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