

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 reagent** (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, CaCl₂ or NaHCO₃?
 - c. Chloride – precept. with **AgNO₃**; 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**; **A₅₉₅**

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. **A₄₇₅**.
 - 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:** DNA Extraction/Quantitation; A260, A280, A260/A280, SDS, Potassium acetate (KOAc), cold temps; Cold alcohol (ethanol or isopropanol); DNA precipitation with alcohol; looping-out DNA. EDTA, chelator, Tris base, alkaline pH.

6. **Ex. 10:** RESTRICTION DIGEST OF Suspected Romanov DNA – Anastasia???: **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** (= ratio 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.

7. **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? _____
 - **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, **2ⁿ** = 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.

8. **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. **CaCl₂** (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.).
9. **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 techniques: paper, column (Gel Filtration; affinity/exchange; fluid phase, solid phase), electrophoresis.
 - Know your **D1S80** Genotype!!!!
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- Question Types: fill-in/terminology; multiple-choice; problem solving and data analysis; lab procedures (manual activity!).