MCB 102 Final Outline

* Nucleic Acids
  + (AG) Purines vs. (TCU) Pyrimidines
  + Nucleotides vs. Nucleosides
    - Difference is in the Phosphate (sans = Nucleoside
  + Chemical Properties
    - Van der Waals
    - Hydrogen Bonding
    - Resonance
    - Absorbance (~260 nm) due to resonance
      * Hyperchromic shift of Denatured DNA
  + RNA vs. DNA
    - Structure – 2’ OH group
    - Stability – RNA is much less stable
      * Single strand, exposed OH groups (alkali liable), weak AU interactions
    - Regulation
      * Similar regulation methods due to the close connection between RNA and DNA
        + DNA is regulated via proteins that duplicate and translate it
        + RNA is regulated in many ways depending on the type of RNA
* Studying DNA
  + Visualization
    - UV
    - X-Ray Crystallography
  + Cellular Localization
    - In the central areas (different Prokaryotes and Eukaryotes)
  + Dynamic Organization
    - Nucleosomes and/or supercoils (more or less depending on the type of cell)
  + “Storage”
    - Information storage in codon (nucleotide triplets) sequences
  + A, B, and Z DNA
    - Formation is dependent on
      * Glycosidic bond conformation
      * Repeating unit base pairs
      * Helix-handedness
    - Regulation and expression of genes
* Replication
  + Origin of replication
  + Okazaki Fragments
  + 5’ 🡪 3’ Synthesis direction
  + DNA Polymerase III
* Repair
  + Mechanisms
    - Nick Translation
  + 3’ 🡪 5’ exonuclease
    - Klenow Fragment contains polymerase and exonuclease activity
  + Limitations
    - Very slow rate of proofreading
  + Enzymes
    - DNA Polymerase I
      * DNA repair + Primer removal
    - Ligase (fixes nicks)
* Transfer of Hereditary Material
  + Vectors and Plasmids
  + Cloning
  + Transformation
    - Phage
* Supercoiling
  + Positive (left-handed) and Negative (right-handed)
  + DNA most stable when there are 10 bases per helical turn
  + Supercoiling alleviates high energy of DNA with the wrong twist (either overwound or underwound)
  + Topoisomerase I:
    - removal of positive/negative supercoils by nicking DNA, allowing swiveling
  + DNA Gyrase = Topoisomerase II
    - Adds negative supercoiling
    - Antibiotics and Anti-Cancer Drugs target Gyrase
  + Negative Supercoiling is easy to unwind – What are the benefits
    - Easier access to unwinding the DNA without supercoiling
    - Allows RNA synthesis
    - Allows DNA Repair
* More on DNA Replication
  + DnaA
    - DnaA Box or TATA Box
  + DnaB = Helicase
    - Binds to DNA, unwind with ATP
  + DnaC
    - Pre-priming protein attaches to DnaB
    - Complex rests on the replication fork
  + DnaG = Primase
  + SSB
    - Single Stranded DNA Binding Protein (Binds to single stranded DNA to prevent reannealing
  + Other proteins (HU, etc.)
    - HU binds with DnaA to DNA near DnaA box, forming a + supercoil
  + Leading and Lagging Strand Synthesis
    - Okazaki Fragments
  + DNA Pol III
    - Alpha-epsilon-omega cores
      * DNA Poly Activity
    - Clamp Loading Complex
    - Beta Clamps
      * Attached to clamp loading complex and alpha-epsilon-omega cores to hold DNA
* Resolution at the terminus
  + Circular DNA
    - Interlinked
    - Topoisomerase IV
      * Similar to Topo II, in relaxing the winds
  + Linear Chromosomes
    - 5’ End of Lagging Strand?
      * Never can be replicated
    - Telomeres and Telomerase
      * Leading strand and elongation by telomerase reverse transcriptase
      * Telomere repeats
      * Telomere length by cell type
* Somatic Cell Mutation Models
  + Linear vs branched mutation hypothesis for cancer
  + Causes of somatic Mutation
* Ames Test
  + Concept of Screening
  + Loss of Function vs. Gain of Function Mutants
  + Revertants
* Source of Genetic Mutations
  + Infidelity of Replication
  + Mutagen
  + Defective Repair
  + Enzymatic Alteration
* Error Rate in Replication Kept low By:
  + 3’ 🡪 5’ exonuclease function of Pol 1 and Pol 3 = Proofreading
  + RNA primers to initiate replication may be error-prone, but are removed
  + Post-replication repair mechanisms
* Damage Sources
  + Oxidative Damage
  + UV Exposure/Radiation
  + Spontaneous Depurination (and to smaller extent Depyrimidination)
* Overview of Repair Types:
  + Direct enzymatic reversal
  + Excision Repair (NER, BER, MMR)
  + Recombination
  + SOS Response
* DNA Photolyase
  + FADH2 coFactor
  + Binds DNA lesion
  + De-cyclizes T=T dimers upon light exposure (370nm)
* Nucleotide Excision Repair (NER)
  + UVr A,B,C,D Mechanism
* Mismatch Repair (MMR)
  + MutS,H,L
  + Parental Strand vs. Newly Synthesized Strand
  + GATC sequence
* Molecular Cloning
  + Plasmids
    - Conjugative Plasmids
      * Rolling Circle Replication
      * Conjugation by pilus
      * “Gender” of cells
    - Non-conjugative Plasmids
  + Plasmid Design
    - Ori
    - Selection (Resistance Gene)
    - Restriction Sites
    - Desired Size of Plasmid
    - Promoters specific to host organism(s)
  + Bacteriophage
    - Lytic v. Lysogenic Life Cycle
  + Transposons
    - Insertion Sequences
    - Composite Transposons
    - LINES and SINES
  + Natural Plasmids
    - Dissimulative (ex. For different C-source metabolism)
    - Pathogenesis
    - R-Factor (Resistance Genes)
  + Restriction Enzymes
    - Class I and Class II
    - Blunt end vs Sticky end endonuclease activity
* Ethidium Bromide
  + Intercalcating agent (Causes DNA helix to stretch)
  + Can cause supercoiling of circular plasmids
  + Allows for DNA visualization under UV exposure
* Reporter Gene
  + LacZ
  + GFP
* Sanger Sequencing
  + ddNTPs
  + Limitations
* PCR
* Some Techniques that can use PCR
  + Diagnosis
  + Identification
  + Introduce site-directed mutations
  + Quantitative measurements of transcript abundance (PCR)
* Sequencing technologies
  + Sanger sequencing
  + Illumina
  + 454
* RNA Synthesis in Prokaryotes
  + Differences between DNA/RNA
  + Sigma Factors
  + Recognition consensus Motifs
    - Pribnow Box (-10) region
    - -35 region
  + RNA Polymerase Initiation
* Operons
  + Lac Operon
    - Repressor = LacI
    - Catabolite Repression
  + Trp Operon
    - Feedback regulation
    - RNA stem-loop structure regulation (terminator loop)
* Flagella synthesis (Transcriptional Regulation Strategies)
  + Catabolite Repression (CRP-cAMP)
  + Feedback regulation = FljB, FljA, and FliC regulatory network
  + Non-coding RNA regulation
* RNA Synthesis Regulation in Prokaryotes and Eukaryots
  + DNA structure (histones, nucleosomes)
  + No sigma-factors, but many possible transcription factors and enhancers
  + Methylation patterns