Lab Concepts

- Pipetting
  - Settings (in microliters):
    - P20: Min: 2 Max: 20
    - P200: Min: 20 Max: 200
    - P1000: Min: 200 Max: 1000

- Enzymes
  - Testing Enzyme Activity: Enzymes usually have an optimal condition of temperature and pH level where it is most effective. This can be determined through testing the enzyme in various environments. See General Concepts: Enzymes.
  - Spec 20: A device that measures the color or clarity of a substance through optical rays.

- Genetic Engineering
  - Transformation and Plasmids: Insertion of foreign DNA into a host cell (usually bacteria)
    1. DNA of both target and host is isolated
    2. DNA samples are cut with the same restriction enzymes (forms “sticky ends”)
    3. DNA is mixed and joined by base pairings. Both recombinant and non-recombinant plasmids are formed.
    4. Recombinant and non-recombinant cells are isolated, usually with an antibiotic which recombinant bacteria are resistant to.
  - DNA Ligase and Restriction Enzymes: DNA ligase is an enzyme that can link two strands of DNA separated with a break on both sides (such as with “sticky ends”) and is necessary to insert DNA during transformation. The restriction enzyme separates two strands at a specific sequence.

- Gel Electrophoresis: Separates DNA based on size and charge
  - Loading Dye: Weights and makes visible the DNA solution during gel electrophoresis. The dye is necessary to keep the DNA from floating and makes it visible to the unaided eye.
  - TAE Buffer: Sodium bromide solution that maintains a constant pH level in the solution/
  - Ethidium Bromide: Makes DNA visible and fluorescent under UV light.
  - Agarose: The gel used during electrophoresis.
  - Measuring Length: Shorter segments of DNA are lighter and are found farther from the starting well, whereas heavier fragments move slower and do not move as far.

- Restriction Analysis
  - Use: Determines where restriction sites for the enzyme being tested are located, usually done with gel electrophoresis.

- Hardy-Weinberg:
- **Use:** Tests whether or not a population is in equilibrium; that is, not evolving. Hardy-Weinberg equilibrium conditions:
  1. Very large population
  2. No gene flow
  3. No mutations
  4. Random mating
  5. No natural selection

- **Chi Square Analysis**
  - **Use:** Determines whether or not the observed results are within 95% range of the expected results.
    
    Equation: \[\text{Sum of } \frac{(\text{expected-observed})^2}{\text{expected}}\]

- **PCR**
  - **Process:** Temperature regulated with a thermocycler
    1. **Denaturing:** Heat separates the two DNA strands
    2. **Annealing:** DNA allowed to cool for primer attachment
    3. **Extension (also “elongation”):** Polymerase completes the replication

<table>
<thead>
<tr>
<th>Process</th>
<th>Temperature (Celsius)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturing</td>
<td>96 °C</td>
</tr>
<tr>
<td>Annealing</td>
<td>68 °C</td>
</tr>
<tr>
<td>Elongation</td>
<td>72 °C</td>
</tr>
</tbody>
</table>

- **TAQ Polymerase:** A type of DNA polymerase that builds on complementing strands for PCR DNA segments. It is used in PCR instead of DNA polymerase because it is able to survive PCR conditions, but is unable to perform mismatch repair.

- **Problems:** Non specific priming: some primers bind to places they aren’t supposed to bind because they are not specific enough. PCR also has no form of mismatch repair allowing mistakes to be ignored and further amplified.

- **Result Analysis:** The PCR product is compared to the DNA ladder with gel electrophoresis, and analyzed for matches.

- **Primer Design:** Small DNA fragments that adhere on ends of DNA strands in PCR that allows TAQ polymerase to bind. In PCR, these must be specially tailored to fit the desired sequence. Primers are ideally long enough to be specific and cut only where they are meant to.

**General Concepts**

- **Chemistry & Biomolecules**
  - **Bonds and Interactions:** Determined by electronegativity
    
    | Nonpolar Covalent | Electrons equally shared |
    |-------------------|--------------------------|
    | Polar Covalent    | Electrons found more on one side than the other |
    | Ionic             | Electrons found only on one side |
    | Hydrogen Bonds    | Bonds formed with hydrogen atoms, where the H is attached to a highly electronegative atom, rendering its charge positive, and then attracting a lone electron pair. Common among water molecules. |
    | Van Der Waals     | Temporary bonds where polar molecules attract briefly an opposite charged molecule. |

- **Molecular Diagrams:** 6 C, 10 H -------->

  Carbons are located at each junction. Each carbon has 4 bonds; bonds not shown are hydrogen molecules.
**Activation Energy**: Initial energy required for a reaction to start.
- **Endergonic**: Endothermic, absorbs heat as a product of reaction.
- **Exergonic**: Exothermic, releases heat as a product of reaction.

**pH Scale**: Concentration of hydrogen ions from a scale of 1-14; 1 being the most acidic and 14 being the most basic.

**Properties of Water**: High specific heat, solid form is less dense than liquid, versatile solvent; evaporative cooling (high heat of vaporization).

**Biomolecules**: Organic molecules part of a living organism

**Condensation and Hydrolysis reactions**:

<table>
<thead>
<tr>
<th>Condensation</th>
<th>Also called dehydration synthesis, losing a water molecule to form a covalent bond between polymers.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis</td>
<td>Adding a water molecule between polymers to break a covalent bond.</td>
</tr>
</tbody>
</table>

**Structure**:

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Lipid</th>
<th>Protein (amino acid)</th>
<th>Nucleic Acid</th>
</tr>
</thead>
</table>

**Carbohydrates**:
- Used for energy, part of other biomolecules, protection.
- Monosaccharides are simplest form, come together to form disaccharides. Always has the 1:2:1 ratio of C:H:O.
- IMPORTANT disaccharides:
  - Fructose + glucose → sucrose
  - Galactose + glucose → lactose
  - Glucose + glucose → maltose
- Glycosidic linkage: condensation that causes covalent bonds to form between monosaccharides.
- Polysaccharides
  - Energy Storage: Starch (plants) and Glycogen (animals)
  - Structural: Cellulose (plants) and Chitin (exoskeleton)
- May be in a ring-form (shown above) or straight form

**Lipids**:
- Used for energy storage, structural, protection for an organism, insulation.
- Mostly made of hydrocarbons (nonpolar and hydrophobic)
- Glycerol + 3 fatty acids
  - Saturated = solid (single bond), unsaturated = liquid (double bond)
  - Saturated | Solid, single bond, straight structure
  - Unsaturated | Liquid, double bond, bent structure
- Phospholipids: hydrophilic phosphate head, hydrophobic tails
- Steroids: chemical messenger, hormones and in cell membrane

**Nucleic Acids**:
- Used to carry genetic materials, form part of ribosomes, and act as energy carriers. Contains the phosphate group, nitrogenous base, and five-sugar base.
• Made up of nucleotides (monomers)
• DNA and RNA are all nucleic acids

Proteins:
• Used for enzymes, structural, hormones, contraction, storage, transport. Contains the H3N+ amino acid functional group.
• Made up of amino acids, which are made up of a same amino and carboxyl group that differs only at the R group.
• Condensation reaction forms a peptide bond in proteins
• Four levels of protein structure:
  1. Primary: Peptide chain, peptide bonds
  2. Secondary: Alpha Helix, Beta Sheet, hydrogen bonds
  3. Tertiary: 3D interaction: disulfide bridge, hydrogen bond, and ionic bond
  4. Quaternary: 2 or more proteins, ionic bond

Enzymes

Affect on reactions: Lowers activation energy, catalyzing the process.

Interactions with substrates: Substrates bind at the active site.

Inhibition:

<table>
<thead>
<tr>
<th>Competitive</th>
<th>Inhibitor binds to active site preventing substrate from attaching.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Competitive</td>
<td>Inhibitor binds to non-active site and alters the enzyme’s active site so that the substrate can no longer bind.</td>
</tr>
</tbody>
</table>

• Cofactors: Non proteins that help catalyze reactions; bind to enzyme and change shape of active site
• Coenzymes: organic (vitamins) molecules that binds to enzyme to improve its function

Enzyme control mechanisms:
• Phosphorylation: Tri-phosphate group from ATP is added into the enzyme changing its shape and possible inactivating it.
• Feedback Inhibition: The product of the process binds to the enzyme inactivating it.
• Allosteric Regulation: Similar to non-competitive inhibition where a molecule binds to a non-active site to change the shape of the enzyme either activating or inactivating it.

Protein Synthesis

Transcription: Creates an mRNA copy of the main DNA in order to synthesize proteins.

Translation: Synthesizes a protein from the information provided by the mRNA copy.

Mutations: Undesirable mistakes in the mRNA copy or the DNA itself. It may be either harmful or unexpressed, and in rare cases beneficial.

Control Mechanisms:

<table>
<thead>
<tr>
<th>Prokaryotes</th>
<th>Transcription factors- Proteins that help RNA Polymerase attach. Enhancers- DNA sequences that help RNA Polymerase as well as promoter sequences activate.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eukaryotes</td>
<td>Operons (inducible- factor added to turn ON, repressible- factor added to turn OFF)</td>
</tr>
</tbody>
</table>

Genetics:

Standard genetic crosses: The basic Punett square, a standard cross consists of a simple 2 alleles and their possible combinations.
- **Dihybrid crosses:** Two dihybrids are mated, and by Mendel’s Law of Independent Assortment, combine as follows:

<table>
<thead>
<tr>
<th></th>
<th>RY</th>
<th>Ry</th>
<th>rY</th>
<th>ry</th>
</tr>
</thead>
<tbody>
<tr>
<td>RY</td>
<td>RRY</td>
<td>RRYy</td>
<td>RrYY</td>
<td>RrYy</td>
</tr>
<tr>
<td>Ry</td>
<td>RRYy</td>
<td>RrYY</td>
<td>RrYy</td>
<td>rrYY</td>
</tr>
<tr>
<td>rY</td>
<td>RrYY</td>
<td>RrYy</td>
<td>rrYY</td>
<td>rrYy</td>
</tr>
<tr>
<td>ry</td>
<td>RrYy</td>
<td>Rrry</td>
<td>rrYy</td>
<td>rrry</td>
</tr>
</tbody>
</table>

- **Dominant, recessive, incomplete dominance, co-dominance:**

<table>
<thead>
<tr>
<th>Dominant</th>
<th>Allele that has priority when in expression. Usually denoted with a capital letter (e.g. “K”)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recessive</td>
<td>Allele that requires both copies to be expressed. Usually denoted with a lower case letter (e.g. “k”)</td>
</tr>
<tr>
<td>Incomplete</td>
<td>Both phenotypes are expressed separately (e.g. black and white cat)</td>
</tr>
<tr>
<td>Co-Dominance</td>
<td>Phenotype falls in the midrange (e.g. gray cat)</td>
</tr>
</tbody>
</table>

- **Sex-Linked Traits:** Traits that are linked with either the X or Y chromosome (e.g. Allele A, located on X chromosome, denoted X<sup>A</sup>)

- **Pedigree:** A family tree based on a specific genetic trait, such as the presence of a disease that can be analyzed for a specific member’s genotype.

- **RFPL Analysis-DNA Probes:** Restriction Fragment Length Polymorphism. The method treats different DNA samples with the same restriction enzyme (which cuts DNA at the same sequence). The digested samples are run on an agarose gel for analysis, where same patterns usually indicate a match or relation.

- **Hardy Weinberg**

  - Allelic Frequencies: Measures the expected frequencies of alleles through the Hardy-Weinberg Equation:
    \[ p^2 + 2pq + q^2 = 1 \]

  - Comparing expected with observed: Chi-Squared analysis where the p, pq, and q frequencies are compared.

- **Evolution**

  - **Lamarck vs. Darwin:**

    | Lamarck | Darwin |
    |---|---|
    | Acquired characteristic are inherited, species are able to will their changes. | “Natural selection” provides mechanism for selecting changes. |

- **Natural Selection:** Individuals with ideal traits are able to better survive and reproduce. Contains 5 observations:
  1. Populations will increase exponentially if all reproduced
  2. Populations, however, tend to remain stable
  3. Resources are limited
  4. There is variation within a population
  5. Unequal ability to survive leads to genetic change

- **Punctuated Equilibrium vs. Gradualism:**

<table>
<thead>
<tr>
<th>Punctuated Equilibrium</th>
<th>Periods of change are interrupted with periods of stable gene pools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gradualism</td>
<td>Change is constant, organisms develop at the same rate</td>
</tr>
</tbody>
</table>

Both theories are “correct”, type depends on conditions.
- **Bottleneck Effect, Genetic Drift, Founder Effect:**
  - **Bottleneck:** The population undergoes a drastic reduction, such as through natural disaster, causing frequencies to be either under or over represented.
  - **Genetic Drift:** Allelic frequencies in a population change overtime, usually as a result of “natural selection”.
  - **Founder Effect:** Allelic frequencies are abnormally common or rare when a small group relocates and “founds” a new population in a geographically isolated area (similar to bottleneck).

- **Geographic Isolation:** Species develop when a population is separated by a geographical feature, such as a river.

- **Mutations:** The random change in DNA sequences, it is the primary driving force behind development of new traits.

- **Fitness:** An organism’s ability to survive and reproduce (contributing to the gene pool) relative to that of others.

- **Selection:**
  - **Directional:** Either one of the extreme alleles are favored, causing an entire shift towards that direction.
  - **Disruptive:** Both extremes are favored, causing an increase in both ends and a decrease in the center. Can possibly cause “speciation”.
  - **Stabilizing:** “Heterozygous advantage”. Conditions favor the mid-ranged alleles causing frequencies to shift towards the middle.
  - **Sexual Selection:** Selection of mates. May be both inter- and intra- gender (male-male competition, female-male selection).

- **Speciation:**
  - **Allopatric:** Occurs when a population is divided physically such as with geographic isolation.
  - **Sympatric:** Occurs when a population still overlaps an area, but are unable to reproduce due to a difference of habit.
  - **Adaptive Radiation:** The “evolution of diverse species” from common ancestor.

- **Gene Flow:** Genetic addition or subtraction of an allele from a population through migration.

- **Heterozygous Advantage:** Greater fitness attributed to those who have one of each allele (e.g. sickle-cell anemia).

- **“Evidence” for evolution:**
  - **Biogeography:** Species are found in ideal geographic locations.
  - **Developmental biology:** Species seems to have “developed” traits ideal for their environment.
  - **Comparative anatomy:** Vestigial structures and other similar structures “suggest” a common ancestor.
  - **Artificial selection:** Human choice of trait by selection ideal mates.

- Mitosis/Meiosis
  - **Cell cycle stages:**
    1. **Interphase:** Chromatin duplicated
Proceeds to Mitotic phase

2. **Prophase:** Mitotic spindle forms, chromatin becomes visible.
3. **Metaphase:** Nuclear envelope dissolves, centrioles move to opposite ends, chromosomes line in the center.
4. **Anaphase:** Spindle pulls apart the sister chromatids
5. **Telophase:** Cell separates, nuclear envelope reforms

**Purpose:**
- **Mitosis:** Forms two diploid, identical daughter cells.
- **Meiosis:** Produces four haploid gametes from the parent cell.

**Identifying animal and plant cells:**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Utilizes the cleavage furrow to separate the cell during telophase.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td>Grows a rigid cell wall in between the two cells during telophase.</td>
</tr>
</tbody>
</table>

**Components:**
- **Centriole:** The two structures that form the mitotic spindle and are found at the opposite ends of the cell during mitosis.
- **Sister Chromatid:** The duplicate pair of chromatin which forms the X shape of the chromosome.
- **Diploid:** Cell containing two chromosome sets
- **Haploid:** Cell containing a single chromosome set (e.g. gamete)

**Recombination frequencies:** The farther apart genes are the greater chance they have of crossing-over. Recombination occurs in Prophase I of meiosis when homologous pairs of chromosomes form tetrads.

**Linked genes:**
- **Epistasis:** One gene product affects the expression of another gene (e.g. black color cannot be expressed without gene for creating pigment)
- **Polygenic inheritance:** Two genes work to create a single phenotype (e.g. eye color)
- **Synapsis:** The formation of tetrads in meiosis, includes crossing-over
- **Chiasma:** The location where crossing-over occurs

**Meiosis errors:**
- **Non-disjunction:** Homologous chromosomes fail to separate properly
- **Polyploidy v. Aneuploidy:**

<table>
<thead>
<tr>
<th>Polyploidy</th>
<th>Abnormal sets of chromosomes (normal set of 2, 2 sets, or 4 chromosomes, received instead).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneuploidy</td>
<td>Abnormal number of chromosomes (normal set of 2, 3 chromosomes received instead).</td>
</tr>
</tbody>
</table>
• Monosomic v. Trisomic:

- DNA Replication
  - Conservative vs. Semi-Conservative Models:

<table>
<thead>
<tr>
<th></th>
<th>Conservative</th>
<th>Semi-Conservative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formed DNA</td>
<td>Consists of one entirely new and one entirely old</td>
<td></td>
</tr>
<tr>
<td>Each of the new DNA</td>
<td>contain one new and one old strand</td>
<td></td>
</tr>
</tbody>
</table>

- Main enzymes involved:
  - Nuclease: Cuts DNA
  - Primase: Forms the initial nucleotide chain ("primer")
  - DNA Polymerase: Synthesizes nucleotides on an existing primer. Polymerase III works the leading strand (5'-3') and Polymerase I the lagging strand (3'-5).
  - Helicase: Untwists and separates the double-helix.
  - Topoisomerase (also "gyrase"): Holds down the untwisted strands relieving stress caused by helicase.
  - Single-stranded binding proteins: Bind to unwound DNA holding it down until DNA polymerase is completed.
  - Ligase: Catalyzes the covalent bond formation among nucleotides. Seals the Okazaki fragments together.

- Okazaki fragments and 5'-3'-direction: DNA synthesis can only copy the 5'-3' direction, however, the opposite direction is possible with Okazaki fragments where segments of nucleotides formed 5'-3' then joined 3'-5' onto the template.

- p53 and cancer: The enzyme p53 is activated by DNA damage where it may activate several repair proteins and possibly cause cellular suicide ("apoptosis"). Telomeres (unused DNA segments in eukaryotes) are especially short in cancerous cells since it shortens with each successive replication. Telomerase, which catalyzes telomere formation, is also especially active during cancer.

- Classification of Organisms
  - Prokaryotes and Eukaryotes:

| Prokaryotes | Does not contain a nucleaus (DNA is free-floating) |
| Eukaryotes  | Contains organelles and a nucleus                 |

- 5 kingdoms and characteristics of each:
  1. Monera: includes all bacteria, single-celled, autotrophs, contain cell-walls
  2. Protista: single celled heterotrophs, usually mobile
  3. Plantae: contain cell walls, autotrophs
  4. Fungi: detriviores, can be either single-celled or multi-cellular
  5. Animalia: multi-cellular, heterotrophs

- 3 domains:
  1. Bacteria: mild conditions
  2. Archaea: generally live in harsh conditions
  3. Eukarya: contains all eukaryotes

- Ecology
  - Biomes:

    1. Tropical Forest: Constant rainfall, high species diversity
    2. Desert: Low precipitation, varying temperatures
    3. Savanna: Dry climate, warm all year-round
    4. Chaparral: Seasonal climate, shrubs and trees (e.g. Mediterranean)
    5. Temperate Grasslands: Includes parries and veldts, seasonal and mostly grass
    6. Coniferous Forest: Largest biome, long winters and hot summers, consists mostly of cone-bearing trees
    7. Temperate Broadleaf Forest: Consists of a canopy and shrub layer, animals usually hibernate during the winter
    8. Tundra: Cold year-round, contains a permafrost
Food webs and energetics:

- 10% of the energy is transferred per step.

Population studies:

- **r/K reproduction strategies:**
  - **r** Short lifespan, rapid reproduction rate, low care for young (e.g. insects)
  - **K** Slow reproductive maturity, care for young (e.g. humans, most mammals)

Primary vs. Secondary succession:

<table>
<thead>
<tr>
<th></th>
<th>Primary</th>
<th>Secondary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lifeless area without soil (e.g. volcanic eruption)</td>
<td>Existing ecosystem cleared, soil intact</td>
</tr>
</tbody>
</table>

Viruses

- **Lytic vs. Lysogenic Cycle:**
  - **Lytic:**
    1. Phage attaches and injects DNA
    2. Phage DNA is synthesized
    3. Cell “lyses”, releasing phages
  - **Lysogenic:**
    1. Phage attaches and integrates DNA in host genome
    2. Host cell reproduces normally, copying viral genomes onto daughter cells
3. The process continues, possibly infecting a large population.

<May switch cycles>

Steps of Virus Infection:
1. **Attachment**: Virus binds to the host cell
2. **Entry**: Virus enters and degrades the host cell’s DNA
3. **Synthesis**: Viral proteins and genomes are synthesized
4. **Assembly**: New viruses are assembled
5. **Release**: Viruses exit cell, possibly killing the cell in the process

- **Bacteriophages**: Viruses that infect bacteria. Undergo the lytic and lysogenic cycles (see Viruses).

Virus Components:
1. **Viral envelope**: Consists of a protein coat. Not always present.
2. **Capsid**: Similar to a nucleus, the structure contains two RNA and reverse transcriptase.
3. **Nucleic Acid**: Consists of two identical strands of RNA which are inserted into the host cell’s genome via reverse transcriptase.