Exam Coverage: Viruses to DNA Sequencing

Viruses:

- Genetic material is RNA or DNA
- Genetic material is surrounded by a protein capsid
- Some virus also have a membrane surrounding the capsid
- Rely on host machinery (e.g. protein synthesis) to replicate.

HIV

- Know the overall life cycle:
  - RNA to dsDNA using reverse transcriptase
  - Intergration into host chromosome
  - expression of proteins, followed by maturation of proteins by HIV protease.
- Why reverse transcriptase and HIV protease are good drug targets (unique to viral life cycle)
- HIV reverse transcriptase inhibitors are both allosteric and competitive. Competitive inhibitor also causes chain termination.

HIV Protease:

- Overall structure of the enzyme (homodimer)
- Function of aspartic acid residues and valine 82 in the active site.
- Determine the relative effectiveness of a protease inhibitor from a reciprocal plot.
- Relate the effectiveness of a protease inhibitor to the interaction between the drug and the enzyme, considering: van der Waals, hydrogen bonding, hydrophobic, electrostatic interactions.
- Come prepared to suggest changes to a drug, based on alteration of residues in HIV protease.

Nucleic Acid Structure:

- Distinguish chemical structure of RNA from DNA (ribose/deoxy ribose, U versus T), reason for chemical instability of RNA.
- Distinguish purine from a pyrimidine
- Recognize hydrogen bond donors and acceptors on bases, both Watson Crick and others.
- Overall structure of double stranded RNA and DNA: backbone=sugar+phosphate, sidechain=bases, antiparallel strands, major and minor grooves (DNA), phosphates and sugars on the outside, bases on the inside. Sugars connected by phosphodiester bonds.
- Nomenclature of DNA sequences, bases written 5' to 3'. Parity to proteins – amino terminus to carboxy terminus.

Polymerases:

- Mechanism of chain elongation:
  - Primer required, anneals to template via Watson Crick hydrogen bonds
  - dNTPs added to 3'OH of primer, growth of chain is in the 5' to 3' direction, based added according to Watson-Crick hydrogen bonds and purine-pyrimidine matching.
- Error correction, some polymerases have 3' to 5' exonuclease activity to correct errors
- HIV reverse transcriptase lacks this proofreading activity, therefore makes mutations in its own genetic material.
- Mutations cause changes in the drug binding sites of HIV protease and reverse transcriptase, reducing the binding of drugs.

Studying Drug resistant HIV Mutations – Overall steps in producing proteins from the mutant gene in E. coli

i. Isolation of viral RNA
ii. Conversion of viral RNA to dsDNA
iii. PCR amplification of desired gene (e.g. protease).
iv. Cutting with restriction enzymes, ligation into plasmid
v. DNA sequencing of mutant gene
vi. Expression and purification of protein (no additional details for this exam)

PCR

- Reversible conversion of double stranded primer-template to single stranded DNA by heat, re-annealing by cooling, primer anneals to the same location.
- Primer design to amplify a region:
    - Left primer is exactly the sequence of the upper strand.
    - Right primer is exactly the sequence of the lower strand.
    - Adding bases (restriction sites) to ends of the PCR product accomplished by placing bases at the 5' end of the primer.
- PCR cycle: denaturation, annealing, polymerization. Each cycle doubles the amount of PCR product.

Restriction Endonucleases & DNA ligase

- Restriction enzymes are homodimeric proteins
    - Recognize DNA sequences that are the same (5'-3') on the top and bottom strand.
    - Cut both strands at the same location.
    - Produce sticky ends if the cut site is not in the center.
- You are **not** required to memorize any restriction sequences.
- Given the shorthand notation (e.g. GGG^CCC) be able to write the double stranded products of the reaction.
- Use of complementary Watson Crick basepairing of sticky ends and DNA ligase to reform phosphodiester bond.
- Using restriction enzymes and DNA ligase to insert PCR product into plasmid.
    - Sites on the PCR product have to match the sites on the plasmid
    - PCR product is digested with enzymes
    - Plasmid is digested with same enzymes
    - DNAs are mixed, and DNA ligase joins the PCR product to the plasmid.

DNA sequencing:

- Primer used to generate DNA fragments that all start at same location.
- Use of "colored" dideoxy nucleotides to identify the last base added when the chain was terminated.
- Separation of terminated DNA fragments by size gives the sequence.
- Come prepared to:
    - Read DNA sequence data of a wild-type (naturally occurring) and mutant protein.
    - Establish the reading frame given the known protein & DNA sequence of the wild-type enzyme
    - Convert the DNA sequence to protein sequence using a codon table.