Methods and Biological Principles

I. DNA
A. Structure:

1. Be able to define cDNA
Complementary DNA (cDNA) is DNA synthesized from a messenger RNA (mRNA) template in a reaction catalyzed by the enzymes reverse transcriptase and DNA polymerase. Therefore, it contains the open reading frame of genes and lacks introns.

B. Function
1. Be familiar with the processes of transcription and translation

2. Be able to define intron, exon, codon, and promotor regions of genes

3. Understand the mechanisms that lead to non-Mendelian inheritance patterns, such as imprinting and mitochondrial gene inheritance

Imprinting:
An epigenetic mechanism that allows gene expression in a parent-of-origin-dependent manner. Genes that are inactivated in the female line are said to be maternally imprinted (only the paternally derived allele is expressed and active), and vice versa. Genetic diseases associated with
Imprinting defects include Prader-Willi, Angelman, Beckwith–Wiedemann syndrome, and Silver-Russell syndromes.

**Mitochondrial inheritance:**
Genetic disorders caused by mutations in the mitochondrial (as opposed to nuclear) genome, and therefore exhibit exclusively maternal inheritance. Individuals may exhibit heteroplasmy, whereby each cell contains both normal and mutated mitochondria. Females with heteroplasmy produce eggs with variable proportions of mutated mitochondria, leading to substantial variation in disease severity in each offspring.

4. **Know the meaning of:**
- **Codon:** group of 3 consecutive mRNA nucleotides that specify one amino acid in a protein sequence.
- **Stop codon:** UAA, UAG, UGA – signal termination of translation
- **Nonsense mutation:** point mutation resulting in a premature stop codon -> truncated protein
- **Missense mutation:** point mutation resulting in a codon that encodes a different amino acid
- **Polymorphism:** natural variation in a DNA sequence, which occurs with relatively high frequency in the general population and have no adverse effects.
- **Single nucleotide polymorphism:** short sequence that varies by one nucleotide among individuals in a population. SNPs can be used as markers for mapping disease genes.
- **Frame-shift mutation:** insertion or deletion of a number of nucleotides not divisible by 3, which leads to alteration of the open reading frame, resulting in translation of a completely different protein sequence downstream of the change.
- **Gene deletion:** loss of genetic material encoding a full gene or multiple neighboring genes.

5. **Understand the following functional categories of mutations:**
- **Loss-of-function mutation:** results in a fully- or partially-inactive gene product
- **Gain-of-function mutation:** results in a more active or constitutively active gene product
- **Null mutation:** results in total absence of the gene product

6. **Know linkage disequilibrium and describe how haplotype mapping aids identification of disease-causing genes.**

In germ cells, the maternal and paternal chromosomes pair up and exchange segments of DNA in a process called recombination. Recombination is more likely to occur between loci that are located further apart on the chromosome. As a consequence, alleles at closely linked loci tend to be co-inherited, a phenomenon called linkage disequilibrium. Haplotypes are groups of alleles spanning about 60,000 bp (containing about 60 SNPs) that are rarely separated by recombination. These can be exploited for mapping of disease genes.

C. **Analytic methods**
1. **Be familiar with the types of enzymes used in molecular biology such as DNA polymerases, RNA polymerases, restriction endonucleases and their properties.**

2. **Know the techniques used to identify and isolate genetic abnormalities:**
- **Northern blot analysis:** used for detection of specific RNA in a sample. mRNA extracted from tissue/cell culture is separated by electrophoresis, then transferred to a membrane, which is then probed with a labeled nucleic acid primer complementary to the RNA sequence of interest.
- **Southern blot analysis:** used for detection of specific DNA sequence in a sample.
- **FISH (Fluorescence in situ hybridization):** Cytogenetic technique that uses fluorescent nucleic acid probes to detect and localize specific DNA sequences on chromosomes.
- **PCR amplification**: technique used for exponentially amplifying a specific segment of DNA using flanking primers and a heat-stable DNA polymerase.

- **RFLP (restriction fragment length polymorphism) analysis**: DNA profiling technique (at one point used in gene mapping and forensics) using restriction endonucleases to generate variable banding patterns from electrophoresed DNA digests from different individuals.

- **DNA methylation analysis**: Treatment of DNA with bisulphite deaminates cytosine residues to uracil but leaves 5-methylcytosine residues unaffected.

- **DNA Sequencing**: Sanger method involves 4 DNA synthesis reaction mixtures, each including DNA polymerase, all four standard deoxynucleotides and one chain-terminating dideoxynucleotide (ddATP, ddCTP, ddGTP, or ddTTP), which produce DNA segments of different lengths, corresponding to the positions of the specific nucleotides in the DNA sequence.

- **Array comparative genomic hybridization**: Total genomic DNA isolated from test and reference cell populations is differentially labeled with fluorescent dyes and hybridized to a microarray. Variation in signal intensity ratios directly correlates to DNA copy number variation within the genome of the test cells.

3. **Know the use of methods to analyze gene function, and mechanisms of transcription:**
   - **DNase footprinting**: detection of docking sequences for DNA binding proteins (e.g. transcription factors) by digestion of unbound (and thus unprotected) DNA with DNAses
   - **Chromatin immunoprecipitation**: Used to determine DNA sequences that bind specific proteins of interest or to map DNA targets of histone modifiers. DNA-binding proteins may be crosslinked to DNA, then chromatin is isolated, DNA is sheared, DNA-protein complex of interest is immunoprecipitated, cross-linking is reversed, and DNA is sequenced or hybridized to a microarray.

4. **Be able to describe chromosome abnormalities:**
   - **Aneuploidy**: abnormal number of chromosomes in a cell
   - **Microdeletion**: relatively small (<5 Mb) loss of DNA sequence
   - **Duplications**: generation of extra copies of chromosomal material by recombination
   - **Translocation**: chromosomal configuration in which part of a chromosome becomes attached to a different chromosome

5. **Describe different applications of DNA (or cDNA) microarrays:**
   - Gene expression profiling, comparative genomic hybridization, ChIP, SNP detection, alternative splicing detection

6. **Understand the inability of standard DNA sequencing assays to detect heterozygous deletion mutations.**

7. **Understand the importance of family studies to determine linkage phase of mutations detected in an individual with a genetic disease.**

II. RNA

A. Structure

1. **Be able to describe the mechanism of alternative splicing and how it leads to different mRNA species:**
B. Function
1. Know the functional differences between different types of RNA:
   - **Messenger RNA (mRNA):** RNA molecule that encodes the amino acid sequence of a protein.
   - **Ribosomal RNA (rRNA):** RNA molecules that form part of the structure of ribosomes, which catalyze the translation of mRNA into protein
   - **Transfer RNA (tRNA):** small RNA molecules that serve as adaptors between specific sets of mRNA codons and specific amino acids during translation
   - **Micro RNA (miRNA):** small non-coding RNA molecules that function in RNA silencing by either promoting degradation or inhibiting translation of specific mRNAs

C. Analytic methods
1. Be able to describe the methods used for detection and quantitation of mRNA
   a) Early approaches
      1) Northern blot
      2) Ribonuclease protection assay
      3) In-situ hybridization: can localize the RNA of interest at the anatomic or cellular level
      4) Spotted cDNA arrays
   b) Contemporary gene expression profiling includes genome-wide approaches such as
      1) Microarrays (oligonucleotide arrays)
      2) Real-time reverse transcription PCR (RT-PCR)
      3) Transcriptome sequencing (RNA sequencing)

2. Understand methods to use RNAs to analyze gene function, such as small interfering RNAs
   Transfect siRNA into cell line -> allow siRNA to bind target mRNA -> mRNA degradation -> gene silencing -> study loss of function phenotype

III. Protein synthesis
A. Protein chemistry
1. Understand the concept of a dominant negative mutation and the mechanisms involved
   Mutation resulting in expression of an altered protein, which forms a complex with and inhibits the activity of its wild-type counterpart.

B. Protein synthetic mechanisms
1. Transcription
   a. Know the general mechanism of action of transcription factors
   Transcription factors bind to cis-acting elements within gene promoters and nearby enhancers and initiate and up- or down-regulate transcription from those promoters, thereby regulating gene
expression. TFs may act by stabilizing RNA polymerase on the DNA, catalyzing histone acetylation or deacetylation, or recruiting coactivator or corepressor proteins.

2. Translation

3. Post-translational modification
   a. Know the meaning of post-translational modification
      - Disulfide bridge formation
      - Cleavage by proteases to release activated protein
      - Regulatory modification: acetylation, carboxylation, phosphorylation, methylation
      - Attachment of lipid anchor
      - Attachment of prosthetic group: heme, biotin

4. Analysis
   a. Know how to perform and interpret Western blot analysis

IV. Manipulation of gene expression
   A. Experimental
      1. Know the fundamentals of altering gene expression in experimental animals
         - Transgenic animals: express cDNA randomly incorporated into genome
         - Single gene knock-out: both alleles of a gene are deleted by homologous recombination
         - Conditional gene modification: tissue or developmental-stage specific
         - Genome editing using zinc finger nucleases and CRISPR system

   B. Therapeutic
      1. Be familiar with methods being developed for gene transfer in humans
         - Insertion of functional genes into host genome via viral vectors to replace non-functional genes
         - Nonsense suppression: read-through compounds allow for production of a functional protein
         - Antisense oligonucleotides to redirect splice site selection
2. Be familiar with endocrine conditions that might be amenable to gene therapy
   - Endocrine hypofunction
   - Endocrine hyperfunction
   - Endocrine tumors
   - Autoimmune disorders

B. Cell biology
1. Growth and metabolism at the cellular level
   a. Be able to describe basic methodologies used to examine mechanisms of growth control at
      the cellular level, such as regulation of replication and apoptosis
   - TUNEL assay: detects DNA fragmentation using terminal deoxynucleotidyl transferase enzyme
   - Flow cytometry: fluorescently labeled cells suspended in fluid and passed through an electronic
     detector apparatus allows for multiparameter analysis, including measurement of DNA degradation,
     mitochondrial membrane potential, permeability changes, and caspase activity
   - Live cell imaging: two-color fluorescence microscopy allows mapping of cell cycle phase