BIOL 230 LAB (CUMULATIVE REVIEW) – Important Concepts 11/26/2018

1. **Ex. 2**: Identification and Separation of Cellular Biomolecules.
   a. Organic compounds: Energy/Calorie (kilocalorie); macromolecules:
   b. Amino acids/Proteins, peptide bonds, **Ninhydrin**, **Rf values**; condensation/hydrolysis; paper chromatography (acetone/acetic acid).
   c. Carbohydrates – boil in **Benedict's reagen**t (red Cu+ precipitate - reducing sugars); Polysaccharides – brown/purple with **Iodine** (KI).
   d. Lipids – water insoluble; → **Sudan IV**, red droplets.

2. **Ex. 2b, 3**: **Gel Filtration; Diffusion and Osmosis**
   a. Mobile, immobile phase; **G-25 Sephadex beads**; Hypothesis; Benedict's reagent, **Lugol's Iodine**; ***Eluant*** Volume; relative rates of large vs. small molecule passage thru column.; 0.9% NaCl
   b. Concentration gradient, simple diffusion; Facilitated diffusion – transporter proteins; Active Transport; **Osmosis** – Osmotic pressure; **isotonic, hypertonic, hypotonic**; *Elodea* plasmolysis, swelling? With range of Sucrose concentrations: 0.2M, 0.4M, 0.6M; Osmotic Lysis –**Blood cells/hemolysis** with 0.3M glucose, fructose, glycerol, urea, $CaCl_2$ or $NaHCO_3$?
   c. Chloride – precept. with **$AgNO_3$**; Protein – **Biuret** turns pinkish-violet; Plasmolysis, Hemolysis (RBC's)

3. **Ex. 4**: Protein concentration and Standard Curve.
   a. BSA, **Standard Curve,** Spectrophotometer; Visible light; Reflected vs. Absorbed light wavelengths (color wheel); **Bradford (Coomassie Brilliant Blue) Dye Reagent**; **A_{595}**

4. **Ex. 5, 6**: Enzyme Activity – Mushroom ***Tyrosinase***
   a. Enzyme, substrate, active site; Tyrosine→ **DOPA** → Dopaquinone→ **DOPAchrome**→ → → Melanin.
   b. Mushroom caps, **sodium phosphate buffer** – pH 4.0, pH 6.0, pH 8.0. **A_{475}**.
   c. Other factors affecting enzyme activity: Concentration of **products**, concentration of **substrates/ reactants**, temperature, (pH), salt concentrations, pressure, biological activators/inhibitors.
   d. **Native PAGE** – polyacrylamide, isoelectric point, loading/sample buffer (bromophenol blue, glycerol, Tris buffer pH 8.6), electrophoresis buffer (Tris buffer & Glycine, pH 8.3), denaturing, native. Substrate (DOPA) concentration, pH and detection conditions.

5. **Ex. 9, 10**: DNA EXTRACTION & RESTRICTION DIGEST OF Suspected Romanov DNA – Anastasia???:
   - Detergents (**SDS**), **Tris** buffer (pH 8.0), **EDTA**, cold **ethanol**, ice, **potassium acetate**, ***aceto-orcein*** stain. A260, A280, **A260/A280 ratio**. 1.0 A260 = 50 µg/ml DNA. Quartz cuvettes. Filtrate, retentate.
   - ***Restriction endonucleases*** cut DNA at specific, **palindromic sites** (read 5'→3'; 3'→5') *– eg: "DNA Land; Evil Olive; Racecar; Yo Banana Boy!";* GAATTC/CTTAAG (for EcoR-I enzyme) – cuts between G and A in each strand; We used **HinD-III** → recognizes AAGCTT//TTCGAA, cutting between each pair of A's. These are our "Molecular Scissors" in DNA analysis and Recombinant DNA Technology." 37°C Incubation.
   - **Agarose Gel electrophoresis**; **Ethidium Bromide** stain. **Rf migration** (= ration of band migration/bromophenol blue dye front migration = $R_{band}/R_{dye\ front}$) values. **Bromophenol Blue**, **Xylene Cyanol** tracking dyes; **TAE** (Tris-Acetate EDTA) ***electrophoresis*** buffer, pH~8.

6. **Ex. 11**: D1S80 PCR Amplification: Molecular markers, **VNTRs**, Marion = 18 and 24 repeat alleles**.** ***Polymorphism***. Calculate Rf and number of repeats → genotype from each of your parents.
   - ***Are you a clone?*** Be able to support your answer!!! What were ***your*** fragment sizes, repeat numbers, and final D1S80 genotypes?_____, _____. What are the *Alien* fragment/allele sizes??_____
     - **Primer Mix; Master Mix.**
     - PCR cycles = 95°C **melt** template, ~60°C **anneal** primers, 72°C **elongate** with ***Taq* DNA polymerase**
     - ***n*** number of cycles, 2^n = number of copies of a single DNA template after n cycles of PCR.
     - ***DNA Acrylamide electrophoresis*** – fine separation of only a few hundred bp fragments; BPB tracking dye, Ethidium Bromide stain. TBE (Tris Borate EDTA) buffer.

7. **Ex. 12**: TRANSFORMATION & OPERON Induction: Plasmids = self-replicating "mini-chromosomes"; Recombinant **pGLO** plasmid has Jellyfish **GFP** ("***reporter gene***") under control of **Arabinose Operon Promoter (P_{BAD})**. Our E. coli bacterial cells keep the plasmid, because we grow them in the presence of Ampicillin – and ampicillin resistance is encoded on pGLO – so they will die without it (selectable marker on the plasmid).
   - **AraC Activator / Inhibitor Protein** binds near the **P_{BAD} promoter,** enhancing transcription of the promoter only when **Arabinose, the substrate of the structural genes, is present (and glucose is absent!!)**. AraC can distinguish between the stereoisomers/ ***enantiomers***, D-arabinose and L-arabinose. **$CaCl_2$** (neutralizes DNA and bacterial cell wall and plasma membrane) and heat-shock at **42°C** loosen the cell wall and cell membrane to facilitate (make cells "competent") for transformation by the pGLO plasmid (***amp^R*** gene & ***ara*** operon promoter connected to **GFP**).
   - Transformed bacteria were selected on nutrient agar plates with **Ampicillin** and D or L arabinose, and with or without the presence of glucose. **UV light (or Black light = long wavelength violet)** was used to visualize colonies expressing Green Fluorescent Protein (produced when P_{BAD} is activated by the proper Arabinose enantiomer.).

8. **Ex. 14, 15**: MITOSIS, MEIOSIS, and FERTILIZATION:
   - Chromosomes, sister chromatids, crossing over, centromeres, kinetochores, microtubule spindle fibers, asters, centrioles/centrosomes,
   - **Mitosis**: Prophase, (prometaphase), Metaphase, Anaphase, Telophase; Interphase.
   - **Meiosis**: Interphase, Pro-I, Meta-I, Ana-I, Telo-I (Interkinesis); Pro-II, Meta-II, Ana-II, Telo-II.
     - ***Recognize all stages of mitosis!!***

   - **Monohybrid cross:** 3:1 phenotypic ratio (1:2:1, AA:Aa:aa, genotypic ratio)
   - **Dihybrid Cross:** 9:3:3:1 phenotypic ratio; but still 3:1 ratio for each gene!!

- *Homozygote, Heterozygote, Allele, Gene, Trait, Genotype, Phenotype, Dominant, Recessive.*
- *Diploid, haploid, Trisomy, Monosomy, **LOCUS**, **independent assortment**, **segregation** of alleles.*

GENERAL:

- **Skills**: (Micro)Pipetting, weighing, measuring, calculating migrations (Rf), PCR, electrophoresis
  - Extracting macromolecules – DNA, Protein
- Comparing Chromatography/**separation techniques**: paper, column (Gel Filtration; affinity/exchange/HIC); fluid phase, solid phase, electrophoresis (polyacrylamide gel, Agarose gel).
- Know your ***D1S80*** Genotype!!!! Do the calculations, if you have not already!!! ☺

➢ Question Types: fill-in/terminology; multiple-choice; problem solving, graphs, and data analysis; lab procedures (manual/hands-on activity!).