approach, we plan to construct a loop-shape hepta-peptide library, which is known to have a stable conformational structure and enhance binding affinity. The loop-shape library can be designed through franking the amino acid sequences between Cys- and -Cys as below: (16)

Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys (Xaa=random amino acid residue)

a) If there is no issue related to chemical synthesis of the DNA fragments to express the above peptide library, what is the maximum number of the random unique peptides generated by the above library construction (5).

This is 7mer peptide library. Therefore, the possible maximum number is 20^7

b) Suppose that we will use the above library to find a trans-dermal peptide through *in vivo* phage display process by applying the library to the skin of a mouse. Describe a possible screening procedure for this *in vivo* phage display screening to discover the trans-dermal peptide (6).

1. Introduce the phage library into the mice skin.
2. Collect skin tissue and remove the epidermis and harvest the phage that are penetrated through skin
3. Wash away weakly bound phage from the skin biopsy sample
4. Elute the bound phage from the skin biopsy samples
5. Amplify and Sequence the eluted phage
6. Check for a consensus sequence, otherwise repeat the process using the eluted phage

d) Suppose that we discover a new peptide sequence that is able to undergo trans-dermal function through phage display using *in vivo* phage screening. The identified peptide sequence is as follows: CAGRGDSAC. Calculate the theoretical probability of finding the isolated sequence from the original library (5).

# of codons: A = 4; G = 4; R = 6; D = 2; S = 6

AGRGDSA – $(4*4*6*4*2*6*4)/64^7 = 4.19\text{x}10^{-9}$

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Table: genetic Code